

LASER POWDER BED FUSION OF Ti_xCu ALLOYS FOR BIOMEDICAL APPLICATIONS

NTHABISENG NHLAPO

Dissertation submitted in Fulfilment of the Requirement for the Degree
DOCTOR OF ENGINEERING IN MECHANICAL ENGINEERING
in the Department of Mechanical and Mechatronics Engineering
Faculty of Engineering and Information Technology
at the Central University of Technology, Free State



Promoter: Dr Thywill Cephas Dzogbewu
Co- Promoter: Prof Olga de Smidt

September 2025
Bloemfontein, South Africa

DECLARATION OF INDEPENDENT WORK

I, Nthabiseng Nhlapo (student number _____), hereby declare that this dissertation, submitted to the Central University of Technology, Free State, for the degree Doctor of Engineering in Mechanical Engineering, is my own independent work. This dissertation complies with the Code of Academic integrity and other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State. It has not been submitted previously to any institution by me or any other person in fulfilment of the requirements for the attainment of any qualification.

Signature of Candidate
Nthabiseng Nhlapo

01/11/2025

Date

ACKNOWLEDGEMENT

- I am eternally grateful to almighty God for my existence, the opportunities and skills He has gifted me, and the people He has brought into my life.
- To my father, Ntate Tello Ben Nhlapo, words cannot even begin to describe how grateful, honoured and proud I am to be your child. Your unconditional love, unwavering support and constant sacrifices have extended my reach further than I could have imagined and enabled me to strive to be the best possible version of myself. You have been the wind beneath my wings.
- I am grateful to my supervisors, Dr Dzogbewu and Prof de Smidt, for going above and beyond the scope of mentorship in bringing this project into existence, seeing it through against all odds and expanding my horizons as a researcher. I will forever treasure how you uplifted me when I felt defeated, for seeing greatness in me and for supervising me as a student and as a person.
- To my mother, Mme Mosele Esther Nhlapo, who left us much too soon, the lessons you imparted to me will forever be my compass as I navigate through life as a woman and a mother. Your words of wisdom echo in the darkest tunnels and harmonise in the brightest sunshine. I am because you were.
- To my girls, Teboho and Tumisho, my everyday motivation.
- To my friends and family at large, the conversations and phone calls, and the relentless asking of me, “When are you finishing?” kept me going.
- I thank all the various chairs that have funded and kept this project alive.
- I thank my colleagues at the CRPM and PDTs for the technical support, at Ya Rona Build for the work-friendly environment and the CUT library staff members.
- Thank you to my collaborators at BITRI, Dr Bella Pule and Dr William Motswainyana, for your patience and consistency over the years.
- Thank you to my collaborators at RUBIC, Prof Earl Prinsloo and Dr Rose Kadye, for your guidance and patience.
- I thank my service providers at UFS, especially Dr Megan Welman–Purchase. Your immense and selfless support to our Department, even during COVID-19, has been invaluable in advancing critical aspects of my research. To Hanlie Grobler of the UFS Centre for Microscopy, the efficiency of your work and the extra miles you went to made my journey easier.

ABSTRACT

Laser powder bed fusion (LPBF) technology is an additive manufacturing (AM) technique used to build objects layer-by-layer from three-dimensional (3D) computer-aided design (CAD) models. This type of technology provides almost unchallenged design freedom and enables the manufacture of patient-specific components while maintaining short turnaround times. Titanium (Ti) and its alloys (particularly Ti6Al4V) are the preferred materials for bioengineering implants in the orthopaedic and dental fields due to their outstanding specific strength, light weight, biocompatibility, and corrosion resistance. However, Ti is bioinert, which limits its biocompatibility. Modifying the surfaces of Ti implants by coating them with polymer nanofibres (NFs) may promote their bioactivity.

Electrospinning is the preferred method for coating surfaces with ultrafine polymer fibres that mimic the extracellular matrix (ECM). Metals and polymers do not inherently possess antibacterial properties and may attract bacterial attachment due to their biocompatibility. Copper (Cu) has broad-spectrum antibacterial activity and, unlike most metal-based antibacterial agents, is an essential element for human health and exhibits low cytotoxicity at controlled concentrations. Thus, adding Cu to Ti-based implants and their polymer coatings may impart bactericidal properties to the biomaterials. The current project aimed to employ LPBF to manufacture Ti6Al4V and commercially pure Ti (cpTi) implant materials in situ alloyed with Cu, and to coat the Ti-based materials with electrospun polymeric NFs and NFs imbued with Cu particles. The mechanical properties of the alloys were then determined, and the functionality of the alloys and coated material was investigated for antibacterial effect and biocompatibility with human cells.

Several process parameters control the characteristics of the final part fabricated using the LPBF procedure; hence, crucial parameters that contribute to obtaining samples with the highest density (namely, layer thickness, laser power, scanning speed and hatching distance) were optimised by conducting the single track experiment. The experiment entailed producing single tracks at various scanning speeds and laser powers, manufacturing double layers at selected speeds and powers while varying the hatch distance, analysing the microstructures and porosity of three-dimensional objects produced at selected speeds, powers, and hatch distances, and finally selecting the optimum parameters. Optimised parameters were applied to manufacture samples from powders of

Ti6Al4V- x Cu, cpTi and Ti- x Cu ($x = 3, 6$ wt.%). Previously determined parameters were used for the Ti6Al4V ELI powder.

Various polymer combinations were explored to determine the most suitable polymer blends for coating Ti6Al4V ELI and cpTi using the electrospinning technique. Suitability was primarily based on adhesion strength at the metal-polymer interface and the potential antibacterial effect. The polymer blends selected were polycaprolactone-polyurethane (PCL/PU) and its copper oxide nanoparticle (CuO NPs)-infused counterpart (PCL/PU/CuO). The PCL/PU and PCL/PU/CuO NPs solutions were used to coat Ti6Al4V ELI and cpTi test specimens successfully by electrospinning. The coatings were characterised. Their adhesion strengths were determined using the direct pull-off adhesion test and were found to be comparable to those reported in the literature.

Mechanical properties (that is, yield strength, ultimate tensile strength, modulus of elasticity and ductility quantifiers) of LPBF-manufactured Ti6Al4V- x Cu, cpTi and Ti- x Cu ($x = 3, 6$ wt.%) specimens were determined using the standard tensile test. The results showed that strength increased and ductility decreased with increasing Cu content. Fractography was conducted to study the fracture behaviour of the tensile-tested surfaces. The effect of Cu was not evident during fracture analysis, and the Ti6Al4V-Cu alloys showed lower ductility than the cpTi alloys with Cu.

The Ti6Al4V- x Cu and Ti- x Cu ($x = 3, 6$ wt.%) and NF-coated Ti6Al4V ELI and cpTi samples were investigated for antibacterial effectiveness and their ability to integrate with human cells without cytotoxicity. The antimicrobial efficacy of the samples was assessed using the agar diffusion assay and plate count method. Using the plate count method, antibacterial activity was evident on NF-coated surfaces and was associated with Cu/CuO-containing samples. C2C12 and MDA-MB-231 cell lines were used to study biocompatibility. Cell toxicity was observed from Ti6Al4V ELI, Ti6Al4V-3Cu, Ti6Al4V-6Cu and Ti-6Cu toward cell lines and was attributed to the Cu content. The NF-coated specimens exhibited the least cytotoxicity and the most cell growth promotion.

The investigations were instrumental in achieving highly dense Ti6Al4V ELI and cpTi LPBF parts and modifying them with Cu alloying and surface modification through coating with PCL/PU and PCL/PU/CuO NFs using electrospinning. The addition of Cu did not adversely affect the renowned

mechanical properties of Ti-based material. Moreover, Cu/CuO particles exhibited antibacterial properties. The NFs enhanced the antibacterial properties and biocompatibility of Ti surfaces with human cells.

LIST OF PUBLICATIONS

- 1) **Nhlapo N.**, Dzogbewu T.C. & de Smidt O. 2022. Nanofiber polymers for coating titanium-based biomedical implants. *Fibers*, 10(4), 36. <https://doi.org/10.3390/fib10040036>
- 2) **Nhlapo N.**, Dzogbewu T.C. & de Smidt O. 2020. The use of nano-modification towards osseointegrative capacity of Ti-based implants: A systematic review, *Proceedings of the 20th International Conference of the Rapid Product Development Association of South Africa*, 6-8 November 2019, Bloemfontein, South Africa, pp. 343-361
- 3) **Nhlapo N.**, Dzogbewu T.C. & de Smidt O. 2020. A systematic review on improving the biocompatibility of titanium implants using nanoparticles, *Manufacturing Review*, 7, 31. <https://doi.org/10.1051/mfreview/2020030>

TABLE OF CONTENTS

DECLARATION OF INDEPENDENT WORK.....	ii
ACKNOWLEDGEMENT.....	iii
ABSTRACT	iv
LIST OF PUBLICATIONS	vii
TABLE OF CONTENTS	viii
LIST OF FIGURES.....	xiii
LIST OF TABLES	xx
LIST OF ABBREVIATIONS	xxi
Chapter 1 INTRODUCTION.....	1
1.1. Background	1
1.2. Aim and objectives of the study	3
1.3. Thesis statements	4
1.4. The scope of the study	4
1.5. Research methodology	4
1.6. Expected contribution	6
1.7. Chapter layout.....	6
References	8
Chapter 2 LITERATURE REVIEW	11
2.1. Introduction.....	11
2.2. Additive manufacturing	12
2.2.1. Laser powder bed fusion	14
2.2.2. Optimising the process parameters of LPBF	16
2.2.2.1. Single tracks and single layers	20
2.2.2.2. Laser scanning strategy	20
2.2.2.3. Defects in LPBF 3D object	22
2.2.2.4. Residual stress.....	25
2.2.2.5. Effect of process parameters on 3D part defects	26
2.3. LPBF for medical implants.....	28
2.4. Biomaterial for medical application.....	28
2.5. Titanium and alloys as implants.....	29

2.5.1. In situ alloying with copper	38
2.5.2. Cu as an antibacterial agent	41
2.5.2.1. Copper oxide nanoparticles.....	43
2.6. Surface features and modification.....	45
2.7. Electrospinning: Fabrication of polymer nanofibres for coating Ti implant surfaces...46	
2.7.1. Principle of electrospinning	46
2.7.2. Electrospinning parameters.....	48
2.7.3. Application of electrospun fibres in tissue engineering.....	53
2.8. Summary	56
References	57
Chapter 3 IN SITU LPBF ALLOYING.....	74
3.1. Introduction.....	74
3.2. Materials and methods	75
3.2.1. EOSINT M 280 machine	75
3.2.1.1. Sieving of the powder	77
3.2.1.2. Platform preparation	78
3.2.1.3. Re-coater blade settings	78
3.2.1.4. Protective atmosphere.....	78
3.2.1.5. CAD modelling.....	79
3.2.1.6. Support structures	80
3.2.1.7. Loading and controlling the LPBF building	81
3.2.2. Powders.....	81
3.2.2.1. In situ alloying	82
3.2.2.2. Characterisation of employed powders.....	83
3.2.2.3. Flowability of employed powders	84
3.2.3. Metallographic preparation.....	84
3.2.4. Optimisation of powders employed using LPBF	86
3.2.4.1. LPBF single tracks.....	87
3.2.4.2. LPBF double layers.....	88
3.2.4.3. LPBF 3D objects.....	90
3.2.4.4. Porosity	91

3.2.4.5. Microstructure.....	92
3.2.5. Statistical analysis.....	92
3.3. Results and discussion	92
3.3.1. Characterisation of employed powders.....	93
3.3.1.1. In situ alloys.....	95
3.3.2. LPBF single tracks.....	96
3.3.2.1. Top view analysis of single tracks.....	99
3.3.2.2. Cross-sectional analysis of single tracks.....	113
3.3.3. LPBF double layers.....	128
3.3.3.1. Top surface analysis of double layers.....	130
3.3.3.2. Cross-sectional analysis of double layers	150
3.3.4. LPBF 3D objects.....	153
3.3.4.1. Porosity	154
3.3.4.2. Microstructure.....	162
3.4. Summary	170
References	171
Chapter 4 EVALUATION OF MECHANICAL PROPERTIES	183
4.1. Introduction.....	183
4.2. Materials and methods	184
4.2.1. Sample preparation and characterisation	184
4.2.2. Surface roughness	185
4.2.3. Microhardness test	185
4.2.4. Tensile test	186
4.2.5. Statistical analysis.....	188
4.3. Results and discussion	188
4.3.1. X-Ray diffractometry (XRD).....	188
4.3.2. Surface roughness	191
4.3.3. Microhardness test	192
4.3.4. Tensile test	195
4.3.4.1. Fractography	199
4.4. Summary	202

References	204
Chapter 5 COATING OF TI SAMPLES WITH POLYMER NANOFIBRES USING ELECTROSPINNING	210
5.1. Introduction.....	210
5.2. LPBF parts	211
5.3. Electrospinning	211
5.3.1. Parameters influencing the electrospinning process	212
5.3.2. Setup... ..	213
5.3.3. Coating technique	214
5.4. Direct pull-off adhesion test	214
5.5. Material	218
5.5.1. Polymers	218
5.5.2. Copper oxide (CuO) nanoparticles	221
5.6. Preliminary studies	223
5.6.1. First preliminary study: PCL/PEG NFs blends.....	223
5.6.2. Second preliminary study: PCL- and PMMA-based polymer NFs	225
5.6.3. Third preliminary study: PCL/PEG/CuO and PCL/PU/CuO blend NF systems	226
5.6.3.1. Application of primer.....	227
5.6.3.2. Coating with fortified NFs	228
5.6.4. Fourth preliminary study: Adhesion of PCL/PU/CuO NFs	229
5.6.5. Fifth preliminary study: Final coating material composition	230
5.7. Final samples: PCL/PU and PCL/PU/CuO nanofibre coatings	232
5.7.1. Characterisation of nanofibres on coated surfaces.....	234
5.7.1.1. Diameters	234
5.7.1.2. X-ray diffraction (XRD)	237
5.7.2. Surface roughness	240
5.7.3. Adhesion strength	242
5.8. Summary	247
References	248
Chapter 6 BIOLOGICAL ASPECTS OF TI BASED IMPLANTS	256
6.1. Introduction.....	256

6.2. Materials and methods	257
6.2.1. Sterilisation	258
6.2.2. Microbiological analyses	259
6.2.2.1. Bacterial strains and their preservation	259
6.2.2.2. Culture media	263
6.2.2.3. Agar diffusion assay	263
6.2.2.4. Plate count method	266
6.2.3. Tissue culture	267
6.2.3.1. Biocompatibility	267
6.2.3.2. Resazurin cell viability assay	270
6.2.3.3. SEM observation of adherent cells	272
6.3. Results and discussion	274
6.3.1. Sterilisation	274
6.3.2. Microbiological analyses	275
6.3.2.1. Agar diffusion assay	275
6.3.2.2. Plate count method	278
6.3.3. Cell culture	290
6.3.3.1. Resazurin assay	306
6.4. Summary	309
References	310
Chapter 7 CONCLUDING REMARKS AND RECOMMENDATIONS	323
7.1. Conclusion	323
7.2. Verification of research objectives	326
7.3. Recommendations for future research	328
APPENDIX A: Stress versus strain curves of LPBF cpTi and Ti64 alloys	330
APPENDIX B: Illustrations of EDS elemental mapping for C2C12	333
APPENDIX C: Illustrations of EDS elemental mapping for MDA-MB-231	334
APPENDIX D: Average relative fluorescence units (RFU) from resazurin assay	335

LIST OF FIGURES

Figure 2.1: Seven process categories of additive manufacturing (adapted from ISO (2021))	14
Figure 2.2: Schematic of a typical LPBF machine (adapted from Leung et al. (2018)).....	16
Figure 2.3: Parameters regulating the LPBF process (adapted from Aboulkhair et al. (2014) and Taghian et al. (2023)).....	17
Figure 2.4: Primary process parameters of LPBF (adapted from Cobbinah et al. (2021)).....	18
Figure 2.5: A schematic presentation of LPBF process (adapted from Islam et al. (2024))	18
Figure 2.6: Scanning strategies of (i) zig-zag scan vectors with no rotation between the layers, (ii) unidirectional scan vectors, and (iii) zig-zag scan vectors with 90° rotation between the layers (adapted from Thijs et al. (2010)).....	21
Figure 2.7: As-built LPBF surface (adapted from Fox et al. (2016))	23
Figure 2.8: Defects of the LPBF process	24
Figure 2.9: Application of cpTi and Ti-based alloys as implants in the human body (adapted from Sarraf et al. (2022)).....	30
Figure 2.10: Titanium phases and main transformation temperatures (adapted from Cao et al. (2021) and Farrokhi (2023))	32
Figure 2.11: Different types of stabiliser in titanium alloys (i) neutral, (ii) α -stabilising, (iii) β -stabilising (isomorphous, for example, Ti6Al4V), and (iv) β -stabilising (eutectoid, for example, binary titanium-copper diagram) (adapted from Ahmad & Zuhailawati (2020)).....	33
Figure 2.12: Microstructure illustrations of LPBF-processed (i) cpTi in the as-printed state (adapted from Pede et al. (2021)), (ii) as-built Ti6Al4V, and (iii) to (v) heat-treated Ti6Al4V (adapted from Lekoadi et al. (2021))	35
Figure 2.13: Optical micrographs illustrating (i) bimodal, and (ii) Widmanstätten microstructures (adapted from Hosseini et al. (2017))	36
Figure 2.14: Microstructure development of Ti6Al4V with variation in heat treatment temperature (adapted from Jamhari et al. (2023)).....	37
Figure 2.15: Effect of heat treatment on the microstructure and mechanical properties of LPBF Ti64 samples (adapted from Jamhari et al. (2023))	37
Figure 2.16: Possible melting process of in situ alloying of Ti and Cu during LPBF process (adapted from Mosallanejad et al. (2021)).....	40

Figure 2.17: A calculated Ti–Al–V–Cu quaternary phase diagram (adapted from Vilardell et al. (2020)).....	41
Figure 2.18: Antibacterial mechanisms of copper nanoparticles in a bacterial cell (adapted from Xu et al. (2022)).....	44
Figure 2.19: Schematic diagram of a basic electrospinning apparatus.....	47
Figure 2.20: Critical entanglement concentration at a particular polymer concentration (adapted from Li & Bou-Akl (2016))	48
Figure 2.21: Changes in morphology of electrospun nanofibres with viscosity shown as (a–d) a schematic (adapted from Al-Abduljabbar & Farooq (2023)), and (e–h) scanning electron microscope (SEM) micrographs of polyethylene oxide with viscosity increased from left to right at 32, 74, 289 and 1835 centipoise (adapted from Fong et al. (1999))	51
Figure 2.22: Influence of voltage on the formation of the Taylor cone (adapted from Al-Abduljabbar & Farooq (2023))	51
Figure 2.23: (i) Schematic illustration of the extracellular matrix (ECM) (adapted from Soles et al. (2023)), and (ii) cells grown in vivo on randomly electrospun poly(L-lactic acid) NF scaffold (adapted from Yin et al. (2015))	54
Figure 3.1: EOSINT M 280 machine installed at CRPM (CUT) and essential components.....	76
Figure 3.2: CAD modelling framework with applicable software (adapted from Dzogbewu (2017))	80
Figure 3.3: Turbula® mixer	83
Figure 3.4: JEOL JSM-6610 Scanning Electron Microscope.....	84
Figure 3.5: (i) CitoPress-1 machine, and (ii) Tegamin-25 polishing machine	85
Figure 3.6: (i) Lab Companion UC-02 Ultrasonic Cleaner, and (ii) ESCO Ductless Fume Hood.....	86
Figure 3.7: ZEISS Axio Scope.A1 Optical Microscope	86
Figure 3.8: The cross-section of the LPBF single track.....	88
Figure 3.9: Mitutoyo SURFTEST SJ-210 profilometer	89
Figure 3.10: Scanning and building coordination system (adapted from Thijs, et al. (2010))	90
Figure 3.11: (i) GE Phoenix Nanotom S nanoCT scanner, and (ii) GE Phoenix VTomeX L240 microCT scanner (adapted from Stellenbosch University (2024)).....	91

Figure 3.12: SEM micrographs (scale = 100 μm) of the employed Ti6Al4V ELI, cpTi and Cu powder illustrated by (i), (iii) and (v), respectively, and particle size distribution curves of the employed Ti6Al4V ELI, cpTi and Cu illustrated by (ii), (iv) and (vi), respectively.....	94
Figure 3.13: SEM micrographs (scale = 100 μm) showing spherical morphology of in situ alloyed (i) Ti6Al4V-3Cu, (ii) Ti6Al4V-6Cu, (iii) Ti-3Cu, and (iv) Ti-6Cu powders (Cu particles are indicated in green).....	95
Figure 3.14: Top view of a substrate illustrating single tracks with continuity and typical metallurgical defects	96
Figure 3.15: Scanning speed and the corresponding linear energy density	98
Figure 3.16: Ti6Al4V-3Cu single tracks at various laser powers and scanning speeds (scale = 100 μm).....	102
Figure 3.17: Ti6Al4V-6Cu single tracks at various laser powers and scanning speeds (scale = 100 μm).....	103
Figure 3.18: cpTi single tracks at various laser powers and scanning speeds (scale = 100 μm)	104
Figure 3.19: Ti-3Cu single tracks at various laser powers and scanning speeds (scale = 100 μm)	105
Figure 3.20: Ti-6Cu single tracks at various laser powers and scanning speeds (scale = 100 μm)	106
Figure 3.21: Process maps of sintered single tracks: top view	108
Figure 3.22: Width of single tracks at different laser powers and scanning speeds	110
Figure 3.23: Width of the single tracks versus linear energy density	112
Figure 3.24: Cross-sectional morphologies of single layer tracks of (i) lack/small penetration, (ii) optimum conduction mode penetration, and (iii) keyhole mode penetration	113
Figure 3.25: Re-melted depths of sintered tracks at varying scanning speeds and laser powers	115
Figure 3.26: Cross-sections of Ti6Al4V-3Cu single tracks at various laser powers and scanning speeds (scale = 20 μm).....	117
Figure 3.27: Cross-sections of Ti6Al4V-6Cu single tracks at various laser powers and scanning speeds (scale = 20 μm).....	118
Figure 3.28: Cross-sections of cpTi single tracks at various laser powers and scanning speeds (scale = 20 μm).....	119

Figure 3.29: Cross-sections of Ti-3Cu single tracks at various laser powers and scanning speeds (scale = 20 μm)	120
Figure 3.30: Cross-sections of Ti-6Cu single tracks at various laser powers and scanning speeds (scale = 20 μm)	121
Figure 3.31: Ratio of re-melted depth to half-width of the single tracks (red dashed line showing rate ratio of 1.0).....	123
Figure 3.32: Heights of the single tracks at varied laser powers and scanning speeds (red dashed line showing powder layer thickness constant at 50 μm)	125
Figure 3.33: Process maps of single tracks produced: cross-sectional view	127
Figure 3.34: Double layers sintered on Ti6Al4V substrate	130
Figure 3.35: Top view of LPBF surfaces from Ti6Al4V-based and Ti-based in situ alloys (scale = 100 μm).....	131
Figure 3.36: Surface roughness R_a values of double layers at varied hatch distances.....	136
Figure 3.37: Surface roughness R_z values of double layers at varied hatch distances.....	138
Figure 3.38: Single scanned and rescanned double layers at 80–100 μm hatch distances (scale = 100 μm).....	141
Figure 3.39: Cu distribution map by SEM-EDS spectra from various powder compositions....	147
Figure 3.40: Cross-sectional view of LPBF double layers of Ti6Al4V and cpTi in situ alloys with Cu (scale = 50 μm).....	151
Figure 3.41: LPBF 3D objects manufactured from Ti64- and cpTi-based powders for optimisation process.....	153
Figure 3.42: Porosity nanoCT scans of Ti6Al4V-3Cu samples produced at (i) $P = 170\text{ W}$, $V = 0.2\text{ m/s}$ ($VED = 212.5\text{ J/mm}^3$), and (ii) $P = 300\text{ W}$, $V = 0.5\text{ m/s}$ ($VED = 150\text{ J/mm}^3$). Building direction is along the Z axis	158
Figure 3.43: Porosity nanoCT scans of Ti6Al4V-6Cu samples produced at (i) $P = 150\text{ W}$, $V = 0.1\text{ m/s}$ ($VED = 375\text{ J/mm}^3$), and (ii) $P = 300\text{ W}$, $V = 0.3\text{ m/s}$ ($VED = 250\text{ J/mm}^3$). Building direction is along the Z axis	158
Figure 3.44: Porosity microCT scans of cpTi samples produced at (i) $P = 150\text{ W}$, $V = 0.3\text{ m/s}$ ($VED = 125\text{ J/mm}^3$), and (ii) $P = 170\text{ W}$, $V = 0.6\text{ m/s}$ ($VED = 70.8\text{ J/mm}^3$). Building direction is along the Z axis.....	159

Figure 3.45: Porosity microCT scans of Ti-3Cu samples produced at (i) $P = 150$ W, $V = 1.4$ m/s and ($VED = 26.8$ J/mm ³), and (ii) $P = 170$ W, $V = 0.6$ m/s ($VED = 70.8$ J/mm ³). Building direction is along the Z axis	159
Figure 3.46: Porosity microCT scans of Ti-6Cu samples produced at (i) $P = 150$ W, $V = 0.4$ m/s and ($VED = 93.8$ J/mm ³), and (ii) $P = 170$ W, $V = 0.6$ m/s ($VED = 70.8$ J/mm ³). Building direction is along the Z axis	160
Figure 3.47: Microstructure of cross-sections of the 3D LPBF samples	163
Figure 4.1: Malvern PANalytical Empyrean X-Ray diffractometer located at the University of the Free State	185
Figure 4.2: FM-700 Digital Vickers Microhardness Tester.....	186
Figure 4.3: Scaled-down tensile test specimens (i) on a Ti6Al4V substrate after LPBF manufacturing and stress relieving, and (ii) after being removed from the substrate and machined to a dumbbell shape according to the dimensions indicated in (iii) (all dimensions are in mm)	187
Figure 4.4: MTS Criterion Model 43 electromechanical universal testing machine	188
Figure 4.5: X-ray diffraction patterns of (i) Ti6Al4V-Cu and (ii) Ti-Cu alloys.....	189
Figure 4.6: Surface roughness measurements of Cu-alloyed Ti64 and cpTi specimens	192
Figure 4.7: Microhardness of Ti-based alloys at different cross-sections	194
Figure 4.8: Tensile properties of titanium/copper alloys	196
Figure 4.9: Correlation between strength and microhardness values of various Ti samples.....	199
Figure 4.10: Tensile fracture surfaces of stress-relieved LPBF Ti-based samples.....	200
Figure 5.1: Test specimens fabricated using LPBF of (i) round discs (10×2 mm), and (ii) rectangular prisms ($30 \times 30 \times 2$ mm) whilst attached to the support structures	211
Figure 5.2: Bioinicia Fluidnatek® LE-100 electrospinning equipment situated at BITRI.....	213
Figure 5.3: DeFelsko PosiTest AT-A automatic Adhesion Tester kit	215
Figure 5.4: (i) Differentiation between the test system fractures, (ii) demonstration of interpreting the nature of a fracture, and (iii) the recording thereof with estimated percentages on the adhesion tester display screen (adapted from DeFelsko (2023))	217
Figure 5.5: In situ production of CuO NPs	222
Figure 5.6: Agar diffusion assay of PCL/PEG nanofiber-coated Ti6Al4V samples	225
Figure 5.7: Micropipette setup for coating of metal substrates with polymer primer	227

Figure 5.8: (i) Shimadzu UV-2600 UV-Vis Spectrophotometer setup, and (ii) UV-vis absorption spectrum of copper oxide nanoparticles solutions at 0.2%, 0.3% and 0.5% w/v	231
Figure 5.9: Electrospun PCL/PU/CuO (3:2:0.5, w/w/w) polymer NF mat peeling off from substrate	232
Figure 5.10: Ti samples coated with electrospun membrane composite of (i) PCL/PU, and (ii) PCL/PU/CuO	234
Figure 5.11: SEM micrographs and fibre diameter distribution curves of (i) PCL/PU, and (ii) PCL/PU/CuO	236
Figure 5.12: X-ray diffraction patterns of (i) Ti6Al4V, and (ii) cpTi coated samples (NF = PCL/PU; NF Cu = PCL/PU/CuO NPs)	239
Figure 5.13: Surface roughness measurements of bare and coated Ti64 and cpTi specimens (NF = PCL/PU; NFCu = PCL/PU/CuO)	241
Figure 6.1: Biosafety cabinets installed at (i) CAFSaB, and (ii) RUBIC	258
Figure 6.2: (i) ALP KT-30L Autoclave, and (ii) Precision Incubator Memmert INB.....	259
Figure 6.3: Microbiology testing equipment sited at CAFSaB (i) LASEC® Shaking Incubator BSI-3, (ii) Interscience easySpiral Pro® automatic plater; (iii) Interscience Scan® standard plate n°3, and (iv) Interscience Scan®1200 HD automatic colony counter and inhibition zone reader.....	265
Figure 6.4: (i) Heal Force CO ₂ incubator, and (ii) Zeiss Axio Vert.A1 FL Inverted Transmitted Light Microscope Lab.....	268
Figure 6.5: (i) Invitrogen™ Countess™ 3 FL Automated Cell Counter with the counter slide, and (ii) addition of cells/trypan blue mixture into the Improved Neubauer haemocytometer	269
Figure 6.6: Invitrogen™ EVOS™ FL Auto 2 Imaging System	270
Figure 6.7: Resazurin assay reaction during cell viability testing (adapted from Creative Bioarray (2025)).....	271
Figure 6.8: Varioskan LUX Multimode Microplate Reader.....	272
Figure 6.9: UFS Centre for Microscopy housed (i) Tousimis SAMDRI®-795 critical point dryer, (ii) Leica EM ACE600 high vacuum sputter coater, and (iii) JEOL JSM-IT200 InTouchScope™ Scanning Electron Microscope and JEOL JSM-7800F Schottky Field Emission Scanning Electron Microscope equipped with EDS	273
Figure 6.10: Inhibition zones from agar diffusion assay	277

Figure 6.11: Colony forming units of test specimens against various bacterial strains (*= statistically/highly statistically significant).....	279
Figure 6.12: <i>p</i> -value matrices for Ti-based alloys against various bacterial strains.....	281
Figure 6.13: Illustration of montages of SEM images collected from (i) an uncoated and (ii) a coated sample disc	292
Figure 6.14: Illustration of stitched EVOS images collected from a single well containing (i) a bare, and (ii) a NF-coated specimen	293
Figure 6.15: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability of C2C12 cells	294
Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability of MDA-MB-231 cells.....	297
Figure 6.17: Relative fluorescence units for cell viability assay of (i) C2C12 (iii) MDA-MB-231 cell lines (red dashed line is the reference line).....	307

LIST OF TABLES

Table 2.1: Relationship between electrospinning process parameters and fibre morphology.....	49
Table 3.1: Chemical composition of Ti6Al4V ELI and cpTi powders (in weight %) (adapted from AZOM (2013a), AZOM, (2013b) and ASM (2023))	82
Table 3.2: Typical physical and mechanical properties of wrought Ti6Al4V ELI, cpTi and Cu (adapted from AZOM (2012), AZOM (2013a), AZOM, (2013b) and ASM (2023))	82
Table 3.3: The recommended polishing procedure for Ti-based alloys	85
Table 3.4: Parameters for X-ray CT scanning of Ti-based samples	92
Table 3.5: Process parameters optimised during single track analysis	128
Table 3.6: Characteristics of pores commonly occurring in LPBF parts.....	155
Table 3.7: Defect analysis of Ti6Al4V and cpTi test samples by CT scans.....	161
Table 3.8: Optimum processing parameters of studied powders	169
Table 4.1: Roughness values of Cu-alloyed Ti64 and cpTi specimen.....	191
Table 4.2: Microhardness values of Ti material at different cross-sections	193
Table 4.3: Mechanical properties of Ti6Al4V and Ti alloys with Cu	195
Table 5.1: Characteristics of the employed polymers.....	218
Table 5.2: Various polymer compositions and their adhesive strengths	226
Table 5.3: Testing of sterilisation, antimicrobial effect and adhesion of various composite NF	228
Table 5.4: Adhesion strength of PCL/PU/CuO on Ti6Al4V substrate with PU primer	229
Table 5.5: Surface roughness values of bare and coated Ti6Al4V and cpTi surfaces.....	241
Table 5.6: Adhesion strength of NF coating on titanium substrate in dry and wet conditions...	244
Table 6.1: List of the test specimens and their descriptions	257
Table 6.2: Bacterial strains and their characteristics	261
Table 6.3: EDS elemental mapping of samples seeded with C2C12 (in wt.%)......	305
Table 6.4: EDS elemental mapping of samples seeded with MDA-MB–231 (in wt.%).....	305

LIST OF ABBREVIATIONS

AFD	Average fibre diameter
Ag	Silver
Al	Aluminium
AM	Additive manufacturing
AOAC	Association of Official Analytical Collaboration
Ar	Argon
ASTM	American Society for Testing and Materials International
ATCC	American Type Culture Collection
BA	Butyl acrylate
bcc	Body-centred cubic
BITRI	Botswana Institute for Technology, Research and Innovation
BSAC	British Society for Antimicrobial Chemotherapy
BSE	Backscatter electron
C	Carbon
CAD	Computer-aided design
CAF	Central Analytical Facilities
CAFSaB	Centre for Applied Food Sustainability and -Biotechnology
CAM	Computer Aided Manufacturing
CA-SFM	Committee on Antibiotic Susceptibility of the French Society of Microbiology
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
CLI	Common Layer Interface
CLSI	Clinical and Laboratory Standard Institute
CNC	Computer numerical control
CRPM	Centre for Rapid Prototyping and Manufacturing
cpTi	Commercially pure titanium
CT	Computer tomography
Cu	Copper
CuO	Copper oxide

CUT	Central University of Technology, Free State
DAPI	Diamidino-2-phenylindole
DC	Direct current
DCM	Dichloromethane
DMEM	Dulbecco's modified Eagle's medium
DMF	N,N-dimethylformamide
DMLS	Direct metal laser sintering
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E	Modulus of elasticity, Elastic modulus, Young's modulus
EBM	Electron beam melting
ECM	Extracellular matrix
EDM	Electrical discharge machining
EDS	Energy-dispersive X-ray spectroscopy
ELI	Extra low interstitials
EOS	Electro Optical Systems
EtOH	Ethanol
FADH	Flavin adenine dinucleotide
FDA	Food and Drug Administration
Fe	Iron
FE-SEM	Field emission scanning electron microscope
FITC	Fluorescein isothiocyanate
FMNH	Flavin mononucleotide hydride
GFP	Green fluorescent protein
<i>h</i>	Hatch distance
H	Hydrogen
hcc	Hexagonal close-packed
HER2	Human epidermal growth factor receptor 2
HF	Hydrofluoric acid
HIFCS	Heat-inactivated fetal calf serum
ISO	Organization for Standardization

KH	Keyhole
<i>L</i>	Length
LB	Luria-Bertani
LoF	Lack-of-fusion
LPBF	Laser powder bed fusion
M	Molar
<i>Ma</i>	Marangoni number
MeOH	Methanol
Mg	Magnesium
Mn	Manganese, Number average molecular weight
MRSA	Methicillin-resistant <i>S. aureus</i>
MSC	Mesenchymal stem cells
MSSA	Methicillin susceptible <i>S. aureus</i>
N	Nitrogen
Na	Sodium
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NF	Nanofibre
NP	Nanoparticle
O	Oxygen
<i>P</i>	Laser power
P	Phosphorus
PAN	Polyacrylonitrile
PBA	Poly(butyl acrylate)
PBF	Powder bed fusion
PBS	Phosphate-buffered saline
PCC	Polymer-cement concrete
PCL	Poly(ϵ -caprolactone) Polycaprolactone
PEG	Poly(ethylene glycol), Polyethylene glycol

PEO	Poly(ethylene oxide)
PGA	Poly(glycolic acid)
PLA	Poly(lactic acid)
PLCL	Poly(L-lactide-co-ε-caprolactone)
PLGA	Poly(lactic-co-glycolic acid)
PMMA	Poly(methyl methacrylate)
PPE	Personal protective equipment
PSW	Process software
PU	Polyurethane
PVA	Poly(vinyl alcohol)
PVP	Polyvinylpyrrolidone
RDA	Recommended Dietary Allowance
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RU	Rhodes University
RUBIC	Rhodes University Biotechnology Innovation Centre
SBF	Simulated body fluids
SE	Secondary electron
SEM	Scanning electron microscope
Si	Silicon
SLA	Sandblast, large-grit, and acid etching
SLI	Specific Layer Interface
SLM	Selective laser melting
SRGA	Swedish Reference Group for Antibiotics
STL	Standard Tessellation Language
t	Powder layer thickness
TCM	Trichloromethane
THF	Tetrahydrofuran
Ti	Titanium
TSA	Tryptone soy agar
TSB	Tryptone soy broth

UTS	Ultimate tensile strength
UV	Ultraviolet
V	Scanning speed
V	Vanadium
v	Volume
VED	Volumetric energy density
w	weight
W	watt
WBA	Water-based acrylic deck adhesive
WHO	World Health Organization
XRD	X-ray diffraction
Yb	Ytterbium
Zn	zinc
α	Alpha, Thermal diffusivity
α'	Alpha prime
β	Beta
Ψ	Sphericity
η	Viscosity
π	Pi
2θ	Bragg angle
%RFU	Percentage relative fluorescence unit
2D	Two-dimensional
3D	Three-dimensional
4IR	Fourth Industrial Revolution
CHCl ₃	Chloroform, Trichloromethane
CO ₂	Carbon dioxide
CuCl ₂ ·2H ₂ O	Copper chloride dihydrate
CuO ₂	Copper peroxide
Cu(OH) ₂	Copper(II) hydroxide
E_a	Area energy density

E_l	Linear energy density
E_v	Volume energy density
HNO_3	Nitric acid
LD_{50}	Lethal dose to 50% animals tested
R_a	Average linear roughness
R_z	Ten-point mean roughness
T_m	Melting temperature
T_g	Glass transition temperature
%	Percentage
at. %	Atomic percentage
wt. %	Weight percentage
°	Degree
°C	Degree Celsius
μg	Microgram
μA	microamperes
μl	Microlitre
μm	Micrometre
g	Gram
GPa	Gigapascal
hr	Hour
HV	Vickers Hardness Number
J	Joule
K	Kelvin
kg	Kilogram
kN	Kilonewton
kPa	Kilopascal
kV	Kilovolt
cm	Centimetre
Da	Dalton
N	Newton

m	Metre
mA	Milliamperes
min	Minute
mg	Milligram
ml	Millilitre
mm	Millimetre
mmol	Millimole
MPa	Megapascal
nm	Nanometers
ref	Relative centrifugal force
rpm	Revolutions per minute
s	Second
U	Unit
W	Watt

CHAPTER 1 INTRODUCTION

1.1. Background

The increase in population and life expectancy over the years has resulted in an increase in cases of bone defects caused by factors such as congenital defects, ageing, diseases and trauma (Weng & Xie, 2015; Zaman, et al., 2015). It is empirical to develop strategies for bone replacement that improve and sustain the quality of life. In addition, factors such as maintaining an optimal bed occupancy rate demand shorter treatment times. With the current world being fast-paced by evolution in technological advancement of the Fourth Industrial Revolution (4IR), remedial strategies should be rapid in manufacturing and restoration of bone function. Implantation is considered the preeminent solution for lost bone. Additive manufacturing (AM), also known as three-dimensional (3D) printing, is a novel approach to fabricating highly complex and customised, near-net shaped metal implants (Baufeld, et al., 2010; Chowdhury, et al., 2022). As a rapid prototyping technique, a fast turnaround is characteristic and design modifications may be effortlessly incorporated. Since parts are built by adding instead of subtracting material, extensive machining is avoided, and the unused feedstock may be reused. Consequently, material costs are reduced and waste is minimised, leading to a lower environmental impact with a decent economic balance (Baufeld, et al., 2010; Chowdhury, et al., 2022).

Additive manufacturing technology was developed in national laboratories, universities and industrial research laboratories. First commercialised in the 1980s, it is now being demonstrated and adopted by industry, as evidenced by the rapid growth in sales of commercial networks (DebRoy, et al., 2018; Srivastava, et al., 2022). The International Organization for Standardization/American Society for Testing and Materials International (ISO/ASTM) 52900:2021 standard classifies AM processes into seven categories, namely: powder bed fusion, vat photopolymerisation, material extrusion, directed energy deposition, sheet lamination, material jetting and binder jetting (de Pastre, et al., 2022). All AM systems have their advantages and disadvantages, and selection should be made based on the objectives and intended use of the finished product. The type of material to be used is one of the main determining factors (Chowdhury, et al., 2022), and for printing metal feedstock, powder bed-based methods, like laser powder bed fusion (LPBF), are preferred due to their superior capability to print dense metallic parts. Laser powder bed fusion involves the interaction of the fed powder with a laser beam that produces a melt pool (DebRoy, et al., 2018). The technique

has rapidly transformed since its development in the 1990s and has been found to be the most versatile AM method for using different materials (Chowdhury, et al., 2022). Therefore, LPBF has become a topic of interest in addressing the increasing demand for implants made from biomaterials.

A biomaterial may be defined as a material that can interact with biological systems to evaluate, treat, augment, restore or replace any tissue, organ or function in the body (Song, et al., 2018). The utilisation of biomaterials for medical applications has become crucial, particularly as artificial implants for the replacement of hard tissue (Zaman, et al., 2015). Moreover, the efficiency of implantable devices depends on their integration with the human body's physiology. In the early 1900s, surgeons identified loss of implant function arising from material and design complications, including mechanical failure, corrosion and biocompatibility issues (Zaman, et al., 2015). As the biomedical industry evolved, titanium (Ti)-based alloys became among the most widely used biomaterials for the production of metallic implants. In the early 1970s, Ti and its alloys began to be at the forefront of implant material for medical applications, even surpassing the renowned stainless steel, owing to its high strength and light weight (Niinomi, 2002; Nag, et al., 2005; BomBač, et al., 2007; Patel & Gohil, 2012). Among the Ti alloys, Ti-6Al-4V – which contains 90 wt.% titanium, 6 wt.% aluminium and 4 wt.% vanadium – is found to be the most biocompatible (Huda, 2010). Ti-6Al-4V ELI (Extra Low Level of Interstitial) is a type of Ti biomaterial suitable for medical implants because of its excellent mechanical strength, corrosion resistance and outstanding biocompatibility (Zaman, et al., 2015; Liu & Shin, 2019). On the other hand, the biocompatibility of Ti and its alloys makes them prone to microbial infection. However, they are also bioinert materials and do not initiate interactions with cells (Al-Khateeb, et al., 2023).

With the extensive advancement in the production of implants through AM, the logical ensuing step towards clinical usage is to functionalise these AM implants by addressing the critical issues of implant-related infection and the separation of implants from the bone (Rasouli, et al., 2018). Alloying conventional Ti implants with metal-based antibacterial agents, such as copper (Cu), not only improves mechanical strength but also introduces antimicrobial properties. Also, Cu is selected because it has more favourable properties for the human body when compared to other extensively researched metal-based antibacterial agents, such as silver (Ashkani, et al., 2023). Furthermore, the surfaces of biometallic alloys require modification to resist bacteria and activate cell interactions (Al-Khateeb, et al., 2023). The surface topography

of Ti-based implants is best controlled at the microscale. Nevertheless, physiological reactions are predominantly associated with processes controlled at the nano level (Rasouli, et al., 2018). Controlling surface topography at a nanometric scale allows for the Ti implant surfaces to emulate the biological environment (Baena, et al., 2017).

Electrical spinning was first documented in the form of eletrospraying in the 17th century. Thereafter, it was first patented as the electrospinning process in 1900 and became prominent as a method for producing nanofibres in the 1990s owing to the emergence of nanotechnology (Weng & Xie, 2015; Kadavil, et al., 2019). The process is used to prepare polymer fibres of diameters ranging from a few nanometres to tens of micrometres and plays an important role in the biomedical industry due to being robust, scalable, versatile and cost-effective (Weng & Xie, 2015; Kadavil, et al., 2019). In comparison to similar fibre-producing techniques, such as wet chemistry methods and molecular beam lithography, electrospinning requires simpler apparatuses for operation but yields significant quantities of products from a wide variety of polymers (Weng & Xie, 2015). Coating the surfaces of Ti implants with bioactive polymer composite NFs with twofold functionalities to encourage tissue formation while preventing bacterial habitation greatly contributes towards shortening rehabilitation periods and extending implant life (Al-Khateeb, et al., 2023). Biocompatibility of biodegradable polymers has made them attractive in biomedical research (Lei, et al., 2019) and coating LPBF Ti implant surfaces with electrospun polymer NFs that mimic the nanofibrous component of the human body may promote tissue interaction. Impregnating the NF coating with Cu particles is a measure to induce the bactericidal effect of implants since antibacterial activity is significantly affected by the surface properties (Zhang, et al., 2013).

1.2. Aim and objectives of the study

The aim of the present study was to optimise the manufacturing of in situ alloys of Ti6Al4V and commercially pure titanium (cpTi) with Cu and polymer NF-coated surfaces of cpTi and Ti6Al4V. The mechanical and biological functionalities of the alloyed and coated specimens were investigated.

The objectives of this study were:

- To determine the process parameters for in situ alloying of Ti6Al4V- x Cu and Ti- x Cu ($x = 3$ and 6 wt.%) using LPBF technology.

- To print Ti6Al4V-xCu, cpTi and Ti-xCu ($x = 3, 6$ wt.%) test samples via LPBF for mechanical and biological analyses.
- To investigate the mechanical properties of uncoated Ti6Al4V, Ti6Al4V-xCu, cpTi and Ti-xCu ($x = 3$ and 6 wt.%) specimens.
- To use the electrospinning apparatus for coating Ti6Al4V and cpTi specimens with polycaprolactone (PCL) and polyurethane (PU) NFs and PCL/PU NFs doped with copper oxide nanoparticles (CuO NPs) and determine the adhesive strength of the NF coatings.
- To employ biological assays in establishing the antibacterial properties and human tissue interactions of Ti6Al4V-xCu and Ti-xCu ($x = 3$ and 6 wt.%) and NF-coated cpTi and Ti6Al4V specimens.

1.3. Thesis statements

Additive manufacturing techniques, such as LPBF, are favoured for the rapid production of metallic implants. Biomedical implants manufactured from titanium alloy have supreme mechanical and biocompatibility. Conversely, their inert surfaces limit bioactivity, and thus coating them with electrospun polymer nanofibres that mimic the morphology of the extracellular matrix would be beneficial. However, neither Ti nor the polymer has been shown to exhibit antimicrobial properties as an antithesis to their ability to attract bacterial cells due to their biocompatibility. The addition of copper particles as an alloying element or additive to the polymer NFs is envisaged to induce the bactericidal effect of Ti-based implants and their NF coating. However, excessive exposure to Cu may be detrimental to cells. Notwithstanding that the exact mechanisms that elucidate the interaction of Cu with cellular structure have not been comprehended, and thus necessitate further research to achieve a balance between the antimicrobial properties of Cu and its promotion of cell attachment.

1.4. The scope of the study

The present study was focused on optimising the functionality of LPBF Ti6Al4V and cpTi in situ alloyed with Cu. It was also directed towards using NFs and NFs imbued with CuO nanoparticles for coating Ti6Al4V and cpTi samples for biomedical applications.

1.5. Research methodology

The research methodology for the current study comprised the following activities:

- Literature review of LPBF of Ti6Al4V, Ti6Al4V- x Cu, cpTi and Ti- x Cu ($x = 3$ and 6 wt.%) alloys, electrospinning of polymer nanofibres and biomedical applications of functionalised Ti and Ti implant alloys.
- Mixing Ti6Al4V- x Cu and Ti- x Cu ($x = 3$ and 6 wt.%) powders and characterising the powders using the scanning electron microscope (SEM).
- Determine the optimum processing parameters of Ti6Al4V- x Cu, cpTi and Ti- x Cu ($x = 3$ and 6 wt.%) powder compositions as follows:
 - form single tracks over a wide range of processing parameters;
 - top view and cross-sectional examination of the tracks using optical microscopy;
 - form single and double layers by varying the hatch distance at optimum scanning speeds and laser powers;
 - examine the layers using optical microscopy and SEM (top view and cross-section); and
 - produce 3D cubes for the examination of porosity (with computed tomography (CT) scanning) and microstructure (with an optical microscope).
- Produce 3D objects using the optimised parameters for subsequent analyses.
- Determine mechanical properties from the tensile test (namely, yield strength, ultimate tensile strength, modulus of elasticity and ductility deduced from %elongation and %reduction in area) and the microhardness of Ti6Al4V- x Cu, cpTi and Ti- x Cu ($x = 3, 6$ wt.%) samples.
- Conduct preliminary experiments to determine the most suitable polymer composition for coating of test samples.
- Electrospinning of PCL/PU and PCL/PU/CuO NPs nanofibres to apply coatings on Ti6Al4V ELI and cpTi sample surfaces and characterise the nanofibre coatings using SEM and X-ray diffraction (XRD).
- Determine the adhesive strength of PCL/PU and PCL/PU/CuO NPs NF coatings using the direct pull-off adhesion test apparatus.
- Conduct antibacterial tests to establish the antibacterial properties of the Cu/CuO particles in Ti6Al4V- x Cu, Ti- x Cu ($x = 3, 6$ wt.%) and the NF-coated samples.
- Use cell lines to conduct cell culture assays and determine the biocompatibility of the Cu-alloyed and NF-coated specimens, and determine the cytotoxicity of the specimens.

- Draw conclusions from the observations and results obtained and make recommendations for future research projects.

1.6. Expected contribution

Additive manufacturing techniques such as LPBF are widely used to fabricate Ti implants in the medical industry. However, they require additional processing to render them functional in the clinical domain. This study establishes the optimum process parameters for in situ alloying of Ti6Al4V ELI and cpTi with Cu. Subsequently, producing LPBF parts containing Cu maintains the advantages of 3D printing – rapidly producing complex, customised devices – while improving mechanical properties and introducing antibacterial functionality to Ti alloys. Coating the Ti6Al4V ELI and cpTi surfaces with polymer NFs using the esteemed electrospinning procedure enhances the biocompatibility of the surfaces, and imbuing the NFs with CuO particles allows for compatibility with human cells while discouraging bacterial cell attachment.

1.7. Chapter layout

The dissertation consists of seven chapters that have been arranged as follows:

Chapter 1 introduces the dissertation topics through their brief background. The features of the chapter include the aim and objectives of the study, the thesis statements, the scope of the study, the research methodology, the expected contribution, and the chapter layout.

Chapter 2 is a literature review of AM processes, with a special focus on producing Ti6Al4V ELI and cpTi specimens using LPBF and on the functionalisation of LPBF parts as biomedical devices.

Chapter 3 details the optimisation of cpTi and in situ alloyed Ti6Al4V- x Cu and Ti- x Cu ($x = 3, 6$ wt.%) powders using LPBF and the manufacturing of the final test samples.

Chapter 4 characterises the in situ alloys and establishes their mechanical properties in terms of the Vickers microhardness and tensile test.

Chapter 5 describes the coating of cpTi and Ti6Al4V with polymer nanofibres using electrospinning and the imbuing of NFs with in situ-manufactured copper oxide nanoparticles.

Chapter 6 documents the biological properties of test samples. The antimicrobial efficacy of the Cu element is examined against various bacterial strains commonly isolated from orthopaedic and dental implant surfaces. The tissue culture analyses highlight the ability of the samples to integrate with mammalian cells without inducing cytotoxicity.

Chapter 7 is the conclusion and provides a summary of the critical findings and the scope for future work.

References

- Al-Khateeb, A., Al-Hassani, E. S., & Jabur, A. R. (2023). Metallic implant surface activation through electrospinning coating of nanocomposite fiber for bone regeneration. *International Journal of Biomaterials*, 2023, Article ID 1332814.
- Ashkani, O., Tavighi, M. R., Karamimoghadam, M., Moradi, M., Bodaghi, M., & Rezayat, M. (2023). Influence of aluminum and copper on mechanical properties of biocompatible Ti-Mo alloys: A simulation-based investigation. *Micromachines*, 14, 1081. doi:10.3390/mi14051081
- Baena, R. R., Rizzo, S., Manzo, L., & Lupi, S. M. (2017). Nanofeatured titanium surfaces for dental implantology: Biological effects, biocompatibility, and safety. *Journal of Nanomaterials*, 2017, 6092895. doi:10.1155/2017/6092895
- Baufeld, B., Van der Biest, O., & Gault, R. (2010). Additive manufacturing of Ti–6Al–4V components by shaped metal deposition: Microstructure and mechanical properties. *Materials and Design*, 31(1), S106–S111. doi:10.1016/j.matdes.2009.11.032
- BomBač, D., Brojan, M., Fajfar, P., Kosel, F., & Turk, R. (2007). Review of materials in medical applications. *RMZ – Materials and Geoenvironment*, 54(4), 471–499.
- Chowdhury, S., Yadaiah, N., Prakash, C., Ramakrishna, S., Dixit, S., Gupta, L. R., & Buddhi, D. (2022). Laser powder bed fusion: a state-of-the-art review of the technology, materials, properties & defects, and numerical modelling. *Journal of Materials Research and Technology*, 20, 2109–2172.
- de Pastre, M. A., Quinsat, Y., & Lartigue, C. (2022). Effects of additive manufacturing processes on part defects and properties: A classification review. *International Journal on Interactive Design and Manufacturing*, 16, 1471–1496. doi:10.1007/s12008-022-00839-8
- DebRoy, T., Wei, H. L., Zuback, J. S., Mukherjee, T., Elmer, J. W., Milewski, J. O., . . . Zhang, W. (2018). Additive manufacturing of metallic components – Process, structure and properties. *Progress in Materials Science*, 92, 112–224. doi:10.1016/j.pmatsci.2017.10.001
- Huda, Z. (2010). Designing fail-safe biomaterials against wear for artificial total hip replacement. *Journal of Biomimetics, Biomaterials and Tissue Engineering*, 6, 45–55. doi:10.4028/www.scientific.net/JBBTE.6.45
- Kadavil, H., Zagho, M., Elzatahry, A., & Altahtamouni, T. (2019). Sputtering of electrospun polymer-based nanofibers for biomedical applications: a perspective. *Nanomaterials*, 9(1), 77. doi:10.3390/nano9010077

- Lei, J., Yao, G., Sun, Z., Wang, B., Yu, C., & Zheng, S. (2019). Fabrication of a novel antibacterial TPU nanofiber membrane containing Cu-loaded zeolite and its antibacterial activity toward Escherichia coli. *Journal of Material Science*, 54, 11682–11693. doi:10.1007/s10853-019-03727-x
- Liu, S., & Shin, Y. C. (2019). Additive manufacturing of Ti6Al4V alloy: a review. *Materials and Design*, 164, 107552.
- Nag, S., Banerjee, R., Stechschulte, J., & Fraser, H. L. (2005). Comparison of microstructural evolution in Ti-Mo-Zr-Fe and Ti-15Mo biocompatible alloys. *Journal of Materials Science: Materials in Medicine*, 16(7), 679–685. doi:10.1007/s10856-005-2540-6
- Niinomi, M. (2002). Recent metallic materials for biomedical applications. *Metallurgical and Materials Transactions A*, 33A, 477–486. doi:10.1007/s11661-002-0109-2
- Patel, N., & Gohil, P. (2012). A Review on biomaterials: Scope, applications & human anatomy significance. *International Journal of Emerging Technology and Advanced Engineering*, 2(4), 91–101.
- Rasouli, R., Barhoum, A., & Uludag, H. (2018). A review of nanostructured surfaces and materials for dental implants: Surface coating, patterning and functionalization for improved performance. *Biomaterials Science*, 6, 1321-1338.
- Song, R., Murphy, M., Li, C., Ting, K., Soo, C., & Zheng, Z. (2018). Current development of biodegradable polymeric materials for biomedical applications. *Drug Design, Development and Therapy*, 12, 3117-3145. doi:10.2147/DDDT.S165440
- Srivastava, M., Rathee, S., Patel, V., Kumar, A., & Koppad, P. G. (2022). A review of various materials for additive manufacturing: Recent trends and processing issues. *Journal of Materials Research and Technology*, 21, 2612-2641. doi:10.1016/j.jmrt.2022.10.015
- Weng, L., & Xie, J. (2015). Smart electrospun nanofibers for controlled drug release: recent advances and new perspectives. *Current Pharmaceutical Design*, 21(15), 1944-1959. doi:10.2174/1381612821666150302151959
- Xu, J., Lu, Y., Pan, X., Zhan, D., Wang, Q., & Zhang, N. (2023). Antibacterial performance of a porous Cu-bearing titanium alloy by laser additive manufacturing. *Frontiers in Bioengineering and Biotechnology*, 11, 1226745. doi:10.3389/fbioe.2023.1226745
- Zaman, H. A., Sharif, S., Idris, M. H., & Kamarudin, A. (2015). Metallic biomaterials for medical implant applications: a review. *Applied Mechanics and Materials*, 735, 19-25. doi:10.4028/www.scientific.net/AMM.735.19

Zhang, E., Li, F., Wang, H., Liu, J., Wang, C., Li, M., & Yang, K. (2013). A new antibacterial titanium–copper sintered alloy: preparation and antibacterial property. *Material Science and Engineering C*, 33, 4280-4287.

CHAPTER 2 LITERATURE REVIEW

2.1. Introduction

The current chapter reviews the literature on additive manufacturing (AM) processes for medical implants, with a special focus on laser powder bed fusion (LPBF) and the use of titanium (Ti). It alloys as the base implant biomaterial, in situ alloying the base materials with copper (Cu) as an antibacterial agent, and improving the biocompatibility of Ti-based implants by the surface modification and nanotechnological technique of coating with polymer biomaterial using electrospinning technology.

The world population has increased by more than 200% over the last three-quarters of the century, from an estimated 2.5 billion people in 1950 to 8.2 billion in 2025, and the United Nations (UN) projects a further increase to approximately 10.3 billion people by the mid-2080s (United Nations, 2024; Iberdrola Group, 2025; Worldometer, 2025). In addition, the current global life expectancy is 73.3 years and the UN projects that by the end of the 2070s, the number of people aged 65 and over will surpass the number of those under 18 years (United Nations, 2024). The exponential increase in population, coupled with extended life expectancy, necessitates greater use of appliances and machinery (Han, et al., 2023). Subsequently, there is an increase in incidences leading to bone damage or loss through natural and birth defects, accidents, trauma and illnesses such as tumour/cancer, diabetes, osteoporosis and osteoarthritis (Kim, et al., 2020; Al-Khateeb, et al., 2023). Thus, the demand for implants to replace and repair damaged and lost bones for improved function and aesthetics has also increased drastically over the course of the century.

Medical implants are artificial mechanical devices that are mounted to the skeletal system of the human body to fulfil various purposes, such as supporting or replacing bones or joints, reattaching tendons or ligaments and providing a post to support and mount replacement teeth (Onuoha, et al., 2019; Kurup, et al., 2021). An implant failure is when there is a need to remove it prematurely before the implant has fulfilled its intended purpose. Implant survival is measured from the date of implantation to the date of its failure (Onuoha, et al., 2019). In most cases of orthopaedic and orthodontic implants, the implants are intended to remain permanently in the body and integrate with surrounding bone tissue.

After blood, bone is the most transplanted tissue in the human body (Kim, et al., 2020). Although implants have a high success rate of 90–98% (Tanner, et al., 1997; Zhang, et al., 2021a), implantation failure continues to plague the medical society. The principal reasons for failure are attributed to bacterial infection and mechanical stress (Tanner, et al., 1997). Geary et al. (2020) compared historic and modern cohorts and found that the most common reason for orthopaedic revision surgery was infection (22.1%), followed by instability (19.2%), aseptic loosening (18.5%), and wear/osteolysis (15.9%). During their study of the period from 1997 to 2000, Sharkey et al. (2002) found that most implants failed and required revision within 2 years of the initial surgery. On the other hand, Geary et al. (2020) found that, for the 1986–2015 cohort, most revisions occurred after 2 years, with a failure rate of 22.8%. The life expectancy of orthopaedic implants was about 15 years previously, but advances in medical technology have extended the survival of implants to an estimated 25 years (Evans, et al., 2019; Yuan, et al., 2021). Through continuous research into the improvement of implantable devices, the ultimate goal of permanent implants that no longer require revision surgery may be realised.

2.2. Additive manufacturing

The current era of Industry 4.0 is characterised by technological advancements and automation with a special focus on the revolution of manufacturing processes and product development (Abd-Elaziem, et al., 2024). An ongoing challenge to be addressed in this era is the smart manufacturing of simultaneously achieving design freedom and creating complex structures with added value to products (Zhang, et al., 2021b). In this context, AM has emerged as an innovative approach to pivotal technology that enables digital production integrating automated and data-driven programming in production processes (Abd-Elaziem, et al., 2024). Because of the inherent material efficiency, energy utilisation, design flexibility and capability for localised production, AM remarkably reduces the consumption of energy or fuel, which in turn lowers carbon emissions and greenhouse gases, a characteristic of Industry 5.0 (Srivastava, et al., 2022; Rahmani, et al., 2025).

Significant advances over the last two decades in the constituent technologies of AM metal processing, including more affordable and reliable industrial lasers, inexpensive high-performance computing hardware and software and metal powder feedstock technology, have enabled it to become a state-of-the-art processing method (DebRoy, et al., 2018; Srivastava, et al., 2022). The process eliminates the requirement of numerous tooling steps of conventional methods, such as dies and punches. Furthermore, unlike with traditional manufacturing

methods (namely, casting, forging or extrusion), intricate features (such as contours, holes, straight cuts and mould cavities) may be implemented in a single step without restraint on the shapes obtained (Chowdhury, et al., 2022; Srivastava, et al., 2022). In that single step, several of only the necessary parts may be manufactured and may not require extensive post-process assembling activities, minimising human intervention (Srivastava, et al., 2022).

The International Organization for Standardization/American Society for Testing and Materials International (ISO/ASTM) 52900:2021 standard groups AM processes into seven categories (ISO/ASTM International, 2021). The categories and the types of processes are outlined in Figure 2.1.

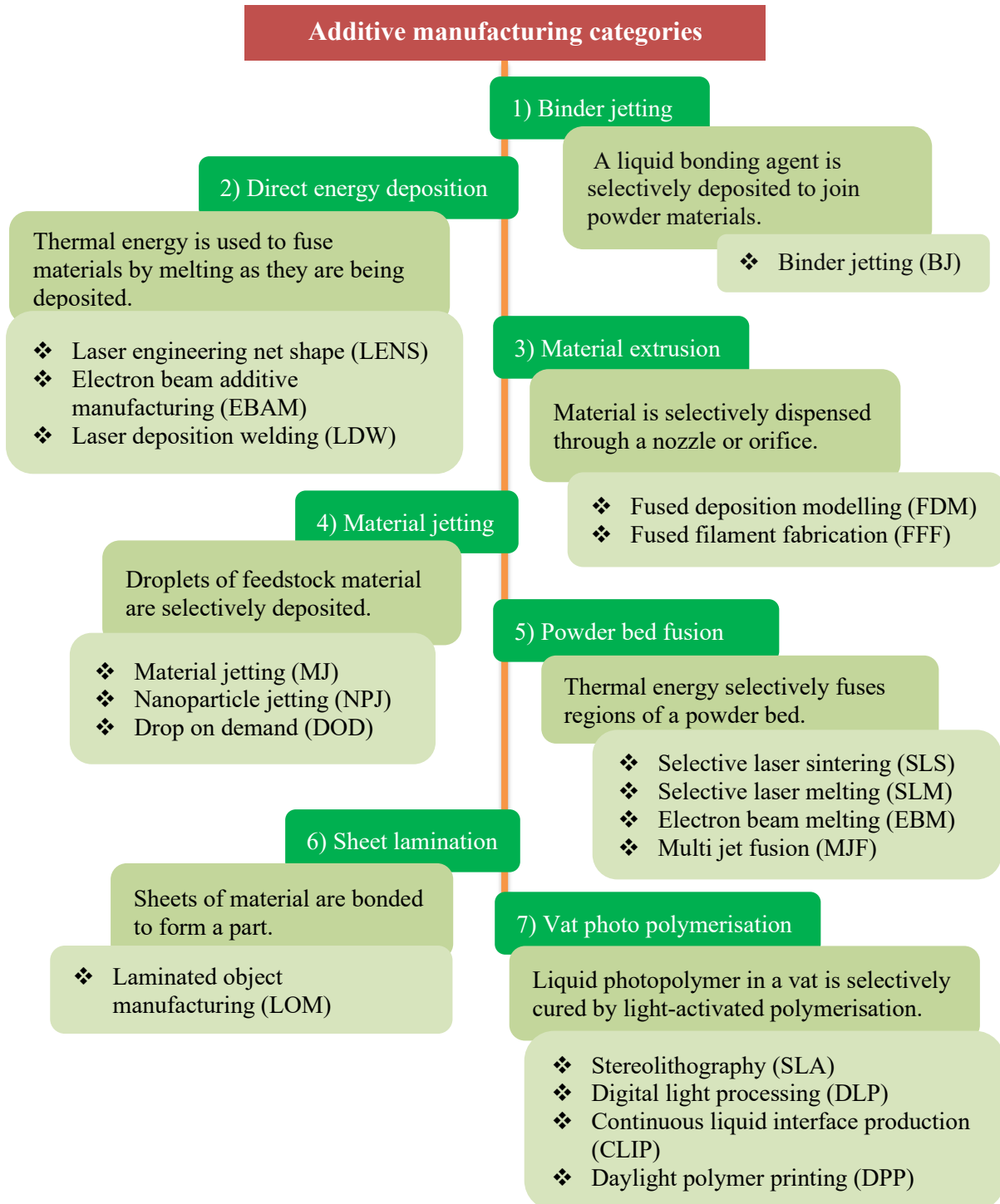


Figure 2.1: Seven process categories of additive manufacturing (adapted from ISO (2021))

2.2.1. Laser powder bed fusion

Powder bed fusion (PBF) based AM processes can fabricate metallic parts directly from numerous engineering alloy powders, such as stainless steel, maraging steel, Ti alloys, cobalt, chromium and more (Srivastava, et al., 2022). Selective laser melting (SLM, also known as laser powder bed fusion) and electron beam melting (EBM) are the most commonly used PBF-

based AM processes (Taghian, et al., 2023). The manufacturing processes of both SLM and EBM involve melting metallic powder feedstock to form a solid part according to well-defined CAD data. The difference is that in SLM, a laser beam selectively melts powder layers in an SLM machine. The beam is controlled by scanning galvanometers, and the process is conducted in an inert gas atmosphere. In contrast, the EBM machine uses an electron beam to melt the metal powder, controlled by electromagnetic lenses, and the fabrication occurs in a vacuum environment (Gong, et al., 2014; Taghian, et al., 2023). The laser beam has a focused spot size and is able to sinter smaller powder particles than the electron beam, which has a diffused spot size. As a consequence, SLM produces parts with finer features, resolution and surface finish than the EBM process (Gong, et al., 2014). Hence, SLM techniques are preferred for producing high-end components with small production volumes (Taghian, et al., 2023).

Laser powder bed fusion is the standard term for the laser-based PBF processes, which are denoted in literature by different commercial names depending on the machine manufacturer, such as direct metal laser sintering (DMLS) for EOS GmbH, LaserCUSING for Concept Laser, direct metal production (DMP) for Phenix Systems (3D Systems) and selective laser melting (SLM) for SLM Solutions GmbH, ReaLizer GmbH, Renishaw Group (Udroiu, 2012; Manfredi & Calignano, 2017). The LPBF process uses a highly energetic laser beam to selectively melt a layer of metallic powder feedstock, which is laid on a substrate (Caprio, et al., 2018). In true AM fashion, the process is repeated layer by layer until a part is built. At the start of the process, a 3D CAD model is digitally sliced into layers of two-dimensional (2D) cross-sections that the equipment interprets as building instructions (Fox, et al., 2016). A thin layer of powder (20 to 100 μm) is spread across a build platform, and a high-power laser is used to selectively melt regions of the powder layer (Fox, et al., 2016). Once a layer is melted and sintered according to the corresponding 2D cross-section, the build platform lowers, a new powder layer is spread across the build platform, and the process repeats until the 3D structure is complete (Fox, et al., 2016). Laser powder bed fusion fabricates intricate and net-shaped metallic parts directly from 3D CAD models (Kasperovich, et al., 2016). This renders it highly applicable for the medical industry, where geometrically complex structures of high-strength materials are extensively utilised (Kasperovich, et al., 2016; Han, et al., 2023). Figure 2.2 illustrates the typical components of an LPBF machine.

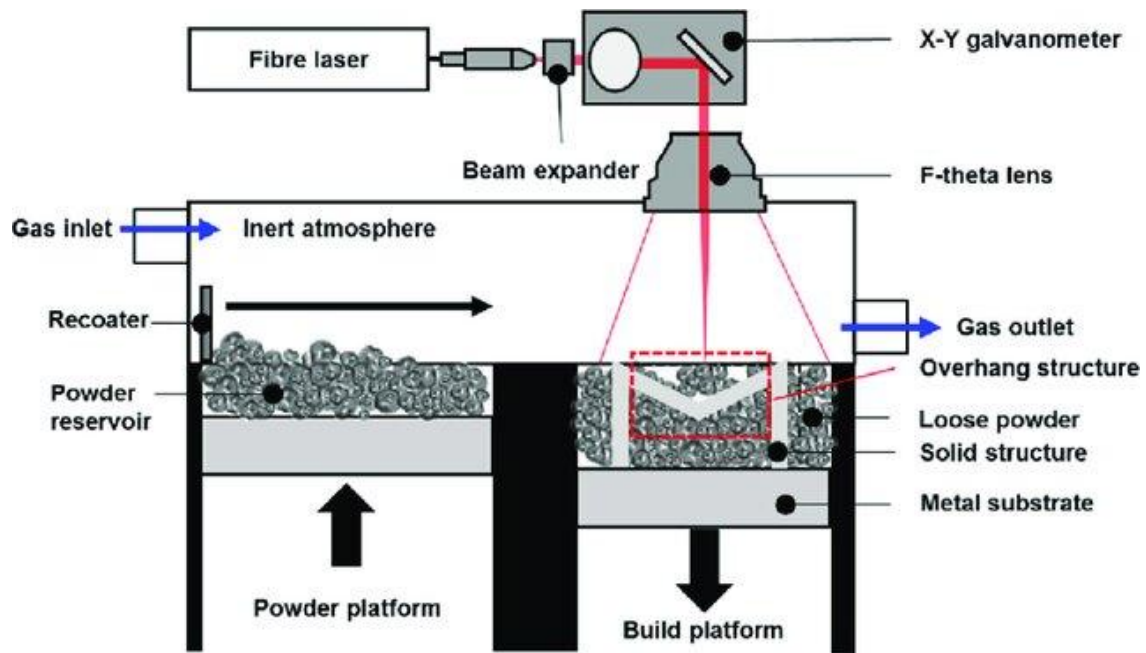


Figure 2.2: Schematic of a typical LPBF machine (adapted from Leung et al. (2018))

The properties of AM parts are considerably influenced by the characteristics of the feedstock materials (Zhao, et al., 2008; Karlsson, et al., 2013). These characteristics include the shape, size distribution and surface morphology of the particles as well as the composition and flowability of the powders. Typical powder particle size for LPBF is in the range of 10–60 μm (DebRoy, et al., 2018). The characteristics of feedstock material are controlled by manufacturing processes, including gas atomisation, water atomisation, rotary atomisation and plasma rotating electrode processes (DebRoy, et al., 2018; Mathias, et al., 2023). Gas atomisation is widely used in metal powder production for use in AM due to its advantages, including high capacity, high flexibility for both elemental and pre-alloyed powder production and capability of rapidly solidified metal powder production (Mathias, et al., 2023).

2.2.2. Optimising the process parameters of LPBF

A crucial research objective in LPBF technology is to improve the productivity and quality of the parts. Processing conditions relating to machine settings are usually predefined. Process parameters are determined mainly based on each powder feedstock composition (Aboulkhair, et al., 2014; Taghian, et al., 2023). For LPBF systems, over 100 processing parameters may be defined (Taghian, et al., 2023) and may be classified into four categories, namely, laser-related parameters, scan-related, powder-related and atmosphere-related (Figure 2.3).

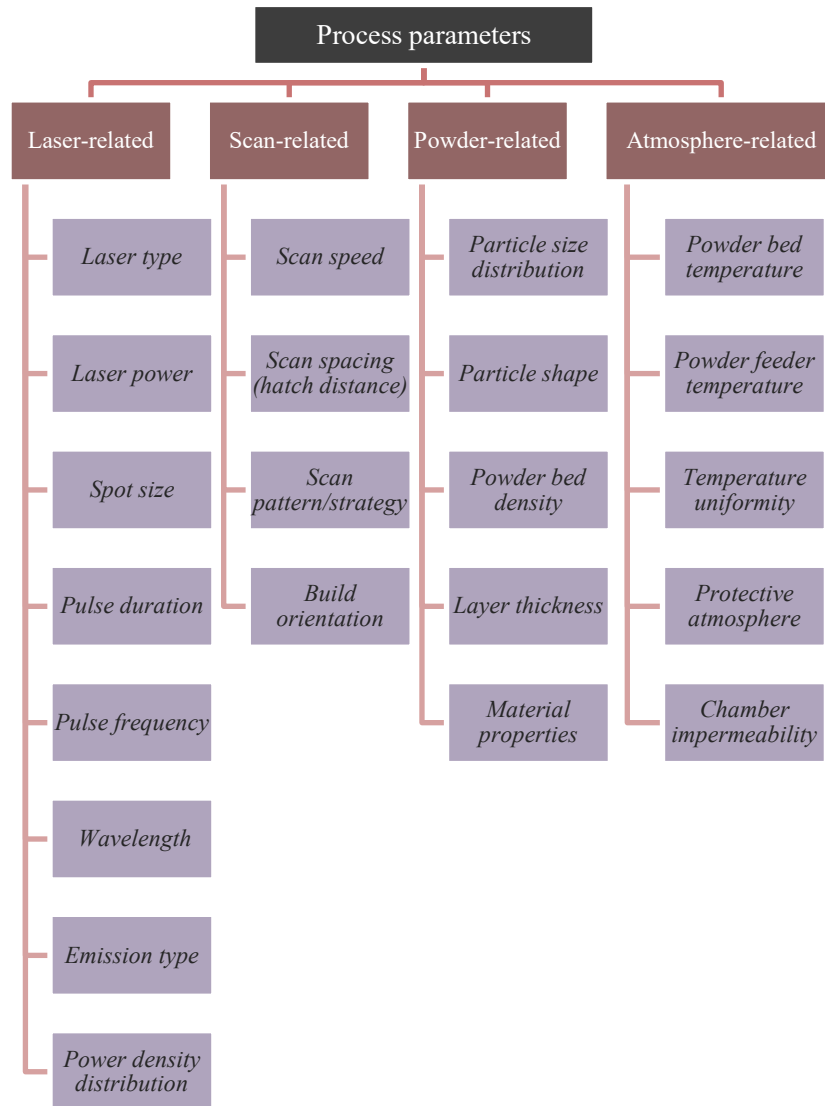


Figure 2.3: Parameters regulating the LPBF process (adapted from Aboulkhair et al. (2014) and Taghian et al. (2023))

Essential parameters that determine various solidification cooling rates and thermal gradients during processing are laser power, scan speed, hatch distance, layer thickness and scanning strategy. As such, these primary parameters considerably impact the resultant microstructure, mechanical characteristics, and overall quality of the LPBF process (Singh, et al., 2020; Strantz, et al., 2021; Taghian, et al., 2023). Optimisation of the primary process parameters provides essential information relating to the features of a scan track (Cobbinah, et al., 2021). The parameters require optimisation for the employed powder alloys and their final applications to obtain optimally dense parts with high dimensional accuracy and the desired mechanical properties (Singh, et al., 2020; Strantz, et al., 2021; Taghian, et al., 2023). Figure 2.4 illustrates the process parameters usually studied and optimised for the LPBF process.

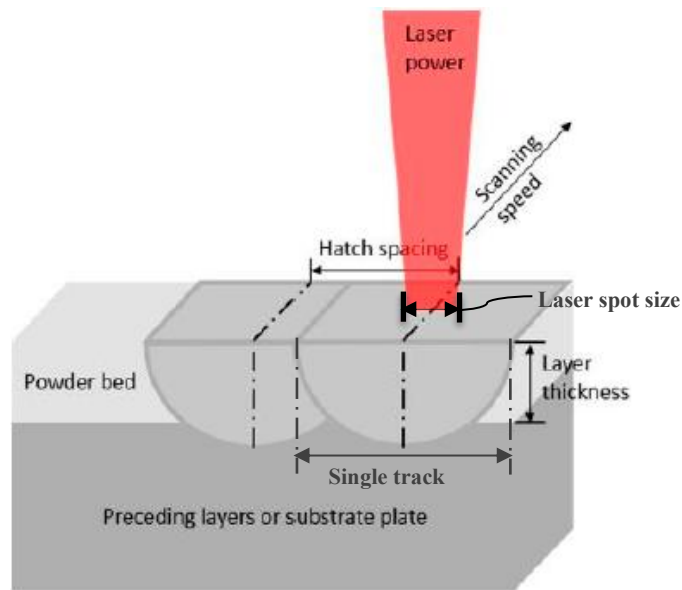


Figure 2.4: Primary process parameters of LPBF (adapted from Cobbinah et al. (2021))

During optimisation, the powder layer thickness and the laser spot size are usually kept constant. Laser power and laser scanning speed are two process variables that control the energy input. Laser power is a flexible parameter that may be easily varied over a broad range. The laser scanning speed is also relatively easy to vary, but it has a quite narrow process window for the successful fabrication of a high-quality track (Krakhmalev & Yadroitsev, 2014). Figure 2.5 shows the laser-scanning process and the associated thermal and physical zones.

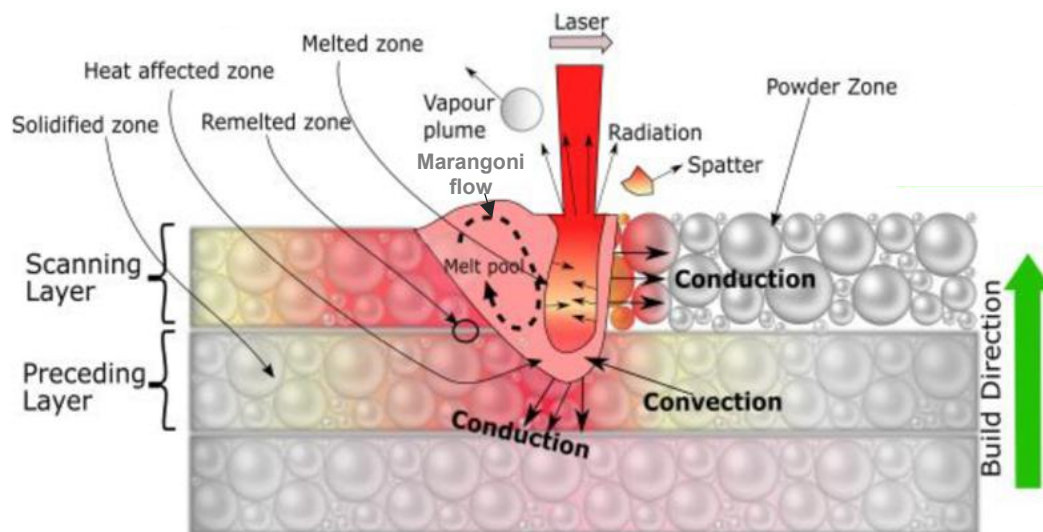


Figure 2.5: A schematic presentation of LPBF process (adapted from Islam et al. (2024))

The LPBF is a heat-driven process. The source of heat for consolidating the powders is the laser energy input and is a paramount factor during the process. The laser energy determines the state of the molten powder, including its melting, vaporisation and sintering states (Dzogbewu, 2017). As the powered laser ray touches the surfaces of the powder particles and travels at a certain speed through the powder layer of a particular thickness in vector format, energy in the form of heat is generated and diffuses inward (Dzogbewu, 2017; Cobbinah, et al., 2021). The melting state is achieved when the temperature of the generated heat reaches the melting point of the material and continuous exposure to the laser beam (Cobbinah, et al., 2021). To obtain stable processing conditions and a highly dense part, the powder bed should be fully melted (Caprio, et al., 2018).

Energy inputs in AM are quantified as linear, area and volume energy densities E_l (J/mm), E_a (J/mm²) and E_v (J/mm³), respectively, and defined as (Kenel, et al., 2017; Wang, et al., 2023a):

$$E_l = \frac{P}{V}; \quad E_a = \frac{P}{V \cdot h}; \quad E_v = \frac{P}{V \cdot h \cdot t} \quad \text{Equation 2.1}$$

Where P is the laser power, V is the scanning speed, h is the hatch distance, and t is the powder layer thickness. Linear and volumetric energy densities are the more commonly considered quantities for the comparison of processing parameter combinations and their influence on the observed properties (Kenel, et al., 2017). While linear energy density is effective when analysing the melt pool geometry of single tracks, volumetric energy density has been shown to have a direct effect on the mass density of the parts (Majumdar, et al., 2019; Khaimovich, et al., 2022; Shrestha & Chou, 2022). Process parameters of the LPBF, in conjunction with the powder energy absorption from the laser irradiation, determine the volumetric energy density available to heat and melt powders (Cobbinah, et al., 2021). The volumetric energy density captures the synergistic effect of the LPBF process parameters, simplifying the optimisation process compared to examining the parameters individually, particularly since they are mutually dependent for achieving a functional final component.

Yadroitsev et al. (2012) investigated the relationships among the principal LPBF parameters (namely, laser power, scanning speed, and layer thickness), powder properties, and the geometrical characteristics of the resultant single tracks. Their results showed that the

behaviour of individual tracks and their geometric characteristics depend on the process parameters and the physical-chemical and granulomorphometrical properties of the powder. Each powder showed particular behaviour during the process of single-track formation. Moreover, Yadroitsev et al. (2012) proposed a hierarchy of process parameters and found that the most influential parameter was the laser power, followed by the powder layer thickness, the scanning speed and the particle size in order of decreasing importance.

2.2.2.1. Single tracks and single layers

The properties for components built by the LPBF process depend on the properties of each single track and each single layer (Dzogbewu, et al., 2017; Cobbinah, et al., 2021). The characteristics of a track are influenced by the substrate material, laser beam power, scanning speed, powder layer thickness and properties of the powder used (Cobbinah, et al., 2021). The width of the scanned tracks is analogous to the laser beam diameter (Yadroitsev, et al., 2010). Since single tracks are the basic units of LPBF-fabricated 3D objects, it is vital to understand their characteristics thoroughly. It has been demonstrated that the formability, microstructure, mechanical properties and residual stress of LPBF parts are all governed by the nature of the single tracks, which is determined by the molten pool dynamics (Fischer, et al., 2003; Yadroitsev, 2009; Körner, et al., 2011; Dzogbewu, et al., 2017).

Sintering of adjacent single tracks of the molten powder formed along the scanning direction of the laser beam results in the formation of a single layer. A part is sequentially filled by single layers of molten powder (Yadroitsev, et al., 2007). Therefore, the surface properties and quality of the final parts fabricated by LPBF greatly depend on the quality of each single track and each single layer, and the quality of the interaction between them (Yadroitsev, et al., 2007; Khorasani, et al., 2020).

2.2.2.2. Laser scanning strategy

When the powder fusion process moves from a single track to a layer, scan spacing in overlapping tracks and scanning patterns are some of the essential factors considered (Cobbinah, et al., 2021). The scanning strategy is the manner in which a material is built up by systematically depositing tracks and layers of the material (Zagade, et al., 2024). It includes the scanning direction and the arrangement of the scan sequence and affects the cooling time of fused layers and the overall material properties of the component (Cobbinah, et al., 2021).

Grain orientations and microstructural texture may be controlled using the laser scanning strategy (Thijs, et al., 2010).

Laser scanning strategies employed in LPBF construction of various alloys include the unidirectional and bidirectional (also known as zig-zag) scanning strategies illustrated in Figure 2.6 (Dzogbewu, 2017; Zagade, et al., 2024). The scanning vectors and consecutive layers in these scan strategies are rotated at 67° , 90° (orthogonal) or 180° ($N + 1$) with the purpose of increasing the homogeneity of the energy input (Cobbinah, et al., 2021). Dewidar et al. (2003) studied different laser scanning strategies for a pre-alloyed steel powder and found that the zig-zag strategy produced coherent, uniform layers. Vrancken et al. (2014) scanned layers in a continuous laser mode, using a zig-zag pattern and a 90° rotation between layers and optimised the scanning strategy to obtain fully dense, high-quality Ti6Al4V products. Hatch distances of below $60\text{ }\mu\text{m}$ have been found to result in considerable pore formation in the boundary regions of the LPBF samples (Kasperovich, et al., 2016). Consequently, hatch distances lower than $60\text{ }\mu\text{m}$ are not recommended when a zig-zag laser strategy is employed (Kasperovich, et al., 2016). Thijs et al. (2010) used three different scanning strategies (Figure 2.6), namely unidirectional scanning, identical scanned layers using zig-zag vectors and the cross-hatching technique (that is, 90° rotation between layers) using zig-zag scan vectors. They found that the orientation of the grains – and hence the microstructural texture – is highly dependent on the scanning strategy.

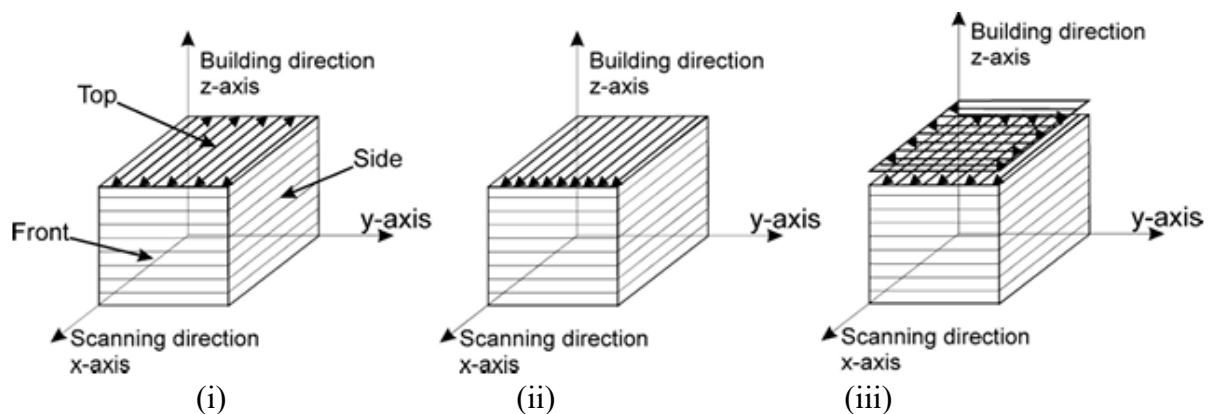


Figure 2.6: Scanning strategies of (i) zig-zag scan vectors with no rotation between the layers, (ii) unidirectional scan vectors, and (iii) zig-zag scan vectors with 90° rotation between the layers (adapted from Thijs et al. (2010))

The scanning strategy for LPBF processing may also include re-scanning or re-melting for some materials. As the name suggests, the re-scanning strategy involves the re-melting of

already scanned layers of a component or only of the top surface (Cobbinah, et al., 2021). The re-scanning strategy during the LPBF process permits the deposition of a higher energy density compared to a single scan strategy (Kenel, et al., 2017). Studies have shown the advantages of using the rescanning strategy, including improving density, enhancing surface quality, increasing wear resistance and releasing entrapped gases (Morgan, et al., 2002; Lamikiz, et al., 2007; Yasa, et al., 2011; Kang, et al., 2017). Rashid et al. (2017) demonstrated that a re-scanning strategy greatly influences the microstructure and density of LPBF components. The authors reported higher density and microhardness in parts printed with a double-scan strategy than in those printed with a single-scan strategy, under both as-fabricated and heat-treated conditions. The differences in density and hardness were attributed to changes in the microstructure during single- to double-scanning. The parameters of re-scanning may vary from the scan parameters. Hence, re-scanning parameters also need to be optimised to be helpful during the LPBF process (Cobbinah, et al., 2021).

2.2.2.3. Defects in LPBF 3D object

Despite the advantages, several challenges are associated with LPBF methods. The majority of current AM processes are at the development stage, and the obstacle to LPBF is understanding the relationship between material properties, processing parameters, and final part properties (Chowdhury, et al., 2022; Srivastava, et al., 2022). There are requirements for highly dense parts obtained from high cooling rates, which affect the microstructures (Chowdhury, et al., 2022). Moreover, variations in material properties such as conductivity and reflectivity complicate the optimisation of process parameters (Khorasani, et al., 2020; Chowdhury, et al., 2022). Figure 2.7 shows the surface of an as-fabricated LPBF part, illustrating common flaws that may occur in solid material due to material variations and inadequate parameter optimisation. These flaws are related to the defects of the final components.

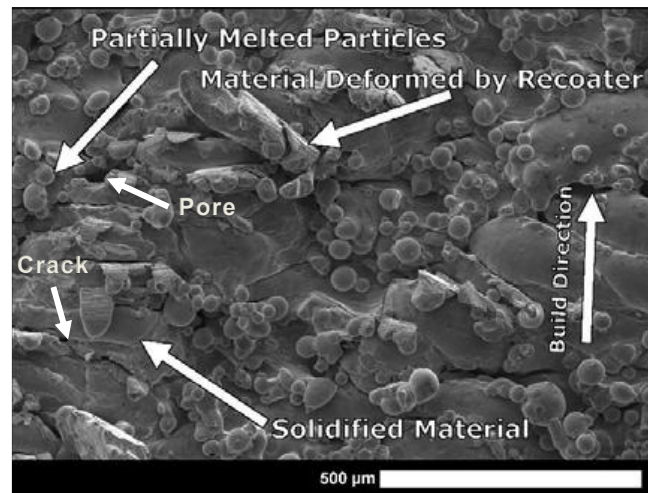


Figure 2.7: As-built LPBF surface (adapted from Fox et al. (2016))

Minor differences between LPBF-manufactured and conventionally produced parts include defects, residual stresses, and anisotropy, which are unique to AM processing and lead to inferior mechanical and other properties of the manufactured parts (Aboulkhair, et al., 2019; Srivastava, et al., 2022). Research has indicated that LPBF-built parts are prone to the presence of defects that influence mechanical properties (Gong, et al., 2014). Non-optimal process parameters are associated with inner defects, such as unmolten powder particles, spherical entrapped gas bubbles, cracks, and other local irregularities (Kasperovich, et al., 2016). Common defects associated with AM processes based on laser sintering are shown in Figure 2.8.

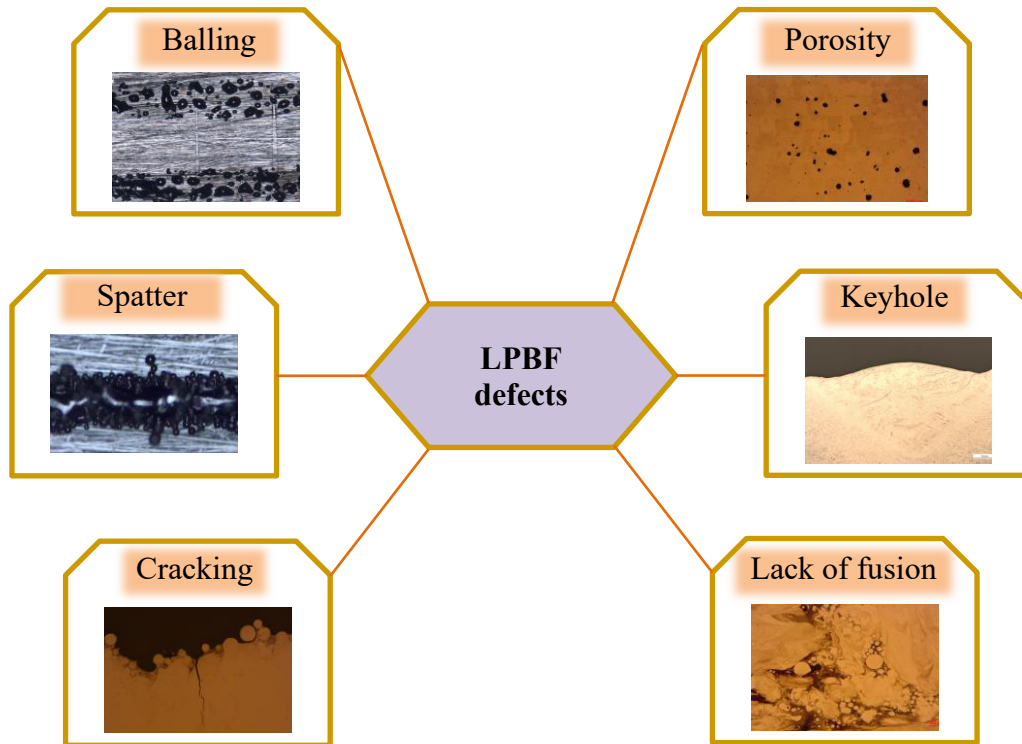


Figure 2.8: Defects of the LPBF process

Porosity is one of the most commonly occurring and detrimental defects to LPBF 3D metal objects, and it directly affects the density of the final part (Khorasani, et al., 2020; Cobbinah, et al., 2021). The causes of pore (or void) defects include the emergence of a keyhole, gas entrapment, and lack of fusion between tracks or layers, leading to microscopic pores; insufficient penetration of subsequent layers into a substrate; and others (Cao, et al., 2021; Wu, et al., 2021). The balling effect, also called bead up, is a defect caused by underlying surfaces that are not wetted by the liquid material, resulting in the formation of beaded scan tracks and thus increasing surface roughness and porosity (Calignano, et al., 2013; Mostafaei, et al., 2022). The laser-induced balling effect influences the densification activity of LPBF-processed parts. The balling effect occurs when the molten powder has low viscosity and is unable to completely wet the substrate due to surface tension, resulting in the formation of isolated spheres with a diameter equal to that of the focused laser beam. Balling hinders deposition, increases porosity and affects the density of the final part (Das, 2003; Cobbinah, et al., 2021). Defects such as porosity, balling and cracking affect the fatigue performance of LPBF-produced parts (Chowdhury, et al., 2022). These need to be addressed for high-performance requirements, such as in clinical applications (Chowdhury, et al., 2022).

2.2.2.4. Residual stress

The rapid melting-solidification transformation during the LPBF process is accompanied by considerable accumulated stresses (Srivastava, et al., 2022). Laser powder bed fusion in situ alloys fabricated from Ti6Al4V powder are able to reach maximum heating and cooling rates of the order of 10^6 °C/s (Cao, et al., 2021; Sing, et al., 2021). Residual stress from the melting and cooling processes and high thermal gradients may lead to the initiation of cracks, distortion and delamination of the final product (Strantz, et al., 2021; Chowdhury, et al., 2022). Cracks are mainly prominent along grain boundaries since the temperature varies for each layer. If stress at the interface exceeds the yield strength of the alloy, mutual separation of layers or delamination occurs (Srivastava, et al., 2022). Therefore, the residual stress prevalent in an LPBF component, combined with an external load, may have a detrimental impact on the performance lifetime of the component (Strantz, et al., 2021).

Heat treatment is often employed to reduce the residual thermal stresses and optimise the microstructure of the fabricated products (ASM International Handbook Committee, 1991). In addition, a post-processing step (such as conventional machining or grinding) is required to obtain a high-quality surface finish of the parts or high-dimensional accuracy (Cobbinah, et al., 2021). Optimisation of the process parameters and control of the melt pool during manufacturing may result in lower temperature gradients, thereby reducing residual stresses (Beuth & Klingbeil, 2001; Shamsaei, et al., 2015). In essence, strategies to control the thermal gradient are effective in preventing the residual stress profile (Strantz, et al., 2021).

The thermal properties are dependent on the employed material (Strantz, et al., 2021). For instance, thermal gradients in a material with low thermal conductivity, high specific heat capacity, high coefficient of thermal expansion and high density yield high thermal stresses (Dieter, 1961). The material is selected based on the intended application of the part, and thus, it is not free to adjust. A better option is to modify the building process. For any given AM technique, the process parameters and build strategy are manipulated to affect the thermal profile and, thus, control the final residual stress profile (Strantz, et al., 2021). Ali et al. (2018) and Strantz et al. (2021) investigated the effect of laser scanning strategy on residual stress distributions in LPBF Ti6Al4V components and found that the scanning strategy greatly influences the residual stress and that a continuous scan strategy with a track orientation rotated from 0° to 90° between layers reduced the residual stress. Robinson et al. (2018) found that the residual stress in LPBF cpTi components was primarily generated parallel to the direction of

scanning. Laser scanning strategy influences the microstructure and overall residual stress within the final component (Cobbinah, et al., 2021).

2.2.2.5. Effect of process parameters on 3D part defects

A combination of low laser power and low scanning speed produces insufficient energy density to generate sufficient liquid phase for the sintering of powder particles (Olakanmi, et al., 2015). This results in partial melting (that is, selective laser sintering) and poor densification. Increasing the laser power while maintaining a low speed means more energy is delivered to the powder bed, causing the powder temperature to increase, followed by a significant formation of the molten phase (Olakanmi, et al., 2015; Cobbinah, et al., 2021). The adequate liquid phase improves densification by reducing surface free energy, surface tension and melt viscosity. Therefore, it may be deduced that proper increments in the laser energy lead to high working temperature and culminate in a reduction in pore size and quantity, hence achieving fully dense components (Yadroitsev et al., 2013).

Overheating a powder particle tends to move it rapidly, generating overpressure in the melted zone and eventually ejecting the particle from the powder bed in the form of spatter and partially melted particles (Das, 2003; Körner, et al., 2013; Yadroitsev, et al., 2013). A high laser power with a low scanning speed may cause vaporisation of the powder particles and the keyhole effect. It may also cause a higher peak melt pool temperature that induces a more intense Marangoni flow, which may lead to the Plateau-Rayleigh instability and balling (Krakhmalev & Yadroitsev, 2014; Cao, et al., 2021). Additionally, increasing the laser energy density beyond the optimum range results in a decreased sintered density. In this condition, higher laser power increases the amount of liquid phase, but a reduced scan speed prolongs its presence, thereby promoting spheroidisation (commonly referred to as the balling effect in LPBF) of the melt pool and its breakdown (Das, 2003).

Unlike laser power, laser scanning speed has a narrow process window for attaining optimum conditions (Krakhmalev & Yadroitsev, 2014). At an optimal laser power, too slow a scanning speed may result in extensive material evaporation and the keyhole effect, while too fast a scanning speed may result in track instability and the occurrence of balling (Krakhmalev & Yadroitsev, 2014). The high scanning rate reduces the interaction time, and high scanning speed with low laser power (that is, low laser energy) forms a small molten pool, limiting the contact area between the molten pool and the substrate. This results in balling due to reduced

wettability, flow and spread characteristics, and Rayleigh instability (Khorasani, et al., 2020; Cobbinah, et al., 2021). On the other hand, when the laser scanning speed is high, heat loss by conduction decreases, and the absorbed laser radiation energy is directed to fusing the material (Figure 2.5) (Cobbinah, et al., 2021). A combination of low laser power, high scanning speed and a thick powder layer also usually results in insufficient energy (Olakanmi, et al., 2011). With a larger powder layer thickness, the energy absorbed in a unit powder volume is inadequate, resulting in a weak flow ability and balling phenomenon (Li, et al., 2012). A thin powder layer is favourable for forming a continuous laser-liquefied scan track and overall success of the LPBF process (Cobbinah, et al., 2021).

Surface properties may be studied to clarify the occurrence of defects, as poor surface finish is common in LPBF parts (Aboulkhair, et al., 2019). Material characteristics and process parameters are prime factors affecting surface quality during the LPBF process (Khorasani, et al., 2020). Comprehending the surface characteristics is required to determine their effects on part properties and in designing parts with improved performance. Additionally, surface roughness has the potential to be used as a process signature (Fox, et al., 2016). The surface roughness in LPBF parts is about 4–5 times that of the machined surfaces (Aboulkhair, et al., 2019). Surface defects arising from surface roughness are also among the major defects of the LPBF process. These defects are caused by high cooling rates and repeated thermal loading (Singh, et al., 2020). The high surface roughness is mainly attributed to the balling phenomenon and the presence of satellites (formed by spattering and/or partial melting of particles in the peripheral zone of the laser spot, illustrated in Figure 2.5), which together result in producing parts with a wide range of surface irregularities (Yadroitsev, et al., 2013; Aboulkhair, et al., 2019). The interaction between the laser beam and the powder bed, and the consequential development of various thermal models arising from the combination of material thermal nonlinearity, latent heat, and laser heat source distribution, generates various surface qualities (Khorasani, et al., 2020).

Using higher powers decreases the surface roughness by increasing the wettability of the melt pool and reducing the formation of balling. However, increasing the scan speed reduces the surface quality of the parts (Khorasani, et al., 2020). Low hatch spacing may result in large overlapping of melt tracks and increase the surface roughness, while larger hatch spaces reduce surface roughness, but may decrease part density (Islam, et al., 2024). Inappropriate scan hatch spacing often results in regular porosity in built parts as adjacent melt lines do not fuse fully

(Cobbinah, et al., 2021). Pores are typically two scan vectors apart and initiate at the single-layer level (Dewidar, et al., 2003). These types of pores may be eliminated by appropriate overlapping of adjacent scan tracks (Cobbinah, et al., 2021). Particle size and distribution also affect the quality of LPBF parts. Powders with coarser particles increase surface roughness and porosity (Khorasani, et al., 2020). Cracks formation is mainly attributed to the scanning strategy due to the band heating. A post-processing treatment may be effective in closing the cracks (Cobbinah, et al., 2021). Kenel et al. (2017) demonstrated that re-scanning at a rotation of 90° reduced cracking by a factor of two. Techniques to process the surface of a part during and after construction exist. However, with complex shapes, the options to successfully post-process the surface decrease. Thus, the quality of the as-built surface is an integral feature for AM (Fox, et al., 2016).

When the process parameters are not optimised, inner defects may occur (Kasperovich, et al., 2016). The defects that occur during LPBF negatively affect the process and surface quality of the produced parts (Khorasani, et al., 2020). Optimisation and selection of appropriate processing parameters for the laser and powder deposition are paramount to attain a suitable process window (Cobbinah, et al., 2021). Therefore, it is compulsory to optimise the process parameters (Khorasani, et al., 2020). Other properties, such as high density, minimal surface roughness and reduction in defects and stresses, should also be maintained, which narrows the available window for optimising the process parameters (Strantz, et al., 2021).

2.3. LPBF for medical implants

Laser powder bed fusion has been increasingly popular in recent years for manufacturing medical implants or devices with higher design freedom and capabilities of producing patient-specific implantable devices from CAD data derived from the patient's own medical imaging (DebRoy, et al., 2018; Haase, et al., 2023). As a result, additively manufactured implants for clinical use are already being approved for commercial exploration (Haase, et al., 2023).

2.4. Biomaterial for medical application

Materials compatibility is the most crucial aspect to be considered for successful medical and dental implantation (Jemat, et al., 2015). Biomaterial science and technology have been rapidly developing since the late 1990s, attesting to a strong vitality and broad development prospects. Biomaterials are not necessarily drugs, and their therapeutic mechanisms are characterised by direct binding and interaction with biological components as a fundamental feature (Han, et

al., 2023). There are numerous types of artificial biomaterials, including metals, polymers, ceramics, composite materials and derived materials (Kurup, et al., 2021; Han, et al., 2023). Substantial research has been conducted in the development of polymers and ceramics for biomedical applications (Kurup, et al., 2021). Biomedical metallic materials are the most widely used load-bearing implant materials in clinical applications due to their superior mechanical properties, resistance to wear and corrosion, high fatigue resistance and potential for biocompatibility (Kurup, et al., 2021; Zhang, et al., 2021a; Han, et al., 2023). In addition, metals and alloys are compatible with most AM techniques. Based on their primary application, metallic biomaterials are classified into four categories as implants: cobalt-based alloys, stainless steels, titanium-based alloys and others (Garcia-Falcon, et al., 2021). Each type of material has its own advantages, particularly suitable for specific biomedical applications (Ribeiro, et al., 2012; Kim, et al., 2020). The use of Ti for biomedical applications was first discovered in 1940 (Jambhulkar, et al., 2023). Over the decades, pure Ti and Ti6Al4V alloy, as one class of the most promising biomaterials, have gradually replaced both stainless steel and cobalt-based alloys in many medical applications, especially in orthopaedic and dental areas (Ren, et al., 2014).

Biomaterials are intended for medical applications in contact with living organisms and should thus be thoroughly studied to ensure their safe use in the human body (Garcia-Falcon, et al., 2021). Biocompatibility requires that biomaterials possess both structural and surface compatibility with the host tissue without triggering an immune response, and hence, mechanical and physico-chemical compatibility are also necessary. Therefore, research on biomaterials represents an interdisciplinary conjugal effort and requires the collaboration of various engineering disciplines, pathologists and clinicians (Ribeiro, et al., 2012; Garcia-Falcon, et al., 2021; Kurup, et al., 2021).

2.5. Titanium and alloys as implants

Commercially pure Ti (cpTi) is divided according to the purity and the processing oxygen content into four grades from Grade 1 to 4 (McCracken, 1999; Liu, et al., 2017). These grades vary in their corrosion resistance, ductility, and strength. Grade 1 cpTi, which is processed with the lowest oxygen content (about 0.18%), has the highest purity, the best corrosion resistance and formability. However, the overall mechanical strength is the lowest. Grade 4 cpTi, which is processed with the highest oxygen content (about 0.4%), is the least pure with moderate formability; however, it has the highest strength (Liu, et al., 2017). The conditions and

parameters may be altered during the manufacturing of Ti and its alloys to produce parts with different mechanical properties for different applications (Kim, et al., 2020).

Of all Ti alloys, Ti6Al4V (titanium–6 weight percentage (wt.%) aluminium–4 wt.% vanadium, may be written as Ti64 or Ti-6Al-4V) or Ti6Al4V ELI (exhibiting an extra low amount of interstitials, hence termed “ELI”) is the most significant, even preferred over cpTi in most implant applications as may be seen in Figure 2.9 (Jambhulkar, et al., 2023). Ti6Al4V is favoured especially for load-bearing orthopaedic implants, while cpTi is a common constituent of dental implants (McCracken, 1999; Pye, et al., 2009; Wang, et al., 2023b). During daily activities such as walking and chewing, heavy loads are transferred to bone tissue; thus, artificial implants intended to replace the bone should be load-bearing to some extent (Bosco, et al., 2012). Additively manufactured Ti6Al4V parts are of broad interest for biomedical implants such as dental and orthopaedic implants (Gong, et al., 2014; Kasperovich, et al., 2016). Furthermore, LPBF of Ti6Al4V has progressively improved with respect to process optimisation and property characterisation, and the components are widely used in various fields, including medicine (Gong, et al., 2014; Vergara-Hernández, et al., 2023). The Ti6Al4V alloy is particularly interesting owing to its high strength-to-weight ratio and low density (Kasperovich, et al., 2016; DebRoy, et al., 2018).

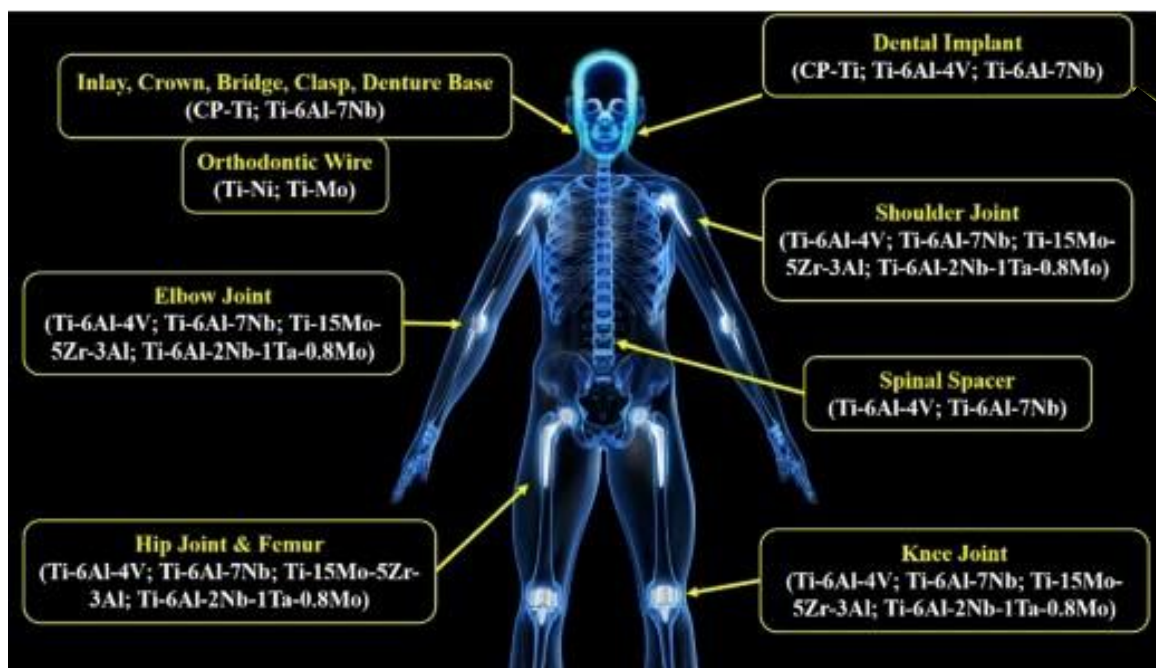


Figure 2.9: Application of cpTi and Ti-based alloys as implants in the human body (adapted from Sarraf et al. (2022))

Titanium and Ti alloys have been extensively studied due to their attractiveness for various applications and have a spectrum of medical applications, including hard tissues such as bone, joints and teeth, and soft tissues such as cardiovascular tissues and artificial organs (Kurup, et al., 2021; Han, et al., 2023). They are used extensively as implant materials due to their outstanding mechanical properties, such as good formability and machinability, high strength, fracture resistance and good fatigue behaviour. Furthermore, their torsional and axial stiffness are closer to those of bone, resulting in less stress shielding of bone compared with other alloys (Ribeiro, et al., 2012; Kurup, et al., 2021; Jambhulkar, et al., 2023; Vergara-Hernández, et al., 2023). Other essential requirements of biomaterial that are met by Ti alloys include excellent biocompatibility, low density (about 60% of that of steel) and excellent corrosion resistance in corporal fluids (Gong, et al., 2014; Donachie, 2020). In addition, the attraction to Ti is augmented by the increasing cost of other biometals, such as palladium (Pd) and gold (Au) (Kurup, et al., 2021). Titanium is readily available since its natural mineral deposits are available in abundance, and it is a commercial material (Jambhulkar, et al., 2023).

Titanium is allotropic and has two elemental crystal structures (

Figure 2.10) (ASM International Handbook Committee, 1991). In the one crystal structure, the atoms are arranged in a hexagonal close-packed (hcp) array, and this structure forms the alpha (α) alloys. In the other crystal structure, the atoms are arranged in a body-centred cubic (bcc) array, and this structure is found in the beta (β) alloys (ASM International Handbook Committee, 1991; Donachie, 2020). At room temperature, cpTi is α in structure. The β structure occurs at high temperatures and when cpTi is alloyed with elements to maintain it at lower temperatures (ASM International Handbook Committee, 1991; Donachie, 2020). The addition of alloying elements to cpTi produces a range of possible microstructures in Ti alloys (Donachie, 2020). Titanium and its alloys are generally classified based on the content of α - and β -phases as α , $\alpha+\beta$ and β alloys, as illustrated in Figure 2.11, with further subdivision into near- α and near- β alloys (Motyka, 2021). The majority of the investigations of cpTi and Ti6Al4V focus on the direct relations between mechanical properties and either processing parameters or microstructure (DebRoy, et al., 2018).

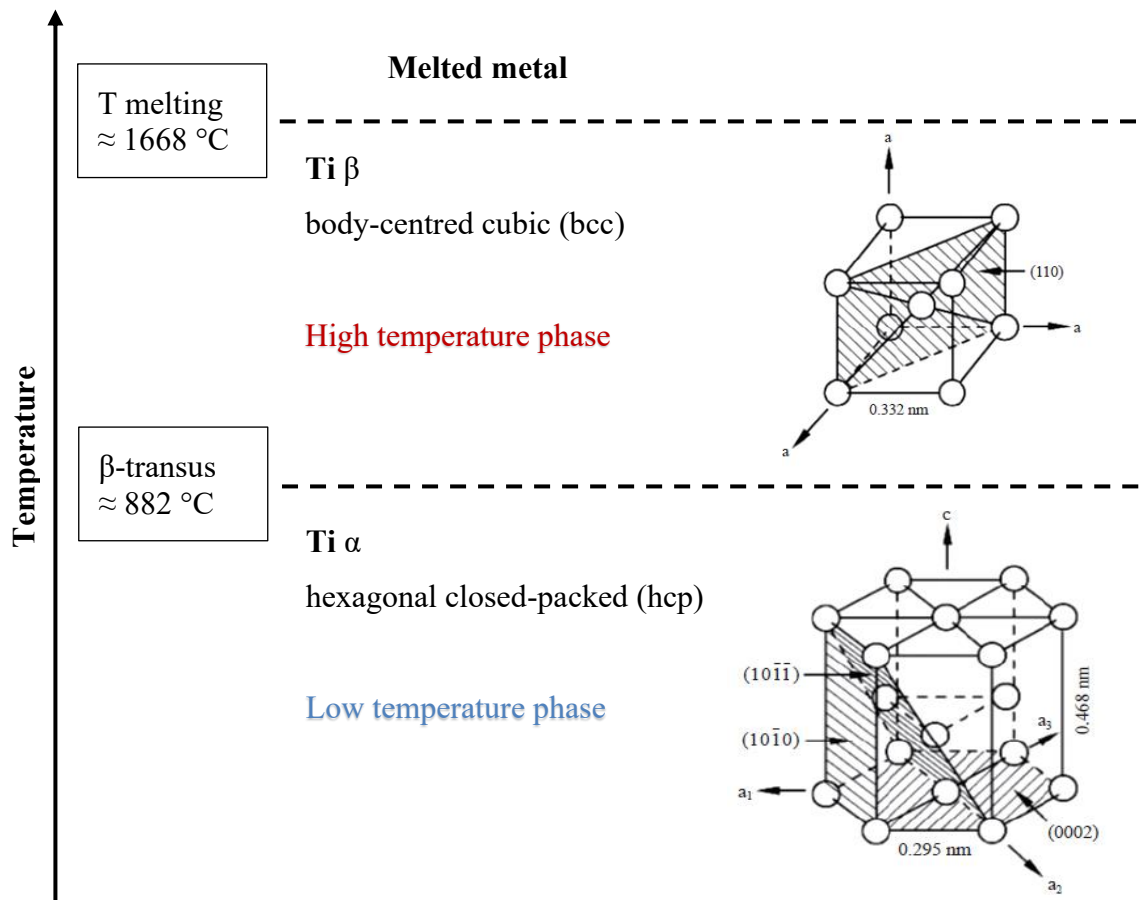


Figure 2.10: Titanium phases and main transformation temperatures (adapted from Cao et al. (2021) and Farrokhi (2023))

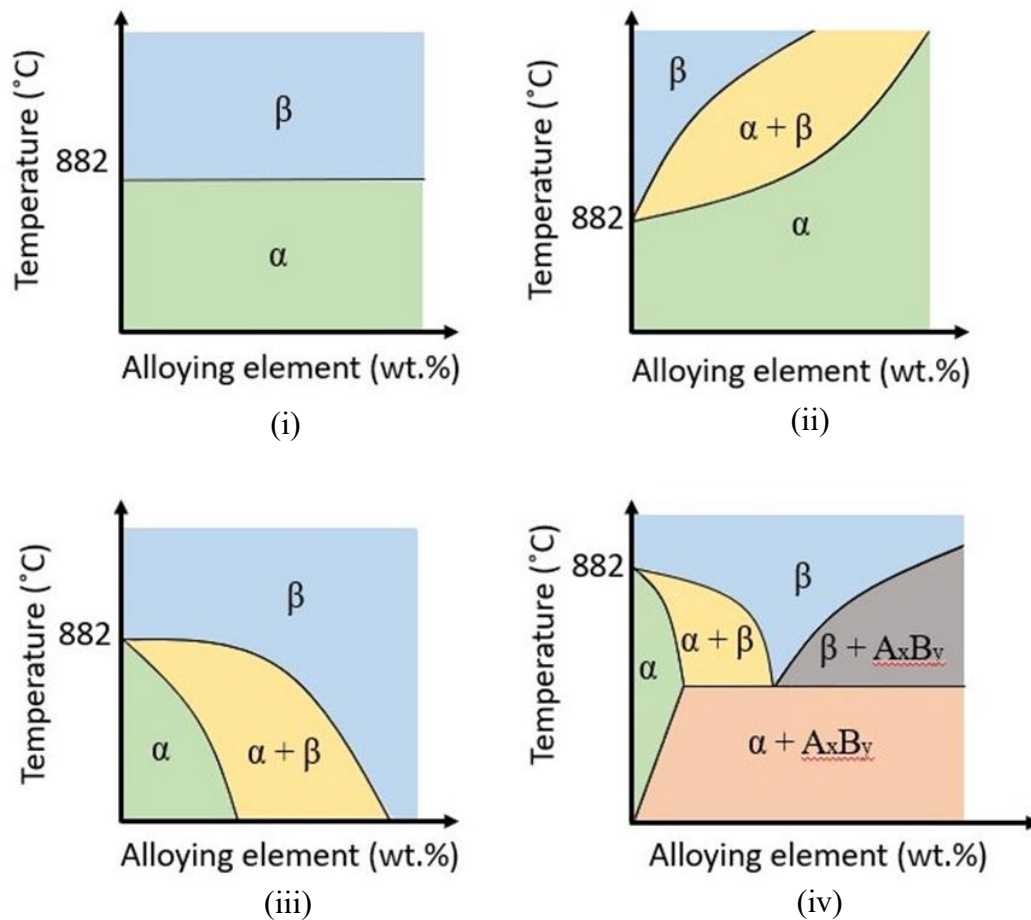


Figure 2.11: Different types of stabiliser in titanium alloys (i) neutral, (ii) α -stabilising, (iii) β -stabilising (isomorphous, for example, Ti6Al4V), and (iv) β -stabilising (eutectoid, for example, binary titanium-copper diagram) (adapted from Ahmad & Zuhailawati (2020))

Allotropic transformation $\alpha \leftrightarrow \beta$ is considered a primary phase transformation in Ti and may result in the possibility of martensite formation in Ti alloys. During heating of Ti above the β -transus temperature of about 882 °C, α (hcp) transforms into β (bcc), and the β phase persists up to the melting point of approximately 1668 °C (ASM International Handbook Committee, 1991; Motyka, 2021). During cooling, the reverse transformation $\beta \rightarrow \alpha$ occurs (Motyka, 2021). When the cooling rate is low, the allotropic transformation $\beta \rightarrow \alpha$ proceeds by nucleation and growth of new phase crystals, whereas at a high cooling rate, it exhibits features of a martensitic transformation (that is, the formation of fine laths of α' acicular martensite) (Motyka, 2021). Needle-shaped α' martensite is a non-equilibrium phase with deformed lattice structures and has been shown to exhibit higher strength and reduced plasticity than other microstructures (Liu & Shin, 2019). Heat treatment may resolve the presence of acicular α' martensite laths. The Ti6Al4V alloy is classified as an $\alpha + \beta$ alloy that contains 6 wt.% aluminium as the α

stabiliser and 4 wt.% vanadium as the β stabiliser. The β -transus temperature of Ti6Al4V is approximately 995 °C under equilibrium conditions, and above this temperature, Ti6Al4V becomes completely β -phase until its melting point of about 1660 °C (Bartolomeu, et al., 2022).

The final microstructures of Ti alloys are described by the size and arrangement of their α - and β -phases and are highly correlated to the cooling rate that occurs from above the β -transus temperature (Fan, et al., 2016; Bartolomeu, et al., 2022). Figure 2.12 illustrates the microstructures of the as-printed LPBF cpTi and Ti6Al4V, and Ti6Al4V that has been heat treated under various conditions. The two extreme cases of phase arrangement are lamellar (Figure 2.12(iv) and (v)) and equiaxed (Figure 2.12(v)) microstructures (Ávila Calderón, et al., 2022). The lamellar microstructure is generated upon cooling from the β -phase and has a greater α/β surface area and more oriented colonies (Fan, et al., 2016). The equiaxed microstructure results from a recrystallisation and globularisation process and consists of a uniform structure composed of α grains and grain boundaries of β (Loier, et al., 1985; Fan, et al., 2016).

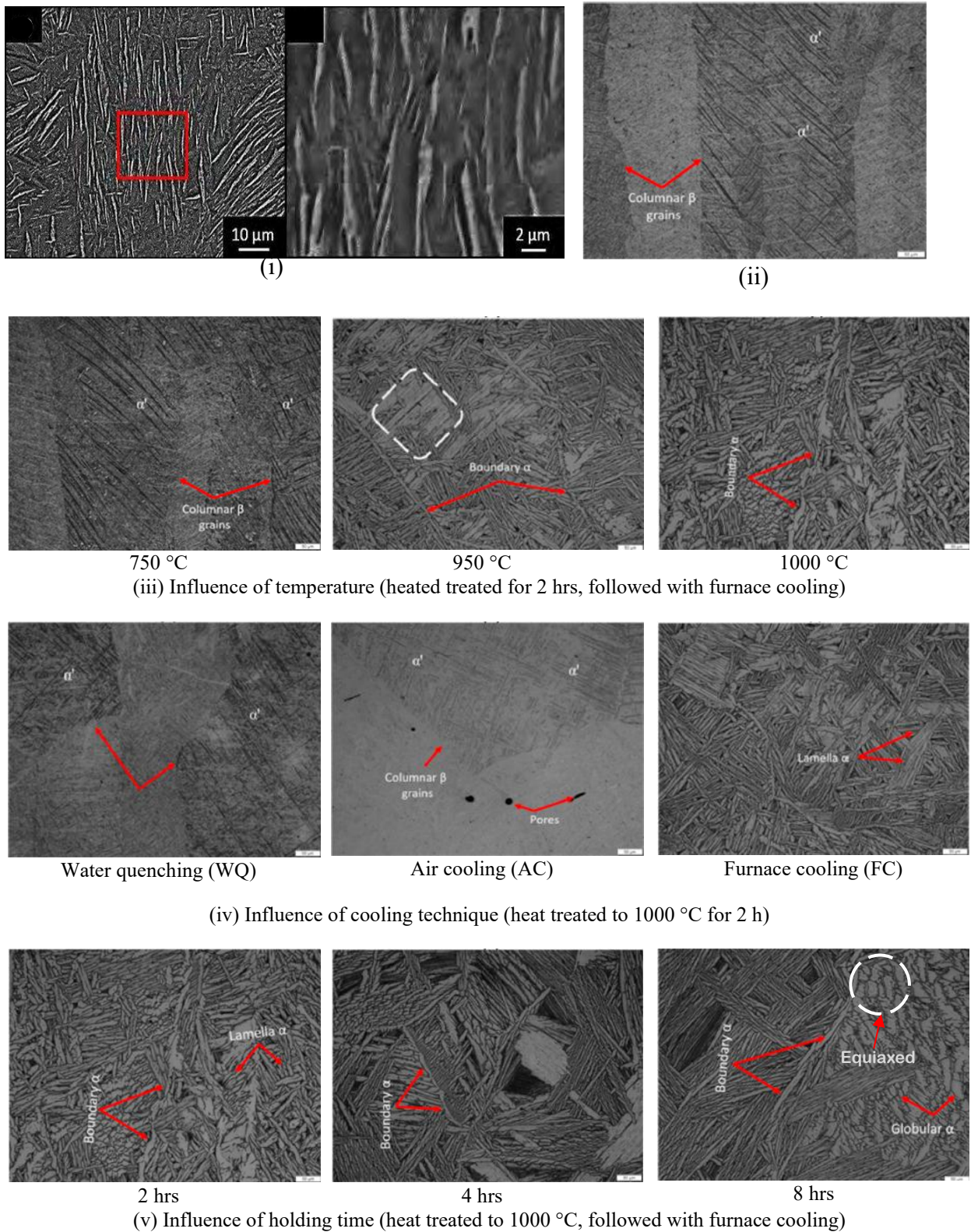


Figure 2.12: Microstructure illustrations of LPBF-processed (i) cpTi in the as-printed state (adapted from Pede et al. (2021)), (ii) as-built Ti6Al4V, and (iii) to (v) heat-treated Ti6Al4V (adapted from Lekoadi et al. (2021))

Research indicates that lamellar microstructure exhibits lower strength and ductility and better fatigue propagation, while equiaxed microstructure provides better fatigue initiation resistance than lamellar microstructure (Loier, et al., 1985; Fan, et al., 2016). A combination of lamellar and equiaxed microstructures is called bimodal microstructure (Figure 2.13(i)) and exhibits a well-balanced fatigue properties profile, since it combines the advantages of both lamellar microstructure (higher fatigue crack propagation resistance) and equiaxed microstructure (higher fatigue crack initiation resistance) (Fan, et al., 2016; Yang & Liu, 2016). Regarding Young's modulus, the β -phase Ti provides a lower elastic modulus compared to the α -phase Ti (Xiao, et al., 2023). A mismatch of elastic modulus between natural bone (10–30 GPa) and bulk metallic biomaterials (~ 110 GPa for Ti) may lead to stress shielding, which may cause bone resorption and loosening of the implant (Zaman, et al., 2015). Widmanstätten microstructure (Figure 2.13(ii)) may be formed in Ti6Al4V during heat treatment when materials are cooled at a critical rate from extremely high temperature (Fan, et al., 2016). For instance, Ahmed & Rack (1998) demonstrated that, for heat treatment at 1050 °C for 30 min and at a low cooling rate (<20 °C s⁻¹), α colonies change into a basket-weave structure (Figure 2.13(ii)), which is a characteristic of the Widmanstätten structure.

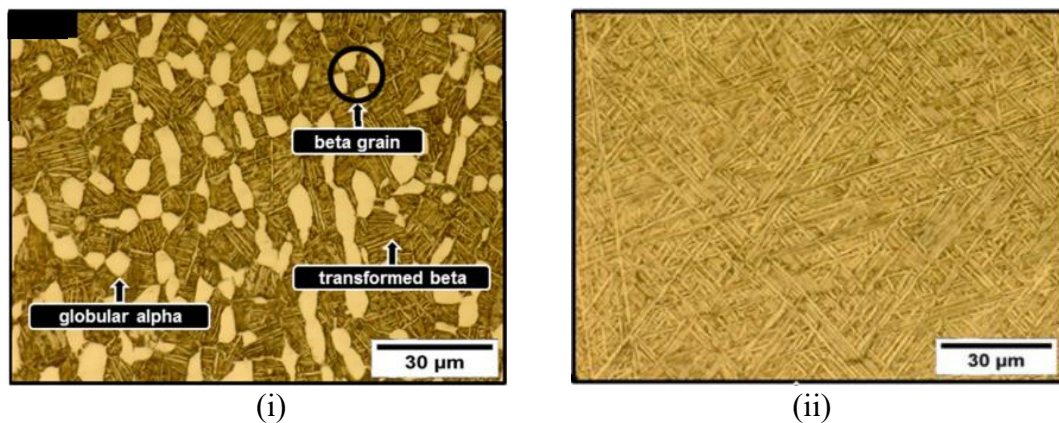


Figure 2.13: Optical micrographs illustrating (i) bimodal, and (ii) Widmanstätten microstructures (adapted from Hosseini et al. (2017))

Figure 2.14 demonstrates the development of the microstructure of LPBF Ti6Al4V with changes in the temperature for heat treatment, while Figure 2.15 illustrates the effect of the heat treatment on the microstructure and resultant mechanical properties. Titanium and its alloys are heat-treated for various benefits. These include reducing residual stresses developed during fabrication (stress relieving), producing an optimum combination of ductility, machinability and dimensional and structural stability (annealing), increasing strength

(solution treating and ageing), and optimising special properties such as fracture toughness, fatigue strength and high-temperature creep strength (ASM International Handbook Committee, 1991).

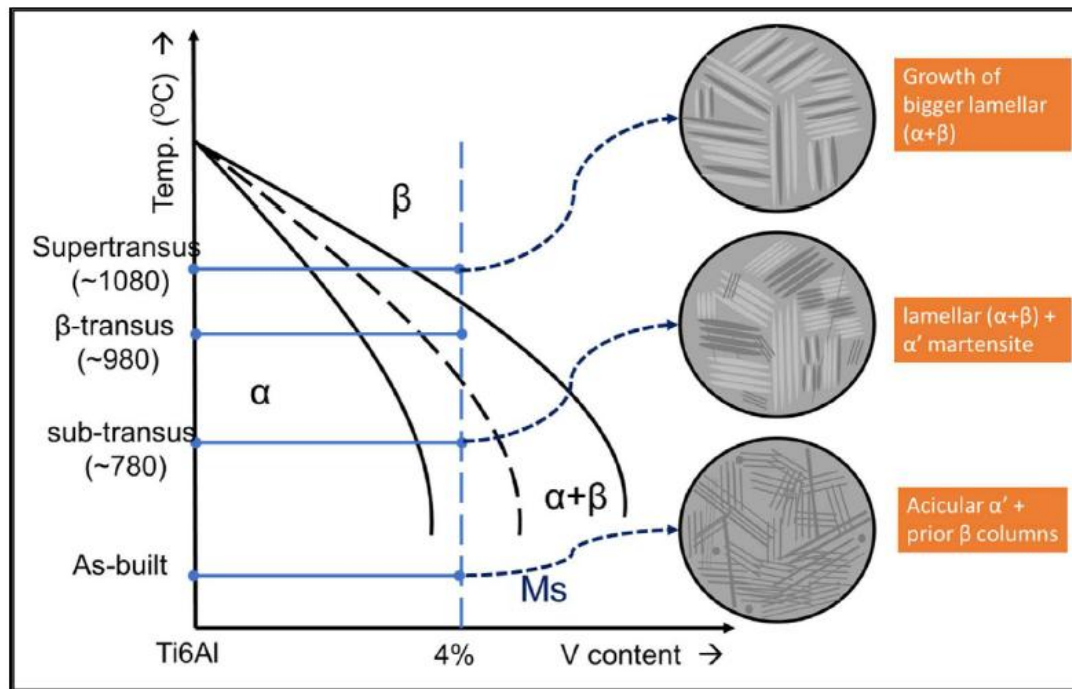


Figure 2.14: Microstructure development of Ti6Al4V with variation in heat treatment temperature (adapted from Jamhari et al. (2023))

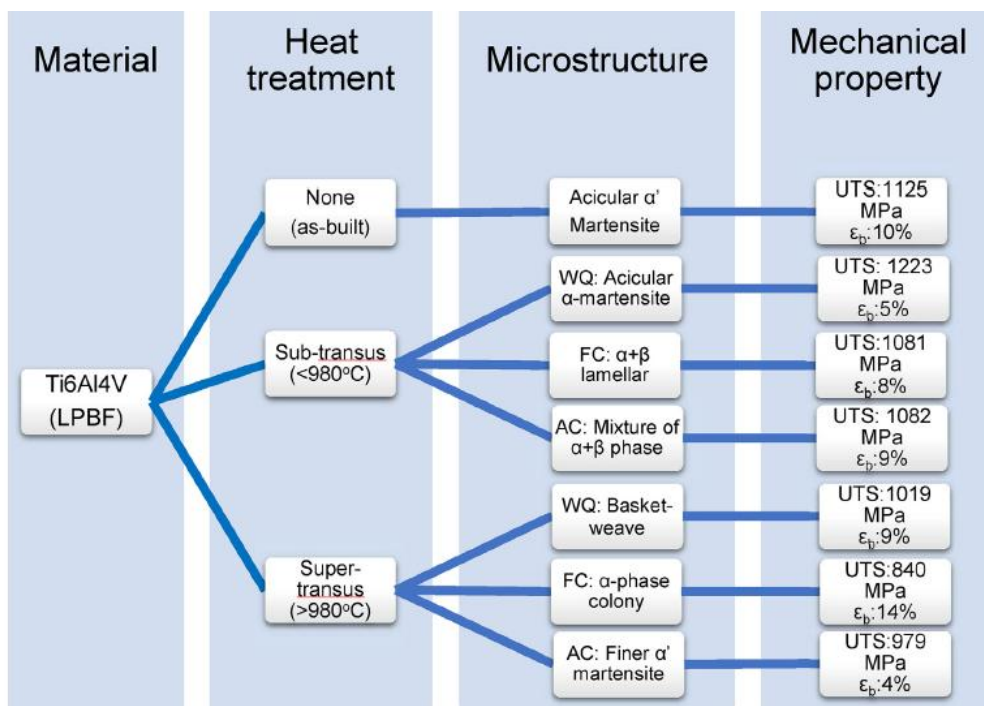


Figure 2.15: Effect of heat treatment on the microstructure and mechanical properties of LPFB Ti64 samples (adapted from Jamhari et al. (2023))

2.5.1. In situ alloying with copper

Manufacturing of some alloys by conventional methods is restricted by immiscibility of elements, narrow solubility ranges, or the risk of precipitation of undesirable intermetallic phases (Krakhmalev, et al., 2017). Achieving alloying in situ during the manufacturing process is a unique opportunity for LPBF (Sing, et al., 2021). The technology combines powder metallurgy: a wide range of powders is blended, then the mixture is fully melted and rapidly cooled, preventing the formation of undesirable phases. Consequently, LPBF offers a greater opportunity to manufacture customised implant materials and design new alloys with compositions often impossible for conventional methods (Krakhmalev, et al., 2017). Selecting processing parameters that guarantee efficient in situ alloying is a challenge that requires a trade-off (Krakhmalev, et al., 2017). When the input energy density is too high, deep re-melting of the substrate may occur, resulting in a keyhole mode and formation of pores in the molten pool (Yadroitsev, et al., 2012; Yadroitsava, et al., 2015a). When the laser power density is low and the scanning speed is high (that is, the input energy is low), the molten pool temperature is low, leading to increased viscosity, which may result in droplet formation and insufficient mixing (Yadroitsev, 2009). For successful in situ alloying, the molten metal should have low viscosity, and the molten state should persist long enough to ensure optimal homogeneity of the elements. Concurrently, the conditions should be within the range of the operation window required to manufacture high-quality 3D objects (Yadroitsev, et al., 2015b).

Laser powder bed fusion has been experimentally proven as a suitable method for manufacturing novel components from elemental powders using in situ alloying (Krakhmalev, et al., 2017). Cui et al. (2024) alloyed steel and Al alloy using LPBF and achieved their aim of fabricating multi-material components by effectively controlling additive manufacturing parameters. Huber et al. (2021) successfully in situ alloyed Ti with four powder elements (namely, tungsten, tantalum, molybdenum and niobium) using LPBF and Aota et al. (2020) in situ alloyed elemental powders (namely, iron, chromium and nickel) to build AISI 304 L steel parts via LPBF. In addition to the general requirements of densification and defect reduction, process parameters for alloying need to be carefully fine-tuned to obtain chemical homogeneity (Aota, et al., 2020). The applied processing parameters should produce large molten pools, while maintaining the energy density below the keyhole porosity threshold, to promote the formation of a chemically homogeneous alloy directly after the LPBF process, without any post-process heat treatment (Aota, et al., 2020). Krakhmalev & Yadroitsev (2014)

demonstrated that optimising process parameters, increasing laser power, or adopting a re-melting strategy, improves the homogeneity of LPBF intermetallic materials.

Since each material has specific laser absorbance and thermal properties, extensive experimental work should be conducted to obtain processing windows for new material systems that allow the production of parts with desired properties (Sing, et al., 2021). Due to the discrete nature of these processing windows, performance degradation may occur when applied to other materials (Sing, et al., 2021; Narasimharaju, et al., 2022). Fischer et al. (2016) study showed that the LPBF process may be utilised to fabricate a homogeneous alloy from mixed powders. The authors also found that high energy levels produce homogeneous and compact samples. Similarly, according to Dadbakhsh & Hao (2014), uniformly distributed ultrafine particles may be consolidated into a net-shape composite part by applying a lower layer thickness, a higher laser power, and a lower scanning speed.

Despite the impressive track record, some advantages of Ti and its alloys have allied disadvantages. For example, the low modulus of elasticity, high strength at elevated temperatures, low thermal conductivity and high chemical reactivity translate to relatively poor machinability or grindability of the Ti material (Ren, et al., 2014). To marginalise these flaws, studies have reported that adding small quantities of Cu to pure Ti and Ti6Al4V alloy during the melting process developed a Ti alloy with better grindability and wear resistance (Ren, et al., 2014; Ju, et al., 2023). Ohkubo et al. (2003) demonstrated that adding Cu improves the wear resistance of as-cast pure Ti and Ti64 alloys as a result of including the eutectoid α -Ti/Ti₂Cu in their microstructure. Figure 2.16 shows a proposed sequence of particles melting during LPBF of in situ alloyed Ti-Cu particles.

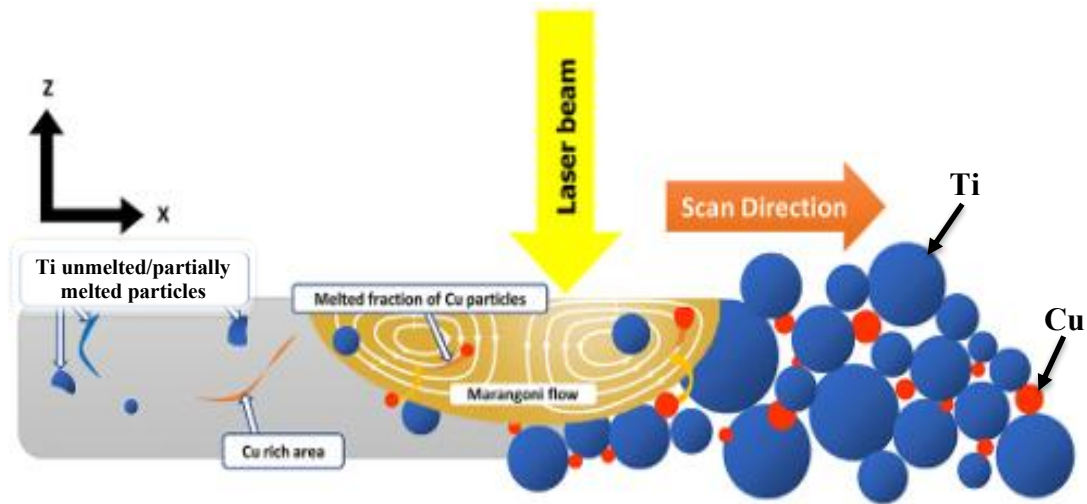


Figure 2.16: Possible melting process of in situ alloying of Ti and Cu during LPBF process (adapted from Mosallanejad et al. (2021))

Using a digital simulation technique, Vilardell et al. (2020) simulated the Ti6Al4V-Cu phase diagram to identify the phases under equilibrium conditions. As illustrated in Figure 2.17, the β -transus of Ti6Al4V was located at a temperature of approximately 920 °C. The diagram also indicates a decrease in β -transus with increasing Cu content, reaching around 820 °C at 4.1 wt.% (3 at.%) Cu due to its β -stabilising effect. Similar microstructural features were observed in material manufactured using LPBF at specified process parameters (Vilardell, et al., 2020). It was postulated that the addition of Cu may enhance the formation of retained β -phase during the LPBF process. Due to the limited solubility of Cu in α -Ti, an intermetallic CuTi_2 phase is formed, leading to a ternary-phase region of $\alpha + \beta + \text{CuTi}_2$. The possibility has been reported of a two-phase eutectoid reaction $\beta \rightarrow \alpha + \text{CuTi}_2$ occurring at higher Cu content in a Ti-Al-V-Cu quaternary system, as in a Ti-Cu binary system (Vilardell, et al., 2020; Ju, et al., 2023).

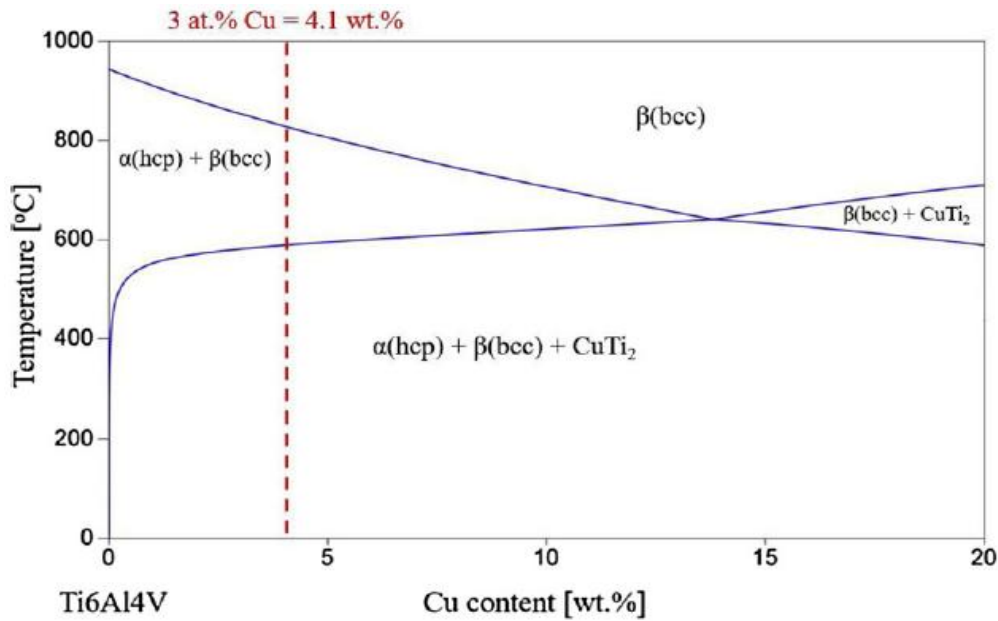


Figure 2.17: A calculated Ti–Al–V–Cu quaternary phase diagram (adapted from Vilardell et al. (2020))

2.5.2. Cu as an antibacterial agent

Despite the high success rates of implant treatment, the risk of postoperative bacterial infection at the surgical site haunts many medical practitioners (Ribeiro, et al., 2012; Li, et al., 2024). Approximately two million nosocomial infections occur in the United States each year, of which about 30–50% are related to implants (Cao, et al., 2019). A ten-year study by Onuoha et al. (2019) identified infection as the leading factor in implant failure, and was associated with 40% of the failed implants. The infection rates of periprosthetic joint infections are 0.5–2%, 2–9% and 0.3–1.7% after total joint replacement of the knee, hip and ankle, respectively (Li, et al., 2016). Therefore, implant-associated infection is considered to be one of the most serious complications of prosthetic implants (Li, et al., 2016). Causes of postoperative implant infection include surgical instruments due to insufficient sanitation, bacterial strains introduced into the surfaces of metallic implants and wound contamination during and after surgery (Al-Khateeb, et al., 2023; ElectrospinTech, 2024). Infection may result in increased healthcare expenses and patient misery arising from long-term hospitalisation, revision surgeries, definitive failure of the implants, and even death (Li, et al., 2016; Al-Khateeb, et al., 2023).

Although Ti and alloys have numerous benefits as commonly used biomaterials in endosseous implants and in orthopaedic applications, Ti is biocompatible and lacks inherent antibacterial characteristics, which allows pathogens to use the implant surface as a substrate for

colonisation and biofilm formation, leading to prolonged infections, particularly in clinical settings because of compromised host defence (Li, et al., 2016; Haq, et al., 2024; Li, et al., 2024). The selection of an antibacterial alloying element should satisfy the basic requirements of having bactericidal activity, should not have a negative impact on the mechanical property of the matrix alloy and should not trigger the host's innate immune response (Zhang, et al., 2013). For biomedical applications, several metal ions, like copper (Cu), silver (Ag) and zinc (Zn) have been described as effective antibacterial agents against a broad spectrum of bacteria (Zhang, et al., 2013; Li, et al., 2016). Copper and Ag are the most prominent elements added to metals to induce the antibacterial properties in alloys (Wu, et al., 2020). Like Ag, Cu has shown success as an alloying metal with a variety of biometals, including Ti and stainless steel, and has been well-established as a potent antibacterial agent with a vast spectrum of activity against numerous bacteria and low incidence of resistance (Ren, et al., 2014; Li, et al., 2016; Wu, et al., 2020; Ju, et al., 2023).

In contrast to Ag, Cu is an essential trace element (that is, a micronutrient) required for human health. Furthermore, Cu has lower toxicity, better cytocompatibility, excellent chemical stability and is more cost-effective than Ag and Zn (Zhang, et al., 2013; Lei, et al., 2019; Wu, et al., 2020; Yuan, et al., 2021; Xu, et al., 2023). Studies reported that a relatively low concentration of Ag^+ and Zn^{2+} ions ($\text{LD}_{50} = 3.5 \times 10^{-3} \text{ mmol/l}$) may be greatly toxic to the tissue cells; however, a relatively higher concentration of Cu^{2+} ions ($\text{LD}_{50} = 2.3 \times 10^{-1} \text{ mmol/l}$) did not exhibit a toxic effect on the cells (Ren, et al., 2014). In other words, the human body is able to tolerate higher amounts of Cu ions than other antibacterial metallic elements (Ren, et al., 2014). Noteworthy, the Recommended Dietary Allowance (RDA) for Cu is 900 μm for a healthy adult human (National Institutes of Health, 2022).

As an essential nutrient vital to the health of all living organisms (including humans, animals, and microorganisms) and at controlled concentrations, Cu is indispensable for the efficient functioning of organs and metabolic processes (Ahire, et al., 2016; Ju, et al., 2023). As such, Cu is essential for the formation of red blood cells, the absorption and utilisation of iron, the metabolism of cholesterol and glucose, and the synthesis and release of life-sustaining proteins and enzymes (Zhang, et al., 2013; Chen, et al., 2021). Furthermore, Cu stimulates the immune system to fight infections and promote healing (Ju, et al., 2023), which emphasises the preference of Cu as a biomedical material. Copper exists in various forms (such as Cu^0 , Cu^+ , Cu^{2+} , CuO , CuO_2 and Cu(OH)_2), and each form behaves differently in different media

(Manakhov, et al., 2021). For instance, it was shown that CuO_2 has considerably higher antibacterial activity compared to Cu(OH)_2 (Manakhov, et al., 2021).

2.5.2.1. Copper oxide nanoparticles

The recent advances in the field of nanotechnology enable the invention of new techniques for the preparation of antimicrobial agents against microbes (Haider, et al., 2015; Muñoz-Escobar, et al., 2019; Li, et al., 2024). Nanomaterials, such as nanoparticles (NPs), provide a higher surface area-to-volume ratio (Muñoz-Escobar, et al., 2019). Studies prompt that inorganic antibacterial agents such as metal/metal oxide NPs have potent roles as antimicrobial agents and preferable thermal stability (Chatterjee, et al., 2014; Chen, et al., 2021). Antimicrobial activity of the NPs depends on their size, stability and concentration of the NPs in the medium (Haider, et al., 2015). Due to their large surface-to-volume ratio and crystalline structure, NPs elicit biological responses distinct from those elicited by the traditional ionic form of the metals. Additionally, metallic NPs were found to be substantially less toxic to mammalian cells than their ionic counterparts (Chatterjee, et al., 2014). Nanoparticles have thus received significant consideration in the medicine field (Haider, et al., 2015).

The particles at the nano-scale tend to enhance antibacterial properties because their smaller particle morphology increases the number of surface atoms available to interact with bacterial membranes (Tahir, et al., 2017; Jan, et al., 2021). Metal oxide NPs offer promise as antimicrobial agents against a broad spectrum of microorganisms, including multidrug-resistant strains of Gram-positive and Gram-negative microbes (Muñoz-Escobar, et al., 2019). Of the oxides of transition metals, copper oxide nanoparticles (CuO NPs) are well-known for antibacterial properties and have a broad-spectrum biocidal effect owed to its effective suppression of growth of bacteria, fungi or algae and its unique chemical, physical and biological properties (Nirmala, et al., 2013; Muñoz-Escobar, et al., 2019; Chen, et al., 2021). During their research, Padil & Cernik (2013) synthesised CuO NPs, which they purified and dried into different CuO NPs sizes. The authors established that small CuO NPs (4.8 ± 1.6 nm) exhibited significantly greater antibacterial activity than larger particles (7.8 ± 2.3 nm). The size of Cu NPs emerged as a major contributing factor in antimicrobial activity.

In addition to the size, the charge also plays a role in the antibacterial mechanisms of metallic ions. Metals generate positive ions in an aqueous environment that allow them to attach to the cell wall of bacteria, which is innately negatively charged, and initiate biocidal activity (Yadav,

et al., 2022). As such, the antimicrobial activity of CuO NPs relies on the ability to release Cu^{2+} metallic ions and the extension of their surface area (Muñoz-Escobar, et al., 2019). The Cu^{2+} ions attach to the negatively charged proteins and lipids of the cell membrane (Muñoz-Escobar, et al., 2019). The Cu nano-ions can operate through various mechanisms, as shown in Figure 2.18, such as cell membrane disruption, diffusion into the cellular matrix and destruction of internal components such as enzymes and nucleic acids (Yadav, et al., 2022). Furthermore, the Cu^{2+} ions have high redox properties and can produce reactive oxygen species, which alter subcellular components, including damaging deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules, to inhibit the genetic copy process and finally induce the apoptosis of bacteria (Ren, et al., 2014; Muñoz-Escobar, et al., 2019). Therefore, Cu nanomaterials are capable of operating through multiple mechanisms simultaneously, making it unlikely that infecting bacteria will develop mutations against the NPs as they do against antibiotics, which usually operate through a single mechanism (Yadav, et al., 2022).

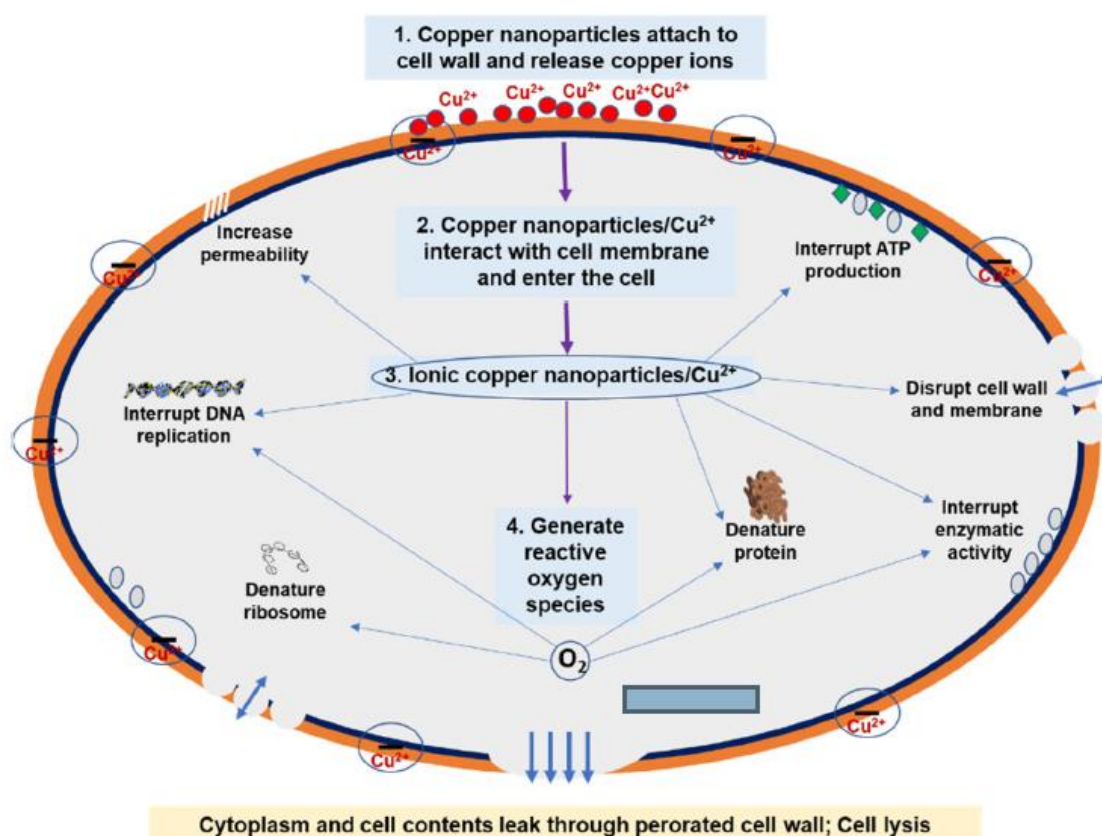


Figure 2.18: Antibacterial mechanisms of copper nanoparticles in a bacterial cell (adapted from Xu et al. (2022))

Facilities and skills to prepare metal/metal oxide NPs of a specific size and shape have been realised (Haider, et al., 2015). The synthesis of stable metallic Cu-NPs is challenging since

they undergo rapid oxidation to Cu^{+2} ions in air or aqueous media (Chatterjee, et al., 2014). Nonetheless, several methods are applicable for the synthesis of CuO NPs, namely physical, chemical and biological methods (Haider, et al., 2015; Muñoz-Escobar, et al., 2019). Chemical reduction is the preferred method because it is simple, cost-effective and efficient since it has optimised experimental factors that allow for the synthesis of NP size and size dispersion. Moreover, most chemical reduction methods are done in an aqueous medium and thus limit the coupling of the synthesised CuO NPs to only hydrophilic polymers (Muñoz-Escobar, et al., 2019).

2.6. Surface features and modification

Titanium and alloys are currently used in clinical orthopaedics and dentistry because of their excellent mechanical properties, and they are additionally preferred materials due to their capacity to form a titanium oxide (TiO_2) layer with a thickness of a few nanometres (Zaman, et al., 2015). The excellent properties of the Ti implants depend on the structure and chemical stability of the TiO_2 layer (Bosco, et al., 2012; Zaman, et al., 2015). It is formed on the surface of a Ti component when it is exposed to air (Kurup, et al., 2021). The TiO_2 layer is generally a very stable oxide layer, which contributes to the inertness of the Ti surface (Bosco, et al., 2012; Zaman, et al., 2015). The biological inertness of the Ti surface is also attributed to its highly corrosion resistant, meaning that it stays relatively unchanged when it is inserted into the human body as an implant. Hence, Ti does not initiate interactions with the human cells (Kurup, et al., 2021). Although the addition of Cu begets antibacterial ability, it does not affect the essence of the Ti alloy as an inert metal (Chen, et al., 2023). Surface modification of the Ti surface is necessary since these vital properties cannot be infused into the metal during the LPBF stage (Kurup, et al., 2021).

Titanium and alloys exhibit poor bioactivity, leading to poor osteointegration (Ho-Shui-Ling, et al., 2018). Surface modifications of implant surfaces are mainly intended to improve wettability, cell-implant adhesion, cell proliferation and osseointegration (that is, fusion with bone), and thus promote faster healing and shorter treatment duration (Jemat, et al., 2015). The techniques usually involve surface treatment or coating the implant structure with a material that promotes bone regeneration (Ho-Shui-Ling, et al., 2018). Various surface-coating techniques have been applied to alter the innate surface properties of Ti implants (Abdal-hay, et al., 2019; Li, et al., 2024). Coating techniques include ion implantation, electrochemical anodisation, ion exchange, sol-gel techniques, plasma spraying, and the inclusion of metal ions

such as Ag, Cu or Zn (Benčina, et al., 2021). Nevertheless, these techniques present several limitations, for example, a restricted long-term antimicrobial effect, insufficient adhesion of the coating to the substrate material, uneven thickness of the deposited layer and disparities in the crystallinity and composition of the coating that compromise their clinical usability (Dion, et al., 1994; He, et al., 2009; Yang, et al., 2010; Nichol, et al., 2021). An effective coating method may significantly improve the mechanical and biological characteristics of implants (Li, et al., 2024). Ideally, modifying the Ti implant surface is proposed to enhance the integration between the material and host tissue. In other words, after coating the surface, the implant material is expected to display improved bioactivity by stimulating direct interactions with the surrounding tissue (Jemat, et al., 2015).

2.7. Electrospinning: Fabrication of polymer nanofibres for coating Ti implant surfaces

One of the most important developments in polymers is the fabrication of nanofibres (NFs) (Mao, et al., 2023). Several methods exist for fabricating polymeric NFs for coating Ti implants, such as chemical vapour deposition, template synthesis, arc discharge, laser ablation, self-assembly phase separation and electrospinning (Nirmala, et al., 2013; Mao, et al., 2023). Among them, electrospinning technology is widely used for advantages of simplicity, an inexpensive experimental setup, wide applicability, relatively rapid production and relatively high production efficiency (Nirmala, et al., 2013; Li & Bou-Akl, 2016; Mao, et al., 2023). First introduced in 1600 by William Gilbert, electrospinning has been developed as a sophisticated, modern and versatile technique since the late 1990s to generate NFs from a range of materials. Electrospinning is a well-known technique to produce continuous ultrathin threads with diameters as small as nanometres (Li & Bou-Akl, 2016; Xue, et al., 2019; Sharma & James, 2022). Coating Ti implants with electrospun polymer NFs has emerged as a promising intervention to overcome the bioinertness of these surfaces. Polymeric NFs fabricated by electrospinning possess rare properties due to their minute diameter, which leads to high surface area, high aspect ratio, electron transport, thermal stability, scalability and better mechanical properties (Nirmala, et al., 2013; Ning, et al., 2020; Kausar, 2021; Manakhov, et al., 2021).

2.7.1. Principle of electrospinning

The electrospinning process basically requires three components, namely a needle tip connected to the positive terminal of a high-voltage power source, a flow rate controller and a grounded collector connected to the negative terminal of the power supply (Leach, et al., 2011;

Li & Bou-Akl, 2016; Hong, et al., 2019; Kausar, 2021). A typical electrospinning apparatus setup is illustrated in Figure 2.19.

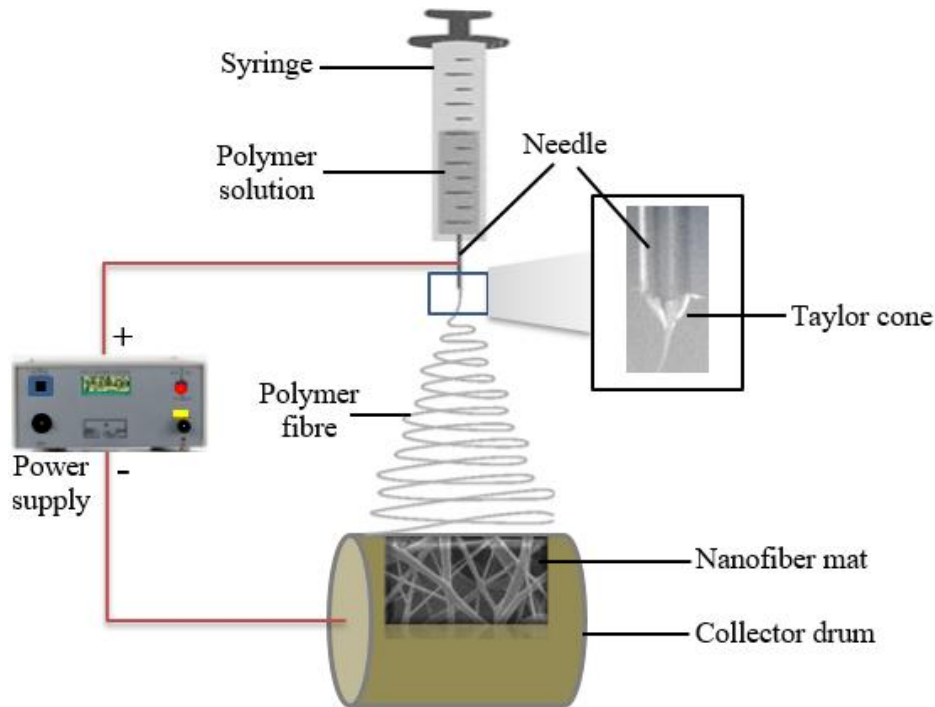


Figure 2.19: Schematic diagram of a basic electrospinning apparatus

The electrospinning process is initiated by dissolving a polymer/polymer blend in a suitable solvent system, followed by pouring the solution into a syringe (Li & Bou-Akl, 2016). The polymer solution is dispensed from the syringe by a syringe pump at a particular flow rate (Leach, et al., 2011). The power source supplies the required charge. When voltage is applied, the repulsive charge accumulates at the tip of the needle. A polymer solution droplet forms a conical shape, called the Taylor cone, at the tip of the needle as the applied electric field strengthens. When the electrical repulsive force within the Taylor cone overcomes the liquid surface tension, a polymer jet ejects from the cone (Hong, et al., 2019; Ni, et al., 2019; Kausar, 2021). The polymer jet forms between the needle tip and collector, and consists of a steady streaming segment and a rapidly oscillating whipping segment (Leach, et al., 2011). To obtain a stable jet, the electric potential, hydrostatic pressure and distance between the needle tip and collector should be adjusted accordingly (Doshi & Reneker, 1995). As the electrified fluid jet travels toward the grounded collector, the solvent evaporates and phase separation occurs, leading to the formation of a fibrous mat consisting of micro/nano-sized fibres deposited onto the collector (Li & Bou-Akl, 2016; Holy, et al., 2001; Ni, et al., 2019; Kausar, 2021). Collectors for electrospinning apparatus are commonly made of metal. Therefore, coating a metal implant with electrospun NFs is relatively straightforward (ElectrospinTech, 2024).

2.7.2. Electrospinning parameters

The minimum concentration for a given polymer solution below which electrospray beads form instead of electrospun fibres is termed the critical entanglement concentration (Figure 2.20) (Leach, et al., 2011; Li & Bou-Akl, 2016). Conversely, a highly concentrated solution may increase viscosity and restrict the flow of fluid to the needle tip, and consequently slow the electrospinning process (Li & Bou-Akl, 2016).

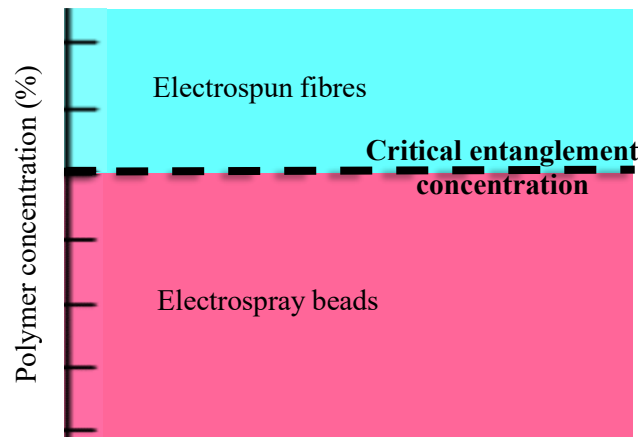


Figure 2.20: Critical entanglement concentration at a particular polymer concentration (adapted from Li & Bou-Akl (2016))

Although a universal model to regulate the morphology of electrospun NFs for a specific polymer/solvent system under different conditions has not been established, general relationships among key parameters may be drawn to guide the electrospinning process (Li & Bou-Akl, 2016).

Table 2.1 outlines these relationships and their influence on NF morphology.

Table 2.1: Relationship between electrospinning process parameters and fibre morphology

Parameters	Relation to fibre morphology	Reference
Solution parameters		
Solvent vapour pressure	Higher solvent volatility or vapour pressure results in higher porosity.	(Leach, et al., 2011; Li & Bou-Akl, 2016)
Solvent permittivity	Bead formation and fibre diameter decrease with increasing solution permittivity.	(Sharma & James, 2022)
Polymer concentration	Increasing fibre diameter with higher concentration (within the optimal range). A consistent fibre morphology and absence of beads at higher concentration.	(Sarabi-Mianeji, et al., 2015; Li & Bou-Akl, 2016)
Solution viscosity	Higher viscosity results in a lower deposition region and increased fibre diameter (within the optimal range).	(Li & Bou-Akl, 2016; Sharma & James, 2022; Mao, et al., 2023)
Solution surface tension	Thin fibres cause smaller surface tension. Surface tension causes bead formation at low polymer concentration.	(Sharma & James, 2022; Mao, et al., 2023)
Solution conductivity	Decreasing diameter and bead formation with higher conductivity	(Sharma & James, 2022; Mao, et al., 2023)
Processing parameters		
Applied voltage or electric field	Diameter and bead formation increase with higher applied voltage (above critical conditions). Smaller, consistent fibre diameter and consistent fibre thickness with uniform electric field distribution.	(Sarabi-Mianeji, et al., 2015; Li & Bou-Akl, 2016; Mao, et al., 2023)
Flow rate	Increasing fibre diameter, continuity and bead formation with higher flow rate (above minimum rate).	(Sarabi-Mianeji, et al., 2015; Li & Bou-Akl, 2016; Sharma & James, 2022)
Needle tip to collector distance	Decreasing fibre diameter with longer distance (within optimal range).	(Sharma & James, 2022; Mao, et al., 2023)
Needle diameter	Small needle diameter produces fibres that are thinner, smoother and bead-free with greater fibre porosity.	(Sharma & James, 2022; Mao, et al., 2023)
Environmental parameters		
Temperature	Higher temperature results in lower solution viscosity and, thus decreases fibre diameter	(Li & Bou-Akl, 2016; Sharma & James, 2022)
Humidity	Higher relative humidity increases porosity, surface roughness and fibre diameter.	(Ni, et al., 2019; Kausar, 2021)

It is essential that the parameters operate in unison towards obtaining a particular fibre morphology. For example, for application in tissue engineering, higher porosity and surface roughness result in higher specific surface area for cell attachment and proliferation (Kausar, 2021). Increased membrane porosity is realised with an increase in NF diameter (Nayl, et al.,

2022). On the other hand, the small diameters and great lengths of electrospun fibres create a large surface-area-to-volume ratio, providing a pillar for tissue regeneration (Kazemzadeh, et al., 2022). A combination of higher applied voltage, smaller needle diameter, and lower flow rate has been shown to produce thinner electrospun NFs (Sharma & James, 2022). Therefore, the parameters of the electrospinning process require optimisation to produce uniform, continuous fibres with desirable morphology and distribution for biological enhancement.

Obtaining the required fibre morphology and distribution largely depends on empirical observations (Li & Bou-Akl, 2016). As the jet flows from the need tip to the collector, the whipping jet is usually invisible to the naked eye (Leach, et al., 2011). However, the streaming segment may be visible under appropriate illumination conditions (Leach, et al., 2011; Li & Bou-Akl, 2016). This is essential because the length, thickness and consistency of the moving segment are useful in predicting the alignment and morphology of the NFs being formed. A stream that is short, non-uniform, inconsistent and/or oscillating is indicative of a variety of defects, including poor fibre alignment, beading, splattering, and curlicue or wavy patterns (Leach, et al., 2011). The stream segment is optimised by adjusting the composition of the solution (mainly in terms of viscosity) and the parameters of the electrospinning apparatus, and thus optimising alignment and morphology of the fibres being produced (Leach, et al., 2011; Li & Bou-Akl, 2016). The polymer material is selected based on the intended function. The parameters to be optimised to obtain the desired fibre morphology and orientation for a particular polymer solution include the voltage from the power supply, the flow rate applied to the solution by the syringe pump, the diameter of the needle and the distance between the needle tip and the collector (Luketich, et al., 2022; Sharma & James, 2022; Mao, et al., 2023).

Viscosity has a substantial influence on the morphology of the resulting polymer nanofibre. Figure 2.21 is the schematic representation and corresponding scanning electron microscope images of electrospun poly(ethylene oxide) to illustrate the development of bead formation leading to the formation of NFs as the viscosity increases (Haider, et al., 2018). A high viscosity results in a slow flow and leads to discontinuity of fibres. When the viscosity is low and the flow rate is too high, beaded fibres are produced and may lead to deposition of droplets (Mao, et al., 2023). The formation of beads during electrospinning reduces the surface area per unit mass of the obtained fibres and, therefore, beads are considered a defect in the electrospinning process (Xue, et al., 2019). Hence, each polymer composition has an optimal viscosity for the fabrication of uniform, continuous fibres. Figure 2.22 shows the effect of the applied voltage

from the power supply. At low voltage, a pendant droplet (orange) is produced at the capillary/needle tip while a Taylor cone (blue) is formed at the pendant droplet tip. By elevating the voltage, a Taylor cone is produced at the capillary tip, and further increases in voltage result in the ejection of the fibre jet through the capillary.

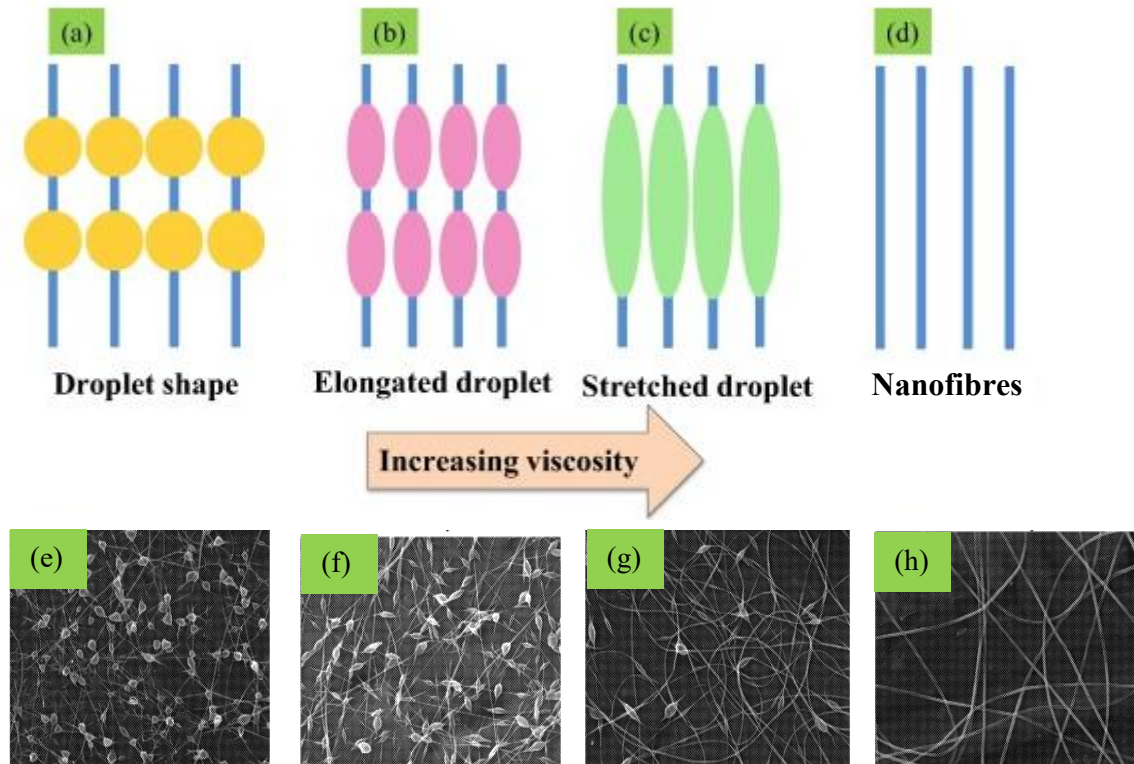


Figure 2.21: Changes in morphology of electrospun nanofibres with viscosity shown as (a–d) a schematic (adapted from Al-Abduljabbar & Farooq (2023)), and (e–h) scanning electron microscope (SEM) micrographs of polyethylene oxide with viscosity increased from left to right at 32, 74, 289 and 1835 centipoise (adapted from Fong et al. (1999))

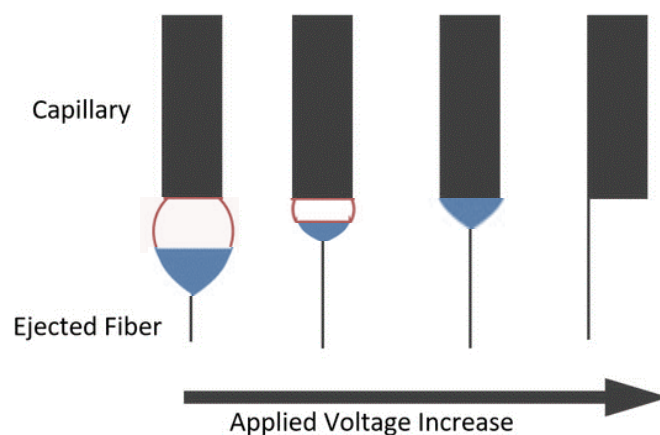


Figure 2.22: Influence of voltage on the formation of the Taylor cone (adapted from Al-Abduljabbar & Farooq (2023))

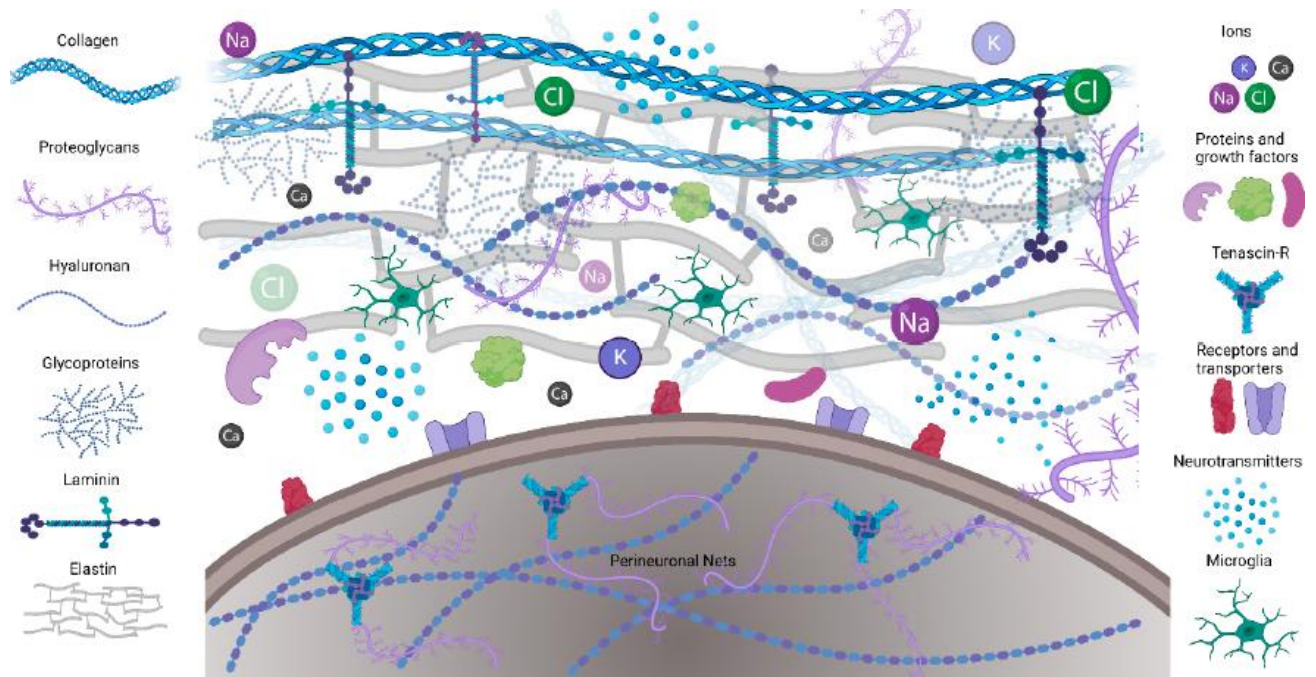
Among the class of materials, polymers are the most widely used in the electrospinning process because they have high molecular weights, which translates into polymeric solutions with relatively high viscosity – a mandatory feature for electrospinnability (ElectrospinTech, 2021). Electrospinning is utilised to generate NFs from various types of organic polymers (Xue, et al., 2019). The majority of organic polymers may be directly applied by electrospinning, provided they are soluble in appropriate solvents to form a solution, or may be melted without degradation (Haider, et al., 2018; Xue, et al., 2019). Over 100 different types of polymers have been successfully explored for solution electrospinning to directly produce NFs (Xue, et al., 2019). A number of these polymers have been used in biomedicine including natural polymers (such as cellulose, silk fibroin, gelatine, chitin, dextrose, chitosan and collagen) and synthetic polymers (such as poly(lactic acid) (PLA), poly(ethylene oxide) (PEO), poly(vinyl alcohol) (PVA), polyacrylonitrile (PAN), poly(ϵ -caprolactone) (PCL), poly(lactic-co-glycolic acid) (PLGA), poly(glycolic acid) (PGA), polyurethane (PU) and poly(methyl methacrylate) (PMMA)) (Ning, et al., 2020; Nhlapo, et al., 2022; Mao, et al., 2023).

Synthetic polymers have numerous advantages in comparison to natural polymers, namely cost-effectiveness and durability, and most of them have stable mechanical properties for applications in load-bearing tissue engineering scaffolds (Nirmala, et al., 2013; Nhlapo, et al., 2022). In addition, biocompatibility, biodegradability and lack of toxicity are common properties of synthetic polymers relevant for coating surfaces of biomaterials. Biodegradation may be defined by hydrolysis in physiological conditions (as in the human body) (Chen, 2013; Maitz, 2015). The hydrolysis of polyester groups produces non-toxic degradation products that are readily eliminated from the body through metabolic pathways (Xue, et al., 2019). Nevertheless, hydrolysis should not be so as to adversely affect the attachment of the polymer coating to the metal surface. It is crucial that the NF matrix remains adherent to the surface of the implant without peeling off *in vivo* until implantation success is attained (ElectrospinTech, 2024). Thus, employing a polymer with elastomeric properties offers an additional advantage and mixing polymers offers a wider spectrum of benefits. Polymers in pure or blend solutions have been electrospun to form NFs (Nirmala, et al., 2013). Polymer blends provide new perspectives in the fabrication of innovative coatings for implantable devices as a strategy to upgrade the biofunctionality of conventional medical devices (Visan, et al., 2020).

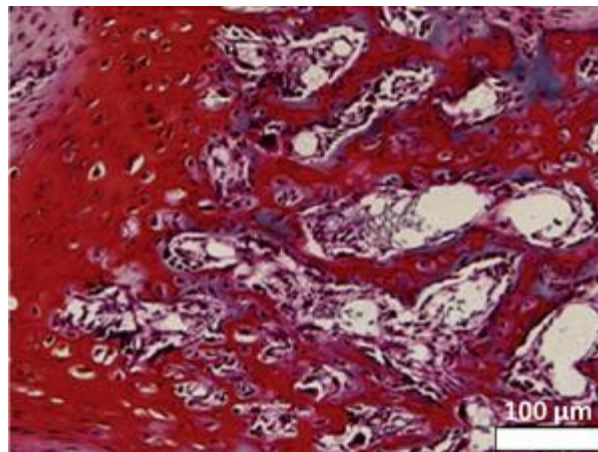
2.7.3. Application of electrospun fibres in tissue engineering

The hierarchical fibrous nanostructures are promising for biomedical applications. The electrospun NFs are used to develop tissue scaffolds to mimic the natural tissues and biological functions, and to facilitate human cell attachment and organisation. Thus, NFs have been used in tissue engineering to form biocompatible 3D membranes (Kausar, 2021). The concept of tissue engineering was initiated in 1987. It is an interdisciplinary discipline involving various fields of medicine, biology, engineering and material science (Ning, et al., 2020). Electrospun polymer NFs have the two distinctive features of minute fibre diameters (generally of 1 μm or less) and large length-to-diameter ratio (usually greater than 1000) (Muñoz-Escobar, et al., 2019; Mao, et al., 2023). Additionally, NFs have a high specific area, high porosity, and controllable size and morphology, with the ability to produce a variety of cross-sectional shapes and variations along their length (Doshi & Reneker, 1995). These characteristics are essential for applications in the biomedical field, including tissue engineering, drug delivery, wound healing and biosensing (Mao, et al., 2023). The physiological environment is composed of nanometre-scale components and thus controlling the surface features of implants at a nanometric level allows for the production of surfaces that readily emulate this environment (Baena, et al., 2017).

Electrospinning is one of the most effective techniques for depositing fibres that replicate the characteristics of fibrous biological materials, both mechanically and structurally (Luketich, et al., 2022). The artificial ultrafine nanofibrous scaffolds produced by electrospinning mimic the innate extracellular matrix (ECM) and are known to induce tuneable favourable features like cell–biomaterial interactions, gene expression and shaping cell morphology (Das, et al., 2018). The main constituents of the ECM exist in the form of NFs as illustrated in Figure 2.23(i) (Al Aboody, 2021). The 3D electrospun porous NF matrices are designed to facilitate nutrient transport, binding, adhesion, proliferation and differentiation of cells during the formation of functional tissue (Das, et al., 2019; Xue, et al., 2019; Ning, et al., 2020). Moreover, electrospun NF scaffolds have been shown to accelerate maturation, improve cell growth and guide cell migration in vitro (Leach, et al., 2011). Figure 2.23(ii) shows in vivo cell attachment and proliferation of a NF scaffold at eight weeks post-surgery.



(i)



(ii)

Figure 2.23: (i) Schematic illustration of the extracellular matrix (ECM) (adapted from Soles et al. (2023)), and (ii) cells grown in vivo on randomly electrospun poly(L-lactic acid) NF scaffold (adapted from Yin et al. (2015))

A bone implant inserted into the body is recognised by human tissues as a foreign body. As a result, the main aim of coating LPBF Ti implants with ultrafine fibres is to avoid the foreign body response as it may hinder osseointegration and promote implant instability (Bosco, et al., 2012). Surface treating implants by coating with NFs has been shown to increase the implant surface roughness and the implant area that interacts with biological fluids and cells to enhance osteogenesis and thereby accelerate peri-implant healing (Jemat, et al., 2015; Al Aboody, 2021; Rampurawala & Patil, 2021). Osseointegration is defined as the direct contact between the

implant and bone at the microscopic level. The ultimate purpose of metal implantation is to achieve stability through osseointegration (Baena, et al., 2017). Surface modification techniques that aid in improving adhesion of soft tissue to the implants are required, as the implant surface's first point of contact is with bodily fluids and soft tissue, which facilitate osseointegration (Kurup, et al., 2021). An implant is required to fully integrate with soft tissue to prevent the occurrence of infectious diseases and inflammation (Kurup, et al., 2021).

The possibility of incorporating one or multiple bioactive factors into the NFs is yet another advantage of the electrospinning process (Li & Bou-Akl, 2016). Electrospinning is efficient for fabricating polymer/metal oxide composites (Muñoz-Escobar, et al., 2019). Surface properties of LPBF implant, particularly roughness and wettability, also contribute to the establishment of implant-related infections (Haq, et al., 2024). The electrospun mats may be easily modified by reinforcing the fibres with organic metallic nanoparticles (Arrieta, et al., 2020). The preparation of Cu/CuO nanofibrous materials has become a prevalent topic because of a vast range of applications of such nanomaterials, including disinfection and antibacterial wound dressings (Manakhov, et al., 2021). The use of anti-adhesive polymers to inhibit bacterial attachment is a popular strategy; however, creating implants with both antibacterial and osteogenic properties remains challenging (Li, et al., 2024). Furthermore, the application of NFs infused with Cu/CuO NPs – particularly applied as coatings for Ti implant – for biomedical applications is not as extensively covered in literature as compared to, for example, Ag nanoparticles (Manakhov, et al., 2021).

The use of the blending solution method for electrospinning allows for the incorporation of multiple additives into selected polymer matrices to form a coating that is conducive to the intended implant environment (ElectrospinTech, 2024). Material selection is crucial in the production of these NFs, as it influences morphology, biocompatibility, mechanical strength, and degradation, and thereby affects the release profile of additives (Sharma & James, 2022). There are mainly two strategies for adding NPs to electrospun NFs, namely, direct incorporation during electrospinning and post-treatment of the as-spun NFs (Xue, et al., 2019). Similar to NPs, NFs have a very high surface area-to-volume ratio. On the other hand, unlike NPs, interlocking NFs by electrospinning means that a carrier is not needed to prevent dispersion (ElectrospinTech, 2021). Electrospun NFs are able to achieve immobilisation, which allows for controlled release in drug delivery applications (Ning, et al., 2020). The drug delivery function may also be applicable for administering the metal-based antibacterial agents.

Therefore, impregnating the NFs with CuO NPs reduced the toxicity factor and provides a controlled and prolonged release of the biocidal NPs (Xue, et al., 2019). Coating the surfaces of LPBF Ti implants with electrospun polymer NFs infused with CuO NPs provides the twofold functionality of promoting bioactivity while inhibiting bacterial colonisation.

2.8. Summary

The literature review chapter discusses the aspects that constitute the body of this dissertation. These aspects include additive manufacturing, with a special focus on laser powder bed fusion technology, optimising the essential process parameters of LPBF, and the application of LPBF in biomedicine. The biomedical applicability is detailed in terms of using Ti and Ti alloys as biomaterials, alloying with Cu to induce antimicrobial activity, coating the Ti implant surfaces with polymer nanofibres by electrospinning to overcome the natural inertness of Ti, and imbuing the electrospun NFs with CuO nanoparticles to provide dual functionality of the NF coating of a biocompatible and biocidal scaffold. This chapter also outlines the electrospinning process and the imperative parameters to optimise to achieve the desired NF morphology and distribution. The important properties required from implantable devices are mentioned throughout the chapter. These include density, corrosion resistance, mechanical properties, antibacterial properties, biocompatibility and negligible cytotoxicity. The subsequent chapters delve into empirical details of these aspects.

References

- Abdal-hay, A., Agour, M., Kim, Y. -K., Lee, M. -H., Hassan, M. K., El-Ainin, H. A., . . . Ivanovski, S. (2019). Magnesium-particle/polyurethane composite layer coating on titanium surfaces for orthopedic applications. *European Polymer Journal*, *112*, 555–568. doi:10.1016/j.eurpolymj.2018.10.012
- Abd-Elaziem, W., Elkatatny, S., Sebaey, T. A., Darwish, M. A., Abd El-Baky, M. A., & Hamada, A. (2024). Machine learning for advancing laser powder bed fusion of stainless steel. *Journal of Materials Research and Technology*, *30*, 4986-5016. doi:10.1016/j.jmrt.2024.04.130
- Aboulkhair, N. T., Everitt, N. M., Ashcroft, I., & Tuck, C. (2014). Reducing porosity in AlSi10Mg parts processed by selective laser melting. *Additive Manufacturing*, *1–4*, 77–86. doi:10.1016/j.addma.2014.08.001
- Aboulkhair, N. T., Simonelli, M., Parry, L., Ashcroft, I., Tuck, C., & Hague, R. (2019). 3D printing of aluminium alloys: additive manufacturing of aluminium alloys using selective laser melting. *Progress in Materials Science*, *106*, 100578. doi:10.1016/j.pmatsci.2019.100578
- Ahire, J. J., Hattingh, M., Neveling, D. P., & Dicks, L. M. (2016). Copper-containing anti-biofilm nanofiber scaffolds as a wound dressing material. *PLoS One*, *11*(3), e0152755.
- Ahmed, T. A., Lee, H. ‘., & Williams, R. C. (2018). Using a modified asphalt bond strength test to investigate the properties of asphalt binders with poly ethylene wax-based warm mix asphalt additive. *International Journal of Pavement Research and Technology*, *11*(1), 28–37. doi:10.1016/j.ijprt.2017.08.004
- Al Aboody, M. S. (2021). Electrospun fabrication and direct coating of bio-degradable fibrous composite on orthopedic titanium implant: synthesis and characterizations. *Materials Research Express*, *8*(1), 015307. doi:10.1088/2053-1591/abd826
- Al-Abduljabbar, A., & Farooq, I. (2023). Electrospun polymer nanofibers: Processing, properties, and applications. 2023, 15, 65. *Polymers*, *15*(1), 65. doi:10.3390/polym15010065
- Al-Khateeb, A., Al-Hassani, E. S., & Jabur, A. R. (2023). Metallic implant surface activation through electrospinning coating of nanocomposite fiber for bone regeneration. *International Journal of Biomaterials*, 2023, Article ID 1332814. doi:10.1155/2023/1332814

- Aota, L. S., Bajaj, P., Sandim, H. R., & Jägle, E. A. (2020). Laser powder-bed fusion as an alloy development tool: Parameter selection for in-situ alloying using elemental powders. *Materials*, *13*, 3922. doi:10.3390/ma13183922
- Arrieta, M. P., Gil, A. L., Yusef, M., Kenny, J. M., & Peponi, L. (2020). Electrospinning of PCL-based blends: processing optimization for their scalable production. *Materials*, *13*, 3853. doi:10.3390/ma13173853
- ASM International Handbook Committee. (1991). *ASM Handbook, Volume 04 - Heat Treating* (Vol. 4). Novelty: ASM International.
- Ávila Calderón, L. A., Graf, B., Rehmer, B., Petrat, T., Skrotzki, B., & Rethmeier, M. (2022). Characterization of Ti–6Al–4V fabricated by multilayer laser powder-based directed energy deposition. *Advanced Engineering Materials*, *24*, 2101333. doi:10.1002/adem.202101333
- Baena, R. R., Rizzo, S., Manzo, L., & Lupi, S. M. (2017). Nanofeatured titanium surfaces for dental implantology: Biological effects, biocompatibility, and safety. *Journal of Nanomaterials*, *2017*, 6092895. doi:10.1155/2017/6092895
- Bartolomeu, F., Gasik, M., Silva, F. S., & Miranda, G. (2022). Mechanical properties of Ti6Al4V fabricated by laser powder bed fusion: A review focused on the processing and microstructural parameters influence on the final properties. *Metals*, *12*(6), 986. doi:10.3390/met12060986
- Benčina, M., Resnik, M., Starič, P., & Junkar, I. (2021). Use of plasma technologies for antibacterial surface properties of metals. *Molecules*, *26*(5), 1418. doi:10.3390/molecules26051418
- Beuth, J., & Klingbeil, N. (2001). The role of process variables in laser-based direct metal solid freeform fabrication. *The Journal of The Minerals, Metals & Materials Society*, *53*, 36–39. doi:10.1007/s11837-001-0067-y
- Bosco, R., Van Den Beucken, J., Leeuwenburgh, S., & Jansen, J. (2012). Surface engineering for bone implants: A trend from passive to active surfaces. *Coatings*, *2*(4), 95–119. doi:10.3390/coatings2030095
- Calignano, F., Manfredi, D., Ambrosio, E. P., Iuliano, L., & Fino, P. (2013). Influence of process parameters on surface roughness of aluminum parts produced by DMLS. *The International Journal of Advanced Manufacturing Technology*, *67*, 2743–2751. doi:10.1007/s00170-012-4688-9

- Cao, S., Zou, Y., Lim, C. V., & Wu, X. (2021). Review of laser powder bed fusion (LPBF) fabricated Ti-6Al-4V: process, post-process treatment, microstructure, and property. *Light: Advanced Manufacturing*, 2(3), 313-332. doi:10.37188/lam.2021.020
- Cao, X. -Y., Tian, N., Dong, X., & Cheng, C. -K. (2019). Implant coating manufactured by micro-arc oxidation and dip coating in resorbable polylactide for antimicrobial applications in orthopedics. *Coatings*, 9(5), 284. doi:10.3390/coatings9050284
- Caprio, L., Chiari, G., Demir , A. G., & Previtali, B. (2018). Development of novel high temperature laser powder bed fusion system for the processing of crack-susceptible alloys. Austin, Texas: Solid Freeform Fabrication 2018: Proceedings of the 29th Annual International. Solid Freeform Fabrication Symposium – An Additive Manufacturing Conference August 13–15. doi:10.26153/tsw/17221
- Chatterjee, A. K., Chakraborty, R., & Basu, T. (2014). Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology*, 25, 135101. doi:10.1088/0957-4484/25/13/135101
- Chen, H., Zhang, J., Wu, H., Li, Y., Li, X., Zhang, J., . . . Cai, X. (2021). Fabrication of a Cu nanoparticles/poly(ϵ -caprolactone)/gelatin fiber membrane with good antibacterial activity and mechanical property via green electrospinning. *ACS Applied Bio Materials*, 4(8), 6137-6147. doi:10.1021/acsabm.1c00485
- Chen, P. (2013). A Preliminary Discourse on Adhesion of Nanofibers Derived from Electrospun Polymers. Akron: University of Akron Mechanical Engineering Faculty Research. Retrieved from http://ideaexchange.uakron.edu/mechanical_ideas/675
- Chen, Y., Yang, W., Zhu, S., & Shi, Y. (2023). Microstructural, mechanical and in vitro biological properties of Ti6Al4V-5Cu alloy fabricated by selective laser melting. *Materials Characterization*, 200, 112858. doi:10.1016/j.matchar.2023.112858
- Cheng, L., Lei , L., & Guo, S. (2010). In vitro and in vivo evaluation of praziquantel loaded implants based on PEG/PCL blends. *International Journal of Pharmaceutics*, 387, 129–138. doi:10.1016/j.ijpharm.2009.12.010
- Chowdhury, S., Yadaiah, N., Prakash, C., Ramakrishna, S., Dixit, S., Gupta, L. R., & Buddhi, D. (2022). Laser powder bed fusion: A state-of-the-art review of the technology, materials, properties & defects, and numerical modelling. *Journal of Materials Research and Technology*, 20, 2109-2172.
- Cobbinah, P. V., Nzeukou, R. A., Onawale, O. T., & Matizamhuka, W. R. (2021). Laser powder bed fusion of potential superalloys: A review. *Metals*, 11(1), 58. doi:10.3390/met11010058

- Das, S. (2003). Physical aspects of process control in selective laser sintering of metals. *Advanced Engineering Materials*, 5, 701–711. doi:10.1002/adem.200310099
- Das, S., Dholam, K., Gurav, S., Bendale, K., Ingle, A., Mohanty, B., . . . Bellare, J. R. (2019). Accentuated osseointegration in osteogenic nanofibrous coated titanium implants. *Scientific Reports*, 9(1), 17638. doi:10.1038/s41598-019-53884-x
- Das, S., Gurav, S., Soni, V., Ingle, A., Mohanty, B. S., Chaudhari, P., . . . Bellare, J. R. (2018). Osteogenic nanofibrous coated titanium implant results in enhanced osseointegration: In vivo preliminary study in a rabbit model. *Tissue Engineering and Regenerative Medicine*, 15(2), 231-247. doi:10.1007/s13770-017-0106-6
- DebRoy, T., Wei, H. L., Zuback, J. S., Mukherjee, T., Elmer, J. W., Milewski, J. O., . . . Zhang, W. (2018). Additive manufacturing of metallic components – Process, structure and properties. *Progress in Materials Science*, 92, 112–224. doi:10.1016/j.pmatsci.2017.10.001
- Dewidar, M. M., Dalgarno, K. W., & Wright, C. S. (2003). Processing conditions and mechanical properties of high-speed steel parts fabricated using direct selective laser sintering. *Proceedings of the Institution of Mechanical Engineers, Part B*, 217(12), 1651-1663. doi:10.1243/095440503772680587
- Dieter, G. E. (1961). *Mechanical Metallurgy*. New York: McGraw-Hill Book Company.
- Dion, I., Bordenave, L., Lefebvre, F., Bareille, R., Baquey, C., Monties, J. R., & Havlik, P. (1994). Physico-chemistry and cytotoxicity of ceramics—Part II Cytotoxicity of ceramics. *Journal of Materials Science: Materials in Medicine*, 5, 18–24. doi:10.1007/BF00121148
- Donachie, M. J. (2020). *Titanium: A Technical Guide* (2nd ed.). Novelty: ASM International. doi:10.31399/asm.tb.ttg2.9781627082693
- Doshi, J., & Reneker, D. H. (1995). Electrospinning process and application of electrospun fibers. *Journal of Electrostatics*, 35(2-3), 151-160. doi:10.1016/0304-3886(95)00041-8
- Dzogbewu, T. C. (2017). Direct Metal Laser Sintering of Titanium Alloys for Biomedical Applications. Doctoral Thesis: Central University of Technology, Free State.
- Dzogbewu, T. C., Yadroitsev, I., Krakhmalev, P., Yadroitsava, I., & Du Plessis, A. (2017). Optimal process parameters for in situ alloyed Ti15Mo structures by laser powder bed fusion. *Proceedings of the 28th Annual International Solid Freeform Fabrication Symposium - an Additive Manufacturing Conference - 2017: August 7-9, 2017, The University of Texas at Austin, Austin, Texas, USA*.

- ElectrospinTech. (2021). *Electrospinning for nanofiber production across materials classes*. Retrieved January 24, 2024, from <https://electrospintech.com/materialclasses.html>
- ElectrospinTech. (2024). *Electrospun Coated Metal Implants*. Retrieved January 24, 2024, from <https://electrospintech.com/coatedmetal.html>
- Erci, F., & Sariipek, F. B. (2023). Fabrication of copper oxide nanoparticles doped PCL/PVP nanofibrous mats by electrospinning and evaluation of their antibacterial activities. *Kahramanmaraş Sütçü İmam University Journal of Engineering Sciences*, 26(4), 823-833. doi:10.17780/ksujes.1290143
- Evans, J. T., Walker, R. W., Evans, J. P., Blom, A. W., Sayers, A., & Whitehouse, M. R. (2019). How long does a knee replacement last? A systematic review and meta-analysis of case series and national registry reports with more than 15 years of follow-up. *The Lancet*, 393(10172), 655–663. doi:10.1016/S0140-6736(18)32531-5
- Fan, Y., Tian, W., Guo, Y., Sun, Z., & Xu, J. (2016). Relationships among the microstructure, mechanical properties, and fatigue behavior in thin Ti6Al4V. *Advances in Materials Science and Engineering*, 2016, Article ID 7278267. doi:10.1155/2016/7278267
- Fischer, M., Joguet, D., Robin, G., Peltier, L., & P, L. (2016). In situ elaboration of a binary Ti–26Nb alloy by selective laser melting of elemental titanium and niobium mixed powders. *Materials Science and Engineering: C*, 62, 852–859. doi:10.1016/j.msec.2016.02.033
- Fischer, P., Romano, V., Weber, H. P., Karapatis, N. P., Boillat, E., & Glardon, R. (2003). Sintering of commercially pure titanium powder with a Nd: YAG laser source. *Acta Materialia*, 51(6), 1651-1662.
- Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, 40(16), 4585-4592. doi: 10.1016/S0032-3861(99)00068-3
- Fox, J. C., Moylan, S. P., & Lane, B. M. (2016). Effect of process parameters on the surface roughness of overhanging structures in laser powder bed fusion additive manufacturing. *Procedia CIRP*, 45, 131-134. doi:10.1016/j.procir.2016.02.347
- Garcia-Falcon, C. M., Gil-Lopez, T., Verdu-Vazquez, A., & Mirza-Rosca, J. C. (2021). Electrochemical characterization of some cobalt base alloys in Ringer solution. *Materials Chemistry and Physics*, 260, 124164. doi:10.1016/j.matchemphys.2020.124164
- Gong, H., Rafi, K., Gu, H., Starr, T., & Stucker, B. (2014). Analysis of defect generation in Ti–6Al–4V parts made using powder bed fusion additive manufacturing processes. *Additive Manufacturing*, 1–4, 87-98. doi:10.1016/j.addma.2014.08.002

- Haase, F., Siemers, C., & Rösler, J. (2023). Laser powder bed fusion (LPBF) of commercially pure titanium and alloy development for the LPBF process. *Frontiers in Bioengineering and Biotechnology*, *11*, 1260925. doi:10.3389/fbioe.2023.1260925
- Haider, A., Haider, S., & Kang, I. -K. (2018). A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arabian Journal of Chemistry*, *11*(8), 1165-1188. doi:10.1016/j.arabjc.2015.11.015
- Haider, A., Kwak, S., Gupta, K. C., & Kang, I. -K. (2015). Antibacterial activity and cytocompatibility of PLGA/CuO hybrid nanofiber scaffolds prepared by electrospinning. *Journal of Nanomaterials*, *2015*, Article ID 832762. doi:10.1155/2015/832762
- Han, X., Ma, J., Tian, A., Wang, Y., Li, Y., Dong, B., . . . Ma, X. (2023). Surface modification techniques of titanium and titanium alloys for biomedical orthopaedics applications: A review. *Colloids and Surfaces B: Biointerfaces*, *227*, 113339. doi:10.1016/j.colsurfb.2023.113339
- Haq, I. U., Khan, T. A., & Krukiewicz, K. (2024). Etiology, pathology, and host-impaired immunity in medical implant-associated infections. *Journal of Infection and Public Health*, *17*(2), 189-203. doi:10.1016/j.jiph.2023.11.024
- He, F. M., Yang, G. L., Li, Y. N., & Wang, X. X. (2009). Early bone response to sandblasted, dual acid-etched and H₂O₂/HCl treated titanium implants: An experimental study in the rabbit. *International Journal of Oral and Maxillofacial Surgery*, *38*(6), 677–681. doi:10.1016/j.ijom.2009.03.716
- Holy, C. E., Cheng, C., Davies, J. E., & Shoichet, M. S. (2001). Optimizing the sterilization of PLGA scaffolds for use in tissue engineering. *Biomaterials*, *22*(1), 25-31. doi:10.1016/S0142-9612(00)00136-8
- Hong, J., Yeo, M., Yang, G. H., & Kim, G. (2019). Cell-electrospinning and its application for tissue engineering. *International Journal of Molecular Sciences*, *20*(24), 6208. doi:10.3390/ijms20246208
- Ho-Shui-Ling, A., Bolander, J., Rustom, L. E., Johnson, A. W., Luyten, F. P., & Picart, C. (2018). Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. *Biomaterials*, *180*, 143-162. doi:10.1016/j.biomaterials.2018.07.017

- Iberdrola Group. (2025). *Evolution of the World Population: The Changing World Population and its Impact on the Future of the Planet*. Retrieved April 12, 2025, from <https://www.iberdrola.com/sustainability/world-population-evolution>
- Islam, S. A., Hao, L., Javaid, Z., Xiong, W., Li, Y., Jamil, Y., . . . Han, G. (2024). Surface roughness of interior fine flow channels in selective laser melted Ti-6Al-4V alloy components. *Micromachines*, 15(3), 348. doi:10.3390/mi15030348
- ISO. (2021). *ISO/ASTM 52900:2021(en) Additive manufacturing — General principles — Fundamentals and vocabulary*. Retrieved April 13, 2025, from <https://www.iso.org/obp/ui/#iso:std:iso-astm:52900:ed-2:v1:en>
- ISO/ASTM International. (2021). *ISO/ASTM 52900: Additive manufacturing — General principles — Fundamentals and vocabulary*.
- Jambhulkar, N., Jaju, S., Raut, A., & Bhoneja, B. (2023). A review on surface modification of dental implants among various implant materials. *Materials Today: Proceedings*, 72(Part 6), 3209-3215. doi:10.1016/j.matpr.2022.12.022
- Jan, H., Gul, R., Andleeb, A., Ullah, S., Shah, M., Khanum, M., . . . Abbasi, B. H. (2021). A detailed review on biosynthesis of platinum nanoparticles (PtNPs), their potential antimicrobial and biomedical applications. *Journal of Saudi Chemical Society*, 25(8), 101297. doi:10.1016/j.jscs.2021.101297
- Jemat, A., Ghazali, M. J., Razali, M., & Otsuka, Y. (2015). Surface modifications and their effects on titanium dental implants. *BioMed Research International*, 6(1), 1-11.
- Ju, J., Zan, R., Shen, Z., Wang, C., Peng, P., Wang, J., . . . Yang, T. (2023). Remarkable bioactivity, bio-tribological, antibacterial, and anti-corrosion properties in a Ti-6Al-4V-xCu alloy by laser powder bed fusion for superior biomedical implant applications. *Chemical Engineering Journal*, 471, 144656. doi:10.1016/j.cej.2023.144656
- Kang, N., Fu, Y., Coddet, P., Guelorget, B., Liao, H., & Coddet, C. (2017). On the microstructure, hardness and wear behavior of Al-Fe-Cr quasicrystal reinforced Al matrix composite prepared by selective laser melting. *Materials & Design*, 132, 105–111. doi:10.1016/j.matdes.2017.06.060
- Karlsson, J., Snis, A., Engqvist, H., & Lausmaa, J. (2013). Characterization and comparison of materials produced by electron beam melting (EBM) of two different Ti-6Al-4V powder fractions. *Journal of Materials Processing Technology*, 213(12), 2109-2118. doi:10.1016/j.jmatprotec.2013.06.010

- Kasperovich, G., Haubrich, J., Gussone, J., & Requena, G. (2016). Correlation between porosity and processing parameters in TiAl6V4 produced by selective laser melting. *Materials and Design*, 105, 160–170. doi:10.1016/j.matdes.2016.05.070
- Kausar, A. (2021). Polymeric nanocomposite via electrospinning: Assessment of morphology, physical properties and applications. *Journal of Plastic Film & Sheeting*, 37(1), 70–92. doi:10.1177/8756087920937344
- Kazemzadeh, G., Jirofti, N., Mohebbi-Kalhor, D., Sarhaddi, F., & Taheri, R. (2022). Pathological examination of blended and co-electrospinning hybrid polycaprolactone/polyurethane nanofibers for soft tissue engineering applications. *Journal of Industrial Textiles*, 51(4S), 6816S–6837S. doi:10.1177/15280837221074070
- Kenel, C., Dasargyri, G., Bauer, T., Colella, A., Spierings, A. B., Leinenbach, C., & Wegener, K. (2017). Selective laser melting of an oxide dispersion strengthened (ODS) γ -TiAl alloy towards production of complex structures. *Materials & Design*, 134, 81-90.
- Khaimovich, A., Balyakin, A., Oleynik, M., Meshkov, A., & Smelov, V. (2022). Optimization of process parameters for powder bed fusion additive manufacturing using a linear programming method: a conceptual framework. *Metals*, 12, 1976. doi:10.3390/met12111976
- Khorasani, A. M., Gibson, I., Ghasemi, A. H., & Ghaderi, A. (2020). Modelling of laser powder bed fusion process and analysing the effective parameters on surface characteristics of Ti-6Al-4V. *International Journal of Mechanical Sciences*, 168, 105299.
- Kim, T., See, C. W., Li, X., & Zhu, D. (2020). Orthopedic implants and devices for bone fractures and defects: Past, present and perspective. *Engineered Regeneration*, 1, 6-18. doi:10.1016/j.engreg.2020.05.003
- Körner, C., Attar, E., & Heinl, P. (2011). Mesoscopic simulation of selective beam melting processes. *Journal of Materials Processing Technology*, 211(6), 978-987.
- Körner, C., Bauereiß, A., & Attar, E. (2013). Fundamental consolidation mechanisms during selective beam melting of powder. *Modelling and Simulation in Materials Science and Engineering*, 21(8), 085011. doi:10.1088/0965-0393/21/8/085011
- Krakhmalev, P., & Yadroitsev, I. (2014). Microstructure and properties of intermetallic composite coatings fabricated by selective laser melting of Ti–SiC powder mixtures. *Intermetallics*, 46, 147-155. doi:10.1016/j.intermet.2013.11.012
- Krakhmalev, P., Yadroitsev, I., Yadroitsava, I., & de Smidt, O. (2017). Functionalization of biomedical Ti6Al4V via in situ alloying by Cu during laser powder bed fusion manufacturing. *Materials*, 10, 1154. doi:10.3390/ma10101154

- Kurup, A., Dhattrak, P., & Khasnis, N. (2021). Surface modification techniques of titanium and titanium alloys for biomedical dental applications: A review. *Materials Today: Proceedings*, 39, 84–90. doi:10.1016/j.matpr.2020.06.163
- Lamikiz, A., Sanchez, J., de Lacalle, L. L., & Arana, J. (2007). Laser polishing of parts built up by selective laser sintering. *International Journal of Machine Tools and Manufacture*, 47(12–13), 2040–2050. doi:10.1016/j.ijmachtools.2007.01.013
- Leach, M. K., Feng, Z. Q., Tuck, S. J., & Corey, J. M. (2011). Electrospinning fundamentals: Optimizing solution and apparatus parameters. *Journal of Visualized Experiments*, 47, e2494. doi:10.3791/2494
- Lei, J., Yao, G., Sun, Z., Wang, B., Yu, C., & Zheng, S. (2019). Fabrication of a novel antibacterial TPU nanofiber membrane containing Cu-loaded zeolite and its antibacterial activity toward Escherichia coli. *Journal of Material Science*, 54, 11682–11693. doi:10.1007/s10853-019-03727-x
- Lekoadi, P., Tlotleng, M., Annan, K., Maledi, N., & Masina, B. (2021). Evaluation of heat treatment parameters on microstructure and hardness properties of high-speed selective laser melted Ti6Al4V. *Metals*, 11, 255. doi:10.3390/met11020255
- Leung, C. L., Marussi, S., Towrie, M., Garcia, J. d., Atwood, R. C., Bodey, A. J., . . . Lee, P. D. (2018). Laser-matter interactions in additive manufacturing of stainless steel SS316L and 13-93 bioactive glass revealed by in situ X-ray imaging. *Additive Manufacturing*, 24, 647-657. doi:10.1016/j.addma.2018.08.025
- Li, M., Ma, Z., Zhu, Y., Xia, H., Yao, M., Chu, X., . . . Mao, C. (2016). Toward a molecular understanding of the antibacterial mechanism of copper-bearing titanium alloys against Staphylococcus aureus. *Advanced Healthcare Materials*, 5(5), 557-566. doi:10.1002/adhm.201500712.
- Li, R., Liu, J., Shi, Y., Wang, L., & Jiang, W. (2012). Balling behavior of stainless steel and nickel powder during selective laser melting process. *The International Journal of Advanced Manufacturing Technology*, 59, 1025–1035. doi:10.1007/s00170-011-3566-1
- Li, Y., & Bou-Akl, T. (2016). Electrospinning in Tissue Engineering. In S. Haider, & A. Haider (Eds.), *Electrospinning*. London: IntechOpen. doi:10.5772/65836
- Li, Y., Stewart, C. A., & Finer, Y. (2024). Advanced antimicrobial and anti-infective strategies to manage peri-implant infection: A narrative review. *Dentistry Journal*, 12(5), 125. doi:10.3390/dj12050125

- Liu, S., & Shin, Y. C. (2019). Additive manufacturing of Ti6Al4V alloy: A review. *Materials & Design*, 164, 107552. doi:10.1016/j.matdes.2018.107552
- Liu, X., Chen, S., Tsoi, J. K., & Matinlinna, J. P. (2017). Binary titanium alloys as dental implant materials-a review. *Regenerative Biomaterials*, 4(5), 315–323. doi:10.1093/rb/rbx027
- Liu, Z., Zhao, J. -h., Liu, P., & He, J. -h. (2016). Tunable surface morphology of electrospun PMMA fiber using binary solvent. *Applied Surface Science*, 364, 516–521. doi:10.1016/j.apsusc.2015.12.176
- Loier, C., Thauvin, G., Hazotte, A., & Simon, A. (1985). Influence of deformation on the $\beta \rightarrow \alpha + \beta$ transformation kinetics of Ti-6wt.%Al-4wt.%V alloy. *Journal of the Less Common Metals*, 108(2), 295-312. doi:10.1016/0022-5088(85)90225-5
- Luketich, S. K., Cosentino, F., Di Giuseppe, M., Menallo, G., Nasello, G., Livreri, P., . . . D'Amore, A. (2022). Engineering in-plane mechanics of electrospun polyurethane scaffolds for cardiovascular tissue applications. *Journal of the Mechanical Behavior of Biomedical Materials*, 128, 105126. doi:10.1016/j.jmbbm.2022.105126
- Maitz, M. F. (2015). Applications of synthetic polymers in clinical medicine. *Biosurface and Biotribology*, 1(3), 161-176. doi:10.1016/j.bsbt.2015.08.002
- Majumdar, T., Bazin, T., Massahud Carvalho Ribeiro, E., Frith, J. E., & Birbilis, N. (2019). Understanding the effects of PBF process parameter interplay on Ti-6Al-4V surface properties. *PLoS ONE*, 14(8), e0221198. doi:10.1371/journal.pone.0221198
- Manakhov, A. M., Sitnikova, N. A., Tsygankova, A. R., Alekseev, A. Y., Adamenko, L. S., Permyakova, E., . . . Solovieva, A. O. (2021). Electrospun biodegradable nanofibers coated homogenously by Cu magnetron sputtering exhibit fast ion release. Computational and experimental study. *Membranes*, 11(12), 965. doi:10.3390/membranes11120965
- Manfredi, D., & Calignano, F. (2017). Laser powder bed fusion of aluminum, titanium and nickel based alloys: Materials and design investigations. *2017 International Conference on Electromagnetics in Advanced Applications (ICEAA)*. Verona: 2017 International Conference on Electromagnetics in Advanced Applications (ICEAA). doi:10.1109/ICEAA.2017.8065546
- Mao, Y., Shen, W., Wu, S., Ge, X., Ao, F., Ning, Y., . . . Liu, Z. (2023). Electrospun polymers: Using devices to enhance their potential for biomedical applications. *Reactive and Functional Polymers*, 186, 105568.

- Mathias, L. E., Andreoli, A. F., & Gargarella, P. (2023). Gas atomization of A2 tool steel: Effect of process parameters on powders' physical properties. *Journal of Alloys and Compounds*, 960, 170696. doi:10.1016/j.jallcom.2023.170696
- McCracken, M. (1999). Dental implant materials: Commercially pure titanium and titanium alloys. *Journal of Prosthodontics*, 8(1), 40–43. doi:10.1111/j.1532-849x.1999.tb00006.x
- Morgan, R. H., Papworth, A. J., Sutcliffe, C., Fox, P., & O'Neill, W. (2002). High density net shape components by direct laser re-melting of single-phase powders. *Journal of Materials Science*, 37, 3093–3100. doi:10.1023/A:1016185606642
- Mostafaei, A., Zhao, C., He, Y., Ghiaasiaan, S. R., Shi, B., Shao, S., . . . Rollett, A. D. (2022). Defects and anomalies in powder bed fusion metal additive manufacturing. *Current Opinion in Solid State and Materials Science*, 26, 2, 2022, , 26(2), 100974. doi:10.1016/j.cossms.2021.100974
- Motyka, M. (2021). Martensite formation and decomposition during traditional and AM processing of two-phase titanium alloys—An overview. *Metals*, 11(3), 481. doi:10.3390/met11030481
- Muñoz-Escobar, A., Ruíz-Baltazar, Á. d., & Reyes-López, S. Y. (2019). Novel route of synthesis of PCL-CuONPs composites with antimicrobial properties. *Dose-Response*, 17(3), 1-11. doi:10.1177/1559325819869502
- Narasimharaju, S. R., Zeng, W., See, T. L., Zhu, Z., Scott, P., Jiang, X., & Lou, S. (2022). A comprehensive review on laser powder bed fusion of steels: Processing, microstructure, defects and control methods, mechanical properties, current challenges and future trends. *Journal of Manufacturing Processes*, 75, 375-414. doi:10.1016/j.jmapro.2021.12.033
- National Institutes of Health. (2022). *Copper – Fact Sheet for Health Professionals*. Retrieved May 02, 2025, from <https://ods.od.nih.gov/factsheets/Copper-HealthProfessional/>
- Nayl, A. A., Abd-Elhamid, A. I., Awwad, N. S., Abdelgawad, M. A., Wu, J., Mo, X., . . . Bräse, S. (2022). Recent progress and potential biomedical applications of electrospun nanofibers in regeneration of tissues and organs. *Polymers*, 14, 1508. doi:10.3390/polym14081508
- Nhlapo, N., Dzogbewu, T. C., & de Smidt, O. (2022). Nanofiber polymers for coating titanium-based biomedical implants. *Fibers*, 10(4), 36. doi:10.3390/fib10040036
- Ni, X., Cheng, W., Huan, S., Wang, D., & Han, G. (2019). Electrospun cellulose nanocrystals/poly(methyl methacrylate) composite nanofibers: Morphology, thermal

- and mechanical properties. *Carbohydrate Polymers*, 206, 29–37. doi:10.1016/j.carbpol.2018.10.103
- Nichol, T., Callaghan, J., Townsend, R., Stockley, I., Hatton, P. V., Le Maitre, C., . . . Akid, R. (2021). The antimicrobial activity and biocompatibility of a controlled gentamicin-releasing single-layer sol-gel coating on hydroxyapatite-coated titanium. *The Bone & Joint Journal*, 103-B(3), 522 - 529. doi:10.1302/0301-620X.103B3.BJJ-2020-0347.R1
- Ning, Y., Shen, W., & Ao, F. (2020). Application of blocking and immobilization of electrospun fiber in the biomedical field. *RSC Advances*, 10(61), 37246-37265. doi:10.1039/d0ra06865a
- Nirmala, R., Jeon, K. S., Lim, B. H., Navamathavan, R., & Kim, H. Y. (2013). Preparation and characterization of copper oxide particles incorporated polyurethane composite nanofibers by electrospinning. *Ceramics International*, 39, 9651–9658. doi:10.1016/j.ceramint.2013.05.087
- Nirwan, V. P., Kowalczyk, T., Bar, J., Buzgo, M., Filová, E., & Fahmi, A. (2022). Advances in electrospun hybrid nanofibers for biomedical applications. *Nanomaterials*, 12, 1829. doi:10.3390/nano12111829
- Olakanmi, E. O., Cochrane, R. F., & Dalgarno, K. W. (2015). A review on selective laser sintering/melting (SLS/SLM) of aluminium alloy powders: Processing, microstructure, and properties. *Progress in Materials Science*, 74, 401-477. doi:10.1016/j.pmatsci.2015.03.002
- Olakanmi, E., Cochrane, R., & Dalgarno, K. (2011). Densification mechanism and microstructural evolution in selective laser sintering of Al–12Si powders. *Journal of Materials Processing Technology*, 211(1), 113–121. doi:10.1016/j.jmatprotec.2010.09.003
- Onuoha, K. M., Omotola, O. E., Bulus, B. B., Alo, M., & Onuoha, C. E. (2019). Orthopaedic implant failure. *Surgery & Case Studies: Open Access Journal*, 2(3), 166-168. doi:10.32474/SCSOAJ.2019.02.000139
- Pede, D., Li, M., Poleske, T., & Mozaffari-Jovein, H. (2021). Influence of additive manufacturing and subsequent treatments on the corrosion behaviour of different titanium alloys. Toronto, Canada: Proceedings of the 59th Conference of Metallurgists, COM 2020: The Canadian Institute of Mining, Metallurgy and Petroleum.
- Pye, A. D., Lockhart, D. E., Dawson, M. P., Murray, C. A., & Smith, A. J. (2009). A review of dental implants and infection. *Journal of Hospital Infection*, 72(2), 104-110. doi:10.1016/j.jhin.2009.02.010

- Rahmani, R., Bashiri, B., Lopes, S. I., Hussain, A., Maurya, H. S., & Vilu, R. (2025). Sustainable additive manufacturing: An overview on life cycle impacts and cost efficiency of laser powder bed fusion. *Journal of Manufacturing and Materials Processing*, 9(1), 18. doi:10.3390/jmmp9010018
- Rampurawala, A., & Patil, A. (2021). Surface Modification of Titanium Orthodontic Implants. In D. Gabrić, & M. Vuletić (Eds.), *Current Concepts in Dental Implantology* (pp. 1-30). London: IntechOpen. doi:10.5772/intechopen.100038
- Ren, J., Ma, Z., Li, M., Zhang, Y., Liu, W., Liao, Z., & Yang, K. (2014). Antibacterial properties of Ti-6Al-4V-xCu alloys. *Journal of Materials Science & Technology*, 30(7), 699-705.
- Ribeiro, M., Monteiro, F. J., & Ferraz, M. P. (2012). Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. *Biomatter*, 2(4), 176-194. doi:10.4161/biom.22905
- Sarabi-Mianeji, S., Scott, J., & Pagé, D. J. (2015). Impact of electrospinning process parameters on the measured current and fiber diameter. *Polymer Engineering & Science*, 55(11), 2576-2582. doi:10.1002/pen.24150
- Shamsaei, N., Yadollahi, A., Bian, L., & Thompson, S. M. (2015). An overview of direct laser deposition for additive manufacturing; Part II: mechanical behavior, process parameter optimization and control. *Additive Manufacturing*, 8, 12-35. doi:10.1016/j.addma.2015.07.002
- Sharma, G. K., & James, N. R. (2022). Electrospinning: The Technique and Applications. In M. Khan, & S. J. Chelladurai (Eds.), *Recent Developments in Nanofibers Research*. London: IntechOpen. doi:10.5772/intechopen.105804
- Shrestha, S., & Chou, K. (2022). Formation of keyhole and lack of fusion pores during the laser powder bed fusion process. *Manufacturing Letters*, 32, 19-23. doi:10.1016/j.mfglet.2022.01.005
- Sing, S. L., Huang, S., Goh, G. D., Goh, G. L., Tey, C. F., Tan, J. H., & Yeong, W. Y. (2021). Emerging metallic systems for additive manufacturing: In-situ alloying and multi-metal processing in laser powder bed fusion. *Progress in Materials Science*, 119, 100795. doi:10.1016/j.pmatsci.2021.100795
- Singh, R., Gupta, A., Tripathi, O., Srivastava, S., Singh, B., Awasthi, A., . . . Saxena, K. K. (2020). Powder bed fusion process in additive manufacturing: An overview. *Materials Today: Proceedings*, 26(2), 3058-3070. doi:10.1016/j.matpr.2020.02.635

- Soles, A., Selimovic, A., Sbrocco, K., Ghannoum, F., Hamel, K., Moncada, E. L., . . . Cvetanovic, M. (2023). Extracellular matrix regulation in physiology and in brain disease. *International Journal of Molecular Sciences*, 24(8), 7049. doi:10.3390/ijms24087049
- Srivastava, M., Rathee, S., Patel, V., Kumar, A., & Koppad, P. G. (2022). A review of various materials for additive manufacturing: Recent trends and processing issues. *Journal of Materials Research and Technology*, 21, 2612-2641. doi:10.1016/j.jmrt.2022.10.015
- Strantza, M., Ganeriwala, R. K., Clausen, B., Phan, T. Q., Levine, L. E., Pagan, D. C., . . . Brown, D. W. (2021). Effect of the scanning strategy on the formation of residual stresses in additively manufactured Ti-6Al-4V. *Additive Manufacturing*, 45, 102003. doi:10.1016/j.addma.2021.102003
- Taghian, M., Mosallanejad, M. H., Lannunziata, E., Greco, G. D., Iuliano, L., & Saboori, A. (2023). Laser powder bed fusion of metallic components: Latest progress in productivity, quality, and cost perspectives. *Journal of Materials Research and Technology*, 27, 6484-6500. doi:10.1016/j.jmrt.2023.11.049
- Tahir, K., Nazir, S., Ahmad, A., Li, B., Khan, A. U., Khan, Z. U., . . . Rahman, A. U. (2017). Facile and green synthesis of phytochemicals capped platinum nanoparticles and in vitro their superior antibacterial activity. *Journal of Photochemistry and Photobiology B: Biology*, 166, 246-251. doi:10.1016/j.jphotobiol.2016.12.016
- Tanner, A., Maiden, M. F., Lee, K., Shulman, L. B., & Weber, H. P. (1997). Dental implant infections. *Clinical Infectious Diseases, Suppl 2*, S213-S217. doi:10.1086/516243
- Thijs, L., Verhaeghe, F., Craeghs, T., Van Humbeeck, J., & Kruth, J. -P. (2010). A study of the microstructural evolution during selective laser melting of Ti-6Al-4V. *Acta Materialia*, 58, 3303-3312. doi:10.1016/j.actamat.2010.02.004
- Udroiu, R. (2012). Powder bed additive manufacturing systems and its applications. *Academic Journal of Manufacturing Engineering*, 10(4), 122-129 .
- United Nations. (2024). *World Population Prospects 2024 Summary of Results*. Retrieved April 12, 2025, from https://population.un.org/wpp/assets/Files/WPP2024_Summary-of-Results.pdf
- Vergara-Hernández, H. J., Olmos, L., Solorio, V. M., Bouvard, D., Villalobos-Brito, J., Chávez, J., & Jimenez, O. (2023). Powder metallurgy fabrication and characterization of Ti6Al4V/xCu alloys for biomedical applications. *Metals*, 13(5), 888. doi:10.3390/met13050888

- Vilardell, A. M., Yadroitsev, I., Yadroitsava, I., Albu, M., Takata, N., Kobashi, M., . . . du Plessis, A. (2020). Manufacturing and characterization of in-situ alloyed Ti6Al4V(ELI)-3 at.% Cu by laser powder bed fusion. *Additive Manufacturing*, 36, 101436.
- Visan, A. I., Popescu-Pelin, G., Gherasim, O., Mihailescu, A., Socol, M., Zgura, I., . . . Socol, G. (2020). Long-term evaluation of dip-coated PCL-blend-PEG coatings in simulated conditions. *Polymers*, 12(3), 717. doi:10.3390/polym12030717
- Wang, J., Zhu, R., Liu, Y., & Zhang, L. (2023a). Understanding melt pool characteristics in laser powder bed fusion: An overview of single- and multi-track melt pools for process optimization. *Advanced Powder Materials*, 2(4), 100137. doi:10.1016/j.apmate.2023.100137
- Wang, L., Wang, F., Ayisen, S., Ren, T., Luo, X., & Wang, P. (2023b). Enhancing the mechanical properties and surface morphology of individualized Ti-mesh fabricated through additive manufacturing for the treatment of alveolar bone defects. *Frontiers in Bioengineering and Biotechnology*, 11, 1284359. doi:10.3389/fbioe.2023.1284359
- Worldometer. (2025). *Current World Population*. Retrieved April 12, 2025, from <https://www.worldometers.info/world-population/>
- Wu, J. -H., Chen, K. -K., Chao, C. -Y., Chang, Y. -H., & Du, J. -K. (2020). Effect of Ti₂Cu precipitation on antibacterial property of Ti-5Cu alloy. *Materials Science and Engineering: C*, 108, 110433. doi:10.1016/j.msec.2019.110433
- Wu, Z., Asherloo, M., Jiang, R., Delpazir, M. H., Sivakumar, N., Paliwal, M., . . . Mostafaei, A. (2021). Study of printability and porosity formation in laser powder bed fusion built hydride-dehydride (HDH) Ti-6Al-4V. *Additive Manufacturing*, 47, 102323. doi:10.1016/j.addma.2021.102323
- Xiao, X., Yang, K., Lei, S., Zhang, D., Guo, L., Dai, Y., & Lin, J. (2023). A β -type titanium alloy with high strength and low elastic modulus achieved by spinodal decomposition. *Journal of Alloys and Compounds*, 963, 171270. doi:10.1016/j.jallcom.2023.171270
- Xu, J., Lu, Y., Pan, X., Zhan, D., Wang, Q., & Zhang, N. (2023). Antibacterial performance of a porous Cu-bearing titanium alloy by laser additive manufacturing. *Frontiers in Bioengineering and Biotechnology*, 11, 1226745. doi:10.3389/fbioe.2023.1226745
- Xu, V. W., Nizami, M. Z., Yin, I. X., Yu, O. Y., Lung, C. Y., & Chu, C. H. (2022). Application of copper nanoparticles in dentistry. *Nanomaterials*, 12(5), 805. doi:10.3390/nano12050805

- Xue, J., Wu, T., Dai, Y., & Xia, Y. (2019). Electrospinning and electrospun nanofibers: Methods, materials, and applications. *Chemical Reviews*, 119(8), 5298–5415. doi:10.1021/acs.chemrev.8b00593
- Yadav, N., Yadav, R. R., & Dey, K. K. (2022). Microwave assisted formation of trimetallic AuPtCu nanoparticles from bimetallic nano-islands: Why it is a superior new age biocidal agent compared to monometallic & bimetallic nanoparticles. *Journal of Alloys and Compounds*, 896, 163073. doi:10.1016/j.jallcom.2021.163073
- Yadroitsava, I., Els, J., Booysen, G., & Yadroitsev, I. (2015a). Peculiarities of single track formation from Ti6Al4V alloy at different laser power densities by selective laser melting. *The South African Journal of Industrial Engineering*, 26(3), 86–95. doi:10.7166/26-3-1185
- Yadroitsev, I. (2009). *Selective Laser Melting: Direct Manufacturing of 3D-objects by Selective Laser Melting of Metal Powders*. Saarbrücken: LAP Lambert Academic Publishing AG & Co. KG.
- Yadroitsev, I., Bertrand, P. h., & Smurov, I. (2007). Parametric analysis of the selective laser melting process. *Applied Surface Science*, 253(19), 8064–8069. doi:10.1016/j.apsusc.2007.02.088
- Yadroitsev, I., Gusarov, A., Yadroitsava, I., & Smurov, I. (2010). Single track formation in selective laser melting of metal powders. *Journal of Materials Processing Technology*, 210, 1624–1631. doi:10.1016/j.jmatprotec.2010.05.010
- Yadroitsev, I., Krakhmalev, P., & Yadroitsava, I. (2015b). Hierarchical design principles of selective laser melting for high quality metallic objects. *Additive Manufacturing*, 7, 45–56. doi:10.1016/j.addma.2014.12.007
- Yadroitsev, I., Krakhmalev, P., Yadroitsava, I., Johansson, S., & Smurov, I. (2013). Energy input effect on morphology and microstructure of selective laser melting single track from metallic powder. *Journal of Materials Processing Technology*, 213(4), 606–613. doi:10.1016/j.jmatprotec.2012.11.014
- Yadroitsev, I., Yadroitsava, I., Bertrand, P., & Smurov, I. (2012). Factor analysis of selective laser melting process parameters and geometrical characteristics of synthesized single tracks. *Rapid Prototyping Journal*, 18(3), 201–208. doi:10.1108/13552541211218117
- Yang, C. Y., Lee, T. M., Lu, Y. Z., Yang, C. W., Lui, T. S., Kuo, A., & Huang, B. W. (2010). The influence of plasma-spraying parameters on the characteristics of fluorapatite coatings. *Journal of Medical and Biological Engineering*, 30(2), 91–98.

- Yang, D., & Liu, Z. (2016). Quantification of microstructural features and prediction of mechanical properties of a dual-phase Ti-6Al-4V alloy. *Materials*, 9(8), 628. doi:10.3390/ma9080628
- Yasa, E., Deckers, J., & Kruth, J. -P. (2011). The investigation of the influence of laser remelting on density, surface quality and microstructure of selective laser melting parts. *Rapid Prototyping Journal*, 17(5), 312–327. doi:10.1108/13552541111156450
- Yin, Z., Chen, X., Song, H. X., Hu, J. J., Tang, Q. M., Zhu, T., . . . Ouyang, H. W. (2015). Electrospun scaffolds for multiple tissues regeneration in vivo through topography dependent induction of lineage specific differentiation. *Biomaterials*, 44, 173–185. doi:10.1016/j.biomaterials.2014.12.027
- Yuan, Z., He, Y., Lin, C., Liu, P., & Cai, K. (2021). Antibacterial surface design of biomedical titanium materials for orthopedic applications. *Journal of Materials Science & Technology*, 78, 51-67. doi:10.1016/j.jmst.2020.10.066
- Zagade, P. R., Gautham, B. P., De, A., & DebRoy, T. (2024). Analytical modelling of scanning strategy effect on temperature field and melt track dimensions in laser powder bed fusion. *Additive Manufacturing*, 82, 104046. doi:10.1016/j.addma.2024.104046
- Zaman, H. A., Sharif, S., Idris, M. H., & Kamarudin, A. (2015). Metallic biomaterials for medical implant applications: A review. *Applied Mechanics and Materials*, 735, 19-25. doi:10.4028/www.scientific.net/AMM.735.19
- Zhang, E., Li, F., Wang, H., Liu, J., Wang, C., Li, M., & Yang, K. (2013). A new antibacterial titanium–copper sintered alloy: preparation and antibacterial property. *Material Science and Engineering C*, 33, 4280-4287.
- Zhang, E., Zhao, X., Hu, J., Wang, R., Fu, S., & Qin, G. (2021a). Antibacterial metals and alloys for potential biomedical implants. *Bioactive Materials*, 6(8), 2569-2612. doi:10.1016/j.bioactmat.2021.01.030
- Zhang, Y., Yang, S., Dong, G., & Zhao, Y. F. (2021b). Predictive manufacturability assessment system for laser powder bed fusion based on a hybrid machine learning model. *Additive Manufacturing*, 41, Article 101946. doi:10.1016/j.addma.2021.101946
- Zhao, X. M., Chen, J., Lin, X., & Huang, W. D. (2008). Study on microstructure and mechanical properties of laser rapid forming Inconel 718. *Materials Science and Engineering A*, 478(1–2), 119-124. doi:10.1016/j.msea.2007.05.079

CHAPTER 3 IN SITU LPBF ALLOYING

3.1. Introduction

The present chapter provides a detailed description of the fundamental principles of the manufacturing machine (that is, the EOSINT M 280), the various powder material characteristics and the analytical equipment used during the experimental work of this research. Furthermore, the determination of the optimum process parameters of Ti6Al4V-3 wt.% copper (Cu), Ti6Al4V-6 wt.% Cu, commercially pure titanium (cpTi), Ti-3 wt.% Cu, and Ti-6 wt.% Cu is discussed in detail.

One of the major advantages of laser powder bed fusion (LPBF) is the freedom in design, which allows for the manufacturing of site-specific biomedical implants. The feasibility of in situ alloying by LPBF for the synthesis of novel materials has been demonstrated, and the quality of final parts depends on the manufacturing parameters (Krakhmalev, et al., 2017; Krakhmalev, et al., 2018; Vilardell, et al., 2020). Therefore, manufacturing strategies and optimal process parameters should be carefully selected to reduce porosity, cracks, surface roughness and residual stresses and prevent deformation of the three-dimensional (3D) part during the LPBF procedure (Yadroitsev, et al., 2015; Vilardell, et al., 2020). Numerous process parameters control the characteristics of the final part produced by LPBF. The most important parameters that contribute to obtaining samples with the highest density include laser power, scanning speed, hatching distance and powder layer thickness (Aboulkhair, et al., 2014; Yadroitsev, et al., 2015; Manfredi & Calignano, 2017; Chowdhury, et al., 2022). The optimal parameter window is effectively determined experimentally, and various approaches in process parameter optimisation have been studied in literature (Laakso, et al., 2016). The most widely used approach to identify optimum process parameters is by conducting single track experiments since the basic building units for LPBF objects are single tracks, which combine to form a single layer and, ultimately, a 3D object is sintered from a sequence of layers (Laakso, et al., 2016; Krakhmalev, et al., 2017). Thus, the quality, reproducibility and reliability of LPBF parts depend on the quality of the sintered single tracks and subsequent layers (Dzogbewu, et al., 2017).

Optimising new metallic powder for LPBF printing involves several steps. In principle, the LPBF process consists of scanning and melting powder particles in a powder layer of a specified thickness with a mobile, high-density laser beam, generating a molten pool that

solidifies into a single track. Firstly, the laser power and scanning speed should be optimised, as they directly influence the formation of single tracks. Secondly, for the formation of a single layer, an appropriate hatch distance and scanning strategy should be selected for the manufacturing of layers with acceptable surface roughness and morphology for the layer-by-layer process (Vilardell, et al., 2020).

For in situ alloying to be achieved, the molten pool should have low viscosity and exist long enough to guarantee sufficient chemical homogeneity of the components (Krakhmalev, et al., 2017; Vilardell, et al., 2020). Simultaneously, the conditions should satisfy the operation window that is required to manufacture high-quality 3D objects (Yadroitsev, et al., 2015). Thus, the careful selection of the optimum process parameters is augmented for new materials manufactured by in situ alloying (Vilardell, et al., 2020). Appropriate scanning and building strategies should be carefully determined and optimised for each type of metallic powder, as only specific combinations of the main process parameters yield optimal results. Such parameters are expected to be different from the parameters for any individual alloy (Yadroitsev, et al., 2015; Vilardell, et al., 2020). Furthermore, understanding the impact of each processing parameter on the specific powder's characteristics would assist in overcoming the various challenges of tailoring the mechanical properties of a particular product for a desired application. Understanding the melt-pool dynamics of each metallic powder is crucial for automating LPBF processes.

3.2. Materials and methods

3.2.1. EOSINT M 280 machine

The Central University of Technology, Free State (CUT) houses an EOSINT M 280 machine (Electro Optical Systems (EOS) GmbH, Krailling, Germany) at the Centre for Rapid Prototyping and Manufacturing (CRPM, Figure 3.1). The main components of the EOSINT M 280 machine are a process chamber with a re-coating system, an optical system with a laser and a scanner, a computer-controlled elevating system, a process gas management system and a process computer with control software.

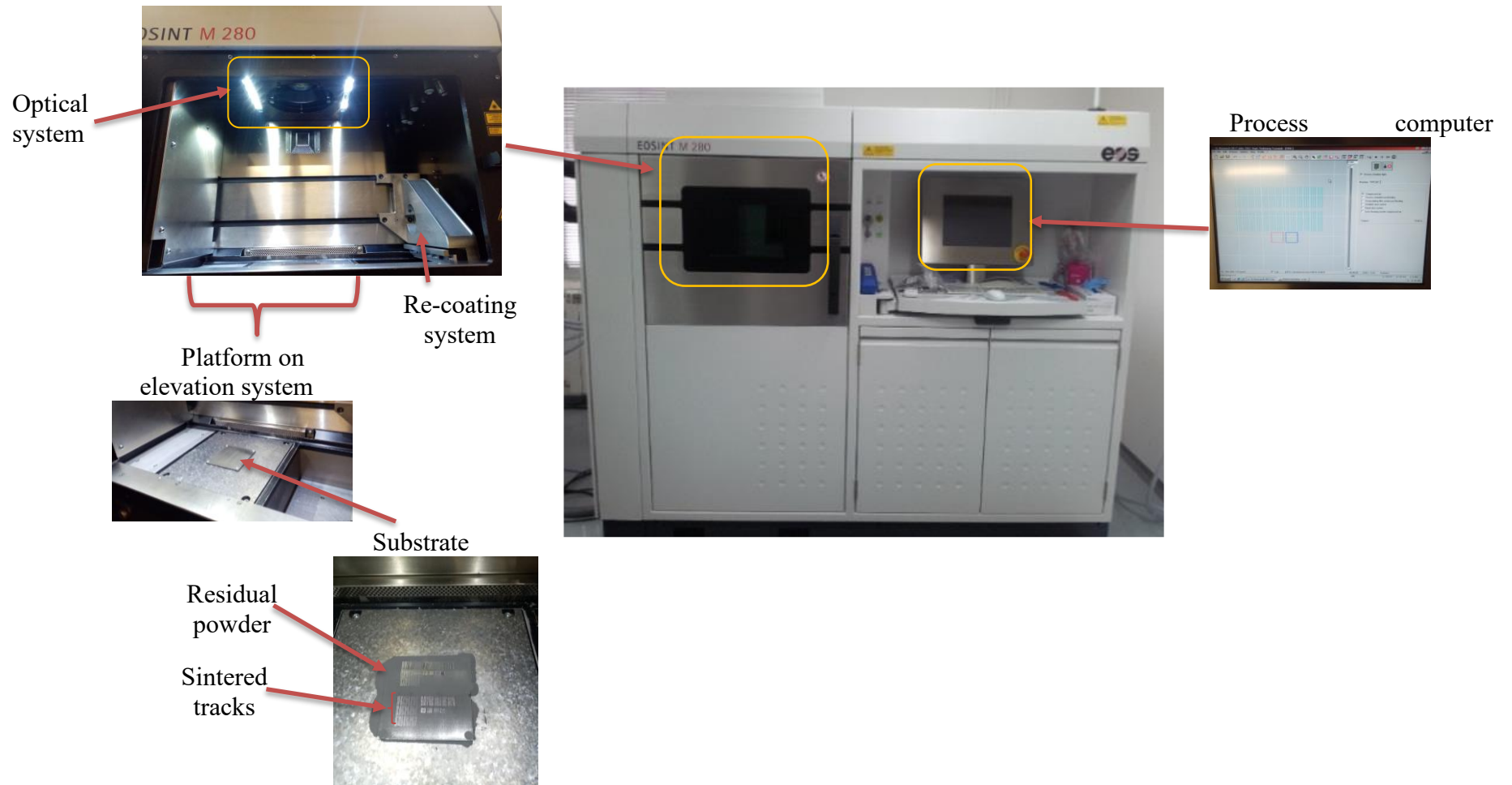


Figure 3.1: EOSINT M 280 machine installed at CRPM (CUT) and essential components

The process chamber (otherwise known as the building chamber) is the section where the physical layer-by-layer construction of a part takes place. The optical system is equipped with a ytterbium (Yb) fibre laser that emits a beam at 1060–1100 nm, capable of generating up to 10 kW of power and an energy efficiency of 10–30% (Cobbinah, et al., 2021). Compared to other commercial lasers – such as the carbon dioxide (CO₂) laser beam – some advantages offered by the Yb-fibre laser include a high electrical-to-optical efficiency (~25%), excellent beam quality, compactness, the ability to operate at lower scanning speeds, and the production of more stable, slightly larger scan tracks. The shorter wavelength of the Yb-fibre laser beam permits light transmission through optical fibre cables instead of the articulated mirror delivery system employed for CO₂ laser (Cobbinah, et al., 2021). The laser beam focuses on a base plate situated on the elevating systems with a spot size of approximately 80 µm.

In the conventional LPBF process, a small Gaussian laser beam (<100 µm in diameter) is used to melt the thin layer of powder (Zhang, et al., 2022). The EOSINT M 280 laser scanner can scan at a maximum speed of 7 m/s and has an exposure area of 250 mm × 250 mm. The operating atmosphere in this chamber is protected by an inert gas, which permits the processing of a wide range of reactive metallic powders by ensuring the minimum acceptable oxygen content (0.07–0.12%). The entire EOSINT M 280 system is virtually controlled by process software (PSW) installed on the process computer. The PSW is used to set the protocol of the building process before manufacturing and to display the graphical layout of the part as it is built on the computerised screen.

For optimal operation of the LPBF machine, a systematic procedure is followed. Each stage of the procedure should be performed with the strictest accuracy to ensure an optimum quality final product. The systematic procedure is carried out using the EOSINT M 280 machine setup. It entails sieving the powder to refill the powder container, preparing the platform, selecting the correct re-coater blade, levelling the re-coater blade with the platform and flushing the building environment with argon or nitrogen gas.

3.2.1.1. Sieving of the powder

The EOSINT M 280 machine has a sieving component. By regulating the elevating system, the dispenser in the collector system can be moved up or down to enable manual collection and sieving of the powder. Non-virgin powders (that is, powder that has been previously used) are sieved before use to remove agglomerates and/or other large particles to ensure even

consistency of the metal powder that is conveyed from the dispenser, building and collector ducts. During the sieving process, it is vital to use appropriate personal protective equipment (PPE), including laboratory coats, masks, gloves and antistatic shoes.

3.2.1.2. Platform preparation

The part(s) may be built directly on the base plate or on a substrate fixed onto the base plate. The base plate is secured to the elevating system in the machine's process chamber. The platform descends to the set value, as predetermined by the desired thickness of each powder layer. For LPBF technology, the thickness of layers is usually 20–100 μm , and the maximum grain size should not be greater than the layer thickness (Kurzynowski, et al., 2012).

3.2.1.3. Re-coater blade settings

The re-coater blade controls the powder dispersion, and hence the accuracy of the space between the re-coater and the base plate is crucial and complex. A gauge clock and a feeler gauge are used to manually level and determine the gap between the re-coater blade and the base plate. The gauge clock is clamped firmly at 90° to the re-coater blade, so that the plunger tip touches the re-coater blade surface. The pointer on the surface of the gauge is zeroed by moving the bezel. The gauge clock is moved across the entire length of the re-coater blade. When the pointer moves from zero as the gauge clock moves, the re-coating is considered unlevelled. The re-coater level is then adjusted, and the process of moving the gauge clock is repeated. This step of setting the re-coater blade is repeated until the pointer remains at zero when the gauge clock is moved across the length of the re-coater blade, indicating that the re-coater blade is set to the desired level for the manufacturing process. The function of the feeler gauge is to determine the size of the gap between the re-coater blade and the base plate, which depends on the required layer thickness of the deposited powder.

3.2.1.4. Protective atmosphere

The atmosphere in which the process takes place is a significant, fixed parameter since high temperature and the presence of oxygen in the building chamber during the LPBF process may cause oxidation of the manufactured part (Kurzynowski, et al., 2012). Therefore, as in most laser processing operations, the inert environment used in the LPBF process serves as a shielding gas for the molten metal against primarily atmospheric oxygen. The high surface area per unit volume of metallic powder particles compared to cast components makes them susceptible to contamination by adsorbed gases, moisture, oxide and nitride films and organics

(Cobbinah, et al., 2021). In addition, the contamination of the sintered part by oxygen molecules may cause porosity by altering the physical and chemical properties of the finished component (AZOM, 2017). Dadbakhsh & Hao (2014) demonstrated that a thin powder layer, higher laser power and lower scanning speed may contribute to the removal of oxygen during LPBF. For repeatability, an optimal measure of gas flow should be monitored, such as maintaining the oxygen level at <1000 ppm (0.1%) throughout the manufacturing process, which may be decreased to approximately 100 ppm (0.01%) oxygen for a highly reactive metallic powder like Ti (AZOM, 2017). An inert atmosphere is necessary for Ti-based alloys, and the most commonly used protective gases in LPBF processes are nitrogen, argon and helium (Kurzynowski, et al., 2012; Cobbinah, et al., 2021). Argon (Ar) gas is the most favoured inert gas for the LPBF process because of its ability to stabilise the molten pool depth and reduce spatter (Cobbinah, et al., 2021).

Temperature within the build chamber is another factor that should be considered since, besides contributing to oxidation, temperature variation in the build chamber affects the properties and microstructure of as-built samples (Zhao, et al., 2016). The general perception is that the higher the build chamber temperature, the less the residual stress affects the sample. If the build chamber temperature is too high, the powder particles begin to agglomerate, affecting the accuracy of the final part. If the build chamber temperature is too low, the curling effect occurs, and the two-phase system of melt and solid material cannot be generated (Wudy & Drummer, 2019). Powder bed preheating may also considerably reduce residual stress. Ali et al. (2017) investigated the effect of powder bed preheating during selective laser melting (SLM) of Ti6Al4V on various mechanical properties. The results showed that a powder bed temperature of approximately 570 °C greatly reduced residual stress formation in components and improved their mechanical properties. Most AM machines offer preheating options that raise the temperature above 500 °C to increase build quality and limit the risk of distortion (Chowdhury, et al., 2022). However, high temperatures are detrimental to the lifespan of the base plate and the entire manufacturing system. Therefore, the recommended operating temperature for the EOSINT M 280 machine is between 60–100 °C (Dzogbewu, 2017).

3.2.1.5. CAD modelling

The first step in most additive manufacturing (AM) processes is designing a three-dimensional (3D) computer-aided design (CAD) model using various CAD software. The selected modelling software should be capable of converting the designed CAD data to a STL (Standard

Tessellation Language) file format. The STL software translates the 3D design structure into a sequence of two-dimensional (2D) layers referred to as “slices”. This conversion process enables the building process by creating the Specific Layer Interface (SLI) and enabling data processing in a computer-aided manufacturing (CAM) platform.

After the 3D CAD data are virtually sliced into 2D layers by the STL software, EOS RP-Tools is applied. The EOS RP-Tools is EOS software specifically designed to generate and manipulate part data in STL format or the Common Layer Interface (CLI). The EOS software converts part data from STL or CLI formats into an EOS-specific CLI format by separating the layer data of the solid bodies into skin and core data. Figure 3.2 depicts the steps and the related software applied.

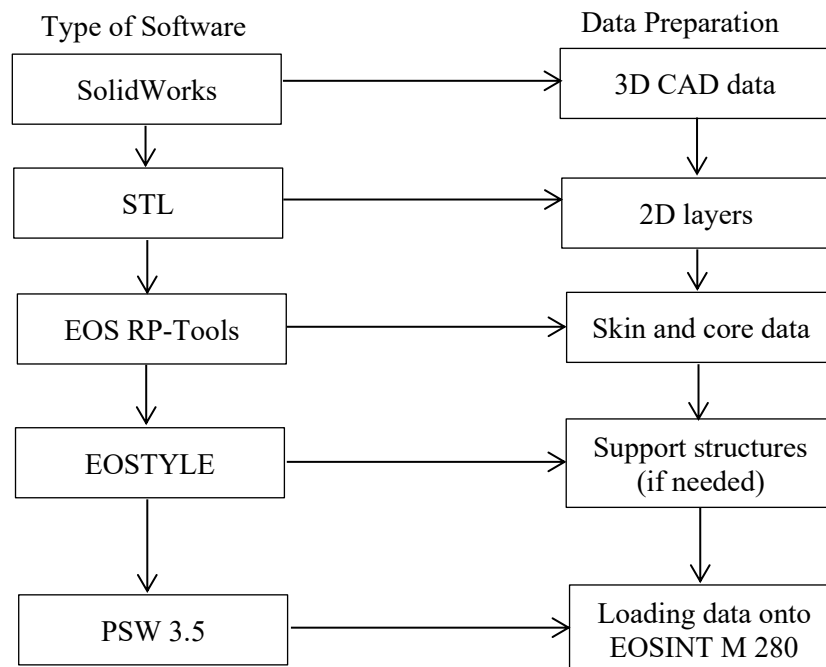


Figure 3.2: CAD modelling framework with applicable software (adapted from Dzogbewu (2017))

3.2.1.6. Support structures

Building support structures for hanging parts is another mandatory step in the LPBF process. The absence of support structures at the required position may cause the built part to severely warp. Support structures are generated using the EOSTYLE software package.

3.2.1.7. Loading and controlling the LPBF building

The support structure and parts are placed on the building platform of the EOSINT M 280 by using the PSW 3.5 computer software interface. The software enables monitoring of the building environment's temperature, oxygen level, and the estimated time to complete a particular job. In addition, the software interface provides information about the vertical movement of the building platform, the horizontal movement of the powder deposition blade and the available powder in the dispensary or collector duct. Thus, the operating parameters, such as laser power, scanning speed, hatch distance, and powder layer thickness, may be controlled and regulated. The optimal values of all processing parameters should be determined to manufacture a part with optimal mechanical properties.

3.2.2. Powders

Input material characterisation and standardisation are vital for the repeatability of the AM process. Powder properties – such as size, morphology, surface area, porosity and impurity content – may directly affect the manufacturing process and the mechanical properties of the final part (Dzogbewu, 2021). Relatively large particle sizes of powder tend to produce parts with a poor surface finish (Ruban, et al., 2014). The morphology of the powder determines the processing of the powder (Kurzynowski, et al., 2012; Krakhmalev, et al., 2016). The granulomorphometry of powders used for the LPBF process is usually spherical, with smooth surfaces and low surface oxidation. These characteristics allow for adequate powder flow properties and reduced levels of contamination prior to handling and processing (Cobbinah, et al., 2021). Three powders were used for this study, namely:

- 1) Pre-alloyed Ti6Al4V Extra Low Interstitial (ELI) powder (also denoted as Ti6Al4V, Ti-6Al-4V or Ti64 in this chapter)
- 2) Elemental commercially pure titanium (cpTi, Grade 2) powder
- 3) Elemental copper (Cu) powder

The employed pre-alloyed Ti6Al4V and elemental cpTi powders comply with the standards of the American Society for Testing and Materials (ASTM) F136 (ASTM International, 2013) and International Organization for Standardization (ISO) 5832-2 (ISO, 2025), respectively, for the acceptable maximum concentration of impurities as shown in Table 3.1. Copper of 99.9% purity was employed. The typical physical and mechanical properties of argon gas atomised Ti6Al4V, cpTi and oxygen-free pure Cu powders are displayed in Table 3.2.

Table 3.1: Chemical composition of Ti6Al4V ELI and cpTi powders (in weight %) (adapted from AZOM (2013a), AZOM (2013b) and ASM (2023))

Ti	Al	V	O	Fe	C	H	N	Si	Mn	Mg
ASTM standard for Ti6Al4V ELI alloy, Grade 23										
88.1-91	5.5-6.5	3.5-4.5	≤0.2	≤0.25	≤0.080	≤0.0125	≤0.030			
Employed Ti6Al4V ELI powder										
89.263	6.31	4.09	0.12	0.20	0.005	0.003	0.009			
International Organization for Standardization (ISO) 5832-2 (cpTi, Grade 2)										
≥ 98.9			≤0.25	≤0.30	≤0.08	≤0.015	≤0.03			
Employed cpTi powder										
Bal			0.17	0.03	0.007	0.002	0.012	0.005	0.01	0.02

Table 3.2: Typical physical and mechanical properties of wrought Ti6Al4V ELI, cpTi and Cu (adapted from AZOM (2012), AZOM (2013a), AZOM (2013b) and ASM (2023))

Property	Ti6Al4V (ELI)	cpTi (Grade 2)	Cu
Density, Kg ^m ⁻³	4430	4510	8890–8940
Melting point, °C	1604–1660	1665	1083
Solidus, °C	1604		1083
Liquidus, °C	1660	1665	1083
Beta transus, °C	980	913	
Specific heat, J·kg ⁻¹ K ⁻¹	0.526	0.523	0.385
Thermal conductivity, Wm ⁻¹ K ⁻¹	6.7	16.4–21.97	350
Ultimate tensile strength, MPa	860	430	221–455
Yield strength, MPa	790	340	69.0–365
Elasticity modulus, GPa	113.8	105–120	115
Shear modulus, GPa	44	38	44.0
Elongation at break, %	14–15	28	55
Notched tensile strength, MPa	1450	720	
Hardness, Vickers	341	145–200	75.0–105
Poisson's ratio	0.342	0.34–0.40	0.310
Fracture toughness, MPa·m ^{1/2}	75		

3.2.2.1. In situ alloying

For in situ alloying, the Ti6Al4V and cpTi powders were each mixed with 3 and 6 wt.% Cu in laboratory scale Turbula[®] mixer machine (Figure 3.3) for 45 min. The Turbula[®] mixes powders by rotation, translation and inversion motions according to the Schatz geometric theory (Mayer, et al., 2015). The resultant powder alloys were Ti6Al4V-3 wt.% Cu, Ti6Al4V-6 wt.% Cu, Ti-

3 wt.% Cu and Ti-6 wt.% Cu and were designated as Ti6Al4V-3Cu, Ti6Al4V-6Cu, Ti-3Cu and Ti-6Cu, respectively.



Figure 3.3: Turbula® mixer

3.2.2.2. Characterisation of employed powders

Due to the large lot sizes, it was not possible to characterise the whole batch of the powders. Therefore, small fractions of the selected powders were examined to represent the characteristics of the bulk powders. The powders were selected under motion to ensure an accurate representation of the entire batch (Dzogbewu, 2021). The powders – namely, Ti6Al4V, cpTi, Cu, Ti6Al4V- x Cu and Ti- x Cu ($x = 3$ and 6 wt.%) – were characterised using the JEOL JSM-6610 Scanning Electron Microscope (SEM, Figure 3.4) with an integrated energy-dispersive X-ray spectroscopy (EDS) detector, situated at the University of the Free State (UFS) Geology Department. The morphological evaluation of the powders was conducted using secondary electron (SE) imaging at 15–20 kV and a working distance of up to 10 mm.



Figure 3.4: JEOL JSM-6610 Scanning Electron Microscope

The particle sizes of the Ti6Al4V, cpTi, and Cu powders were measured on SEM micrographs (scale = 100 μm) using the ImageJ software ($n = \sim 1500$), and the particle size distributions were analysed using the IBM SPSS Statistics software. The particle size distribution of powders used for AM has a Gaussian distribution, and this trend can influence the melt pool stability along with the processing parameters (Viale, et al., 2022). Elemental mapping of the Ti6Al4V, cpTi, and Cu powders was conducted with the EDS, concomitantly with the SEM imaging, to determine the chemical composition of each powder.

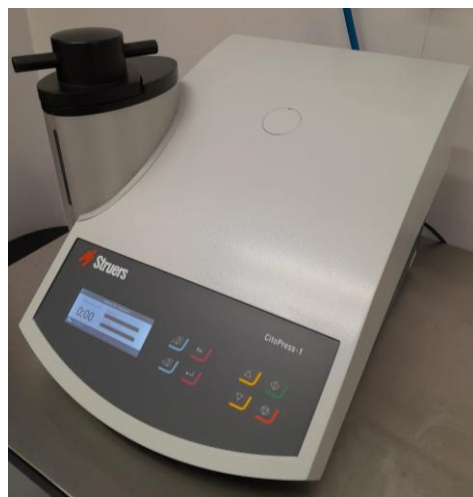
3.2.2.3. Flowability of employed powders

Powder flowability has a momentous effect on the LPBF build product. Dzogbewu (2017) applied the Hausner ratio and Carr index to demonstrate that the Ti6Al4V ELI, cpTi, and Cu powders used in this research exhibit good to excellent flow, as required for the LPBF process. During the present study and before the laser processing, the powders were dried in a vacuum oven at 80 °C overnight, then cooled to room temperature to increase powder flowability and prevent agglomeration.

3.2.3. Metallographic preparation

The specimens for metallographic analysis were prepared in accordance with ASTM E3 (ASTM International, 2017). A Struers CitoPress-1 machine (Figure 3.5(i)) was used to mount units in MultiFast non-conductive resin for 3.5 min at a temperature of 180 °C and a pressure

of 250 bar, followed by rapid cooling with water for 2 min. The mounted samples were then ground and polished with a Struers Tegramin-25 machine (Figure 3.5(ii)) according to the protocol suggested by the manufacturer for Ti-based alloys as detailed in Table 3.3.



(i)



(ii)

Figure 3.5: (i) CitoPress-1 machine, and (ii) Tegramin-25 polishing machine

Table 3.3: The recommended polishing procedure for Ti-based alloys

Process	Wheel/Cloth	Lubricant	Force (N)	Speed (rpm)	Time (min)
Coarse grinding	320 SIC	Water	25	300	1.50
Fine grinding	MD-Largo	DiaPro All/Lar.9	30	150	5.00
First polishing	MD-Mol	DiaPro Mol.3	25	150	2.20
Final polishing	MD-CHEM	OP-S Suspension	20	150	1.50

The OP-S colloidal silica suspension applied at the final stage of polishing, along with the MD-CHEM polishing disc, ensured that the sample surface had a mirror finish and was free of mechanical deformation. Each sample was rinsed with tap water, followed by rinsing in Lab Companion UC-02 Ultrasonic Cleaner for 3 min (Figure 3.6(i)). The sample was washed with alcohol and dried by air at room temperature. Subsequently, the sample was etched in ESCO Ductless Fume Hood (Figure 3.6(ii)) with Kroll's reagent, which is commonly used for etching Ti alloys. The Kroll etchant is a dilute aqueous solution containing 10 ml of 48% hydrofluoric acid (HF), 5 ml of nitric acid (HNO₃) and 85 ml of distilled water. Etching prepared the samples for microscopic examinations. A ZEISS Axio Scope.A1 Optical Microscope with AxioVision SE64 software (Figure 3.7) was used to capture the images for metallographic analysis.



(i)



(ii)

Figure 3.6: (i) Lab Companion UC-02 Ultrasonic Cleaner, and (ii) ESCO Ductless Fume Hood

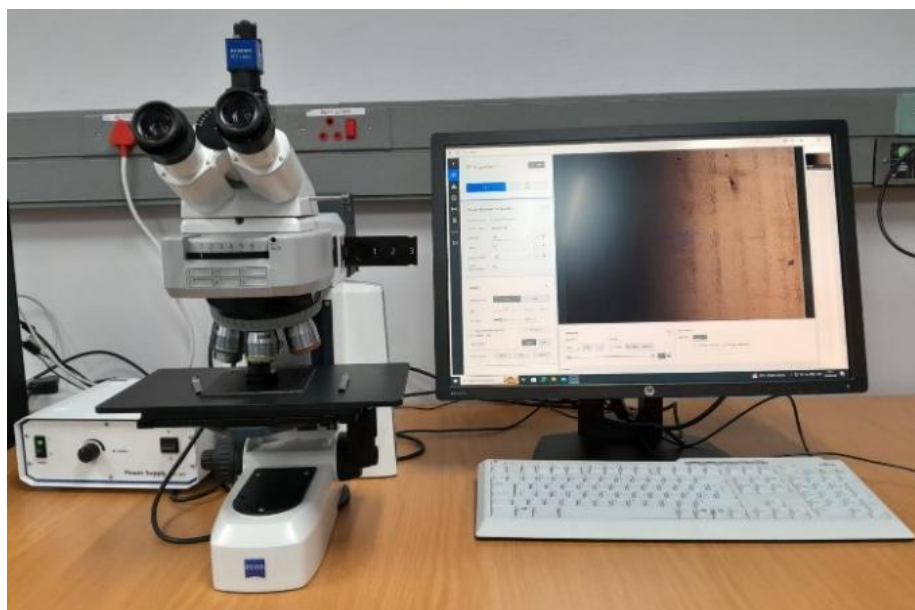


Figure 3.7: ZEISS Axio Scope.A1 Optical Microscope

3.2.4. Optimisation of powders employed using LPBF

The optimum process parameters for Ti6Al4V-3Cu, Ti6Al4V-6Cu, cpTi, Ti-3Cu, and Ti-6Cu powder compositions were determined individually. Optimisation entailed optimising the process parameters of scanning speed, laser power and hatch distance. The EOSINT M 280

machine (Figure 3.1) was used for the optimisation process and thus, to fabricate all the metal samples used in the current study. The base plate was fixed to the machine platform in the process chamber of the machine by screwing its four corners to the platform. A Ti6Al4V (not necessarily an ELI) substrate with a chemical composition similar to the powders was used for the optimisation process. The substrate was machined by grinding to smooth its surface, then cleaned with ethanol. Thereafter, the substrate was secured onto the base plate. Powder processing commenced with the sieving of the powder onto the platform. A powder layer thickness of 50 μm was applied for the current investigations. Therefore, a feeler gauge of 0.05 mm was used to set the gap between the base plate and the re-coater blade for the layer thickness of 50 μm . The re-coated blade was made of a high-speed steel alloy, and its operating speed was set at 150 mm/s. An inert environment was maintained by continuously flushing argon gas into the building chamber of the EOSINT M 280 system.

3.2.4.1. LPBF single tracks

For optimising the powders, the starting process parameters for feedstock material were chosen based on previous studies of optimisation using a similar system (Krakhmalev, et al., 2017; Vilardell, et al., 2020; Dzogbewu, 2021; Oyesola, et al., 2021; Ramosena, et al., 2022). For all powder compositions, a uniform powder layer of 50 μm thickness was deposited on a Ti6Al4V substrate, which was screwed onto the building platform of the LPBF machine. For Ti6Al4V-3Cu and Ti6Al4V-6Cu, single tracks were sintered at laser powers of 100, 150, 170 and 300 W and at a scanning speed range of 0.08–2.2 m/s. For cpTi, tracks were sintered at laser powers of 100, 150, 170 and 300 W and at a scanning speed range of 0.08–1.2 m/s. The single tracks for Ti-3Cu and Ti-6Cu were sintered at laser powers of 100, 150 and 170 W and at a scanning speed range of 0.4–2.2 m/s. For each scanning speed, a set of three tracks, each 20 mm long and separated by a 1 mm gap, was produced using a laser spot of $\sim 80 \mu\text{m}$. A gap of 2 mm was placed between each set of single tracks produced at a particular scanning speed to distinguish between the sets.

After the single tracks were produced, the loose powder was removed from the plates. The substrate with the sintered tracks was unscrewed from the LPBF platform. The optical microscope (Figure 3.7) was used to examine the prepared samples from the top view to observe the continuity and measure the width of the tracks. After the top view analysis of the single tracks, the plates were sectioned into smaller units ($<30 \text{ mm}$) using the JiangSu

Fangzheng computer numerical control (CNC) wire electrical discharge machine (EDM). Each cross-section was mounted, polished and etched in preparation for metallographic analysis to evaluate the cross-sections of the sintered single tracks as described in Section 3.2.3. Metallographic preparation The optical microscope was used to examine the prepared samples from the cross-sectional view to measure the height of the tracks above the substrate and their penetration depth into the substrate, and to identify tracks with adequate material deposition (Figure 3.8). From the single track analysis, two sets of optimum laser powers and corresponding scanning speeds were selected for each of the powders of Ti6Al4V-3Cu, Ti6Al4V-6Cu, cpTi, Ti-3Cu and Ti-6Cu.

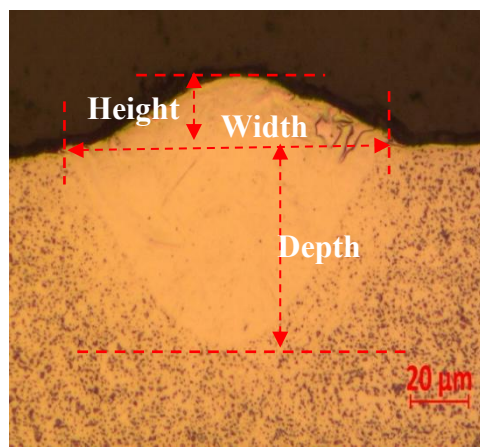


Figure 3.8: The cross-section of the LPBF single track

3.2.4.2. LPBF double layers

The succeeding step of the current investigation was to optimise the hatch distance towards obtaining steady layers in the layer-by-layer construction, since the hatch distance and the scanning strategy govern the quality of a single LPBF layer (Yadroitsev, et al., 2015). A uniform powder layer of 50 μm thickness was deposited on a newly prepared Ti6Al4V substrate in the building chamber of the LPBF machine. The single tracks were printed side-by-side at the optimised scanning speeds and laser powers with a laser spot size of 80 μm, to form square single layers and subsequent double layers with dimensions 5 × 5 mm. The zig-zag (bidirectional) strategy was followed during the scanning of adjacent tracks. The double layers were sintered to optimise the hatch distance; hence, the tracks were produced at hatch distances of 80, 90 and 100 μm. Rescanning was applied, whereby each double layer was scanned twice with the laser. The double scanning entailed that the laser beam first melted the powder layer to form adjacent (and possibly overlapping) tracks at the specified hatch distance until a layer was formed, followed by the laser beam passing between sintered tracks and re-

melting the adjoining tracks. This approach is envisaged to improve the surface quality of the layers and enhance the density and surface quality of sintered parts (Yadroitsev, et al., 2015). After the double layers were produced using single scanning and rescanning strategies at each hatch distance, the loose powder was removed from the plate.

The substrate was unscrewed from the LPBF platform, and the optical microscope (Figure 3.7) was used to examine the double layers from the top view. A portable Mitutoyo SURFTEST SJ-210 profilometer (Figure 3.9) was used to measure the surface roughness of the double layers manufactured using a single and double scanning strategy. The roughness tester was set to record measurements in accordance with ISO 1997. The stylus was set to travel to a cut-off length of $\lambda_c = 0.8$ mm at a measuring speed of 0.5 mm/s and perpendicular to the scanning direction to account for the “staircase effect” common in layer-wise AM processes (Chen, et al., 2018; Metelkova, et al., 2021). The surface roughness measurements were recorded at three randomly selected locations for each double layer. The top surfaces of the single and rescanned layers were examined for defects using SEM (Figure 3.4), and EDS mapping was used to assess the homogeneity of Cu.



Figure 3.9: Mitutoyo SURFTEST SJ-210 profilometer

Upon completion of top-surface analyses, the substrates with double layers were sectioned into smaller units (<30 mm) using the CNC wire EDM and prepared for metallographic analyses, as described in Section 3.2.3. Metallographic preparation, to evaluate the cross-sections of the sintered layers. Usually, single layers are produced and analysed first, followed by double layers; however, the results obtained from the analysis of double layers are often preferred in literature since the morphology of single and double layers has been observed to be similar (Ramosena, et al., 2022). Therefore, in this study, only the double layers have been examined.

The optimum hatch distance to manufacture 3D objects using LPBF was deduced from the double-layer examination data for each powder composition.

3.2.4.3. LPBF 3D objects

Three-dimensional objects were fabricated on newly prepared Ti6Al4V substrates. For parts made of cpTi powder (including Ti-3Cu and Ti-6Cu), an additional 1 mm of the support structure was built to compensate for compositional differences between the cpTi alloys and the Ti6Al4V substrate. For porosity and microstructure analyses, $10 \times 10 \times 10$ mm 3D cube samples were built at the optimised sets of parameters of laser power and scanning speed combinations, as well as at the optimised hatch distance, while maintaining the powder layer thickness of 50 μm and using a zig-zag alternating strategy with rescanning of the last layer. To minimise uneven thickness in the synthesised layers resulting from surface irregularities, the scanning direction in each single layer was rotated by 90° relative to the previous layer. The strategy for building the cubes is illustrated in Figure 3.10.

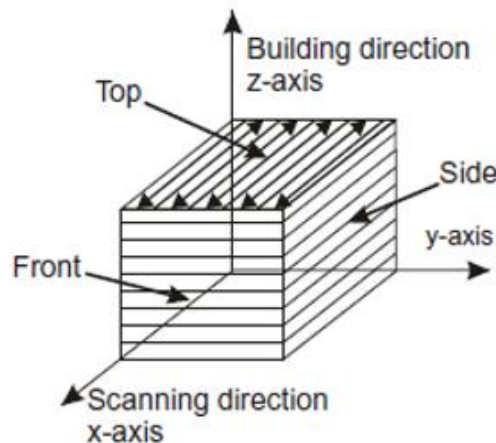


Figure 3.10: Scanning and building coordination system (adapted from Thijs, et al. (2010))

When the LPBF construction of the 3D objects was complete, the loose powder was removed from the base plate, and the substrate was unscrewed and removed from the platform. The 3D samples were utilised in their as-built condition with no additional heat treatment. The cubes were removed from the substrate and sectioned into smaller units using EDM wire cutting. One unit was reserved for porosity testing, and two were prepared for microstructure analysis. The combination of parameters that produced the most dense objects (determined by the part with the least porosity) was delineated as the optimum process parameters for the particular powder.

3.2.4.4. Porosity

To study internal defects in the samples, reconstruction, visualisation and analysis were performed at the CT Scanner Unit of the Central Analytical Facilities (CAF) at Stellenbosch University according to the procedure described by du Plessis et al. (2018a, b). X-ray computed tomography (CT) scanning was executed on General Electric (GE) Phoenix instruments, illustrated in Figure 3.11, using the parameters listed in Table 3.4 for the various test samples. The selection of the equipment and software was subject to availability. A global threshold was selected based on the grey-value histogram of the entire volumetric data set, and a local optimisation was performed at each point to find the steepest grey-value gradient at the interface between two materials.



(i)



(ii)

Figure 3.11: (i) GE Phoenix Nanotom S nanoCT scanner, and (ii) GE Phoenix VTomeX L240 microCT scanner (adapted from Stellenbosch University (2024))

Table 3.4: Parameters for X-ray CT scanning of Ti-based samples

Test samples	Ti6Al4V-3Cu and Ti6Al4V-6Cu	cpTi, Ti-3Cu and Ti-6Cu
Equipment (type of imaging)	GE Phoenix Nanotom S (nanoCT)	GE Phoenix VTomeX L240 (microCT)
Voltage	140 kV	100 kV
Current	100 μ A	50 μ A
Copper beam filter size	0.5 mm	0.1 mm
Voxel size set	5 μ m	5 μ m
Rate of imaging	500 ms	1000 ms
Images captured during 360-degree rotation	3000	3000
Image acquisition and reconstruction software	Datos	Datos
3D image analysis software	VGSTUDIO MAX 3.5	Dragonfly 2022.2

3.2.4.5. Microstructure

The samples were prepared for microstructure analysis by conventional steps of metallographic preparation according to ASTM E3 (ASTM International, 2017), as described in Section 3.2.3. Metallographic preparation, for evaluation the microstructure at the top (transverse, in XY horizontal plane parallel to the laser scanning direction and perpendicular to the build direction) and side (longitudinal, in the Z direction parallel to the build direction) views of the 3D units.

3.2.5. Statistical analysis

All measured data were collected in triplicate, unless otherwise stated. The values were reported as mean $\pm$ standard deviation. The Student's t-test was performed, and values of $p \leq 0.05$ (95% confidence interval) and $p \leq 0.01$ (99% confidence interval) were considered as indicating statistically significant and highly statistically significant, respectively.

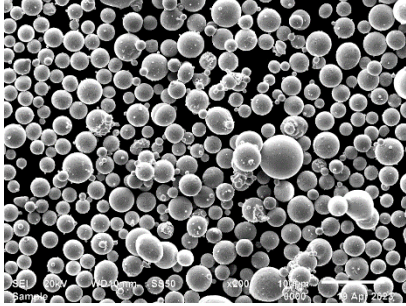
3.3. Results and discussion

The quality, reproducibility and reliability of LPBF-built parts depend on the quality of individual sintered single tracks and layers. Yadroitsev et al. (2015) elucidated that a hierarchical approach, including a systematic analysis of LPBF parameters, is necessary to control final product quality at every level during track and layer formation.

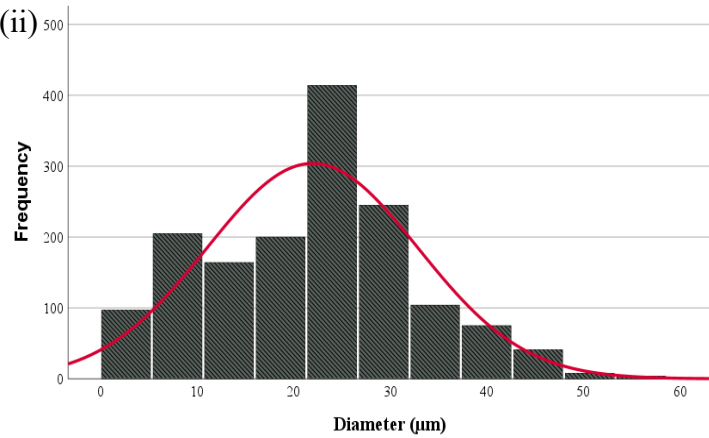
3.3.1. Characterisation of employed powders

Figure 3.12 shows the morphology and the particle size distribution curves of the gas atomised Ti6Al4V, cpTi and oxygen-free Cu powders. From the SEM micrographs, it may be seen that the powder particles were smooth and highly spherical. Irregular particle morphology negatively impacts LPBF processing and is unfavourable for efficient powder packing and flowability. Thus, the deposition of a uniform layer of powder would be poor, and a skewed particle size distribution could be a consequence of such morphology (Aboulkhair, et al., 2014). Dzogbewu & Du Preez (2022) also observed Ti powder particles that were spherical and had smooth surfaces without a distinct tendency towards aggregation. The average particle size of the employed Ti6Al4V, cpTi and Cu powders was $21.9 \pm 10.9 \mu\text{m}$, $22.3 \pm 10.5 \mu\text{m}$ and $8.9 \pm 6.8 \mu\text{m}$, respectively.

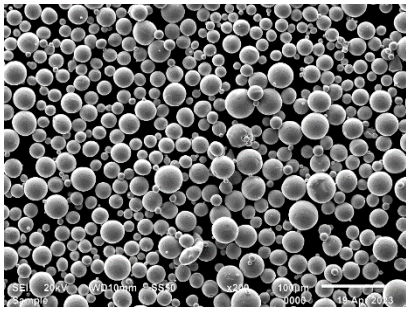
(i)



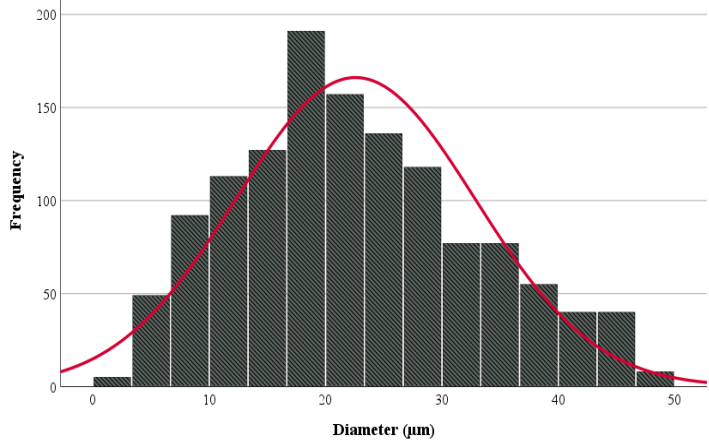
(ii)



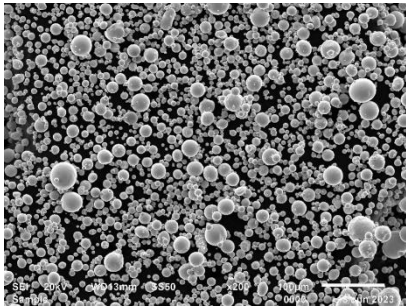
(iii)



(iv)



(v)



(vi)

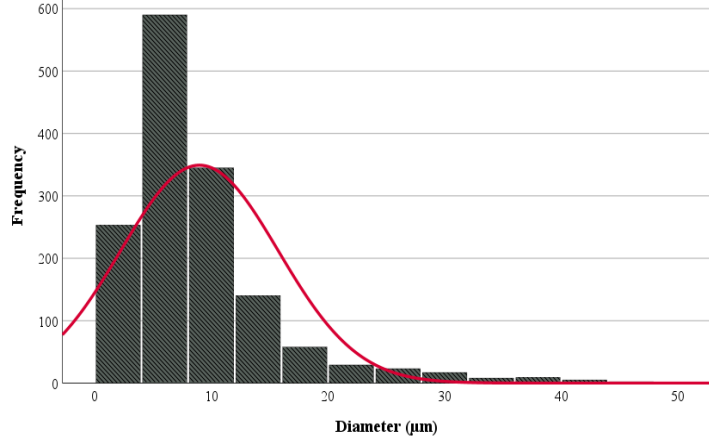


Figure 3.12: SEM micrographs (scale = 100 μm) of the employed Ti6Al4V ELI, cpTi and Cu powder illustrated by (i), (iii) and (v), respectively, and particle size distribution curves of the employed Ti6Al4V ELI , cpTi and Cu illustrated by (ii), (iv) and (vi), respectively

3.3.1.1. In situ alloys

In situ mixing and alloying during the LPBF to manufacture Ti-Cu alloys combines both the biocompatibility of Ti material and antibacterial properties of Cu at the bone-implant interface (Zhang, et al., 2013; Vilardell, et al., 2020). Titanium implant material alloyed with Cu may stimulate biological characteristics required for cell proliferation and promote differentiation of stem cells in the field of tissue engineering, since Cu is an essential element (Shen, et al., 2022). Song et al. (2014) found that Ti alloyed with up to 10 wt.% Cu substantially increased cell viability, followed by a gradual decrease as the concentration of the alloying element increased further. Although a higher percentage of Cu may guarantee bactericidal effect, Ti-Cu alloys with lower amounts of Cu may not adversely affect the mechanical and corrosion performances of Ti and its alloys (Vilardell, et al., 2020). The powders were analysed using the SEM equipped with EDS, and the images thereof are shown in Figure 3.13. As with the elemental and pre-alloyed powders, the particles of the in situ alloys were spherical and smooth. The micrographs show an even distribution of Cu particles (in green) in the powder mixtures.

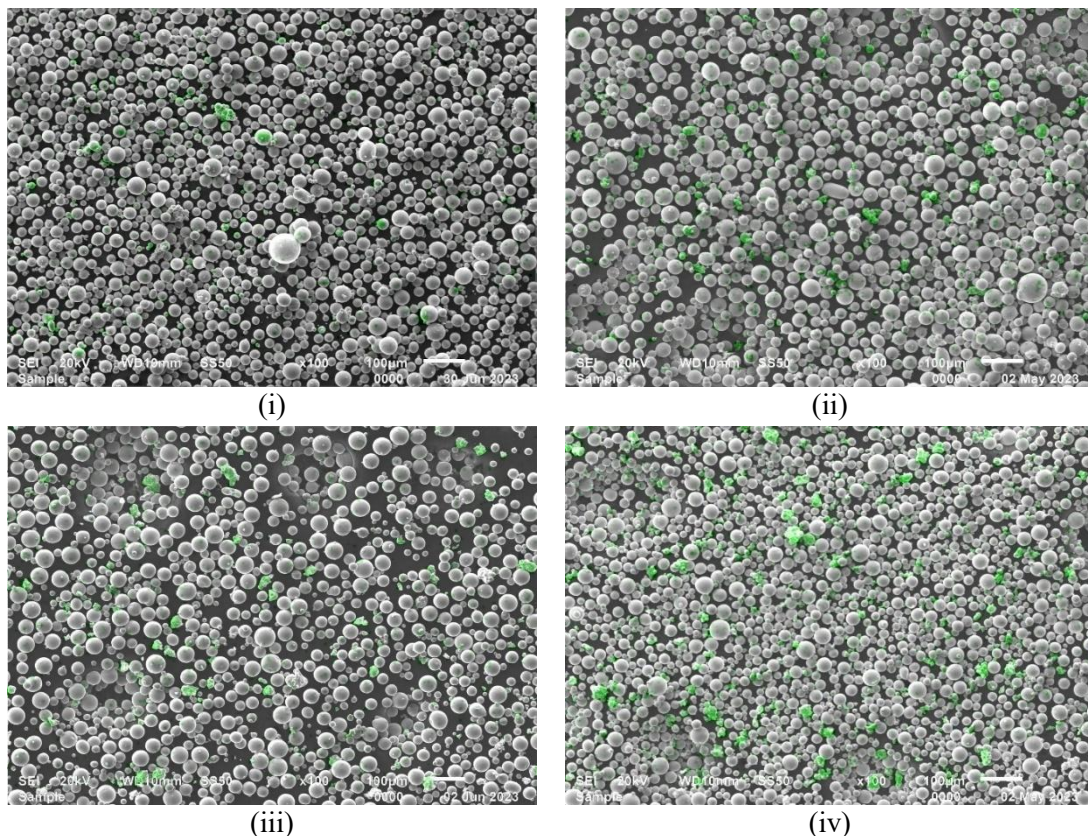


Figure 3.13: SEM micrographs (scale = 100 μm) showing spherical morphology of in situ alloyed (i) Ti6Al4V-3Cu, (ii) Ti6Al4V-6Cu, (iii) Ti-3Cu, and (iv) Ti-6Cu powders (Cu particles are indicated in green)

3.3.2. LPBF single tracks

The optimisation of LPBF process parameters by fabricating single tracks from metal powders has been the focus of several publications (Yadroitsev, et al., 2015; Kinnear, et al., 2016; Dzogbewu & Du Preez, 2022; Ramosena, et al., 2022). The initiating step in the algorithm for finding optimal LPBF process parameters is the fabrication of high-quality, stable single tracks. For each powder material, there are sets of process parameters that yield stable or unstable tracks. A stable track is distinguished by continuous geometrical characteristics without irregularities and with adequate penetration depth for providing a strong metallurgical bond between the track and the substrate (Dzogbewu, et al., 2017). Discontinuities in tracks produced by LPBF are characterised by the balling effect, irregular geometrical characteristics from satellites, insufficient or absent substrate penetration, and keyhole mode (Figure 3.14) (Dzogbewu, 2021).

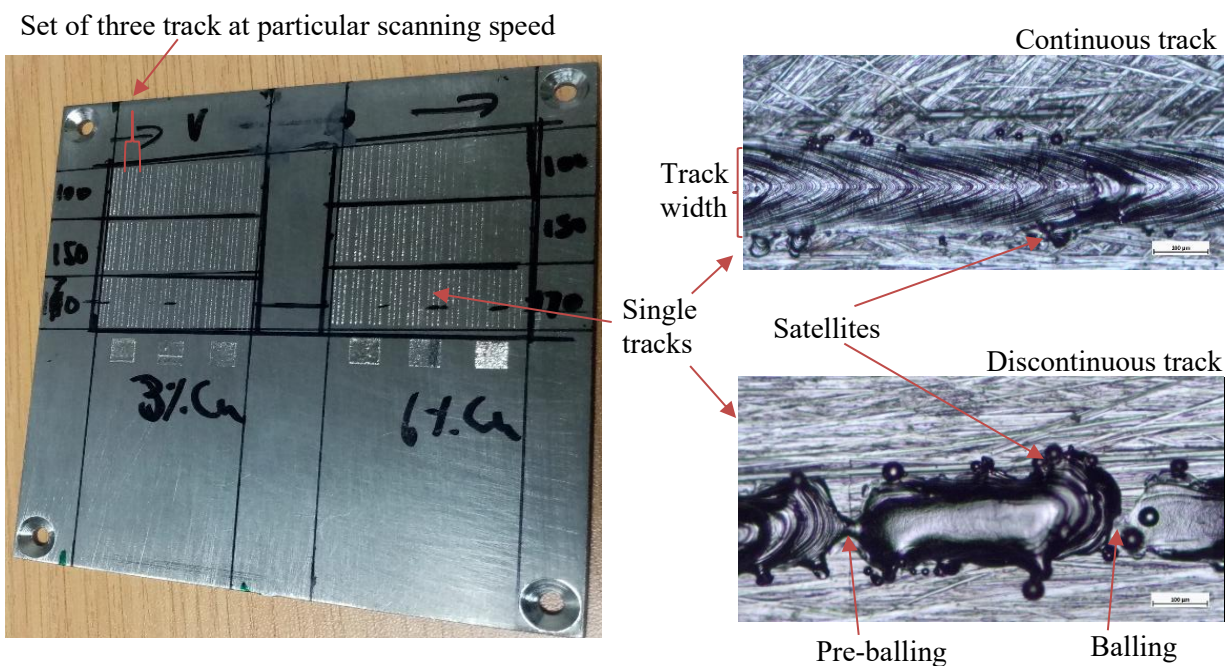
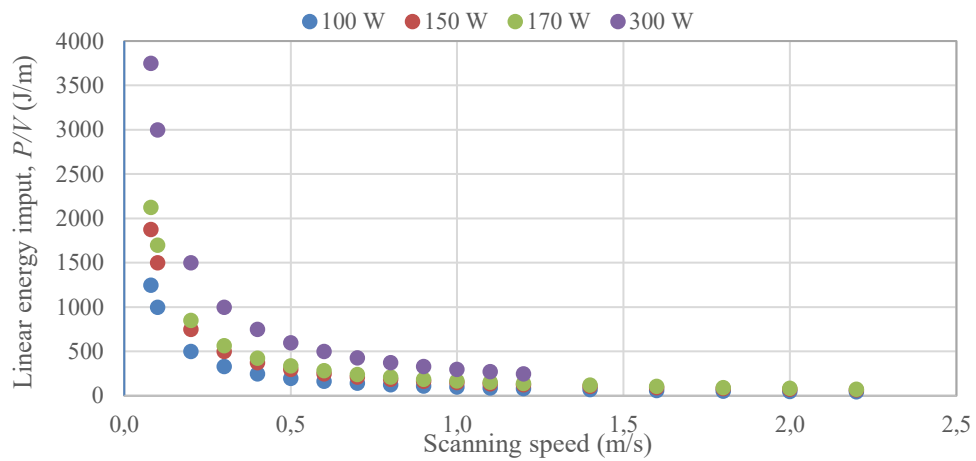


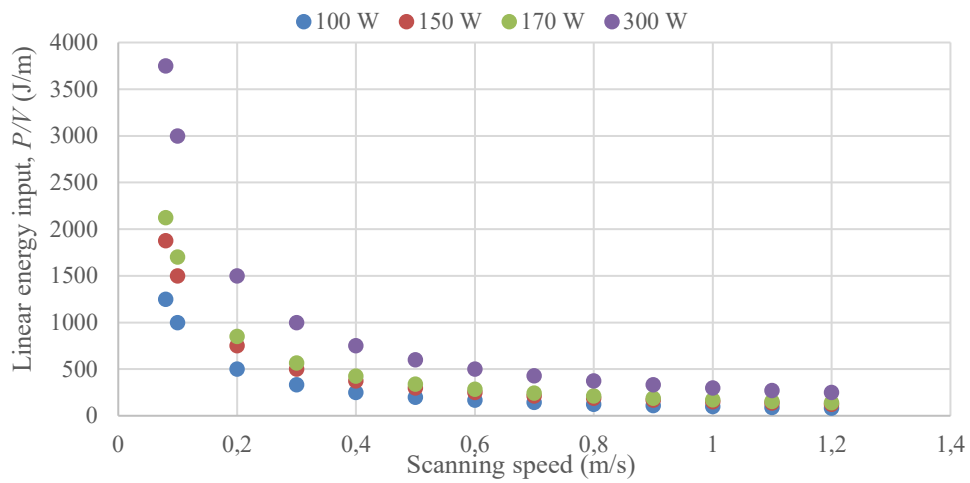
Figure 3.14: Top view of a substrate illustrating single tracks with continuity and typical metallurgical defects

To form tracks that have a metallurgical bond with the substrate, the hierarchy of the process parameters, in order of importance, is as follows: laser power density, thickness of the deposited powder layer, scanning speed and powder particle size (Yadroitsev, et al., 2012; Krakhmalev, et al., 2017). Statistical factor analysis of laser process parameters shows that geometric characteristics of a track, such as width and penetration depth, are determined mainly

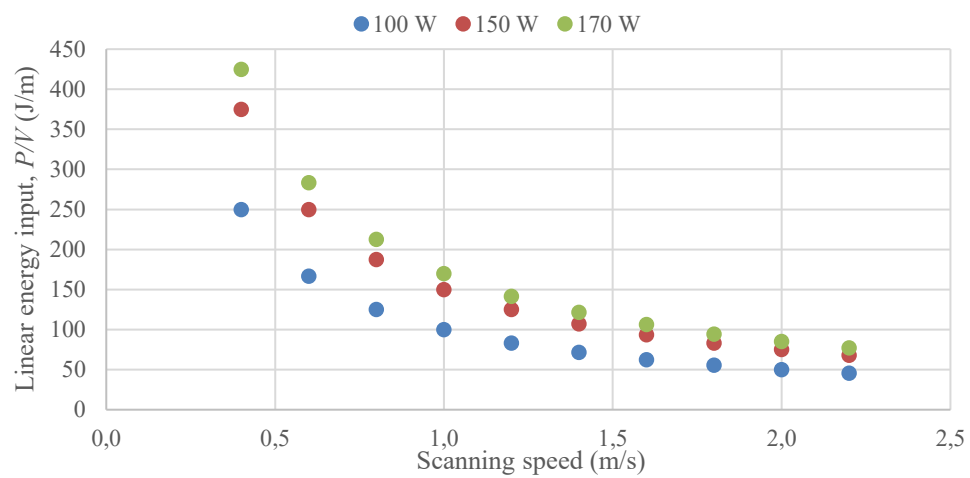
by the laser power density (Yadroitsev, et al., 2012). The powder layer thickness determines the height of the track. Scanning speed is the most flexible parameter and simplest to adjust during LPBF. Hence, by providing adequate layer thickness for the employed powder particle size distribution, fixing the laser power density and optimising the scanning speed, the stability of the tracks is achievable (Yadroitsev, et al., 2015). It is necessary to find a process parameter window that ensures the production of high-quality, regular tracks (Dzogbewu, 2021). Figure 3.15 indicates laser powers (P), scanning speeds (V) and the linear energy density as the ratio of laser power and scan speed (P/V).



(i) Ti6Al4V-3Cu and Ti6Al4V-6Cu



(ii) cpTi



(iii) Ti-3Cu and Ti-6Cu

Figure 3.15: Scanning speed and the corresponding linear energy density

3.3.2.1. Top view analysis of single tracks

The morphology of a single track is determined by the energy input and the interaction time of the laser beam with the powder/substrate material (Vilardell, et al., 2020). The surface tension causes the molten material to form a cylindrical track (Yadroitsev, et al., 2010; Yadroitsev, et al., 2015). Various aspects may be associated with the mechanisms of distortion, irregularities and balling effect. These aspects include inhomogeneity in powder layer thickness and the material's granulomorphometric characteristics, thermo-physical properties, and melt hydrodynamics, as well as the energy input parameters (that is, laser power, spot size and scanning speed) (Yadroitsev, et al., 2015; Krakhmalev, et al., 2017).

For the present investigations, it was observed that the molten pool plunges into the powder bed and forms droplets at the edge of the tracks (Figure 3.14). During laser melting, the molten liquid accumulates in a bow wave ahead of the laser spot as the laser beam scans the powder layer. The widening molten liquid may spill over onto the powder particles ahead of the laser beam and pinched off onto the powder bed as spatter particles (Matilainen, et al., 2015). Yadroitsev et al. (2010, 2013a) also observed spatter particles of the stainless steel molten pool and termed them satellites. Satellites are surface defects that form at the edge of a sintering powder due to melt spattering and partial powder melting in the peripheral zone of the laser spot (Dzogbewu, 2017). These satellite particles stick to the surface of the scanned track and occur when the energy input is excessive due to high laser power or low scanning speed (Yadroitsev, et al., 2013a). Ramosena et al. (2022) blended cpTi and Al-V master-alloy (MA) powders to in situ alloy Ti6Al4V for optimisation using the EOSINT M 280 system. The authors observed satellites forming at the edges of single tracks, leading to their formation on the surfaces of single and double layers. Vilardell et al. (2020) observed many satellites at the periphery of single tracks of Ti6Al4V(ELI)-3 at.% Cu that were LPBF-fabricated at laser powers of 170 and 340 W and scanning speeds between 0.4–2.8 m/s on a 50 µm thick powder layer. The formation of satellites was attributed to the high thermal conductivity and lower melting point of Cu particles in the powder mixture. This high conductivity caused the heat from the sintered track to redistribute into the surrounding powder particles, and they partially melted and stuck to the solidified track (Vilardell, et al., 2020).

Periodic fragmentation has been documented in laser-based manufacturing processes as early as 1968 (Zhang, et al., 2022). Fragmentation into bead chains of the re-melted tracks is a

common LPBF shortcoming, indicating discontinuity of the single tracks and is referred to as the balling effect (Yadroitsev, et al., 2010). During the layer-by-layer process, the balling effect results in discontinuous scan tracks and large inter-track pores as layers are processed. This results in a severe impediment to a uniform deposition of the next powder on the previously processed layer and tends to produce porosity induced by poor interlayer bonding (Gu, 2015). The balling effect was observed by Vilardell et al. (2020) at 170 W laser power and high scanning speeds. Gu (2015) reported that the occurrence of balling decreased the linear laser energy density, thereby lowering the densification rate of SLM-processed TiC/Ti nanocomposite parts. The author postulated that interlayer pores may also result from the balling effect.

The use of the Plateau-Rayleigh instability of a liquid jet to explain the balling (or humping) phenomenon has been proposed in literature (Rayleigh, 1892; Zhang, et al., 2022). It states that the instability of a free liquid cylinder initiates when the length-to-width ratio of the molten pool exceeds π (Lee & Zhang, 2015). The Plateau-Rayleigh effect explains the underlying physics of the balling effect and is used to determine the breaking time of cylindrical molten metals above their liquidus temperature (Rayleigh, 1892). Hence, as the laser scanning speed increases, the molten pool increases in length and decreases in width, leading to instability (Chandrasekar, 1961; Lee & Zhang, 2015). Zhang et al. (2022) employed the Plateau-Rayleigh theory in laser-based powder bed fusion of metals to show the initial instability of the molten track and its growth rate. Viale et al. (2022) stated that Plateau-Rayleigh instability may occur during the solidification of the molten track and cause the balling effect, resulting in very high surface roughness in the as-built part. To minimise the impact of the Plateau-Rayleigh instability and improve the homogeneity of the powder, Viale et al. (2022) recommended using a thinner layer thickness and a slower scanning speed at a constant volumetric energy density. Other factors that may be responsible for the balling phenomenon in LPBF include the local powder particle arrangement, underlying substrate, wettability of the powder, gravity, capillary forces, low laser beam power, non-Gaussian laser beams and Marangoni convection (Körner, et al., 2011; Lee & Zhang, 2015; Dzogbewu, 2017; Zhang, et al., 2022).

The Gaussian distribution of the laser beam intensity and scanning motion during processing results in a non-uniform temperature distribution within the melt pool, which prompts consideration of convection, keyhole formation and evaporation in the melting pool (Mirkoohi, et al., 2018; Viale, et al., 2022). As a result, these temperature-surface tension gradients lead

to the formation of a flow in the melt pool, known as “Marangoni convection” (Lee, et al., 1998). The Marangoni convection of fluids, also known as thermo-capillary convection, is associated with surface tension gradient whereby fluids flow from regions of low surface tension to regions of high surface tension (Cox, et al., 2023; Abd-Elaziem, et al., 2024). The Marangoni number (Ma) is defined as (Dzogbewu, 2017):

$$Ma = \frac{d\gamma_{LV}}{dT} \frac{dT}{dr} \frac{L}{2\eta\alpha} \quad \text{Equation 3.1}$$

where $\left(\frac{d\gamma_{LV}}{dT}\right)$ is the surface tension gradient, $\frac{dT}{dr}$ is the temperature gradient, L is the length of the melt pool, η is the viscosity, and α is the thermal diffusivity.

While the gradient in surface tension directs the flow, the subsequent Marangoni stress of thermocapillary-driven flow influences the shape of the molten pool (Cox, et al., 2023). Generally, the temperature at the centre of the melting pool is higher due to the laser intensity distribution. Thus, it is important to focus on the surface tension properties of the materials. Should the surface tension be higher in the centre (that is, the surface tension gradient is positive), the Marangoni flow tends to form a deep, narrow melt pool with chemical homogeneity. Conversely, a negative gradient exists for a surface tension that is higher at the melt pool boundaries, and the Marangoni flow forms a wider and shallower pool (Aggoune, et al., 2021; Viale, et al., 2022). The Marangoni convection may be useful as a turbulence effect to break the oxides on the surface of reactive materials such as aluminium and titanium alloys. The keyhole effect is, however, directly related to the evaporation of alloying elements, particularly in a deep melt pool where the gas is entrapped in the depths of the molten pool and creates a vapour cavity defect (Viale, et al., 2022). The majority of pure metals and alloys have been reported to exhibit a negative surface tension gradient (Keene, 1993) and Ti and its alloys are accordingly expected to form shallow, wide molten pools of cylindrical liquids with high surface energy. Disintegration of the thermocapillary-driven flow cylinder is required to reduce the free surface energy of the molten metal (Dzogbewu, 2017).

The top-view micrographs of the single tracks are illustrated in FiguresFigure 3.16–Figure 3.20. The Ti6Al4V samples seemingly produce more spatter particles than the cpTi samples. The spatter particles of Ti6Al4V-3Cu and Ti6Al4V-6Cu were similar, while those of cpTi samples decreased with an increase in Cu content, with those of Ti-6Cu being the least. Uniform tracks were distinguished in terms of their consistent width and continuity in length.

The continuity of the initial track is due to the optimal combination of powder layer thickness, laser power and scanning speed (Yadroitsev, 2009).

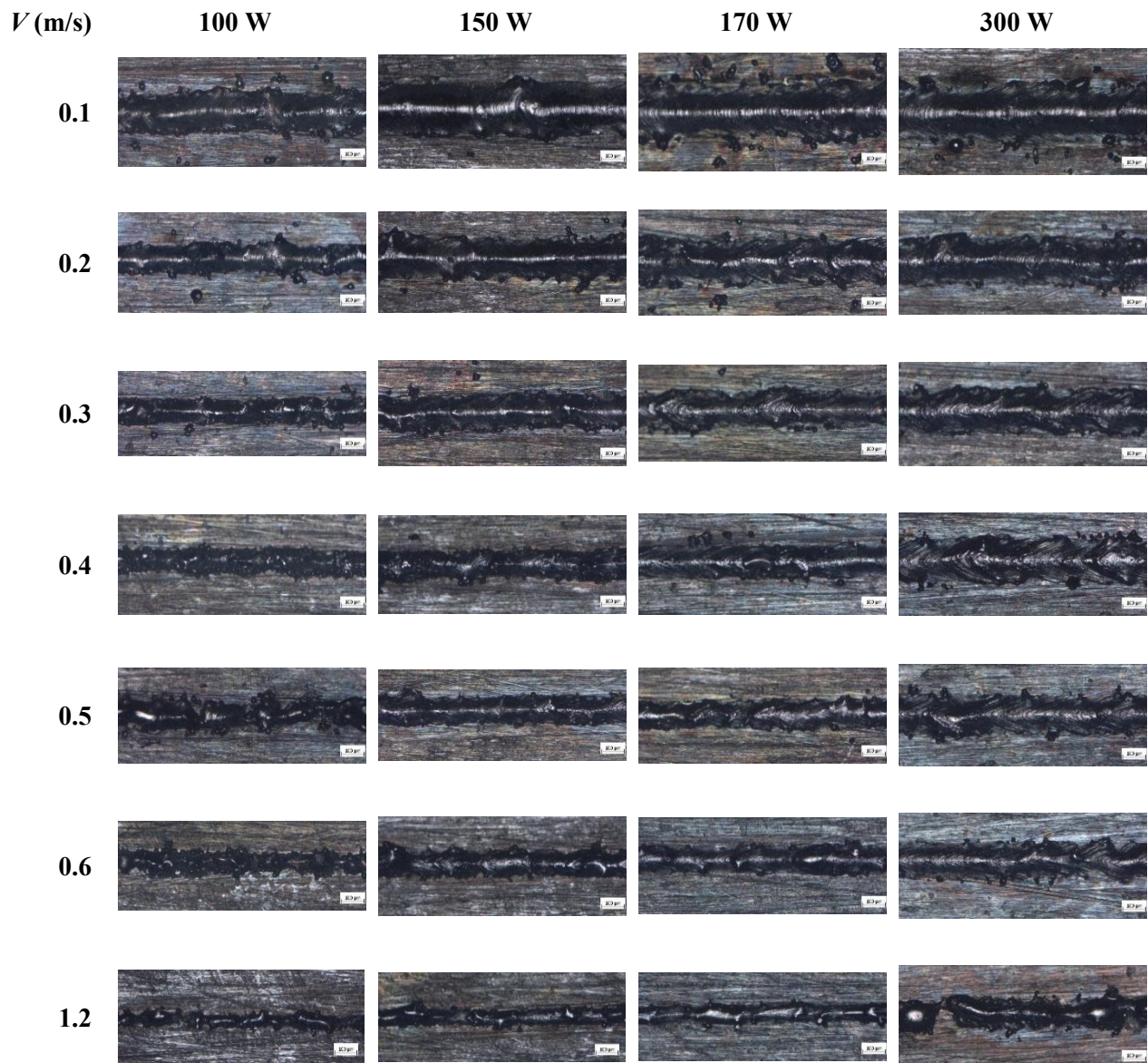


Figure 3.16: Ti6Al4V-3Cu single tracks at various laser powers and scanning speeds (scale = 100 μm)

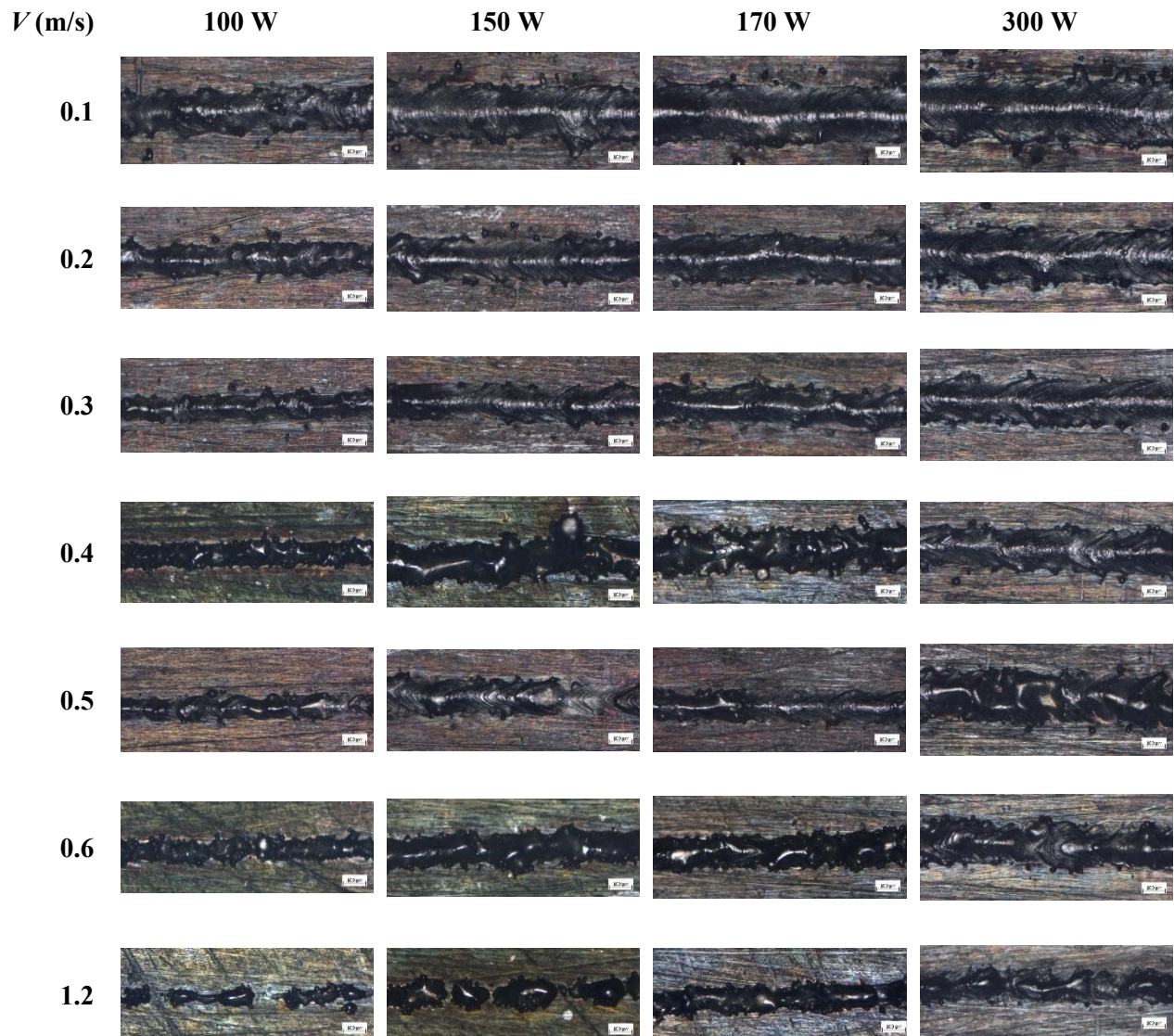


Figure 3.17: Ti6Al4V-6Cu single tracks at various laser powers and scanning speeds (scale = 100 μm)

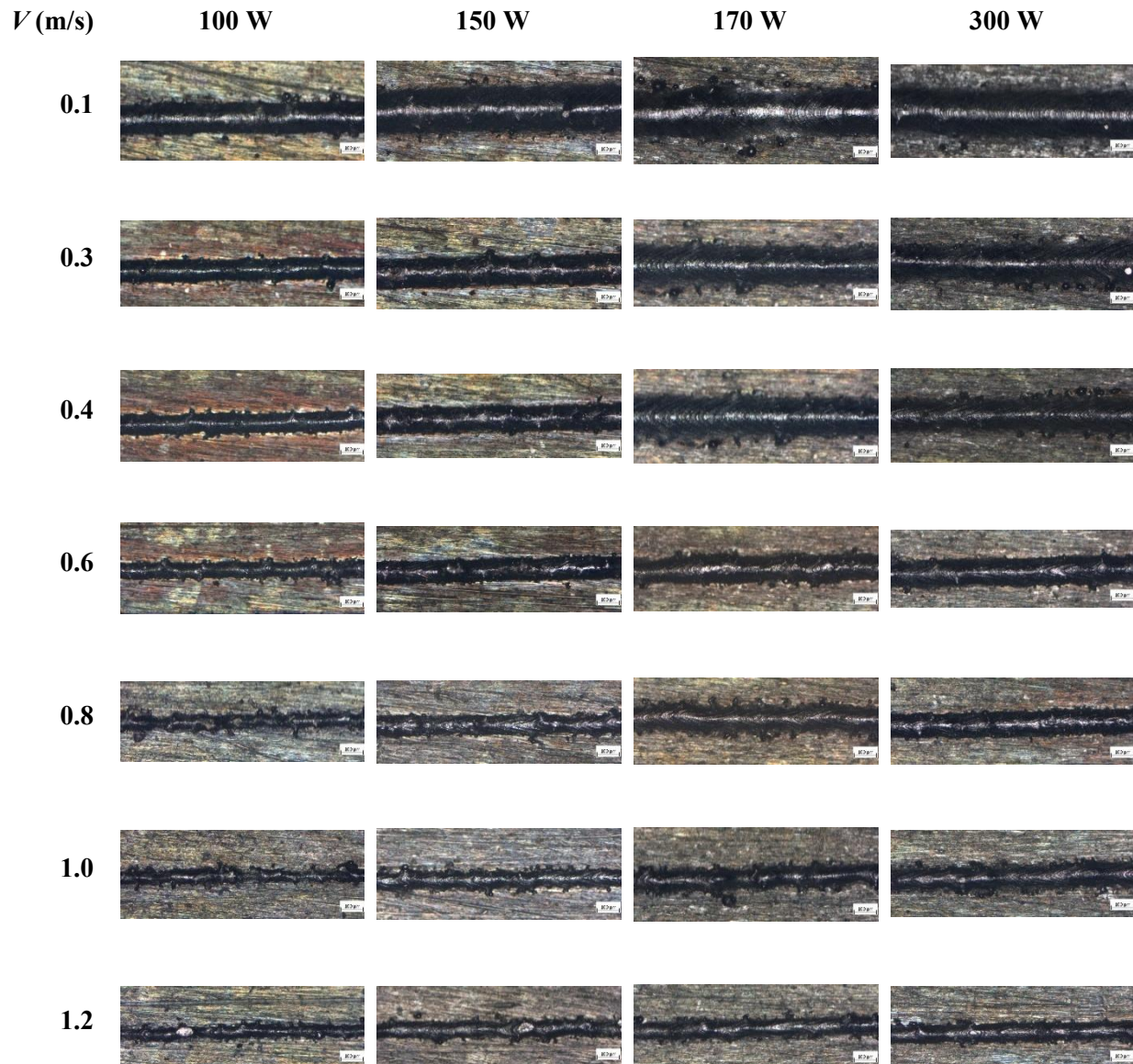


Figure 3.18: cpTi single tracks at various laser powers and scanning speeds (scale = 100 μm)

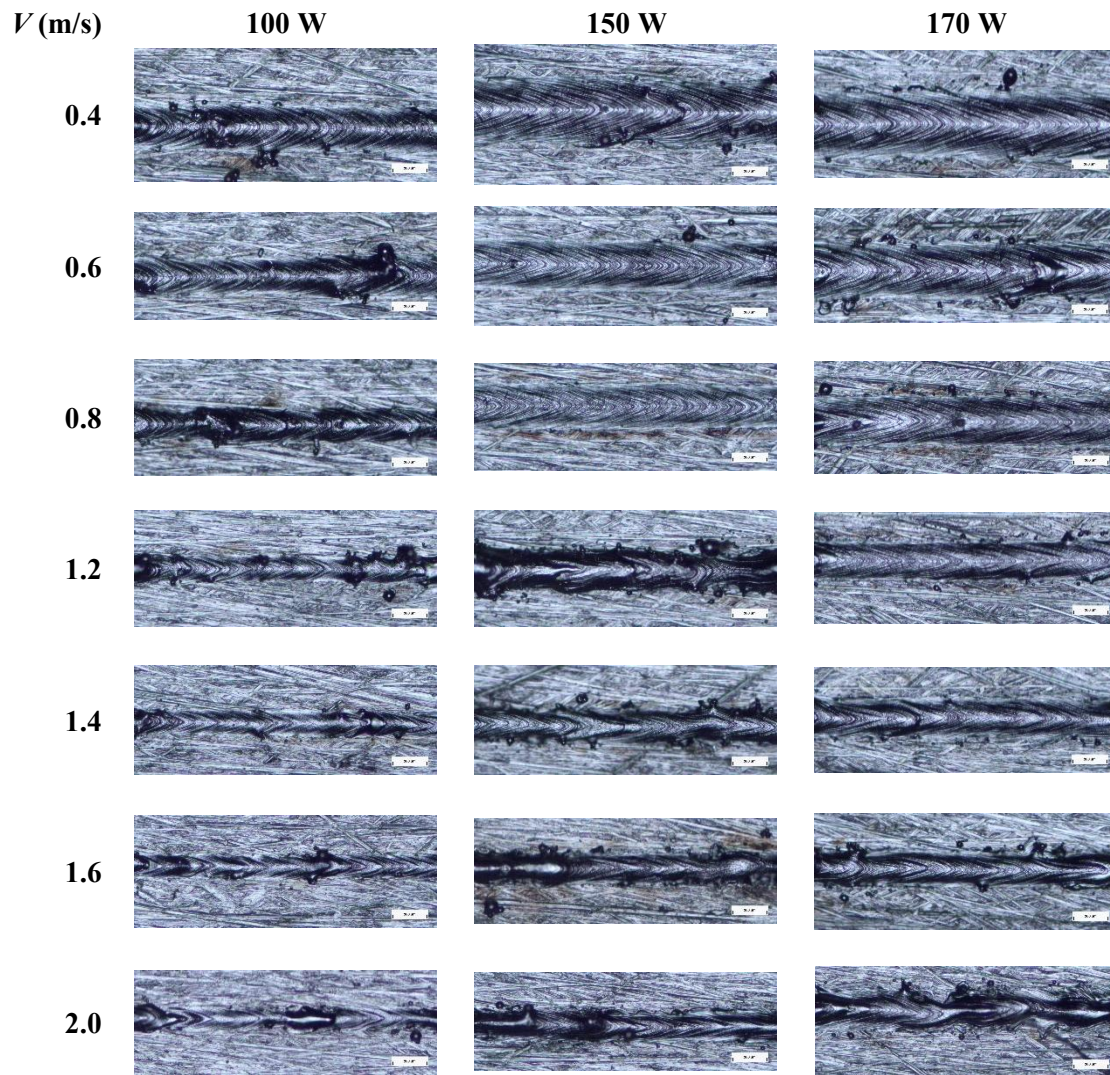


Figure 3.19: Ti-3Cu single tracks at various laser powers and scanning speeds (scale = 100 μm)

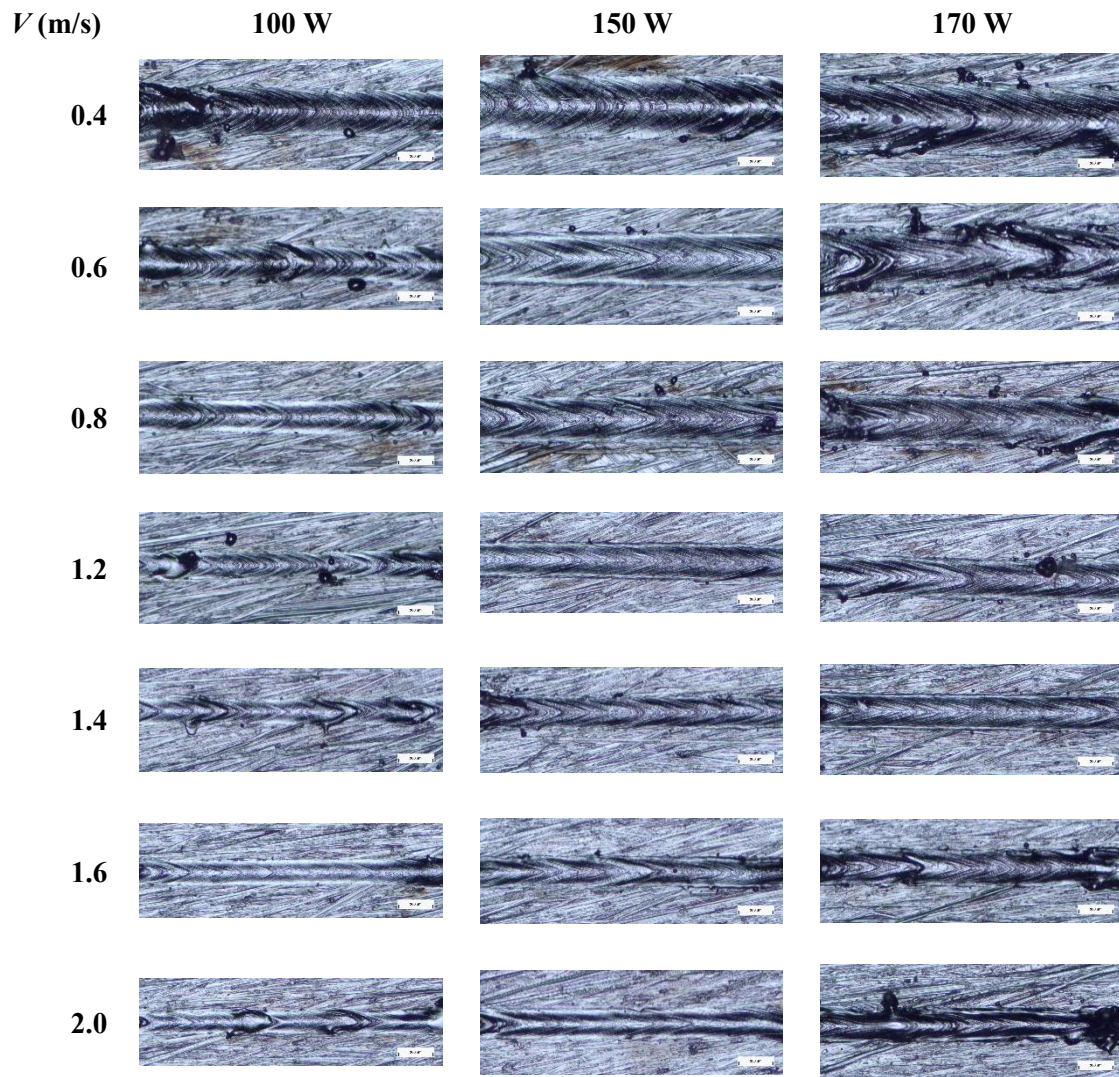
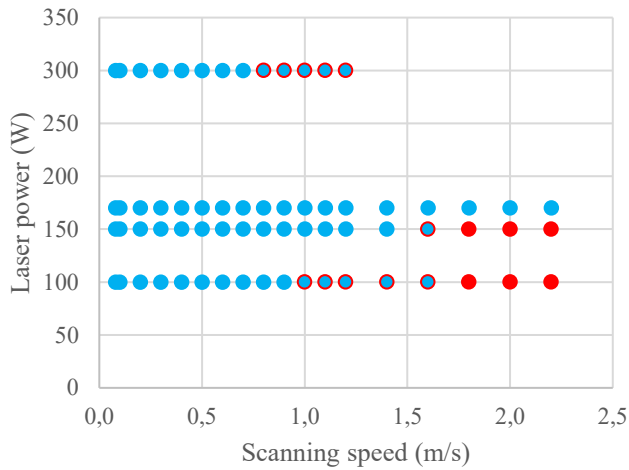
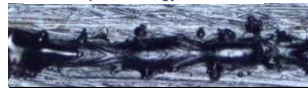


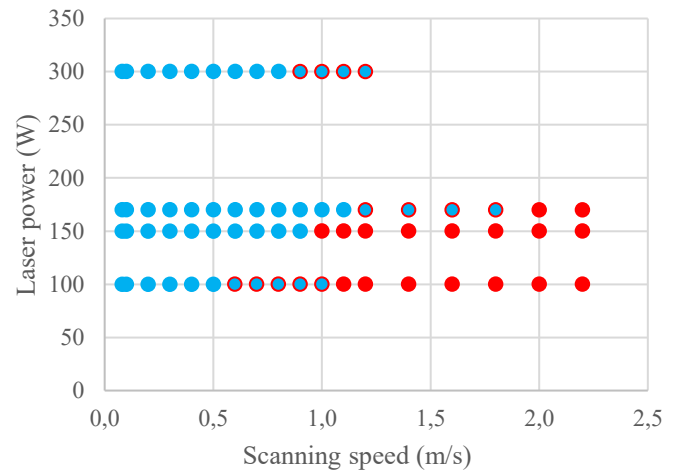
Figure 3.20: Ti-6Cu single tracks at various laser powers and scanning speeds (scale = 100 μm)

Balling started at a higher scanning speed for the various laser powers, with the alloys of Ti6Al4V experiencing a greater balling effect than those of cpTi, which may be traced on the process maps for continuous and/or discontinuous single tracks illustrated in Figure 3.21. Similarly, Yadroitsev et al. (2015) observed a balling effect at high laser power density and high scanning speed, and the findings indicated a balling effect starting at scanning speeds above 1.4 m/s for a 45 μm powder layer. Vilardell et al. (2020) top-view investigation revealed that stable single track occurred at scanning speeds below 1.4 m/s and 1.6 m/s with laser powers of 170 W and 340 W, respectively. The authors also observed humping and balling at higher laser scanning speeds, but the tracks remained continuous with ~ 50 μm penetration into the substrate at 340 W and up to 2.8 m/s. The initially selected scanning speeds and corresponding laser powers for this study provided sufficient energy for continuous track formation across all powder combinations within established processing windows. It was noted that a gradual

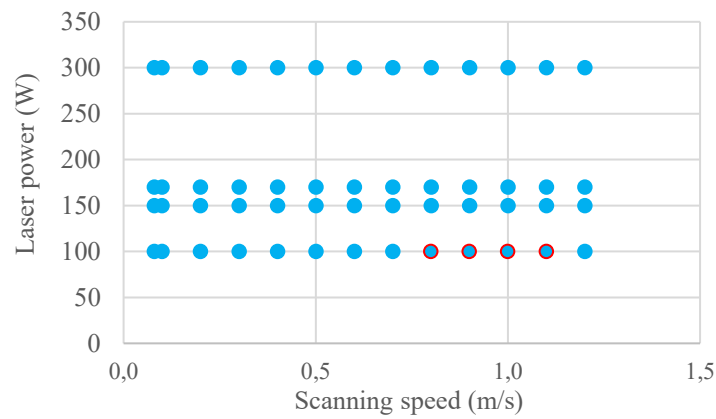
increase in the scanning speed has a considerable effect on the capillarity instability of the molten laser cylindrical liquid tracks (Simchi & Asgharzadeh, 2004; Yadroitsev, et al., 2007).



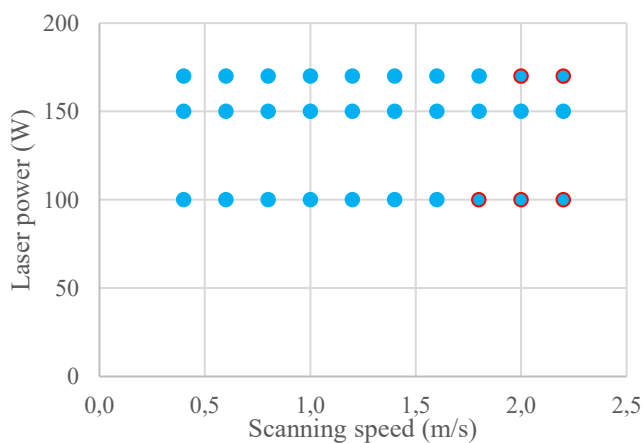
(i) Ti6Al4V-3Cu ($P = 100, 150, 170$ and 300 W, $V = 0.08$ – 2.2 m/s)



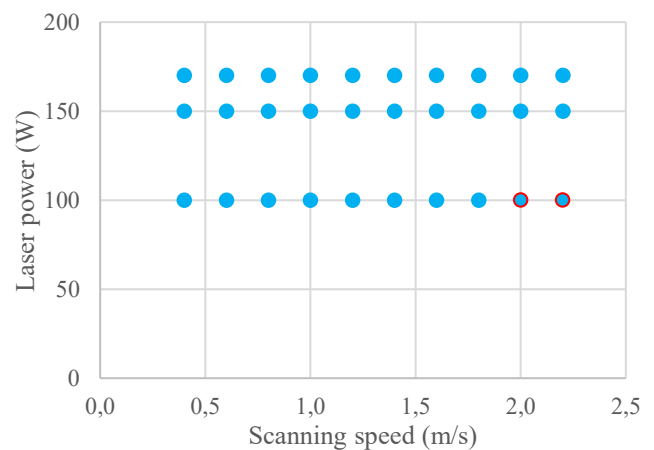
(ii) Ti6Al4V-6Cu ($P = 100, 150, 170$ and 300 W, $V = 0.08$ – 2.2 m/s)



(iii) cpTi ($P = 100, 150$ and 170 W, $V = 0.4$ – 2.2 m/s)



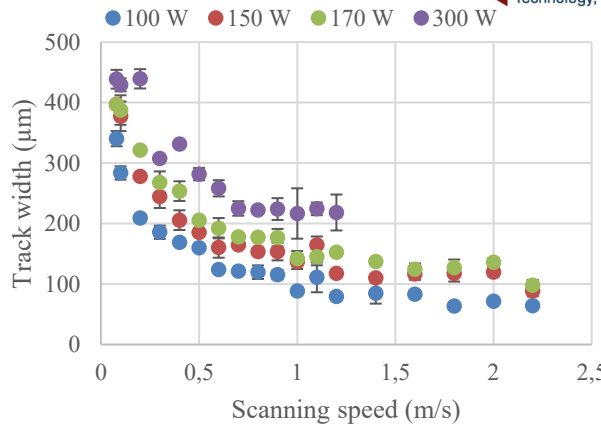
(iv) Ti-3Cu ($P = 100, 150$ and 170 W, $V = 0.4$ – 2.2 m/s)



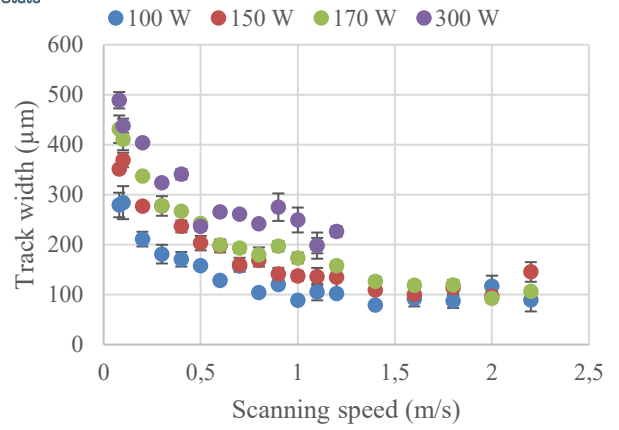
(v) Ti-6Cu ($P = 100, 150$ and 170 W, $V = 0.4$ – 2.2 m/s)

Figure 3.21: Process maps of sintered single tracks: top view

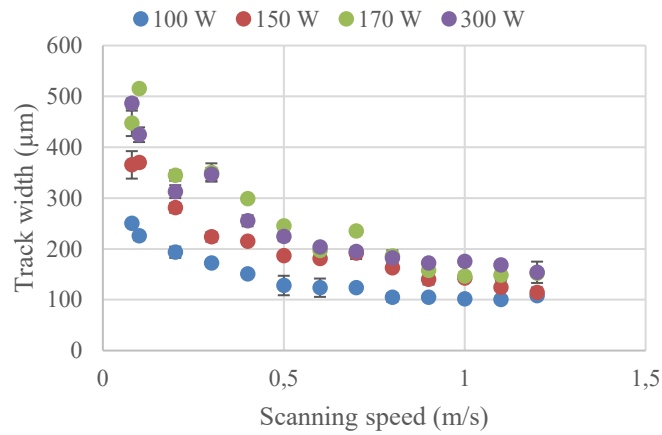
Yadroitsev et al. (2015) investigated the influence of laser scanning speed on single track quality of Ti6Al4V, showing that the track width decreased from 150 μm to 125 μm as the scanning speed decreased from 0.16 m/s to 0.08 m/s at a laser beam power and a powder layer thickness of 50 W and 50 μm . Conversely, during the current experiment, for cpTi and all in situ alloyed powders, a general reduction in track width at the same laser power was observed as the scanning speed increased, as illustrated in Figure 3.22. The track width values were higher at the 170 W and 300 W laser powers and lowest at 100 W across the various speed points. Likewise, Yadroitsev et al. (2010) studied the effect of power input on track formation and found that track width decreased with decreasing power. For laser powers of 50 W (0.06–0.24 m/s scanning speed range) and 25 W (0.03–0.12 m/s scanning speed range), the width of the fusion line varied from 125 μm to 85 μm and from 80 μm to 60 μm , respectively. Bidare et al. (2018) reported that other authors had made similar observations. This information is crucial for determining hatch distance to ensure accurate overlapping between the tracks.



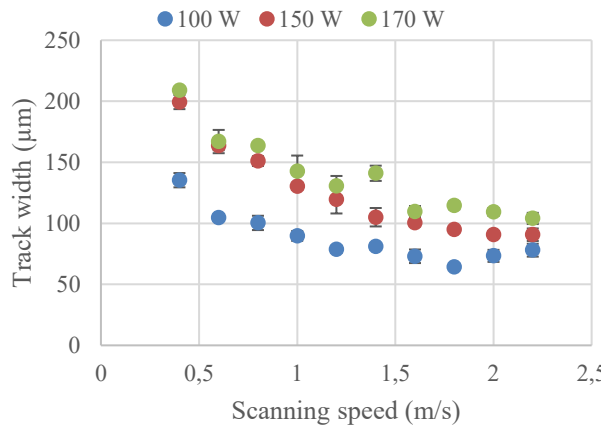
(i) Ti6Al4V-3Cu



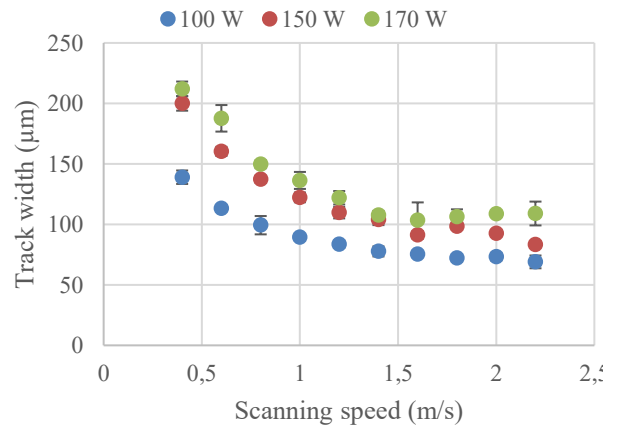
(ii) Ti6Al4V-6Cu



(iii) cpTi



(iv) Ti-3Cu



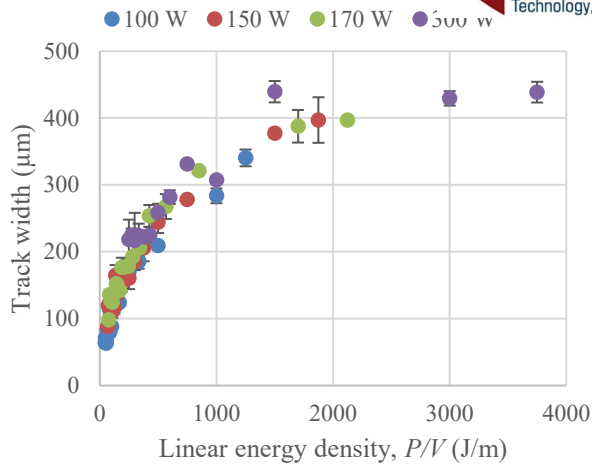
(v) Ti-6Cu

Figure 3.22: Width of single tracks at different laser powers and scanning speeds

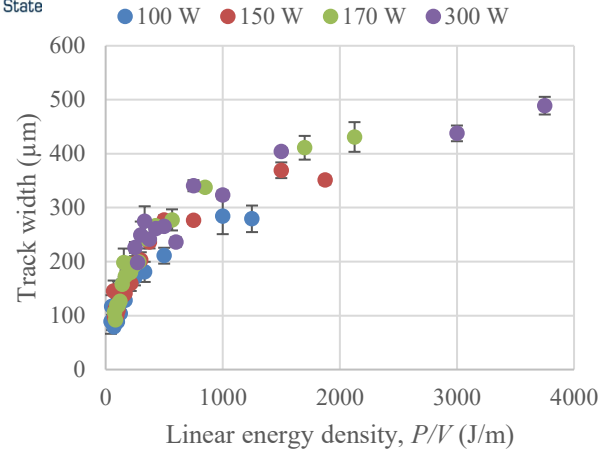
Vilardell et al. (2020) studied single tracks of Ti6Al4V(ELI)-3 at.% Cu manufactured using an EOSINT M 280 system and found that an increase in laser power from 170 W to 340 W led to an increase in single track width of approximately 25–50 μm wider with the same scanning

speed. The scanning speed range was between 0.4–2.8 m/s on a 50 μm thick powder layer., Using a similar manufacturing system during this study and increasing the laser power from 100 W up to 170 W (for Ti-3Cu and Ti-3Cu) and up to 300 W (for cpTi, Ti6Al4V-3Cu and Ti6Al4V-6Cu) also showed an increase in track width at the same scanning speed. A minimal difference in track width was observed with the addition of Cu. Thus, the Cu content in the current samples did not affect the conductivity of the materials since the track width for higher conductive materials is expected to be extensively greater than for low conductive materials because heat is conducted away from the neighbouring areas (Chowdhury, et al., 2022). The laser-related parameters (namely, laser power and absorption coefficient of the material) are suspected to have a greater effect on track width and maximum temperature than other parameters, such as powder size, apparent density of the powder layer, and so forth (Yadroitsev, et al., 2015). The single track width may be physically limited by flows within the molten pool, which are more prominent at higher laser power.

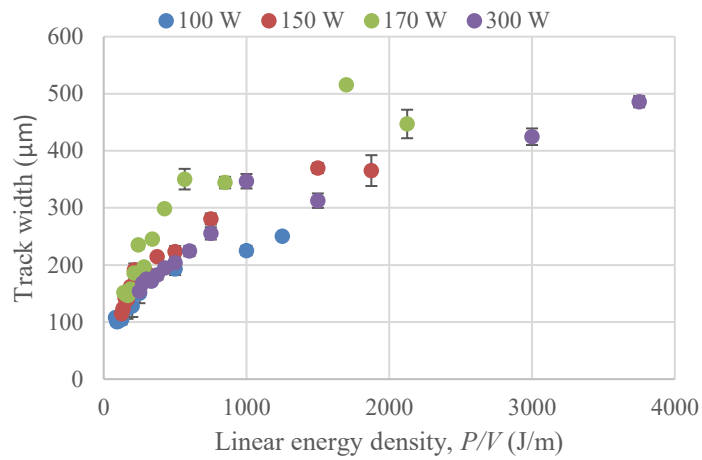
The impact of the selected process parameters on track geometry may be analysed with respect to linear laser energy input P/V , where P is the laser power and V is the scanning speed. Figure 3.23 shows that increasing the scanning speed reduced the linear energy density. Consequently, a reduction in energy density (for instance, when the laser power is low and the scanning speed is high) lowers the molten pool temperature. At this low sintering temperature, a minimal and highly viscous molten fluid forms, which leads to the formation of semi-discontinuous tracks known as pre-balls and subsequent spherical drops on the substrate, which is the balling effect (Krakhmalev, et al., 2017). On the other hand, higher input energy increases the sintering temperature and promotes the formation of a large molten pool with a low viscosity, encouraging the flow of the liquid phase. Moreover, at low scanning speeds, the conduction phenomena promote heating of the substrate and powder material; thus, creating ideal conditions for incorporating new material into the fusion process (Dzogbewu, 2017). However, several factors involving complex mechanisms of interaction between laser radiation and a powder system may complicate the continuity of the single tracks. These factors include the substrate, the flow in the molten pool, the possible variable thickness of the powder layer and non-homogeneous distribution of particles in the layer (Dzogbewu, 2017).



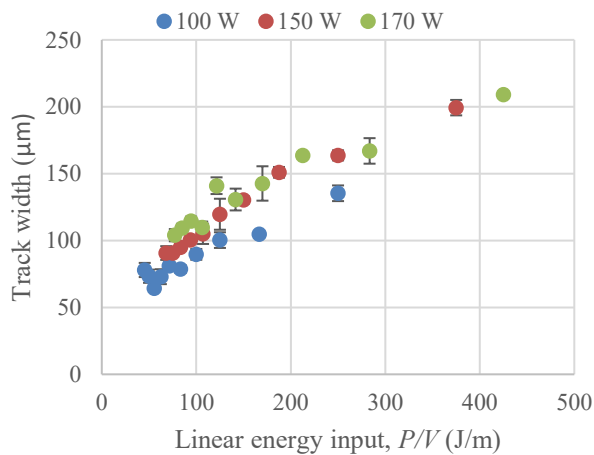
(i) Ti6Al4V-3Cu



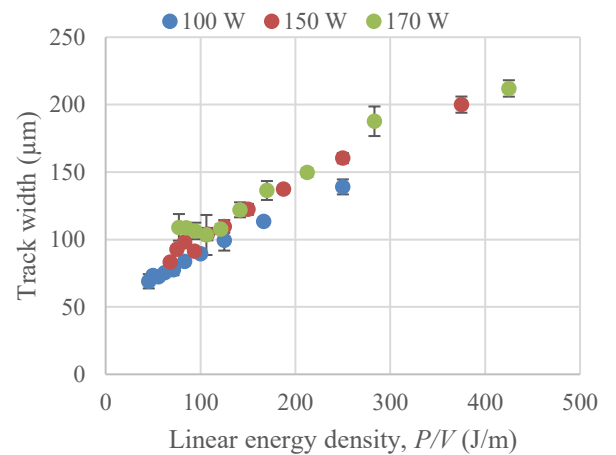
(ii) Ti6Al4V-6Cu



(iii) cpTi



(iv) Ti-3Cu



(v) Ti-6Cu

Figure 3.23: Width of the single tracks versus linear energy density

3.3.2.2. Cross-sectional analysis of single tracks

During the layer-by-layer LFBF process, if the specified process parameters provide adequate laser energy density to melt the powder and penetrate the pre-existing layer, metallurgical bonding within the substrate would occur. The molten pool formed during laser scanning of the powder layer should remain stable to generate continuous single tracks with optimal penetration depth, since it plays a significant role in the quality and mechanical properties of 3D-manufactured parts (Krakhmalev, et al., 2017; Vilardell, et al., 2020). Penetration into the substrate, or the previously sintered layer, provides additional stability for continuous track formation (Yadroitsev, et al., 2015). Re-melting of the substrate is necessary to bind the current layer to the previous layer. This re-melting of the previous layer further enhances the stability of the molten cylindrical liquid and dismantles the oxide films to ensure a clean surface between the liquid and the solid at an atomic level (Yadroitsev, et al., 2010; Cobbinah, et al., 2021). Figure 3.24 presents three possible cases – namely, lack/small penetration, optimum penetration and keyhole-like penetration into the substrate – that may be observed when analysing the cross-section of the single tracks.

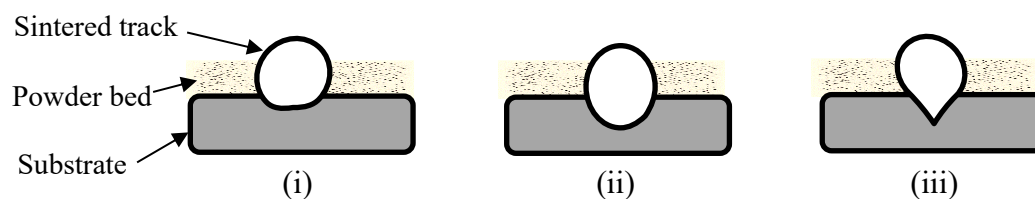


Figure 3.24: Cross-sectional morphologies of single layer tracks of (i) lack/small penetration, (ii) optimum conduction mode penetration, and (iii) keyhole mode penetration

The case of minimal or lack of penetration is characterised by the formation of a rounded free surface (circular cylinder) with a minimal contact zone with the substrate (Yadroitsev, et al., 2010). Optimum penetration is achieved by the conduction mode and is characterised by a semi-cylindrical “U-shape”, and the keyhole penetration mode is characterised by a deep, narrow “V-shape” (Makoana, et al., 2016; Yang, et al., 2016). A major difference between these two distinctive modes of laser processing lies in the laser energy density (power/spot size) applied to the processing area. In conduction mode, the applied power density does not cause substantial evaporation; thus, the energy density is just enough to melt the powder completely and form a strong metallurgical bond with the previous layer. Whereas in keyhole mode, there is excess power density to vaporise the material to such an extent that a hole is created in the melt pool (Makoana, et al., 2016). The vapour formed enhances laser energy

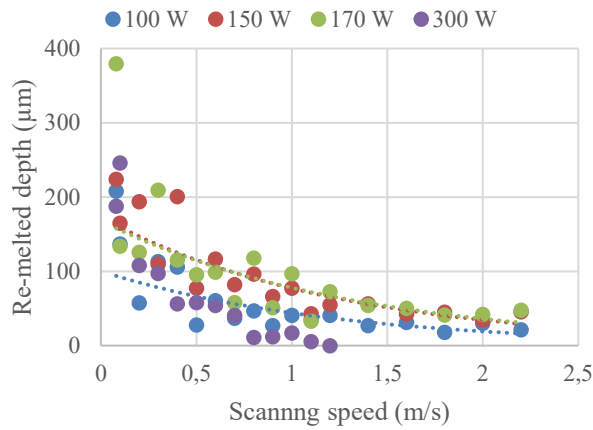
absorption into the substrate and subsequent “drilling” of the substrate to a far deeper depth than is possible in conduction mode (King, et al., 2014).

When the combination of process parameters exceeds a certain threshold during LPBF, the mode of thermal conduction in the molten pool may change from conduction mode to the keyhole mode (King, et al., 2014). Complete re-melting of the powder layer and a portion of the substrate is required for high-quality and pore-free objects to be manufactured (Krakhmalev, et al., 2017). Conversely, keyhole-like penetration is unacceptable in LPBF as it may provoke pores in the final 3D object due to the collapse of the molten pool and gas bubbles trapped in the material. Penetration deeper than the powder layer thickness is also not suitable for energy and resource efficiency (Yadroitsava, et al., 2015; Khairallah, et al., 2016).

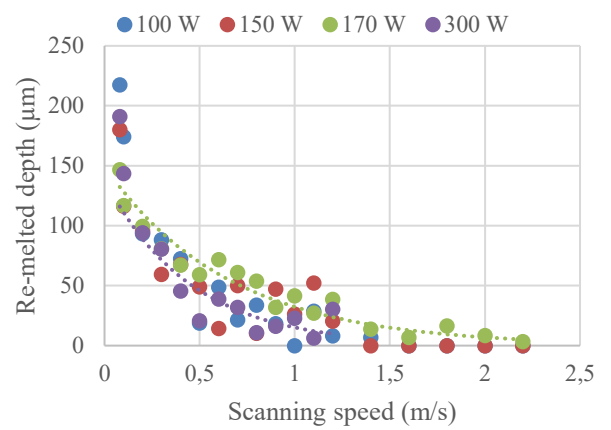
Hann et al. (2011) investigated the effect of the processing parameter on various materials in order to optimise the processing strategy and were able to demonstrate that the threshold for transition from conduction to keyhole mode depends on power, speed and beam size for a specific powder layer thickness. It has also been indicated that the layer thickness and, possibly, the powder size distribution may influence the threshold (King et al., 2014; Yang et al., 2016). King et al. (2014) cautioned that insufficient melting may result if the parameters are excessively below the threshold, and that keyhole mode melting may occur if they are excessively above the threshold. They concluded that the keyhole mode may be expected when the penetration depth is greater than half the width of the tracks.

For the current study, investigations of the individual track cross-sections perpendicular to the scanning direction were conducted to estimate the extent of the re-melted penetration depth into the substrate and the height of the tracks above the substrate. Analysis of cross-sections indicated that for the chosen layer thickness, the laser energy was sufficient to penetrate the substrate for all the powder compositions, as shown in Figure 3.25. The re-melted depth decreased as the scanning speed increased at the various laser powers. Increasing the scanning speed at constant laser power decreased the linear energy density, leading to a reduction in penetration depth (Figure 3.25). For the Ti6Al4V samples, the highest laser power of 300 W produced lower penetration depths than the lower laser powers, indicating that a high laser energy density is not necessarily required to melt the powder and penetrate the substrate. However, the values produced by the cpTi samples were in favour of the notion that high laser energy density, elevated by high laser power, results in

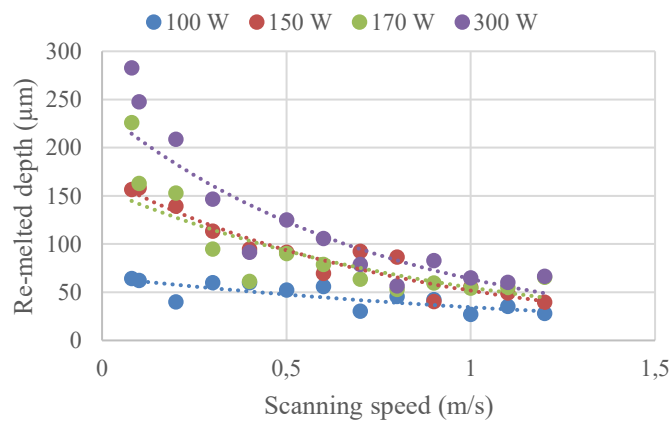
deeper penetration depths.



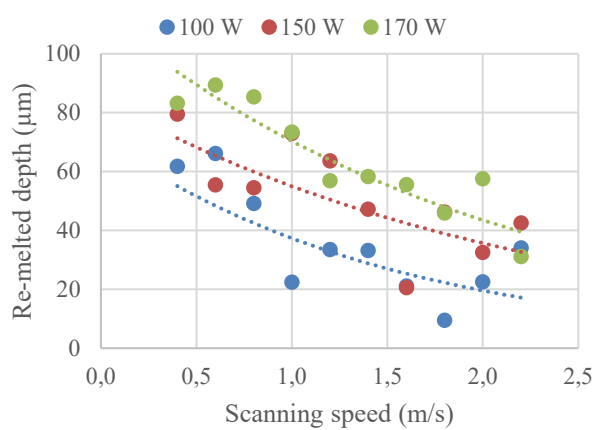
(i) Ti6Al4V-3Cu



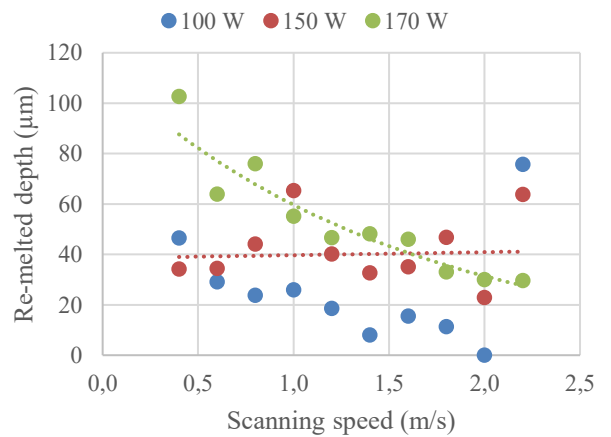
(ii) Ti6Al4V-6Cu



(iii) cpTi



(iv) Ti-3Cu



(v) Ti-6Cu

Figure 3.25: Re-melted depths of sintered tracks at varying scanning speeds and laser powers

The cross-sections of the single tracks of Ti6Al4V- x Cu and Ti- x Cu ($x = 3, 6$ wt.%) alloys and cpTi are shown in FiguresFigure 3.26–Figure 3.30. There was enough laser energy to melt the powder completely and to achieve a sufficient laser energy density to melt the substrate material, forming a metallurgical bond. Keyhole penetration was prevalent at low power and speed, particularly in Ti6Al4V-3Cu and Ti6Al4V-6Cu, where the keyhole mode occurred at 100 and 150 W at scanning speeds below 0.6 m/s. Low penetration was mainly observed at elevated power and speed. The highest scanning speed of 1.2 m/s for Ti6Al4V-3Cu and Ti6Al4V-6Cu, illustrated in FiguresFigure 3.26 andFigure 3.27, respectively, exhibited the least penetration depths. Furthermore, at the laser power of 300 W, the lowest re-melt depth was observed at the majority of the scanning speeds when compared to the laser powers of 100, 150 and 170 W, as was demonstrated in Figure 3.25(i) and (ii). High surface tension very rapidly (in about 5 μ s) reverses the direction of the molten flow vector toward the centre of the pool and traps gas bubbles within the melt, resulting in pores forming at the rear of the track (Khairallah, et al., 2016; Dzogbewu, 2017). Under the chosen process parameters, no pores were observed in the keyhole mode during the current experiments.

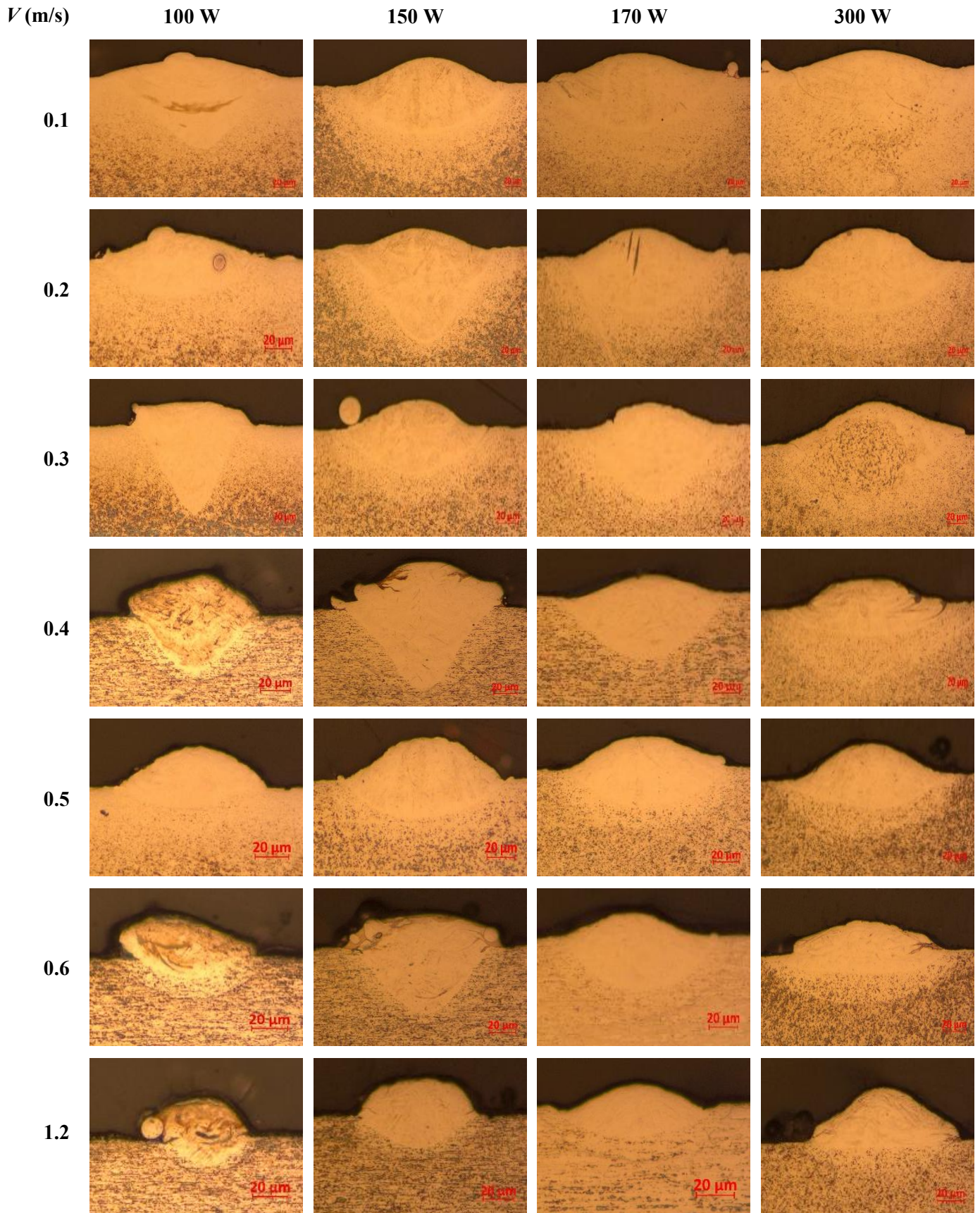


Figure 3.26: Cross-sections of Ti6Al4V-3Cu single tracks at various laser powers and scanning speeds (scale = 20 μm)

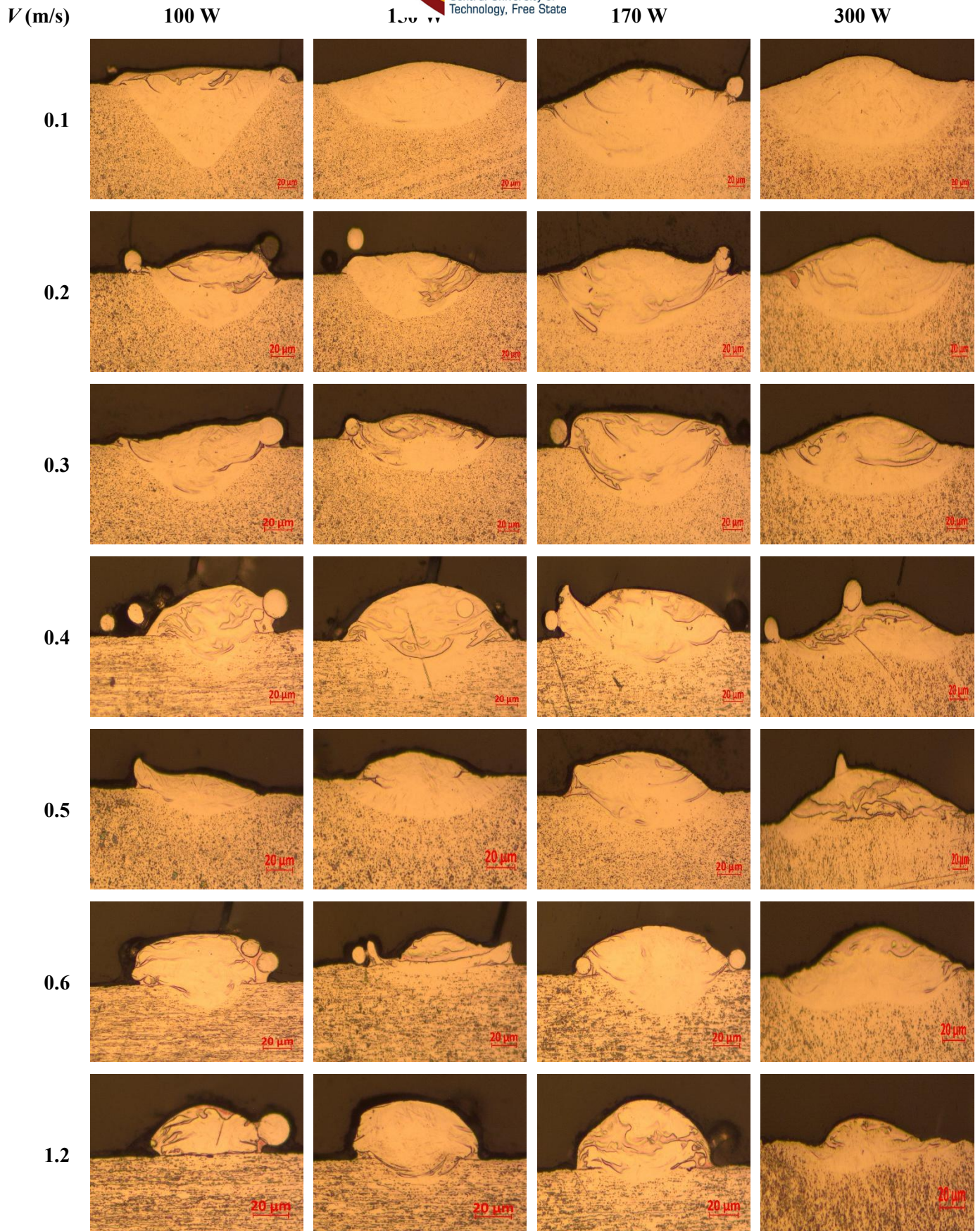


Figure 3.27: Cross-sections of Ti6Al4V-6Cu single tracks at various laser powers and scanning speeds (scale = 20 μm)

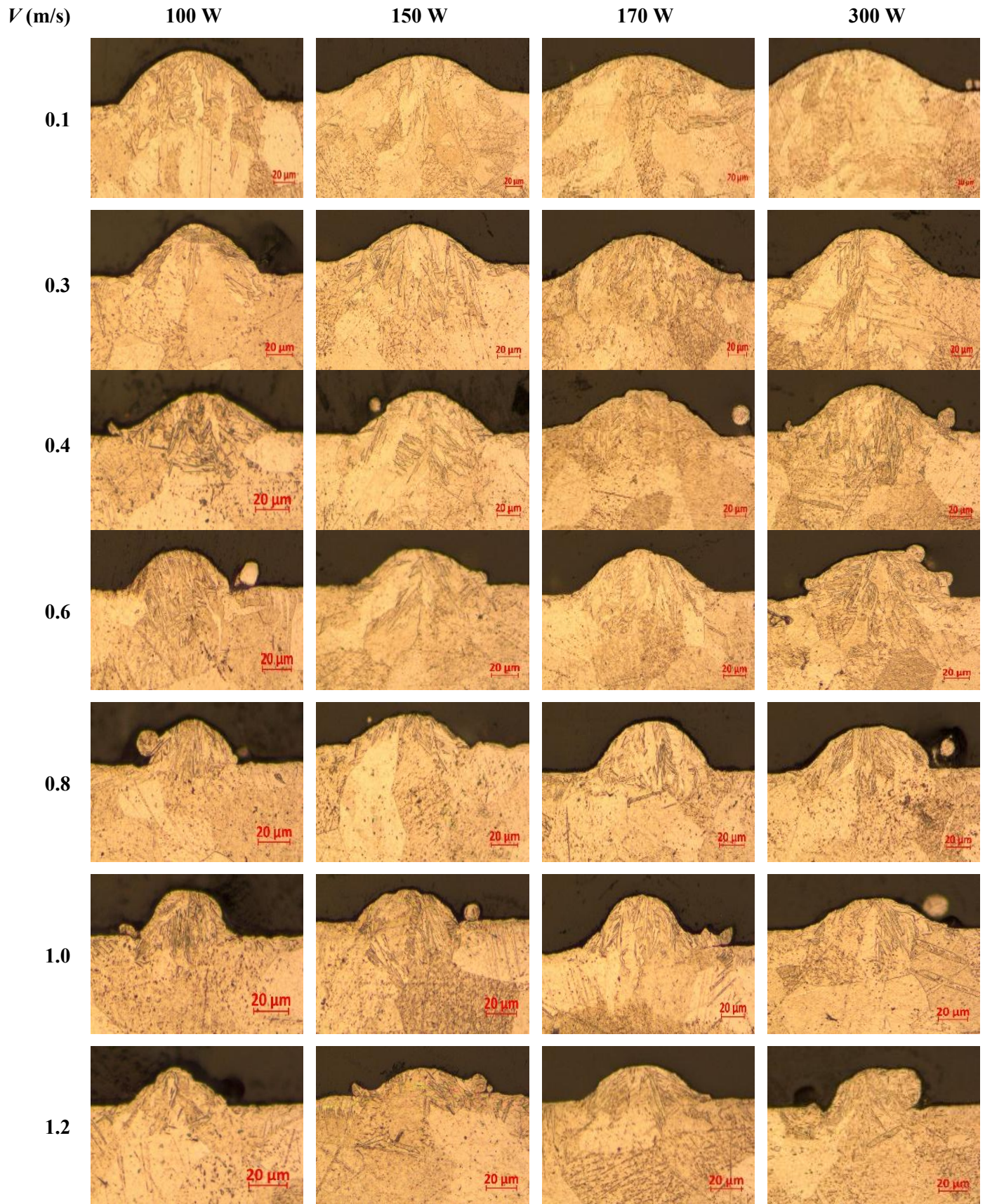


Figure 3.28: Cross-sections of cpTi single tracks at various laser powers and scanning speeds (scale = 20 μm)

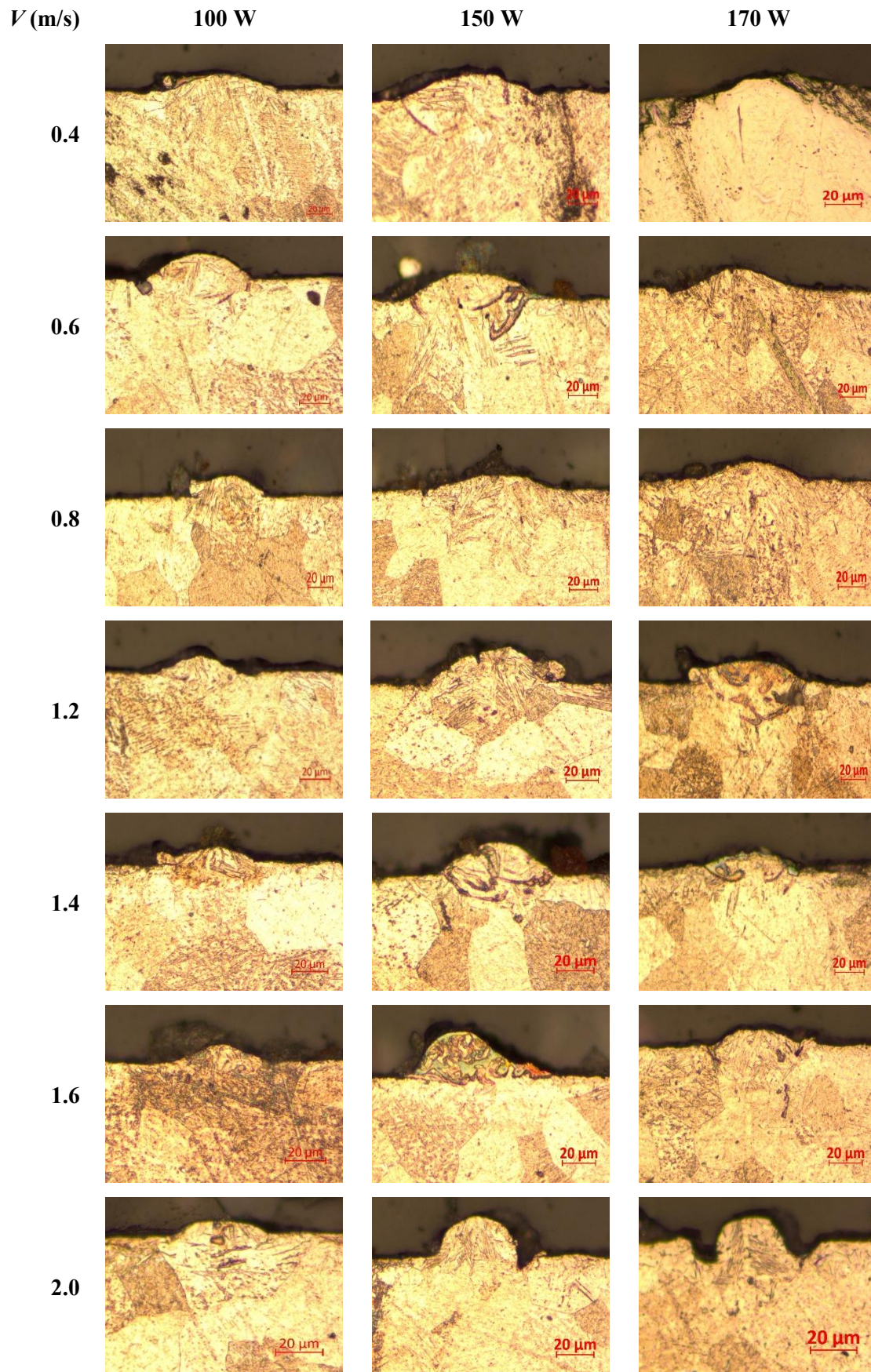


Figure 3.29: Cross-sections of Ti-3Cu single tracks at various laser powers and scanning speeds (scale = 20 μm)

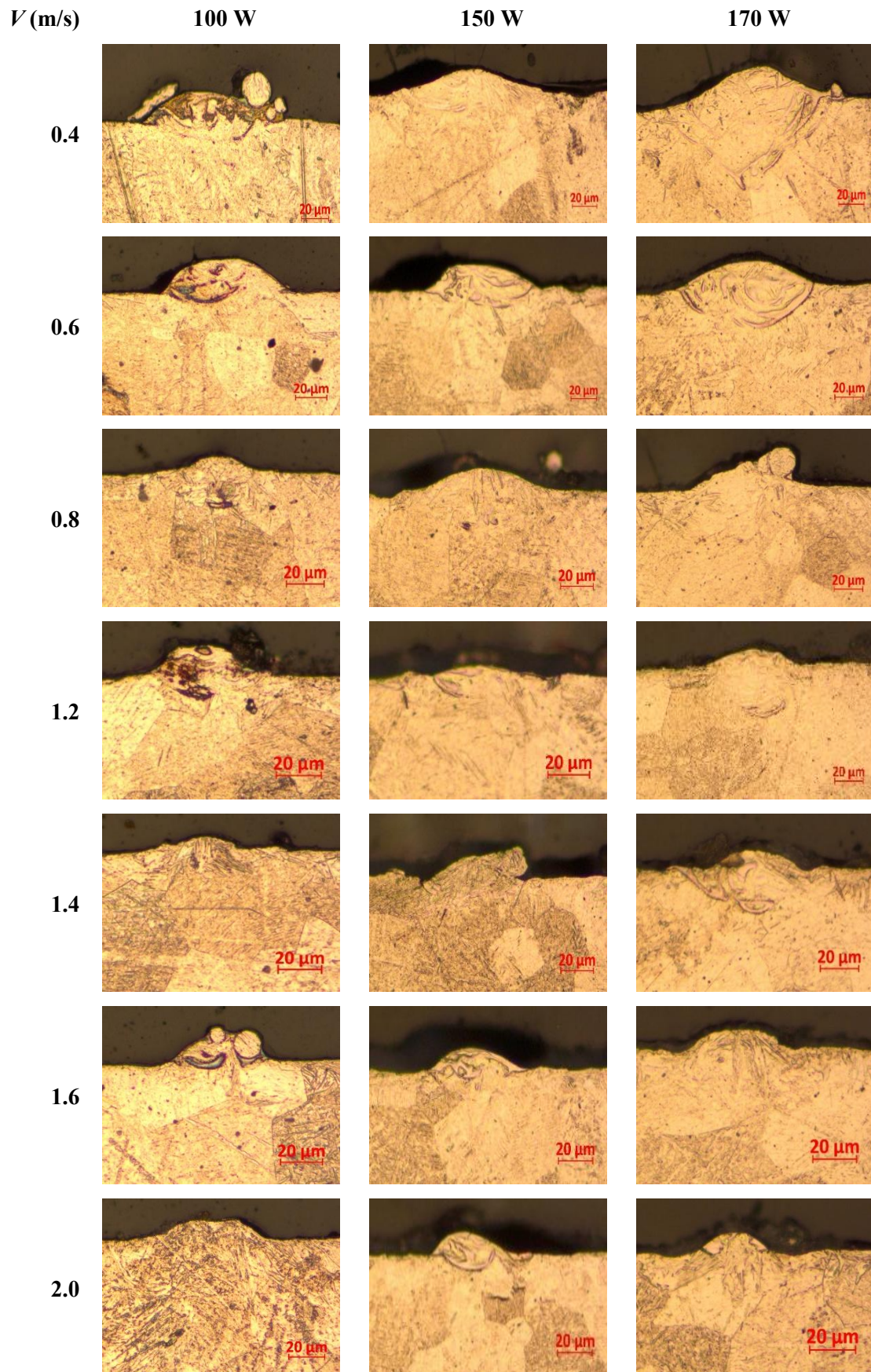


Figure 3.30: Cross-sections of Ti-6Cu single tracks at various laser powers and scanning speeds (scale = 20 μm)

King et al. (2014) and Yang et al. (2016) determined keyhole melting by identifying melt pools with a penetration depth greater than half the width, as illustrated in Figure 3.31. For Ti6Al4V-3Cu and Ti6Al4V-6Cu, the highest laser power of 300 W yielded the lowest re-melted depth-to-width ratios, all below one, and did not cause the keyhole mode. On the contrary, for cpTi, the laser power of 300 W produced the highest ratios, with some indicating keyhole penetration, while the lowest power of 100 W had the lowest ratios, with none indicating keyhole penetration. This is expected as the keyhole mode is associated with high laser energy. Similarly, for Ti-3Cu and Ti-6Cu, the higher laser powers exhibited higher ratios of re-melted depths to half the width.

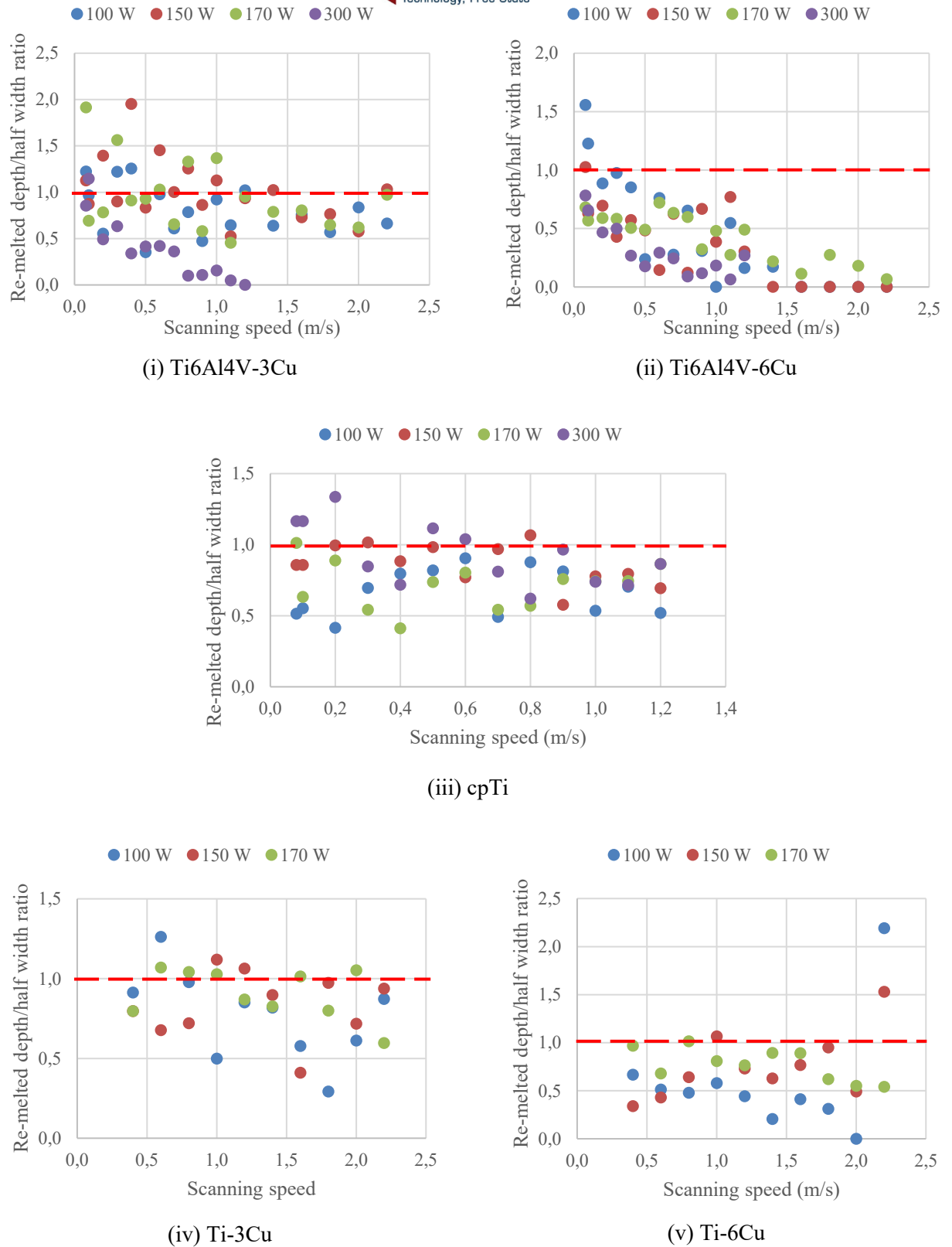


Figure 3.31: Ratio of re-melted depth to half-width of the single tracks (red dashed line showing rate ratio of 1.0)

The ratios at 150 W and 170 W primarily indicated conduction mode. Excessive laser energy may have caused the laser beam's topological depression in the substrate, creating a keyhole (Khairallah, et al., 2016). The keyhole phenomenon causes the laser beam to penetrate too deeply into previously melted/solidified layers, leading to pore formation and other metallurgical defects (Dzogbewu, 2017). Vilardell et al. (2020) found that the depth of the molten pool increased by almost double with increased laser power at the same laser scanning speed. Moreover, during their analysis of the cross-sections of the tracks, a keyhole mode was observed at and below scanning speeds of 0.4–0.6 m/s and 0.8 m/s with laser power of 170 W and 340 W, respectively. Yadroitsev et al. (2010) studied the influence of power input on track formation. They observed that the single track width and re-melted depth showed a linear dependence, decreasing with decreases in laser power and scanning speed, and that the shape of the liquid pool was substantially affected. For laser powers of 50 W (0.06–0.24 m/s scanning speed range) and 25 W (0.03–0.12 m/s scanning speed range), the re-melted depth was 55–30 μm and 30–20 μm , respectively. The authors made similar observations in subsequent studies (Yadroitsev, et al., 2015).

The measured heights of the tracks shown in

Figure 3.32 noticeably demonstrate the uneven nature of the track heights. Yadroitsava et al. (2015) made similar observations with the track heights of Ti6Al4V and attributed the irregularities in track heights to inhomogeneous deposition of powder layer and melt hydrodynamics. Yang et al. (2016) demonstrated that the track heights of Ti6Al4V were nearly constant at approximately 30 μm across various scanning speeds and ascribed the consistency in track heights to low layer thicknesses (20–40 μm at 100–400 W). As shown in

Figure 3.32, the track heights varied from the powder layer thickness of 50 μm . For Ti6Al4V-3Cu, the difference between track heights and layer thickness at various scanning speeds at 100 W was statistically significant ($p = 0.0326$), was highly significant at 150 W ($p = 0.00006$) and 300 W ($p = 0.0001$) and was insignificant at 170 W ($p = 0.6360$). For Ti6Al4V-6Cu, an insignificant difference was observed for 100 W ($p = 0.8929$) and 300 W ($p = 0.9075$), and a highly significant difference was observed for 150 W ($p = 0.0048$) and 170 W ($p = 0.000001$). For cpTi, the differences were all highly significant at $p = 0.0015$, 0.0006, 0.00001 and 0.0000002, respectively, for 100, 150, 170 and 300 W. For Ti-3Cu, at 100 W ($p = 0.00001$) and 170 W ($p = 0.0017$), the differences were highly significant and for 150 W, there was a significant ($p = 0.0194$) difference between track heights and layer thickness. For Ti-6Cu and

for all the laser powers, values of the track heights and layer thickness displayed high significance at $p = 0.0040$, 0.0001 , and 0.0005 for 100, 150, and 170 W, respectively.

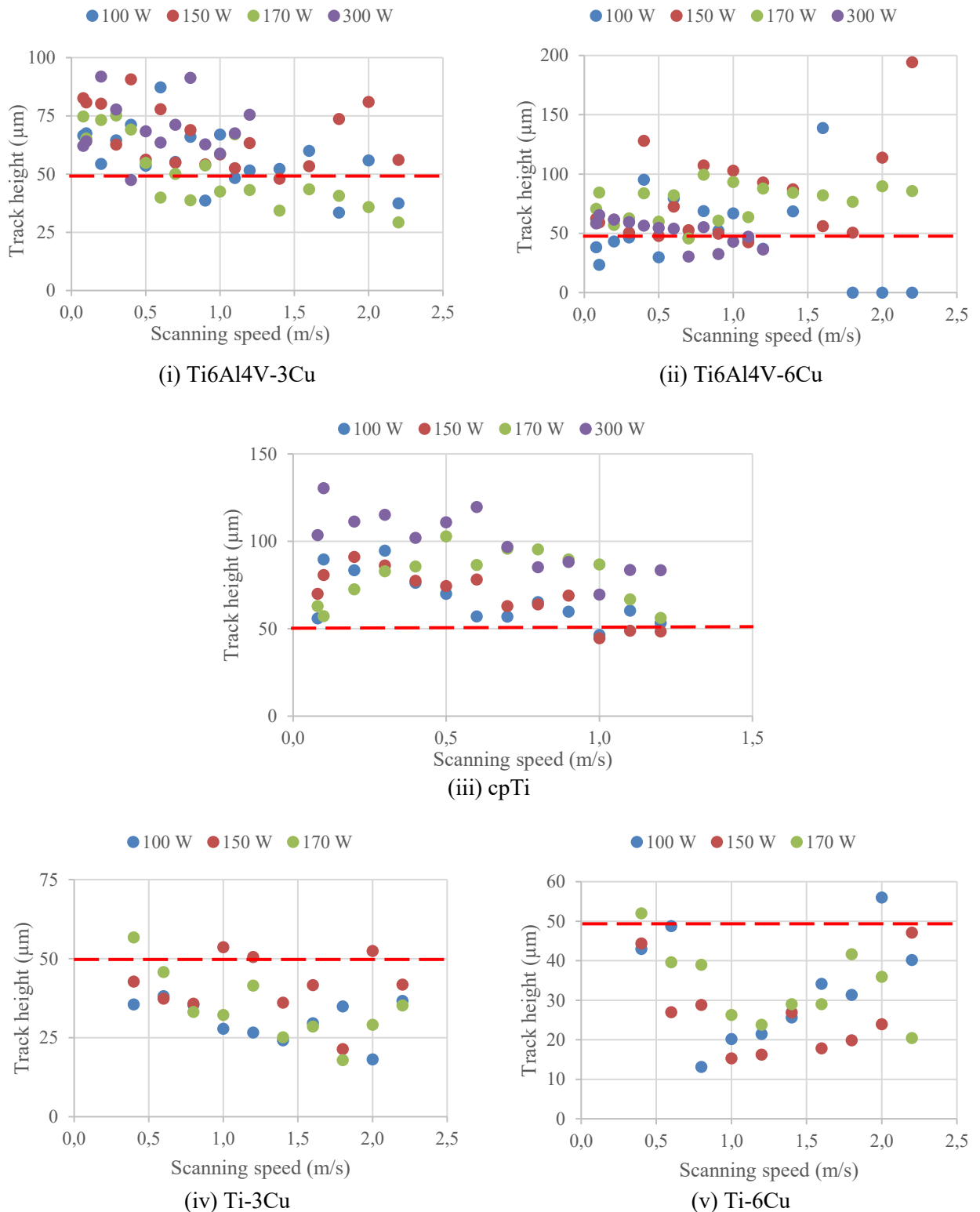
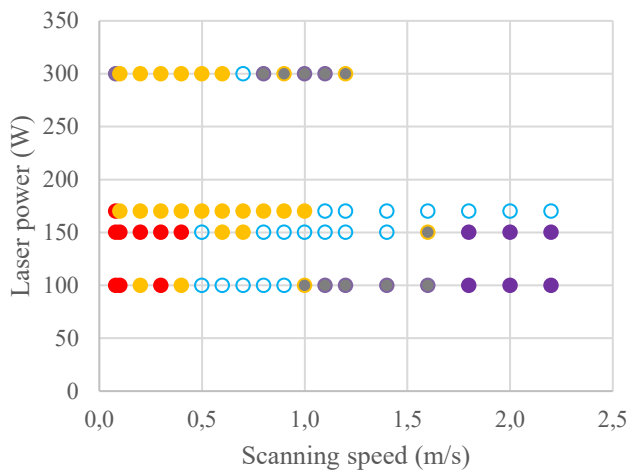


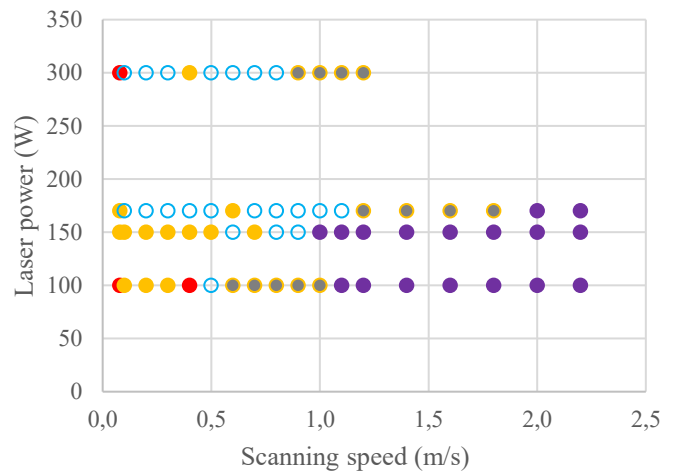
Figure 3.32: Heights of the single tracks at varied laser powers and scanning speeds (red dashed line showing powder layer thickness constant at 50 μm)

Following the cross-sectional process maps of the single tracks illustrated in Figure 3.33, the current process parameters generally start in keyhole mode, followed by optimum conduction mode (or skip keyhole mode and start in conduction mode), then proceed to pre-balling and end with balling effect. In the present experiment, it may be posited that for each laser power, there exists a specific scanning speed range that yields optimal results. Therefore, there may be a defined range of scanning speeds for a particular laser power to produce the desired mechanical properties.

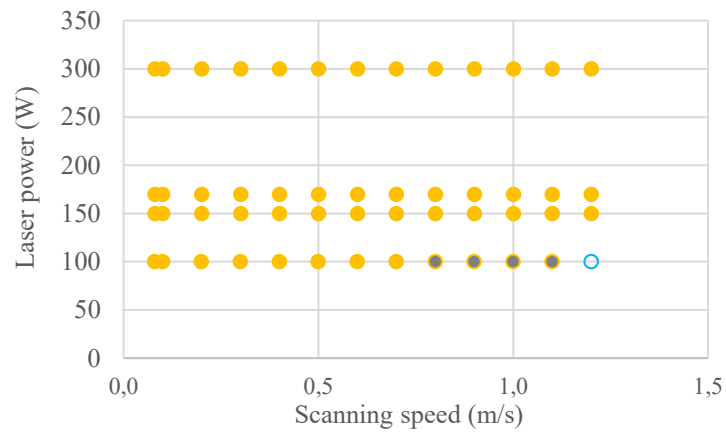
○ Low penetration
 ● Excessive
 ● Optimum
 ● Pre-balling
 ● Balling



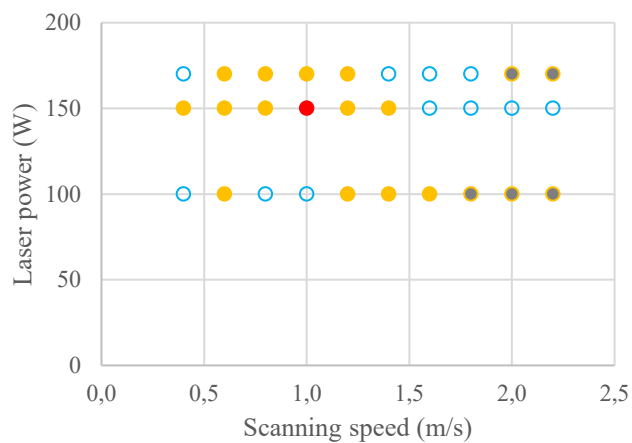
(i) Ti6Al4V-3Cu ($P = 100, 150, 170$ and 300 W, $V = 0.08\text{--}2.2$ m/s)



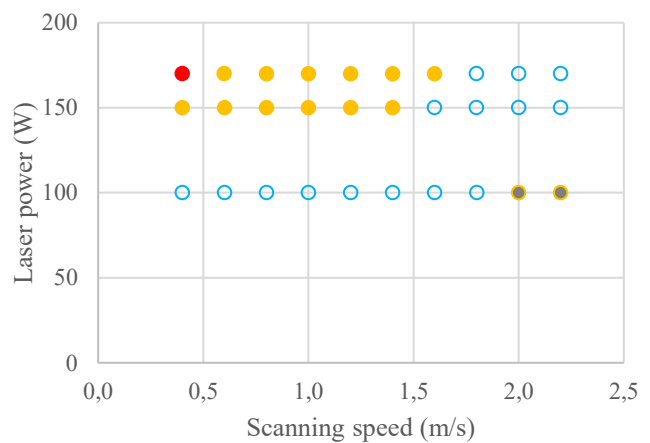
(ii) Ti6Al4V-6Cu ($P = 100, 150, 170$ and 300 W, $V = 0.08\text{--}2.2$ m/s)



(iii) cpTi ($P = 100, 150$ and 170 W, $V = 0.4\text{--}2.2$ m/s)



(iv) Ti-3Cu ($P = 100, 150$ and 170 W, $V = 0.4\text{--}2.2$ m/s)



(v) Ti-6Cu ($P = 100, 150$ and 170 W, $V = 0.4\text{--}2.2$ m/s)

Figure 3.33: Process maps of single tracks produced: cross-sectional view

It has been suggested that, at elevated laser powers, the range of optimal scanning speeds for the formation of stable, continuous, single tracks, as indicated by metallurgical bonding, would expand (Yadroitsev, 2009). However, at excessively high laser power density, keyholes occur at low scanning speeds, and instability of long shallow molten pools occurs at high scanning speeds (Yadroitsev, et al., 2015). Single tracks are not only formed from the powder directly under the laser spot, but also from adjacent particles due to the scattering of radiation, conduction through the substrate and neighbouring particles, capillary phenomena, and so forth (Yadroitsev, et al., 2015). The denudation zone, that is, the area without powder after laser scanning, may be double the width of the track (Yadroitsev, 2009). This effect defines the geometrical characteristics of the single tracks and the eventual morphology of layers (Yadroitsev, et al., 2015). Krakhmalev et al. (2017) investigated Ti6Al4V (ELI)-1 at.%Cu material, manufactured by LPBF from a mixture of Ti6Al4V (ELI) and pure Cu powders and found that at the laser spot size of 80 μm and laser power of 170 W, stable tracks were formed at a scanning speed range of 0.7–1.3 m/s. For the present study, the optimal scanning speeds at the corresponding laser powers are listed in Table 3.5. At these parameters, stable tracks were formed. Consequently, the manufacturing of double layers and 3D specimens was done at these process parameters.

Table 3.5: Process parameters optimised during single track analysis

Powder	Laser power, P (W)	Scanning speed, V (m/s)
Ti6Al4V-3Cu	170	0.2
	300	0.5
Ti6Al4V-6Cu	150	0.1
	300	0.3
cpTi	150	0.3
	170	0.6
Ti-3Cu	150	1.4
	170	0.6
Ti-6Cu	150	0.4
	170	0.6

3.3.3. LPBF double layers

The quality of individual sets of single tracks determines the morphology and mechanical properties of the final object. Single tracks with regular shapes allow for homogeneity of the layers onto which powder layers may be evenly deposited and result in a dense final part (Vilardell, et al., 2020). According to Yadroitsev et al. (2015), the hierarchical design algorithm states that the basic building block of laser-sintered objects is a set of single tracks that are

placed side-by-side to form a single layer. Furthermore, it has been demonstrated that the surface morphology of a single layer depends on the characteristics of the set of conjoining single tracks that produce the layer using LPBF (Ramosena, et al., 2022).

A previously solidified layer serves as the substrate for the subsequent layer in the layer-by-layer formation. This layer-wise process continues until the object is manufactured. In other words, the superposition of various tracks and layers results in the manufacture of 3D objects. The interaction between radiation and properties of the powder and the substrate has a substantial effect on the molten pool dynamics and thus on the solidified layers (Childs, et al., 2004; Kruth, et al., 2005). Fundamental parameters such as laser power, spot diameter, scanning speed, hatch distance, powder layer thickness, and powder characteristics also have a decisive effect on the surface morphology of LPBF parts (Dzogbewu, 2021; Ruban, et al., 2014). Optimal hatch distances are essential for the reduction of the process time and control of thermal cycling during LPBF (Krakhmalev, et al., 2017). Moreover, information on the geometric characteristics of single tracks is useful for establishing the hatch distance for laser beam scanning. The relationship between the numerous parameters has been proven to be non-linear (Yadroitsev, et al., 2010). Hence, it is necessary to conduct an experimental investigation to identify the combination of process parameters required to achieve the desired surface quality.

Determining the effect of a hatch distance and scanning strategy on the surface quality and distribution of elements in the in situ sintered alloy is vital (Krakhmalev, et al., 2017). Shah & Dey (2019) found hatch spacing to be the most important process parameter affecting relative density and also had a major influence on the ultimate tensile stress of built parts. The repeated powder deposition and scanning during the layer-by-layer LPBF process may amplify undulations associated with defects, such as cracks, high surface roughness, and possible building failures (Zhang, et al., 2022). Studying the morphology of a single layer is essential in the selection of the optimal strategy to manufacture pore-free 3D objects (Yadroitsev, et al., 2015). The double layers produced for the present investigation are shown in Figure 3.34.



Figure 3.34: Double layers sintered on Ti6Al4V substrate

3.3.3.1. Top surface analysis of double layers

The width of single tracks that formed the scan and rescan samples ranged from 63 to 515 μm for the various powders. Thus, the selected hatch distances allowed efficient overlapping of the individual tracks that formed the layers (Figure 3.35). Ramosena et al. (2022) postulated that at average track widths of 152 and 145 μm , a hatch distance of 80 μm would allow for a more efficient overlap of single tracks as compared to those of 90 and 100 μm hatch distances. It may be observed from the images obtained with the optical microscope shown in Figure 3.35 that, across all samples, the tracks completely overlapped at the various process parameters, forming metallurgical bonding and fully dense sintered surfaces.

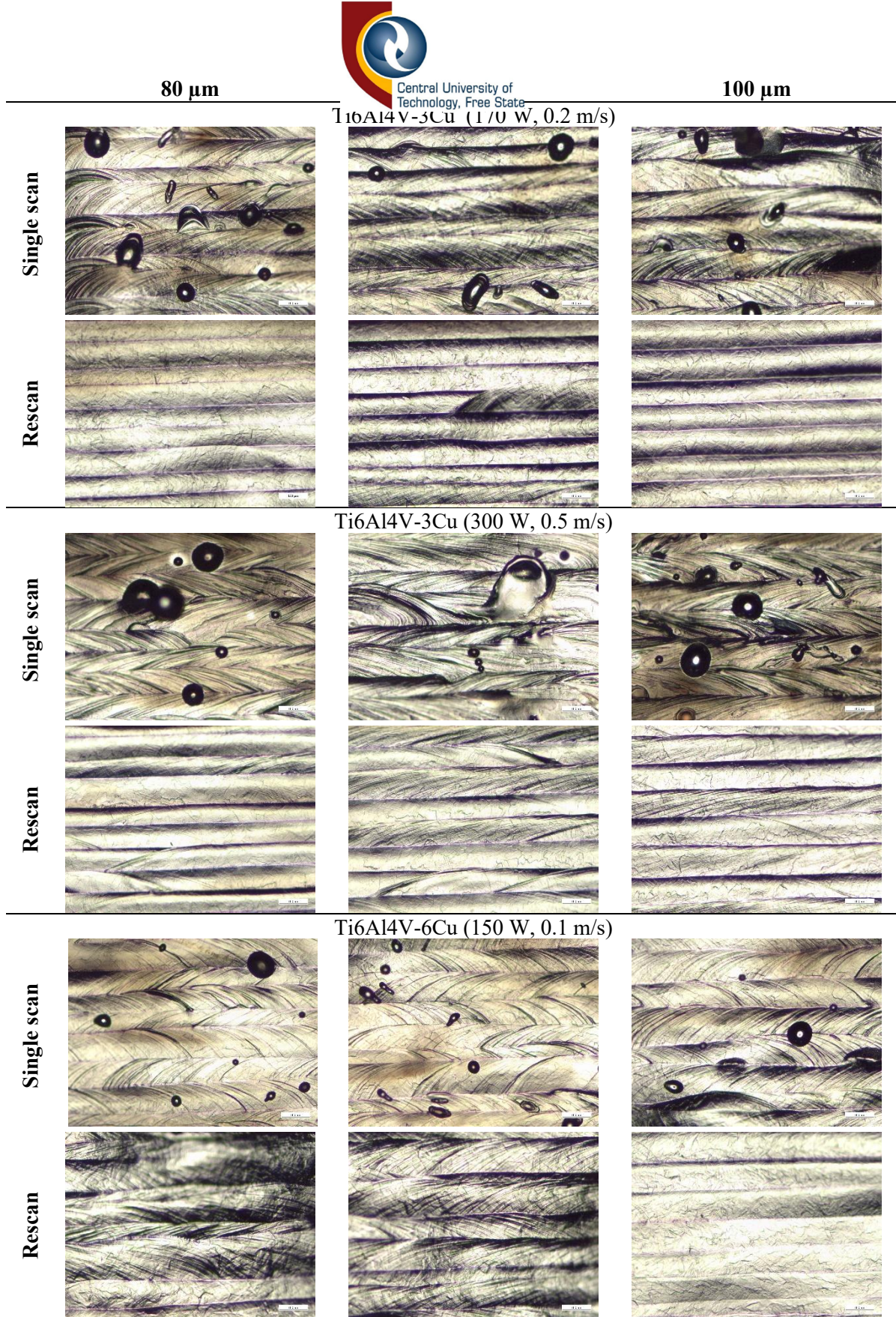


Figure 3.35: Top view of LPBF surfaces from Ti6Al4V-based and Ti-based in situ alloys (scale = 100 μm)

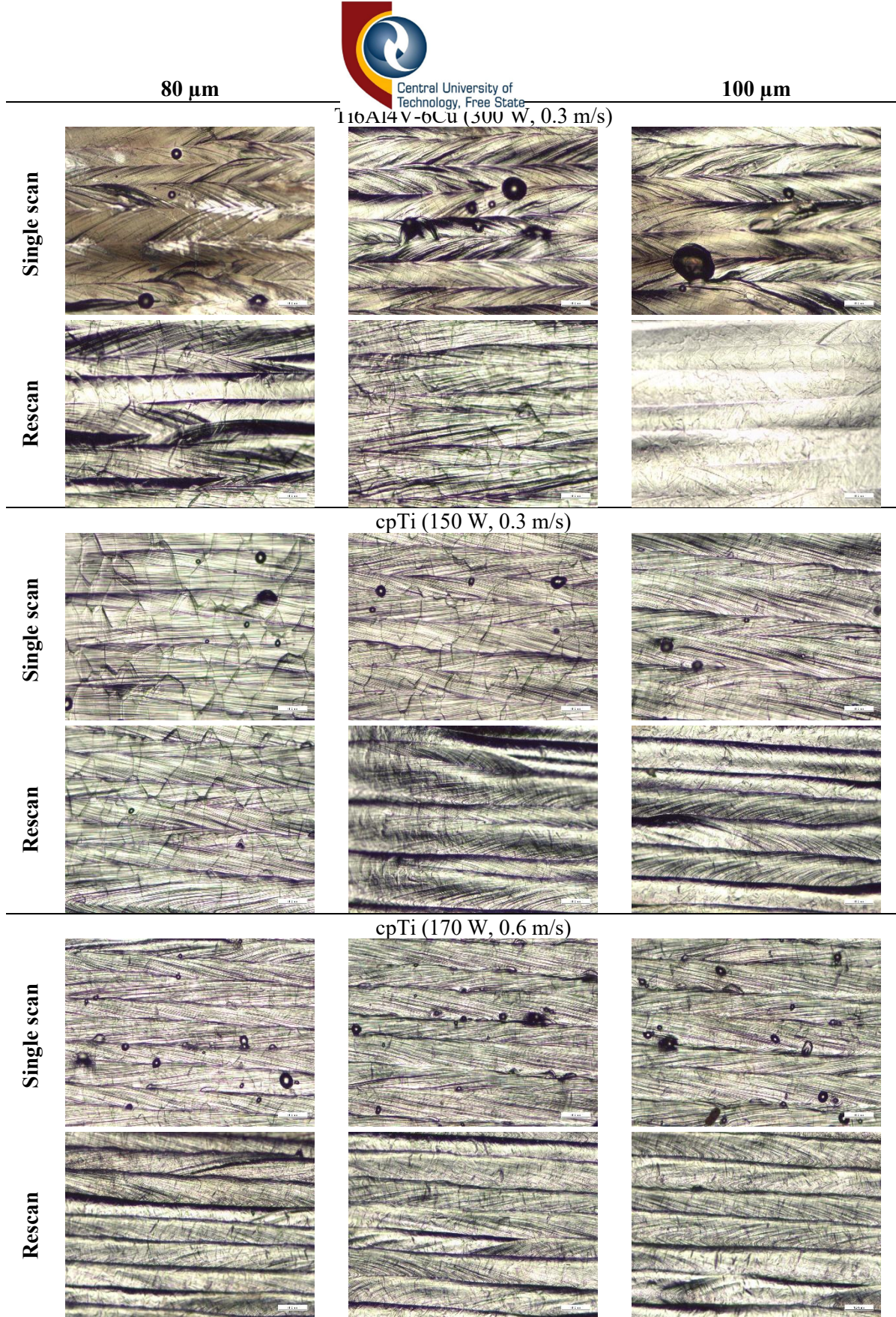


Figure 3.35: Top view of LPBF surfaces from Ti6Al4V-based and Ti-based in situ alloys (scale = 100 μm) (cont.)

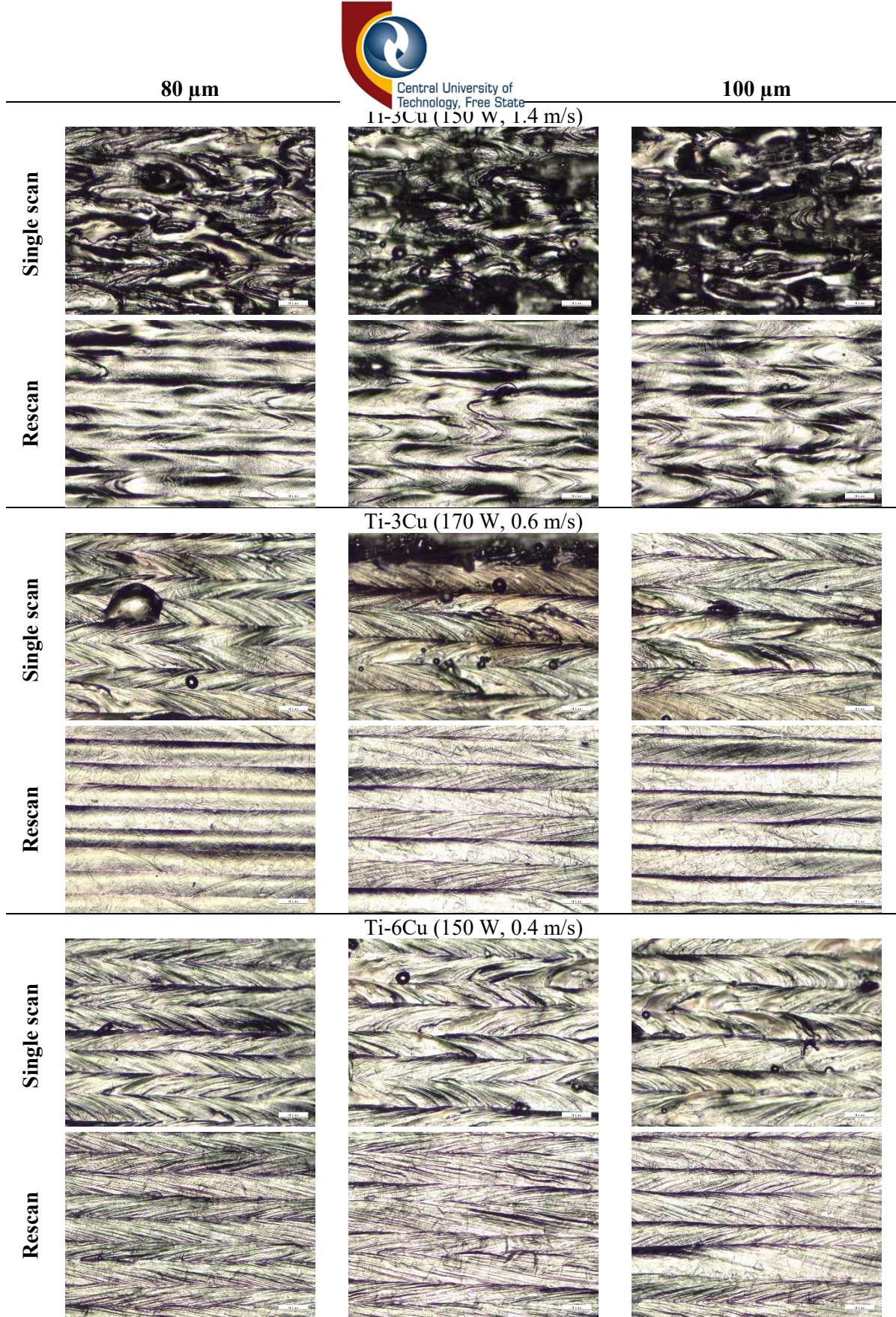


Figure 3.35: Top view of LPBF surfaces from Ti6Al4V-based and Ti-based in situ alloys (scale = 100 μm) (cont.)

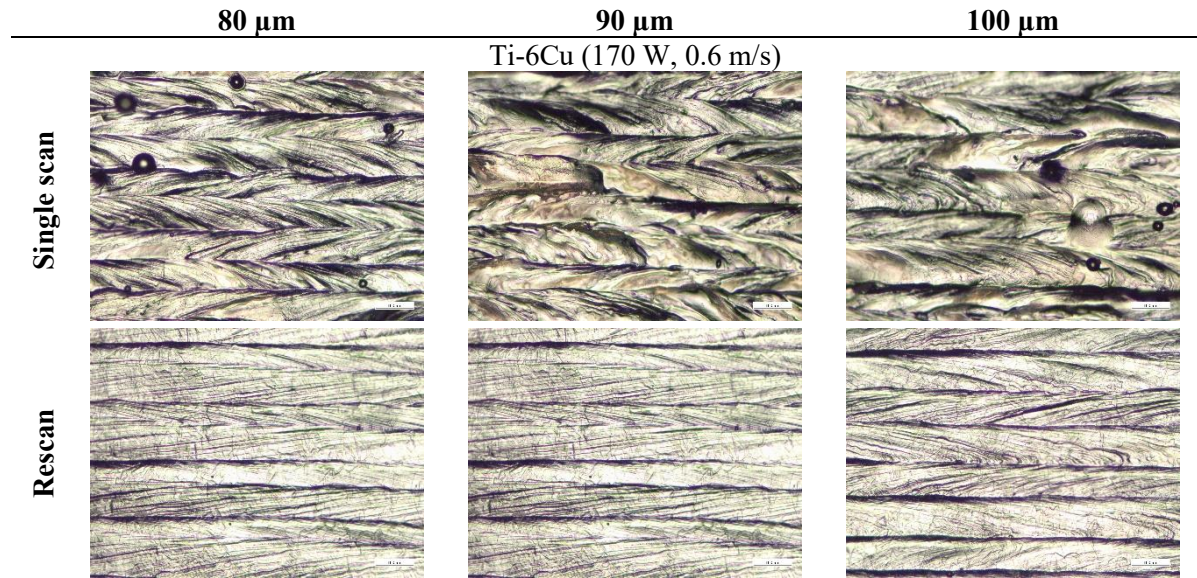


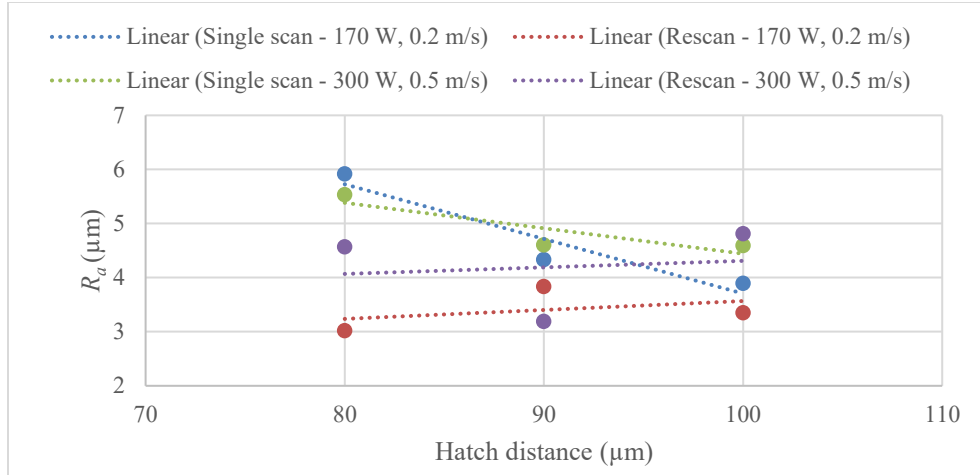
Figure 3.35: Top view of LPBF surfaces from Ti6Al4V-based and Ti-based in situ alloys (scale = 100 μm) (cont.)

Surface roughness

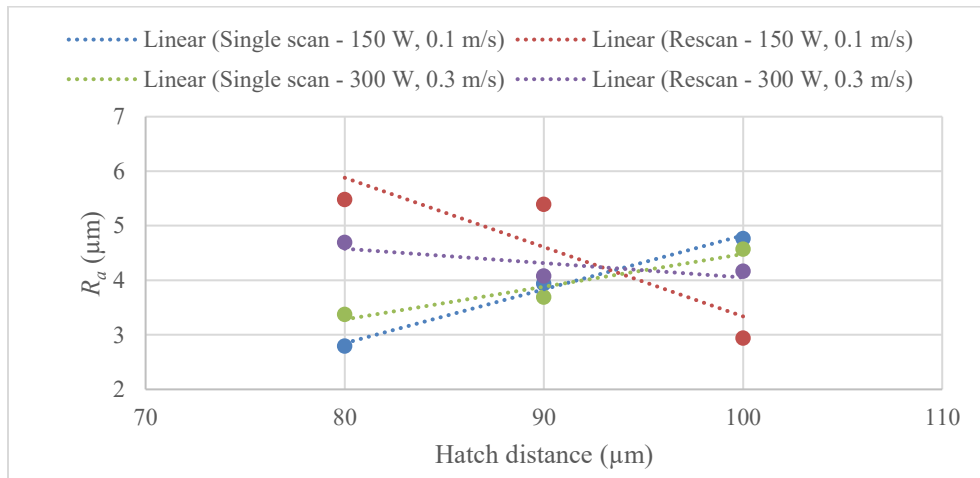
The primary purpose of analysing the surface topography of a LPBF layer is to examine the roughness of the surface. Surface roughness is a factor of great importance influencing the quality of LPBF parts and evaluating the accuracy of the manufacturing strategy (Ruban, et al., 2014; Vilardell, et al., 2020). Measurements of surface roughness are usually quantified as values of average linear roughness (R_a) and ten-point mean roughness (R_z). R_a value is the arithmetic average of the absolute values of the profile height deviations from the mean line and recorded within the evaluation length (Krakhmalev, et al., 2017). R_z value is an average of the vertical distance between the highest and lowest points (that is, a combination of peak heights and valley depths) of the profile within a sampling length (Krakhmalev, et al., 2017; Ramosena, et al., 2022). Krakhmalev et al. (2017) argued that the R_z value is more applicable for the assessment of surface roughness of LPBF objects since every surface defect may result in a bulk defect detrimental to the quality of the final object, and R_z values describe extremes in heights or depths responsible for surface roughness.

The surface roughness of single scanned and rescanned layers of cpTi and in situ alloys was measured at hatch distances of 80, 90 and 100 μm and the R_a and R_z values are illustrated in FiguresFigure 3.36 andFigure 3.37, respectively. The R_a and R_z values of Ti6Al4V-3Cu decreased

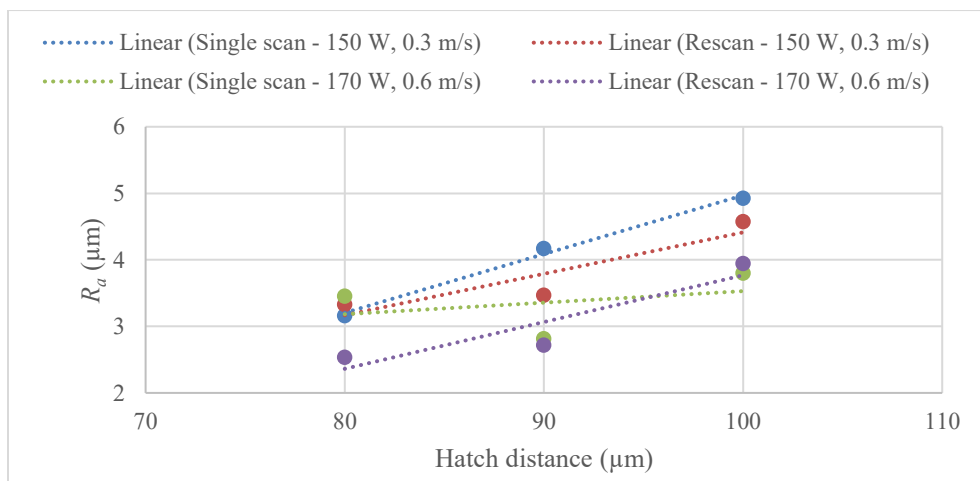
during the single scan and generally increased during the rescan, although the single scan values were higher than those of the rescan. The opposite trend was observed for Ti6Al4V-6Cu, whereby the roughness parameter values increased for the single scan and decreased for the rescan. Rescanned layers were rougher at 80 and 90 μm hatch distances, and smoother at 100 μm . For cpTi and Ti-3Cu, R_a and R_z increased with hatch distance, and the single scan layers had higher roughness than the rescan layers. The trend for Ti-6Cu indicated a small change in roughness, particularly in R_z values. It has been reported that, for LPBF parts, top-surface roughness is 6–10 μm for R_a and >30 μm for R_z value (Dzogbewu, 2017). The experimental results were in proximity to the expected values for both R_a and R_z .



(i) Ti6Al4V-3Cu

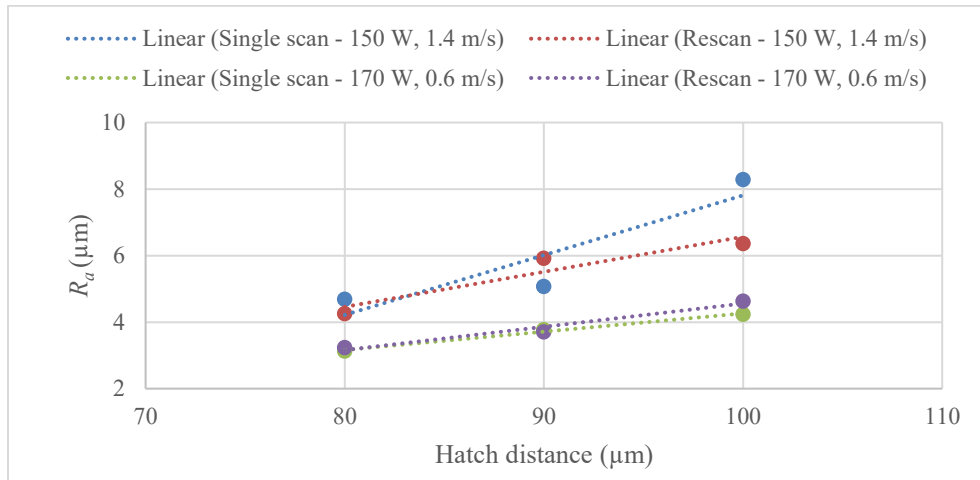


(ii) Ti6Al4V-6Cu

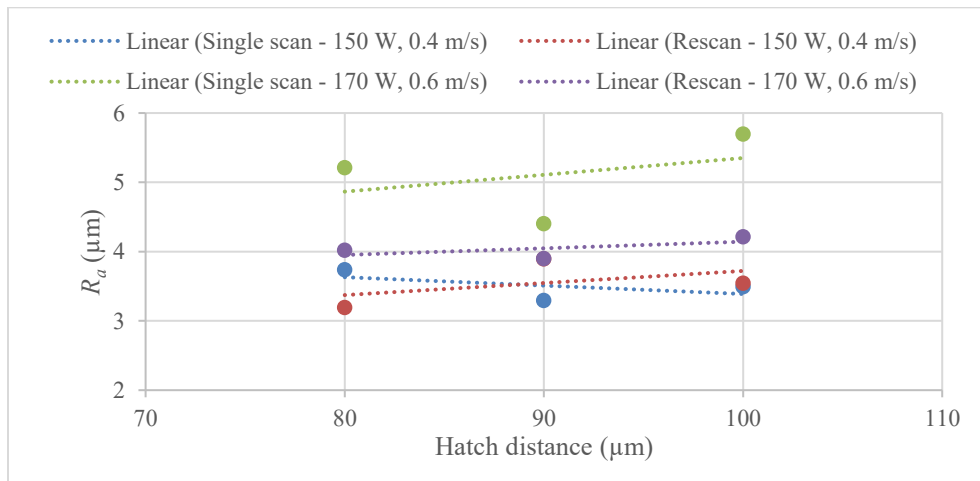


(iii) cpTi

Figure 3.36: Surface roughness R_a values of double layers at varied hatch distances

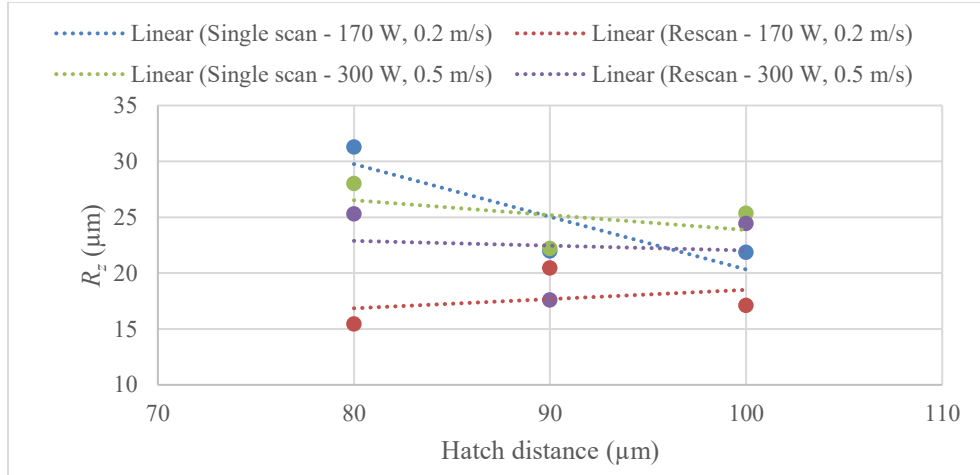


(iv) Ti-3Cu

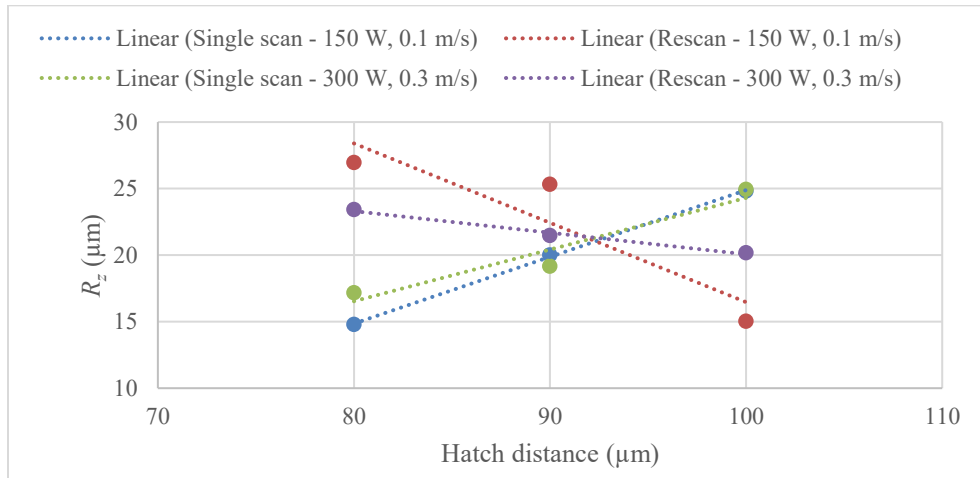


(v) Ti-6Cu

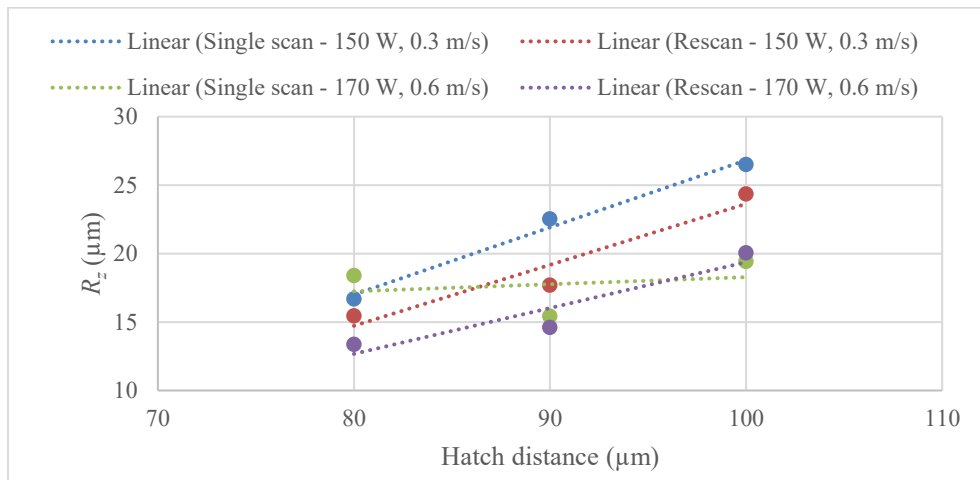
Figure 3.36: Surface roughness R_a values of double layers at varied hatch distances (cont.)



(i) Ti6Al4V-3Cu

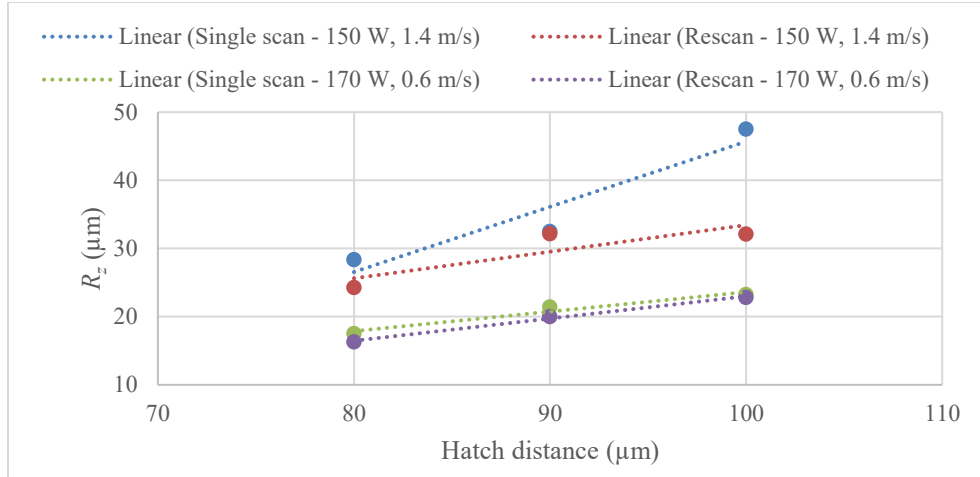


(ii) Ti6Al4V-6Cu

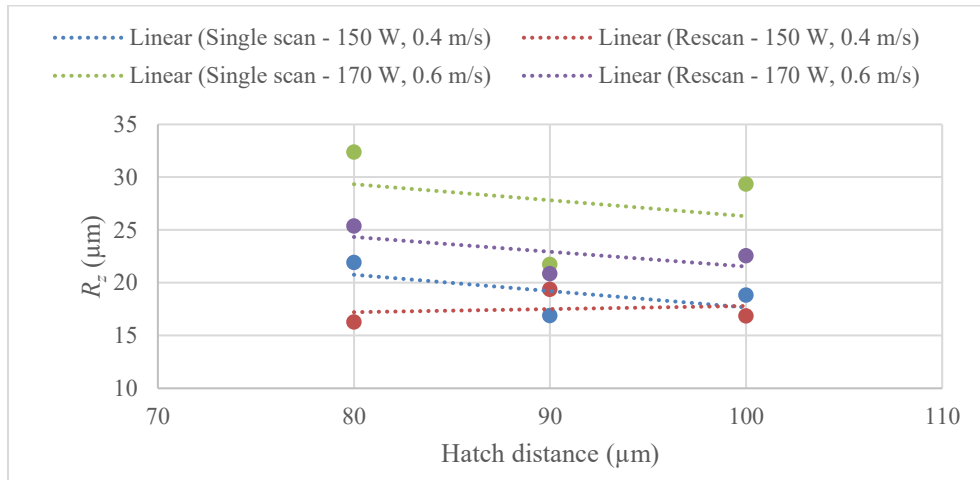


(iii) cpTi

Figure 3.37: Surface roughness R_z values of double layers at varied hatch distances



(iv) Ti-3Cu



(v) Ti-6Cu

Figure 3.37 : Surface roughness R_z values of double layers at varied hatch distances (cont.)

Since the molten surface area increases as the hatch distance decreases, it is expected that surface roughness would increase with increasing hatch distance. At a lower hatch distance, a larger surface area of the previously solidified layer is re-melted, which could melt any unmelted powder particles (Dzogbewu, et al., 2017). A higher roughness of the rescanned surfaces may be due to machine properties, thermo-physical properties, and laser absorptance of the powder material. In addition, the rougher surface before rescanning may experience a more substantial Marangoni effect, resulting in a smoother layer post-rescanning. The two-zone strategy involves rescanning without deposition of powder; therefore, a layer of solidified or partially solidified molten pool tracks is scanned during rescanning.

For their study, Oyesola et al. (2021) examined the effects of laser power and scanning speed on the surface roughness of Ti6Al4V metal specimens fabricated via SLM. They found that surface roughness decreased with increasing laser power and increased with increasing scanning speed. Similarly, Vilardell et al. (2020) and Krakhmalev et al. (2017) characterised in situ alloyed Ti6Al4V (ELI)-3 at.% Cu and Ti6Al4V (ELI)-1 at.% Cu, respectively, and found that the surface roughness increased with an increase in scanning speed. Krakhmalev et al. (2017) further discovered that surface roughness increased with increasing hatch distance. Likewise, Ramosena et al. (2022) found that the R_z of in situ alloyed Ti-6Al4V increased with increasing hatch distance. The findings of Dzogbewu et al. (2017) were in disagreement, stating that the surface roughness increased with decreasing hatch distance for in situ alloyed Ti15Mo. According to Dzogbewu et al. (2017), a lower hatch distance resulted in a larger surface area of the previously solidified layer being re-melted, potentially melting previously unmelted powder particles. The authors also found that surface roughness increased with increasing laser power and scanning speed. Thijs et al. (2010) found that varying the hatch spacing also alters the energy density and may influence the overlap between two neighbouring scan vectors. There were no noteworthy relationships between the roughness values and either the laser power/scanning speed combinations or the different Cu contents across the various alloys in this study.

Variations in surface roughness may provoke porosity resulting from the lack of fusion of layers within the 3D object, particularly since the previously melted single layer is the substrate for the subsequent layer (Vilardell, et al., 2020). Possible causes of surface defects that may increase roughness include satellites, denudation, balling effect and pores. For the current study, many satellite particles were found adhering to the solidified surfaces after the single scan process, as displayed on the SEM micrographs in Figure 3.38. Satellite particles formed from spluttered liquid droplets from the molten pool are considered peaks in surface roughness measurements. Krakhmalev et al. (2017) observed more satellites on the surface at higher scanning speeds and hatch distances that led to a dramatic increase in the measured surface roughness parameters. Similarly, double layers produced by Ramosena et al. (2022) exhibited more satellites at a hatch distance of 100 μm than those produced at a hatch distance of 90 μm . These satellite particles have been shown to adversely affect the mechanical properties of sintered parts (Andani, et al., 2017).

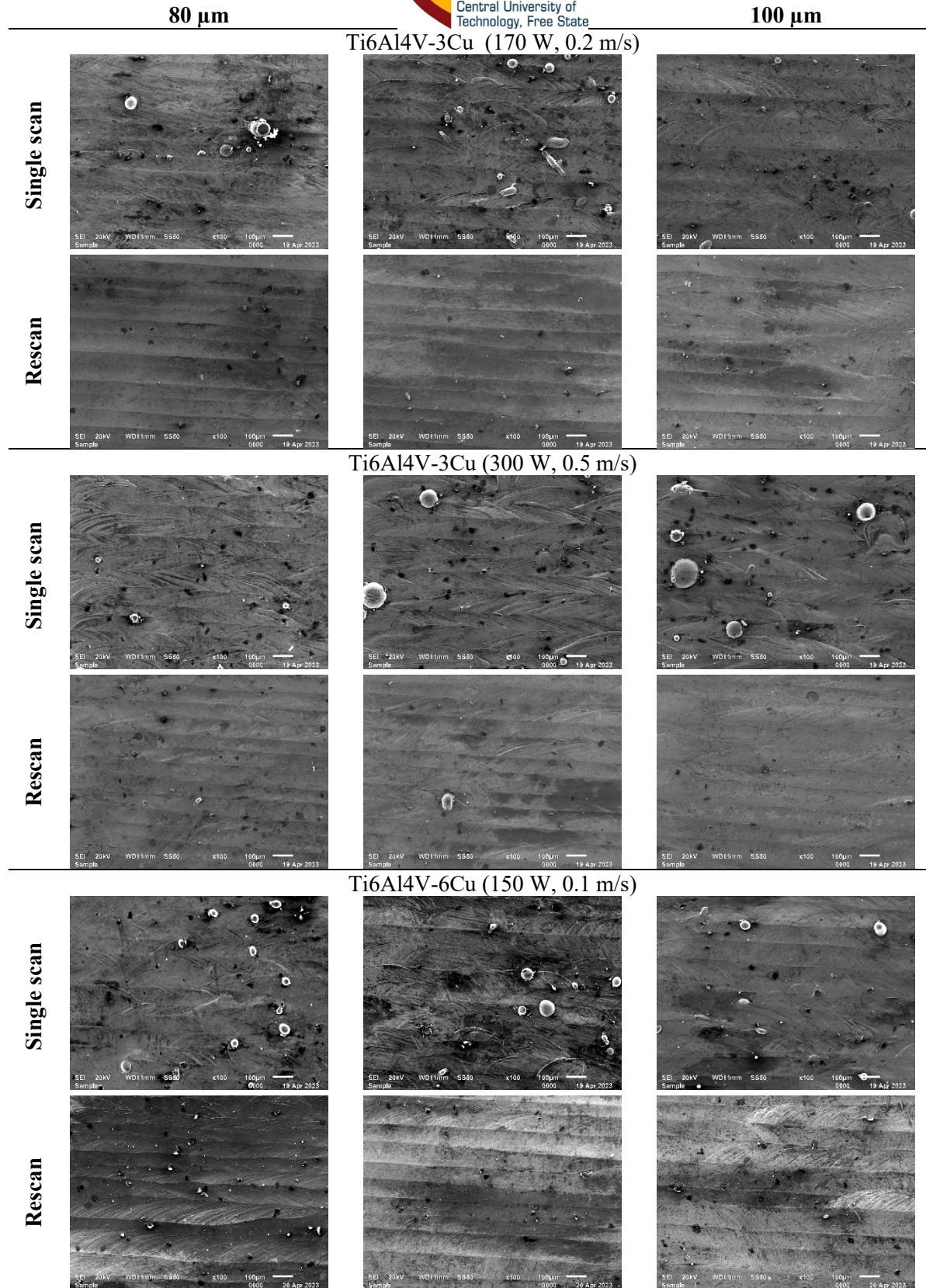
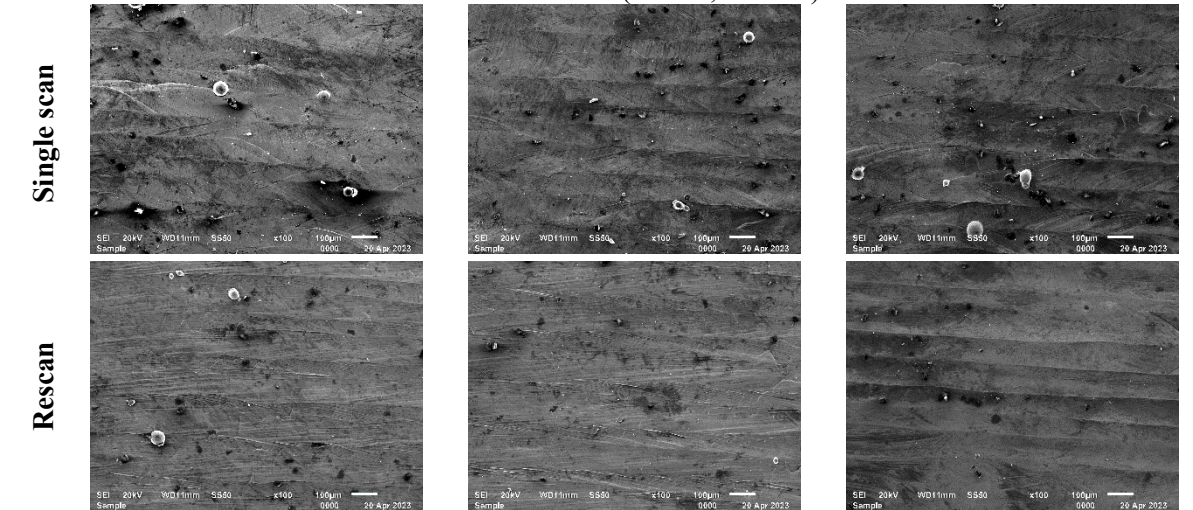
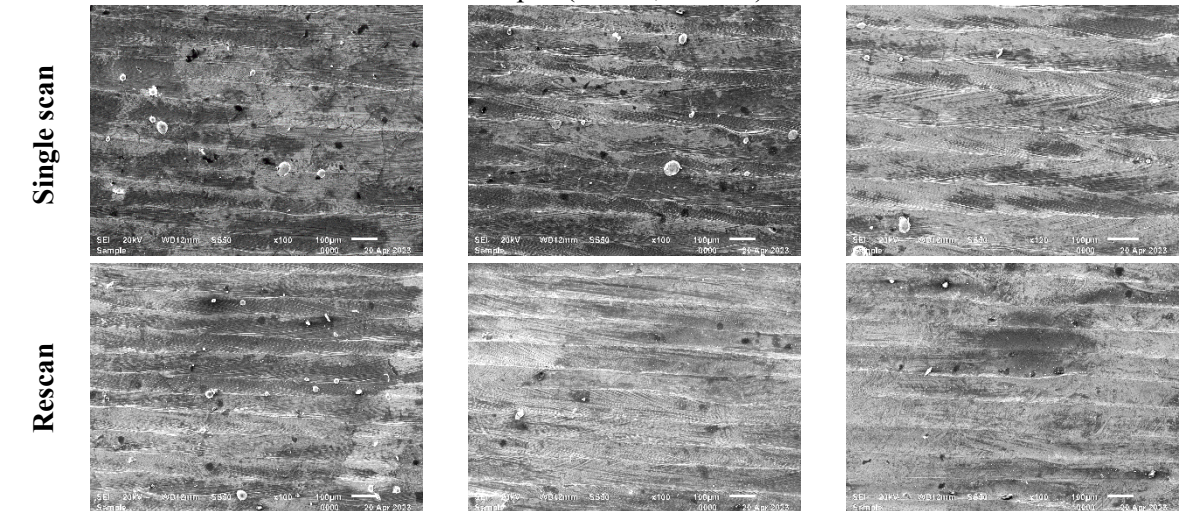


Figure 3.38: Single scanned and rescanned double layers at 80–100 μm hatch distances (scale = 100 μm)

Ti6Al4V-6Cu (300 W, 0.3 m/s)



cpTi (150 W, 0.3 m/s)



cpTi (170 W, 0.6 m/s)

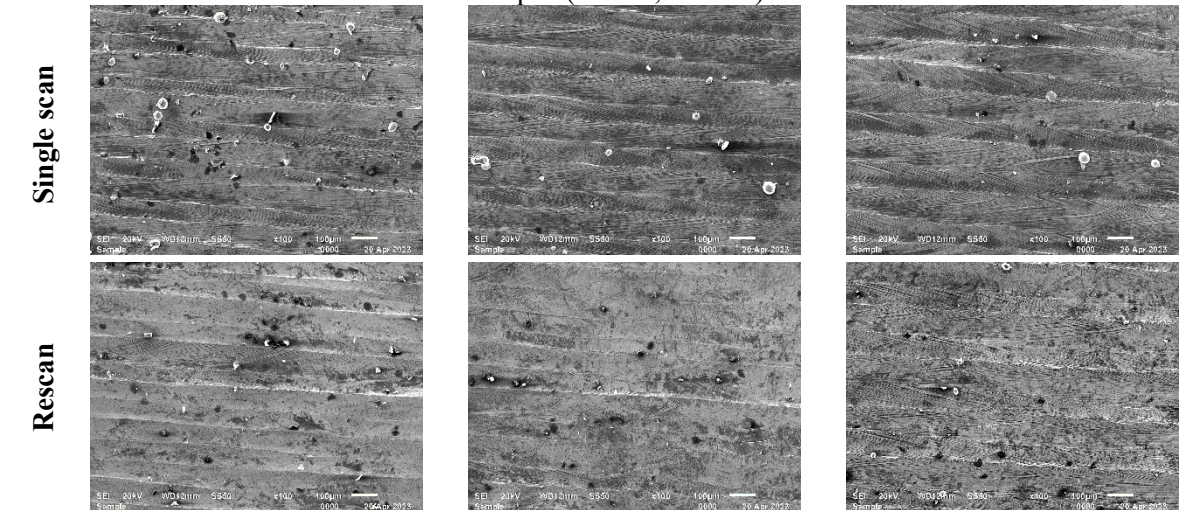


Figure 3.38: Single scanned and rescanned double layers at 80–100 μm hatch distances (scale = 100 μm) (cont.)

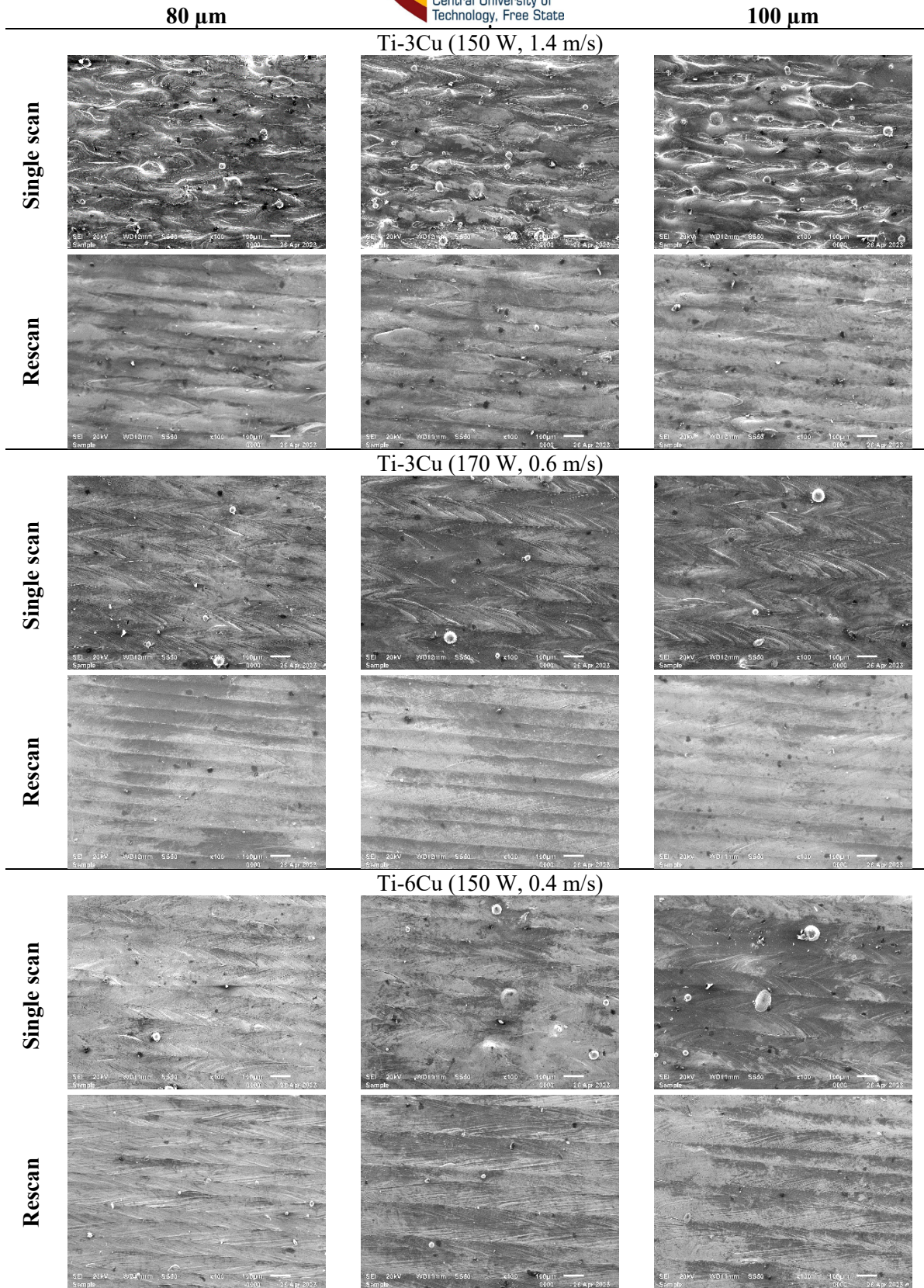


Figure 3.38: Single scanned and rescanned double layers at 80–100 μm hatch distances (scale = 100 μm) (cont.)

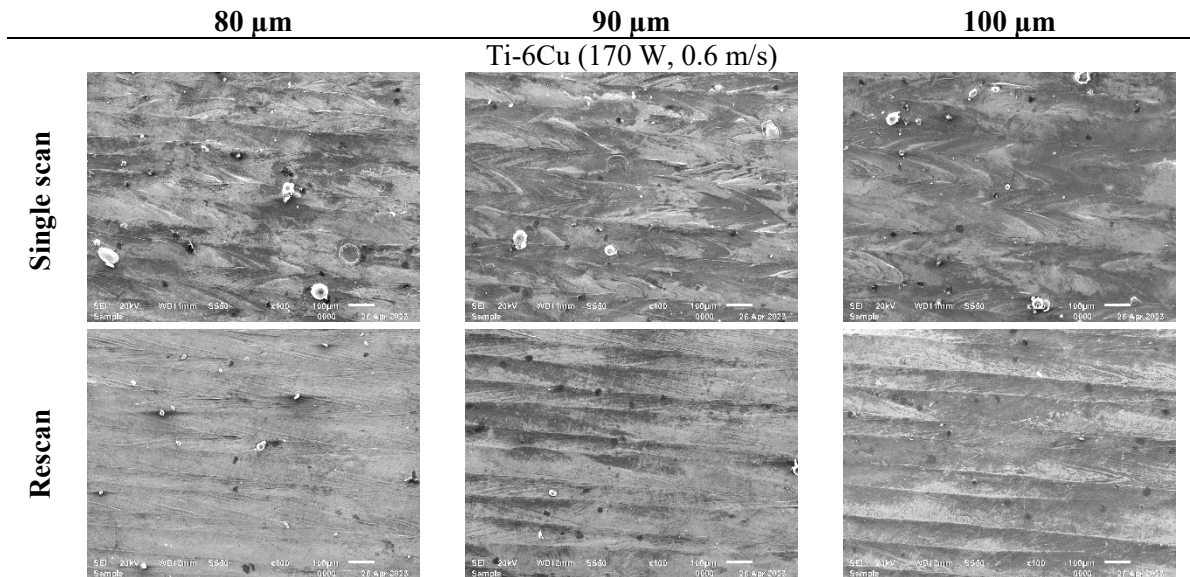


Figure 3.38: Single scanned and rescanned double layers at 80–100 μm hatch distances (scale = 100 μm) (cont.)

Excessive balling also causes a surface to be irregular and improper deposition and inhomogeneity of the subsequent layer as a result of various regions having different melting and solidifying behaviours (Aboulkhair, et al., 2014). Osakada & Shiomi (2006) suggested that these balls form due to non-linear solidification. Aboulkhair et al. (2014) observed balling when the scanning speed increased and credited it to capillary instability in the molten pool, promoted by high speed, leading to the splashing of small liquid droplets onto the surface. If the hatch distance exceeds the single track width, the powder is sintered in a sequence of individual tracks, and gaps occur between tracks in a layer, which causes chains of pores in the final LPBF component (Yadroitsev, et al., 2015). When the hatch distance is less than the width of the track, overlapping occurs – that is, one part of the molten pool solidifies in contact with the previous synthesised track, and the other part solidifies in contact with the substrate (Yadroitsev, et al., 2015). Vilardell et al. (2020) suggested that irregularities and undercuts may lead to rough surfaces on single layers and to porosity in the final 3D parts. The authors detected pores and deeper penetrations into the substrate during the formation of single tracks and layers at slow scanning speeds of 0.4–0.6 m/s, and the humping/balling effects, which started at 1.4 m/s.

Despite contradictory findings on the influence of process parameters on surface roughness, the majority of studies (including the current one) agreed that rescanning decreased surface roughness. Previous studies have shown that rescanning of LPBF layers improves surface quality (Kinnear, et al., 2016; Dzogbewu, et al., 2017; Vilardell, et al., 2020; Ramosena, et al., 2022). Spatter particles are powder particles that did not efficiently melt; therefore, the second laser scan may melt them sufficiently to fuse them into the molten layer. Ramosena et al. (2022) stated that after rescanning, the value of R_z decreased by an average of 51% due to the reduction in satellites formed by single scanning. Dzogbewu et al. (2017) also observed a decrease in the R_z value after rescanning; thus, rescanning considerably reduces surface roughness. The authors had hypothesised that the rescanning strategy may ensure that the laser melts satellites and enhances the homogeneity of in situ alloyed material. During their investigations, Yadroitsev et al. (2015) and Krakhmalev et al. (2017) discovered that the application of the rescan strategy removed the majority of satellites from the surface and reduced the roughness of the manufactured layer. After the first scanning during the study by Yadroitsev et al. (2015), a lack of symmetry in the cross-section of the tracks was observed, and a non-uniform thickness of the sintered layer resulted from the denudation of the substrate-powder material used to produce the track. Aboulkhair et al. (2014) found that scanning each layer twice at a scanning speed of 500 mm/s effectively reduced porosity. The reduction (if not elimination) of keyhole pores, however, was at the expense of introducing metallurgical pores because of the excessive energy required to double scan at slower scanning speeds.

When rescanning is applied and as the laser beam interacts with the solid material during the second laser pass, thermo-physical conditions of LPBF are different: absorptivity, reflectivity, thermal conductivity and heat transfer of the powder differ from those of the solid material, and the geometrical characteristics of the tracks change (Yadroitsev et al., 2013a,b). The solid material has a lower laser absorbance than the powder material (Yang, et al., 2018), and therefore, during the second laser scan, the partially melted powders absorbed more laser radiation, thus leading to complete melting and redistribution into the bulk material. In addition, the lowered temperature by the solid surface influences the penetration depth and width of the molten pool (Yadroitsev, et al., 2013b). These observations from previous investigations were confirmed by those obtained in the current study. The effect of this strategy is also illustrated in the SEM micrographs in Figure

3.38, showing the double layers before and after rescanning, with fewer to no satellites and greater surface homogeneity observed after rescanning.

Other thermo-physical differences to be considered are those between the Ti6Al4V substrate and the new powder material consisting of copper, which also interacts with the laser beam to form a single track. The Ti with Cu has a greater thermal conductivity and reflectivity, and a high concentration of Cu at the edges may increase the reflection coefficient of laser radiation (Vilardell, et al., 2020). Furthermore, in metals with low absorptivity in their flat and smooth state, such as Cu, the absorptance increases considerably with surface roughness (Kinnear, et al., 2016). The scan that follows passes over a relatively regular powder bed that leads to the formation of a larger molten pool and a larger denudation zone. This condition is repeated numerous times during the manufacturing of a layer and results in a variation of the penetration depth and track width (Vilardell, et al., 2020).

Homogeneity of Cu

Scanning electron microscopy with EDS elemental map analysis was performed to verify the distribution of Cu at the surfaces of the Ti6Al4V and cpTi alloys. It was revealed that the Cu was inhomogeneously distributed across all samples during the single scan (Figure 3.39). It is noteworthy that the Cu concentration appeared higher on cpTi surfaces than on Ti6Al4V surfaces, particularly after the single scan. Rescanning improved the distribution of the Cu in all the alloys by re-melting concentrations of Cu that formed at the boundaries of the single tracks. The Cu appeared most evenly distributed at the hatch distance of 80 μm , and no substantial difference was observed between 90 μm and 100 μm hatch distances after the single scan.

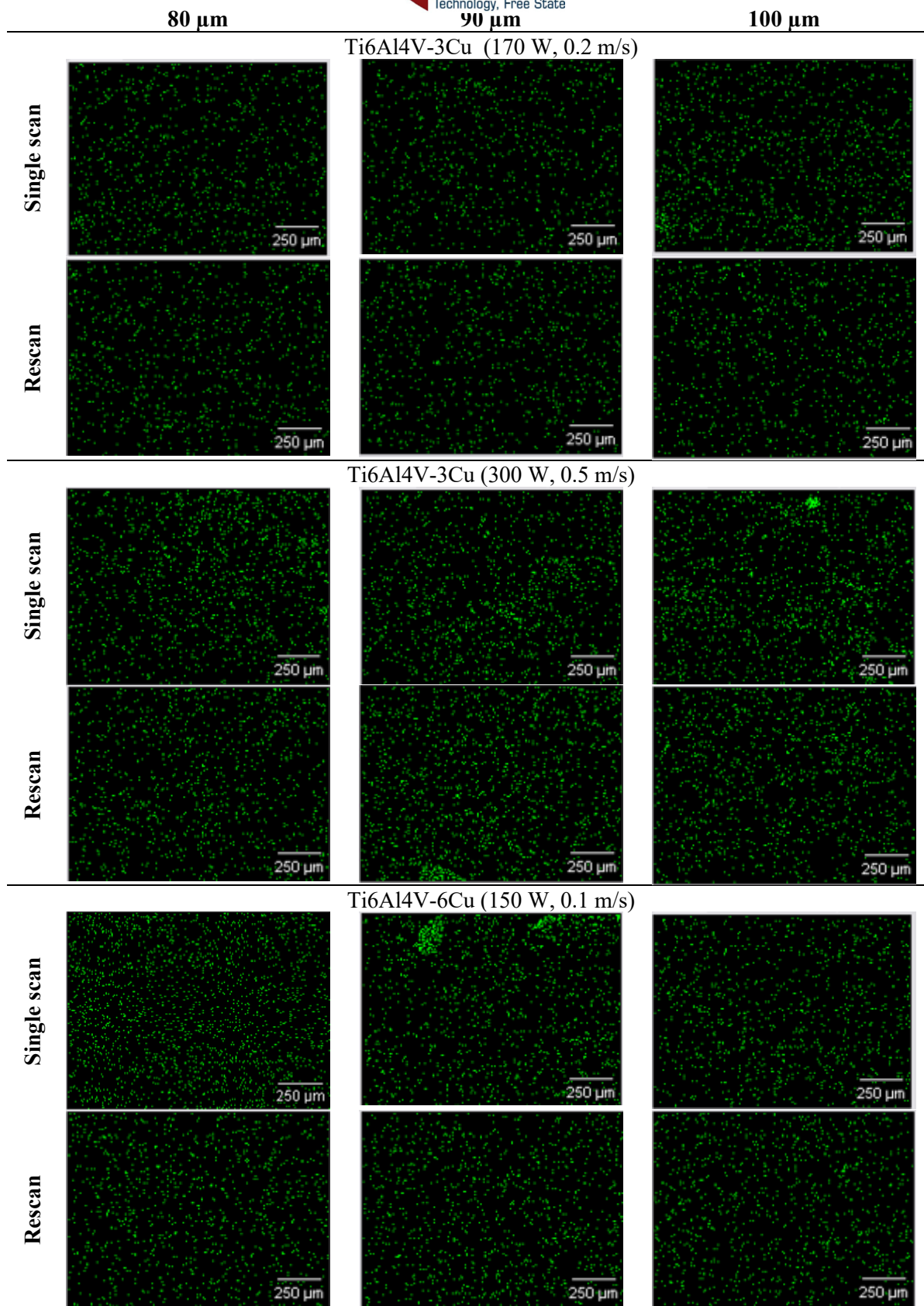


Figure 3.39: Cu distribution map by SEM-EDS spectra from various powder compositions

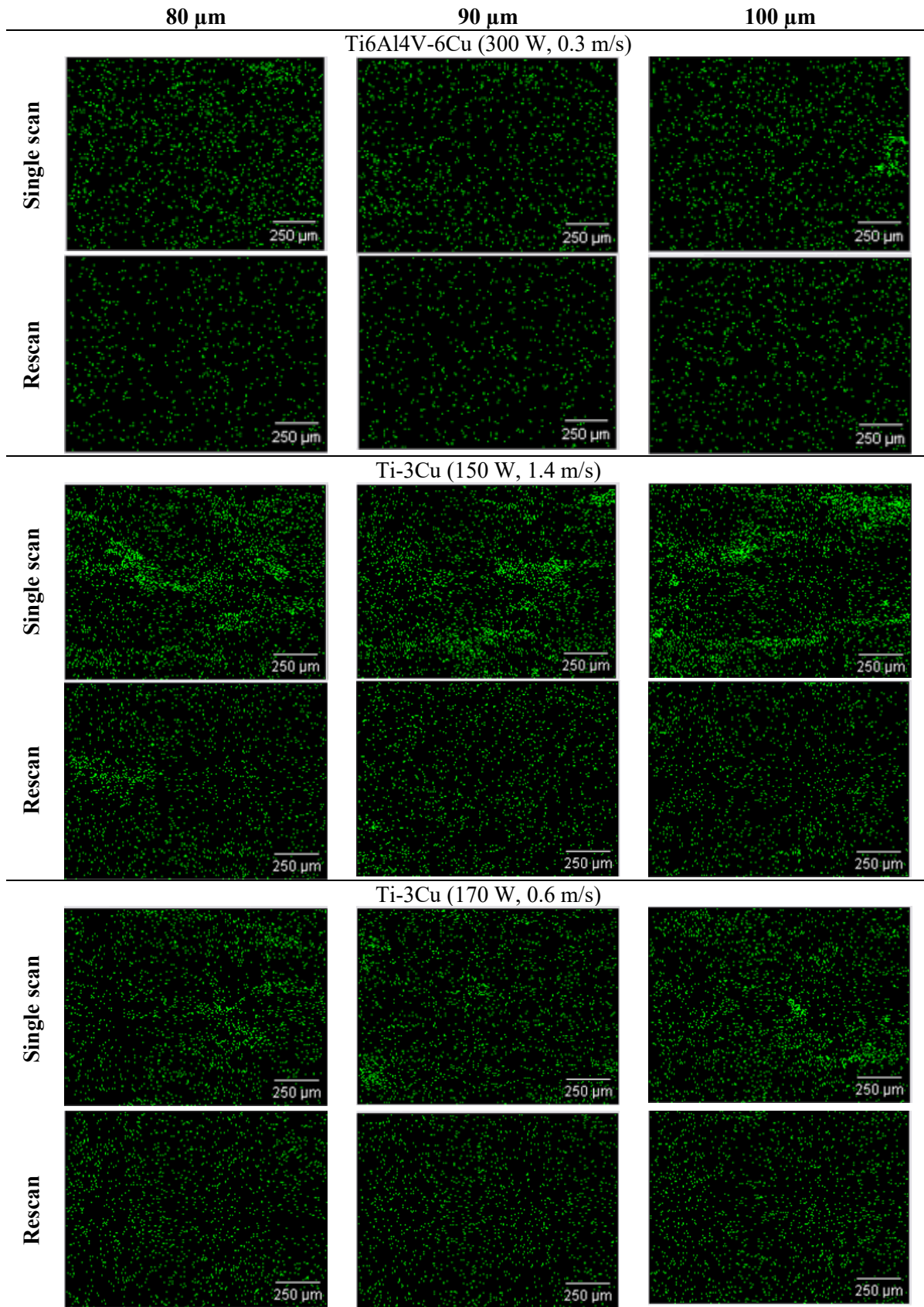


Figure 3.39: Cu distribution map by SEM-EDS spectra from various powder compositions (cont.)

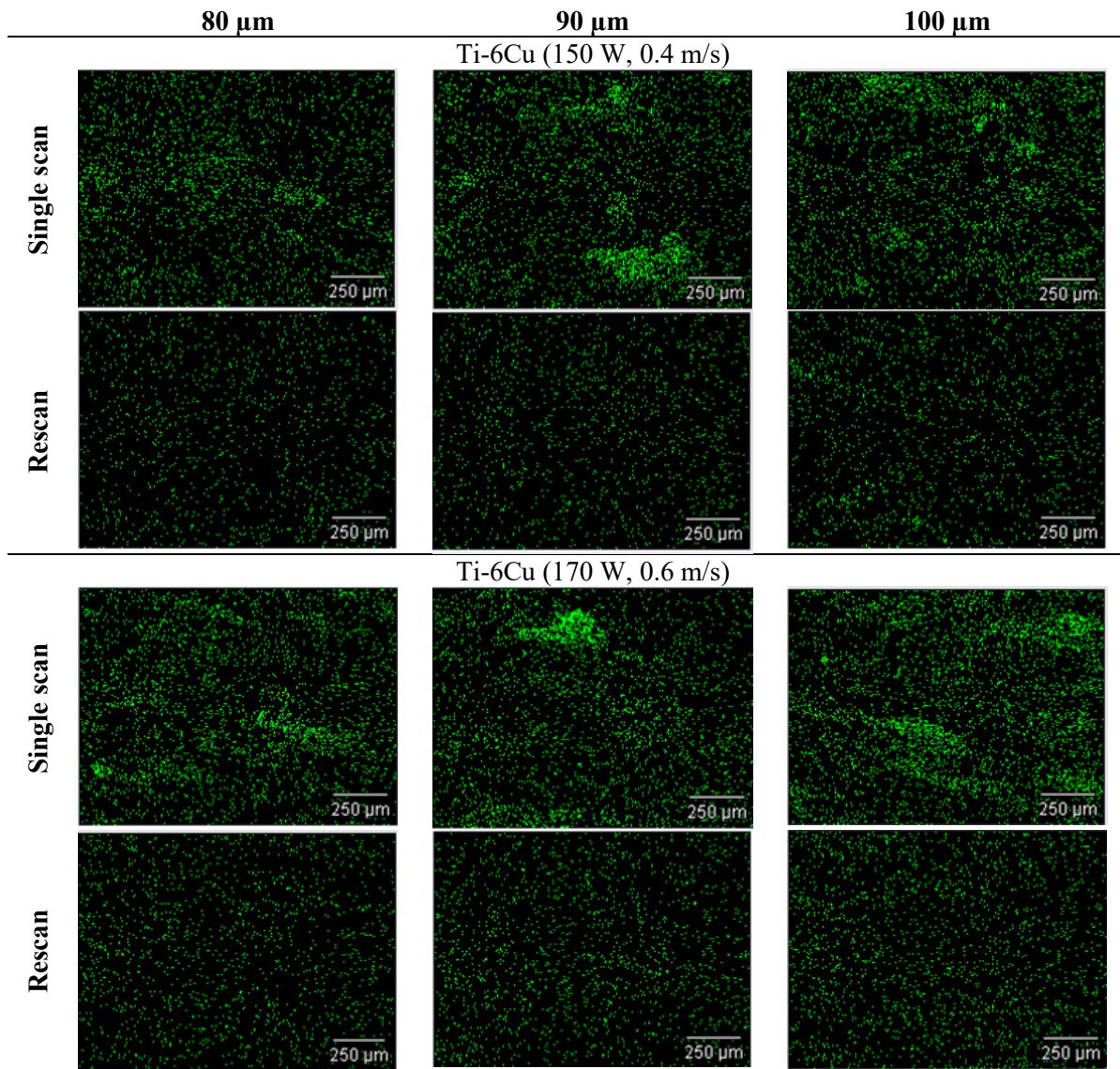


Figure 3.39: Cu distribution map by SEM-EDS spectra from various powder compositions (cont.)

Kinnear et al. (2016) and Krakhmalev et al. (2016) performed SEM-EDS analyses to assess the homogeneity of Cu in Ti6Al4V on the surface of a single layer manufactured from in situ alloyed Ti6Al4V-xCu material using the single and rescan strategies at various scanning speeds (0.7–1.3 m/s) and hatch distances (70–100 μm). The studies revealed that Cu homogeneity decreased as the scanning speed and hatch distance increased (that is, a decrease in energy input) under both scan strategies. A smaller hatch distance helps to re-melt a larger portion (40–50 % of single track width) of the previously manufactured single track, ensuring better in situ homogenisation of the material (Vilardell, et al., 2020). Nonetheless, changing from a single scan to a rescan strategy removed the majority of the Cu segregations that formed at the peripheries of the single tracks,

possibly due to the complete re-melting of the material by the repeating scan. Rescanning also enhances the Cu composition on the surface of a sintered layer. This variation in the Cu concentration is relatable to the vast deviation in the material properties of Ti materials and Cu, as illustrated in Table 3.2.

3.3.3.2. Cross-sectional analysis of double layers

The cross-sections of the double layers of the studied sample alloys are shown in Figure 3.40. Copper particles melted and repeatedly created islands of solidification lines of the material after the single scan, as noted in the Cu containing samples in Figure 3.40. Rescanning seemingly increased the re-melt depths of the double layers. This observation is in agreement with the study of Krakhmalev et al. (2017), during which the cross-sections of single layers revealed that the average re-melted depth was about 60–70 μm after a single scan, and the total re-melted depth into the substrate increased to approximately 110 μm after the rescan. At this depth, sufficient metallurgical bonding between layers to provide fully dense LPBF parts was achieved.

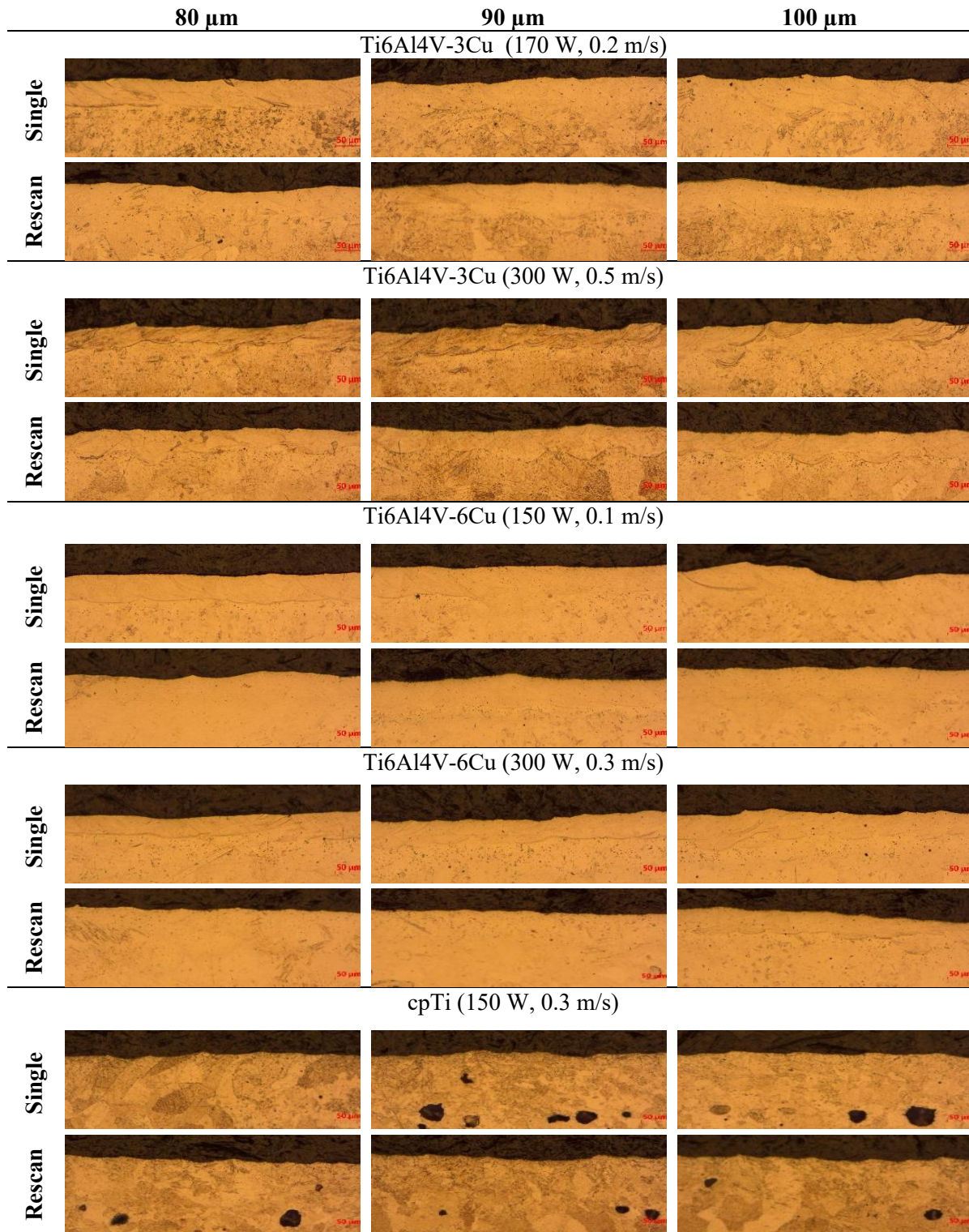


Figure 3.40: Cross-sectional view of LPBF double layers of Ti6Al4V and cpTi in situ alloys with Cu (scale = 50 μm)

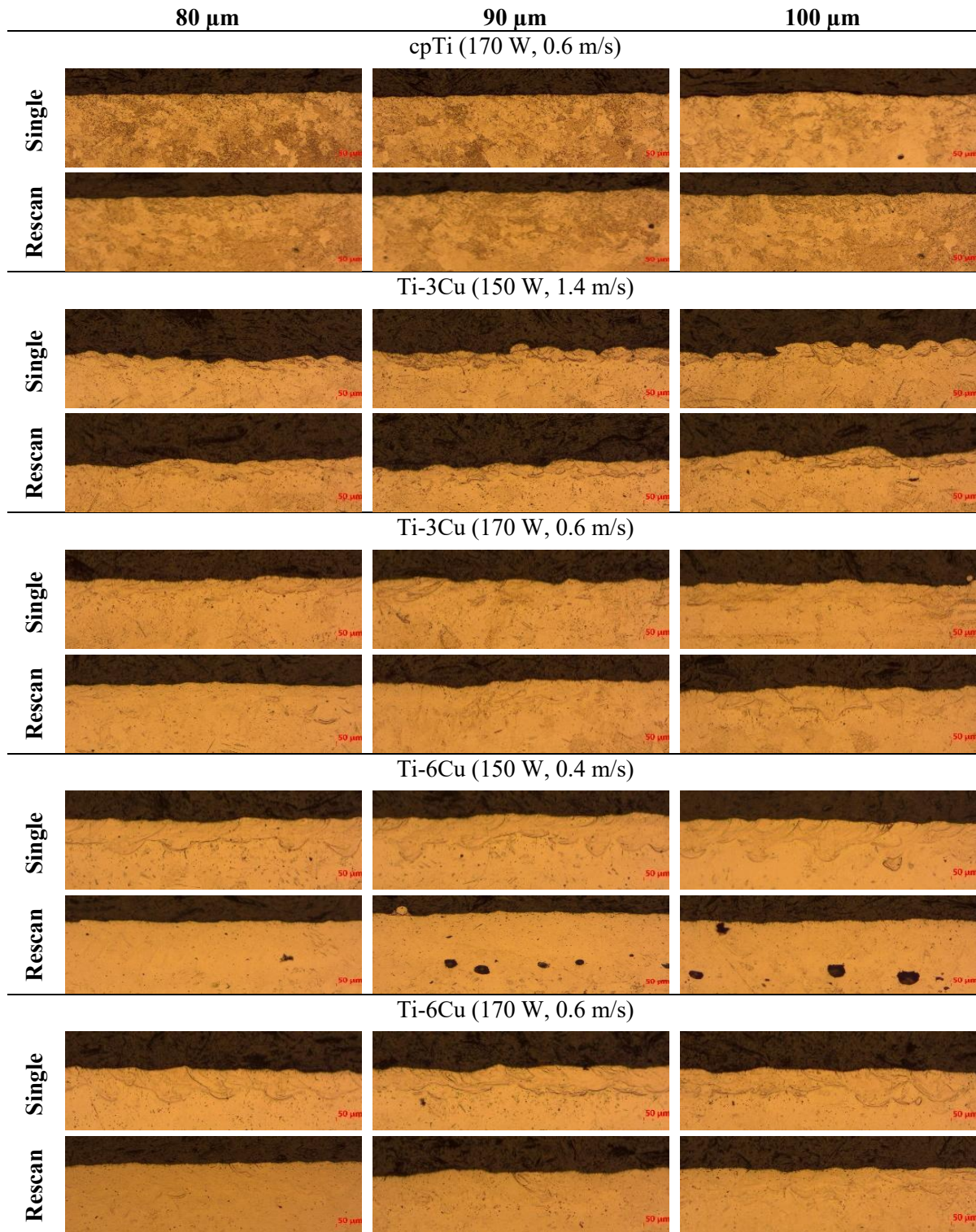


Figure 3.40: Cross-sectional view of LPBF double layers of Ti6Al4V and cpTi in situ alloys with Cu (scale = 50 μm) (cont.)

During the rescanning process, the previously rough surface would enhance laser radiation absorbance of the samples (Bergström, et al., 2007). The increase in laser absorptivity due to the rougher surface triggers both an increase in temperature and a more pronounced flow of the molten pool. During the cross-sectional analyses of the double layers by Ramosena et al. (2022), layers produced at a hatch distance of 80 μm showed better track-to-track overlap than those produced at 90 or 100 μm when different process parameters were applied. Furthermore, Kinnear et al. (2016) and Ramosena et al. (2022) found neither gaps between tracks nor delamination between layers at the various hatch distances during the production of single and double layers. Similarly, for the present study, defects such as porosity, delamination of layers and solidification cracks were not observed in LPBF double layers before and after rescanning for all powder compositions at a hatch distance of 80 μm . Therefore, this hatch distance was used to fabricate the 3D objects.

3.3.4. LPBF 3D objects

The line-by-line and layer-by-layer building process applied during LPBF manufacturing results in the microstructure of the built parts differing when viewed from different planes, forming the top, side, and front views (Thijs, et al., 2010). A commonly used coordinate system is the scanning plane XY in the Z build direction. During the LPBF process, the single scan tracks are arranged in the scanning direction, and the layers are arranged in the build direction (Yadroitsev, et al., 2010; Yadroitsev, et al., 2015). The 3D cubes produced for the present study on a Ti6Al4V substrate are presented in Figure 3.41.

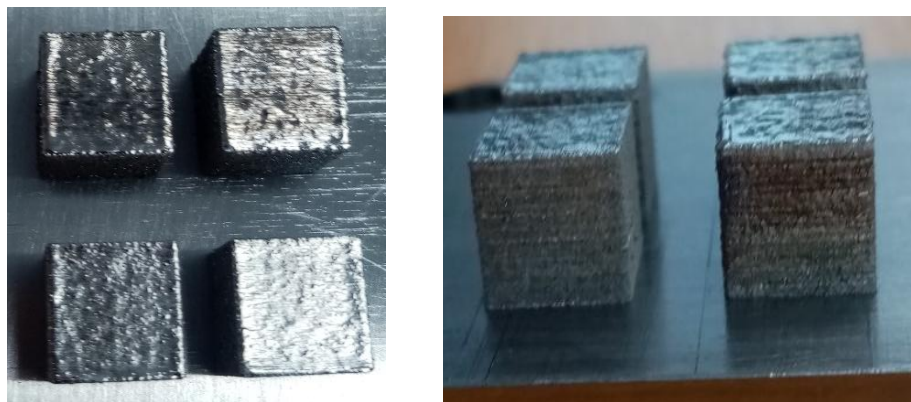


Figure 3.41: LPBF 3D objects manufactured from Ti64- and cpTi-based powders for optimisation process

3.3.4.1. Porosity

Porosity has a crucial impact on the final structural properties and performance. Defects (porosity-type) occurring in LPBF AM are primarily classified into three categories based on their formation mechanisms and physical characteristics: gas, keyhole and lack-of-fusion pores (du Plessis, 2019; Snell, et al., 2020; Jost, et al., 2021; Nudelis & Mayr, 2021). Gas pores are also known as metallurgical or hydrogen-induced pores because their formation is credited to either defects induced on surfaces of the inception powder particles when gaseous argon is entrapped during the atomisation process or the hydrogen readily entrapped in LPBF parts during the melting and solidification process (Gong, et al., 2014; Snell, et al., 2020). They are the smallest and most spherical of all pores, with diameters up to 100 μm , and are randomly distributed within the LPBF part (Aboulkhair, et al., 2014; Snell, et al., 2020; Ramosena, et al., 2022). Metallurgical pores are usually the most prevalent pore type in AM processes (Snell, et al., 2020), however, they are the least deleterious. Luo et al. (2022) prepared Ti6Al4V samples using LPBF followed by heat treatment to relieve residual stress. Their observations revealed internal pore size distributions consisting of small, spherical gaseous pores with equivalent diameters below 50 μm in all LPBF samples with no preferred locations. The researchers found that although there was a large number of these small pores, they made a negligible contribution to the overall porosity due to their small size. These gaseous pores accounted for an average of 60% of the total pores, but contributed only ~5–15% of the total pore volume of a sample. Moreover, Luo et al. (2022) found that defects smaller than 50 μm in diameter retained an elongation-to-failure of 12% and thus had no impact on the ductility of the material.

Keyhole (KH) pores are characterised by their near-spherical shape and pore diameters typically less than 100 μm . These pore types are caused by high input energy (high power, low speed and low hatch distance) resulting in excessive penetration of the metal powder during the melting process, which leaves a pore near the bottom of the melt pool after solidification because of the Marangoni effect (Snell, et al., 2020; Shrestha & Chou, 2022; Suryawanshi, et al., 2023). The resultant pore is a relatively large pore that is either circular horizontally and elongated vertically or has a wider top with a narrow bottom resembling a keyhole (Snell, et al., 2020). Keyhole pores form within the track boundary where the energy density is high and deep penetration melting occurs (Shrestha & Chou, 2022).

The so-called lack-of-fusion (LoF) porosity originates from insufficient overlap of successive melt pools caused by incomplete melting of powder particles and/or individual scan tracks (Tang, et al., 2017; Shrestha & Chou, 2022). Lack-of-fusion pores are caused by a low input energy (low power, high speed and high hatch distance) applied to the powder bed during the melting process, which fails to melt the metal powder fully and so leaves voids in the final part (du Plessis, 2019; Snell, et al., 2020; Zhao, et al., 2023). Lack-of-fusion pores show a highly irregular morphology and are usually larger in size, with diameters of above 100 μm (Xiao & Zhang, 2014; Aboulkhair, et al., 2019). The sizes of LoF pores may vary greatly depending on the process parameters (Shrestha & Chou, 2022). These pore types are the largest and least spherical (Snell, et al., 2020). Energy density levels below the LoF threshold may not successfully build parts due to severe thermal deformation (Gong, et al., 2014). Lack-of-fusion pores may be found to enclose non-molten powder particles (Aboulkhair, et al., 2019). Smearing or polishing during common metallographic techniques may obstruct the observation of the non-molten powder (Aboulkhair, et al., 2019). Keyhole and LoF defects may be influenced and controlled to a degree by process parameter optimisation, while the distribution of gas pores is mainly dictated by raw material quality (Jost, et al., 2021). Table 3.6 summarises the characteristics of the pore types form during the sintering procedure.

Table 3.6: Characteristics of pores commonly occurring in LPBF parts

Pore type	Gas pores	Keyhole pores	Lack-of-fusion pores
Cause	Trapped gas/moisture	Excess of input energy	Insufficient input energy
Size	<100 μm	<100 μm	>100 μm
Morphology	Spherical	Near-spherical, keyhole shape	Irregular
Sphericity	$\Psi > 0.6$	$\Psi > 0.6$	$\Psi \leq 0.6$
Orientation in the final part	Randomly distributed	Within track boundaries	Planar to the layers, in between tracks and layers

Since the formation of the KH and LoF pore is dictated by energy density, there should be a correlation between energy density and pore frequency. An increase in energy density should increase the occurrence of KH pores and decrease the number of LoF pores (Snell, et al., 2020). Part density (or porosity) is typically measured experimentally with respect to energy density

(Tang, et al., 2017). While linear energy density (P/V) is effective when analysing the melt pool geometry of single tracks, volumetric energy density (VED) has been shown to have a direct effect on the mass density of the parts (Majumdar, et al., 2019; Khaimovich, et al., 2022; Shrestha & Chou, 2022). A correlation between VED values and internal porosity in the final parts has been established, demonstrating the possibility of minimising residual porosity should deposition take place within a precise VED window (Gong, et al., 2014; Kasperovich, et al., 2016; Bertoli, et al., 2017). The volumetric energy density describes the amount of energy delivered per unit volume of powder deposited on the bed, and is expressed as a ratio between laser power (P , in J/s), scanning velocity (V , in m/s), hatch distance (h , in m) and layer thickness (t , in m) (Kasperovich, et al., 2016; Bertoli, et al., 2017):

$$VED = \frac{P}{Vht}, [J/m^3] \quad \text{Equation 3.2}$$

Volumetric energy density affects the melting pool, and Bertoli et al. (2017) demonstrated that values below $100 J/mm^3$ are insufficient to fully melt an alloy. Volume energy is currently the most established measure to quantify continuous wave laser sources and is employable to analyse and adjust the process-material relationships (Kasperovich, et al., 2016). The dominant parameter in determining pore density, size, and shape is laser power, followed by scanning speed and hatch spacing. Thus, the variation of pore properties is related to the power and volume energy. In general, with increasing power and volume energy, pore density and mean diameter decrease and sphericity increases (Zhao, et al., 2023).

The sphericity, Ψ , is defined by (Wadell, 1935) as:

$$\Psi = \frac{\text{surface area of the volume equivalent sphere}}{\text{surface area of the particle}} = \frac{\pi d_{eq}^2}{SA} = \frac{\sqrt[3]{36\pi V_p^2}}{SA} \quad \text{Equation 3.3}$$

where d_{eq} is the diameter of the sphere having the equivalent volume as the particle, V_p is the volume of the particle, and SA is the surface area of the sphere. The measure of sphericity approximates the degree to which the shape of a particle approaches the shape of a sphere and is independent of the particle's size (Cruz-Matías, et al., 2019). Sphericity values range from 0 to 1. The maximum value of one corresponds to a particle with a perfectly spherical shape. The closer

the sphericity value is to 1, the better the sphericity of that shape, and it becomes closer to 0 for a more concave, elongated or irregular shape of the void (Bagheri & Bonadonna, 2016; Kasperovich, et al., 2016; Li, et al., 2024). For sphericity, standards for the criteria of spherical and irregular shapes were not discovered during the current project and values in literature were open to interpretation (Snell, et al., 2020). Snell et al. (2020) and Nudelis & Mayr (2021) identified defects with a sphericity of 0.6 or less as LoF pores and classified those with a sphericity above 0.6 as gas/KH pores. Thus, these criteria were applied to describe the sphericity of defects identified in this study.

Figures Figure 3.42–Figure 3.46 illustrate CT scans of the pore distribution within the test samples. The typical pore size in as-built Ti6Al4V ELI samples ranged from 30 to 200 μm during the study of Yadroitsev et al. (2018). Zhao et al. (2023) investigated the relationships between printing parameters, pore properties and porosity in 316L stainless steel samples additively manufactured by LPBF. The results of their study showed that 65 W and 43.33 J/mm^3 volume energy were insufficient to liquefy the previous layer and the hatch line, which led to pores forming due to incomplete binding of the powder layer or hatch line. These pores were large, aligned parallel to layers or hatch lines, and exhibited elongated voids with a sphericity of 0.37 (LoF porosity). When Zhao et al. (2023) increased the power to 85 W and the volume energy to 56.67 J/mm^3 , smaller, rounder KH pores formed, with a sphericity of 0.56. Baumgärtner et al. (2023) observed a decrease in pore diameter with an increase in the measured pore sphericity, which was ascribed to the rounding effect and a reduction in LoF pores.

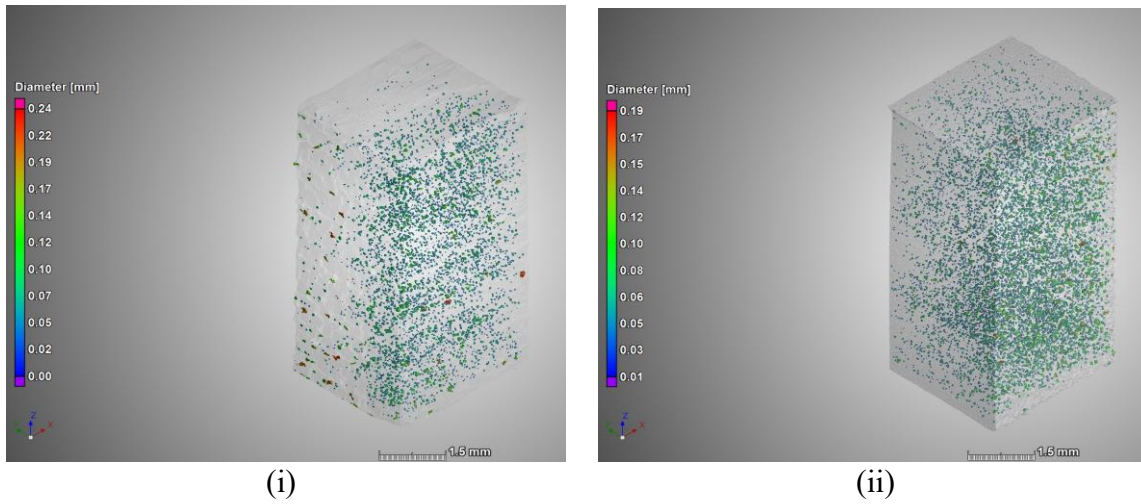


Figure 3.42: Porosity nanoCT scans of Ti6Al4V-3Cu samples produced at (i) $P = 170$ W, $V = 0.2$ m/s ($VED = 212.5$ J/mm³), and (ii) $P = 300$ W, $V = 0.5$ m/s ($VED = 150$ J/mm³). Building direction is along the Z axis

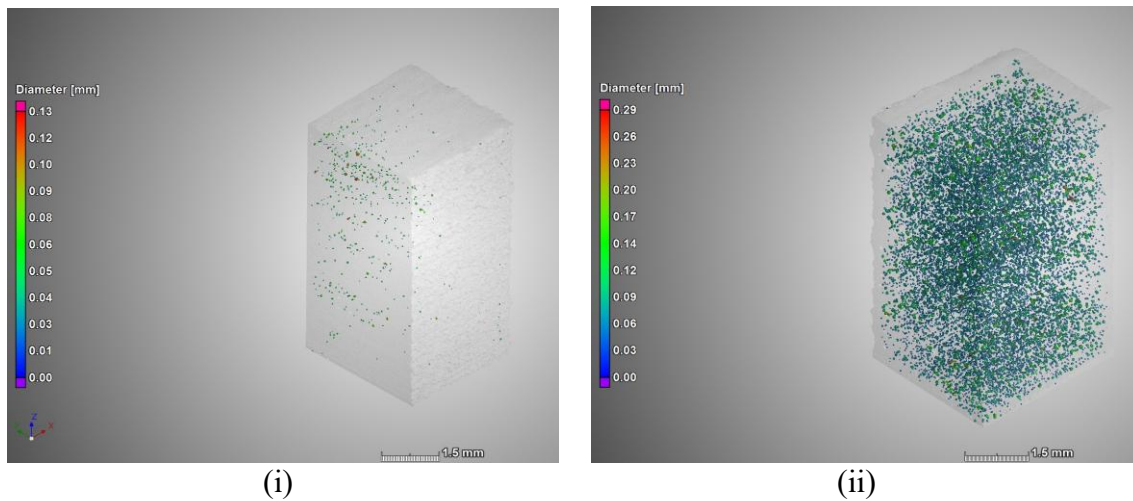


Figure 3.43: Porosity nanoCT scans of Ti6Al4V-6Cu samples produced at (i) $P = 150$ W, $V = 0.1$ m/s ($VED = 375$ J/mm³), and (ii) $P = 300$ W, $V = 0.3$ m/s ($VED = 250$ J/mm³). Building direction is along the Z axis

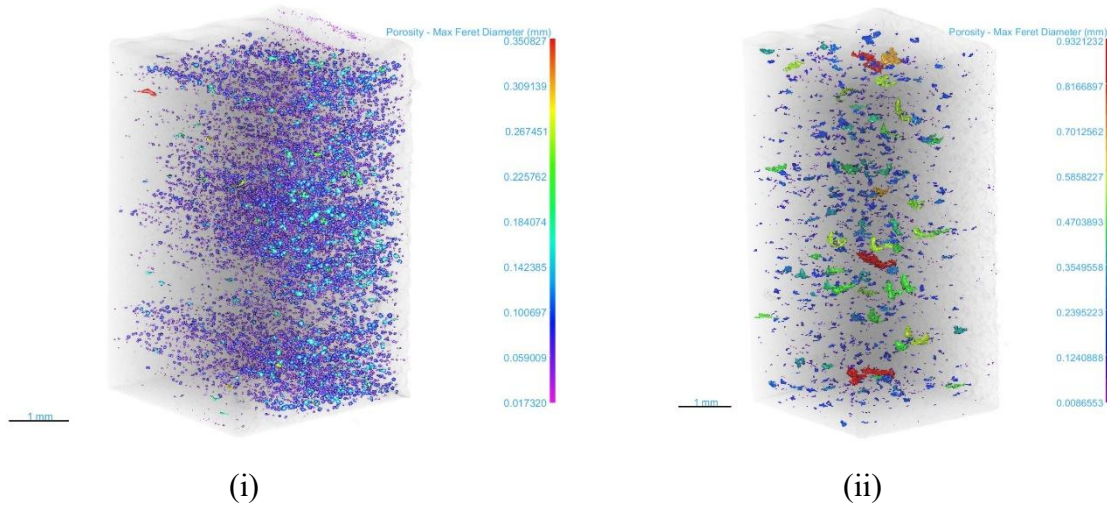


Figure 3.44: Porosity microCT scans of cpTi samples produced at (i) $P = 150$ W, $V = 0.3$ m/s ($VED = 125$ J/mm³), and (ii) $P = 170$ W, $V = 0.6$ m/s ($VED = 70.8$ J/mm³). Building direction is along the Z axis

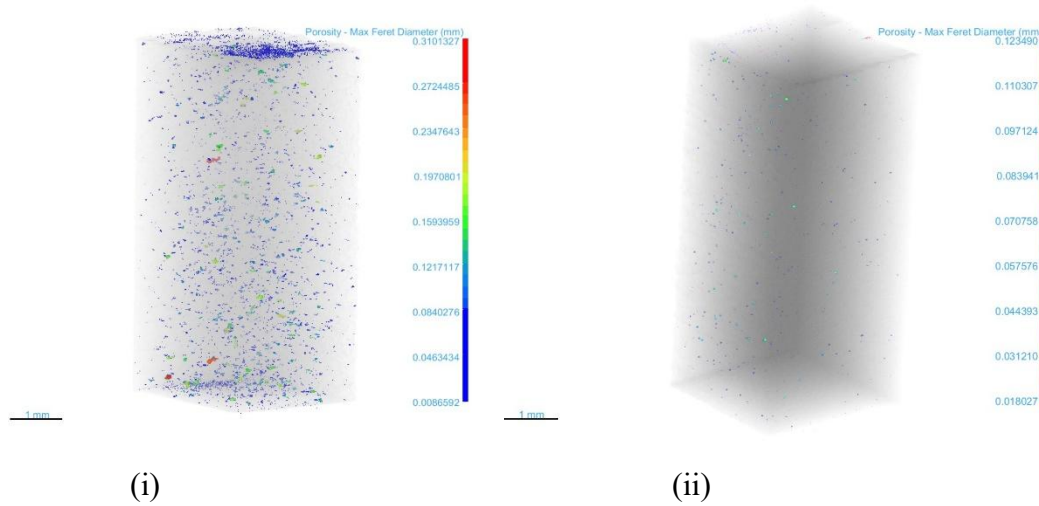


Figure 3.45: Porosity microCT scans of Ti-3Cu samples produced at (i) $P = 150$ W, $V = 1.4$ m/s and ($VED = 26.8$ J/mm³), and (ii) $P = 170$ W, $V = 0.6$ m/s ($VED = 70.8$ J/mm³). Building direction is along the Z axis

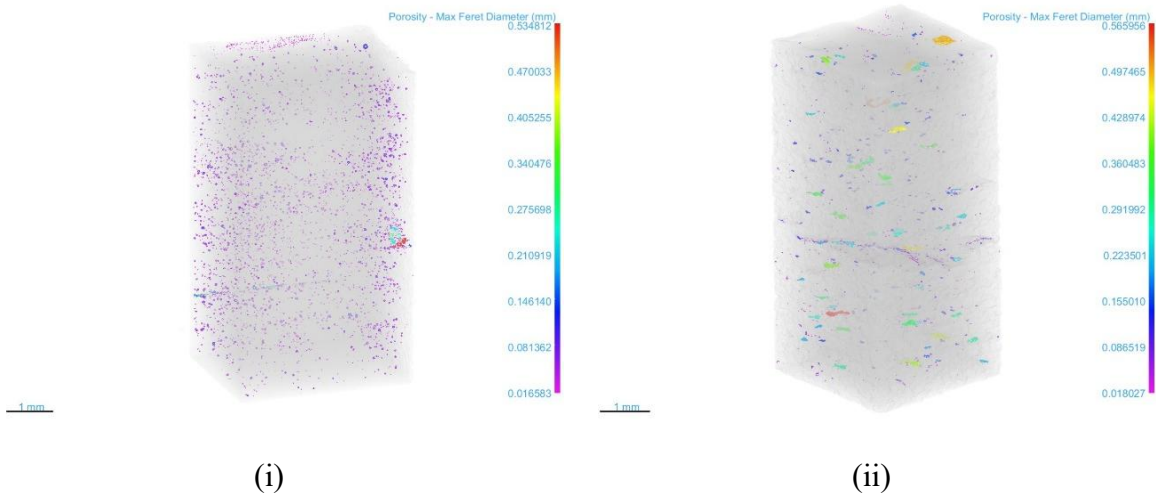


Figure 3.46: Porosity microCT scans of Ti-6Cu samples produced at (i) $P = 150$ W, $V = 0.4$ m/s and ($VED = 93.8$ J/mm³), and (ii) $P = 170$ W, $V = 0.6$ m/s ($VED = 70.8$ J/mm³). Building direction is along the Z axis

The CT scanning results are tabulated in Table 3.7, and it is evident from the values of the cpTi-based samples that the same VED did not produce the same volume of defects across the different samples. Also, when comparing each pair consisting of the same powder composition processed using the two different parameters, it may be seen that those with more defects did not necessarily contain defects with the highest diameter or lowest sphericity. Hence, the importance of analysing not only the overall quantity of pores, but also the morphology of the pores. It is noteworthy that there was generally a low positive correlation ($r = 0.2532$) between the diameter and sphericity of all test samples, indicating that the two did not have a strong relationship. Also noteworthy is that the VED for samples produced with cpTi powder was generally significantly ($p = 0.0261$) lower than that for samples produced with Ti6Al4V powder. From FiguresFigure 3.42–Figure 3.46 and Table 3.7, it may be concluded that samples mainly consisted of metallurgical and/or KH pores. The samples manufactured by Gong et al. (2014) through SLM of pre-alloyed Ti6Al4V powder experienced rapid growth in porosity at a laser power of 160 W and scanning speed of below 600 mm/s (high energy density) and at a laser power of 40 W and scanning speed of 1200 mm/s (low energy density). There was no apparent porosity at 160 W when the scan speed was between 600 and 1600 mm/s, and at 80 W for approximately 600 mm/s. Consequently, since high and low energy density produce KH and LoF pores, respectively, the minimal porosity volume in most of

the current test samples indicates that porosity may be attributed to metallurgical pores. This observation is further supported by the predominantly random distribution of pores as shown in FiguresFigure 3.42–Figure 3.46, which is characteristic of gas pores.

Table 3.7: Defect analysis of Ti6Al4V and cpTi test samples by CT scans

Sample (P in W, V in m/s)	Defect characteristics					
	Material volume (mm ³)	Defect volume (mm ³)	Defect volume ratio (%)	Diameter <100 μ m (%)	Sphericity >0.6 (%)	VED (J/mm ³)
Ti6Al4V- 3Cu (170, 0.2)	97.83	0.20	0.20	96.90	62.77	212.50
Ti6Al4V- 3Cu (300, 0.5)	96.84	0.44	0.45	98.15	75.78	150
Ti6Al4V- 6Cu (150, 0.1)	104.86	0.01	0.01	98.59	60.92	375
Ti6Al4V- 6Cu (300, 0.3)	115.64	0.98	0.84	94.54	66.44	250
cpTi (150, 0.3)	90.31	0.55	0.60	96.01	95.39	125
cpTi (170, 0.6)	93.88	0.26	0.27	87.92	79.14	70.83
Ti-3Cu (150, 1.4)	74.28	0.05	0.06	99.46	98.34	26.79
Ti-3Cu (170, 0.6)	71.04	0.005	0.01	99.86	97.41	70.83
Ti-6Cu (150, 0.6)	104.66	0.05	0.04	99.54	94.71	62.50
Ti-6Cu (170, 0.4)	81.35	0.05	0.06	88.72	78.54	106.25

At high-energy-density conditions, Luo et al. (2022) observed intermediate-sized pores with diameters of 50–200 μ m in Ti6Al4V samples and identified them as KH pores. The KH porosity was designated as the main contributor to porosity in the high energy density regime and was shown to result in reduced elongation and strength of material compared to material processed in dense regimes (Luo, et al., 2022). Large defects with large diameters from 200 μ m to over 500 μ m were observed from two processing regimes, namely: those with insufficient energy input for producing dense samples and those with excessively high energy input, where bead formation leads to irregular defects. The consequence of these two regimes is evident in the lack of fusion

among melt pools. Vilardell et al. (2020) microCT results showed that the maximum pore size was approximately 400 μm , and was found at the bottom part of the sample produced with a laser power of 170 W and scanning speed of 0.9 m/s. Samples produced at a higher laser power of 340 W had less porosity in comparison with the ones produced at 170 W. Their small pores were spherical in shape and were identified as possibly metallurgical pores (du Plessis, et al., 2018b). Porosity values of the current samples did not reach 400 μm diameter, and thus the occurrence of LoF pore was either non-existent or minimal, which is evident with the low volumes of defects. Suryawanshi et al. (2023) employed the EOSINT M 280 system to print samples from Ti6Al4V ELI powder and found that the samples mostly exhibited porosities due to gas entrapment and lack of fusion. Their detailed porosity analysis using X-ray showed that for optimal *VED* of 37–93 J/mm^3 , the porosity fraction was less than 1%. The porosity fraction for all the current test samples was also below 1% at the various *VED* values.

Shrestha & Chou (2022) investigated the formation of pores at different energy densities during LPBF and found that porosity decreased as the *VED* decreased to a certain level and attributed this observation to a change in the melting mode from keyhole to conduction. Shrestha & Chou, (2022) also observed that the formation of LoF pores caused a further decrease in the number of pores due to a lower energy. Conversely, because of the larger size of LoF pores, the pore volume was higher at low energy density. Therefore, the lowest energy density resulted in considerably large, interconnected pores, which increased the pore volume, although the pore count decreased (Shrestha & Chou, 2022). Similarly, for this study, the Ti6Al4V-based samples with the highest *VED* of 375 J/mm^3 produced the least %volume of defects. In contrast, sphericity values in the study by Kasperovich et al. (2016) revealed irregular shapes at *VED* = 292 J/mm^3 , resulting in a broad distribution of lower sphericity values within the 3D structure. The researchers investigated the influence of SLM parameters on the type and amount of porosity formed in TiAl6V4 to define a process window for structural components.

3.3.4.2. Microstructure

Studying the microstructure of LPBF printed parts is essential in predicting their mechanical properties (Suryawanshi, et al., 2023). The microstructure images obtained using the optical

microscopy after etching with Kroll's reagent, as captured from the transverse and longitudinal sections of the sectioned 3D objects, are illustrated in Figure 3.47.

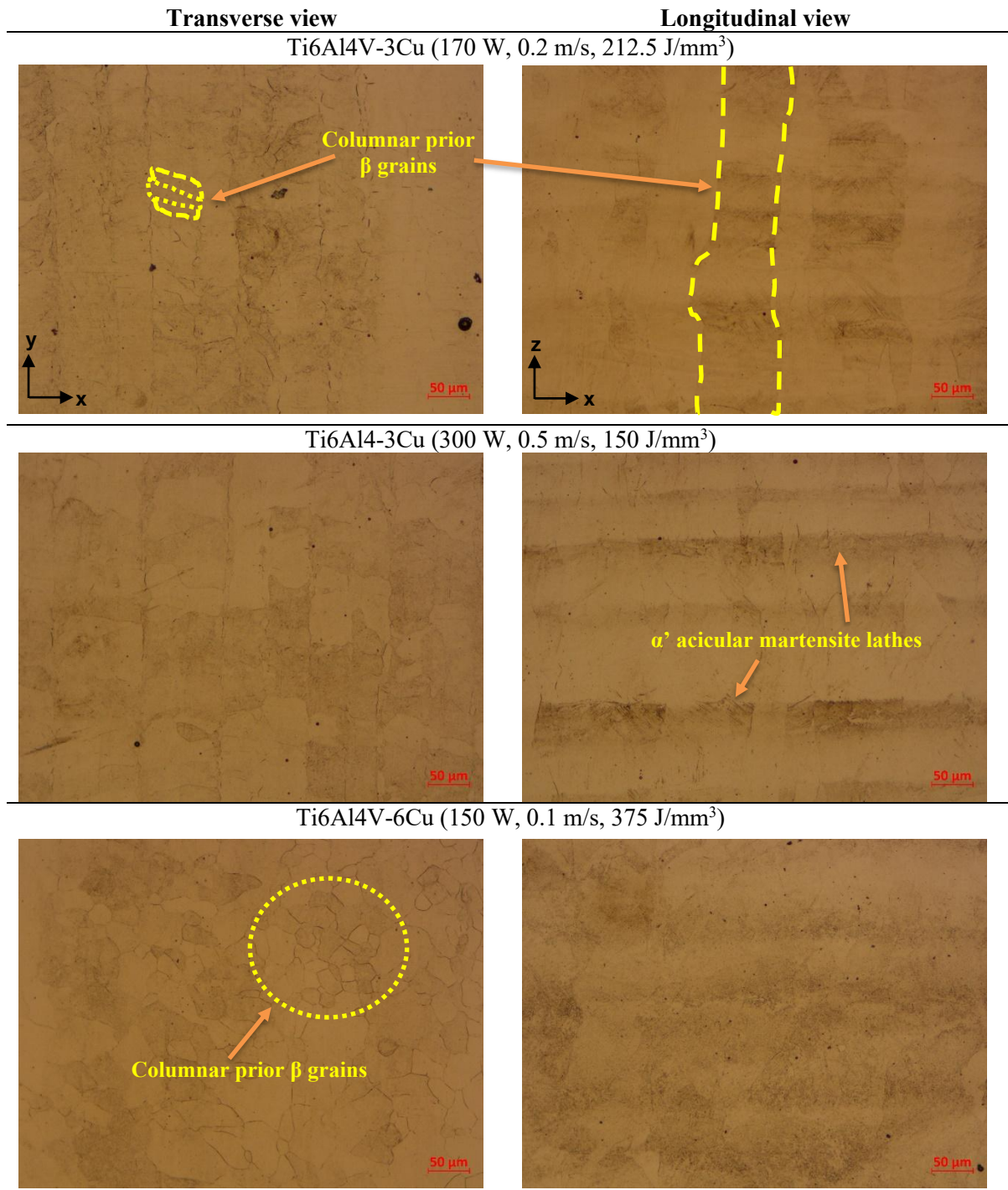


Figure 3.47: Microstructure of cross-sections of the 3D LPBF samples

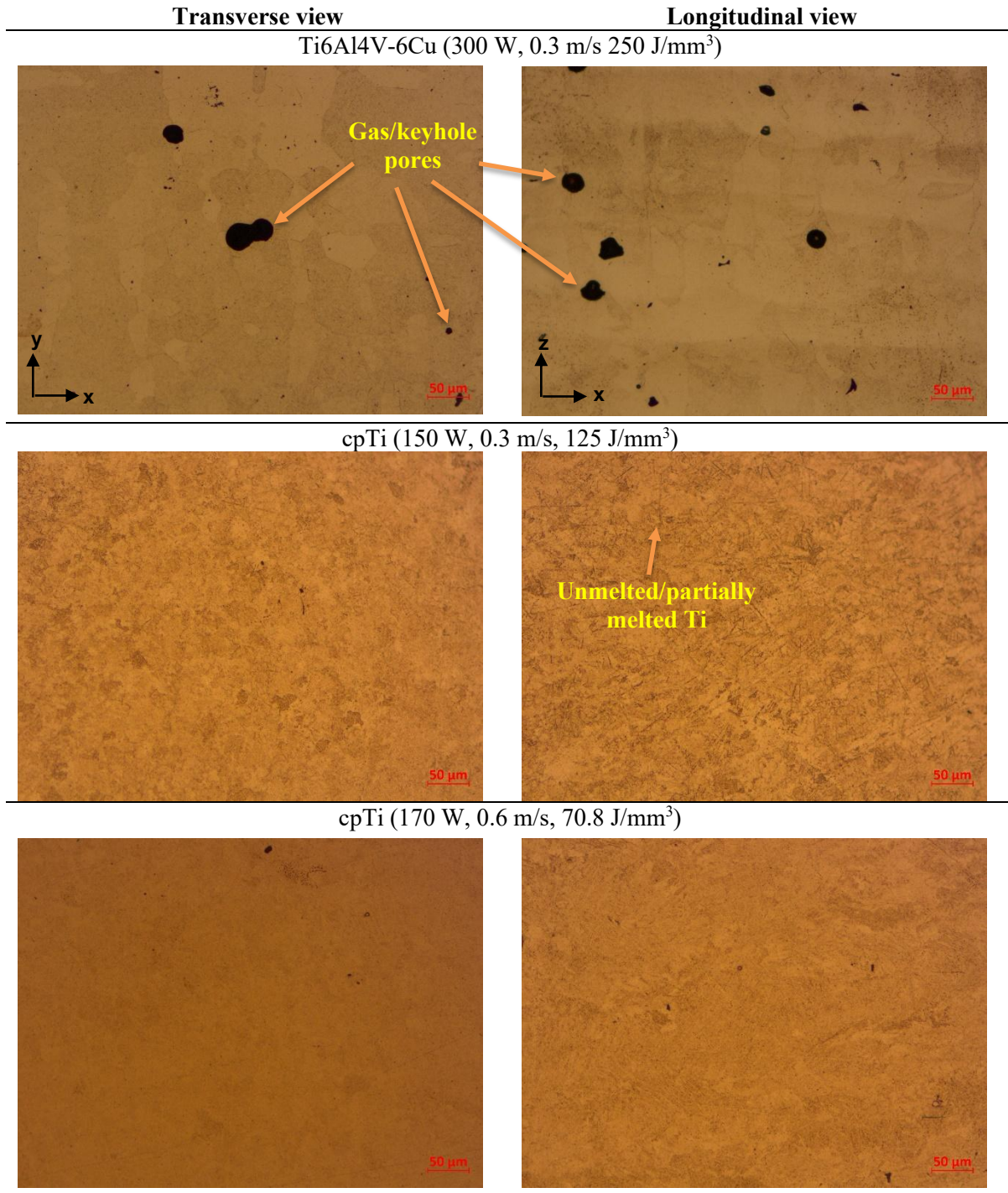


Figure 3.47: Microstructure of cross-sections of the 3D LPBF samples (cont.)

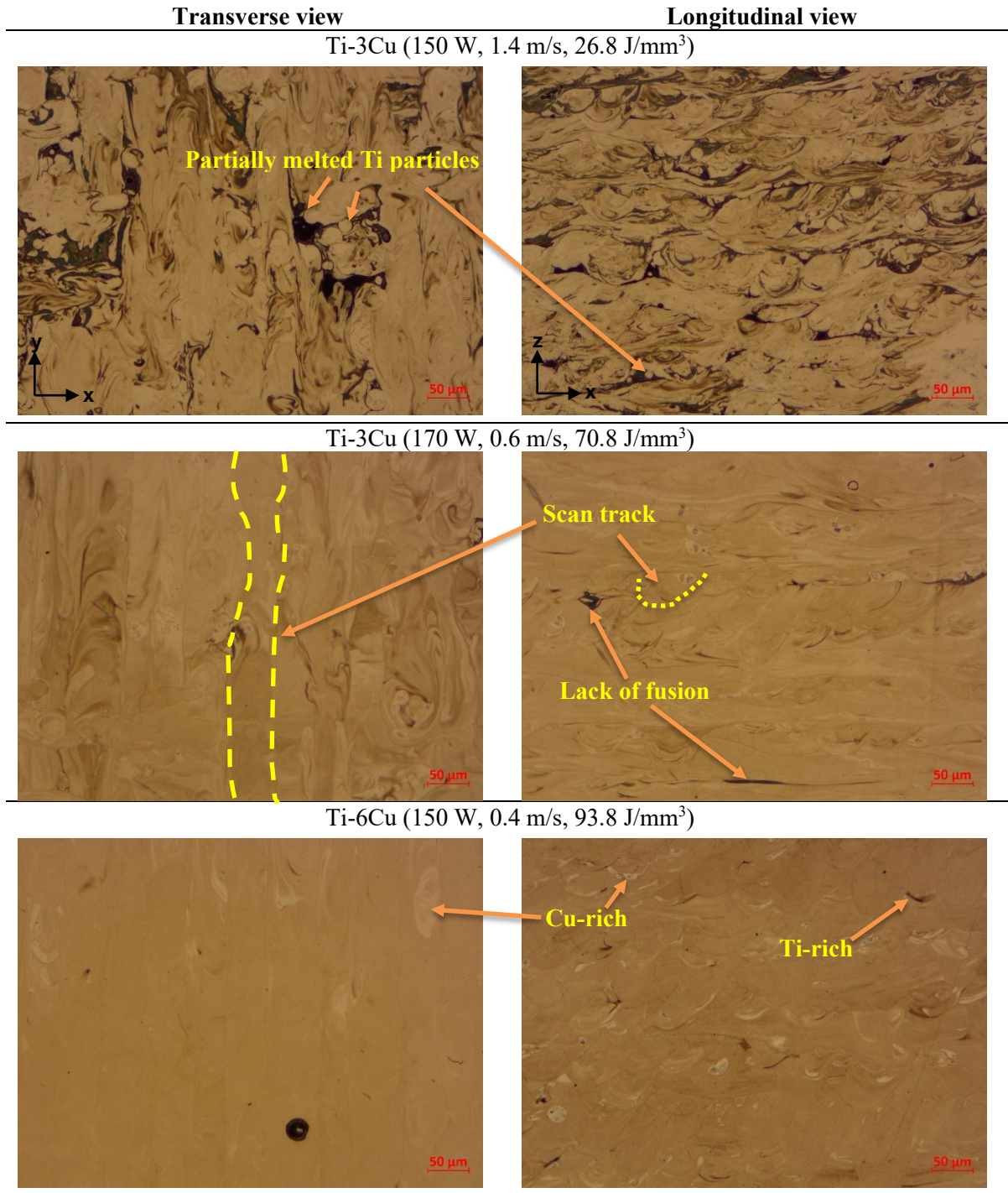


Figure 3.47: Microstructure of cross-sections of the 3D LPBF samples (cont.)

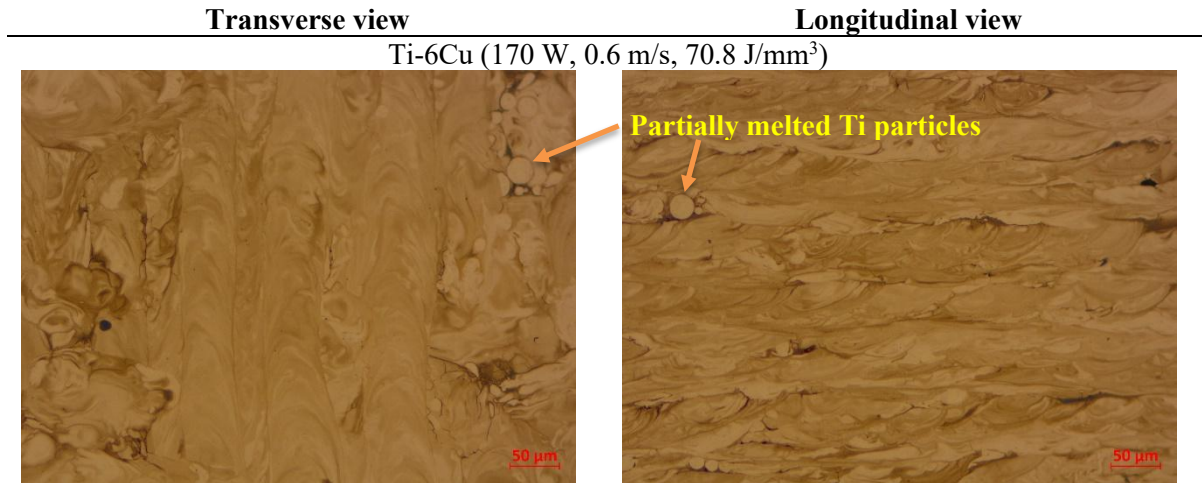


Figure 3.47: Microstructure of cross-sections of the 3D LPBF samples (cont.)

The microstructure of Ti6Al4V specimens exhibited well-defined grains at the transverse plane. Boundaries of vertical colonies may be seen on the specimens of Ti6Al4V-3Cu and Ti6Al4V-6Cu. Micrographs for Ti6Al4V-3Cu and Ti6Al4V-6Cu reveal fine laths of α' acicular martensite woven inside columnar prior- β grains. Likewise, Yadroitsev et al. (2018) found that the microstructure of as-built Ti6Al4V ELI consisted of metastable structures of α' hexagonal martensite with pronounced prior β -texturisation without β precipitations. The prior β -phase showed prominent columnar growth, while the texture of the hexagonal α' -phase was weak. A needle-like morphology is characteristic of the hexagonal α' martensite phase observed in Ti alloys after the LPBF process due to the rapid solidification of the liquefied metal pool (Simonelli, et al., 2018; Dzogbewu, 2020a). This martensitic morphology reduces the ductility of the LPBF-manufactured samples and induces undesirable factors such as residual stress (Dzogbewu, 2020b; Vilardell, et al., 2020).

According to Zhao et al. (2016), the as-fabricated microstructure of laser-melted Ti6Al4V parts is dominated by columnar β grains and α' martensite due to the rapid cooling rate. Ramosena et al. (2022) also credited the high cooling and solidification rates (up to 10^8 K/s) for the formation of a binary ($\alpha+\beta$) microstructure, consisting of acicular martensitic α' laths within columnar prior- β grains, of LPBF Ti6Al4V pre-alloy. Vilardell et al. (2020) identified α' acicular martensite phase on SEM micrographs of the Ti6Al4V (ELI)-3 at.% Cu microstructure consisting of finer needles as compared to the martensite observed in Ti6Al4V (ELI) manufactured using a similar LPBF

process. This was ascribed to differences in solidification rates resulting from Cu alloying and varying process parameters (Krakhmalev, et al., 2016). The microstructural analysis of Vilardell et al. (2020) exemplified chemical inhomogeneity with Cu exhibiting a trend of segregation at the bottom of layer fusion boundaries. This trend was more pronounced in materials manufactured with a laser power of 170 W than of 340 W. In this study, Cu segregation to the bottom of the layer was observed in Ti-Cu samples.

Unmolten Ti particles were most detectable on optical micrographs obtained for Ti-3Cu and Ti-6Cu microstructures. In addition, the grain structures of deposited samples were not clear, and the expected α/α' acicular grains and prior- β columnar grains were not clearly delineated. Alternatively, eddy regions that etched at different rates are shown in the micrographs (Dzogbewu, 2020b). This suggests substantial variations in the constituents resulting in elemental segregation in the molten pool (Simonelli, et al., 2018), particularly since unmolten particles were not visible on the cpTi surfaces, indicating a sufficient melting of the powder-bed. The incomplete melting of Ti powder particles is associated with the thermo-physical differences (Table 3.2) between the two elemental powders. Inhomogeneity in chemical dispersion is likely to have caused over-etching in some areas of the microstructure (Simonelli, et al., 2018). It may be noted that the Ti-3Cu specimen fabricated using the lowest *VED* of 26.8 J/mm³ was the most inhomogeneous, containing the most unmolten particles of all test samples, which is in line with the findings of Vilardell et al. (2020), who observed that when the laser power was elevated, the microstructure of single layers was more homogeneous.

The mixing and dispersion of partially melted Ti particles in the solidified melt pool may be associated with the convectional flow in the liquid melt pool (that is, the Marangoni flow) (Kou, et al., 2011). The solid melt pool with a convective melt flow pattern is attributed to the intrinsic non-uniform distribution of the Gaussian laser beam while it melts the metallic powders, leading to a non-uniform temperature gradient in the molten pool (Dzogbewu, 2020b). The temperature gradient in the molten pool governs the hydrodynamic movement of the liquid metal and induces recirculation of flow within the molten pool, resulting in the random dispersion of partly melted Ti particles within the molten network (Dzogbewu, 2020b). Körner et al. (2011) and Körner et al. (2013) alluded that the complexities of the Gaussian laser beam melting process are dependent on

factors such as laser beam absorption, Marangoni flow, viscosity, surface tension, capillary effects, gravity, and so forth, which results in stochastic melt flow of the molten liquid and the resultant random distribution of the partially melted Ti particles in the solidified alloy (Dzogbewu, 2020b).

Based on the experimental observations and the thermo-physical properties of the powders listed in Table 3.2, the mechanisms leading to the formation of the molten pool may be postulated. As the laser beam scans the powder bed during LPBF, the Ti particles readily absorb the laser radiation. The laser absorptivity of Ti (at 0.77) is higher than that of Cu (at 0.59) (Tolochko, et al., 2000), hence Ti would absorb more of the laser radiation than Cu. The absorbed energy heats the Ti powder particles. Through conduction (and convection through the gas bubbles entrapped between the loosely packed powder particles), the Cu powder particles absorb the heat from the surrounding Ti particles. As the heat homogenises, the transferred heat reaches those Cu particles which were shielded from the full radiation intensity of the laser beam due to the gravitational pull to the bottom of the molten pool, since the density of Cu at approximately 8900 Kg/m^3 is double that of Ti and its alloys at approximately 4500 Kg/m^3 . The elevated temperatures and the homogenisation of the heat cause the surfaces of the powder particles to melt, and a molten pool is accordingly expected to form in the laser-irradiated region.

The operating temperature of the Cu particles is likely to rapidly increase since Cu has a considerably higher thermal conductivity of $350 \text{ W/m}\cdot\text{K}$ when compared to Ti and its alloys, with a thermal conductivity of below $22 \text{ W/m}\cdot\text{K}$. Thus, the rate of heat conduction among Cu particles should be faster than among Ti particles. Moreover, the liquidus temperatures of the Ti6Al4V and cpTi powders ($\sim 1660^\circ\text{C}$) are higher than that of the Cu powder ($\sim 1083^\circ\text{C}$). This may translate to the complete melting of Cu powder particles if the rate of solidification is monitored to occur gradually, leading to a homogenous microstructure (Dzogbewu, 2020b). The LPBF high cooling and solidification rates of up to 10^8 K/s (Ramosena, et al., 2022) may result in incomplete melting of the Ti particles at the end of the solidification stage. The rapid thermal cycles in the process chamber of the LPBF machine may also contribute to the partial melting of Ti particles (Roberts, et al., 2009). There was no clear correlation between the homogeneity of molten powders and the Cu quantity or the applied parameters.

Ye et al. (2024) distinguished two distinct phases from SEM images of LPBF Ti–4Cu alloy: a large amount of acicular α -Ti phase (darker region) and a small amount of Ti₂Cu (bright region), and the grain size of α -Ti generally increased with the increase of *VED*. These findings were supported by Qin et al. (2018) and Mosallanejad et al. (2021), who studied LPBF Ti-5Cu alloy. In addition to the five-times larger particle size of pure Ti than Cu, Mosallanejad et al. (2021) also attributed the differences in thermo-physical characteristics of absorption coefficient and thermal conductivity between Ti and Cu to the different melting behaviours in the constituents upon exposure to the laser beam.

Delamination of layers was not identified in any of the studied microstructures. There seemed to be minor pores on both the Ti6Al4V-3Cu specimens and the Ti6Al4V-6Cu specimen produced at 150 W and 0.1 m/s. The Ti6Al4V-6Cu specimen produced at 300 W and 0.3 m/s exhibited the most excessive porosity. Pores were not established on the cpTi-based samples. Contours of the single tracks are evident in the transverse microstructures, illustrating complete overlap between adjacent tracks, and hence optimisation of the hatch spacing was achieved. The optimum processing parameters of the metallic alloys investigated during the current study are listed in Table 3.8.

Table 3.8: Optimum processing parameters of studied powders

Metal powder	Laser power (W)	Scanning speed (m/s)	Hatch distance (μm)	Powder layer thickness (μm)
*Ti6Al4V (ELI)	170	1.25	80	30
Ti6Al4V-3Cu	170	0.2	80	50
Ti6Al4V-6Cu	150	0.1	80	50
cpTi	170	0.6	80	50
Ti-3Cu	170	0.6	80	50
Ti-6Cu	150	0.6	80	50

* The parameters for Ti6Al4V were obtained from literature (Yadroitsava, et al., 2015; Manfredi & Calignano, 2017; Dzogbewu, 2020a)

3.4. Summary

The earlier sections of the chapter provide a detailed description of the components of the EOSINT M 280 machine, which was used for LPBF manufacturing of test samples required to complete this project. The functioning of each element and the essential mechanisms of 3D printing design were also focused on. The subsequent sections characterised the employed elemental powders, cpTi and Cu, and the pre-alloyed powder Ti6Al4V in terms of their chemical composition, physical and mechanical properties, and morphology. In the last sections, the hierarchical development of the optimum process parameters for the employed powders for LPBF manufacturing is followed. The approach involved the fabrication and analysis of single tracks by varying the laser powder and scanning speed at a constant layer powder thickness, followed by the production and analysis of double layers at various hatch distances and then the 3D cubes were manufactured at selected process parameters to study the porosity and microstructure at the different planes and build direction. Ultimately, the optimum process parameters for the alloys Ti6Al4V-3Cu, Ti6Al4V-6Cu, cpTi, Ti-3Cu and Ti-6Cu are documented.

References

- Aboulkhair, N. T., Everitt, N. M., Ashcroft, I., & Tuck, C. (2014). Reducing porosity in AlSi10Mg parts processed by selective laser melting. *Additive Manufacturing*, 1–4, 77-86. doi:10.1016/j.addma.2014.08.001
- Aboulkhair, N. T., Simonelli, M., Parry, L., Ashcroft, I., Tuck, C., & Hague, R. (2019). 3D printing of aluminium alloys: additive manufacturing of aluminium alloys using selective laser melting. *Progress in Materials Science*, 106, 100578. doi:10.1016/j.pmatsci.2019.100578
- Aggoune, S., Hamadi, F., Kheloufi, K., Tamsaout, T., Amara, E. -H., & Boughrara, K. (2021). The Marangoni convection effect on melt pool formation during selective laser melting process. *Defect and Diffusion Forum (Online)*, 412, 107-114. doi:10.4028/www.scientific.net/DDF.412.107
- Ali, H., Ma, L., Ghadbeigi, H., & Mumtaz, K. (2017). In-situ residual stress reduction, martensitic decomposition and mechanical properties enhancement through high temperature powder bed pre-heating of selective laser melted Ti6Al4V. *Materials Science & Engineering A*, 695, 211-220. doi:10.1016/j.msea.2017.04.033
- Al-Khateeb, A., Al-Hassani, E. S., & Jabur, A. R. (2023). Metallic implant surface activation through electrospinning coating of nanocomposite fiber for bone regeneration. *International Journal of Biomaterials*, 2023, Article ID 1332814. doi:10.1155/2023/1332814
- Andani, M. T., Dehghani, R., Karamooz-Ravari, M. R., Mirzaeifar, R., & Ni, J. (2017). Spatter formation in selective laser melting process using multi-laser technology. *Materials & Design*, 131, 460–469. doi:10.1016/j.matdes.2017.06.040
- ASM. (2023). *Titanium*. Retrieved April 24, 2023, from <https://www.aerospacemetals.com/titanium-distributor.html>
- Assael, M. J., Kalyva, A. E., Antoniadis, K. D., Michael Banish, R., Egry, I., Wu, J., . . . Wakeham, W. A. (2010). Reference data for the density and viscosity of liquid copper and liquid tin. *Journal of Physical and Chemical Reference Data*, 39, 033105. doi:10.1063/1.3467496
- ASTM International. (2013). ASTM F136-13: Standard Specification for Wrought Titanium-6Aluminum-4Vanadium ELI (Extra Low Interstitial) Alloy for Surgical Implant Applications (UNS R56401). West Conshohocken, Pennsylvania, USA: ASTM International.

- ASTM International. (2017). ASTM E3-11: Standard Guide for Preparation of Metallographic Specimens. West Conshohocken, Pennsylvania, USA: ASTM International.
- AZOM. (2012). *Oxygen Free Low Phosphorus Copper – UNS C10800* . Retrieved April 25, 2023, from <https://www.azom.com/article.aspx?ArticleID=6319>
- AZOM. (2013a). *Grade 23 Ti 6Al 4V ELI Alloy (UNS R56401) 2013*. Retrieved April 24, 2023, from <https://www.azom.com/article.aspx?ArticleID=9365>
- AZOM. (2013b). *Grade 2 Unalloyed Ti ("Pure") 50A (UNS R50400)*. Retrieved April 24, 2023, from <https://www.azom.com/article.aspx?ArticleID=9413>
- AZOM. (2017). *Heat Treatment of 3D Additive Manufactured Parts*. Retrieved April 15, 2023, from <https://www.azom.com/article.aspx?ArticleID=14320>
- Bagheri, G., & Bonadonna, C. (2016). Aerodynamics of Volcanic Particles: Characterization of Size, Shape, and Settling Velocity. In S. Mackie, K. Cashman, H. Ricketts, A. Rust, & M. Watson (Eds.), *Volcanic Ash: Hazard Observation* (pp. 39-52). Amsterdam: Elsevier.
- Battezzati, L., & Greer, A. L. (1989). The viscosity of liquid-metals and alloys. *Acta Metallurgica*, 37(7), 1791-1802. doi:10.1016/0001-6160(89)90064-3
- Baumgärtner, B., Hussein, J., & Hausotte, T. (2023). Investigation of the shape and detectability of pores with x-ray computed tomography. *Journal of Manufacturing and Materials Processing*, 7(3), 103. doi:10.3390/jmmp7030103
- Bergström, D., Powell, J., & Kaplan, A. F. (2007). A ray-tracing analysis of the absorption of light by smooth and rough metal surfaces. *Journal of Applied Physics*, 101(11), 113504. doi:10.1063/1.2738417
- Bertoli, U. S., Wolfer, A. L., Matthews, M. J., Delplanque, J. -P., & Schoenung, J. M. (2017). On the limitations of Volumetric Energy Density as a design parameter for Selective Laser Melting. *Materials & Design*, 113, 331-340. doi:10.1016/j.matdes.2016.10.037
- Bidare, P., Bitharas, I., Ward, R. M., Attallah, M. M., & Moore, A. J. (2018). Laser powder bed fusion at sub-atmospheric pressures. *International Journal of Machine Tools and Manufacture*, 130–131, 65–72. doi:10.1016/j.ijmachtools.2018.03.007
- Chandrasekar, S. (1961). *Hydrodynamic and Hydromagnetic Stability*. New York: Clarendon Press.

- Chen, Z., Wu, X., Tomus, D., & Davies, C. H. (2018). Surface roughness of Selective Laser Melted Ti-6Al-4V alloy components. *Additive Manufacturing*, 21, 91–103. doi:10.1016/j.addma.2018.02.009
- Childs, T. H., Hauser, C., & Badrossamay, M. (2004). Mapping and modelling single scan track formation in direct metal selective laser melting. *CIRP Annals-Manufacturing Technology*, 53(1), 191-194. doi:10.1016/S0007-8506(07)60676-3
- Chowdhury, S., Yadaiah, N., Prakash, C., Ramakrishna, S., Dixit, S., Gupta, L. R., & Buddhi, D. (2022). Laser powder bed fusion: a state-of-the-art review of the technology, materials, properties & defects, and numerical modelling. *Journal of Materials Research and Technology*, 20, 2109-2172.
- Cobbinah, P. V., Nzeukou, R. A., Onawale, O. T., & Matizamhuka, W. R. (2021). Laser powder bed fusion of potential superalloys: A review. *Metals*, 11(1), 58. doi:10.3390/met11010058
- Cox, B., Ghayoor, M., Pasebani, S., & Gess, J. (2023). Tracking of Marangoni driven motion during laser powder bed fusion. *Powder Technology*, 425, 118610. doi:10.1016/j.powtec.2023.118610
- Cruz-Matías, I., Ayala, D., Hiller, D., Gutsch, S., Zacharias, M., Estradé, S., & Peiró, F. (2019). Sphericity and roundness computation for particles using the extreme vertices model. *Journal of Computational Science*, 30, 28–40. doi:10.1016/j.jocs.2018.11.005
- Dadbakhsh, S., & Hao, L. (2014). Effect of layer thickness in selective laser melting on microstructure of Al/5wt.%Fe₂O₃ powder consolidated parts. *The Scientific World Journal*, 2014, 106129. doi:10.1155/2014/106129
- DebRoy, T., Wei, H. L., Zuback, J. S., Mukherjee, T., Elmer, J. W., Milewski, J. O., . . . Zhang, W. (2018). Additive manufacturing of metallic components – Process, structure and properties. *Progress in Materials Science*, 92, 112–224. doi:10.1016/j.pmatsci.2017.10.001
- du Plessis, A. (2019). Effects of process parameters on porosity in laser powder bed fusion revealed by X-ray tomography. *Additive Manufacturing*, 30, 100871. doi:10.1016/j.addma.2019.100871
- du Plessis, A., Sperling, P., Beerlink, A., Kruger, O., Tshabalala, L., Hoosain, S., . . . le Roux, S. G. (2018b). Standard method for microCT-based additive manufacturing quality control 1: Porosity analysis. *MethodsX*, 5, 1102–1110. doi:10.1016/j.mex.2018.09.005

- du Plessis, A., Yadroitsev, I., Yadroitsava, I., & Le Roux, S. G. (2018a). X-ray microcomputed tomography in additive manufacturing: a review of the current technology and applications. *3D Printing and Additive Manufacturing*, 5(3), 227–247. doi:10.1089/3dp.2018.0060
- Dzogbewu, T. C. (2017). Direct Metal Laser Sintering of Titanium Alloys for Biomedical Applications. Doctoral Thesis: Central University of Technology, Free State.
- Dzogbewu, T. C. (2020a). Laser powder bed fusion of Ti6Al4V lattice structures and their applications. *Journal of Metals, Materials and Minerals*, 30(4), 68-78. doi:10.14456/jmmm.2020.53
- Dzogbewu, T. C. (2020b). Laser powder bed fusion of Ti15Mo. *Results in Engineering*, 7, 100155. doi:10.1016/j.rineng.2020.100155
- Dzogbewu, T. C. (2021). Laser powder bed fusion of Ti6Al4V-xCu: process parameters. *Journal of Metals, Materials and Minerals*, 31(2), 62-70.
- Dzogbewu, T. C., & Du Preez, W. B. (2022). Producing Ti5Mo-fused tracks and layers via laser powder bed fusion. *Metals*, 12, 950. doi:10.3390/met12060950
- Dzogbewu, T. C., Yadroitsev, I., Krakhmalev, P., Yadroitsava, I., & Du Plessis, A. (2017). Optimal process parameters for in situ alloyed Ti15Mo structures by laser powder bed fusion. *Proceedings of the 28th Annual International Solid Freeform Fabrication Symposium - an Additive Manufacturing Conference - 2017: August 7-9, 2017, The University of Texas at Austin, Austin, Texas, USA*.
- Fischer, P., Romano, V., Weber, H. P., Karapatis, N. P., Boillat, E., & Glardon, R. (2003). Sintering of commercially pure titanium powder with a Nd: YAG laser source. *Acta Materialia*, 51(6), 1651-1662.
- Gong, H., Rafi, K., Gu, H., Starr, T., & Stucker, B. (2014). Analysis of defect generation in Ti–6Al–4V parts made using powder bed fusion additive manufacturing processes. *Additive Manufacturing*, 1–4, 87-98. doi:10.1016/j.addma.2014.08.002
- Gu, D. (2015). 7 - New Metallic Materials Development by Laser Additive Manufacturing. In J. Lawrence, & D. G. Waugh (Eds.), *Laser Surface Engineering: Processes and Applications* (pp. 163-180). Sawston: Woodhead Publishing. doi:10.1016/B978-1-78242-074-3.00007-6

- Hann, D. B., Iammi, J., & Folkes, J. (2011). A simple methodology for predicting laser-weld properties from material and laser parameters. *Journal of Physics D: Applied Physics*, 44(44), 445401. doi:10.1088/0022-3727/44/44/445401
- ISO. (2025). *ISO 5832-2:2025(en) Implants for surgery—Metallic materials—Part 2: Unalloyed titanium*. Retrieved October 27, 2025, from <https://www.iso.org/obp/ui/en/#iso:std:iso:5832:-2:ed-5:v1:en>
- Jost, E. W., Miers, J. C., Robbins, A., Moore, D. G., & Saldana, C. (2021). Effects of spatial energy distribution-induced porosity on mechanical properties of laser powder bed fusion 316L stainless steel. *Additive Manufacturing*, 39, 101875. doi:10.1016/j.addma.2021.101875
- Kasperovich, G., Haubrich, J., Gussone, J., & Requena, G. (2016). Correlation between porosity and processing parameters in TiAl6V4 produced by selective laser melting. *Materials and Design*, 105, 160–170. doi:10.1016/j.matdes.2016.05.070
- Keene, B. J. (1993). Review of data for the surface tension of pure metals. *International Materials Reviews*, 38(4), 157-192. doi:10.1179/imr.1993.38.4.157
- Khaimovich, A., Balyakin, A., Oleynik, M., Meshkov, A., & Smelov, V. (2022). Optimization of process parameters for powder bed fusion additive manufacturing using a linear programming method: a conceptual framework. *Metals*, 12, 1976. doi:10.3390/met12111976
- Khairallah, S. A., Anderson, A. T., Rubenchik, A., & King, W. E. (2016). Laser powder-bed fusion additive manufacturing: physics of complex melt flow and formation mechanisms of pores, spatter, and denudation zones. *Acta Materialia*, 108, 36-45. doi:10.1016/j.actamat.2016.02.014
- King, W. E., Barth, H. D., Castillo, V. M., Gallegos, G. F., Gibbs, J. W., Hahn, D. E., . . . Rubenchik, A. M. (2014). Observation of keyhole-mode laser melting in laser powder-bed fusion additive manufacturing. *Journal of Materials Processing Technology*, 214, 2915–2925. doi:10.1016/j.jmatprotec.2014.06.005
- Kinnear, A., Dzogbewu, T. C., Yadroitsava, I., & Yadroitsev, I. (2016). In-situ alloying process of ti6al4v-xcu structures by direct metal laser sintering. *Proceedings of the 17th International conference Rapid Product Development Association of South Africa, 2-4 November 2016*(Vaal University of Technology Vanderbijlpark, South Africa).

- Körner, C., Attar, E., & Heinl, P. (2011). Mesoscopic simulation of selective beam melting processes. *Journal of Materials Processing Technology*, 211(6), 978-987.
- Körner, C., Bauereiß, A., & Attar, E. (2013). Fundamental consolidation mechanisms during selective beam melting of powder. *Modelling and Simulation in Materials Science and Engineering*, 21(8), 085011. doi:10.1088/0965-0393/21/8/085011
- Kou, S., Limmaneevichitr, C., & Wei, P. S. (2011). Oscillatory Marangoni flow: A fundamental study by conduction-mode laser spot welding. *Welding Journal*, 90(12), 229s-240s.
- Krakhmalev, P., Fredriksson, G., Yadroitsava, I., Kazantseva, N., du Plessis, A., & Yadroitsev, I. (2016). Deformation behavior and microstructure of Ti6Al4V manufactured by SLM. *Physics Procedia*, 83, 778-788. doi:10.1016/j.phpro.2016.08.080
- Krakhmalev, P., Yadroitsev, I., Baker, I., & Yadroitsava, I. (2018). Manufacturing of intermetallic Mn-46%Al by laser powder bed fusion. *Procedia CIRP*, 74, 64-67. doi:10.1016/j.procir.2018.08.031
- Krakhmalev, P., Yadroitsev, I., Yadroitsava, I., & de Smidt, O. (2017). Functionalization of biomedical Ti6Al4V via in situ alloying by Cu during laser powder bed fusion manufacturing. *Materials*, 10, 1154. doi:10.3390/ma10101154
- Kruth, J. P., Mercelis, P., Van Vaerenbergh, J., Froyen, L., & Rombouts, M. (2005). Binding mechanisms in selective laser sintering and selective laser melting. *Rapid Prototyping Journal*, 11(1), 26-36. doi:10.1108/13552540510573365
- Kurzynowski, T., Chlebus, E., Kuźnicka, B., & Reiner, J. (2012). Parameters in selective laser melting for processing metallic powders. *International Society for Optics and Photonics, In SPIE LASE*, 823914. doi:10.1117/12.907292
- Laakso, P., Riipinen, T., Laukkanen, A., Andersson, T., Jokinen, A., Revuelta, A., & Ruusuvaori, K. (2016). Optimization and simulation of SLM process for high density H13 tool steel parts. *Physics Procedia*, 83, 26-35. doi:10.1016/j.phpro.2016.08.004
- Lebea, L., Desai, D., Ngwangwa, H., & Nemavhola, F. (2024). Evaluation of 3D printing orientation on volume parameters and mechanical properties of as-build Ti64ELI. *Metals*, 14, 447. doi:10.3390/met14040447
- Lee, P. D., Quested, P. N., & McLean, M. (1998). Modelling of Marangoni effects in electron beam melting. *Philosophical Transactions of The Royal Society A Mathematical Physical and Engineering Sciences*, 356(1739), 1027-1043. doi:10.1098/rsta.1998.0207

- Lee, Y. S., & Zhang, W. (2015). Mesoscopic Simulation of Heat Transfer and Fluid Flow in Laser Powder Bed Additive Manufacturing. *In International Solid Free Form Fabrication Symposium, Austin, Texas.*
- Leung, C. L., Marussi, S., Towrie, M., del Val Garcia, J., Atwood, R. C., Bodey, A. J., . . . Lee, P. D. (2018). Laser-matter interactions in additive manufacturing of stainless steel SS316L and 13-93 bioactive glass revealed by in situ X-ray imaging. *Additive Manufacturing*, 24, 647-657. doi:10.1016/j.addma.2018.08.025
- Li, H., Zhang, S., Chen, Q., Du, Z., Chen, X., Chen, X., . . . Li, Z. (2024). High-quality spherical silver alloy powder for laser powder bed fusion using plasma rotating electrode process. *Micromachines*, 15, 396. doi:10.3390/mi15030396
- Luo, Q., Yin, L., Simpson, T. W., & Beese, A. M. (2022). Effect of processing parameters on pore structures, grain features, and mechanical properties in Ti-6Al-4V by laser powder bed fusion. *Additive Manufacturing*, 56, 102915. doi:10.1016/j.addma.2022.102915
- Majumdar, T., Bazin, T., Massahud Carvalho Ribeiro, E., Frith, J. E., & Birbilis, N. (2019). Understanding the effects of PBF process parameter interplay on Ti-6Al-4V surface properties. *PLoS ONE*, 14(8), e0221198. doi:10.1371/journal.pone.0221198
- Makoana, N. W., Moller, H., Burger, H., Tlotleng, M., & Yadroitsev, I. (2016). Evaluation of single tracks of 17-4PH steel manufactured at different power densities and scanning speeds by selective laser melting. *South African Journal of Industrial Engineering*, 27(3), 210-218. doi:10.7166/27-3-1668
- Manfredi, D., & Calignano, F. (2017). Laser powder bed fusion of aluminum, titanium and nickel based alloys: Materials and design investigations. *2017 International Conference on Electromagnetics in Advanced Applications (ICEAA)*. Verona: 2017 International Conference on Electromagnetics in Advanced Applications (ICEAA). doi:10.1109/ICEAA.2017.8065546
- Matilainen, V. P., Piili, H., Salminen, A., & Nyrhilä, O. (2015). Preliminary investigation of keyhole phenomena during single layer fabrication in laser additive manufacturing of stainless steel. *Physics Procedia*, 78, 377-387. doi:10.1016/j.phpro.2015.11.052
- Matthews, M. J., Guss, G., Khairallah, S. A., Rubenchik, A. M., Depond, P. J., & King, W. E. (2016). Denudation of metal powder layers in laser powder bed fusion processes 10.1016/j.actamat.2016.05.017. *Acta Materialia*, 114(2016), 33-42.

- Mayer, C., Gatumel, C., & Berthiaux, H. (2015). Mixing dynamics for easy flowing powders in a lab scale Turbula® mixer. *Chemical Engineering Research and Design*, 95, 248-261. doi:10.1016/j.cherd.2014.11.003
- Metelkova, J., Vanmunster, L., Haitjema, H., & Van Hooreweder, B. (2021). Texture of inclined up-facing surfaces in laser powder bed fusion of metals. *Additive Manufacturing*, 42, 101970. doi:10.1016/j.addma.2021.101970
- Mirkoochi, E., Ning, J., Bocchini, P., Fergani, O., Chiang, K. -N., & Liang, S. Y. (2018). Thermal modeling of temperature distribution in metal additive manufacturing considering effects of build layers, latent heat, and temperature-sensitivity of material properties. *Journal of Manufacturing and Materials Processing*, 2, 63. doi:10.3390/jmmp2030063
- Mosallanejad, M. H., Niroumand, B., Aversa, A., Manfredi, D., & Saboori, A. (2021). Laser Powder Bed Fusion in-situ alloying of Ti-5%Cu alloy: Process-structure relationships. *Journal of Alloys and Compounds*, 857, 157558. doi:10.1016/j.jallcom.2020.157558
- Nudelis, N., & Mayr, P. (2021). A novel classification method for pores in laser powder bed fusion. *Metals*, 11, 1912. doi:10.3390/met11121912
- Osakada, K., & Shiomi, M. (2006). Flexible manufacturing of metallic products by selective laser melting of powder. *International Journal of Machine Tools and Manufacture*, 46(11), 1188-1193. doi:10.1016/j.ijmachtools.2006.01.024
- Oyesola, M., Mpofu, K., Mathe, N., Fatoba, S., Hoosain, S., & Daniyan, I. (2021). Optimization of selective laser melting process parameters for surface quality performance of the fabricated Ti6Al4V. *The International Journal of Advanced Manufacturing Technology*, 114(4), 1585–1599. doi:10.1007/s00170-021-06953-3
- Paradis, P. -F., Ishikawa, T., & Yoda, S. (2002). Non-contact measurements of surface tension and viscosity of niobium, zirconium, and titanium using an electrostatic levitation furnace. *International Journal of Thermophysics*, 23, 825-842. doi:10.1023/A:1015459222027
- Qin, P., Liu, Y., Sercombe, T. B., Li, Y., Zhang, C., Cao, C., . . . Zhang, L. -C. (2018). Improved corrosion resistance on selective laser melting produced Ti-5Cu alloy after heat treatment. *ACS Biomaterials Science & Engineering*, 4(7), 2633–2642. doi:10.1021/acsbmaterials.8b00319
- Ramosena, L. A., Dzogbewu, T. C., & du Preez, W. (2022). Direct metal laser sintering of the Ti6Al4V alloy from a powder blend. *Materials*, 15, 8193. doi:10.3390/ma15228193

- Rayleigh, L. (1892). On the instability of a cylinder of viscous liquid under capillary force. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science*, 34(207), 145-154.
- Roberts, I. A., Wang, C. J., Esterlein, R., Stanford, M., & Mynors, D. J. (2009). A three-dimensional finite element analysis of the temperature field during laser melting of metal powders in additive layer manufacturing. *International Journal of Machine Tools and Manufacture*, 49(12–13), 916-923. doi:10.1016/j.ijmachtools.2009.07.004
- Ruban, W., Vijayakumar, V., Dhanabal, P., & Pridhar, T. (2014). Effective process parameters in selective laser sintering. *International Journal of Rapid Manufacturing*, 4(2/3/4), 148-164. doi:10.1504/IJRAPIDM.2014.066036
- Shah, R. K., & Dey, P. P. (2019). Process parameter optimization of DMLS process to produce AlSi10Mg components. *Journal of Physics Conference Series*, 1240(1), 012011. doi:10.1088/1742-6596/1240/1/012011
- Shen, Q., Qi, Y., Kong, Y., Bao, H., Wang, Y., Dong, A., . . . Xu, Y. (2022). Advances in copper-based biomaterials with antibacterial and osteogenic properties for bone tissue engineering. *Frontiers in Bioengineering and Biotechnology*, 9, 795425. doi:10.3389/fbioe.2021.795425
- Shrestha, S., & Chou, K. (2022). Formation of keyhole and lack of fusion pores during the laser powder bed fusion process. *Manufacturing Letters*, 32, 19-23. doi:10.1016/j.mfglet.2022.01.005
- Simchi, A., & Asgharzadeh, H. (2004). Densification and microstructural evaluation during laser sintering of M2 high speed steel powder. *Materials Science and Technology*, 20(11), 1462-1468. doi:10.1179/026708304X3944
- Simonelli, M., Aboulkhair, N. T., Cohen, P., Murray, J. W., Clare, A. T., Tuck, C., & Hague, R. J. (2018). A comparison of Ti-6Al-4V in-situ alloying in Selective Laser Melting using simply-mixed and satellited powder blend feedstocks. *Materials Characterization*, 143, 118-126.
- Snell, R., Tammas-Williams, S., Chechik, L., Lyle, A., Hernández-Nava, E., Boig, C., . . . Todd, I. (2020). Methods for rapid pore classification in metal additive manufacturing. *Journal of The Minerals, Metals & Materials Society*, 72, 101–109. doi:10.1007/s11837-019-03761-9

- Song, Y. -H., Kim, M. -K., Park, E. -J., Song, H. -J., Anusavice, K. J., & Park, Y. -J. (2014). Cytotoxicity of alloying elements and experimental titanium alloys by WST-1 and agar overlay tests. *Dental Materials*, 30(9), 977–983. doi:10.1016/j.dental.2014.05.012
- Suryawanshi, C. M., Gaur, B., Singh, A. K., Soman, D., Sagar, S., Bhallamudi, R., & Mishra, S. (2023). Microstructure and mechanical characterization of direct metal laser sintered Ti-6Al-4V alloy for orthopedic implant applications. *Materials Today Communications*, 35, 105770. doi:10.1016/j.mtcomm.2023.105770
- Tang, M., Pistorius, P. C., & Beuth, J. L. (2017). Prediction of lack-of-fusion porosity for powder bed fusion. *Additive Manufacturing*, 14, 39–48. doi:10.1016/j.addma.2016.12.001
- Thijs, L., Verhaeghe, F., Craeghs, T., Van Humbeeck, J., & Kruth, J. -P. (2010). A study of the microstructural evolution during selective laser melting of Ti-6Al-4V. *Acta Materialia*, 58, 3303–3312. doi:10.1016/j.actamat.2010.02.004
- Tolochko, N. K., Khlopkov, Y. V., Mozzharov, S. E., Ignatiev, M. B., Laoui, T., & Titov, V. I. (2000). Absorptance of powder materials suitable for laser sintering. *Rapid Prototyping Journal*, 6(3), 155-161. doi:10.1108/13552540010337029
- Verhaeghe, F., Craeghs, T., Heulens, J., & Pandelaers, L. (2009). A pragmatic model for selective laser melting with evaporation. *Acta Materialia*, 57, 6006–6012. doi:10.1016/j.actamat.2009.08.027
- Viale, V., Stavridis, J., Salmi, A., Bondioli, F., & Saboori, A. (2022). Optimisation of downskin parameters to produce metallic parts via laser powder bed fusion process: an overview. *The International Journal of Advanced Manufacturing Technology*, 123, 2159–2182. doi:10.1007/s00170-022-10314-z
- Villardell, A. M., Yadroitsev, I., Yadroitsava, I., Albu, M., Takata, N., Kobashi, M., . . . du Plessis, A. (2020). Manufacturing and characterization of in-situ alloyed Ti6Al4V(ELI)-3 at.% Cu by laser powder bed fusion. *Additive Manufacturing*, 36, 101436.
- Wadell, H. (1935). Volume, shape, and roundness of quartz particles. *The Journal of Geology*, 43(3), 250–280. doi:10.1086/624298
- Wu, Y., Zhou, H., Zeng, Y., Xie, H., Ma, D., Wang, Z., & Liang, H. (2022). Recent advances in copper-doped titanium implants. *Materials*, 15, 2342. doi:10.3390/ma15072342

- Wudy, K., & Drummer, D. (2019). Aging effects of polyamide 12 in selective laser sintering: Molecular weight distribution and thermal properties. *Additive Manufacturing*, 25, 1–9. doi:10.1016/j.addma.2018.11.007
- Xiao, R., & Zhang, X. (2014). Problems and issues in laser beam welding of aluminum–lithium alloys. *Journal of Manufacturing Processes*, 16, 166–175. doi:10.1016/j.jmapro.2013.10.005
- Yadroitsava, I., Els, J., Booysen, G., & Yadroitsev, I. (2015). Peculiarities of single track formation from Ti6Al4V alloy at different laser power densities by selective laser melting. *The South African Journal of Industrial Engineering*, 26(3), 86–95. doi:10.7166/26-3-1185
- Yadroitsev, I. (2009). *Selective Laser Melting: Direct Manufacturing of 3D-objects by Selective Laser Melting of Metal Powders*. Saarbrücken: LAP Lambert Academic Publishing AG & Co. KG.
- Yadroitsev, I., Bertrand, P., & Smurov, I. (2007). Parametric analysis of the selective laser melting process. *Applied Surface Science*, 253(19), 8064–8069. doi:10.1016/j.apsusc.2007.02.088
- Yadroitsev, I., Bertrand, P., Antonenkova, G., Grigoriev, S., & Smurov, I. (2013b). Use of track/layer morphology to develop functional parts by selective laser melting. *Journal of Laser Applications*, 25, 052003. doi:10.2351/1.4811838
- Yadroitsev, I., Gusarov, A., Yadroitsava, I., & Smurov, I. (2010). Single track formation in selective laser melting of metal powders. *Journal of Materials Processing Technology*, 210, 1624–1631. doi:10.1016/j.jmatprotec.2010.05.010
- Yadroitsev, I., Krakhmalev, P., & Yadroitsava, I. (2015). Hierarchical design principles of selective laser melting for high quality metallic objects. *Additive Manufacturing*, 7, 45–56. doi:10.1016/j.addma.2014.12.007
- Yadroitsev, I., Krakhmalev, P., Yadroitsava, I., & du Plessis, A. (2018). Qualification of Ti6Al4V ELI alloy produced by laser powder bed fusion for biomedical applications. *JOM*, 70(3), 372–377. doi:10.1007/s11837-017-2655-5
- Yadroitsev, I., Krakhmalev, P., Yadroitsava, I., Johansson, S., & Smurov, I. (2013a). Energy input effect on morphology and microstructure of selective laser melting single track from metallic powder. *Journal of Materials Processing Technology*, 213(4), 606–613. doi:10.1016/j.jmatprotec.2012.11.014

- Yadroitsev, I., Yadroitsava, I., Bertrand, P., & Smurov, I. (2012). Factor analysis of selective laser melting process parameters and geometrical characteristics of synthesized single tracks. *Rapid Prototyping Journal*, 18(3), 201-208. doi:10.1108/13552541211218117
- Yang, J., Han, J., Yu, H., Yin, J., Gao, M., Wang, Z., & Zeng, X. (2016). Role of molten pool mode on formability, microstructure and mechanical properties of selective laser melted Ti-6Al-4V alloy. *Materials & Design*, 110, 558-570. doi:10.1016/j.matdes.2016.08.036
- Yang, Y., Gu, D., Dai, D., & Ma, C. (2018). Laser energy absorption behavior of powder particles using ray tracing method during selective laser melting additive manufacturing of aluminum alloy. *Materials & Design*, 143, 12–19. doi:10.1016/j.matdes.2018.01.043
- Zhang, E., Li, F., Wang, H., Liu, J., Wang, C., Li, M., & Yang, K. (2013). A new antibacterial titanium–copper sintered alloy: preparation and antibacterial property. *Materials Science and Engineering C*, 33, 4280-4287.
- Zhang, W., Hou, W., Deike, L., & Arnold, C. (2022). Understanding the Rayleigh instability in humping phenomenon during laser powder bed fusion process. *International Journal of Extreme Manufacturing*, 4(2022), 015201. doi:10.1088/2631-7990/ac466d
- Zhao, R., Shmatok, A., Fischer, R., & Prorok, B. C. (2023). Investigation of causal relationships between printing parameters, pore properties and porosity in laser powder bed fusion. *Metals*, 13(2), 330. doi:10.3390/met13020330
- Zhao, X., Li, S., Zhang, M., Liu, Y., Sercombe, T. B., Wang, S., . . . Murr, L. E. (2016). Comparison of the microstructures and mechanical properties of Ti–6Al–4V fabricated by selective laser melting and electron beam melting. *Materials and Design*, 95, 21–31.

CHAPTER 4 EVALUATION OF MECHANICAL PROPERTIES

4.1. Introduction

The present chapter fundamentally delineates the investigations of the mechanical properties of the copper (Cu) alloyed Ti6Al4V ELI and commercially pure titanium (cpTi) specimens in terms of the microhardness and tensile properties (including fractography). The mechanical properties of a biomedical material are fundamental in the clinical application (Zhang, et al., 2013). An additive manufacturing process with optimised parameters is crucial for attaining the mechanical stability of implants necessary to provide the intended functionality inside the human body throughout its lifetime (Mierzejewska, et al., 2019; Suryawanshi, et al., 2023). Pure titanium (Ti) and Ti6Al4V alloy have been widely applied as hard-tissue replacement metallic implants in the orthopaedic and dental professions because of their exceptional mechanical properties (Okazaki & Ishino, 2020; Suryawanshi, et al., 2023). The biological functioning of Ti and its alloys may be augmented by adding antibacterial agents such as Cu particles (Shen, et al., 2022).

4.2. Materials and methods

4.2.1. Sample preparation and characterisation

The samples were built using the laser powder bed fusion (LPBF) technique on the EOS EOSINT M 280 machine (Figure 3.1). The optimised process parameters listed in Table 3.8 were employed to manufacture all test specimens using the pre-alloyed Ti6Al4V ELI (also referred to as Ti6Al4V or Ti64 in this chapter) powder, the elemental commercially pure Ti (cpTi) powder and the in situ alloys of Ti6Al4V-3 wt.% Cu, Ti6Al4V-6 wt.% Cu, Ti-3 wt.% Cu and Ti-6 wt.% Cu (denoted as Ti6Al4V-3Cu/Ti64-3Cu, Ti6Al4V-6Cu/Ti64-Cu, Ti-3Cu and Ti-6Cu in this chapter). The elemental compositions and crystalline phases of the as-built samples were analysed using a Malvern PANalytical Empyrean X-Ray diffractometer (XRD) housed at the University of the Free State (UFS) Geology Department (Figure 4.1). The diffractometer consisted of a Cu side window tube and an X'Celerator detector. The XRD patterns were recorded using monochromatic CuK_α radiation ($\lambda = 0.1541 \text{ nm}$) at a generator voltage of 45 kV and a current of 40 mA. The measurements were conducted within a two theta (2θ , also known as the Bragg angle) range of 20° to 80° at a step size of 0.017° and a scanning rate of $0.02^\circ/\text{s}$ and were analysed with HighScore software from Malvern PANalytical and the ICDD-PDF-2 2021 database of the International Centre for Diffraction Data (ICDD).



Figure 4.1: Malvern PANalytical Empyrean X-Ray diffractometer located at the University of the Free State

4.2.2. Surface roughness

The portable Mitutoyo SURFTEST SJ-210 profilometer (Figure 3.9) was utilised to measure the surface roughness of LPBF-manufactured samples (10×2 mm) of Ti6Al4V, Ti6Al4V- x Cu, cpTi and Ti- x Cu ($x = 3, 6$ wt.%) in their as-built condition, without any surface treatment or polishing. The tester was set to measure in accordance with ISO 1997. The stylus was set to travel to a cut-off length of $\lambda_c = 0.8$ mm at a measuring speed of 0.5 mm/s for all samples. Average surface roughness measurements were taken perpendicular to the laser scanning direction at 5 randomly selected surfaces for each sample.

4.2.3. Microhardness test

Microhardness of the as-built LPBF-manufactured specimens (namely, Ti6Al4V- x Cu, cpTi and Ti- x Cu ($x = 3, 6$ wt.%)) was determined to study the anisotropic behaviour typical of LPBF parts (Thijs, et al., 2010; Dzogbewu, et al., 2017). The specimens were prepared by conventional steps

of metallographic preparation, according to ASTM E3 (ASTM International, 2017a; Chen, et al., 2017). The microhardness test was conducted with the FM-700 Digital Vickers Microhardness Tester, shown in Figure 4.2. The samples were placed in succession in the specimen holder, and the pyramid-shaped diamond indenter was pressed into the surface of the material at a constant load of 200 g with a dwell time of 10–15 s. Ten indentations were made and measured at 20 μm intervals along the length of each specimen. The Vickers microhardness of Ti6Al4V ELI has been examined in previous LPBF optimisation studies; hence, the hardness values were obtained from literature (Dzogbewu, 2020).

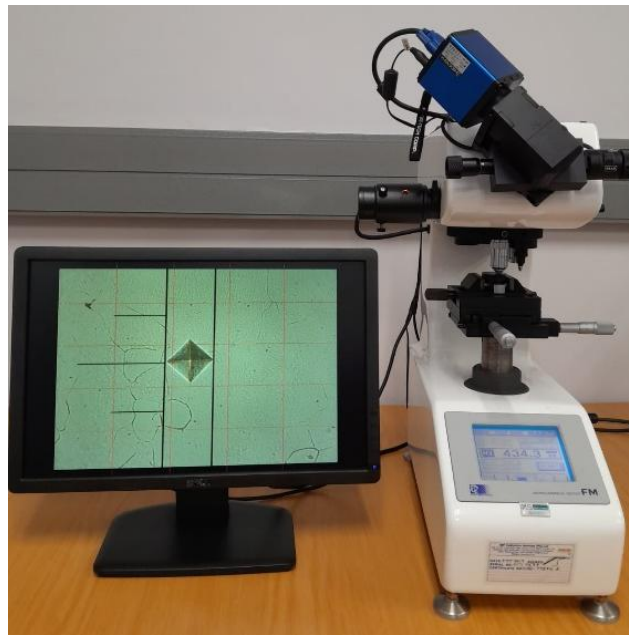


Figure 4.2: FM-700 Digital Vickers Microhardness Tester

4.2.4. Tensile test

Laser powder bed fusion was employed to manufacture four scaled-down tensile test specimens for each of the powders Ti6Al4V-3Cu, Ti6Al4V-6Cu, cpTi, Ti-3Cu and Ti-6Cu. The scaled-down specimens, as shown in Figure 4.3, were fabricated in compliance with the ASTM E8/E8M standard. The tensile test specimens underwent a stress-relief treatment in a vacuum furnace at 650 °C for a soaking period of 3 hrs. The samples were then allowed to cool in the furnace to room temperature. Thereafter, they were removed from the substrate by wire electrical discharge machining (EDM). The dumbbell-shaped specimens were threaded at the grip sections and were machined along the gauge length (10 mm in Figure 4.3(iii)). The tensile properties for Ti6Al4V

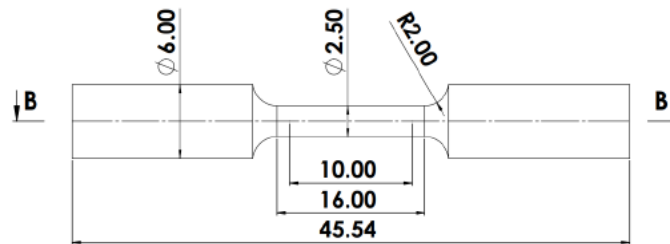
have been extensively evaluated in previous research studies, and the values of tensile test properties of Ti6Al4V were derived from literature (Kasperovich & Hausmann, 2015; Okazaki & Ishino, 2020; Bartolomeu, et al., 2022).



(i)



(ii)



(iii)

Figure 4.3: Scaled-down tensile test specimens (i) on a Ti6Al4V substrate after LPBF manufacturing and stress relieving, and (ii) after being removed from the substrate and machined to a dumbbell shape according to the dimensions indicated in (iii) (all dimensions are in mm)

Tensile testing was conducted with the MTS Criterion Model 43 electromechanical universal testing machine (Figure 4.4). Clamping the scaled-down samples into position for tensile testing was achieved using customised inserts. For accurate strain measurement, a 10 mm extensometer was attached along the gauge length of the dumbbell specimen during tensile testing. Samples were pulled at room temperature at a constant, quasi-static strain rate of 0.03/min. Samples were deformed by a machine-applied tensile load until fracture, which marked the end of the tensile test. The load (in kN) and extension (in mm) were instantaneously recorded using the MTS TestSuite™ TW Elite Software. The software plotted stress versus strain curves and determined the yield strength at 0.2% offset, ultimate tensile strength (UTS), modulus of elasticity (E), %reduction in area and %total elongation to failure. The initial and final diameters and lengths were measured

manually using a Vernier calliper. Upon completion of the tensile tests, the fractured surfaces were preserved for fractography analysis to study the fracture behaviour of the material. The surfaces at the fracture points were investigated with the assistance of a JEOL JSM-6610 Scanning Electron Microscope (SEM, Figure 3.4).



Figure 4.4: MTS Criterion Model 43 electromechanical universal testing machine

4.2.5. Statistical analysis

The quantitative results were expressed as mean value with standard deviation. The Student's *t*-test was performed, and values of $p \leq 0.05$ were considered statistically significantly different and of $p \leq 0.01$ were regarded as statistically highly significantly different.

4.3. Results and discussion

4.3.1. X-Ray diffractometry (XRD)

X-ray diffraction (XRD) is employed to investigate the phase structures of alloys (Nirmala, et al., 2013). It is also a highly reliable technique utilised to investigate the nature of any crystalline compounds (Sheikh, et al., 2011). Figure 4.5 shows the XRD diffraction patterns of as-built

Ti6Al4V-xCu and Ti-xCu ($x = 3, 6$ wt.%) alloys and ascertains that in situ alloying of Ti6Al4V and cpTi with the different compositions of Cu was successful.

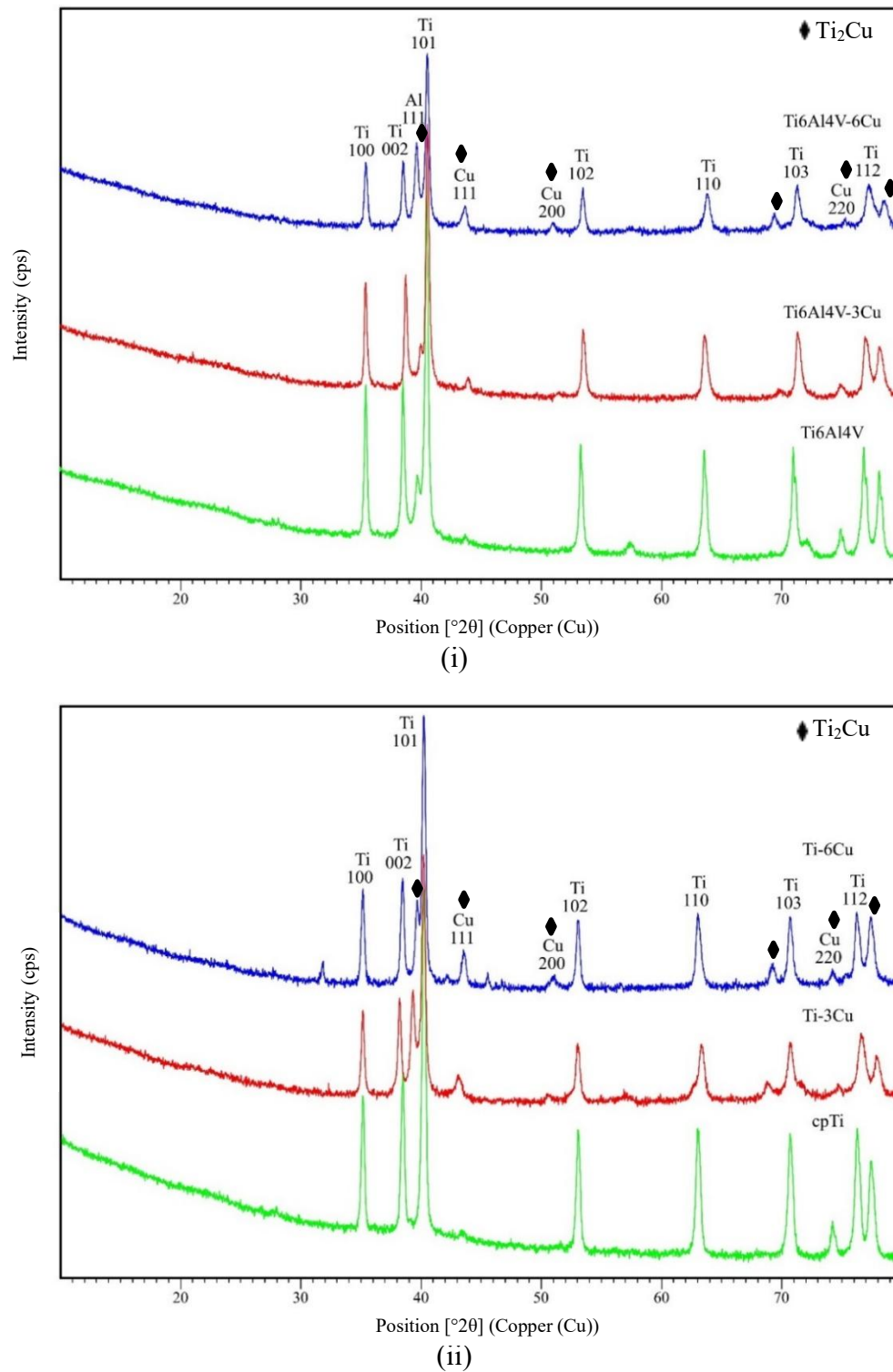


Figure 4.5: X-ray diffraction patterns of (i) Ti6Al4V-Cu and (ii) Ti-Cu alloys

Similar to the research of Bolzoni & Yang (2024), no substantial differences may be delineated between the patterns of Ti6Al4V and cpTi alloys, with only minor deviations in peak intensities. The Ti diffraction peaks represented the α/α' -phase, and the α -Ti (101) plane peak at 2θ of 40.42° was the major XRD peak with the highest intensity. The rapid cooling rate during the LPBF process results in an acicular martensitic α -phase or the α' -phase (Kasperovich & Hausmann, 2015). Although they are two different phases, it is challenging to differentiate peaks of α and α' because they both have hcp crystal structure (Rafi, et al., 2013; Kasperovich & Hausmann, 2015). The XRD (002) peak of the α/α' -phase overlaps with the XRD (110) peak of cubic β -Ti, making it difficult to confidently identify the β -phase by XRD (Vilardell, et al., 2020). Moreover, the cubic β -phase was not detected by XRD due to its low content. Numerous studies have demonstrated that the detectable phase on Ti6Al4V- x Cu and Ti- x Cu XRD patterns is the α/α' -phase, and the absence of peaks related to the β -phase in near-alpha Ti alloys is common (Rafi, et al., 2013; Kasperovich & Hausmann, 2015; Peng, et al., 2019; Vilardell, et al., 2020; Wang, et al., 2021; Muiruri, et al., 2023; Bolzoni & Yang, 2024).

The intermetallic phase of Ti_2Cu was also identified (Souza, et al., 2009; Hesse, et al., 2021). The Cu diffraction peaks were identified at Bragg angles of 43.38° , 50.52° , and 74.25° for (111), (200), and (220) crystalline planes, respectively. Congruently, the XRD pattern of Chen et al. (2021) distinguished clear diffraction peaks at 43.46° , 50.60° , and 74.24° characteristic of the analysed Cu nanoparticles, corresponding to the (111), (200), and (220) reflections of copper. Sheikh et al. (2011) denoted the presence of crystalline Cu from diffraction peaks at 2θ equivalent to 43.29° , 50.43° , 74.12° , 89.92° , 136.49° and 144.70° corresponding to (111), (200), (220), (311), (311) and (420) crystal planes. These coincide with the diffraction peaks of the Cu-alloyed Ti6Al4V and cpTi samples of the present study. The intensity of the Cu-rich phases increased with the increase in Cu content, as also reported by Sheikh et al. (2011) and Chen et al. (2021). Incorporation of Cu particles did not alter or shift the peaks of Ti; however, it diminished the intensity of the peaks. The XRD patterns of Ti- x Cu ($x = 3, 7.1, 12$ wt.%) samples of Pina et al. (2016) also indicated that the volume fraction of Ti_2Cu increased with the Cu content in the alloy. Additional Cu dissolves in the α/α' -phase during the LPBF process, resulting in greater lattice distortion (Wang, et al., 2021). Vilardell et al. (2020) performed XRD measurements on LPBF Ti6Al4V (ELI)-3 at.% Cu material after stress-relieving heat treatment, and found low-intensity peaks of $CuTi_2$ phase.

Conversely, Wang et al. (2021) were not able to detect the Cu-containing intermetallic phase by XRD analysis of Ti-xCu ($x = 1.25, 2.5$ and 5 wt.%) samples fabricated by selective laser melting (SLM), and this may be attributed to a lower concentration of the β -stabilising elements in cpTi.

4.3.2. Surface roughness

Surface roughness may influence the mechanical performance of parts due to multiple stress concentration points (Greitemeier, et al., 2016). The roughness of as-printed surfaces influences the selection of the post-process regime and parameters. Laser powder bed fusion produces two types of roughness: a primary roughness induced from the solidification of the molten pool and secondary roughness induced by partly melted powder particles (Greitemeier, et al., 2016). The roughness values of R_a and R_z are presented in Table 4.1 and Figure 4.6. The surface roughness of the Ti6Al4V specimens increased with increasing Cu content. Ti6Al4V roughness was significantly lower than that of Ti6Al4V-3Cu ($p = 0.0177$ for R_a and $p = 0.0395$ for R_z) and highly significantly lower than that of Ti6Al4V-6Cu ($p = 0.00001$ for R_a and $p = 0.0002$ for R_z). The values of the unalloyed cpTi were significantly higher than those of Ti-3Cu ($p = 0.0181$ for R_a and $p = 0.0369$ for R_z) and Ti-6Cu ($p = 0.0322$ for R_a and $p = 0.0625$ for R_z). Ti-3Cu and Ti-6Cu had a similar surface roughness. Thus, the addition of Cu particles roughened Ti6Al4V but smoothed cpTi. Commercially pure Ti had a highly significantly ($p = 0.0033$ for R_a and $p = 0.0041$ for R_z) higher surface roughness than Ti6Al4V.

Table 4.1: Roughness values of Cu-alloyed Ti64 and cpTi specimens

Sample	R_a (μm)	R_z (μm)
Ti6Al4V	10.22 ± 1.18	54.64 ± 6.17
Ti6Al4V-3Cu	14.09 ± 2.12	73.02 ± 12.48
Ti6Al4V-6Cu	19.22 ± 0.52	91.45 ± 0.87
cpTi	16.45 ± 2.34	83.46 ± 11.40
Ti-3Cu	12.17 ± 0.92	66.37 ± 4.04
Ti-6Cu	12.48 ± 1.94	67.92 ± 8.44

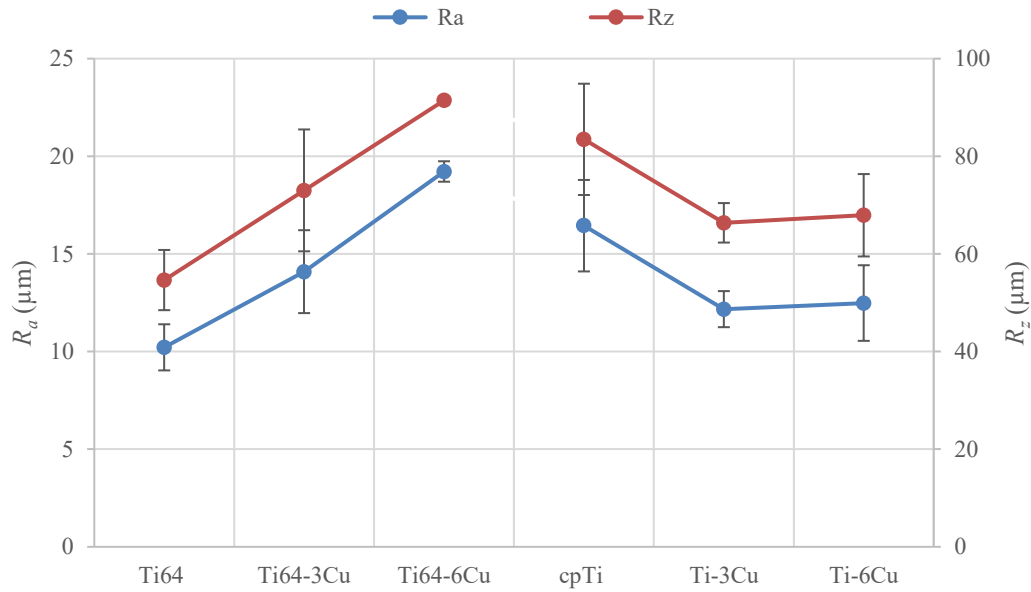


Figure 4.6: Surface roughness measurements of Cu-alloyed Ti64 and cpTi specimens

According to Urlea & Brailovski (2017), as-built surface finish of Ti6Al4V components produced using EOS M 270/280/290 SLM machines is expected to have a surface roughness R_a of 9–12 μm . Greitemeier et al. (2016) and Cabrini et al. (2022) studied the surface roughness of as-built LPBF Ti6Al4V and measured R_a values of 13.0 and 14.5 μm , respectively. Mhlanga et al. (2022) reported R_a and R_z values in the ranges of 9–10 μm and 50–70 μm , respectively, for Ti6Al4V ELI. These roughness values for Ti6Al4V are consistent with those found for the present investigation. The findings from the roughness tests of Wang et al. (2023) of SLMed cpTi were R_a of ~6 μm and R_z of ~32.5 μm , and those of laser-treated pure Ti samples by Kibria et al. (2018) ranged between 3.57 and 14.64 μm for R_a , and 21.54 and 75.54 μm for R_z . Rougher surfaces were achieved for cpTi during the present study.

4.3.3. Microhardness test

The microhardness gives an indication of the ductility of the material microstructure and the work hardening introduced into the material during sintering and by post-sintering treatments (Benedetti, et al., 2017). According to Benedetti et al. (2017), microhardness values of as-built and stress-relieved conditions present a martensitic α' microstructure. Vickers hardness numbers are presented in Table 4.2, and Figure 4.7 indicates a significant ($p \leq 0.05$) difference in hardness of

the different cross-sections for Ti6Al4V-6Cu, cpTi and Ti-3Cu, and no significance ($p > 0.05$) among the cross-sectional hardness values of Ti6Al4V-3Cu and Ti-6Cu. The microhardness values of Ti6Al4V-Cu samples were higher than those of Ti6Al4V, as shown in Table 4.2. Therefore, alloying Ti6Al4V with Cu caused the material to become harder. Several studies report on the increase in hardness of Ti6Al4V with an increase in Cu concentration (Aoki, et al., 2004; Vilardell, et al., 2020). However, the current results showed slight changes in hardness with an increase in Cu content, with no clear trend observed between Ti6Al4V-3Cu and Ti6Al4V-6Cu (Figure 4.7). The value of Ti6Al4V-3Cu was significantly ($p = 0.0020$) higher than that of Ti6Al4V-6Cu in the longitudinal direction and was insignificant ($p = 0.6764$) in the transverse view. During their characterisation, Krakhmalev et al. (2017) measured hardness of 383 ± 13 HV in as-built LPBF Ti6Al4V and 456 ± 20 HV in Ti6Al4V (ELI)-1 at % Cu. The value for Ti6Al4V was in agreement with that of Moletsane et al. (2016) and Benedetti et al. (2017), respectively, at 389 ± 14.8 HV and 380 ± 10 HV. As shown in Table 4.2, these literature values are in the vicinity of the present investigation.

Table 4.2: Microhardness values of Ti material at different cross-sections

Specimen	Transverse view (HV)	Longitudinal view (HV)
Ti6Al4V*	361 ± 12	372 ± 17
Ti6Al4V-3Cu	416 ± 12	417 ± 14
Ti6Al4V-6Cu	424 ± 16	390 ± 10
cpTi	195 ± 9	211 ± 2
Ti-3Cu	272 ± 14	294 ± 10
Ti-6Cu	358 ± 14	350 ± 16

*Hardness values for Ti6Al4V ELI were obtained from literature (Dzogbewu, 2020).

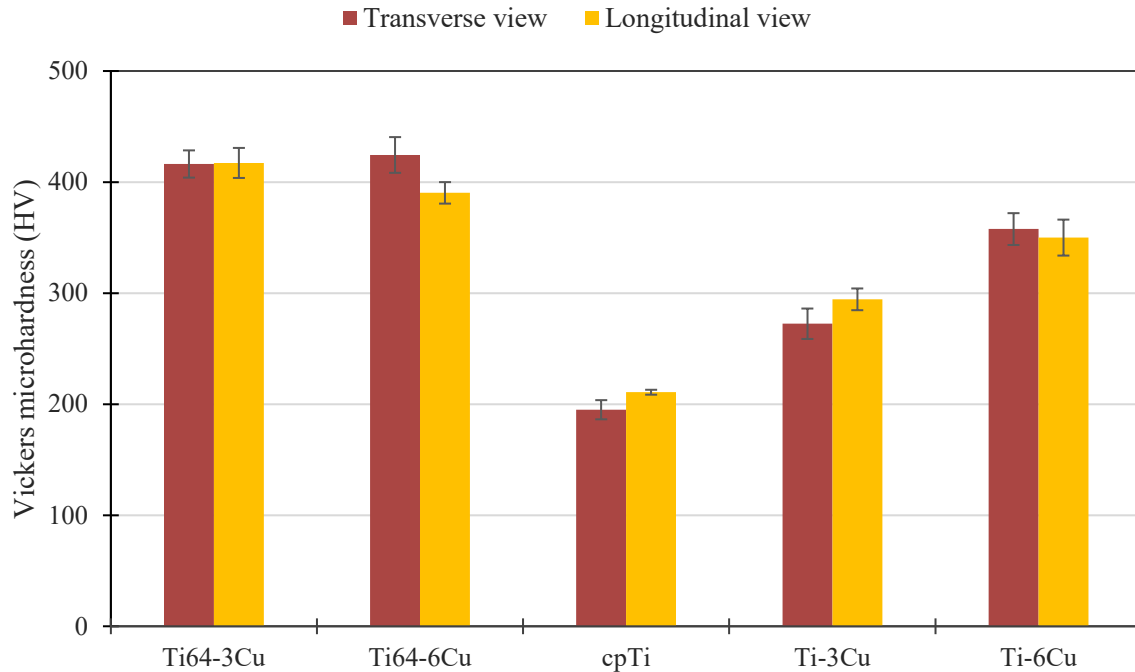


Figure 4.7: Microhardness of Ti-based alloys at different cross-sections

For cpTi samples, there was a distinct increase in hardness with an increase in Cu mass. The microhardness values for cpTi were highly significantly ($p \leq 0.01$) lower than those of Ti-3Cu and Ti-6Cu in both the transverse and longitudinal views, and the hardness values of Ti-3Cu highly significantly ($p \leq 0.01$) lower than those of Ti-6Cu in both views. The present hardness values matched those of Wang et al. (2021), who in situ alloyed cpTi with 1.25, 2.5 and 5 wt.% Cu contents for fabrication by SLM and recorded mean hardness values of 258, 299 and 356 HV for Ti-1.25Cu, Ti-2.5Cu and Ti-5Cu, respectively. Likewise, the hardness values reported by Pina et al. (2016) for cpTi, Ti-3Cu, Ti-7.1Cu and Ti-12Cu were 260 ± 34 , 297 ± 20 , 313 ± 22 and 369 ± 19 HV, respectively. Zhang et al. (2013) found that the microhardness of cpTi at 160.3 ± 2.6 HV was almost half that of Ti-10 wt.% Cu at 369.5 ± 12.8 HV, indicating that the addition of Cu increased the hardness considerably. It may be deduced that the increase in hardness was caused by solid-solution hardening of α -Ti and the precipitation of Ti_2Cu phase. The difference in the diameters of Ti and Cu atoms causes severe distortion of the solid-solution crystal lattice, resulting in a rough slip surface and increased resistance to dislocation motion, thereby increasing the hardness of the alloy (Yi, et al., 2020). The higher hardness for Cu fortified specimens may also be attributed to the formation of very fine martensite phase and Ti_2Cu intermetallic phase with Cu-

rich phases, and suggests that the wear behaviour of the alloys with Cu should be improved in comparison with Ti6Al4V and pure Ti (Zhang, et al., 2013; Vilardell, et al., 2020).

4.3.4. Tensile test

The great temperature gradients characteristic of LPBF manufacturing of Ti parts tend to induce thermal residual stresses in the fabricated part. These residual stresses may cause the parts to warp and/or deform upon removal from the base plate (Matthews, et al., 2016; Ramosena, et al., 2022). For the current specimens, the stress-relief treatment was effective in alleviating internal thermal residual stresses, as the tensile test specimens did not exhibit warping or deformation. Similarly, Benedetti et al. (2017) demonstrated, using residual stress profiling, that stress-relief treatment of Ti6Al4V ELI at 670 °C for 5 hrs was effective in nearly eliminating the high residual stresses after SLM. The stress-strain curves of the alloy variants illustrated in Appendix B demonstrate that all tested samples underwent plastic deformation, indicating ductility. Table 4.3 and Figure 4.8 constitute the mechanical properties derived from the tensile test.

Table 4.3: Mechanical properties of Ti6Al4V and Ti alloys with Cu

Specimen	Yield strength (Offset 0.2%) (MPa)	Ultimate tensile strength (MPa)	Elastic modulus (GPa)	Elongation (%)	Reduction in area (%)
Ti64*	≥ 760	≥ 825		≥ 8	≥ 20
Ti64**	1051 ± 2.3	1115 ± 0.3	117.4 ± 1.7	11.3 ± 0.7	31 ± 3
Ti64-3Cu	1131 ± 13	1195 ± 13	121 ± 1	2.95 ± 0.90	20.72 ± 2.83
Ti64-6Cu	1209 ± 22	1286 ± 18	118 ± 3	1.79 ± 1.16	14.10 ± 1.84
cpTi*	≥ 275	≥ 345		≥ 20	≥ 30
cpTi	369 ± 16	501 ± 6	110 ± 6	8.84 ± 0.70	41.29 ± 6.54
Ti-3Cu	608 ± 7	763 ± 10	122 ± 15	7.04 ± 0.88	44.23 ± 7.29
Ti-6Cu	759 ± 16	904 ± 16	120 ± 3	4.62 ± 1.50	29.24 ± 7.47
Human cortical bone**	71.56 ± 10.19	93–190	10–30	0.67 ± 0.04	

* Values as per ASTM F3001 standard (for Ti6Al4V ELI) (ASTM International, 2021) and ASTM F67 standard (for cpTi) (ASTM International, 2017b).

**Values were derived from literature (Kasperovich & Hausmann, 2015; Morgan, et al., 2018; Okazaki & Ishino, 2020; Bartolomeu, et al., 2022).

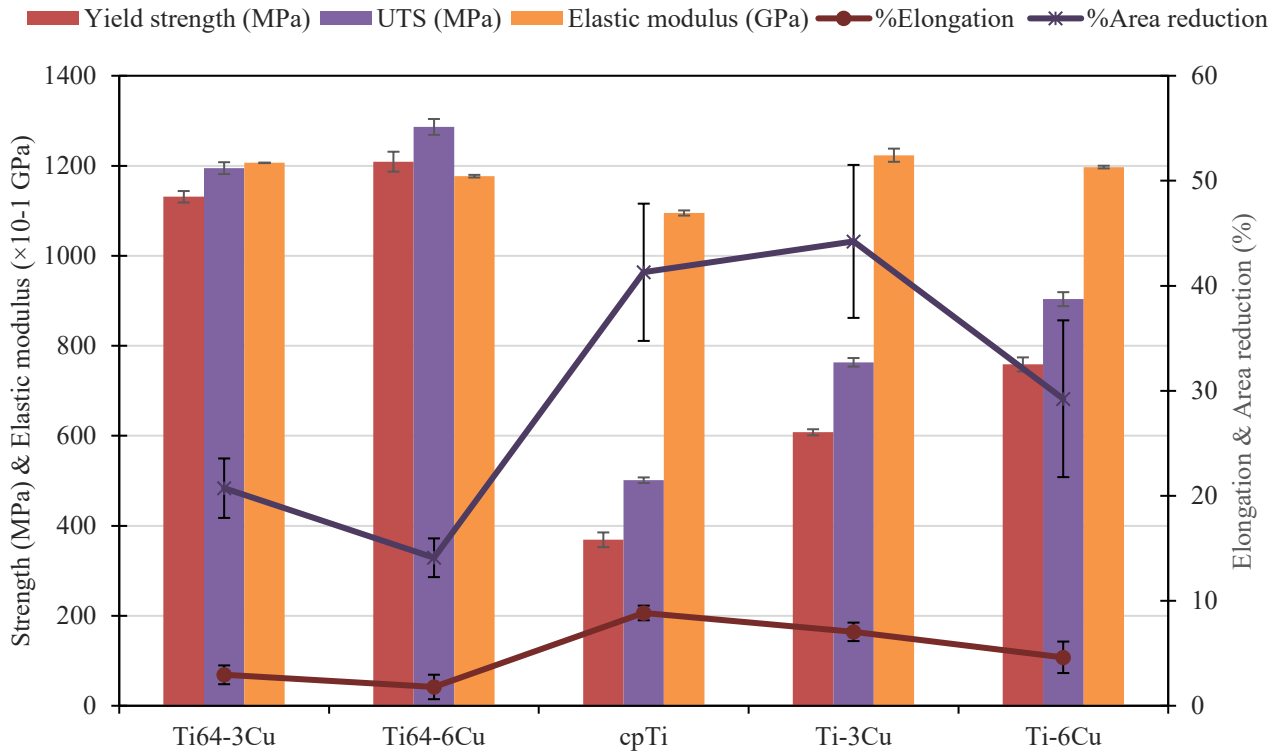


Figure 4.8: Tensile properties of titanium/copper alloys

The literature values for Ti6Al4V complemented the standard tensile test values. When compared to the literature values of Ti6Al4V (Table 4.3), the yield strength and the UTS of Ti6Al4V were lower than those of Ti6Al4V-3Cu and Ti6Al4V-6Cu. Moreover, the strength values of Ti6Al4V-3Cu were highly significantly lower than those of Ti6Al4V-6Cu at $p = 0.0043$ for yield strength and $p = 0.0005$ for UTS. The elastic moduli of Ti6Al4V-3Cu and Ti6Al4V-6Cu were slightly above the elastic modulus of Ti6Al4V and were not significantly ($p = 0.1048$) different between Ti6Al4V-3Cu and Ti6Al4V-6Cu. The %elongation and %reduction in area of Ti6Al4V were notably higher than those of Ti6Al4V-3Cu and Ti6Al4V-6Cu. The %elongation of Ti6Al4V-3Cu was insignificantly ($p = 0.2666$) higher than that of Ti6Al4V-6Cu, and the %reduction in area was significantly ($p = 0.0184$) higher than that of Ti6Al4V-6Cu. The parameters of %elongation and %reduction in area describe the ductility. Therefore, it may be observed from Table 4.3 and Figure 4.8 that the addition of Cu increased the strength and reduced the ductility of Ti6Al4V.

Commercially pure Ti test samples complied with the ASTM F67 standard values for the tensile test properties. The yield strength for cpTi was highly significantly lower than Ti-3Cu ($p = 0.000002$) and Ti-6Cu ($p = 0.0000001$), and was highly significantly ($p = 0.00001$) lower for Ti-3Cu than for Ti-6Cu. Likewise, UTS for cpTi was highly significantly lower than for Ti-3Cu ($p = 0.0000001$) and Ti-6Cu ($p = 0.000002$), and was also highly significantly ($p = 0.00004$) lower for Ti-3Cu than for Ti-6Cu. The difference in elastic modulus was insignificant between cpTi and Ti-3Cu ($p = 0.9426$), cpTi and Ti-6Cu ($p = 0.9081$) and Ti-3 and Ti-6 Cu ($p = 0.7786$). Percentage elongation was insignificantly different between cpTi and Ti-3Cu ($p = 0.1146$) and Ti-3Cu and Ti-6Cu ($p = 0.0621$), but %elongation of cpTi was significantly ($p = 0.0232$) higher than that of Ti-6Cu. For the %reduction in area, there was no significant difference between cpTi and Ti-3Cu ($p = 0.6217$) and cpTi and Ti-6Cu ($p = 0.0810$), but the %reduction in area of Ti-3Cu was significantly ($p = 0.0473$) higher than that of Ti-6Cu. Therefore, as with Ti6Al4V, Table 4.3 and Figure 4.8 indicate that alloying with Cu increased the strength and decreased the ductility of cpTi. High strength and low ductility limits are required for material in high-loaded components (Popovich, et al., 2015). The quest to establish a suitable alloy for producing implants for direct load bearing has directed focus on β -type titanium alloys since bone tissue responds more favourably in β -type alloys than in $(\alpha+\beta)$ -type Ti alloys (Dzogbewu, et al., 2017). Copper is a eutectoid β -stabilising element (Ahmad & Zuhailawati, 2020). Beta phase produces good mechanical properties, such as high strength (Mierzejewska, et al., 2019).

The compressive test of Zhang et al. (2013) demonstrated that cpTi was considerably more ductile than Ti-10 wt.% Cu, but the Ti-Cu alloy showed much higher yield strength. During their investigation, Wang et al. (2021) found the mechanical properties of Ti-2.5 wt.% Cu were: yield strength = 826 ± 18 MPa, UTS = 1002 ± 3 MPa, elongation = $5.3 \pm 1.0\%$; and of Ti-5 wt.% Cu were: yield strength = 1036 ± 9 MPa, UTS = 1242 ± 10 MPa, elongation = $2.5 \pm 1.6\%$. These values were comparable to those of Ti-3%Cu and Ti-6%Cu in Table 4.3. Benedetti et al. (2017) reported tensile properties for SLM Ti6Al4V ELI of 113 GPa, 1015 MPa, 1090 MPa, and 10% for elastic modulus, yield strength, UTS, and total elongation, respectively, which were almost equal to those of Greitemeier et al. (2016) in the as-built condition. The tensile figures of the Ti6Al4V- x Cu ($x = 3, 6$ wt.%) of this research were higher than those of Ti6Al4V ELI for yield strength and UTS, and were lower for % elongation. Aoki et al. (2004) examined the tensile properties of

Ti6Al4V- x Cu ($x = 1, 4, 10$ wt.%) alloys manufactured by casting and found that the tensile strength decreased from 1016 MPa for the 1% Cu alloy to 387 MPa for the 10% Cu alloy. Elongation also reduced with an increase in Cu content, while bulk hardness increased with the increase in Cu, such that the alloy with 10% Cu had the highest hardness value at 506 HV and undertook the most brittle fracture. From these results, it may be deduced that Cu addition should be kept minimal at about 1–4 wt.% to achieve an optimal combination of mechanical properties.

Copper addition during the present study did not adversely affect the elastic modulus of Ti6Al4V and cpTi. Pina et al. (2016) obtained Young's modulus values that were almost equal to those of the present experiment at 110.9 ± 3 , 109.2 ± 4 , 111.9 ± 3 , and 101.9 ± 3 GPa, respectively, for cpTi, Ti-3Cu, Ti-7.1Cu, and Ti-12Cu. The incompatibility in elastic modulus between Ti implants and bone tissue is one of the major causes of stress shielding, bone resorption, and implant loosening (Dzogbewu, et al., 2017). Stress shielding is among the most probable culprits responsible for bone-implant failure. Stress shielding typically arises when the modulus of elasticity of the implant is substantially greater than that of the surrounding bone (Al-Khateeb, et al., 2023). This considerable discrepancy in the elastic modulus shields the adjacent bone from supporting its share of the load, thus preventing the necessary stress from being transferred to the bone and leading to bone resorption around the implant and, ultimately, loosening and failure (Dzogbewu, et al., 2017). Porous structures and nontoxic alloying elements with low modulus of elasticity are part of the substantial developments toward combating stress shielding by lowering the modulus of elasticity values of metal implants to levels comparable to those of natural bone (Wu, et al., 2015; Lai, et al., 2021).

The relationship between strength and Vickers microhardness values is shown in Figure 4.9. There was no clear correlation between the hardness and strengths of Ti6Al4V-Cu samples. On the contrary, a distinct correlation was observed for the cpTi-Cu samples, such that the microhardness was directly proportional to strength and both increased with Cu addition. Likewise, Wang et al. (2021) confirmed a linear relationship between the strength and microhardness for SLM-fabricated Ti-Cu samples. Overlapping of Figures Figure 4.8 and Figure 4.9 would illustrate a minimal change in microhardness and ductility among the Ti64 samples, whereas ductility decreased with increasing hardness and strength in the cpTi-fabricated samples.

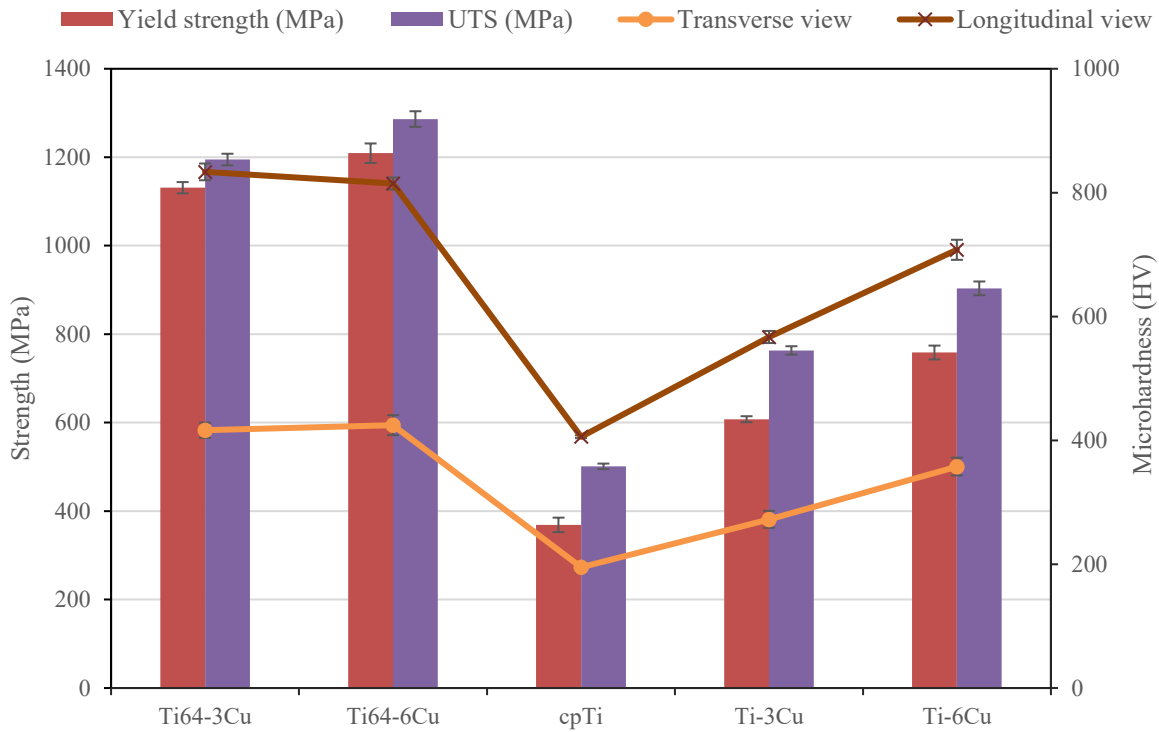
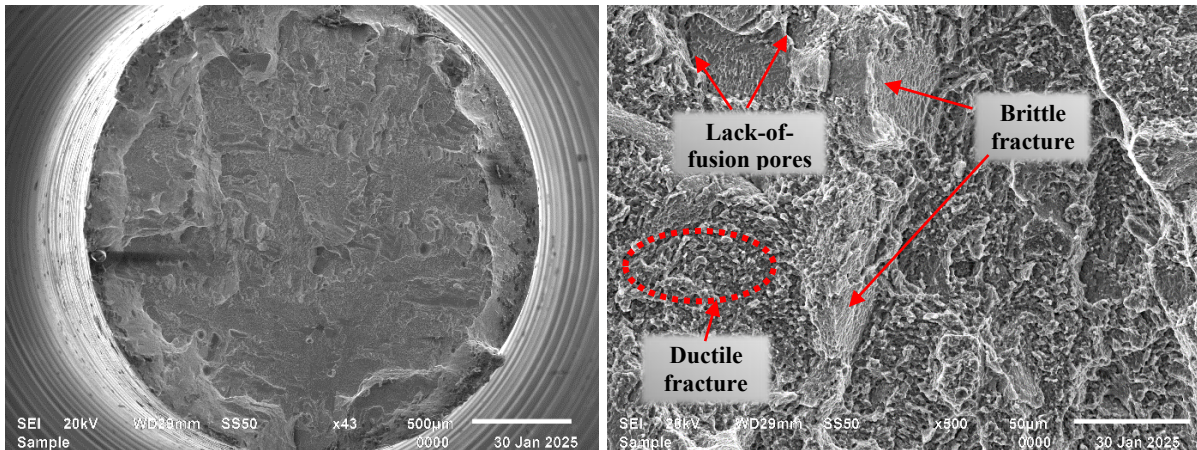


Figure 4.9: Correlation between strength and microhardness values of various Ti samples

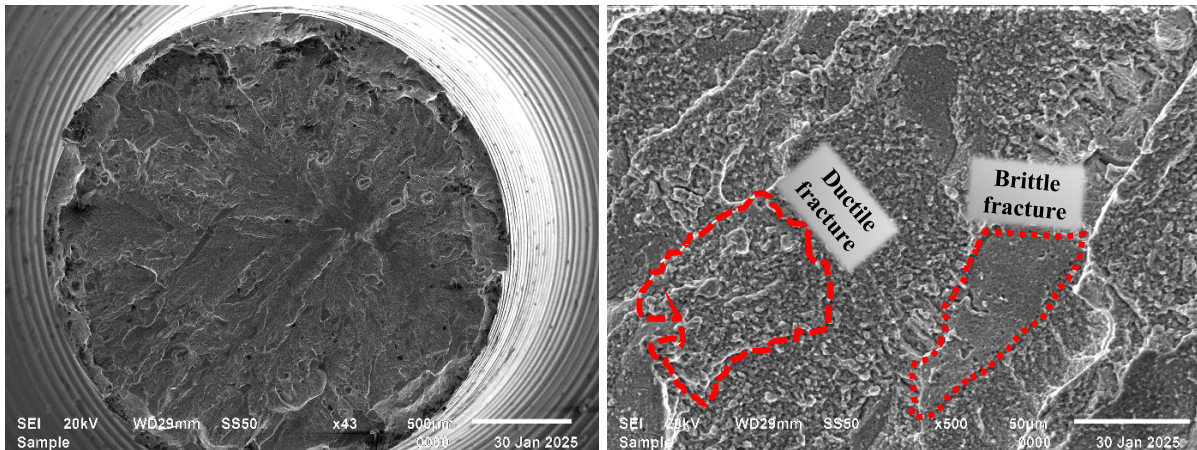
4.3.4.1. Fractography

Fractured surfaces resultant from the tensile test were examined using the SEM, and the fractographs are illustrated in Figure 4.10. The stress-relieved specimens showed cup-and-cone shapes and evident necking at the point of fracture. The necking tendency was supported by the stress/strain curves in Appendix B and is typically associated with a ductile fracture. The shear lips on the periphery of the cup or cone developed in the early stage of the application of the tensile force because the ductility of the hardened layer at the surface was far lower than that of the inner bulk mass and may be characterised by several transverse cracks developed near the ruptured site on the exterior wall (Aoki, et al., 2004). Images from the lower-magnification micrograph in Figure 4.10 show pores in the necking region. These enlarged pores may act as nucleation points for cracks and lead to final failure (Krakhmalev, et al., 2016; Moletsane, et al., 2016). According to Krakhmalev et al. (2017), crack nucleation may also be initiated by Cu concentrated regions.

Ti6Al4V-3Cu



Ti6Al4V-6Cu



cpTi

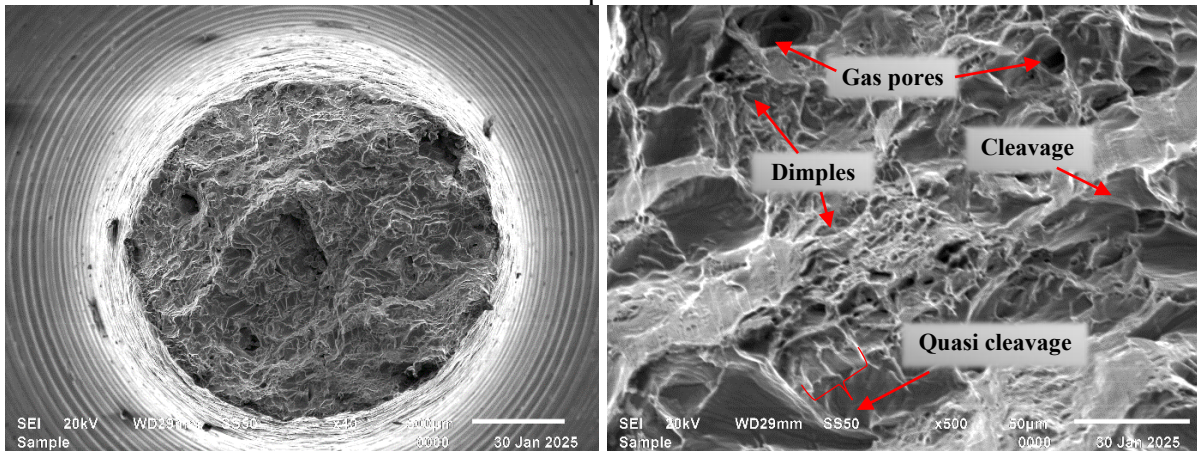
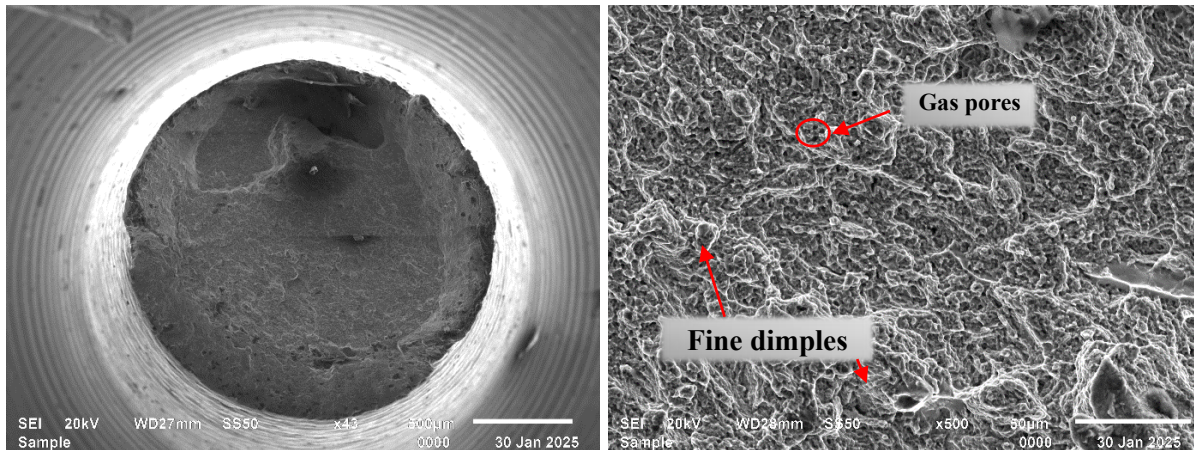


Figure 4.10: Tensile fracture surfaces of stress-relieved LPBF Ti-based samples

Ti-3Cu



Ti-6Cu

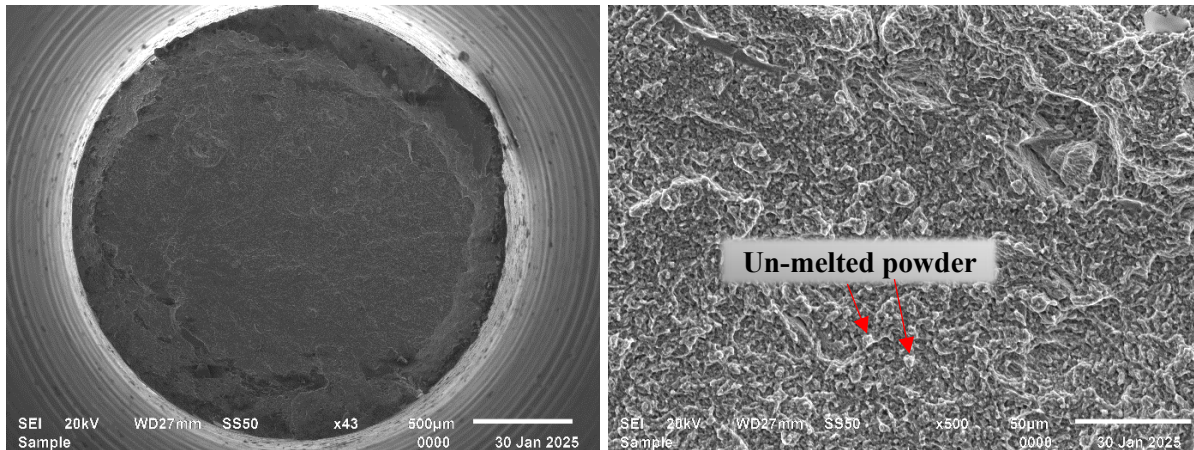


Figure 4.10: Tensile fracture surfaces of stress-relieved LPBF Ti-based samples (cont.)

The study of Yadroitsev et al. (2018) of the fractured surface of LPBF Ti6Al4V ELI demonstrated a cup-and-cone fracture resulting from necking with a distinct inner fibrous region dominated by dimpled rupture. Quasi cleavage was observed for Ti6Al4V ELI and harboured martensite needles on stress-relieved samples, verifying that these regions form during crack propagation along a martensite colony (Yadroitsev, et al., 2018). A cleavage fracture is a brittle fracture that propagates along low-index crystallographic planes and is caused by low energy density (Rafi, et al., 2013). The Ti6Al4V- x Cu ($x = 3, 6$ wt.%) fractured surfaces in this study exhibited a mixed mode of brittle and ductile fracture with the occurrence of gas and lack-of-fusion porosity. The Ti- x Cu ($x = 3, 6$ wt.%) samples presented predominantly ductile fracture surfaces characterised by dimples and minute gas pores. The higher ductility of Ti- x Cu ($x = 3, 6$ wt.%) than that of Ti6Al4V- x Cu ($x = 3,$

6 wt.%) is in agreement with the %elongation values (Table 4.3) and is supported by the stress-strain curves (Appendix B). Contrary to the tensile test values and curves, cpTi displayed the most brittle fracture with larger pores, fewer dimples and the prevalence of cleavage and quasi-cleavage facets on the central surface. Okazaki & Ishino (2020) observed dimples dominating the fractured surfaces of laser-sintered cpTi specimens.

Although LPBF processes enable high freedom of design, making them the prime choice for biomedical applications, their fast rates of heating and cooling lead to the formation of the martensitic phase which greatly reduces the ductility of the material (Dzogbewu, et al., 2017). Hard martensite accentuates high tensile strengths but poor ductility in as-fabricated LPBF products (Zhao, et al., 2016). In this study, partially melted Ti particles were randomly distributed in the alloys. Inhomogeneity in sintered parts and residual stress may reduce ductility. Nevertheless, the fractography results suggested that Cu improved the ductility of Ti-based materials.

4.4. Summary

The experiments of Chapter 4 began with the characterisation of Ti6Al4V- x Cu and Ti- x Cu ($x = 0, 3, 6$ wt.%) using XRD, which confirmed successful alloying with Cu particles at 3 and 6 wt.% using LPBF. The surface roughness examination of these specimens showed that Cu increased the roughness of Ti6Al4V and decreased the roughness of cpTi surfaces. The central focus of the chapter was to explore the mechanical properties of Ti6Al4V-3Cu, Ti6Al4V-6Cu, cpTi, Ti-3Cu and Ti-6Cu parts built using LPBF. Two tests were conducted, namely the microhardness test and the tensile test. The microhardness was determined using the Vickers microhardness tester in the transverse and longitudinal directions of the materials. The hardness of Ti6Al4V- x Cu ($x = 3, 6$ wt.%) was higher than that of Ti6Al4V ELI. The difference in hardness of Ti6Al4V was not established with an increase in Cu content from 3 wt.% to 6 wt.%. On the other hand, for Ti- x Cu ($x = 3, 6$ wt.%), the microhardness distinctively increased from 0 to 6 wt.% Cu. The effect of Cu concentration on cpTi samples and its minimal impact on Ti6Al4V was also observed with %elongation and %area reduction during the tensile test. This reinforces the notion that hardness predicts ductility. The stress-relief treatment before tensile testing effectively alleviated the effects of residual stresses in all materials, as evidenced by the absence of warping and deformation. The

strength increased with increasing Cu addition, but ductility decreased. Nonetheless, analysis of the fracture surface revealed higher ductility, characterised by dimples and gas pores, in copper-containing samples than in cpTi, which predominantly displayed cleavage and quasi-cleavage facets.

References

- Ahmad, F. N., & Zuhailawati, H. (2020). A brief review on the properties of titanium as a metallic biomaterials. *International Journal of Electroactive Materials*, 8, 63-67.
- Aoki, T., Okafor, I. C., Watanabe, I., Hattori, M., Oda, Y., & Okabe, T. (2004). Mechanical properties of cast Ti-6Al-4V-XCu alloys. *Journal of Oral Rehabilitation*, 31(11), 1109–1114. doi:10.1111/j.1365-2842.2004.01347.x
- ASTM International. (2017a). ASTM E3-11: Standard Guide for Preparation of Metallographic Specimens. West Conshohocken, Pennsylvania, USA: ASTM International.
- ASTM International. (2017b). ASTM F67-13: Standard Specification for Unalloyed Titanium, for Surgical Implant Applications (UNS R50250, UNS R50400, UNS R50550, UNS R50700). West Conshohocken, PA, USA: ASTM International
- ASTM International. (2021). ASTM F3001-14: Standard Specification for Additive Manufacturing Titanium-6 Aluminum-4 Vanadium ELI (Extra Low Interstitial) with Powder Bed Fusion. West Conshohocken, PA, USA: ASTM International .
- Bartolomeu, F., Gasik, M., Silva, F. S., & Miranda, G. (2022). Mechanical properties of Ti6Al4V fabricated by laser powder bed fusion: A review focused on the processing and microstructural parameters influence on the final properties. *Metals*, 12(6), 986. doi:10.3390/met12060986
- Benedetti, M., Torresani, E., Leoni, M., Fontanari, V., Bandini, M., Pederzoli, C., & Potrich, C. (2017). The effect of post-sintering treatments on the fatigue and biological behavior of Ti-6Al-4V ELI parts made by selective laser melting. *Journal of the Mechanical Behavior of Biomedical Materials*, 71, 295-306. doi:10.1016/j.jmbbm.2017.03.024
- Bolzoni, L., & Yang, F. (2024). X-ray diffraction for phase identification in Ti-based alloys: benefits and limitations. *Physica Scripta*, 99(6), 065024. doi:10.1088/1402-4896/ad4785
- Cabrini, M., Carrozza, A., Lorenzi, S., Pastore, T., Testa, C., Manfredi, D., . . . Scenini, F. (2022). Influence of surface finishing and heat treatments on the corrosion resistance of LPBF-produced Ti-6Al-4V alloy for biomedical applications. *Journal of Materials Processing Technology*, 308, 117730. doi:10.1016/j.jmatprotec.2022.117730
- Chen, H., Zhang, J., Wu, H., Li, Y., Li, X., Zhang, J., . . . Cai, X. (2021). Fabrication of a Cu nanoparticles/poly(ϵ -caprolactone)/gelatin fiber membrane with good antibacterial activity

- and mechanical property via green electrospinning. *ACS Applied Bio Materials*, 4(8), 6137-6147. doi:10.1021/acsabm.1c00485
- Chen, Y., Frith, J. E., Dehghan-Manshadi, A., Attar, H., Kent, D., Dominique, N., . . . Dargusch, M. S. (2017). Mechanical properties and biocompatibility of porous titanium scaffolds for bone tissue engineering. *Journal of the Mechanical Behavior of Biomedical Materials*, 75, 169-174. doi:10.1016/j.jmbbm.2017.07.015
- Dzogbewu, T. C. (2020). Laser powder bed fusion of Ti6Al4V lattice structures and their applications. *Journal of Metals, Materials and Minerals*, 30(4), 68-78. doi:10.14456/jmmm.2020.53
- Dzogbewu, T. C., Yadroitsev, I., Krakhmalev, P., Yadroitsava, I., & Du Plessis, A. (2017). Optimal process parameters for in situ alloyed Ti15Mo structures by laser powder bed fusion. *Proceedings of the 28th Annual International Solid Freeform Fabrication Symposium - an Additive Manufacturing Conference - 2017: August 7-9, 2017, The University of Texas at Austin, Austin, Texas, USA*.
- Greitemeier, D., Dalle Donne, C., Syassen, F., Eufinger, J., & Melz, T. (2016). Effect of surface roughness on fatigue performance of additive manufactured Ti–6Al–4V. *Materials Science and Technology*, 32(7), 629–634. doi:10.1179/1743284715y.0000000005
- Hesse, F. A., Verissimo, N. C., Soyama, J., & Bertazzoli, R. (2021). High-energy ball milling of intermetallic Ti-Cu alloys for the preparation of oxide nanoparticles. *Advanced Powder Technology*, 32(12), 4609-4620. doi:10.1016/j.appt.2021.10.009
- Kasperovich, G., & Hausmann, J. (2015). Improvement of fatigue resistance and ductility of TiAl6V4 processed by selective laser melting. *Journal of Materials Processing Technology*, 220, 202-214. doi:10.1016/j.jmatprotec.2015.01.025
- Kibria, G., Chatterjee, S., Shivakoti, I., Doloi, B., & Bhattacharyya, B. (2018). RSM based experimental investigation and analysis into laser surface texturing on titanium using pulsed nd:YAG laser. *IOP Conference Series.Materials Science and Engineering*, 377(1), 012203. doi:10.1088/1757-899X/377/1/012203
- Krakhmalev, P., Fredriksson, G., Yadroitsava, I., Kazantseva, N., du Plessis, A., & Yadroitsev, I. (2016). Deformation behavior and microstructure of Ti6Al4V manufactured by SLM. *Physics Procedia*, 83, 778-788. doi:10.1016/j.phpro.2016.08.080

- Krakhmalev, P., Yadroitsev, I., Yadroitsava, I., & de Smidt, O. (2017). Functionalization of biomedical Ti6Al4V via in situ alloying by Cu during laser powder bed fusion manufacturing. *Materials*, *10*, 1154. doi:10.3390/ma10101154
- Lai, T., Xu, J. L., Cao, X., Bao, L. Z., Luo, J. M., & Huang, Y. Z. (2021). Bioactive CaTiO₃ film prepared on the biomedical porous Ti–15Mo alloy by one-step hydrothermal treatment. *Journal of Materials Research and Technology*, *14*, 202–209. doi:10.1016/j.jmrt.2021.06.065
- Matthews, M. J., Guss, G., Khairallah, S. A., Rubenchik, A. M., Depond, P. J., & King, W. E. (2016). Denudation of metal powder layers in laser powder bed fusion processes. *Acta Materialia*, *114*(2016), 33–42. doi:10.1016/j.actamat.2016.05.017
- Mhlanga, M., Masheane, L. R., Yadroitsava, I., & Yadroitsev, I. (2022). Surface roughness of Ti6Al4V samples produced by laser powder bed fusion for bone implants. *MATEC Web of Conferences. 2022 RAPDASA-RobMech-PRASA-CoSAAMI Conference*, *370*, 11001. doi:10.1051/mateconf/202237011001
- Mierzejewska, Ź. A., Hudák, R., & Sidun, J. (2019). Mechanical properties and microstructure of DMLS Ti6Al4V alloy dedicated to biomedical applications. *Materials*, *12*(1), 176. doi:10.3390/ma12010176
- Moletsane, M. G., Krakhmalev, P., Kazantseva, N., du Plessis, A., Yadroitsava, I., & Yadroitsev, I. (2016). Tensile properties and microstructure of direct metal laser-sintered Ti6Al4V (ELI) alloy. *South African Journal of Industrial Engineering*, *27*(3), 110–121. doi:10.7166/27-3-1667
- Morgan, E. F., Unnikrisnan, G. U., & Hussein, A. I. (2018). Bone mechanical properties in healthy and diseased states. *Annual Review of Biomedical Engineering*, *20*, 119–143. doi:10.1146/annurev-bioeng-062117-121139
- Muiruri, A., Maringa, M., & du Preez, W. (2023). X-ray diffraction profile analysis of martensitic Ti6Al4V (ELI) parts produced by laser powder bed fusion. *MATEC Web of Conferences*, *388*(8), 10003. doi:10.1051/mateconf/202338810003
- Nirmala, R., Jeon, K. S., Lim, B. H., Navamathavan, R., & Kim, H. Y. (2013). Preparation and characterization of copper oxide particles incorporated polyurethane composite nanofibers by electrospinning. *Ceramics International*, *39*, 9651–9658. doi:10.1016/j.ceramint.2013.05.087

- Okazaki, Y., & Ishino, A. (2020). Microstructures and mechanical properties of laser-sintered commercially pure Ti and Ti-6Al-4V alloy for dental applications. *Materials*, 13(3), 609. doi:10.3390/ma13030609
- Peng, C., Liu, Y., Liu, H., Zhang, S., Bai, C., Wan, Y., . . . Yang, K. (2019). Optimization of annealing treatment and comprehensive properties of Cu-containing Ti6Al4V-xCu alloys. *Journal of Materials Science & Technology*, 35(10), 2121-2131. doi:10.1016/j.jmst.2019.05.020
- Pina, V. G., Amigó, V., & Muñoz, A. I. (2016). Microstructural, electrochemical and tribo-electrochemical characterisation of titanium-copper biomedical alloys. *Corrosion Science*, 109, 115-125. doi:10.1016/j.corsci.2016.02.014
- Popovich, A., Sufiiarov, V., Borisov, E., & Polozov, I. (2015). Microstructure and mechanical properties of Ti-6Al-4V manufactured by SLM. *Key Engineering Materials*, 651-653, 677-682. doi:10.4028/www.scientific.net/KEM.651-653.677
- Rafī, H. K., Karthik, N. V., Gong, H., Starr, T. L., & Stucker, B. E. (2013). Microstructures and mechanical properties of Ti6Al4V parts fabricated by selective laser melting and electron beam melting. *Journal of Materials Engineering and Performance*, 22(12), 3872–3883. doi:10.1007/s11665-013-0658-0
- Ramosena, L. A., Dzogbewu, T. C., & du Preez, W. (2022). Direct metal laser sintering of the Ti6Al4V alloy from a powder blend. *Materials*, 15, 8193. doi:10.3390/ma15228193
- Ren, J., Ma, Z., Li, M., Zhang, Y., Liu, W., Liao, Z., & Yang, K. (2014). Antibacterial properties of Ti-6Al-4V-xCu alloys. *Journal of Materials Science & Technology*, 30(7), 699-705.
- Sheikh, F. A., Kanjwal, M. A., Saran, S., Chung, W. -J., & Kim, H. (2011). Polyurethane nanofibers containing copper nanoparticles as future materials. *Applied Surface Science*, 257, 3020–3026. doi:10.1016/j.apsusc.2010.10.110
- Shen, Q., Qi, Y., Kong, Y., Bao, H., Wang, Y., Dong, A., . . . Xu, Y. (2022). Advances in copper-based biomaterials with antibacterial and osteogenic properties for bone tissue engineering. *Frontiers in Bioengineering and Biotechnology*, 9, 795425. doi:10.3389/fbioe.2021.795425
- Souza, S. A., Afonso, C. R., Ferrandini, P. L., Coelho, A. A., & Caram, R. (2009). Effect of cooling rate on Ti-Cu eutectoid alloy microstructure, 29, 3, . *Materials Science and Engineering: C*, 29(3), 1023-1028. doi:10.1016/j.msec.2008.09.007

- Suryawanshi, C. M., Gaur, B., Singh, A. K., Soman, D., Sagar, S., Bhallamudi, R., & Mishra, S. (2023). Microstructure and mechanical characterization of direct metal laser sintered Ti-6Al-4V alloy for orthopedic implant applications. *Materials Today Communications*, 35, 105770. doi:10.1016/j.mtcomm.2023.105770
- Thijs, L., Verhaeghe, F., Craeghs, T., Van Humbeeck, J., & Kruth, J. -P. (2010). A study of the microstructural evolution during selective laser melting of Ti-6Al-4V. *Acta Materialia*, 58, 3303–3312. doi:10.1016/j.actamat.2010.02.004
- Urlea, V., & Brailovski, V. (2017). Electropolishing and electropolishing-related allowances for powder bed selectively laser-melted Ti-6Al-4V alloy components. *Journal of Materials Processing Technology*, 242, 1-11. doi:10.1016/j.jmatprotec.2016.11.014
- Vilardell, A. M., Yadroitsev, I., Yadroitsava, I., Albu, M., Takata, N., Kobashi, M., . . . du Plessis, A. (2020). Manufacturing and characterization of in-situ alloyed Ti6Al4V(ELI)-3 at.% Cu by laser powder bed fusion. *Additive Manufacturing*, 36, 101436. doi:doi.org/10.1016/j.addma.2020.101436
- Wang, L., Wang, F., Ayisen, S., Ren, T., Luo, X., & Wang, P. (2023). Enhancing the mechanical properties and surface morphology of individualized Ti-mesh fabricated through additive manufacturing for the treatment of alveolar bone defects. *Frontiers in Bioengineering and Biotechnology*, 11, 1284359. doi:10.3389/fbioe.2023.1284359
- Wang, Q., Zhang, K., & Niu, W. (2021). Microstructural characteristic and mechanical properties of titanium-copper alloys in-situ fabricated by selective laser melting. *Journal of Alloys and Compounds*, 885, 161032. doi:10.1016/j.jallcom.2021.161032
- Wysocki, B., Maj, P., Sitek, R., Buhagiar, J., Kurzydłowski, K. J., & Świąszkowski, W. (2017). Laser and electron beam additive manufacturing methods of fabricating titanium bone implants. *Applied Sciences*, 7(7), 657. doi:10.3390/app7070657
- Yadroitsev, I., Krakhmalev, P., Yadroitsava, I., & du Plessis, A. (2018). Qualification of Ti6Al4V ELI alloy produced by laser powder bed fusion for biomedical applications. *The Journal of The Minerals, Metals & Materials Society*, 70(3), 372–377. doi:10.1007/s11837-017-2655-5
- Yi, C., Ke, Z., Zhang, L., Tan, J., Jiang, Y., & He, Z. (2020). Antibacterial Ti-Cu alloy with enhanced mechanical properties as implant applications. *Materials Research Express*, 7(10), 105404. doi:10.1088/2053-1591/abc371

- Zhang, E., Li, F., Wang, H., Liu, J., Wang, C., Li, M., & Yang, K. (2013). A new antibacterial titanium–copper sintered alloy: preparation and antibacterial property. *Material Science and Engineering C*, 33, 4280-4287.
- Zhao, X., Li, S., Zhang, M., Liu, Y., Sercombe, T. B., Wang, S., . . . Murr, L. E. (2016). Comparison of the microstructures and mechanical properties of Ti–6Al–4V fabricated by selective laser melting and electron beam melting. *Materials and Design*, 95, 21–31.

CHAPTER 5 COATING OF TI SAMPLES WITH POLYMER NANOFIBRES USING ELECTROSPINNING

5.1. Introduction

Chapter 5 outlines the electrospinning procedure for coating titanium (Ti)-based implant specimens with polymer nanofibres (NFs) and preliminary investigations for the optimisation of conventional electrospun fibres to fulfil the adhesion requirements of the NF mats on Ti6Al4V ELI and commercially pure Ti (cpTi) substrates produced using laser powder bed fusion (LPBF). The optimal amount of copper oxide nanoparticles (CuO NPs) to embed in the NFs, without hindering the electrospinning of the polymer solution while retaining antimicrobial properties, was also the focus of the investigations.

The electrospinning technique allows for the production of fibres that replicate the characteristics of fibrous biological materials, and its processing variables may be altered to enable the production of tissue engineering scaffolds with a broad array of fibre morphologies and properties (Kausar, 2021; Luketich, et al., 2022). Coating Ti-based implants with electrospun NFs of a polymer may enhance their bioactivity. Furthermore, adding nanoparticles to electrospun fibres generates enhanced functional characteristics (Duygulu, 2024). The challenge of electrospinning technology is to adjust the spinning conditions to produce fibres that will satisfy the application requirements after selecting the appropriate polymer composition (Mao, et al., 2023). Among the various factors involved in the electrospinning procedure, the three main parameters include those associated with the electrospinning process itself, polymeric solution parameters and environmental parameters (Haider, et al., 2018; Arrieta, et al., 2020; Mao, et al., 2023).

5.2. LPBF parts

For the purpose of coating using the electrospinning technology, Ti6Al4V ELI (also referred to as Ti6Al4V or Ti64 in this chapter) and cpTi test samples were manufactured using the LPBF technique and the parameters as specified in Table 3.8. Figure 5.1 illustrates two types of test specimens manufactured: discs dimensioned 10×2 mm and rectangular samples dimensioned $30 \times 30 \times 2$ mm. The sample discs were fabricated on a Ti6Al4V substrate, and the rectangular samples were built on the base plate. The samples were used in their as-built condition without polishing or treating the surfaces. After being removed from the support structures, the samples were ultrasonically cleaned with acetone, anhydrous ethanol and distilled water for 15 min each in turn to remove surface impurities and dried under natural air (Wang, et al., 2022). The discs were utilised during biological investigations, and the rectangular samples were used for adhesion testing.

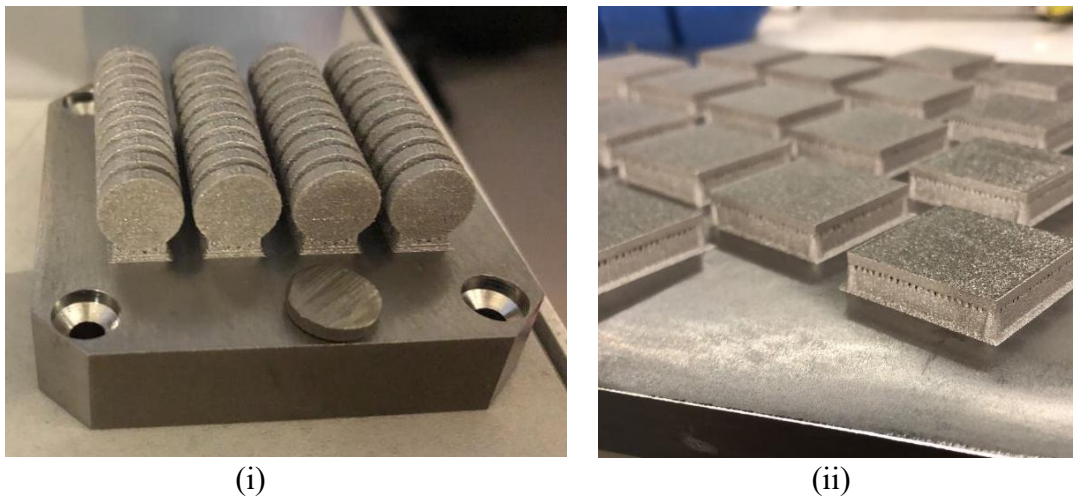


Figure 5.1: Test specimens fabricated using LPBF of (i) round discs (10×2 mm), and (ii) rectangular prisms ($30 \times 30 \times 2$ mm) whilst attached to the support structures

5.3. Electrospinning

Solution electrospinning is the most commonly used method and involves dissolving the polymer in an appropriate solvent to an appropriate concentration (Sharma & James, 2022). Blend or single jet electrospinning is the most common and simplest type of solution electrospinning method, and it involves the combining of all chemical elements with polymer components by mixing in a single-solvent system to form a homogeneous solution for electrospinning (Parham, et al., 2020; Abadi,

et al., 2022; Mao, et al., 2023). Thus, blend solution electrospinning allows the mixing of different polymers or polymers with functional agents to prepare a single fluid to be electrospun. In addition, encapsulation of CuO NPs within the resultant NF may be achieved in a single step, which leads to a prolonged release of the Cu ions (Abadi, et al., 2022).

5.3.1. Parameters influencing the electrospinning process

Although a universal model to regulate the morphology of electrospun NFs for a specific polymer/solvent system under different conditions has not been established, general relationships among key parameters may be drawn to guide the electrospinning process (Li & Bou-Akl, 2016). Table 2.1 outlines these relationships and their influence on NF morphology. It is essential that these parameters operate in unison to achieve a specific fibre morphology. For example, a combination of a higher applied voltage, a smaller needle diameter, and a lower flow rate has been shown to produce thinner electrospun NFs (Sharma & James, 2022). For application in tissue engineering, higher porosity and surface roughness result in a higher specific surface area for cell attachment and proliferation (Kausar, 2021). Increased membrane porosity is realised with an increase in NF diameter (Nayl, et al., 2022). On the other hand, the small diameters and great lengths of electrospun fibres create a large surface-area-to-volume ratio, providing a pillar for tissue regeneration (Kazemzadeh, et al., 2022). Conversely, the formation of beads during electrospinning of NFs reduces the surface area per unit mass of the obtained fibres and, thus, beads are considered a defect in the electrospinning process (Xue, et al., 2019). Therefore, the parameters of the electrospinning process require optimisation to produce uniform, continuous fibres with desirable morphology and distribution for biological enhancement.

5.3.2. Setup

The Fluidnatek® LE-100 electrospinning equipment by Bioinicia (Figure 5.2), located at the Botswana Institute for Technology, Research and Innovation (BITRI) was used for this project. The machine is mainly comprised two syringe pumps, a needle holder, a high voltage power supply and a collector.

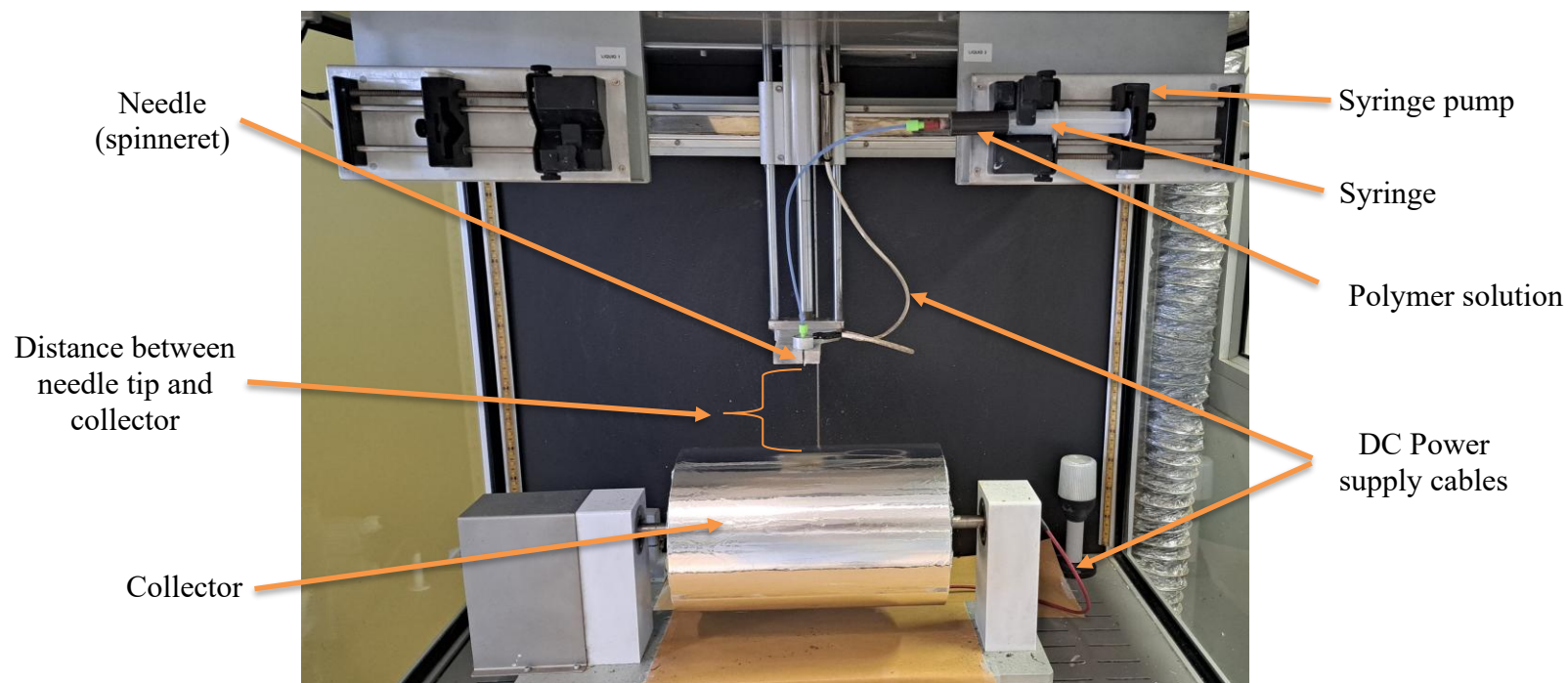


Figure 5.2: Bioinicia Fluidnatek® LE-100 electrospinning equipment situated at BITRI

5.3.3. Coating technique

For the present investigations, the solution electrospinning method was applied using a blend electrospinning approach. A polymer solution was prepared by dissolving the polymer composition with agitation in an appropriate solvent system. A 20 ml plastic syringe was used to load the polymer solution, which was then placed in the syringe pump section of the machine. The Ti6Al4V and cpTi samples to be coated were placed on a stationary collector drum covered with aluminium foil. The collector should remain isolated from its axle in such a way that it may be grounded without also grounding adjacent objects (Leach, et al., 2011). The syringe pump was used to apply a constant flow rate to the polymer solution from the syringe to the needle. A needle-based single nozzle electrospinning equipment, in which only one needle is used as the spinneret, is the simplest and most basic form. Mao et al. (2023) found that this is the most frequently used electrospinning equipment in studies. A single nozzle electrospinning with a stainless steel blunt-end needle tip was adopted for this project.

A high-voltage direct current (DC) power supply housed in the base of the machine was used to supply the required charge to the solution. The needle was connected to the positive terminal of the power supply to charge the polymer solution, while the negative terminal was connected to the collector to ground it. When the electric field from the power supply overcame the liquid surface tension of the polymer solution, a Taylor cone formed at the tip of the needle-like spinneret. With continuous application of flow rate and charge to the polymer solution, the conical shape led to fibre jet ejection through the needle tip, with the jet travelling a specific distance to the collector to coat the Ti6Al4V and cpTi surfaces with polymer nanofibres. Since the collector was stationary, the specimens were coated with random fibres. Aligned fibres may be collected on a rapidly rotating collector wheel (Leach, et al., 2011). To obtain continuous bead-free NFs, the voltage, flow rate and distance between the needle tip and the top of the collector should be optimised. These parameters were arbitrarily adjusted until NFs with the desired morphology were obtained.

5.4. Direct pull-off adhesion test

For a coating to perform, it is critical that it sufficiently adheres to the substrate (Ahmed, et al., 2018). Pull-off adhesion testing has become the industry standard for most applications because it is simple, repeatable and provides valuable results. A pull-off adhesion tester quantifies the force

perpendicular to the substrate required to pull a specified diameter of coating away from the substrate (DeFelsko, 2020; Ibrahim & Hamad, 2023). The DeFelsko PosiTest AT-A Automatic Adhesion Tester kit (

Figure 5.3) was employed as per the manufacturer's instructions in accordance with ASTM D4541 to test the adhesion strength of the electrospun polymeric NF-coated onto Ti6Al4V and cpTi samples (dimensions: $30 \times 30 \times 2$ mm).



Figure 5.3: DeFelsko PosiTest AT-A automatic Adhesion Tester kit

The following steps were used to complete the direct pull-off adhesion test (DeFelsko, 2020):

1. The surface of the loading fixture (also known as the dolly) was prepared by cleaning and roughening with the abrasive pad to help the glue form a tight bond with the dolly surface. For the present investigation, 20 mm dollies were used.
2. Equal amounts of the two-part epoxy glue were stirred on the pallet with mixing sticks until thoroughly combined.
3. A uniform layer of the glue was applied to the dolly, and it was pressed vertically on the coating. Any excess glue was removed with a swab.
4. The glue was left to cure for 24 hrs at room temperature.

5. The coating around the dolly was cut with the cutting tool to isolate the test area. This is particularly essential for elastomers.
6. The actuator was attached to the dolly, ensuring that quick coupling was completely engaged.
7. An electronically controlled hydraulic pump applied a continuous pressure at a rate-of-pull of 1.0 MPa/s to the dolly until it detached from the substrate surface, which marked the completion of the test.

The adhesion tester measured the maximum pressure required to pull the fixture from the surface. The measurement data was automatically recorded using the PosiSoft Software on the tester. In addition to pull-off strength, ASTM D4541 and ISO 4624 require the nature of the fracture to be recorded based on visual inspection of the test system at fracture (DeFelsko, 2023). According to ASTM International (2022), the glue and coating layers constitute the test system. The scheme that describes the total test system and its fractures is illustrated in Figure 5.4 and is as follows (ASTM International, 2022; DeFelsko, 2023):

- » The coated specimen is designated as substrate *A*, upon which successive coating layers *B*, *C*, *D*, and so forth have been applied, including the glue *Y* that secures the dolly *Z* to the top coat.
- » A cohesive fracture occurs within a coating layer (the coating on the dolly face is identical to that on the substrate). It is designated by the layer within which it appears as *B*, *C*, *D*, *Y*, and so forth, and the visually estimated percentage.
- » An adhesive fracture occurs at the interface between layers (the coating on the dolly face differs from the substrate). Glue failure is the visible separation of the glue from itself, the coating or the dolly (no coating is visible on the dolly face during glue failure). Adhesive fractures and glue failure are designated by the interfaces at which they occur as *A/B*, *B/C*, *C/D*, *Y/Z*, and so forth, and the visually estimated percentage.

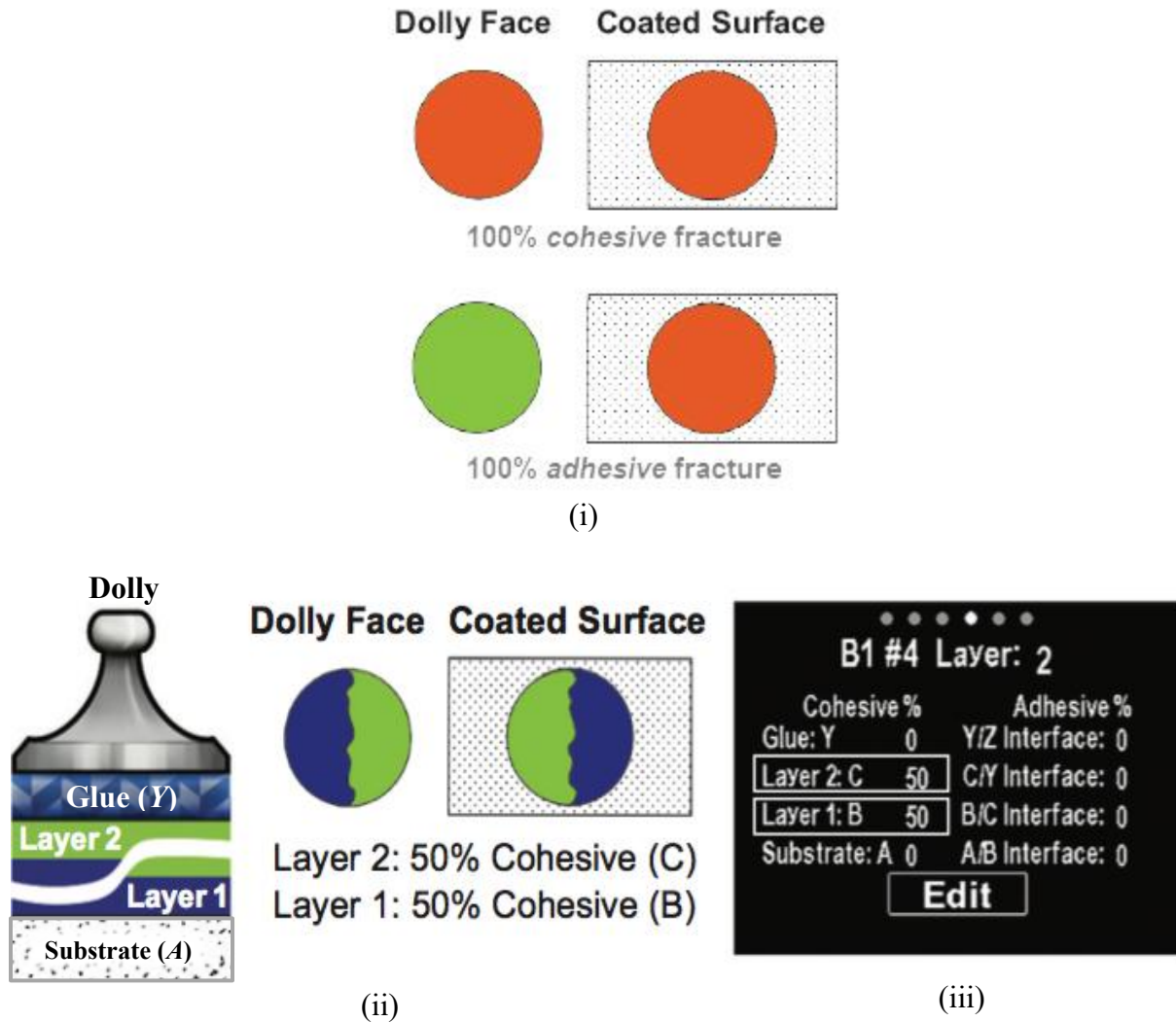


Figure 5.4: (i) Differentiation between the test system fractures, (ii) demonstration of interpreting the nature of a fracture, and (iii) the recording thereof with estimated percentages on the adhesion tester display screen (adapted from DeFelsko (2023))

The Ti6Al4V and cpTi substrates in the current investigations were coated with a single layer of an electrospun NF matrix. Therefore, each test system consisted of the glue *Y* and layer *B* (NF layer) on the substrate *A*. In cases where a primer was applied at the nanofibre-metal interface, the primer layer was also a polymer and/or was too thin to be considered as a separate layer. The criteria for acceptable adhesion strength and fracture were not established from the standards. Therefore, the experimental data were compared with those found in the literature.

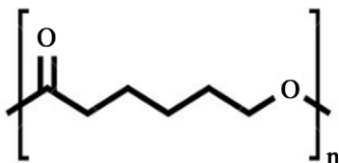
5.5. Material

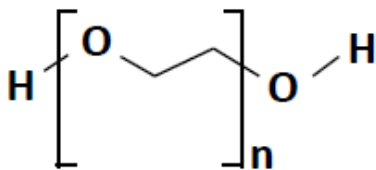
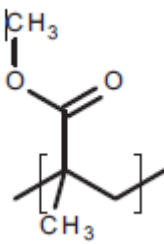
5.5.1. Polymers

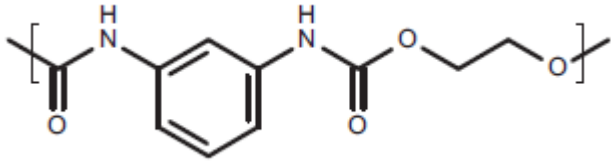
Material selection is crucial for producing biodegradable, bioadhesive polymer NFs for biomedical applications. The selected polymer composition determines the morphology, mechanical strength, degradation rate and biocompatibility of the NFs (Sharma & James, 2022).

Table 5.1 highlights the synthetic polymers selected to prepare solutions for electrospinning into NFs as coatings for the LPBF Ti6Al4V and cpTi surfaces, the solvents in which they are soluble, and their features applicable to biomedicine.

Table 5.1: Characteristics of the employed polymers

Polymer details		Ref.
<i>Poly(ε-caprolactone) or polycaprolactone (PCL)</i>		(Cheng, et al., 2010; Baker, et al., 2016; Ashtikar & Wacker, 2018; Song, et al., 2018; Kiran, et al., 2019; Arrieta, et al., 2020; Stewart, et al., 2020; Bikuna-Izagirre, et al., 2022; Mao, et al., 2023; Quilez-Molina, et al., 2023)
Structure:		
Characteristics:	- Semi-crystalline, aliphatic polyester - Biocompatible and biodegradable - Slow degradation rate (2–4 years) - Hydrophobic - Low melting temperature (T_m, ~ 60°C) and glass transition temperature (T_g, ~-60°C), - Exceptional mechanical properties [high tensile strength (10.5–16.1 MPa), yield strength (8.2–10.1 MPa) and elastic modulus (343.9–364.3 MPa)] - Unique combination of polyolefin-like mechanical properties and polyester-like hydrolysability; - Distinct rheological and viscoelastic properties - Thermoelastic - Non-toxic	
Solvents:	N,N-dimethylformamide (DMF), dichloromethane (DCM), methylene chloride, trichloromethane (TCM, also known as chloroform (CHCl ₃))	
Molecular weight:	Average 3,000 to 80,000 Da	
Applications:	Tissue engineering; drug-delivery systems; wound dressings; biosensors; fixation devices; cardiovascular stents; implants	
<i>Poly(ethylene glycol) (PEG)</i>		

Structure:		(Cheng, et al., 2010; Leung, 2014; Bîrcă, et al., 2019; Visan, et al., 2020)
Characteristics:	- Hydrophilic (water soluble) - Biocompatible and biodegradable - Linear or branched-chain polyether - Low melting temperature (40–60 °C) - Highly flexible - At low molecular weight: liquid, amorphous - At high molecular weight: solid, crystalline - Non-toxic	
Solvents:	Water and most organic solvents (such as methanol (MeOH), ethanol (EtOH), acetonitrile, benzene, and TCM)	
Molecular weight:	200 Da to several million Da	
Applications:	Tissue engineering; drug-delivery systems; implants	
Poly(methyl methacrylate) (PMMA)		
Structure:		(Ottenbrite & Javan, 2005; Maitz, 2015; Liu, et al., 2016; Banoriya, et al., 2017; Ni, et al., 2019; Nathanael & Oh, 2020; Kausar, 2021; Yuan, et al., 2022)
Characteristics:	- Amorphous - Hydrophobic - Biocompatible - Non-biodegradable, slow degradation - Glassy transparent polymer - Thermoplastic - Poor thermal and mechanical properties (brittle, tensile strength of ~ 59 MPa, elastic modulus of ~2.55 GPa) - Low toxicity	
Solvents:	Acetone, DMF, tetrahydrofuran (THF), ethyl acetate, CHCl ₃	
Molecular weight:	100,000 to 1,000,000 Da	
Applications:	Bone tissue engineering; drug-delivery systems, sensors, orthodontic retainers, fixation cement for the orthopaedic prosthetics	
Polyurethane (PU)		

Structure:		(Sheikh, et al., 2011; Nirmala, et al., 2013; Maitz, 2015; Banoriya, et al., 2017; Jordi, 2018; Abdal-hay, et al., 2019; Nathanael & Oh, 2020; Kazemzadeh, et al., 2022)
Characteristics:	- Amorphous - Hydrophobic - Biocompatible - Non-biodegradable, low degradation rate - Favourable mechanical properties - Physical and mechanical properties similar to natural tissue (tensile strength of ~ 35 MPa, elastic modulus of ~ 20 MPa) - Self-healing property - Thermoplastic elastomer - Resistant to abrasion - Excellent hydrolytic stability - Non-toxic	
Solvents:	DMF, THF, dimethyl sulfoxide (DMSO)	
Molecular weight:	50,000–300,000 Da	
Applications:	Wound dressing, coating matrix, pacemaker lead coatings, breast implant coatings, vascular devices, sensors, implants	

To improve the properties of polymer materials and broaden their applications, biopolymers are often blended to create unique multifunctional polymeric structures (Arrieta, et al., 2020). The chief purpose of hybrid polymers is to optimise essential characteristics, such as mechanical stability, degradation rates and water solubility. Among most polymers, PCL possesses a bulk of essential properties, easily forms copolymers with other compounds, is highly permeable to various drugs and presents excellent electrospinnable properties, making it an ideal base polymer in hybrid materials (Cheng, et al., 2010; Stewart, et al., 2020; Quilez-Molina, et al., 2023). A major shortcoming of PCL is its poor wettability, resulting from its intrinsic hydrophobicity, which slows its degradation kinetics and hinders cellular attachment, scaffold infiltration, and drug delivery. Hence, there is a necessity to combine PCL with other polymers and promote success in clinical applications (Cheng, et al., 2010; Kiran, et al., 2019; Bikuna-Izagirre, et al., 2022). Poly(ε-caprolactone), PEG and PMMA are Food and Drug Administration (FDA) approved biomedical materials (Stewart, et al., 2020; Bhogal, et al., 2024). Because of its unique combination of flexibility, toughness, and biocompatibility, PU is also among the polymers commonly used in

biomedicine as both degradable and non-degradable materials (Brzeska, et al., 2014; Nathanael & Oh, 2020; Mao, et al., 2023).

Studies have shown that molecular weight affects the miscibility of compounds and the degradation rates of polymers (Stewart, et al., 2020). The relative molecular weight of the polymer is directly proportional to solution viscosity (Mao, et al., 2023). Of the properties of precursor polymer solution listed in

Table 5.1, the viscosity of the solution is the basis of fibre formation during electrospinning. It determines the structure and morphology of the resultant fibres (Mao, et al., 2023). Solution viscosity depends on the selection of the most suitable solvent ratio for preparing the electrospinning solution to obtain the desired fibre diameter, consistency, continuity and uniformity of fibres. Binary solvents are preferred as opposed to single solvent systems as they allow for the electrospinning of porous structures and hence have an effect on the formation of specific surface structures (Liu, et al., 2016).

5.5.2. Copper oxide (CuO) nanoparticles

Among the commonly used metal/metal oxide antibacterial agents, copper (Cu) is an essential metal in living organisms for supporting metabolic processes (Ahire, et al., 2016). Adding Cu particles to polymer NFs facilitates the development of multifunctional nanocomposites for tissue regeneration by enhancing the mechanical and biological properties (Kausar, 2021). At high concentrations, Cu displays a highly efficient antibacterial effect and thermal stability (Chen, et al., 2021). Furthermore, a high copper content increases the electric conductivity of a polymer solution, which results in the production of high charge values and acceleration of jetting polymer during the electrospinning process (Sheikh, et al., 2011; Chen, et al., 2021). Sheikh et al. (2011) observed an increase in the rate of nanofibre production by electrospinning as the Cu content was increased from 0 to 10% in a polymeric solution. However, excessively high Cu concentrations may cause cytotoxicity. Hence, the Cu content should be maintained at an optimal level such that it is neither too high to be toxic nor too low to exhibit a bactericidal effect. Moreover, within the optimal range, the Cu added to the polymer solution should not interfere with the electrospinnability of the polymer jet and the subsequent fibre morphology. Studies have shown

that the maximum concentration of Cu particles that may be electrospun into NFs was 150 mg/ml (Ahire, et al., 2016).

For this study, Cu in the form of copper oxide nanoparticles (CuO NPs, also referred to as CuO) was employed as an additive to induce the antibacterial properties of the NF coatings. Copper oxide nanoparticles were synthesised in situ by aqueous precipitation method using copper chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 98%) as a precursor and sodium hydroxide (NaOH) as a reducing agent (Figure 5.5). Copper (II) chloride solution (0.2 M) and polyvinylpyrrolidone (PVP) (0.1 M) were placed in a round-bottomed flask and heated to boiling under magnetic stirring. Then, 1.0 M NaOH solution was poured into the flask. The colour of the solution immediately changed from blue to black and resulted in the formation of a black suspension. The black suspension was stirred and heated to boiling for 2.5 hrs. The mixture was then cooled to room temperature and centrifuged to obtain a wet CuO precipitate. The precipitate was filtered and washed with distilled water and absolute ethanol several times. The resulting product was dried (at 60 °C for 6 hrs) to obtain the dry powder of CuO NPs as shown in Figure 5.5. The chemical reactions involved in the preparation of CuO nanoparticles are outlined below:

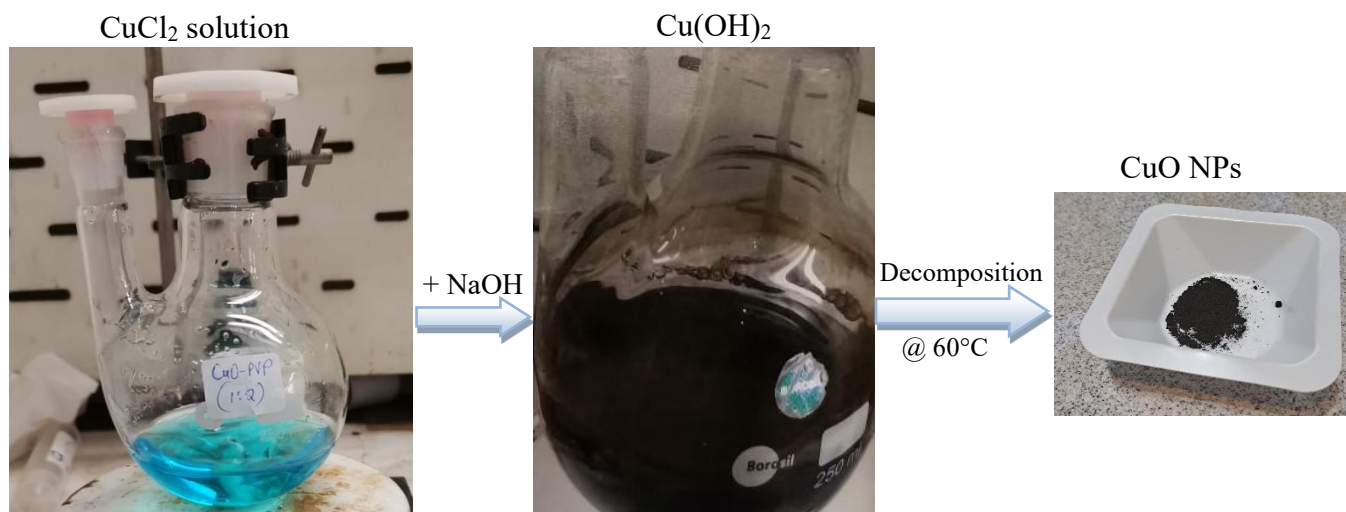
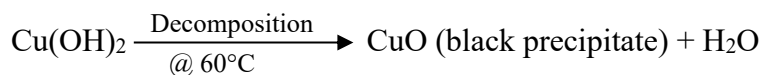


Figure 5.5: In situ production of CuO NPs

5.6. Preliminary studies

Because of the novelty of the current experiments, information on the composition of polymer nanofibres containing an acceptable amount of CuO NPs for electrospinning and Ti surface adhesion was found to be lacking during the current investigations. Accordingly, preliminary studies were conducted to determine the most suitable material for further analyses. Poly(ϵ -caprolactone) was selected as the base material due to its ability to be miscible with other polymers and readily form copolymers and blends (Stewart, et al., 2020). Poly(ϵ -caprolactone) and its copolymers have gained popularity as biomaterials in the design of various controlled/prolonged drug delivery systems such as nanoparticles and implants (Cheng, et al., 2010). All reagents used in this work were analytical grade and were used as received without additional purification. Due to the toxic nature of the solvents, all solutions were prepared under a fume hood. Only Ti6Al4V specimens manufactured using LPBF were employed in the preliminary investigations.

5.6.1. First preliminary study: PCL/PEG NFs blends

The primary advantages of polycaprolactone/polyethylene glycol (PCL/PEG) blends include the enhanced hydrophilicity of PCL and the mechanical properties of PEG. Wettability measurements of Visan et al. (2020) evidenced a distinct hydrophilic character of the PCL/PEG blend coatings as compared to those of the PCL-coated and bare Ti samples. Moreover, the PCL/PEG contact angle value of the 3:1 (weight/weight (w/w)) blend at 62.1 was almost double that of the 1:3 (w/w) blend at 36.2. Douglas et al. (2016) discovered a detrimental effect on the tensile strength and stiffness of PCL with the addition of PEG, reducing them by 20–40% as the concentration of PEG was increased from 5% to 25% in the PCL/PEG blend.

Subsequent benefits of PCL/PEG composite coatings have been realised. Polyethylene glycol has been reported to increase the degree of crystallinity of PCL as a result of the mobile PEG chains that improve molecular chain mobility, thus elevating crystallisation rate and serving as sites for the growth of PCL crystals due to the plasticising effect of PEG (Stewart, et al., 2020). Stewart et al. (2020) demonstrated an additional crystalline peak characteristic of PEG crystalline domains in addition to those characteristics of PCL on an x-ray diffraction (XRD) plot of a PCL/PEG

coating film intended for AM implant devices. Also, the melting point of PCL was reduced by the addition of PEG. The results of Douglas et al. (2016) were contrary to those of Stewart et al. (2020) regarding crystallinity, as their XRD showed no new peaks for PCL-PEG blends, which was attributed to immiscibility between the polymers (An et al., 2001). However, both studies agreed that PEG contributed to the improvement in the thermal stability of the blends. It is noteworthy that molecular weight has an effect on the miscibility of compounds (Stewart, et al., 2020). For their study, Visan et al. (2020) focused on the long-term degradation of PCL-blend-PEG coatings fabricated for coating Ti implants, and found that the PEG component had completely degraded after 16 weeks of immersion in simulated body fluids (SBF). Their fluorescence microscopy studies showed that the PCL-blend-PEG coating was an adequate substrate for the adhesion and proliferation of human mesenchymal stem cells and endothelial cells, and preserved their phenotype.

For the current project, Ti6Al4V sample discs (10×2 mm) were coated with a blend of PCL ($M_n = 80,000$ Da) and PEG ($M_n = 6,000$ Da) NFs using the electrospinning process. It was envisaged that the mechanical strength of PCL and the hydrophilicity of PEG would provide a suitable scaffold for guided tissue regeneration. The PCL/PEG (1:1 w/w) blend was prepared by dissolving PCL and PEG in 20 ml of $\text{CHCl}_3/\text{MeOH}$ (3:1 v/v) solvent system. Complete dissolution was achieved after 4 hrs of continuous stirring at room temperature; thereafter, the polymer solution was electrospun onto Ti6Al4V samples. Electrospinning was conducted at applied voltages ranging from 20 to 24 kV, driven by a high-voltage DC power supply. The polymer solution was loaded into a 20 ml syringe equipped with a blunt steel needle with a 30 mm tip diameter. The collector was covered with aluminium foil, and the Ti6Al4V samples were placed on the foil as the collecting substrates. The distance between the needle tip and the collector with samples was maintained at 10–20 cm. The syringe was placed in the pump section of the machine (Figure 5.2), and the polymer solution was pumped at a flow rate of 1.6–3.0 ml/hr. Electrospinning was carried out at room temperature and ambient relative humidity. The Ti6Al4V plates coated with the polymer films were kept at room temperature for 24 hrs to allow for a gradual solvent evaporation.

Three different collection times (10 min, 20 min, and 1 hr) were applied to obtain three samples in duplicate. Uncoated Ti6Al4V discs were used as controls. Polymers have a low glass transition

temperature of $\sim 60^\circ\text{C}$; therefore, sterilising by autoclaving may adversely affect the structural and functional properties of the electrospun NFs. Sterilisation using 70% (v/v) ethanol (EtOH) solution and agar diffusion assay were conducted as described in Chapter 6. The antimicrobial efficacy of the three samples was tested against *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and *Escherichia coli* (*E. coli*) ATCC 25922.

The sterilisation effectiveness of 70% EtOH was qualitatively assessed by the absence of microbial growth in the medium after a 24-hour culture period for all samples. Subsequently, the agar diffusion assay was carried out. The PCL/PEG nanofibre coatings did not exhibit any antibacterial properties against the *S. aureus* strain, as shown in Figure 5.6. The same was observed for the *E. coli* strain (results not shown, as they were similar to those of *S. aureus*). Furthermore, after extensive treatment with 70% EtOH, re-sterilisation of the samples was not established, and the NFs peeled off from the Ti6Al4V surfaces. Therefore, further modifications to the NF polymer solution and its application on the Ti6Al4V surface were required to improve the microbiological properties and adhesion of the electrospun NFs.

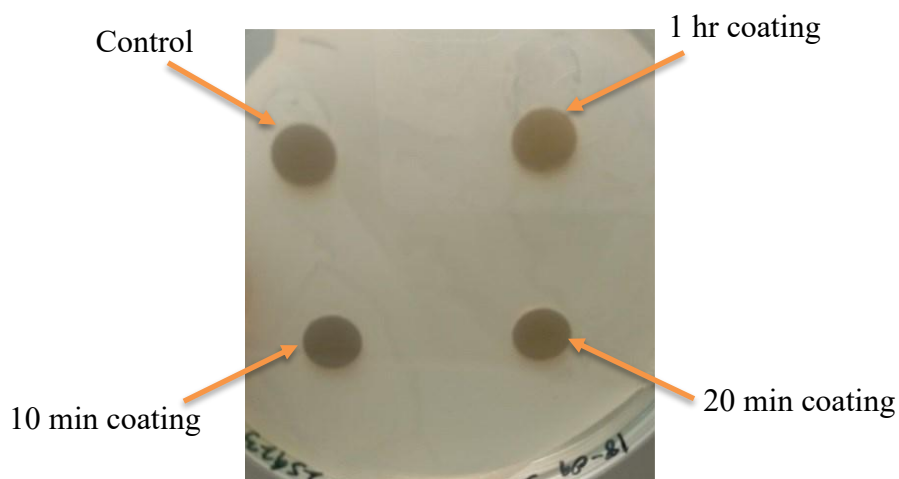


Figure 5.6: Agar diffusion assay of PCL/PEG nanofiber-coated Ti6Al4V samples

5.6.2. Second preliminary study: PCL- and PMMA-based polymer NFs

Poly(methyl methacrylate) is an FDA-approved biomaterial that is tough, heat resistant, compatible with human tissue, hydrophobic, and used extensively as a medical adhesive (Ottenbrite & Javan, 2005; Liu, et al., 2016; Bhogal, et al., 2024). Hence, PMMA is worth

exploring as a base material for electrospinning-coating of medical implants with polymeric NFs. One issue associated with coating a metal surface with organic materials is poor adhesion between the two materials. To remedy this, polymers may be covalently anchored to the metal substrate surface to generate an adhesive interlayer (Nathanael & Oh, 2020). To explore strategies to improve the adhesion strength of electrospun NFs, various polymer NF compositions and adhesives were applied to Ti6Al4V samples ($30 \times 30 \times 2$ mm). Base polymers were PMMA ($M_n = 12,000$ Da) and PCL ($M_n = 80,000$ Da). The direct pull-off adhesion test was conducted. Sample coating descriptions and adhesion test results are shown in Table 5.2.

Table 5.2: Various polymer compositions and their adhesive strengths

Sample no.	Sample description	Adhesion test results (MPa)
A	PMMA + WBA Glue	-
B	PMMA/PCC + BA	0.39
C	PMMA + BA	0.50
D	PMMA	-
E	PCL + BA	0.75

WBA = water-based acrylic deck adhesive; PCC = polymer-cement concrete; BA = butyl acrylate

The coatings of Samples A and D peeled off from the Ti6Al4V surface after the coating process. Therefore, the adhesion test could not be conducted and indicated a lack of adhesion to the Ti6Al4V substrate, hence discouraging the use of glue- or epoxy-type adhesives. As shown in Table 5.2, the adhesion strengths of all tested samples were lower than those reported in the literature (Abdal-hay, et al., 2012). The coating containing PCL was more adherent than that containing PMMA. This may be attributed to the higher stiffness and amorphousness of PMMA. In consequence, PCL was used for further investigations towards establishing the most suitable polymer solution for coating biomedical Ti-based implants.

5.6.3. Third preliminary study: PCL/PEG/CuO and PCL/PU/CuO blend NF systems

The extracellular matrix (ECM) of soft tissue is comprised of elastic components (Kazemzadeh, et al., 2022). Accordingly, polyurethane (PU) is a flexible polymer that may simulate the ECM elasticity in support of the excellent mechanical properties of PCL (Kazemzadeh, et al., 2022). Poly(ϵ -caprolactone) and PU have been shown to form neat structures suitable for soft tissue

engineering due to their high biocompatibility and controllable degradation rates. Metal oxide-containing polymer NFs may possess improved properties in terms of thermal stability, flexibility and solubility (Nirmala, et al., 2013). To continue with the investigation of the effectiveness of ethanol sterilisation, determine the antimicrobial efficacy and observe adhesion of NFs on Ti6Al4V substrates, polymer blends of PCL/PEG and PCL/PU were imbued with the in situ produced CuO NPs to enhance the antimicrobial properties of the NF coatings with the application of a primer to promote adhesion of the NF mats onto the Ti6Al4V surfaces.

5.6.3.1. Application of primer

The flexibility of PU and the adhesive nature of the acrylates, such as PMMA and poly(butyl acrylate) (PBA), make them ideal primers to promote adhesion between the metal surface and the electrospun polymerous NF coating. Furthermore, among the studied materials, PU and PMMA are the amorphous, and amorphous materials tend to exhibit better adhesive qualities than their semi-crystalline counterparts (Polymershapes, 2025). Since the electrospun nanofibrous coating is porous, fluids may come into contact with the metal surface and initiate corrosion; however, when the polymeric material completely covers the implant surface, the metal will only start to corrode after the polymer coating has degraded (ElectrospinTech, 2024). Before coating the Ti6Al4V surfaces with polymer NFs by electrospinning, the primer solutions were applied on the surfaces of some substrates using a manual micropipette coating method (Figure 5.7) (Fuglsby, et al., 2020). The specimens were subsequently electrospun with NFs directly after primer application, along with those without primer.



Figure 5.7: Micropipette setup for coating of metal substrates with polymer primer

5.6.3.2. Coating with fortified NFs

For the electrospinning solutions, the solvent systems used for the preparation of PCL/PEG/CuO and PCL/PU/CuO blends were $\text{CHCl}_3/\text{MeOH}$ (3:1 v/v) and DMF/THF (1:1 v/v), respectively. The polymer solutions were prepared by first dispersing 0.2 g of CuO NPs in 20 ml of each solvent system for 5 min in a sonicator at room temperature, followed by dissolving PCL/PEG (3:1 w/w) and PCL/PU (3:2 w/w) in the designated solvent mixture and agitated on a magnetic stirrer for 3 hrs at 40 °C in a fume hood. The PCL/PEG/CuO and PCL/PU/CuO blends produced dark brown solutions, characteristic of CuO NPs in solution. Ti6Al4V discs (10×2 mm) were coated with PCL/PEG/CuO and PCL/PU/CuO by electrospinning.

Sterilisation using 70% EtOH solution and agar diffusion assay were conducted as described in Chapter 6. The description of the samples, the results from the sterilisation, the antimicrobial testing using *S. aureus* ATCC 25923, and the adhesion condition of the NF coatings on the Ti6Al4V specimens throughout the analysis are outlined in Table 5.3. The adhesion was not measured; instead, it was visually inspected to assess its condition and determine whether the coating remained firmly on the sample (designated as intact) or peeled from the sample surface, and the stage of analysis at which it peeled.

Table 5.3: Testing of sterilisation, antimicrobial effect and adhesion of various composite NF

Sample description	Initial sterilisation	Re-sterilisation of fails	Antimicrobial properties against <i>S. aureus</i>	Re-sterilisation for reuse	Adhesion of NF coat
PCL/PEG/CuO	Pass	-	None	Fail	Intact
PCL/PU/CuO	Pass	-		Fail	Intact
PCL/PEG/CuO (PU primer)	Fail	Fail		-	Intact
PCL/PU/CuO (PU primer)	Pass	-		Pass	Intact
PCL/PEG/CuO (PMMA primer)	Fail	Pass		Fail	Peeled on 1st re-sterilisation
PCL/PU/CuO (PMMA primer)	Pass	-		Fail	Intact
PCL/PU/CuO (PBA primer)	Fail	Fail		-	Peeled on arrival

The samples that failed the sterility test after the initial sterilisation were re-sterilised before continuing antimicrobial testing of the sterile samples against the *S. aureus* strain. Those that failed the re-sterilisation before antibacterial testing were excluded from the test. As shown in Table 5.3, none of the tested specimens displayed bactericidal effects. This observation corresponds to that of the first preliminary study, where PCL/PEG amalgam was used without CuO and thus may be considered a negative control for the samples in this preliminary study. Therefore, the antibacterial effect of the CuO NPs was not evident. The coating consisting of PCL/PU/CuO NFs with PU as the primer is the only sample which passed all the stages of sterilisation and remained adherent throughout. This coating and primer combination was used in ensuing examinations.

5.6.4. Fourth preliminary study: Adhesion of PCL/PU/CuO NFs

Since the PCL/PU/CuO has been shown to adhere to the Ti6Al4V surface during sterilisation and re-sterilisation procedures pre- and post-antimicrobial testing, respectively, the adhesion strength of the polymer composite was explored. Nine Ti6Al4V samples ($30 \times 30 \times 2$ mm) were coated with PU primer solution at different concentrations using the micropipette, followed by electrospinning coating with the PCL/PU/CuO composite as previously described at varying electrospinning deposition times. After the polymer mats had dried, the direct pull-off adhesion test was performed (as described in Section 5.4. Direct pull-off adhesion test), and the results are shown in Table 5.4.

Table 5.4: Adhesion strength of PCL/PU/CuO on Ti6Al4V substrate with PU primer

Polymer solution for NFs	PU primer (%w/v)	Electrospinning deposition time (hrs)	Adhesion strength (MPa)
PCL/PU/CuO	30	2	7.13
		1	2.72
		0.5	2.27
	16	2	2.43
		1	2.54
		0.5	-
	8	2	7.65
		1	2.81
		0.5	-

For adhesion test results to be reliable, the coating should be evenly distributed on the test surface, which was not the case for the samples whose adhesion strengths were not determined as indicated in Table 5.4. The adhesion strength values were evidently higher than those observed during the second preliminary study; hence, the PU primer considerably improved the adhesion properties of the electrospun polymer coating. This may be attributed to the thermoplastic elastomeric property of the PU in both the primer and the NF composition. There was no clear correlation among the various PU primer concentrations. The primer coating concentrations of 30% PU and 8% PU showed high adhesion strengths of 7.13 MPa and 7.65 MPa, respectively, at 2 hrs electrospinning deposition period, suggesting that longer coating times may optimise the adhesion strength of the polymer coating.

5.6.5. Fifth preliminary study: Final coating material composition

The ensuing step after establishing adhesion strength was to improve the antimicrobial activity of the PCL/PU/CuO nanofibres by adjusting the concentration of CuO nanoparticles in the polymer hybrid. An excessive increase in the amount of additives in the polymer solution may adversely affect the quality of the electrospun NFs and thus render the polymer solution unsuitable for electrospinning. Four sets of coating materials were prepared, namely:

- 1) PCL/PU polymer composite solution at 3:2 (w/w), respectively
- 2) PCL/PU/CuO NPs polymer composite solution at 3:2:0.2 (w/w/w), respectively
- 3) PCL/PU/CuO NPs polymer composite solution at 3:2:0.3 (w/w/w), respectively

4) PCL/PU/CuO NPs polymer composite solution at 3:2:0.5 (w/w/w), respectively

The solutions containing CuO nanoparticles were characterised by ultraviolet visible (UV-vis) spectroscopy to confirm the formation of nanoparticles. The UV-vis spectra were measured on a Shimadzu UV-2600 UV-Vis Spectrophotometer (Figure 5.8) in a wavelength range of 200–900 nm. UV-visible absorption spectroscopy plays a crucial role in investigating the optical properties of nanoparticles. The optical property of CuO NPs powder suspended in THF solvent was analysed in the wavelength range of 200–900 nm. The analysis was performed in a quartz cell, using THF as a reference solvent. From the UV-vis spectra in Figure 5.8, two peaks of maximum absorption were exhibited. A strong peak occurred at about 275 nm, while the other was weak at 339 nm. This may be attributed to the formation of CuO NPs (Nirmala, et al., 2013). An increase in the absorbance peak intensity was observed with increasing CuO concentration in solution, demonstrating a substantial enhancement of light absorption in the visible region. These results indicated that CuO NPs had been successfully synthesised.



(i)

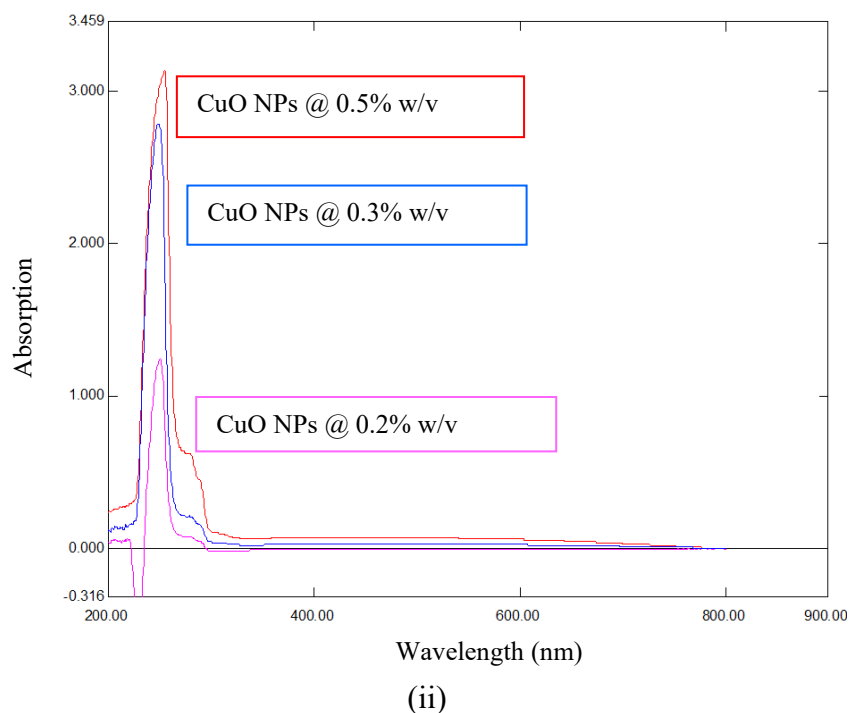


Figure 5.8: (i) Shimadzu UV-2600 UV-Vis Spectrophotometer setup, and (ii) UV-vis absorption spectrum of copper oxide nanoparticles solutions at 0.2%, 0.3% and 0.5% w/v

The samples containing 0.2% w/v CuO NPs have been shown not to exhibit antimicrobial characteristics in preceding preliminary analyses. During electrospinning of the solution containing 0.5% w/v CuO NPs, black spots were produced by the spattering of agglomerated CuO NPs and the fibre network that formed at various voltages and spinning distances was non-uniform, discontinuous and not firmly adhered to the Ti6Al4V surface (Figure 5.9). This may be due to a high concentration of CuO NPs, a solution that is too viscous or insufficient mixing. For these reasons, the 0.3% w/v CuO NPs solution was selected as the final polymer solution to be used in this project.



Figure 5.9: Electrospun PCL/PU/CuO (3:2:0.5, w/w/w) polymer NF mat peeling off from substrate

5.7. Final samples: PCL/PU and PCL/PU/CuO nanofibre coatings

The selected polymer amalgamations were utilised to prepare solutions for electrospinning into NF coatings. The substrates were produced with LPBF of the Ti6Al4V and cpTi discs (10×2 mm) and rectangular samples ($30 \times 30 \times 2$ mm). Substrates were ultrasonically cleaned with acetone, anhydrous ethanol and distilled water for 15 min each in turn to remove surface impurities and dried under natural air (Wang, et al., 2022). The polymer compositions were PCL/PU at a ratio of 3:2 (w/w) and PCL/PU/CuO NPs at 3:2:0.3 (w/w/w). Before coating with electrospun NFs, the substrates were coated with a PU primer solution. The primer was prepared by dissolving 0.613 g PU polymer in a 2 ml mixed solvent system containing 1:1 (v/v) of DMF/THF at room temperature and continuous magnetic stirring for 10 min. A 30% w/v PU primer solution was obtained. Thereafter, one side of the substrates was fully coated with the primer solution using a micropipette (as shown in Figure 5.7) to pour 10 μ l of primer on the small discs and 100 μ l on the rectangular ones. The electrospinning parameters for the manufacturing of NF scaffolds were varied until continuous, bead-free fibre jets were obtained. Optimal electrospinning conditions for coating with NFs were:

- » Voltage: 17 kV
- » Flow rate: 0.7 ml/hr
- » Collecting time: 2 hrs
- » Distance between needle tip and collector: 10 cm
- » Needle tip diameter: 600 μ m
- » Solvent volume: 20 mL

» Relative humidity: ambient

Typically, samples coated with PCL/PU were white, while those coated with PCL/PU/CuO were brown due to the CuO NPs (Figure 5.10). Sheikh et al. (2011) also noticed visible colour changes between pristine PU and Cu-containing formulations after electrospinning, with the pristine PU retaining its original white colour and the Cu-containing NF mats exhibiting a reddish-brown colour. According to Chen et al. (2021), several research papers have reported that the colour of electrospun polymer NFs changes to reddish brown upon the addition of Cu particles. The rectangular samples were used for adhesion strength testing in the current chapter, and the disc samples were used for biological analyses in Chapter 6. For nanofibre characterisation in this chapter, the most suitable sample shape and size for the particular analysis was employed.



(i)



(ii)

Figure 5.10: Ti samples coated with electrospun membrane composite of (i) PCL/PU, and (ii) PCL/PU/CuO

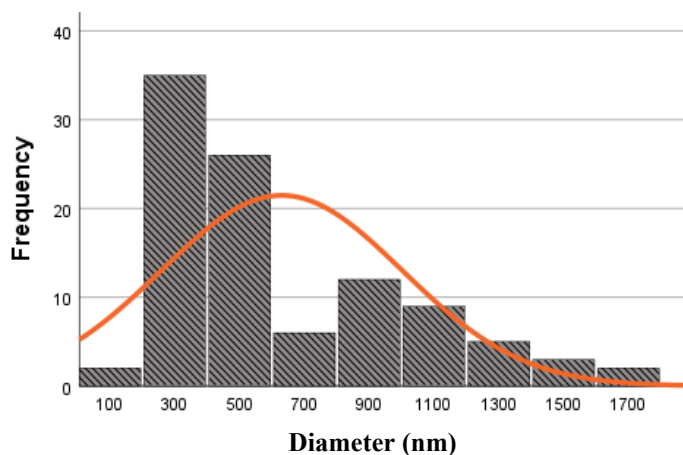
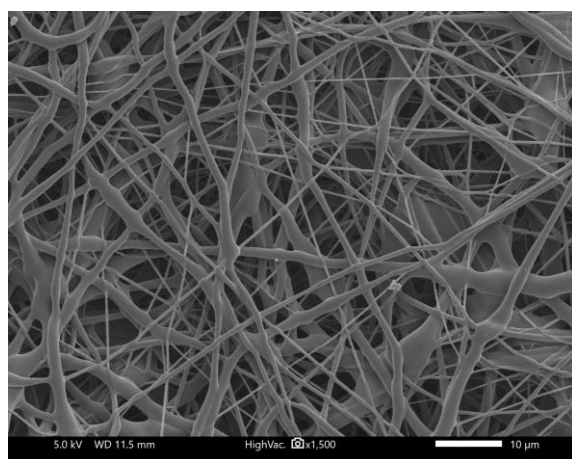
5.7.1. Characterisation of nanofibres on coated surfaces

5.7.1.1. Diameters

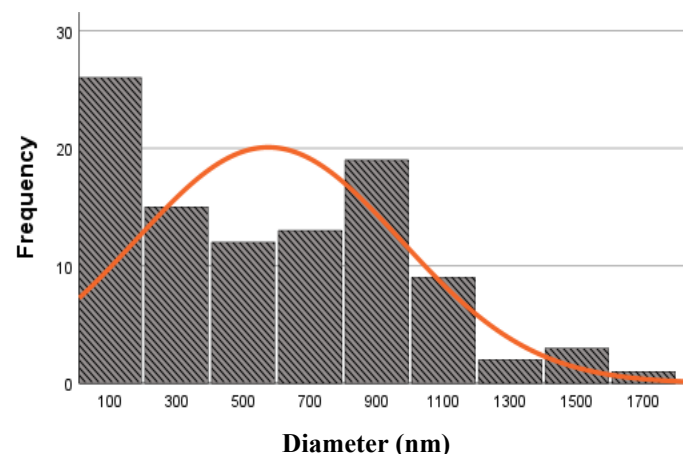
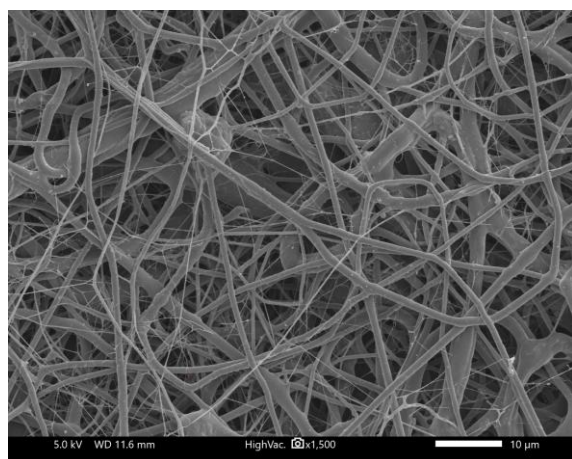
The morphologies of the as-spun PCL/PU and PCL/PU/CuO fibre membranes coated on the substrate (10×2 mm) surfaces were investigated using JEOL JSM-IT200 InTouchScope™ Scanning Electron Microscope (SEM) equipped with an energy-dispersive X-ray spectroscopy (EDS). Specimens were coated with iridium and imaged at 5 kV at a working distance of 10 mm. The diameters of one hundred ($n = 100$) randomly selected fibres on the SEM micrographs of the corresponding coating scaffold were measured using the ImageJ image analysis software (FIJI

version) and analysed using IBM SPSS Statistics software. The results were recorded at average fibre diameter (AFD) $\pm$ standard deviation.

The morphologies of the as-fabricated PCL/PU and PCL/PU/CuO nanofibre mats coated on Ti surfaces and the corresponding fibre diameter distribution plots are shown in Figure 5.11. The electrospinning process produced scaffolds composed of randomly distributed, well-defined NFs, which were smooth, bead-free, and continuous, with varying diameters. The morphology of the PCL/PU NFs was not affected by the addition of CuO. However, NFs modified with CuO were visibly thinner than those without the NPs. The AFD of PCL/PU and PCL/PU/CuO NFs were 632 ± 371 nm and 575 ± 397 nm, respectively. The values are comparable to those reported by Sarabi-Mianeji et al. (2015) and Chen et al. (2021). Sarabi-Mianeji et al. (2015) optimised the electrospinning conditions for the fabrication of PCL NFs and found that the fibre diameters were 546 ± 173 nm under optimal conditions. While the results of Chen et al. (2021) showed that the AFD for electrospun PCL/gelatine NFs infused with 0.3 wt.% of Cu NPs was 501 ± 121 nm, which was less than the fibre diameter of NFs without Cu NPs of 686 ± 151 nm. The diameter decreased with increasing additive mass of Cu NPs.



(i)



(ii)

Figure 5.11: SEM micrographs and fibre diameter distribution curves of (i) PCL/PU, and (ii) PCL/PU/CuO

The fibre diameter distribution plot in Figure 5.11(i) shows PCL/PU nanofibers with diameters ranging from 160–1700 nm, with about 60% of the fibres having diameters between 200–600 nm. The NFs produced using PCL/PU/CuO (Figure 5.11(ii)) had a diameter distribution ranging from 60–1700 nm, and 49% of the fibres were in the range of from below 100 nm to 500 nm. This reduction in diameter with the addition of copper is a general trend observed amongst similar studies of polymers permeated with Cu NPs for electrospinning (Sheikh, et al., 2011; Ahire, et al., 2016; Chen, et al., 2021; Erci & Sariipek, 2023). The enhanced solution conductivity due to Cu in the polymer solution affects the Taylor cone size and decreases the diameter of PCL/PU NFs. Also,

the increased electrical conduction accelerates jetting of the solution from the Taylor cone and results in the formation of thinner fibres because of a faster electrospinning process (Sheikh, et al., 2011; Nirmala, et al., 2013; Ahire, et al., 2016; Chen, et al., 2021; Erci & Sariipek, 2023). On the contrary, Quilez-Molina et al. (2023) observed an increase in the diameter of PCL-Cu NFs as the content of Cu nanoparticles was increased and credited this to a rise in the viscosity of the spun fibre due to the presence of Cu NPs. Their SEM micrograph further revealed white spots ubiquitously distributed throughout the electrospun fibres, consistent with the copper concentration; hence, the inhomogeneity of the PCL/Cu mixture possibly contributed to the higher solution viscosity and the ultimate increase in the as-spun fibre diameter.

Numerous studies have reported a wide range of diameters for PCL and PU NFs. Manakhov et al. (2021) and Erci & Sariipek (2023) electrospun PCL fibres with diameter values within the range of 40-300 nm. Scanning electron microscope analysis by Quilez-Molina et al. (2023) exhibited PCL NF mats of fibre diameters ranging from approximately 0.2 to 7.5 μm . Kazemzadeh et al. (2022) determined AFDs of PCL, PU and PCL/PU scaffolds fabricated by the blend electrospinning method. The AFDs of PCL and PU were 433 ± 80 nm and 470 ± 95 nm, respectively. The PCL/PU scaffolds produced diameters ranging between 420 ± 75 nm and 436 ± 84 nm, and the diameters increased with the addition of PU and corresponding reduction of PCL in PCL/PU scaffolds. The diameters of pristine PU and PU/CuO composite NFs studied by Nirmala et al. (2013) were similar and in the range of 100–300 nm along their lengths. Zhang et al. (2020) demonstrated that electrospinning produced fibres of different diameters on substrates with varying conductivity, and aluminium foil yielded the thinnest NF. Increasing the electrospinning voltage also resulted in thinner fibre diameters.

5.7.1.2. X-ray diffraction (XRD)

The elemental compositions and crystalline phases of the PCL/PU and PCL/PU/CuO blend coatings on Ti6Al4V and cpTi surfaces were analysed using the XRD equipment and parameters as described in Section 4.2.1. Sample preparation and characterisation. The XRD diffraction patterns are illustrated in Figure 5.12. In agreement with UV-vis spectra in Figure 5.8, the XRD data confirmed the successful synthesis of CuO NPs and incorporation thereof in PCL/PU nanofibres. The Cu peaks were identified at 2θ values of 43.38° , 50.52° , and 74.25° corresponding

to the (111), (200), and (220) crystalline planes. Similarly, Xu et al. (2018) analysed the diffraction pattern of CuO NPs deposited onto a polymer blend containing PCL and discovered Cu peaks located at 43.5° and 50.5° , corresponding to the (111) and (200) planes. Nirmala et al. (2013) prepared CuO particles incorporated PU nanofibres by electrospinning. Their intense diffraction peaks at 35° , 38° , 44° , 48° , 64.5° and 78° corresponded to the (002), (111), (200), (202), (113) and (220) crystal planes of CuO.

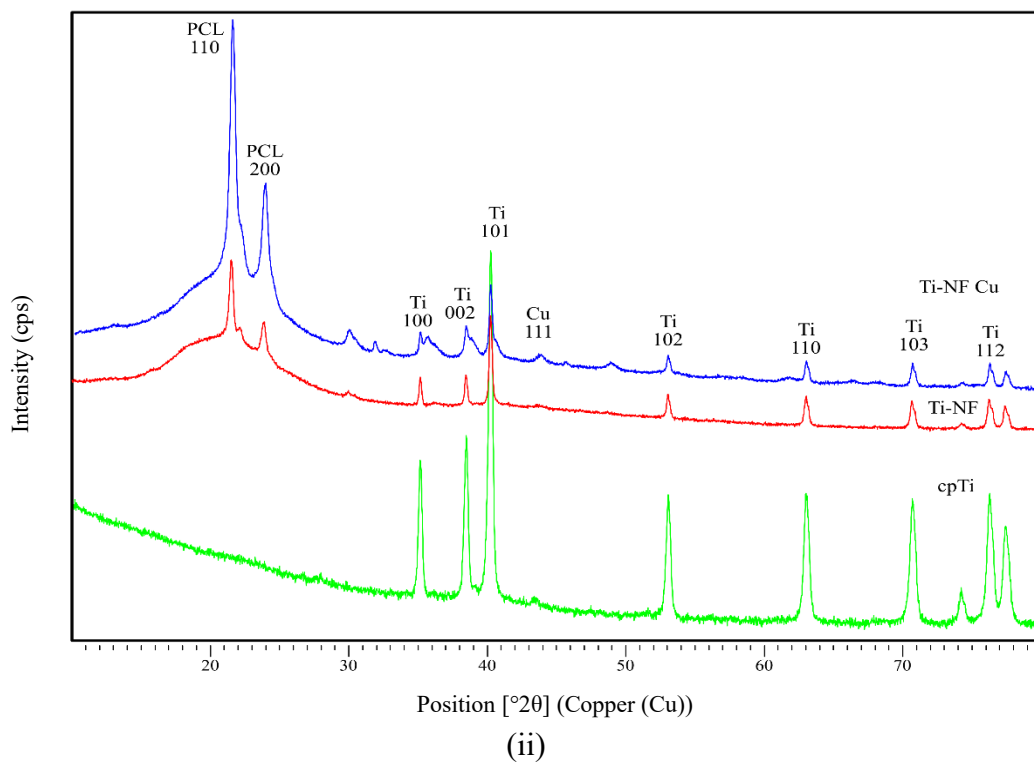
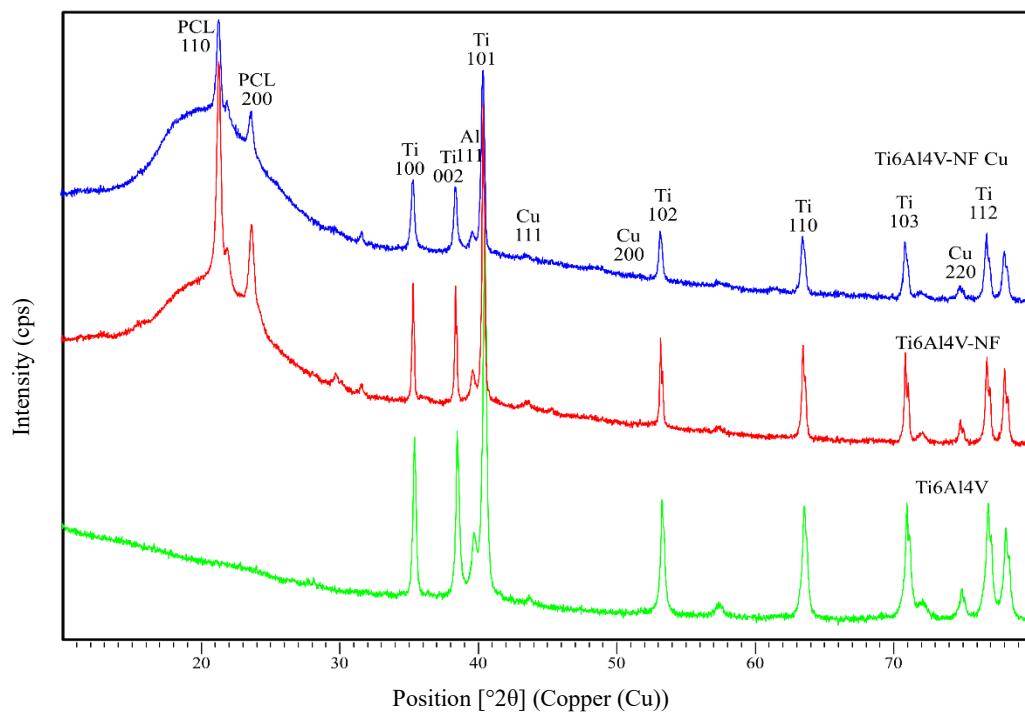


Figure 5.12: X-ray diffraction patterns of (i) Ti6Al4V, and (ii) cpTi coated samples (NF = PCL/PU; NF Cu = PCL/PU/CuO NPs)

Two diffraction peaks were distinctive from the Ti6Al4V and cpTi patterns (Figure 5.12(i) and (ii), respectively), which were formed at transmittance angles of 21.9° and 24.2° at (110) and (200) crystalline planes, respectively. They were identified as the peaks of semi-crystalline PCL (Balu, et al., 2011). Several studies also observed the characteristic peaks of pristine PCL at Bragg angles of approximately 21.5° and 24° and associated them with the crystal planes of (110) and (200) (Arrieta, et al., 2020; Stewart, et al., 2020; Kandi, et al., 2021; Alyamani, et al., 2023; Qian, et al., 2023; Yaseri, et al., 2023). As shown in Figure 5.12, a broad peak formed over a 2θ range of $17-20^\circ$, which is characteristic of the amorphous nature of pristine PU. Kandi et al. (2021)'s XRD of pure PU also revealed a broad peak between 15° and 25° , while Nirmala et al. (2013) reported a broad diffraction peak at 20° for pristine PU nanofibres. Polyurethane is innately amorphous; thus, the spectrum of pure PU does not possess any reasonable peaks to indicate its noncrystalline nature (Sheikh, et al., 2011).

For the coated Ti6Al4V samples (Figure 5.12(i)), the diffraction patterns for the Ti element in Ti6Al4V and Ti6Al4V-PCL/PU were similar, with the patterns differentiated by the polymeric peaks. There seemed to be a slight reduction in the Ti peaks, a great reduction in the PCL peaks, and a larger hump in the PU amorphous peak with the addition of CuO NPs to the polymer composite. The cpTi samples (Figure 5.12(ii)) showed a great difference in peak intensity between the bare and the coated surfaces. A major change in peak intensity of the Ti element was not observed between Ti-PCL/PU and Ti-PCL/PU/CuO. Nonetheless, a large increase in PCL intensity occurred with the addition of CuO NPs to the NF solution, and thus, coupled with the weak intensity peaks of Cu in Figure 5.12(i) and (ii), raises questions about the purity and orientation of the NPs within the NFs. Literature exploring the properties of polymeric NFs as coatings on metal surfaces is sparse. Hence, it may be postulated that properties of the NF mat (such as thickness and porosity) may affect the detectability of some elements and the orientation of the constituents of the NFs scaffolds when applied as metallic coatings.

5.7.2. Surface roughness

The portable surface roughness tester was utilised to measure the surface roughness of LPBF-manufactured samples ($30 \times 30 \times 2$ mm) before and after electrospinning coating with PCL/PU and PCL/PU/CuO NFs mats. The profilometer was set to record measurements in accordance with

ISO 1997. The stylus was set to travel to a cut-off length of $\lambda_c = 2.5$ mm at a measuring speed of 0.5 mm/s for all samples, and perpendicular to the laser scanning direction for the uncoated samples. The average surface roughness measurements were collected at five randomly selected locations on each plate. The roughness values of R_a and R_z were captured and presented as mean $\pm$ standard deviation, as shown in Table 5.5 and Figure 5.13. For statistical analysis, the Student's t-test was performed, and values of $p \leq 0.05$ were considered statistically significant, and values of $p \leq 0.01$ were considered statistically highly significant.

Table 5.5: Surface roughness values of bare and coated Ti6Al4V and cpTi surfaces

Sample	R_a (μm)	R_z (μm)
Ti6Al4V	12.85 ± 0.97	78.30 ± 4.11
Ti6Al4V-PCL/PU	4.37 ± 0.61	27.90 ± 4.76
Ti6Al4V-PCL/PU/CuO	11.03 ± 1.28	65.25 ± 7.30
cpTi	26.64 ± 2.50	143.34 ± 13.18
Ti-PCL/PU	3.36 ± 0.48	18.52 ± 1.86
Ti-PCL/PU/CuO	10.19 ± 3.60	54.17 ± 13.62

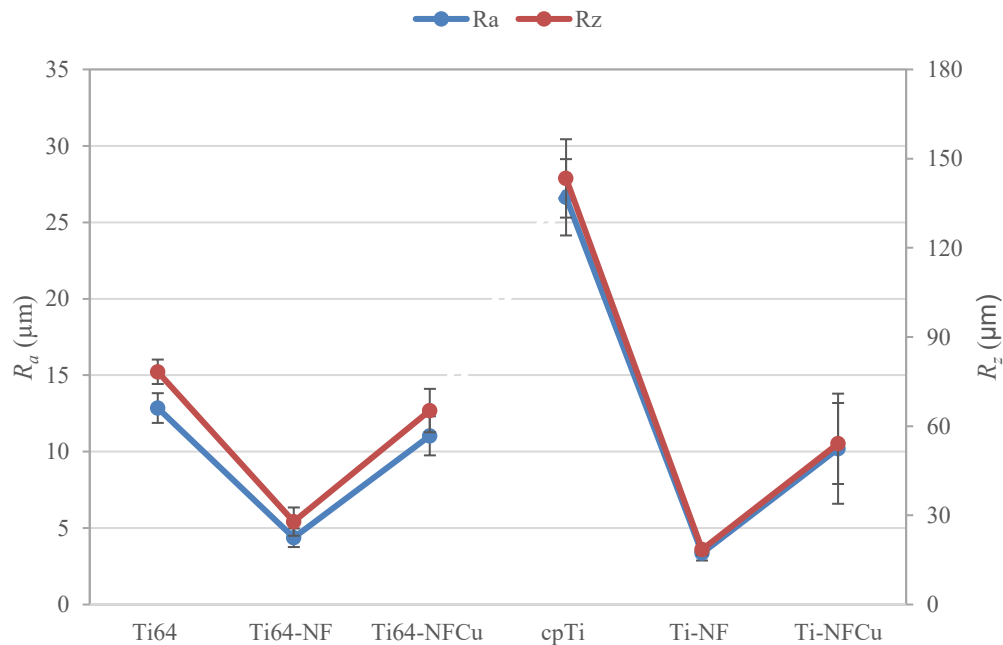


Figure 5.13: Surface roughness measurements of bare and coated Ti64 and cpTi specimens (NF = PCL/PU; NFCu = PCL/PU/CuO)

The surface roughness values of bare Ti6Al4V ($p = 0.000002$ for R_a and $p = 0.0000003$ for R_z) and Ti6Al4V-PCL/PU/CuO ($p = 0.0001$ for R_a and R_z) were highly significantly higher than of Ti6Al4V-PCL/PU, and of Ti6Al4V was insignificantly ($p = 0.0556$ for R_a and $p = 0.0194$ for R_z) higher than of Ti6Al4V-PCL/PU/CuO. Similarly, roughness values of bare cpTi and were highly significantly higher than of Ti-PCL/PU ($p = 0.00003$ for R_a and $p = 0.00004$ for R_z) and Ti-PCL/PU/CuO ($p = 0.0001$ and 0.00001 for R_a and R_z , respectively), and of Ti-PCL/PU/CuO was highly significantly higher than of Ti-PCL/PU ($p = 0.0186$ for R_a and $p = 0.0059$ for R_z). Therefore, coating with PCL/PU NFs smoothened the substrate surfaces, whereas CuO increased the roughness of the NF mats, almost restoring it to the uncoated value for Ti6Al4V.

The surface of bare cpTi was highly significantly ($p = 0.0001$ for R_a and $p = 0.0003$ for R_z) rougher than that of bare Ti6Al4V. Conversely, as expected, significance ($p > 0.05$) was not established between Ti6Al4V and cpTi surfaces coated with NFs of the same composition, as shown in Figure 5.13. Havlíček et al. (2020) measured an average surface roughness of $\sim 1.5 \mu\text{m}$ for electrospun PU, and Kichi et al. (2020) studied the roughness of Ti6Al4V substrate coated with a PCL-based polymer blend and found roughness values of $R_a = 3.43\text{--}5.97 \mu\text{m}$ and $R_z = 39.45\text{--}52.04 \mu\text{m}$. These values are analogous to those obtained in this research, and it may be postulated that PCL was the primary contributor to the roughness of the PCL/PU amalgam. Electrospun NFs with a high surface roughness may be preferred for applications in tissue engineering because they result in a larger specific surface area (Ni, et al., 2019).

5.7.3. Adhesion strength

A solid interfacial bonding strength between the NF coating and Ti implants is mandatory to prevent disruption, delamination or folding of the NF coatings on the Ti implant surface during implantation (Song, et al., 2017; Sopcak, et al., 2018; Zhang, et al., 2020; ElectrospinTech, 2024). Detachment of the coating from the substrate accelerates the accumulation of water molecules and corrosive ions at the substrate surface and may facilitate the easy release of the coated layer (Abdallah, et al., 2012). Early detachment of the coating may disrupt cell integration with Ti during clinical applications, which is the primary objective of coating Ti implant surfaces with NFs that mimic the ECM. The bonding strength between a polymer matrix and metal substrate depends on various factors such as surface chemistry, microstructure, surface roughness of the substrate, and

processing parameters of the applied technique (Sopcak, et al., 2018). Electrospun coatings are known to provide insufficient adhesion on a substrate, and thus, electrospinning of polymer NFs directly onto metallic surfaces leads to complications at the polymer/metal interface, which may pose a threat to the longevity of the implants (Abdal-hay, et al., 2012). A possible solution to strengthen the adhesion of electrospun NFs is to first coat the metallic substrate with a polymeric film that adheres to metallic surfaces, then electrospin the nanofibrous mat onto the film. The resultant nanofibre-film-substrate interfaces may be more adherent than a nanofibre-metallic interface (Abdal-hay, et al., 2012).

For the present experiment, the Ti ($30 \times 30 \times 2$ mm) surfaces were coated with PU primer using the micropipette method before electrospinning coating with PCL/PU and PCL/PU/CuO NF. Prior to the pull-off test, the coated substrates were visually inspected, and the polymeric coatings were found to be rigidly adherent to the Ti surfaces, with negligible risk of peeling off by hand. The adhesion test was conducted under both dry and wet conditions, similar to Abdal-hay et al. (2012, 2019). For the wet adhesion test, the samples were immersed in Ringer's solution (Sigma-Aldrich) at 37 °C to simulate the physiological environment. After 8 hrs of immersion, the coated samples were removed from the solution, the surfaces were patted dry with paper tissue, and the adhesion test was immediately conducted as described earlier in this chapter. The adhesion strengths were tested in triplicate and reported as the mean $\pm$ standard deviation. The adhesion strengths of the NF scaffolds on Ti6Al4V and cpTi substrates in dry and wet conditions are presented in Table 5.6. The recorded nature of fracture was the most occurring and/or the most representative among the repeats of each sample.

Table 5.6: Adhesion strength of NF coating on titanium substrate in dry and wet conditions

Sample	Adhesion strength (MPa)		Nature of fracture		Examples of fracture types
	Dry	Wet	Dry	Wet	
Ti6Al4V-PCL/PU	2.78 ± 0.73	7.76 ± 0.41	Cohesive: 100% <i>B</i>	Cohesive: 100% <i>B</i>	Cohesive: 100% <i>B</i> 
Ti6Al4V-PCL/PU/CuO	2.52 ± 0.05	5.71 ± 2.66	Cohesive: 100% <i>B</i>	Cohesive: 70% <i>B</i> Adhesive: 30% <i>A/B</i>	Adhesive: 100% <i>A/B</i> 
Ti-PCL/PU	1.66 ± 0.28	5.87 ± 2.35	Adhesive: 100% <i>A/B</i>	Cohesive: 100% <i>B</i>	Cohesive: 70% <i>B</i> Adhesive: 30% <i>A/B</i> 
Ti-PCL/PU/CuO	3.45 ± 0.16	4.87 ± 2.07	Cohesive: 100% <i>B</i>	Cohesive: 60% <i>B</i> Adhesive: 40% <i>A/B</i>	

**A* = metal substrate; *B* = polymer coating

The most occurring failure mode for all coatings was cohesive. Cohesive fracture is an indication of a strong interfacial adhesion of the coating onto the substrate (Fu, et al., 2001). This is also evident in Table 5.6, where the higher adhesive strength values were obtained from cohesive fractures under each test condition. The addition of CuO particles to the PCL/PU coating on Ti6Al4V substrates reduced adhesive strength, particularly under wet conditions. The effect of CuO NPs on the adhesion strength of PCL/PU was not well established for the cpTi samples. This was similar to that reported by Abdal-hay et al. (2019), who did not detect adverse effects on the adhesion performance of a PU composite layer after incorporating magnesium particles and coating on Ti. The surface roughness of cpTi was highest (Table 5.5); however, the PCL/PU and

PCL/PU/CuO coatings on the cpTi displayed the weakest adhesion strength in dry and wet conditions, respectively. For all coated substrates, the adhesion strengths were higher in wet than in dry conditions. During their investigation, Abdal-hay et al. (2012) dip-coated a cpTi specimen in polylactic acid (PLA), followed by electrospinning coating with PCL NFs. The researchers then tested the adhesion strength of the hybrid polymer in dry and wet conditions. The samples were immersed in standard SBF for the wet conditions, and the pull-off test was conducted on the dry and wet samples. Contrary to the current study, the dry adhesion strengths were stronger than the wet adhesion strengths.

Samples of Abdal-hay et al. (2012) coated with electrospun PCL layer and PLA as an interfacial layer on the Ti substrates produced higher adhesion strength values of 3.8 ± 0.75 and 1.9 ± 0.42 MPa than those with PCL fibres electrospun directly on the Ti surface, which produced adhesion strength values of 2.67 ± 0.8 and 0.8 ± 0.33 MPa for dry and wet conditions, respectively. Other studies have shown adhesion strength values of PCL at 1.8 MPa (Xu & Yamamoto, 2012), 1.52 MPa (Ibrahim & Hamad, 2023) and 1.3 MPa (Bou-Francis, et al., 2020) and of PU at 1.46 MPa (Duong, et al., 2024), 1.128–11.73 MPa (Passos, et al., 2021) and ~ 4.8 MPa (Skosana, et al., 2021). As a result, it may be concluded that the application of the PU as a primer and as a constituent of the electrospun NF coating greatly enhanced the adhesive strengths of the NF mats.

Poly(ϵ -caprolactone) has been reported to exhibit insufficient bonding to metallic surfaces due to its low oxygen content in its molecular structure (Abdal-hay, et al., 2012; Grewal, et al., 2023). Consequently, the higher adhesion strength values obtained in this experiment were ascribed to PU, as it contains a higher ratio of oxygen in its weight than PCL. Therefore, PU provided stronger electrostatic interactions on Ti substrate surfaces, particularly because the primer consisted of PU only (Xu & Yamamoto, 2012). In addition, titanium dioxide (TiO_2), naturally present on Ti surfaces, facilitates the formation of stronger bonds.

Brzeska et al. (2014) demonstrated that PU undergoes hydrolysis and degrades in Ringer's solutions, eventually leading to physical crosslinking of macromolecules. Along with hydrolysis of the ester linkages of PCL, which are able to incorporate into the soft segments of PU, the crosslinking of PU molecules after being soaked in Ringer's solutions may have resulted in

stronger bonds and higher adhesion of the polymer layer with the metal surface (Brzeska, et al., 2014). For their study, Song et al. (2017) deposited polycaprolactone/polyvinyl alcohol (PCL/PVA) NFs directly onto a Ti implant surface during electrospinning and measured the bonding strength of the coating using a standard single-pass scratch test. The scratch test demonstrated strong bonding between the NF coating and the Ti pins. The authors also confirmed this in an *ex vivo* porcine bone implantation model, in which the NF coating remained non-delaminated during implantation. Furthermore, they found that >80% of PCL NFs remained bound to the Ti implant surface after 30 min of ultrasound sonication and identified a potential reason as that hydrogen bonds form between PCL and TiO₂ on the Ti metal surface. The hydrogen bonding of PCL NFs to the Ti implant surface may be difficult to break. In addition, adhesion between electrospun NFs and Ti surface generally results from the van der Waals force induced by the nanoscale structures of the as-spun fibres, which are characterised by a high surface area-to-volume ratio, highly active chains at the surface and the electrostatic force involved in the electrospinning process (Ifkovits, et al., 2009; Song, et al., 2017; Zhang, et al., 2020). Consequently, the adhesion force may differ due to the different number of fibres deposited per unit area, such that the more fibres on the same area, the larger the adhesion force (Zhang, et al., 2020).

The adhesion strength at the NF-primer-metal interfaces may be improved by thinner coatings with an average thickness of ~50 µm (Sopcak, et al., 2018); the occurrence of pores adds to a larger surface area for embedded fibres and thus increases the adhesive strength of the coating (Abdal-hay, et al., 2012); and infusing NFs with particles that have irregular shapes (Abdal-hay, et al., 2019). Although the effect of the as-fabricated surface roughness was not established in the present investigation, pre-treating the substrates to roughen the surface by, for example, grit blasting, acid etching, alkaline treatment, and sandblasting, large-grit, and acid etching (SLA) has been suggested to improve the adhesion strength of NF on metallic surfaces (Jemat, et al., 2015).

5.8. Summary

Chapter 5 elucidated the manufacturing of Ti6Al4V ELI and cpTi test using laser power bed fusion. The electrospinning setup, parameters, and technique were discussed, along with the selected polymer materials and the in situ fabrication of CuO nanoparticles. Preliminary studies were conducted using various polymers and CuO concentrations to identify the most efficient polymer blends that may exhibit antibacterial properties and form strong adhesive bonds with substrates. The final polymer composites found to be optimal for coating the titanium substrates were PCL/PU (3:2, w/w) and PCL/PU/CuO NPs (3:2:0.3, w/w/w) with the use of PU as a primer to promote adhesion of electrospun NFs. After applying the PU primer by micropipetting and the ensuing composite NFs by electrospinning, the polymeric coatings were characterised using SEM for diameters and XRD for elemental compositions and crystalline phases. The surface roughness and adhesion strength of the coated samples were also examined. The XRD demonstrated the successful infusion of PCL/PU with in situ-manufactured CuO NPs. The addition of CuO reduced the diameters, increased the surface roughness and reduced the adhesion strength of PCL/PU NF coatings on Ti6Al4V and cpTi samples. A correlation between the surface roughness and the adhesive strength was not observed. The polymers evaluated during the preliminary investigations did not display antibacterial properties even with the addition of CuO. Optimisation of polymeric coatings and an in-depth assessment of antimicrobial properties requires further investigation.

References

- Abadi, B., Goshtasbi, N., Bolourian, S., Tahsili, J., Adeli-Sardou, M., & Forootanfar, H. (2022). Electrospun hybrid nanofibres: Fabrication, characterization, and biomedical applications. *Frontiers in Bioengineering and Biotechnology*, *10*, 986975. doi:10.3389/fbioe.2022.98697
- Abdal-hay, A., Agour, M., Kim, Y. -K., Lee, M. -H., Hassan, M. K., El-Ainin, H. A., . . . Ivanovski, S. (2019). Magnesium-particle/polyurethane composite layer coating on titanium surfaces for orthopedic applications. *European Polymer Journal*, *112*, 555–568. doi:10.1016/j.eurpolymj.2018.10.012
- Abdal-hay, A., Hwang, M. -G., & Lim, J. K. (2012). In vitro bioactivity of titanium implants coated with bicomponent hybrid biodegradable polymers. *Journal of Sol-Gel Science and Technology*, *64*(3), 756-764. doi:10.1007/s10971-012-2912-6
- Ahire, J. J., Hattingh, M., Neveling, D. P., & Dicks, L. M. (2016). Copper-containing anti-biofilm nanofibre scaffolds as a wound dressing material. *PLoS One*, *11*(3), e0152755.
- Ahmed, T. A., Lee, H. ‘., & Williams, R. C. (2018). Using a modified asphalt bond strength test to investigate the properties of asphalt binders with poly ethylene wax-based warm mix asphalt additive. *International Journal of Pavement Research and Technology*, *11*(1), 28–37. doi:10.1016/j.ijprt.2017.08.004
- Alyamani, A. A., Al-Musawi, M. H., Albukhaty, S., Sulaiman, G. M., Ibrahim, K. M., Ahmed, E. M., . . . Mohammed, M. K. (2023). Electrospun polycaprolactone/chitosan nanofibres containing Cordia myxa fruit extract as potential biocompatible antibacterial wound dressings. *Molecules*, *28*(6), 2501. doi:10.3390/molecules28062501
- Arrieta, M. P., Gil, A. L., Yusef, M., Kenny, J. M., & Peponi, L. (2020). Electrospinning of PCL-based blends: processing optimization for their scalable production. *Materials*, *13*, 3853. doi:10.3390/ma13173853
- Ashtikar, M., & Wacker, M. G. (2018). Nanopharmaceuticals for wound healing – Lost in translation? *Advanced Drug Delivery Reviews*, *129*, 194-218. doi:10.1016/j.addr.2018.03.005
- ASTM International. (2022). ASTM International ASTM D4541-22 Standard Test Method for Pull-Off Strength of Coatings Using Portable Adhesion Testers. West Conshohocken, Pennsylvania: ASTM International.

- Baker, S. R., Banerjee, S., Bonin, K., & Guthold, M. (2016). Determining the mechanical properties of electrospun poly- ϵ -caprolactone (PCL) nanofibres using AFM and a novel fiber anchoring technique. *Materials Science and Engineering C*, 59, 203–212. doi:10.1016/j.msec.2015.09.102
- Balu, R., Kumar, T. S., Ramalingam, M., & Ramakrishna, S. (2011). Electrospun polycaprolactone/poly(1,4-butylene adipate-co-polycaprolactam) blends: Potential biodegradable scaffold for bone tissue regeneration. *Journal of Biomaterials and Tissue Engineering*, 1(1), 30–39. doi:10.1166/jbt.2011.1004
- Banoriya, D., Purohit, R., & Dwivedi, R. K. (2017). Advanced application of polymer based biomaterials. *Materials Today: Proceedings*, 4(2, Part A), 3534-3541. doi:10.1016/j.matpr.2017.02.244
- Bhogal, S. S., D'Auria, J. L., Gehrman, S. V., Baratz, M. E., & Kaufmann, R. A. (2024). Polymethyl methacrylate for elbow arthroplasty: Is there another way? *Journal of Hand Surgery Global Online*, 6(2), 233-235. doi:10.1016/j.jhsg.2023.11.006
- Bikuna-Izagirre, M., Aldazabal, J., & Paredes, J. (2022). Gelatin blends enhance performance of electrospun polymeric scaffolds in comparison to coating protocols. *Polymers*, 14, 1311. doi:10.3390/polym14071311
- Bîrcă, A., Gherasim, O., Grumezescu, V., & Grumezescu, A. M. (2019). Chapter 1 - Introduction in thermoplastic and thermosetting polymers. In V. Grumezescu, & A. M. Grumezescu (Eds.), *Materials for Biomedical Engineering* (pp. 1–28). Alpharetta: Elsevier. doi:10.1016/b978-0-12-816874-5.00001-3
- Bou-Francis, A., Piercey, M., Al-Qatami, O., Mazzanti, G., Khattab, R., & Ghanem, A. (2020). Polycaprolactone blends for fracture fixation in low load-bearing applications. *Journal of Applied Polymer Science*, 137(32), 48940. doi:10.1002/app.48940
- Brzeska, J., Heimowska, A., Janeczek, H., Kowalczyk, M., & Rutkowska, M. (2014). Polyurethanes based on atactic poly[(R,S)-3-hydroxybutyrate]: Preliminary degradation studies in simulated body fluids. *Journal of Polymers and the Environment*, 22, 176–182. doi:10.1007/s10924-014-0650-2
- Chen, H., Zhang, J., Wu, H., Li, Y., Li, X., Zhang, J., . . . Cai, X. (2021). Fabrication of a Cu nanoparticles/poly(ϵ -caprolactone)/gelatin fiber membrane with good antibacterial activity

- and mechanical property via green electrospinning. *ACS Applied Bio Materials*, 4(8), 6137-6147. doi:10.1021/acsabm.1c00485
- Cheng, L., Lei, L., & Guo, S. (2010). In vitro and in vivo evaluation of praziquantel loaded implants based on PEG/PCL blends. *International Journal of Pharmaceutics*, 387, 129–138. doi:10.1016/j.ijpharm.2009.12.010
- DeFelsko. (2020). *How to Measure Adhesion Using the PosiTest® AT Adhesion*. Retrieved January 10, 2025, from https://www.youtube.com/watch?v=7D1yzGPdc_Q
- DeFelsko. (2023). *PosiTest® AT Pull-off Adhesion Testers*. Retrieved January 10, 2025, from <https://www.defelsko.com/positest-at#at-a>
- Douglas, P., Albadarin, A. B., Sajjia, M., Mangwandi, C., Kuhs, M., Collins, M. N., & Walker, G. M. (2016). Effect of poly ethylene glycol on the mechanical and thermal properties of bioactive poly(ϵ -caprolactone) melt extrudates for pharmaceutical applications. *International Journal of Pharmaceutics*, 500(1–2), 179-186. doi:10.1016/j.ijpharm.2016.01.036
- Duong, T. T., Le, M. L., & Kim, J. (2024). Improving adhesion strength between steel and 2K polyurethane containing organic–inorganic hybrid nanoparticles and its performance optimization. *Materials Today Communications*, 41, 110453. doi:10.1016/j.mtcomm.2024.110453
- Duygulu, N. E. (2024). Functionalization of PLA nanofibres with PCL blending and TiO₂ nanoparticle addition. *Materials Research Express*, 11(7), 075403. doi:10.1088/2053-1591/ad62bd
- ElectrospinTech. (2024). *Electrospun Coated Metal Implants*. Retrieved January 24, 2024, from <https://electrospintech.com/coatedmetal.html>
- Erci, F., & Sariipek, F. B. (2023). Fabrication of copper oxide nanoparticles doped PCL/PVP nanofibrous mats by electrospinning and evaluation of their antibacterial activities. *Kahramanmaraş Sütçü İmam University Journal of Engineering Sciences*, 26(4), 823-833. doi:10.17780/ksujes.1290143
- Fu, L., Khor, K. A., & Lim, J. P. (2001). The evaluation of powder processing on microstructure and mechanical properties of hydroxyapatite (HA)/yttria stabilized zirconia (YSZ) composite coatings. *Surface and Coatings Technology*, 140(3), 263-268. doi:10.1016/S0257-8972(01)01168-9

- Fuglsby, K., Anderson, J. A., Engebretson, D., & Lamichhane, S. (2020). Development of an automated micropipette coating method for drug-coated balloons. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 108(5), 2258-2275. doi:10.1002/jbm.b.34563
- Ghasemi-Mobarakeh, L., Semnani, D., & Morshed, M. (2007). A novel method for porosity measurement of various surface layers of nanofibres mat using image analysis for tissue engineering applications. *Journal of Applied Polymer Science*, 106(4), 2536-2542. doi:10.1002/app.26949
- Grewal, N. S., Kumar, K., & Batra, U. (2023). Polycaprolactone/hydroxyapatite coating on spark generated Mg surfaces to promote anticorrosion and interfacial adhesion strength. *Materials Today Communications*, 36, 106440. doi:10.1016/j.mtcomm.2023.106440
- Haider, A., Haider, S., & Kang, I. -K. (2018). A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibres in biomedical and biotechnology. *Arabian Journal of Chemistry*, 11(8), 1165–1188. doi:10.1016/j.arabjc.2015.11.015
- Havlíček, K., Svobodová, L., Bakalova, T., & Lederer, T. (2020). Influence of electrospinning methods on characteristics of polyvinyl butyral and polyurethane nanofibres essential for biological applications. *Materials & Design*, 194, 108898. doi:10.1016/j.matdes.2020.108898
- Ibrahim, S. W., & Hamad, T. I. (2023). Electrospun nano-barium titanate/polycaprolactone composite coatings on titanium and Ti13Nb13Zr alloy. *Composites and Advanced Materials*, 32, 1–17. doi:10.1177/26349833231203742
- Ifkovits, J. L., Sundararaghavan, H. G., & Burdick, J. A. (2009). Electrospinning fibrous polymer scaffolds for tissue engineering and cell culture. *Journal of visualized experiments : JoVE*, 32, 1589. doi:10.3791/1589
- Jemat, A., Ghazali, M. J., Razali, M., & Otsuka, Y. (2015). Surface modifications and their effects on titanium dental implants. *BioMed Research International*, 6(1), 1-11.
- Jordi, M. (2018). *Typical Molecular Weights of Common Polymers*. Retrieved January 13, 2024, from <https://www.rqmplus.com/blog/typical-polymer-molecular-weights>
- Kandi, R., Pandey, P. M., Majood, M., & Mohanty, S. (2021). Fabrication and characterization of customized tubular scaffolds for tracheal tissue engineering by using solvent based 3D

- printing on predefined template. *Rapid Prototyping Journal*, 27(2), 421-428. doi:10.1108/RPJ-08-2020-0186
- Kausar, A. (2021). Polymeric nanocomposite via electrospinning: Assessment of morphology, physical properties and applications. *Journal of Plastic Film & Sheeting*, 37(1), 70–92. doi:10.1177/8756087920937344
- Kazemzadeh, G., Jirofti, N., Mohebbi-Kalhari, D., Sarhaddi, F., & Taheri, R. (2022). Pathological examination of blended and co-electrospinning hybrid polycaprolactone/ polyurethane nanofibres for soft tissue engineering applications. *Journal of Industrial Textiles*, 51(4S), 6816S–6837S. doi:10.1177/15280837221074070
- Kichi, M. K., Torkaman, R., Mohammadi, H., Toutounchi, A., Kharaziha, M., & Alihosseini, F. (2020). Electrochemical and in vitro bioactivity behavior of poly (ϵ -caprolactone) (PCL)-gelatin-forsterite nano coating on titanium for biomedical application. *Materials Today Communications*, 24, 101326. doi:10.1016/j.mtcomm.2020.101326
- Kiran, A. S., Kizhakeyil, A., Ramalingam, R., Verma, N. K., Lakshminarayanan, R., Kumar, T. S., . . . Ramakrishna, S. (2019). Drug loaded electrospun polymer/ceramic composite nanofibrous coatings on titanium for implant related infections. *Ceramics International*, 45(15), 18710-18720. doi:10.1016/j.ceramint.2019.06.097
- Leach, M. K., Feng, Z. Q., Tuck, S. J., & Corey, J. M. (2011). Electrospinning fundamentals: Optimizing solution and apparatus parameters. *Journal of Visualized Experiments*, 47, e2494. doi:10.3791/2494
- Leung, H. -W. (2014). Polyethylene Glycol. In P. Wexler (Ed.), *Encyclopedia of Toxicology* (3rd ed., pp. 1043-1044). Cambridge: Academic Press. doi:10.1016/B978-0-12-386454-3.00050-6
- Liu, Z., Zhao, J. -h., Liu, P., & He, J. -h. (2016). Tunable surface morphology of electrospun PMMA fiber using binary solvent. *Applied Surface Science*, 364, 516–521. doi:10.1016/j.apsusc.2015.12.176
- Luketich, S. K., Cosentino, F., Di Giuseppe, M., Menallo, G., Nasello, G., Livreri, P., . . . D'Amore, A. (2022). Engineering in-plane mechanics of electrospun polyurethane scaffolds for cardiovascular tissue applications. *Journal of the Mechanical Behavior of Biomedical Materials*, 128, 105126. doi:10.1016/j.jmbbm.2022.105126

- Maitz, M. F. (2015). Applications of synthetic polymers in clinical medicine. *Biosurface and Biotribology*, 1(3), 161-176. doi:10.1016/j.bsbt.2015.08.002
- Manakhov, A. M., Sitnikova, N. A., Tsygankova, A. R., Alekseev, A. Y., Adamenko, L. S., Permyakova, E., . . . Solovieva, A. O. (2021). Electrospun biodegradable nanofibres coated homogenously by Cu magnetron sputtering exhibit fast ion release. Computational and experimental study. *Membranes (Basel)*, 11(12), 965.
- Mao, Y., Shen, W., Wu, S., Ge, X., Ao, F., Ning, Y., . . . Liu, Z. (2023). Electrospun polymers: Using devices to enhance their potential for biomedical applications. *Reactive and Functional Polymers*, 186, 105568.
- Nathanael, A. J., & Oh, T. H. (2020). Biopolymer coatings for biomedical applications. *Polymers*, 12(12), 3061. doi:10.3390/polym12123061
- Ni, X., Cheng, W., Huan, S., Wang, D., & Han, G. (2019). Electrospun cellulose nanocrystals/poly(methyl methacrylate) composite nanofibres: Morphology, thermal and mechanical properties. *Carbohydrate Polymers*, 206, 29–37. doi:10.1016/j.carbpol.2018.10.103
- Nirmala, R., Jeon, K. S., Lim, B. H., Navamathavan, R., & Kim, H. Y. (2013). Preparation and characterization of copper oxide particles incorporated polyurethane composite nanofibres by electrospinning. *Ceramics International*, 39, 9651–9658. doi:10.1016/j.ceramint.2013.05.087
- Ottenbrite, R. M., & Javan, R. (2005). Biological Structures. In F. Bassani, G. L. Liedl, & P. Wyder (Eds.), *Encyclopedia of Condensed Matter Physics* (pp. 99–108). Elsevier. doi:10.1016/b0-12-369401-9/00698-7
- Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Ghayour, H., Ismail, A. F., Nur, H., & Berto, F. (2020). Electrospun nano-fibers for biomedical and tissue engineering applications: A comprehensive review. *Materials*, 13(9), 2153. doi:10.3390/ma13092153
- Passos, A. C., Arouche, M. M., Aguiar, R. A., Costa, H. R., de Barros, S., & Sampaio, E. M. (2021). Adhesion of epoxy and polyurethane adhesives in pultruded composite material. *Journal of Advanced Joining Processes*, 3, 100045. doi:10.1016/j.jajp.2021.100045
- Polymershapes. (2025). *Amorphous vs Semicrystalline*. Retrieved January 19, 2025, from <https://www.polymershapes.com/amorphous-vs-semicrystalline/>

- Qian, C., Liu, Y., Chen, S., Zhang, C., Chen, X., Liu, Y., & Liu, P. (2023). Electrospun core-sheath PCL nanofibres loaded with nHA and simvastatin and their potential bone regeneration applications. *Frontiers in Bioengineering and Biotechnology*, *11*, 1205252. doi:10.3389/fbioe.2023.1205252
- Quilez-Molina, A. I., Barroso-Solares, S., Hurtado-García, V., Heredia-Guerrero, J. A., Rodriguez-Mendez, M. L., Rodríguez-Pérez, M. Á., & Pinto, J. (2023). Encapsulation of copper nanoparticles in electrospun nanofibres for sustainable removal of pesticides. *ACS Applied Materials & Interfaces*, *15*(16), 20385-20397. doi:10.1021/acsami.3c00849
- Sarabi-Mianeji, S., Scott, J., & Pagé, D. J. (2015). Impact of electrospinning process parameters on the measured current and fiber diameter. *Polymer Engineering & Science*, *55*(11), 2576-2582. doi:10.1002/pen.24150
- Sharma, G. K., & James, N. R. (2022). Electrospinning: The Technique and Applications. In M. Khan, & S. J. Chelladurai (Eds.), *Recent Developments in Nanofibres Research*. London: IntechOpen. doi:10.5772/intechopen.105804
- Sheikh, F. A., Kanjwal, M. A., Saran, S., Chung, W. -J., & Kim, H. (2011). Polyurethane nanofibres containing copper nanoparticles as future materials. *Applied Surface Science*, *257*, 3020–3026. doi:10.1016/j.apsusc.2010.10.110
- Skosana, S. J., Khoathane, C., & Malwela, T. (2021). Enhancing the adhesion strength of polyurethane coatings by dispersing layered silicates via sonication and high-shear mixing method. *Polymer Bulletin*, *78*, 203–221. doi:10.1007/s00289-020-03100-y
- Song, R., Murphy, M., Li, C., Ting, K., Soo, C., & Zheng, Z. (2018). Current development of biodegradable polymeric materials for biomedical applications. *Drug Design, Development and Therapy*, *12*, 3117-3145. doi:10.2147/DDDT.S165440
- Song, W., Seta, J., Chen, L., Bergum, C., Zhou, Z., Kanneganti, P., . . . Yu, X. (2017). Doxycycline-loaded coaxial nanofibre coating of titanium implants enhances osseointegration and inhibits *Staphylococcus aureus* infection. *Biomedical Materials*, *12*(4), 045008.
- Sopcak, T., Medvecky, L., Zagyva, T., Dzupon, M., Balko, J., Balázs, K., & Balázs, C. (2018). Characterization and adhesion strength of porous electrosprayed polymer–hydroxyapatite composite coatings. *Resolution and Discovery*, *3*(2), 17-23. doi:10.1556/2051.2018.00057

- Stewart, S. A., Domínguez-Robles, J., McIlorum, V. J., Gonzalez, Z., Utomo, E., Mancuso, E., . . . Larrañeta, E. (2020). Poly(caprolactone)-based coatings on 3D-printed biodegradable implants: A novel strategy to prolong delivery of hydrophilic drugs. *Molecular Pharmaceutics*, 17(9), 3487–3500. doi:10.1021/acs.molpharmaceut.0c00515
- Visan, A. I., Popescu-Pelin, G., Gherasim, O., Mihailescu, A., Socol, M., Zgura, I., . . . Socol, G. (2020). Long-term evaluation of dip-coated PCL-blend-PEG coatings in simulated conditions. *Polymers*, 12(3), 717. doi:10.3390/polym12030717
- Wang, A., Yuan, W., Song, Y., Zang, Y., & Yu, Y. (2022). Osseointegration effect of micro-nano implants loaded with kaempferol in osteoporotic rats. *Frontiers in Bioengineering and Biotechnology*, 10, Article 842014. doi:10.3389/fbioe.2022.842014
- Xu, L., & Yamamoto, A. (2012). Characteristics and cytocompatibility of biodegradable polymer film on magnesium by spin coating. *Colloids and Surfaces B: Biointerfaces*, 93, 67-74. doi:10.1016/j.colsurfb.2011.12.009
- Xu, T., Jin, W., Wang, Z., Cheng, H., Huang, X., Guo, X., . . . Yang, H. (2018). Electrospun CuO-nanoparticles-modified polycaprolactone @polypyrrole fibers: An application to sensing glucose in saliva. *Nanomaterials*, 8(3), 133. doi:10.3390/nano8030133
- Yaseri, R., Fadaie, M., Mirzaei, E., Samadian, H., & Ebrahimezhad, A. (2023). Surface modification of polycaprolactone nanofibres through hydrolysis and aminolysis: A comparative study on structural characteristics, mechanical properties, and cellular performance. *Scientific Reports*, 13(1), 9434. doi:10.1038/s41598-023-36563-w
- Yuan, M., Huang, D., Wang, Z., Chen, X., & Yang, M. (2022). Synthesis of ultra-high molecular weight poly(methyl methacrylate) with hydrosilane as initiator. *Materials Today Communications*, 33, 104927. doi:10.1016/j.mtcomm.2022.104927
- Zhang, B., Yan, X., Xu, Y., Zhao, H. -S., Yu, M., & Long, Y. -Z. (2020). Measurement of adhesion of in situ electrospun nanofibres on different substrates by a direct pulling method. *Advances in Materials Science and Engineering*, 2020, 7517109. doi:10.1155/2020/7517109

CHAPTER 6 BIOLOGICAL ASPECTS OF TI BASED IMPLANTS

6.1. Introduction

The current chapter discussed the evaluation of functionalised laser powder bed fusion (LPBF) Ti6Al4V ELI and commercially pure titanium (cpTi) samples for antibacterial efficacy against bacterial strains commonly associated with implants and biocompatibility with cell lines without cytotoxicity. The titanium (Ti)-based specimens were functionalised in two forms: 1) by in situ alloying with copper (Cu) particles during the LPBF process; and 2) by coating their surfaces with electrospun polymer nanofibres (NFs) imbued with copper oxide nanoparticles.

Titanium alloys have been widely used as orthopaedic and dental bone implant materials because of biocompatibility with the human physiology (Zhang, et al., 2013). Laser powder bed fusion has been used to successfully manufacture Ti-base implants with a spectrum of biocompatible features (Krakhmalev, et al., 2017). However, because Ti implant surfaces are bioinert, it is necessary to design surfaces that can stimulate interactions with human tissue, and coating these surfaces with NFs has been identified as the ideal intervention. Fibrous polymeric materials derived from electrospinning have the ability to promote adhesion and proliferation of tissue cells by mimicking the extracellular matrix (ECM) (Rodríguez-Tobías, et al., 2019). Nonetheless, materials such as Ti and synthetic polymers do not innately possess antibacterial properties, and their biocompatibility allows for bacteria to easily breed under the appropriate conditions, posing a great risk in their clinical applications (Lei, et al., 2019). Human implant material requires optimum and long-lasting antibacterial activity, and Cu is an essential trace element in the human body with known antimicrobial abilities (Zhang, et al., 2013; Abdal-hay, et al., 2019; Al-Khateeb, et al., 2023).

6.2. Materials and methods

The Ti6Al4V ELI (Grade 23, also denoted as Ti6Al4V or Ti64 in this chapter), commercially pure Ti (cpTi, Grade 2), and in situ alloyed Ti6Al4V- x Cu and Ti- x Cu ($x = 3, 6$ wt.%) test specimen discs (10 mm $\times$ 2 mm) were manufactured by LPBF technique using the procedure and parameters detailed in Chapter 3. The samples were used in as-built condition without further heat or surface treatment. After removing the discs from the support structures, they were ultrasonically cleaned with acetone, anhydrous ethanol and distilled water for 15 min each in turn to remove surface impurities and dried under natural air (Wang, et al., 2022). The Ti6Al4V and cpTi substrates were coated with polycaprolactone/polyurethane (PCL/PU) NFs and PCL/PU NFs imbued with copper oxide nanoparticles (CuO NPs, also referred to as CuO in this chapter) using the electrospinning technique described in Chapter 5. The test samples are as listed and described in Table 6.1. Uncoated and Cu-free Ti6Al4V and cpTi discs were used as controls. Unless otherwise specified, all analyses were conducted in duplicate. All reagents were analytical grade and were used as received without additional purification.

Table 6.1: List of the test specimens and their descriptions

Sample name	Description
Ti6Al4V or Ti64	Control
Ti6Al4V-3Cu or Ti64-3Cu	Ti6Al4V alloyed with 3 wt.% Cu
Ti6Al4V-6Cu or Ti64-6Cu	Ti6Al4V alloyed with 6 wt.% Cu
Ti6Al4V-NF or Ti64-NF	Ti6Al4V coated with PCL/PU (3:2, w/w)
Ti6Al4V-NFCu or Ti64-NFCu	Ti6Al4V coated with PCL/PU/CuO (3:2:0.3, w/w/w)
cpTi	Control
Ti-3Cu	cpTi alloyed with 3 wt.% Cu
Ti-6Cu	cpTi alloyed with 6 wt.% Cu
Ti-NF	cpTi coated with PCL/PU (3:2, w/w)
Ti-NFCu	cpTi coated with PCL/PU/CuO (3:2:0.3, w/w/w)

The microbiological analyses to examine the antibacterial efficacy of the Cu particles in the test specimens were conducted at the Centre for Applied Food Sustainability and -Biotechnology (CAFSaB) at the Central University of Technology, Free State (CUT). The cell culture

experiments to evaluate the interaction between the test specimens and cell lines were conducted at Rhodes University Biotechnology Innovation Centre (RUBIC) at Rhodes University (RU). All experiments in the present study were performed under aseptic conditions in a class II biosafety cabinet to prevent contamination. Figure 6.1 shows the biosafety cabinets situated in CAFSaB and RUBIC.



(i)



(ii)

Figure 6.1: Biosafety cabinets installed at (i) CAFSaB, and (ii) RUBIC

6.2.1. Sterilisation

All the tools, reagents, and samples without coating were sterilised by steam autoclaving (Figure 6.2(i)) at 121 °C and ~120 kPa for 15 min, and allowed to cool to room temperature before the experiments. The NF-coated samples were sterilised with 70% (v/v) ethanol (EtOH) and tested for sterility. The ethanol sterilisation method used was derived from Shearer et al. (2006) and Naderi et al. (2016). The discs were placed in glass petri dishes in their respective groups with the coated side facing upwards. They were then submersed in 70% ethanol (diluted from 99% (v/v) EtOH with distilled water) for 30 min, followed by three rinse cycles in sterile phosphate-buffered saline (PBS). Two McCartney bottles containing only sterile tryptone soy broth (TSB) were used as

controls. One tube was kept closed, and the other was kept open and treated the same as the samples (excluding the sterilising with EtOH) throughout the sterilisation procedure. Samples were tested for sterilisation effectiveness by placing one disc from each group in a McCartney bottle containing 5 ml of TSB. All the bottles were then placed in an incubator (Figure 6.2(ii)) at 37 °C overnight. The broths were examined macroscopically, and broth turbidity indicated inefficient sterilisation; a clear broth indicated efficient sterilisation and no contamination. In cases where the macroscopic examination of the broth was inconclusive, the broth was plated onto tryptone soy agar (TSA) (WHO, 2024) and incubated at 37 °C overnight. Clear agar without any growth observed during macroscopic examination indicated a lack of contamination and efficient sterilisation. The sterilisation and sterility testing processes were repeated for the samples that the sterility assay indicated bacterial growth, until those samples displayed adequate bacterial inactivation.



Figure 6.2: (i) ALP KT-30L Autoclave, and (ii) Precision Incubator Memmert INB

6.2.2. Microbiological analyses

6.2.2.1. Bacterial strains and their preservation

The bactericidal effect of the specimens was tested against reference strains of pure cultures obtained using KWIK-STIKs (Microbiologics, Minnesota, USA). The strains are listed and discussed in Table 6.2. The bacterial strains were selected because of their prevalence in

orthopaedic and dental implants. The Microbank™ system was used to preserve and retrieve individual bacterial strains according to the guidelines provided by the manufacturer. Microbank™ is a ready-to-use system designed for the long-term storage and retrieval of bacterial isolates (Pro-lab Diagnostic, 2011). The Microbank™ platform consists of storage vials containing approximately 25 sterile single-coloured beads per vial and a specifically formulated cryopreservative for low-temperature storage. The specially treated Microbank™ beads are porous, allowing microorganisms to readily adhere onto the bead surface (Pro-lab Diagnostic, 2011; David, et al., 2023). For the present study, each strain was streaked onto TSA using the KWIK-STIK (as per the manufacturer's instructions) and incubated overnight at 37 °C. A single colony was then picked with a sterile inoculating loop, streaked onto a new TSA plate and incubated under the same conditions to obtain a bacterial lawn. The bacterial growth was harvested using a sterile loop for inoculation in the Microbank™ vials and stored at –80 °C for cryopreservation as part of the collection of bacterial strains at the CAFSaB at the CUT. Fresh culture may be grown by inoculating a single bead from the Microbank™ vial directly into culture medium (David, et al., 2023).

Table 6.2: Bacterial strains and their characteristics

Bacteria strains	Properties	Common implant association	Noteworthy	Ref.
<i>Staphylococcus aureus</i> (<i>S. aureus</i>) ATCC 25923 <i>S. aureus</i> ATCC 43300	Gram-positive Aerobic	Orthopaedic Dental	- <i>S. aureus</i> ATCC 25923 is known as methicillin-susceptible <i>S. aureus</i> (MSSA) strain. - <i>S. aureus</i> ATCC 43300 is known as a methicillin-resistant <i>S. aureus</i> (MRSA) strain. - Implant-associated infections are mainly caused by staphylococcal species, with <i>S. aureus</i> and <i>Staphylococcus epidermidis</i> being the main causative agents. - Highly reported medical implant-associated pathogens. - Staphylococci comprise up to two-thirds of all pathogens in orthopaedic implant infections. - Implicated in peri-implantitis or periodontal diseases. - <i>S. aureus</i> has been demonstrated to have the ability to adhere to titanium surfaces. - Significant nosocomial, or hospital-acquired, pathogen. - Biofilm-forming and antibiotic-resistant.	(Tanner, et al., 1997; Pye, et al., 2009; Ribeiro, et al., 2012; Zhang, et al., 2013; Arciola, et al., 2018; Bassous, et al., 2019; Dhaliwal, et al., 2021; Lu, et al., 2022; Zhao, et al., 2023; Haq, et al., 2024)
<i>Staphylococcus epidermidis</i> (<i>S. epidermidis</i>) ATCC 35984	Gram-positive Aerobic	Orthopaedic Dental	- Associated with nosocomial infections. - Highly reported medical implant-associated pathogens. - Have been found to be more antibiotic resistant than <i>S. aureus</i>. - Resistant to antibiotic treatment when in biofilm.	(Tanner, et al., 1997; Ribeiro, et al., 2012; Prinzi & Rohde, 2023; Haq, et al., 2024)
<i>Escherichia coli</i> (<i>E. coli</i>) ATCC 25922	Gram-negative Aerobic	Orthopaedic	- Associated with nosocomial infections. - Highly reported medical implant-associated pathogens. - Extensively isolated opportunistic pathogens from implants. - The most etiologic gram-negative organism, followed by <i>P. aeruginosa</i> and <i>Klebsiella</i> spp.	(Raquid, et al., 2018; Haq, et al., 2024)

<i>Klebsiella pneumoniae</i> (<i>K. pneumoniae</i>) ATCC 700603	Gram-negative Facultative anaerobic	Orthopaedic	- Resistant to antibiotic treatment when in biofilm. - Biofilm-forming on orthopaedic implants. - Highly reported medical implant-associated pathogens. - Extensively isolated opportunistic pathogen from implants. - Found in the normal flora of the mouth, skin, and intestines.	(Guo, et al., 2012; Chouhan, 2016; Prinzi & Rohde, 2023; Haq, et al., 2024)
<i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>) ATCC 27853	Gram-negative Aerobic	Orthopaedic Dental	- Opportunistic bacteria, A major cause of nosocomial infections. - Highly reported medical implant-associated pathogens. - Extensively isolated opportunistic pathogens from implants. - Biofilm-forming and antibiotic-resistant.	(Tanner, et al., 1997; Buyck, et al., 2017; Haq, et al., 2024; Manhas, et al., 2024)

Antibiotic-resistant *P. aeruginosa* and *S. aureus* were listed among high-priority pathogens on the World Health Organization (WHO) Bacterial Priority Pathogens List in 2024 due to their global threat, especially in health-care settings (WHO, 2024). Furthermore, among the antimicrobial-resistant pathogens typically found to burden healthcare, methicillin-resistant *S. aureus* (MRSA), multidrug-resistant (MDR) *P. aeruginosa* and carbapenem-resistant Enterobacterales (CRE) (including *E. coli* and *K. pneumoniae*) were classified as presenting urgent, serious and concerning threats by the Centers for Disease Control and Prevention (CDC) (2024) and may produce severe soft tissue infections (Muñoz-Escobar, et al., 2019).

6.2.2.2. Culture media

The media used were commercially obtained TSA and TSB. The broth employed for *P. aeruginosa* was Luria-Bertani (LB) broth (1% w/v tryptone, 1% w/v sodium chloride, 0.5% w/v yeast extract, pH = ~ 6.76). Tryptone soy agar was obtained by adding agar bacteriological to TSB. The TSA and TSB were prepared according to the manufacturer's instructions. After preparing its solution, the TSA was sterilised by steam autoclaving at 121 °C and 121 kPa for 15 min, then to 55 °C in a water bath before pouring into sterile 90 ml Petri dishes. The agar plates were then allowed to cool and solidify at room temperature overnight. Subsequently, the plates were spot-checked for any growth, which would indicate contamination. Contaminated agar plates were discarded, and the remaining plates were stored in the refrigerator and used within 1 month of preparation. The TSB and LB were prepared and sterilised as needed. Enumeration of bacterial suspensions was performed using the agar diffusion assay and the plate count method.

6.2.2.3. Agar diffusion assay

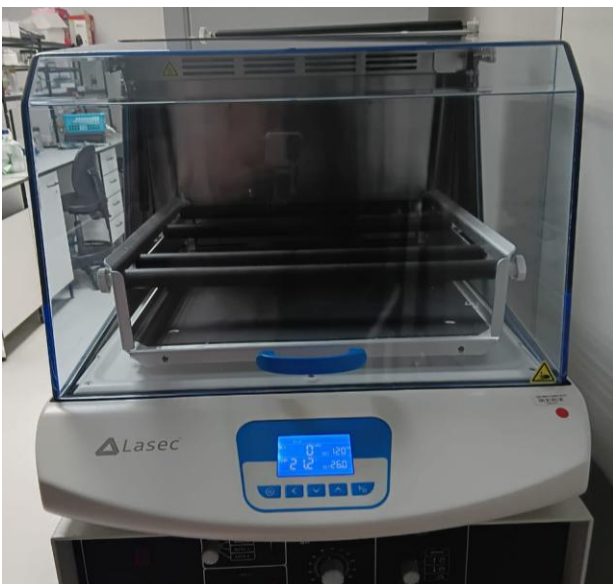
Preparation of antibiotic filter paper discs

The antibiotic used was erythromycin. Whatman number one (no 1) filter papers (6 mm diameter) were selected for preparing the discs because of their common use in disc preparation (Ukwuoma, et al., 2014). Before impregnation with erythromycin, the filter papers were sterilised by autoclaving for 15 minutes at 121°C and allowed to cool. The antibiotic stock solution for filter paper disc impregnation was prepared according to the method described by Epoke et al. (2003) with minor modifications. The filter paper discs were impregnated with 15 µg of erythromycin. Impregnation was achieved by submerging the sterilised filter paper discs in the working solution

for 10 min to allow absorption. The discs dried in the oven at 40 °C for 15 min and were used immediately.

Preparation and seeding of inoculum

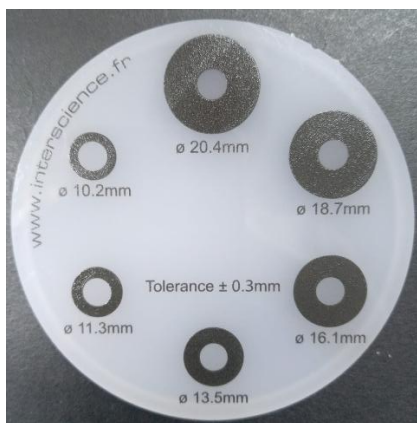
Each bacterial strain (Table 6.2) was revived from cryopreservation by plating a single Microbank™ bead on TSA and incubating at 37 °C overnight. A single colony was then inoculated into an Erlenmeyer flask containing 20 ml of sterile TSB and incubated overnight in a shaking incubator at 37 °C (Figure 6.3(i)). The resulting bacterial suspension was plated on a TSA plate using the easySpiral Pro® automatic plater (Figure 6.3(ii)) at the constant mode setting. After leaving the suspension to dry on the agar plate (~15 min), the Ti sample discs (Table 6.1) were placed in a specific configuration with the guidance of an Interscience Scan® standard plate (Figure 6.3(iii)). The NF-coated samples were placed with the coated surface in contact with the agar. Uncoated Ti6Al4V and cpTi discs were used as negative controls for the respective samples, and a filter paper disc imbued with erythromycin was used as a positive control on each plate. The plates were incubated at 37 °C overnight. The antibacterial property was determined by measuring the diameter of the zone of inhibition around the sample disc. Inhibition zones were measured using the Scan® HD inhibition zone reader (Figure 6.3(iv)) and analysed with Scan 1200® version 7.0.2.0 software. The recommendations of the Committee on Antibiotic Susceptibility of the French Society of Microbiology (CA-SFM) interpretation system were used according to the CA-SFM 2013 standard (Dubreuil, et al., 2020; Duque, et al., 2024; Lee, et al., 2025). The size of the zone of inhibition is directly proportional to antibacterial capacity.



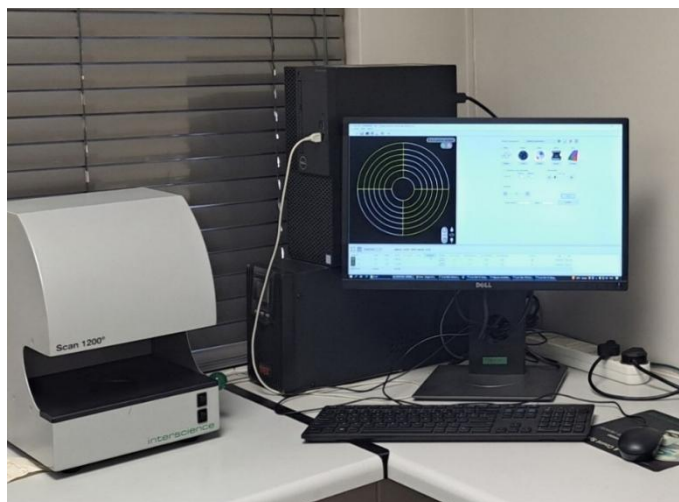
(i)



(ii)



(iii)



(iv)

Figure 6.3: Microbiology testing equipment sited at CAFSaB (i) LASEC® Shaking Incubator BSI-3, (ii) Interscience easySpiral Pro® automatic plater; (iii) Interscience Scan® standard plate n°3, and (iv) Interscience Scan®1200 HD automatic colony counter and inhibition zone reader

6.2.2.4. Plate count method

Preparation and seeding of inoculum

The bacterial strains were revived from cryopreservation and inoculated in 20 ml of sterile TSB as described for the agar diffusion assay. The TSB containing the bacteria, which had been cultivated as a single colony for 12 hrs at 37 °C, was diluted through a serial dilution process using TSB (or LB for *P. aeruginosa*) to a dilution factor of 10^{-6} . The dilutions at 10^{-5} and 10^{-6} were plated on separate TSA plates using the easySpiral Pro[®] automatic plater on the exponential mode (that is, the Spiral[®] method). The Spiral[®] method is a normalised method by the standards of International Organization for Standardization (ISO) 4833-2, ISO 7218 and Association of Official Analytical Collaboration (AOAC) International 977.27 as a method for bacterial enumeration (rapidmicrobiology.com, 2010). After spiral plating and incubating at 37 °C for 12 hrs (or until the colonies were macroscopically observable), the bacterial counts were enumerated using the Interscience Scan[®]1200 HD automatic colony counter (Figure 6.3(iii)). The counts aid in determining the number of colony forming units (CFU) in the original 20 ml inoculum from a single isolated colony and establish the dilution factor required to cultivate the bacterial suspension of 10^5 CFU/ml (Ren, et al., 2014; Wang, et al., 2019).

The Ti6Al4V and cpTi sample discs (Table 6.1) were individually placed in 24-well plates. The coated samples were placed with the coated side facing upwards. Each well was inoculated with 1 ml of the bacterial suspension. The control wells contained: bacterial suspension only (in duplicate), broth only and broth with the control disc (either Ti6Al4V or cpTi). The 24-well plates were closed with their lids, sealed with Parafilm, weighted and incubated at 37 °C for 24 and 48 hrs. At the 24- and 48-hr incubation periods, the plates were weighed to monitor evaporation. The suspension from each well was rinsed three times in the respective well and placed in a Corning[®] Cryogenic Vial. Each suspension was then diluted to 10^{-6} for bacterial enumeration. The 10^{-5} and 10^{-6} dilutions were plated on TSA plates using the Spiral[®] method. Following incubation at 37 °C for 12 hrs (or until colonies were macroscopically visible), the colonies were enumerated using a colony counter.

Statistical analysis

The enumerated colony counts were expressed as mean $\pm$ standard deviation in CFU/ml. The counts from the 10^{-5} and 10^{-6} dilution factors produced a total of four readings per sample, including the bacterial-suspension-only control. The recorded measurements were log-transformed. The significance of cell growth relative to the bacterial suspension was analysed using the Student's t-test, with $p \leq 0.05$ (95% confidence interval) and $p \leq 0.01$ (99% confidence interval) considered statistically significant and highly statistically significant, respectively.

6.2.3. Tissue culture

6.2.3.1. Biocompatibility

All tissue culture experiments were conducted at RUBIC using C2C12 (a kind donation by Prof Carola Niesler from the University of KwaZulu-Natal, South Africa) and MDA-MB-231 (a kind donation from Prof Adrienne Edkins from RU) cell lines. The cells were maintained in 75 cm² tissue culture (TC) flasks containing 10 ml of complete media comprising Dulbecco's Modified Eagle's Medium (DMEM) High Glucose, 10 % (v/v) heat-inactivated fetal calf serum (HIFCS) and supplemented with 1% 50/50 penicillin/streptomycin (100 U/ml) antibiotic solution. The flasks containing suspended cells were kept at 37 °C in a humidified incubator under a 5% (v/v) CO₂ atmosphere (Figure 6.4(i)). An inverted light microscope (Figure 6.4(ii)) was used to observe the confluence of the cells. Once the cells reached a confluence of approximately 80%, the media was removed from the flask and discarded. The cells were rinsed with 5 ml PBS, trypsinised by adding 2 ml 1× trypsin (prepared from 10× trypsin in PBS) and incubated for approximately 5 min. Trypsinisation was confirmed under the light microscope – indicated by detached cells in suspension – followed by the addition of 10 ml complete media to arrest the trypsinisation.



(i)



(ii)

Figure 6.4: (i) Heal Force CO₂ incubator, and (ii) Zeiss Axio Vert.A1 FL Inverted Transmitted Light Microscope Lab

The cell suspension was transferred to sterile 15 ml Falcon[®] centrifuge tubes and centrifuged at 2040 rpm (or 400 rcf) for 5 min. The supernatant was poured out, and the pelleted cells were re-suspended in 10 ml complete media. For viable cell enumeration, an aliquot of 20 μ l of the cell suspension was mixed with 20 μ l of trypan blue (that is, at a ratio of 1:1) to stain dead cells. The cells/trypan blue mixture (10 μ l) was added to each cell side of an Invitrogen[™] Countess[™] Cell Counting Chamber Slide, and the cells were counted using the Invitrogen[™] Countess[™] 3 Automated Cell Counter (Figure 6.5(i)). The cells were also manually counted by adding 20 μ l of the cells/trypan blue mixture to the upper and lower chambers of the Improved Neubauer haemocytometer (Figure 6.5(ii)) and enumerated with the assistance of the inverted light microscope and the online Cell Dilution Calculator by Omni Calculator (Omni Calculator, 2024) to obtain the total cell count.

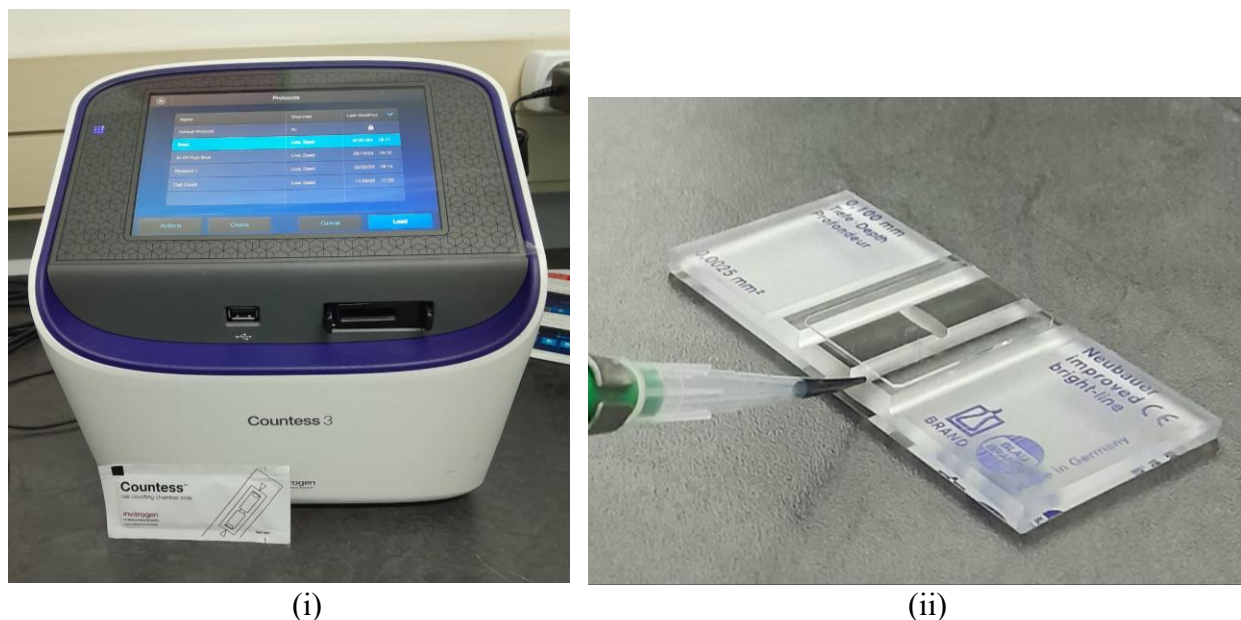


Figure 6.5: (i) Invitrogen™ Countess™ 3 FL Automated Cell Counter with the counter slide, and (ii) addition of cells/trypan blue mixture into the Improved Neubauer haemocytometer

The sterile sample discs (Table 6.1) were placed in 24-well plates with one disc/well. The harvested cells were then seeded at a seeding density of 1×10^5 cells/well into the corresponding wells. The control wells contained cell suspension only (in duplicate), media only and media with control disc (Ti6Al4V or cpTi). The plates were spot-checked using an inverted light microscope to assess the presence/absence of cells in the respective wells. The 24-well plates were incubated in the 5% CO₂ incubator at 37 °C for 24 hrs, 48 hrs and 5 days. At each time interval, the 24-well plate was removed from the incubator, and the samples from the individual wells were rinsed twice in 1 ml PBS to test cell retention. One sample from each sample group, as listed in Table 6.1, at the 24-hr and 5-day incubation intervals, was preserved for imaging using a scanning electron microscope (SEM). The remaining duplicate specimens at 24-hr, 48-hr and 5-day intervals were transferred to a new 24-well plate, and 1 ml of complete media was added to each sample. For imaging and viability, ReadyProbes™ Cell Viability Imaging Kit (Blue/Green) by Invitrogen™ was used. Five microliters each of the NucBlue® Live reagent and the NucGreen® Dead reagent were added to each well, and the plates were incubated for 30 min. Images of the fluorescence-stained cells were captured on the Invitrogen™ EVOS™ FL Auto 2 Imaging System (Figure 6.6) at 10× magnification using a standard diamidino-2-phenylindole (DAPI) filter set and DAPI EVOS light cube (357/44 nm excitation; 447/60 nm emission) for the blue stain and a standard fluorescein

isothiocyanate/green fluorescent protein (FITC/GFP) filter set and GFP EVOS light cube (482/25 nm excitation; 524/24 nm emission) for the green stain. When the fluorescence imaging was completed, samples in their respective plates were analysed for cytotoxicity using the resazurin assay.

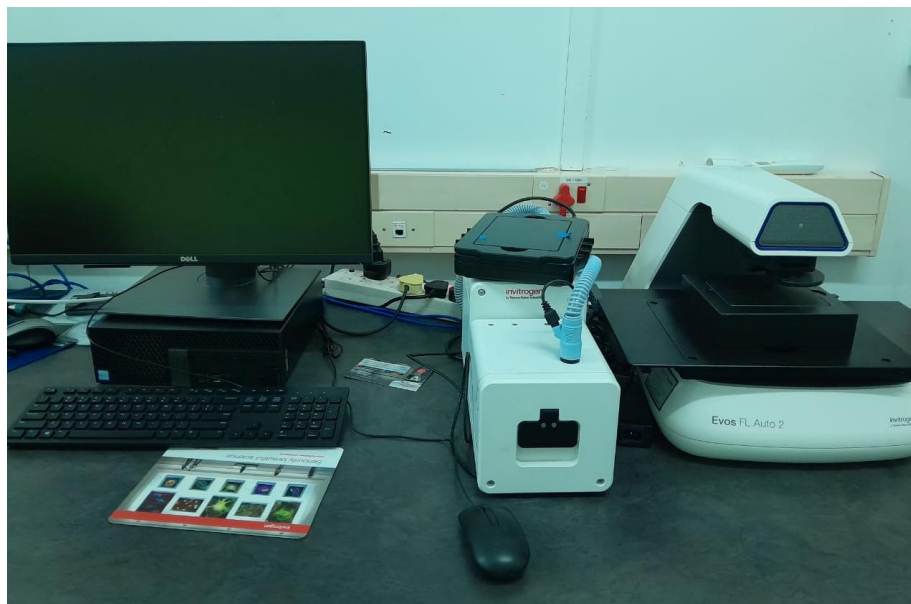


Figure 6.6: Invitrogen™ EVOS™ FL Auto 2 Imaging System

6.2.3.2. Resazurin cell viability assay

Resazurin (7-hydroxy-10-oxido-phenoxazin-10-ium-3-one) is a redox dye used during the resazurin assay (Khatua, et al., 2022). The assay is a colorimetric fluorescence test based on the reduction of the oxidised resazurin implemented by living cells (Sanchez Armengol & Laffleur, 2021). As illustrated in Figure 6.7, the non-fluorescent blue-coloured resazurin enters the cytosol where it accepts an electron from nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide hydride (FMNH), flavin adenine dinucleotide (FADH) and cytochromes, and is reduced to the red-coloured, highly fluorescent pink compound named resorufin (Sanchez Armengol & Laffleur, 2021; Khatua, et al., 2022). Hence, the resazurin assay is a cell viability test and has been recognised as a standard assay due to its simplicity of use and good reproducibility (Sanchez Armengol & Laffleur, 2021).

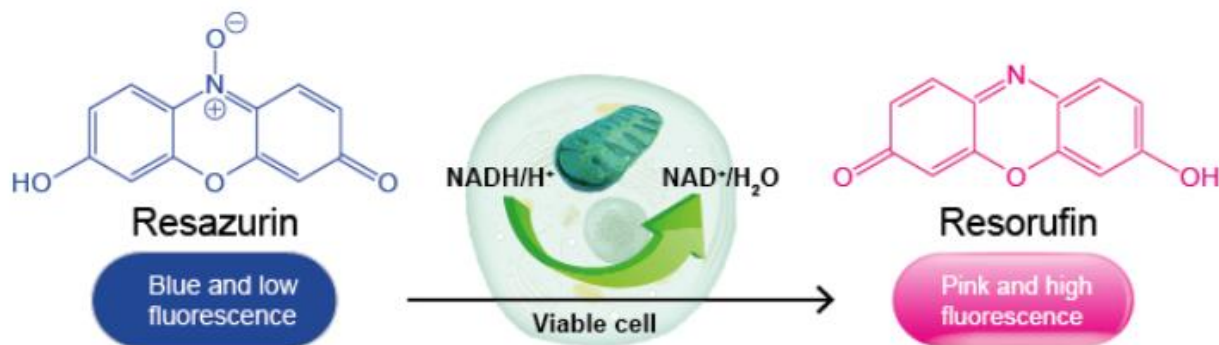


Figure 6.7: Resazurin assay reaction during cell viability testing (adapted from Creative Bioarray (2025))

The viability of C2C12 and MDA-MB-231 cells seeded onto the titanium sample discs was evaluated for cytotoxicity using resazurin sodium salt. The resazurin reagent was prepared by dissolving 0.01 g of resazurin powder in 1 ml PBS, resulting in a 44 mM (1000×) stock solution, from which a 44 μM (10×) dye solution was prepared in PBS. The resazurin dye is sensitive to light and temperature. Hence, light exposure was minimised during preparation, and the solution was stored in a light-resistant container at −20 °C for a year or at 4°C for 6 months. The 10× resazurin dye (100 μl) was added to the respective wells and incubated for 4–5 hrs at 37 °C in 5% CO₂ for analysis of metabolic activity. Approximately 150 μl of the liquid from each well was transferred to a 96-well plate for colorimetric (absorbance) and fluorescence plate reading. Measurements of the resazurin reduced to resorufin were conducted using a Varioskan LUX Multimode Microplate Reader by Thermo Scientific (Figure 6.8) at a wavelength of 600 nm for absorbance, and for fluorescence at the excitation and emission wavelengths of 560 nm and 600 nm, respectively. The readings were taken at a temperature of 37 °C and recorded as relative fluorescence unit (RFU). For cell viability, the RFU was calculated using Equation 6.1.

$$\text{RFU} = \frac{\text{RFU}_{\text{sample}} - \text{RFU}_{\text{blank}}}{\text{RFU}_{\text{cells}} - \text{RFU}_{\text{blank}}} \quad \text{Equation 6.1}$$

where RFU_{sample}, RFU_{cells} and RFU_{blank}, are RFU values obtained from control/test sample, control well with cells only and media blank, respectively. The RFU values were presented as a fold change by normalising the cell suspension control to 1 (representing cell viability of 100%).



Figure 6.8: Varioskan LUX Multimode Microplate Reader

6.2.3.3. SEM observation of adherent cells

The morphology of adherent C2C12 and MDA-MB-231 cells on the surfaces of the test specimens was observed for the 24-hr and 5-day samples. After rinsing twice in PBS as described above (that is, samples that were not subjected to any staining), each disc was submerged in a fixative solution consisting of cold 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer and transported to the Centre for Microscopy at the University of the Free State (UFS) for imaging and analyses. The samples were transferred to a 3% glutaraldehyde fixative in 0.1 M sodium phosphate buffer. After the fixation, the materials were washed with PBS, followed by gradient dehydration in varying concentrations of ethanol (30%, 50%, 70% and 95%) for 30 min, and the dehydration was completed in two changes of 100% EtOH for 1 hr per change. The samples were dried using a Tousimis SAMDRI®-795 critical point dryer (Figure 6.9(i)). The dry specimens were mounted onto aluminium stubs using epoxy glue and coated with iridium in a Leica EM ACE600 high vacuum sputter coater (Figure 6.9(ii)). JEOL JSM-IT200 InTouchScope™ Scanning Electron Microscope (SEM, Figure 6.9(iii)) was used for imaging, and the elemental analysis was conducted using the JEOL JSM-7800F Schottky Field Emission Scanning Electron Microscope (FE-SEM, Figure 6.9(iii)) equipped with the Oxford Instruments X-Max 80 energy-dispersive X-ray spectrometer (EDS) detector with AZtec software. The specimens were imaged using the secondary electron detector at 5 kV for 40 min per sample, with a working distance of 10 mm.



(i)



(ii)



(iii)

Figure 6.9: UFS Centre for Microscopy housed (i) Tousimis SAMDRI®-795 critical point dryer, (ii) Leica EM ACE600 high vacuum sputter coater, and (iii) JEOL JSM-IT200 InTouchScope™ Scanning Electron Microscope and JEOL JSM-7800F Schottky Field Emission Scanning Electron Microscope equipped with EDS

6.3. Results and discussion

6.3.1. Sterilisation

Establishing an efficient and non-destructive sterilisation protocol for polyester scaffolds that preserves their three-dimensional (3D) structure remains a challenge (Holy, et al., 2001). Rogers (2005) defines sterilisation as the destruction of viable microorganisms that are capable of surviving in most environmental conditions. To be approved for use in the human body, polymer scaffolds are required to demonstrate sterility (Rediguieri, et al., 2016; Bhaskar, et al., 2017). Sterility is mandatory for all medical devices and should be incorporated during the product development stage (Bhaskar, et al., 2017). Steam sterilisation accomplished in an autoclave is the most widely used and most dependable of all the available sterilisation methods (CDC, 2023). Moreover, heat is the oldest and primarily recommended terminal sterilant by health authorities (Rediguieri, et al., 2016). However, autoclaving is not a viable option for sterilising polymers with low melting points.

Polycaprolactone has a low melting point (T_m , around 60 °C) and glass transition temperature (T_g , around -60 °C) (Cheng, et al., 2010; Domańska, et al., 2020). Rediguieri et al. (2016) identified literature that describes the deleterious effect on the scaffold structure of PCL NFs after autoclaving. In addition, biocompatible polymers are hydrolytically unstable and, thus, exposure to heated moisture may accelerate degradation, compromise longevity and alter mechanical properties (Yoganarasimha, et al., 2014). Other common traditional methods are sterilisation using ultraviolet (UV) radiation, ethylene oxide, gamma radiation and ethanol. Ultraviolet radiation is not suitable for sterilising medical devices with undulating surfaces (ElectrospinTech, 2022). Ethylene oxide may be explosive and inflammable, and leads to toxic residues that require extensive degassing after sterilisation, which may be difficult to completely remove from woven NF mats (Rogers, 2005; Rediguieri, et al., 2016). Gamma radiation may cause alterations in the molecular structures of the polymer, resulting in deterioration and reduced ductility (Shearer, et al., 2006; Rediguieri, et al., 2016).

Alcohol sterilisation is not approved as a sterilisation method by health authorities (Rediguieri, et al., 2016). However, integrating various aspects of the sterilisation of polymer materials for healthcare applications without adversely affecting their end use expands the scope of sterilisation

(Rogers, 2005). Sterilisation of medical products using ethanol is widely used in research laboratory settings due to its low cost, availability, apparent effectiveness and ease of use (Shearer, et al., 2006; Bhaskar, et al., 2017). Bhaskar et al. (2017) demonstrated that EtOH sterilisation of an electrospun PCL scaffold did not alter cellular response to the scaffold. Similarly, Holy et al. (2001) studied the sterilisation behaviour of poly(D,L lactic-co-glycolic acid) (PLGA) and found that ethanol was less degrading than other common sterilisation techniques (namely, ethylene oxide and gamma radiation). Bhaskar et al. (2017) acknowledged this behaviour of PLGA as an indicator for the reaction of other biodegradable polyesters such as PCL. Hence, EtOH sterilisation was applied for the present coated samples and was found to be effective in sterilising the NF-coated test specimens.

6.3.2. Microbiological analyses

Infection at a bone-implant interface is the most plausible cause of implant failure directly after implantation (Krakhmalev, et al., 2017). Consequently, utilising implant materials with embedded antibacterial agents has been proven to effectively reduce bacterial infection by acting locally and long-term at the infection site (Zhang, et al., 2013; Krakhmalev, et al., 2017; Kiran, et al., 2019). Metal or oxide based on Cu is well established as an antibacterial agent (Manfredi & Calignano, 2017; Muñoz-Escobar, et al., 2019). The antibacterial performance of Ti–Cu alloys is critical for reducing inflammation caused by bacterial infection (Xin, et al., 2022). Furthermore, coating the Ti implant surface with NFs permeated with Cu particles allows for the controlled release of Cu ions (Muñoz-Escobar, et al., 2019).

6.3.2.1. Agar diffusion assay

The agar diffusion assay (also known as the disc diffusion method) is favoured for its simplicity and rapidity (Joshi, et al., 2008). The technique was first utilised in the late 1800s and is performed according to standardised methodologies issued by reference groups, such as Clinical and Laboratory Standard Institute (CLSI), British Society for Antimicrobial Chemotherapy (BSAC) and the Swedish Reference Group for Antibiotics (SRGA) (Tenover, 2009; Ukwuoma, et al., 2014). In the agar disc diffusion method of antimicrobial susceptibility testing, the antimicrobial agent diffuses from a focus (which is the sample disc in this study) through a solid medium, inhibiting the growth of an organism to a distance depending on the sensitivity of the organism to

the agent (Ukwuoma, et al., 2014). Thus, the method measures the qualitative action of an antimicrobial agent against pathogenic microorganisms (Bayot & Bragg, 2024).

Figure 6.10 illustrates the results of the agar diffusion assay for the test specimens (Table 6.1) against the bacterial strains (Table 6.2). From these results, it may be seen that an inhibition zone was not formed by any of the test specimens, as with the negative control, including those containing Cu/CuO particles in alloy form and as imbued in the electrospun PCL/PU NFs coating. The erythromycin showed inhibition against *S. aureus* ATCC 25923 (inhibition diameter = 27.1 ± 0.3 mm), *E. coli* (inhibition diameter = 14.8 ± 0.5 mm), *K. pneumoniae* (inhibition diameter = 8.8 ± 0.8 mm) and *P. aeruginosa* (inhibition diameter = 11.7 ± 0.4 mm).

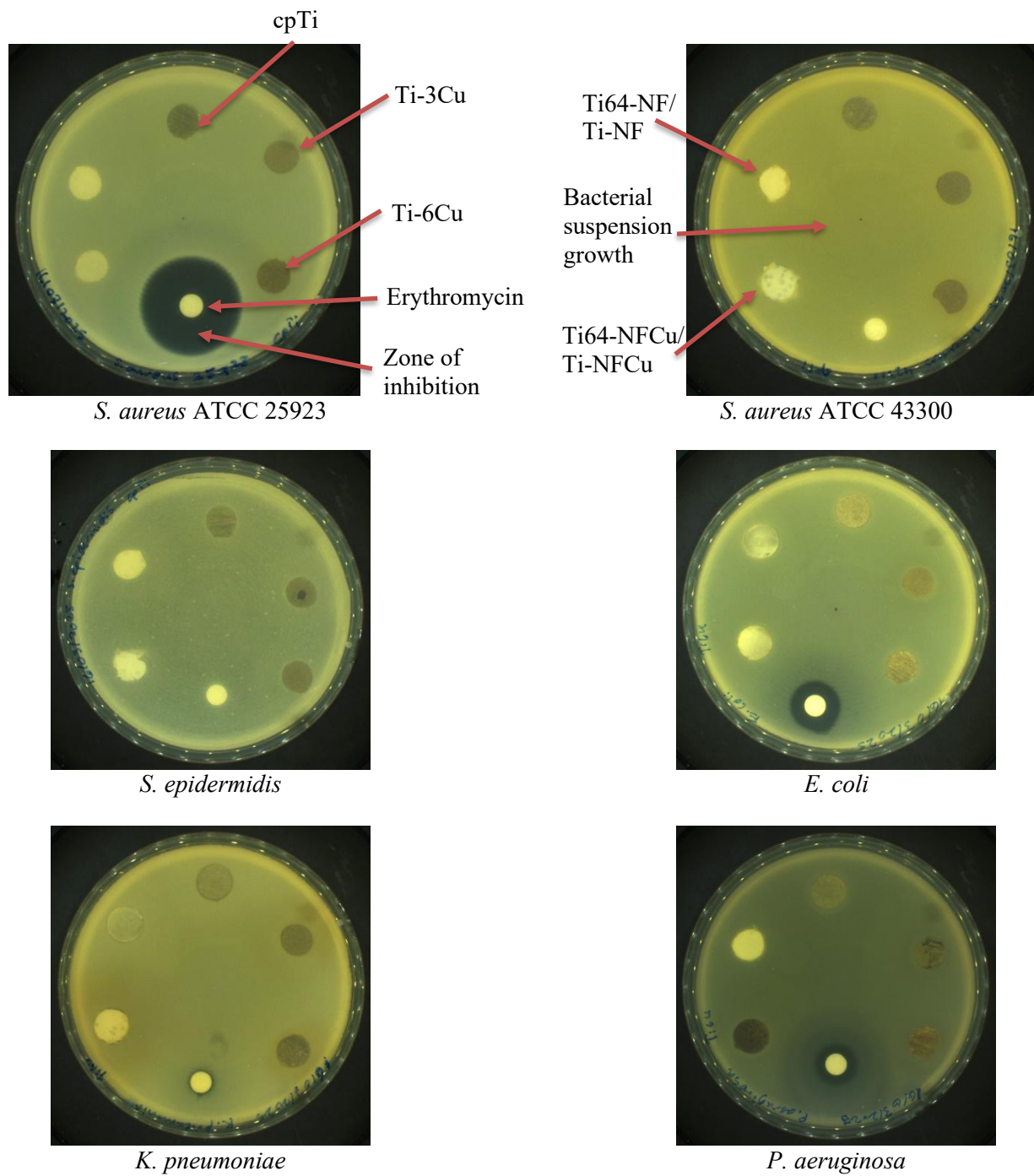


Figure 6.10: Inhibition zones from agar diffusion assay

Based on the agar diffusion assay, the Cu content did not exhibit antibacterial properties against the tested strains. For their research, Zhang et al. (2013) fabricated a Ti-10 wt.% Cu alloy with a

diameter of 25 mm by powder metallurgy to determine the antibacterial efficacy Cu against of *S. aureus* and *E. coli*. Similar to the current study, their agar diffusion assay did not show inhibition zones with Ti–Cu samples against both bacteria. On the contrary, Haider et al. (2015) and Sarwar et al. (2022) observed zones of inhibition against both Gram-positive and Gram-negative bacteria produced by electrospun polymer NF scaffolds impregnated with CuO NPs. For both studies, the inhibition was attributed to the bactericidal activity of CuO.

Muñoz-Escobar et al. (2019) performed the disc-diffusion method to examine the antibacterial activity of PCL-CuO NPs nanofibres prepared by electrospinning. The analysis was performed against three Gram-positive (*S. aureus*, *Bacillus subtilis* and *Streptococcus mutans*) and three Gram-negative (*E. coli*, *P. aeruginosa* and *Klebsiella oxytoca*) bacteria. The best inhibition result was against the *P. aeruginosa*, showing effectiveness for the lowest concentration of CuO NPs at 25 mM, with an inhibition zone of 7.00 mm, and the inhibition significantly increased to 11.20 mm at the highest CuO NP concentration of 200 mM. *Klebsiella oxytoca* and *S. aureus* showed only a small inhibition zone at the highest concentration (200 mM), producing inhibition halos of 6.69 mm and 6.79 mm, respectively. Zones of inhibition in the other tested bacteria were not observable at any PCL-CuO NPs concentration. Varying Cu/CuO concentrations may influence the antimicrobial behaviour of test specimens.

6.3.2.2. Plate count method

Figure 6.11 illustrates the enumerated CFUs of the test samples for the various strains, and Figure 6.12 shows matrices of *p*-values comparing the bacterial counts among the test samples. On each matrix, the lower triangle represents the results after 24 hours of incubation, and the upper triangle represents the results after 48 hours of incubation. Comparing the counts obtained from the bacterial suspension only (incubated without a sample disc and designated as “Suspension” in Figure 6.11 and Figure 6.12) with those obtained from the test samples indicated the antibacterial property of the samples (or samples’ ability to promote cell growth), and statistical significance/high significance thereof is indicated in Figure 6.11.

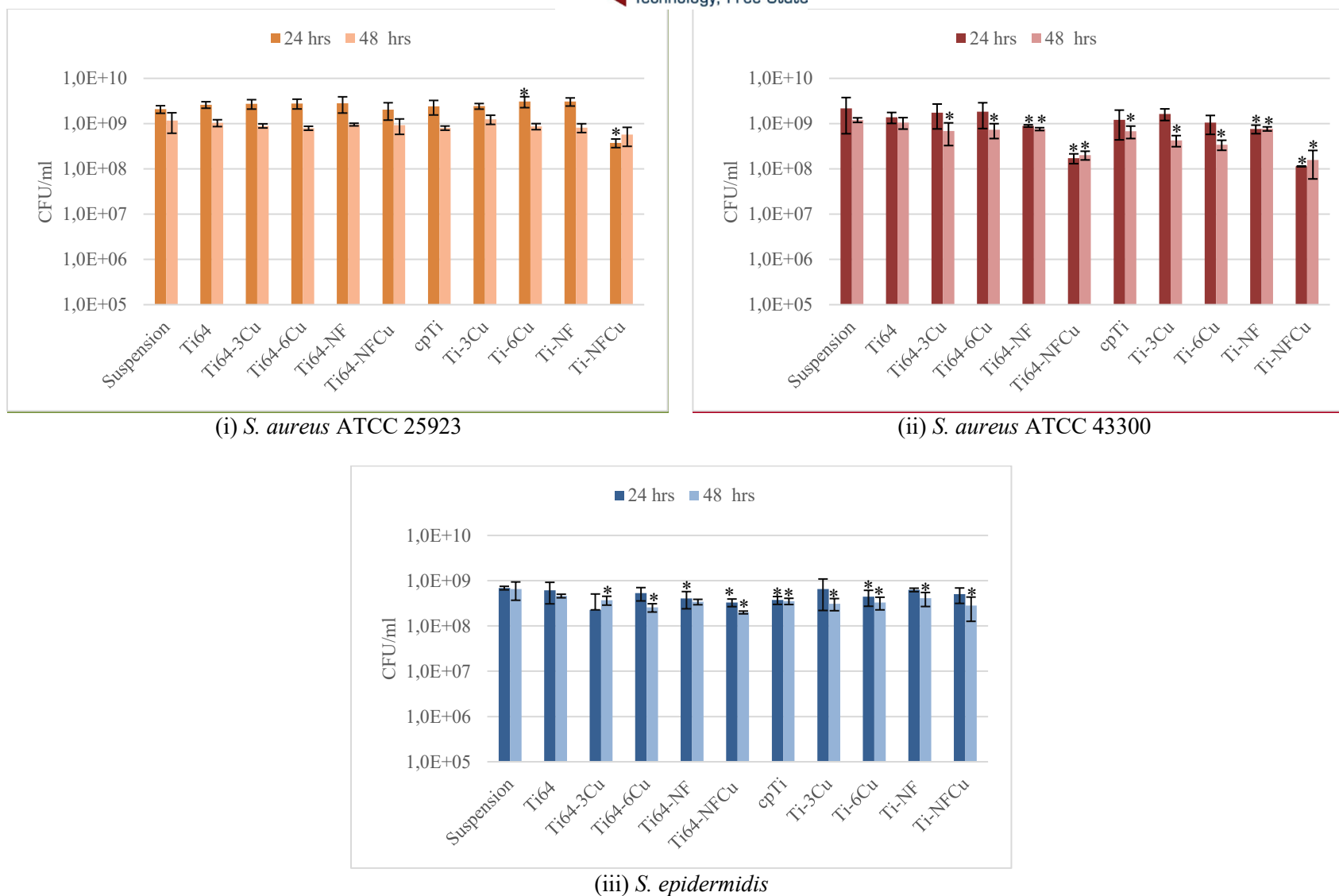


Figure 6.11: Colony forming units of test specimens against various bacterial strains (*= statistically/highly statistically significant)

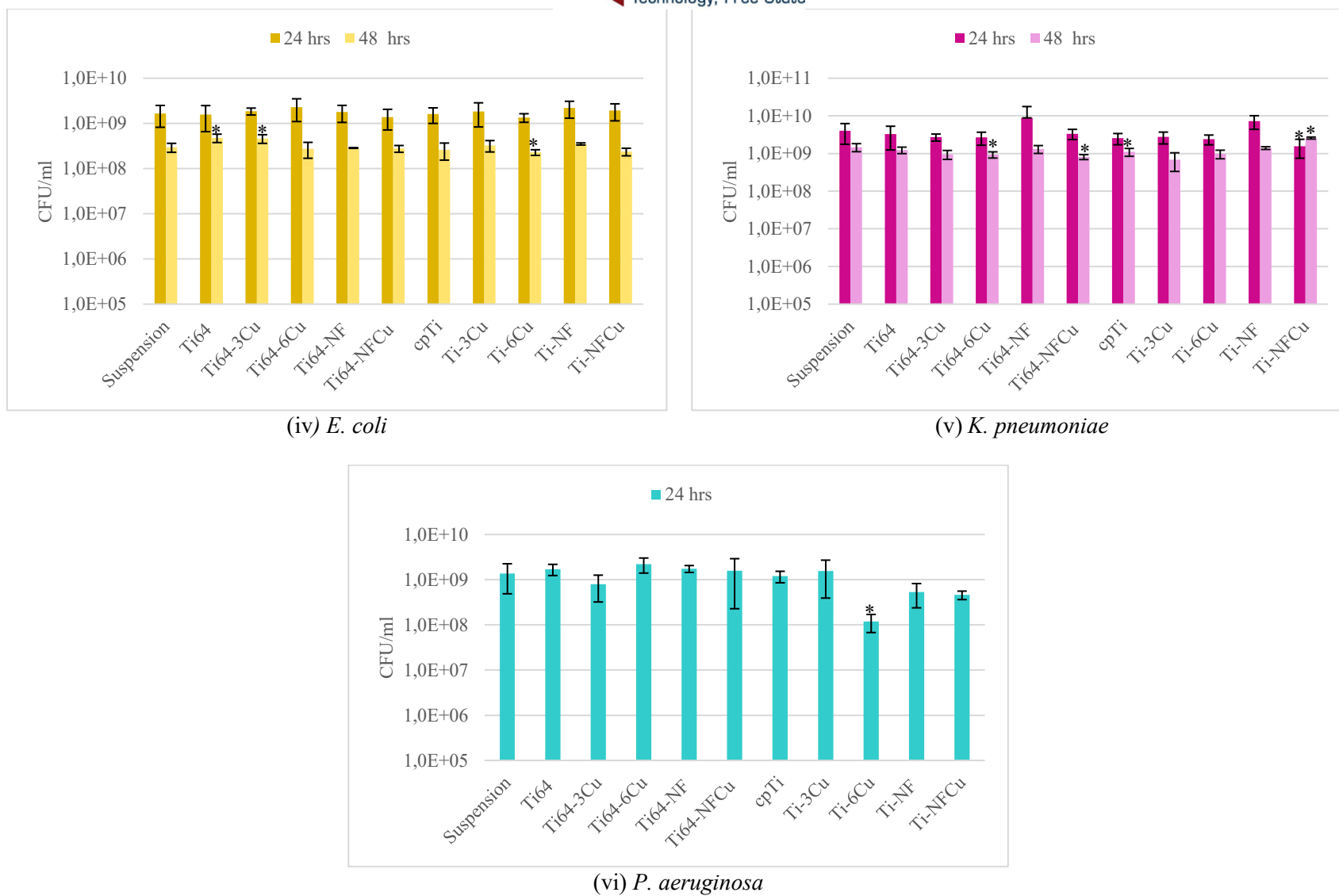


Figure 6.11: Colony forming units of test specimens against various bacterial strains (*= statistically/highly statistically significant)
(cont.)

		48 hrs										
		Suspension	Ti64	Ti64-3Cu	Ti64-6Cu	Ti64-NF	Ti64-NFCu	cpTi	Ti-3Cu	Ti-6Cu	Ti-NF	Ti-NFCu
<i>S. aureus</i> ATCC 25923	Suspension	1	0.6583	0.2604	0.2886	0.2771	0.3833	0.3496	0.7548	0.9594	0.9054	0.6540
	Ti64	0.2522	1	0.1211	0.1427	0.1285	0.2188	0.1713	0.8308	0.7945	0.6730	0.7201
	Ti64-3Cu	0.1083	0.6730	1	0.8992	0.8507	0.9371	0.8645	0.0657	0.4833	0.6110	0.5394
	Ti64-6Cu	0.0963	0.3161	0.4537	1	0.8196	0.9955	0.8163	0.1067	0.4713	0.5907	0.5319
	Ti64-NF	0.3213	0.3473	0.4542	0.4857	1	0.8865	0.9437	0.0713	0.5033	0.6344	0.5454
	Ti64-NFCu	0.9806	0.2098	0.1129	0.1037	0.1877	1	0.8685	0.2250	0.5038	0.6164	0.5340
	cpTi	0.3709	0.3699	0.2249	0.1966	0.2524	0.2603	1	0.1221	0.5327	0.6608	0.5501
	Ti-3Cu	0.2445	0.3986	0.1865	0.1613	0.2509	0.1973	0.9282	1	0.8877	0.7514	0.6905
	Ti-6Cu	0.0479	0.1546	0.2354	0.2711	0.3464	0.0615	0.1724	0.1507	1	0.8893	0.6691
	Ti-NF	0.0591	0.1902	0.2446	0.2839	0.3671	0.0712	0.1896	0.1778	0.9679	1	0.6350
	Ti-NFCu	0.0003	0.0008	0.0001	0.0001	0.0044	0.0074	0.00005	0.00002	0.0007	0.0046	1
		24 hrs										
		48 hrs										
		Suspension	Ti64	Ti64-3Cu	Ti64-6Cu	Ti64-NF	Ti64-NFCu	cpTi	Ti-3Cu	Ti-6Cu	Ti-NF	Ti-NFCu
<i>S. aureus</i> ATCC 43300	Suspension	1	0.1210	0.0100	0.0047	0.0017	0.00001	0.0006	0.00004	0.00002	0.0019	0.000005
	Ti64	0.1157	1	0.0790	0.0588	0.0166	0.00001	0.0062	0.0001	0.00003	0.0225	0.000005
	Ti64-3Cu	0.2837	0.4705	1	0.8135	0.6628	0.0287	0.9596	0.1750	0.0864	0.6316	0.0201
	Ti64-6Cu	0.3450	0.3959	0.8794	1	0.8346	0.0058	0.6859	0.0505	0.0201	0.7869	0.0033
	Ti64-NF	0.0398	0.0044	0.1098	0.1023	1	0.00001	0.2909	0.0007	0.00003	0.8779	0.0003
	Ti64-NFCu	0.0089	0.000003	0.0155	0.0169	0.0160	1	0.00005	0.0062	0.0156	0.0002	0.5173
	cpTi	0.1737	0.7825	0.6882	0.5888	0.1426	0.0065	1	0.0124	0.0010	0.2840	0.0001
	Ti-3Cu	0.2023	0.3237	0.8547	0.7223	0.0153	0.0009	0.7424	1	0.2311	0.0012	0.0098
	Ti-6Cu	0.5553	0.5751	0.8343	0.9082	0.3175	0.1293	0.6651	0.7560	1	0.0002	0.0369
	Ti-NF	0.0305	0.0021	0.0758	0.0728	0.2985	0.0062	0.0871	0.0071	0.1979	1	0.0002
	Ti-NFCu	0.0080	0.000003	0.0135	0.0149	0.0408	0.0941	0.0051	0.0008	0.1122	0.0063	1
		24 hrs										

Figure 6.12: *p*-value matrices for Ti-based alloys against various bacterial strains (■ = significantly; ■ = highly significantly)

48 hrs												
<i>S. epidermidis</i>		Suspension	Ti64	Ti64-3Cu	Ti64-6Cu	Ti64-NF	Ti64-NFCu	cpTi	Ti-3Cu	Ti-6Cu	Ti-NF	Ti-NFCu
	Suspension	1	0.5642	0.0177	0.0040	0.0117	0.0021	0.0218	0.0081	0.0107	0.0470	0.0118
	Ti64	0.3801	1	0.2706	0.2114	0.2537	0.1863	0.2798	0.2378	0.2480	0.2953	0.2252
	Ti64-3Cu	0.6756	0.7699	1	0.0291	0.5290	0.0048	0.7675	0.2981	0.4967	0.6858	0.4015
	Ti64-6Cu	0.0835	0.5221	0.3727	1	0.0508	0.0633	0.0117	0.2949	0.1981	0.1540	0.8068
	Ti64-NF	0.0349	0.1729	0.1459	0.3562	1	0.0100	0.3443	0.5495	0.8333	0.4716	0.5565
	Ti64-NFCu	0.0003	0.0240	0.0456	0.0528	0.4998	1	0.0007	0.0438	0.0358	0.0802	0.4280
	cpTi	0.0009	0.0571	0.0730	0.1308	0.7667	0.5495	1	0.1970	0.3551	0.8117	0.3325
	Ti-3Cu	0.7467	0.8564	0.9750	0.5702	0.2908	0.1580	0.2132	1	0.7611	0.3291	0.7790
	Ti-6Cu	0.0169	0.1980	0.1712	0.4371	0.7838	0.2258	0.4762	0.3446	1	0.4299	0.6492
	Ti-NF	0.2191	0.9043	0.7949	0.2863	0.1030	0.0009	0.0021	0.8927	0.0619	1	0.3264
	Ti-NFCu	0.1360	0.4723	0.3624	0.8420	0.5357	0.2120	0.3371	0.5133	0.6655	0.3375	1
24 hrs												
48 hrs												
<i>E. coli</i>		Suspension	Ti64	Ti64-3Cu	Ti64-6Cu	Ti64-NF	Ti64-NFCu	cpTi	Ti-3Cu	Ti-6Cu	Ti-NF	Ti-NFCu
	Suspension	1	0.0008	0.0116	0.5357	0.6722	0.6641	0.4182	0.5474	0.0247	0.0576	0.2005
	Ti64	0.8331	1	0.4806	0.2060	0.0007	0.0024	0.2247	0.0094	0.0001	0.0064	0.0007
	Ti64-3Cu	0.8700	0.9895	1	0.3710	0.0110	0.0136	0.0050	0.0485	0.0025	0.0575	0.0047
	Ti64-6Cu	0.3268	0.2691	0.3078	1	0.4710	0.4407	0.3644	0.7429	0.2295	0.9256	0.2666
	Ti64-NF	0.8251	0.6995	0.7376	0.4657	1	0.8141	0.5009	0.3853	0.0103	0.0327	0.2623
	Ti64-NFCu	0.5896	0.7180	0.7406	0.2036	0.5156	1	0.7519	0.3957	0.2855	0.1445	0.4356
	cpTi	0.8778	0.9254	0.9522	0.2674	0.7249	0.6367	1	0.2607	0.4207	0.0373	0.6303
	Ti-3Cu	0.7396	0.6238	0.6662	0.5310	0.9168	0.4605	0.6405	1	0.0674	0.5675	0.1351
	Ti-6Cu	0.9184	0.9129	0.9373	0.2973	0.7627	0.6489	0.9729	0.6816	1	0.0008	0.8311
	Ti-NF	0.4976	0.4355	0.4562	0.9054	0.6121	0.3421	0.4499	0.6723	0.4666	1	0.0528
	Ti-NFCu	0.6419	0.5382	0.5822	0.6086	0.8194	0.3992	0.5500	0.9026	0.5896	0.7449	1
24 hrs												

Figure 6.12: *p*-value matrices for Ti-based alloys against various bacterial strains (■ = significantly; ■ = highly significantly) (cont.)

48 hrs

		Suspension	Ti64	Ti64-3Cu	Ti64-6Cu	Ti64-NF	Ti64-NFCu	cpTi	Ti-3Cu	Ti-6Cu	Ti-NF	Ti-NFCu
K. pneumoniae	Suspension	1	0.3698	0.2506	0.0020	0.4891	0.0004	0.0230	0.9021	0.6165	0.6576	0.0486
	Ti64	0.5483	1	0.5333	0.0150	0.9768	0.0027	0.1526	0.9363	0.9293	0.2920	0.0295
	Ti64-3Cu	0.1813	0.5379	1	0.3939	0.5959	0.2025	0.8435	0.7703	0.7454	0.1895	0.0161
	Ti64-6Cu	0.1942	0.5396	0.9406	1	0.1208	0.2862	0.1688	0.5557	0.3383	0.0326	0.0124
	Ti64-NF	0.4169	0.3582	0.3174	0.3150	1	0.0614	0.3453	0.9305	0.9471	0.3502	0.0289
	Ti64-NFCu	0.5478	0.9315	0.3644	0.3848	0.3641	1	0.0248	0.4587	0.2126	0.0200	0.0101
	cpTi	0.2346	0.2032	0.1801	0.1789	0.6146	0.2059	1	0.7128	0.6093	0.0776	0.0179
	Ti-3Cu	0.2159	0.5898	0.9380	0.9002	0.3203	0.4317	0.1818	1	0.9091	0.7981	0.2635
	Ti-6Cu	0.3912	0.8253	0.6836	0.6778	0.3399	0.7229	0.1930	0.7431	1	0.4707	0.0457
	Ti-NF	0.2436	0.1764	0.1519	0.1459	0.7874	0.1874	0.4348	0.1508	0.1615	1	0.0776
	Ti-NFCu	0.0312	0.1088	0.0897	0.1338	0.2507	0.0565	0.1427	0.1055	0.1216	0.0997	1

24 hrs

	Suspension	Ti64	Ti64-3Cu	Ti64-6Cu	Ti64-NF	Ti64-NFCu	cpTi	Ti-3Cu	Ti-6Cu	Ti-NF	Ti-NFCu
<i>P. aeruginosa</i>	Suspension	1									
	Ti64	0.7585	1								
	Ti64-3Cu	0.1583	0.0942	1							
	Ti64-6Cu	0.7631	0.4899	0.1155	1						
	Ti64-NF	0.7946	0.9012	0.0798	0.5136	1					
	Ti64-NFCu	0.7315	0.8767	0.4065	0.5388	0.8351	1				
	cpTi	0.3151	0.1824	0.3630	0.2112	0.0796	0.6657	1			
	Ti-3Cu	0.6901	0.8345	0.3632	0.4865	0.7854	0.9819	0.6414	1		
	Ti-6Cu	0.0325	0.0097	0.1793	0.0671	0.0022	0.1579	0.0110	0.3555	1	
	Ti-NF	0.9716	0.8595	0.4780	0.8999	0.8792	0.8102	0.6259	0.7949	0.2925	1
	Ti-NFCu	0.0658	0.0175	0.4253	0.0911	0.0032	0.2475	0.0287	0.2017	0.0041	0.3740

24 hrs

Figure 6.12: *p*-value matrices for Ti-based alloys against various bacterial strains (■ = significantly; ■ = highly significantly) (cont.)

The CFUs of *S. aureus* ATCC 25923 enumerated from Ti64-*x*Cu ($x = 0, 3, 6$ wt.%) and NF-coated Ti64 samples did not illustrate a significant ($p > 0.05$, Figure 6.12) change after 24 and 48 hrs when compared to the bacterial suspension and when compared amongst the samples. The cpTi control sample, Ti-3Cu, and Ti-NF did not show a significant ($p > 0.05$) difference in *S. aureus* ATCC 25923 counts after 24 and 48 hrs when compared to the bacterial suspension or to each other. Ti-6Cu significantly ($p = 0.0479$) promoted bacterial growth after 24 hrs compared to the bacterial suspension and did not differ significantly ($p > 0.05$) from cpTi or Ti-3Cu. Ti-NFCu highly significantly ($p \leq 0.01$) inhibited the growth of CFUs when compared to the bacterial suspension ($p = 0.0003$) and to all other samples, including the control sample cpTi ($p = 0.00005$) and Ti-NF ($p = 0.0046$). Overall, the counts of all the samples after 48 hrs against *S. aureus* ATCC 25923 did not show significance ($p > 0.05$, Figure 6.12).

For *S. aureus* ATCC 43300, the Cu-alloyed samples of Ti64 and cpTi did not show significant ($p > 0.05$, Figure 6.12) changes in bacterial growth after 24 hrs. A reduction in CFU growth was observed for all coated samples with significance (Ti64-NF ($p = 0.0398$) and Ti-NF ($p = 0.0305$)) and with high significance for those containing CuO NPs (Ti64-NFCu ($p = 0.0089$) and Ti-NFCu ($p = 0.0080$)) in comparison with the bacterial suspension after the 24-hr incubation period. Furthermore, Ti64-NFCu showed a highly significant ($p = 0.000003$) decrease in the growth of *S. aureus* ATCC 43300 when compared to the control Ti64 sample and significantly ($p = 0.0160$) retarded growth more than Ti64-NF. Likewise, Ti-NFCu displayed a highly significant decrease in cell growth compared to the control cpTi sample ($p = 0.0051$) and Ti-NF ($p = 0.0063$). Therefore, the decrease in the growth of *S. aureus* ATCC 43300 after 24 hrs may be attributed to CuO in the NFs. After 48 hrs, highly significant decrease in *S. aureus* ATCC 43300 cell growth was observed for all specimens – except for Ti64 – of Ti64-3Cu ($p = 0.0100$), Ti64-6Cu ($p = 0.0047$), Ti64-NF ($p = 0.0017$), Ti64-NFCu ($p = 0.00001$), cpTi ($p = 0.0006$), Ti-3Cu ($p = 0.00004$), Ti-6Cu ($p = 0.00002$), Ti-NF ($p = 0.0019$) and Ti-NFCu ($p = 0.000005$) when compared to the bacterial suspension. The counts for the Ti64 control did not differ significantly ($p = 0.1210$) from those in the suspension. In addition, the colony counts, compared with the cpTi control, showed a highly significant decrease for Ti-3Cu ($p = 0.0124$) and Ti-6Cu ($p = 0.0010$). Therefore, it may be postulated that the Cu content of these alloyed samples showed inhibited growth of *S. aureus* ATCC 43300 after 48 hrs. Likewise and similar to the 24 hrs period, CuO in the NFs at 48

hrs exhibited antimicrobial activity against *S. aureus* ATCC 43300 as evidenced by counts for Ti64-NFCu that had highly significantly ($p = 0.00001$) decreased below those of the suspension, Ti64 control and Ti64-NF, and the counts for Ti-NFCu has also highly significantly decreased further than those of the suspension ($p = 0.000005$), cpTi control ($p = 0.0001$) and Ti-NF ($p = 0.0002$) after 48 hrs. The antibacterial effect of Cu/CuO particles against *S. aureus* ATCC 43300 was more prominent at the 48-hr incubation period.

Staphylococcus epidermidis produced insignificant ($p > 0.05$, Figure 6.12) differences in the counts for Ti64-xCu ($x = 0, 3, 6$ wt.%), Ti-3Cu, Ti-NF and Ti-NFCu when compared to the bacterial suspension after 24 hrs. A significant decrease in CFUs for Ti64-NF ($p = 0.0349$) and Ti-6Cu ($p = 0.0169$), and highly significantly decreased counts for Ti64-NFCu ($p = 0.0003$) and cpTi ($p = 0.0009$) were evident in comparison with the bacterial suspension after 24 hrs. For the coated Ti64 samples, the decrease in cell growth may be attributed to the CuO particles, as the control sample did not show a significant ($p = 0.3801$) difference in cell count compared to the suspension. The Ti64-NFCu had significantly ($p = 0.0240$) lower counts than the control, whereas the Ti64-NF did not ($p = 0.1729$). After 48 hrs, Ti64 ($p = 0.5642$) did not demonstrate a significant difference in the number of CFUs, while Ti64-3Cu ($p = 0.0177$) showed a significant decrease. Ti64-6Cu ($p = 0.0040$), Ti64-NF ($p = 0.0117$) and Ti64-NFCu ($p = 0.0021$) showed a highly significant reduction in cell growth in comparison to the suspension, and the decrease was highly significant ($p = 0.0100$) for Ti64-NFCu than for Ti64-NF (Figure 6.12). Hence, for the Ti64 and similar to the 24-hr incubation period, bactericidal effect against *S. epidermidis* after 48 hrs was ascribed to the Cu/CuO particles. The same could not be said for the cpTi samples, as the control cpTi exhibited a highly significant decrease in counts after 24 hrs ($p = 0.0009$) and a significant decrease after 48 hrs ($p = 0.0218$). Moreover, a significant difference in CFUs was not established between the control and test samples after 24 hrs (Ti-3Cu ($p = 0.2132$), Ti-6Cu ($p = 0.4762$) and Ti-NFCu ($p = 0.3371$)) and after 48 hrs (Ti-3Cu ($p = 0.1970$), Ti-6Cu ($p = 0.3551$) and Ti-NFCu ($p = 0.3325$)). Thus, growth inhibition of *S. epidermidis* was not observed for any Cu/CuO content in the cpTi samples after 24 or 48 hrs.

None of the specimens displayed significant ($p > 0.05$, Figure 6.12) antibacterial activity against *E. coli* after 24 hrs, compared to the bacterial suspension or among the samples. Instead, a highly

significant increase in cell growth was observed after 48 hrs for Ti64 ($p = 0.0008$) and Ti64-3Cu ($p = 0.0116$) samples compared to the suspension. Only Ti-6Cu showed significant ($p = 0.0247$) growth inhibition of *E. coli* after 48 hrs, which may be attributed to the presence of Cu. For *K. pneumoniae*, only Ti-NFCu showed a significant ($p = 0.0312$) decrease in cell count after 24 hrs, but it also significantly ($p = 0.0486$) promoted growth of the bacterium after 48 hrs. The remaining specimens did not exhibit a significant ($p > 0.05$, Figure 6.12) effect on *K. pneumoniae* cell counts compared with the bacterial suspension or with the samples after 24 hrs. After 48 hrs, the Ti64 specimens displayed antimicrobial activity against *K. pneumoniae* related to the Cu/CuO particles. Ti64, Ti64-3Cu, and Ti64-NF did not significantly affect bacterial counts in the suspension at $p = 0.3698$, 0.2506 , and $p = 0.4891$, respectively. However, Ti64-6Cu ($p = 0.0020$) and Ti64-NFCu ($p = 0.0004$) showed highly significant reductions in bacterial counts. The control sample cpTi promoted a significant ($p = 0.0230$) reduction in the suspended cells, and Ti- x Cu samples ($x = 3, 6$ wt.%) did not show significance ($p > 0.05$, Figure 6.12) after 48 hrs. Thus, any change in the cell counts could not be associated with the Cu/CuO content for these samples. Notably, as with the suspension, the significance/high significance designated in Figure 6.12 for the *K. pneumoniae* counts of Ti-NFCu is an increase when compared to all the specimens except Ti-3Cu ($p = 0.2635$) and Ti-NF ($p = 0.0776$). This suggests that *K. pneumoniae* had an affinity for growing in the presence of Ti-NFCu discs. For *P. aeruginosa*, only Ti-6Cu displayed a significant ($p = 0.0325$) decrease in cell growth post 24 hrs, and the decrease was by one order of magnitude from $1.37 \times 10^9 \pm 8.83 \times 10^8$ CFU/ml in the bacterial suspension to $1.19 \times 10^8 \pm 5.14 \times 10^7$ CFU/ml after incubation on Ti-6Cu. Biofilm formation by the bacterial suspension in the relevant wells prevented the enumeration of *P. aeruginosa* after 48 hrs. The viscosity of the suspension, due to the biofilm, impeded the extraction of the required volume for enumeration.

Overall, specimens produced using cpTi exhibited better antimicrobial activity than those manufactured using Ti6Al4V ELI, such that the control sample Ti64 did not greatly reduce counts of any of the examined bacterial strains and instead promoted the growth of *E. coli* cells. In contrast, the cpTi control did not promote cell growth in any strain and decreased the growth of some strains. Therefore, it may be postulated that the components of Ti64 may enhance bacterial growth. The study of Wang et al. (2020) showed that a low level of vanadium could promote bacterial growth, and high microbial diversity and richness were found in samples with a moderate

vanadium level. The samples coated with PCL/PU/CuO NFs appeared to exhibit more efficient antibacterial properties than those alloyed with Cu particles during LPBF for both cpTi and Ti64 test samples. *Staphylococcus aureus* ATCC 25923 is a well-known antibiotic-susceptible strain and referred to as a methicillin-susceptible *S. aureus* (MSSA) strain, while *S. aureus* ATCC 43300 is a well-known antibiotic-resistant strain and referred to as a methicillin-resistant *S. aureus* (MRSA) strain (Zhao, et al., 2023). This was also observed in the agar diffusion assay, in which *S. aureus* ATCC 25923 showed the highest susceptibility to erythromycin and *S. aureus* ATCC 43300 showed resistance. However, the findings of the plate count method revealed *S. aureus* ATCC 25923 as the resistant strain and *S. aureus* ATCC 43300 as the susceptible strain to metal/metal oxide-based antibacterial agents.

Numerous studies have successfully used the plate count method to demonstrate the antimicrobial properties of Cu/CuO particles alloyed with Ti and Ti64, as well as those alloyed with a polymer matrix. The plate count method of Zhang et al. (2013) using a Ti–10 wt.% Cu alloy demonstrated strong antibacterial activity, with antibacterial rates above 99% against both *S. aureus* and *E. coli* after 24 hrs. According to the authors, their results indicated that only the bacteria in direct contact with the Ti–Cu alloy were killed. Zhu et al. (2012) showed that the antimicrobial activity of Cu increased with increasing Cu content and incubation duration. In addition, it has been reported that the antibacterial properties of a liquid containing Cu ions are related to the Cu^{2+} concentration and the initial bacteria concentration (Zhang, et al., 2013). Likewise, Ju et al. (2023) fabricated a series of novel Ti-6Al-4V-xCu ($x = 3, 5, 8, 10$ wt.%) using LPBF and evaluated their antibacterial properties against *E. coli* and *S. aureus* using the plate count method. Their results also showed that the number of colonies decreased with increasing Cu content. Several researchers observed a similar tendency of increasing bactericidal effect with increasing Cu concentrations alloyed with Ti64 (Ren, et al., 2014; Guo, et al., 2017; Li, et al., 2025) and cpTi (Shirai, et al., 2009; Zhang, et al., 2016; Liang, et al., 2025). Conversely, Fowler et al. (2019) demonstrated that an increase in Cu ion concentration does not guarantee improved antibacterial activity. In support of this, Xin et al. (2022) and Li et al. (2024) illustrated a nonlinear relationship between %Cu and %antibacterial rate of Ti-Cu and Ti64-Cu samples, respectively.

Krakhmalev et al. (2017) manufactured Ti6Al4V (ELI)-1 at.% Cu material by LPBF and investigated its bactericidal impact against *E. coli* and *S. aureus* ATCC 25923 after 24 hrs of exposure. The Ti6Al4V discs alloyed with pure Cu resulted in a one order of magnitude decrease in growth of Gram-negative *E. coli* (from 1.48×10^9 to 1.27×10^8 CFU/ml) and a two order of magnitude decrease in growth of Gram-positive *S. aureus* (from 8.46×10^8 to 1.22×10^6 CFU/ml). Ghasemian Lemraski et al. (2021) also observed that Gram-positive bacteria were more sensitive compared to Gram-negative bacteria when exposed to an electrospun polymer composite impregnated with Cu NPs. Similarly, in this study, Gram-positive *S. aureus* ATCC 43300 and *S. epidermidis* were more susceptible to the antibacterial activity of the Cu/CuO particles than the Gram-negative *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Moreover, the test specimens appeared to promote the growth of Gram-negative strains, as observed with *E. coli* and *K. pneumoniae*. The basic differences in cell wall structure between Gram-negative and Gram-positive bacteria may be the likely reason for the Gram-positive bacteria being more susceptible to destruction by Cu ions than Gram-negative bacteria. Copper is able to cause reactive oxygen species (ROS) production to increase to a level that is toxic to the cell, and may disrupt the cell membrane and cause cell death (Jin, et al., 2015; Ghasemian Lemraski, et al., 2021). Copper also has the ability to induce cytoplasmic leakage by interacting with the bacterial outer membrane, causing the membrane to rupture and damaging the DNA stem (Ghasemian Lemraski, et al., 2021; Mahmoudi, et al., 2022).

Copper alloys are efficient in rapidly eliminating bacteria on contact (Zhu, et al., 2012), and the inactivation is enhanced by low moisture conditions, minimal media and high Cu corrosion rates. Living organisms, including bacteria, require Cu as an essential micronutrient at low concentrations; however, at high concentrations, Cu can inhibit cell growth or even cause cell death (Zhang, et al., 2013). Nonetheless, different bacterial strains exhibit different sensitivity towards Cu (Manakhov, et al., 2021). Bacteria have numerous defence mechanisms against the toxic effects of Cu ions, namely (Ryan, et al., 2001; Manakhov, et al., 2021):

- » the relative impermeability of the outer and inner cell membranes (sequestration), which restricts intake of Cu into the cell, and of the inner bacterial membranes to Cu ions (intracellular sequestration);
- » efflux pumps that allow the microorganisms to regulate their internal environment by removing toxic substances, including antimicrobial agents, and are intrinsic to Gram-negative bacteria;

- » absorption of Cu by metallothionein-like proteins in the cytoplasm and periplasm;
- » precipitation as copper sulphide (CuS);
- » extracellular complexation; and
- » the minimum inhibitory concentration (MIC) of the bacterium to copper ions may be higher than the Cu^{2+} concentration released from materials, and thus the Cu^{2+} leached from the alloys and polymer layers were insignificant in inducing the antibacterial effects against the bacterial strains.

Liu et al. (2020) demonstrated that surface treatment of Ti, such as sandblasting and acid etching (SLA), facilitated the sustained release of Cu ions, thereby enhancing the bone integration and antibacterial properties of the Ti–Cu alloy. The experiments by Hu et al. (2020) demonstrated that ultrasonic micro-arc oxidation treatment could significantly improve the antibacterial activity of Ti-3 wt.% Cu alloy by approximately 20–40 % against *S. aureus* and attributed it to the formation of Cu_2O and CuO . Heat treatment (such as annealing and ageing) has a strong correlation with the microstructure of the Ti/Ti64-Cu alloy, and Ti_2Cu precipitates resultant from the treatment have been found to influence the antibacterial properties (Ng, et al., 2015; Wu, et al., 2020). Wu et al. (2020) subjected a Ti-5 wt.% Cu alloy to various heat treatments and observed fewer *E. coli* colonies with increasing ageing time. The ageing method with the most extended period and highest temperature (850 °C for 1 hr followed by 700 °C for 4 hrs) had the highest antibacterial rate of 99.9%. Zhang et al. (2016) employed Ti- $x\text{Cu}$ ($x = 5, 10$ wt.%) and *S. aureus* to illustrate that heat treatment had a greater influence on antimicrobial rate than the Cu concentration. Xin et al. (2022)'s results proved that the Ti_2Cu phase was able to improve the antibacterial properties of Ti–Cu alloys. The Cu^{2+} ion release rate in lamellar Ti_2Cu was 55% higher than that of granular Ti_2Cu , and the antibacterial effect of the needle-like phase was twice that of the fine phase.

In vivo research has been conducted to investigate the antibacterial capacity of Ti–Cu implant material. Wang et al. (2019) conducted an antibacterial experiment against *S. aureus* for Ti–10 wt.% Cu sintered alloy using rabbits and displayed strong in vivo antibacterial properties of the Ti–10Cu and also showed that prolonged exposure increased the antimicrobial effect as numerous colonies were found in the cpTi group at all intervals (which were 1, 4, 7 and 14 days), fewer

bacteria colonies cultivated in the Ti–Cu group after 1–4 days postimplantation and no bacteria colonies were found after 7 days postimplantation. The *in vivo* study of Shirai et al. (2009) using a rabbit model showed that the Ti-1% Cu alloy significantly ($p < 0.05$) inhibited inflammation and infection.

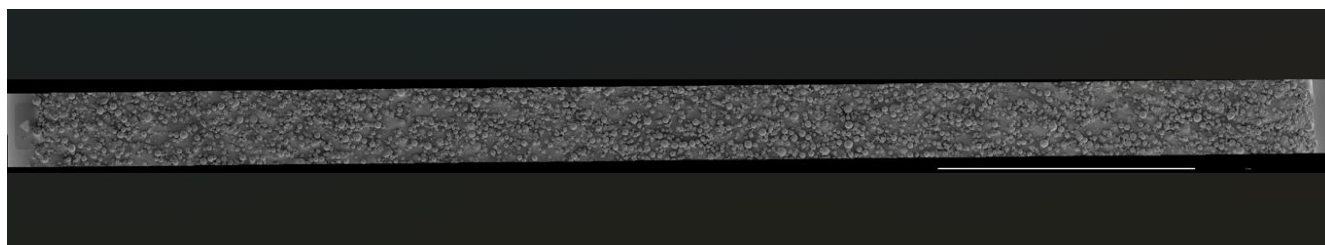
Titanium and its alloys are well known for their biocompatibility, and the race-to-surface concept between bacterial and human cells is a leading motive for antibacterial investigation of implant surfaces (Mahmoudi, et al., 2022). As such, it is noteworthy that some enumerations from the control and test samples of this study were higher than those of the bacterial suspension at each incubation period. Approximately 35% and 12% of the CFU/ml at 24 hrs and 48 hrs, respectively, were higher than those in the incubated bacterial suspension, attesting to the biocompatible nature of Ti surfaces and electrospun NFs.

6.3.3. Cell culture

The intimate relationship between living tissue and the Ti surface ensures a high degree of implantation stability (Rampurawala & Patil, 2021). Due to the difficulty of accessing primary cells, cell lines are often used in cell culture research (Jang, et al., 2022). The cell lines used for the present research are murine myoblast cell lineage (C2C12) and MD Anderson-Metastatic Breast Cancer-231 (MDA-MB–231). The C2C12 is an immortalised mouse skeletal myoblast cell line (Jang, et al., 2022; Katayama, et al., 2023). C2C12 cells are commonly used as a model for studying skeletal muscle differentiation because they may be utilised to recognise cellular state during differentiation and investigate the metabolism of muscle cells (Niioka, et al., 2018; Jang, et al., 2022; Katayama, et al., 2023). They have also been shown to have capabilities in osteogenesis (formation of bone) and adipogenesis (formation of fat cells) by specific stimuli (Liu, et al., 2011; Katayama, et al., 2023). Osteogenesis on titanium surfaces is a prerequisite for osseointegration (Al Mustafa, et al., 2015). The C2C12 cell line can change its phenotype during differentiation from a globular shape to elongated tubular shape, or spindle-like shape, as the cells adhere to the substrate surface, followed by fusion of the mononucleated myoblast cells to become multinucleated myotubes and ultimately resulting in the formation of myofibers (muscle fibres) (Niioka, et al., 2018; Jang, et al., 2022). In favourable conditions, the myogenic differentiation period has been proven to take place within 5 days (Jang, et al., 2022; Katayama, et al., 2023).

The MDA-MB-231 cells are epithelial, human breast cancer cells and are one of the highly invasive, aggressive, poorly differentiated, and metastatic human breast carcinoma cell lines (Lin, et al., 2011; Xu, et al., 2014; Khan, et al., 2023; Culture Collections, 2025). In addition, MDA-MB-231 cells are one of the most commonly used breast cancer cell lines in research laboratories (Culture Collections, 2025). MDA-MB-231 cell line represents a typical triple negative breast cancer that lacks estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2) protein expression, and is known to undergo epithelial-to-mesenchymal transition (EMT) (Guo, et al., 2013; Wei, et al., 2018).

The SEM and live/dead fluorescence images were used to assess cell attachment and biocompatibility of the test samples (Table 6.1) using the C2C12 and MDA-MB-231 cell lines at 24 hrs and 5 days post-seeding. The SEM images were captured as a montage on each sample disc. Each montage consisted of 2 rows of 26 images per row, captured from one side to the other of each disc, and was captured at approximately the same location on each sample disc. The montage images were stitched together as shown in Figure 6.13.



(i)



(ii)

Figure 6.13: Illustration of montages of SEM images collected from (i) an uncoated and (ii) a coated sample disc

For fluorescence imaging, the ReadyProbes® Cell Viability Imaging Kit (Blue/Green) was used to assess cell viability. NucBlue® Live reagent stains the nuclei of all cells and emits blue fluorescence. Hoechst 33342 dye in NucBlue® Live reagent binds to the minor groove of the DNA double helix, specifically in regions rich in adenine and thymine (AT-rich) regions. NucGreen® Dead reagent stains only the nuclei of dead cells with compromised plasma membranes and emits green fluorescence (Modica-Napolitano, et al., 2019; Kaczmarek, et al., 2025). From each well of the 24-well plate, a series of images was taken at 10× magnification on the EVOS system using the DAPI, GFP and TRANS (transmitted light or brightfield) channels. The images were stitched together to create a single merged image. Figure 6.14 instantiates the images of single wells, each composed of a tiled composite of 220 individual fields of view.

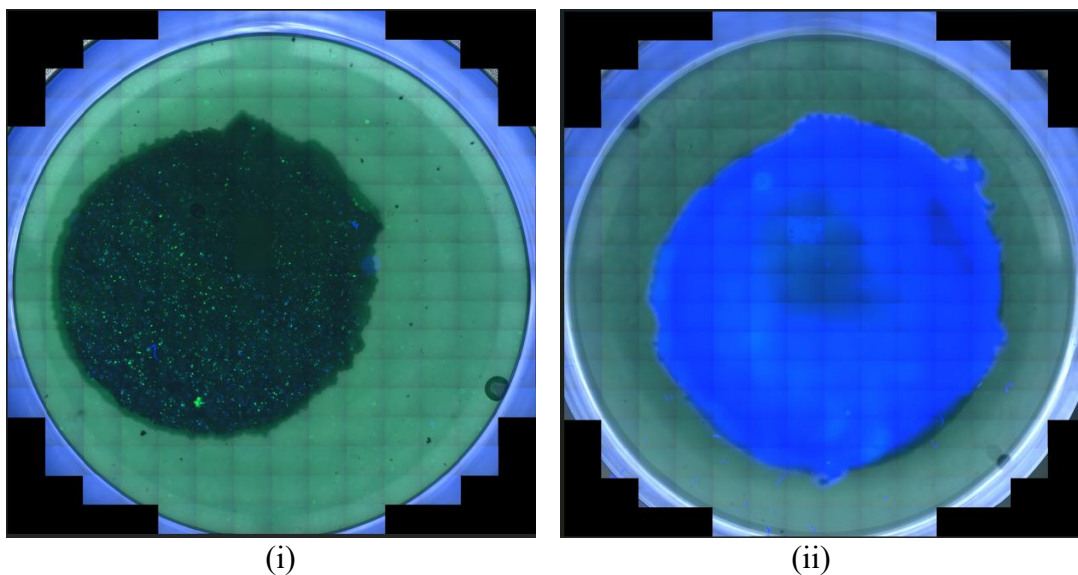


Figure 6.14: Illustration of stitched EVOS images collected from a single well containing (i) a bare, and (ii) a NF-coated specimen

Representative SEM micrographs and individual tiles were selected from the montages and the stitched EVOS images and are presented in

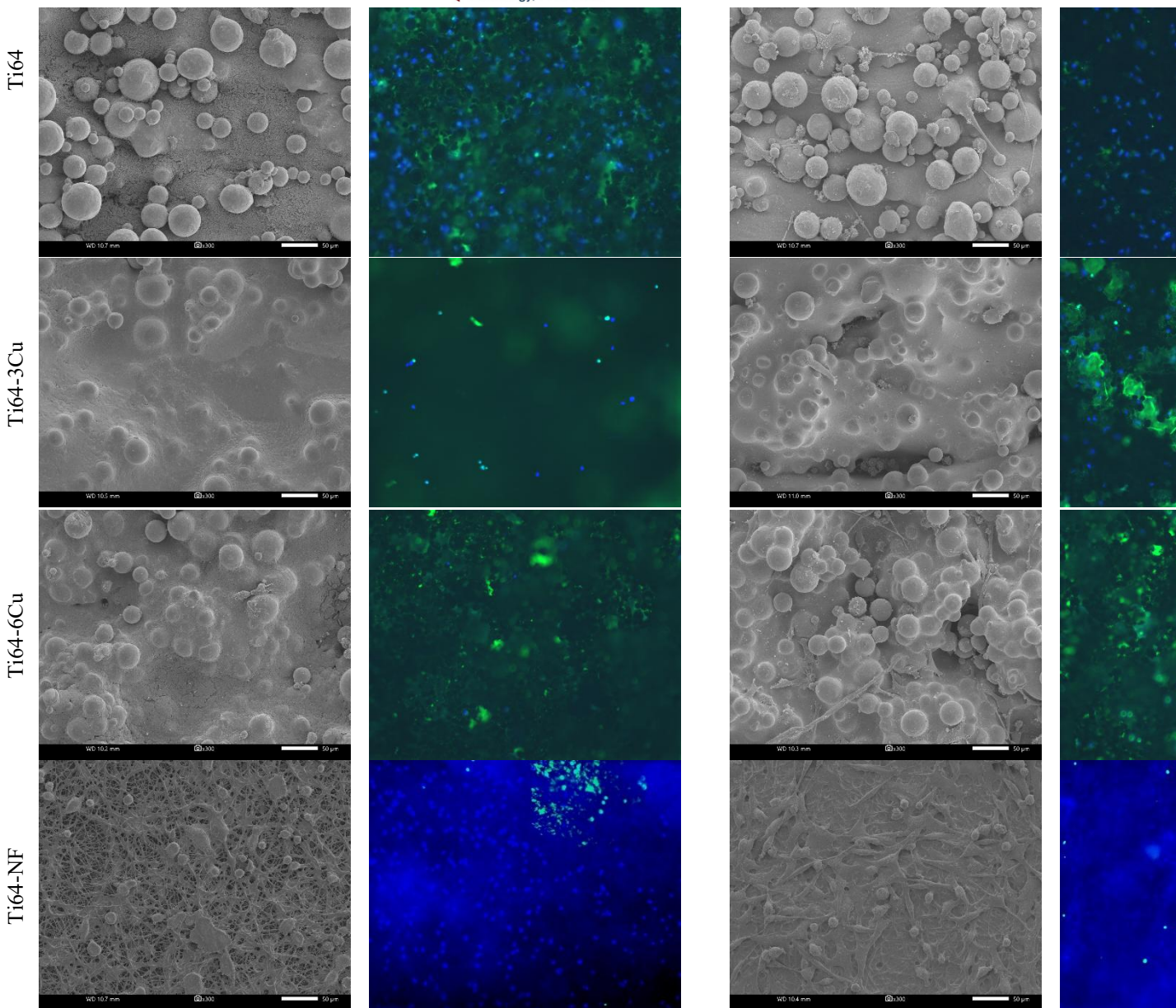
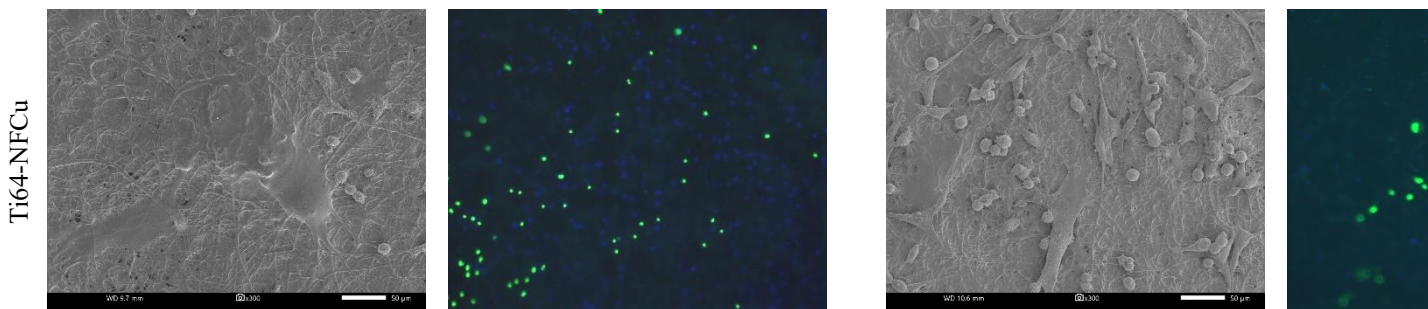


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability



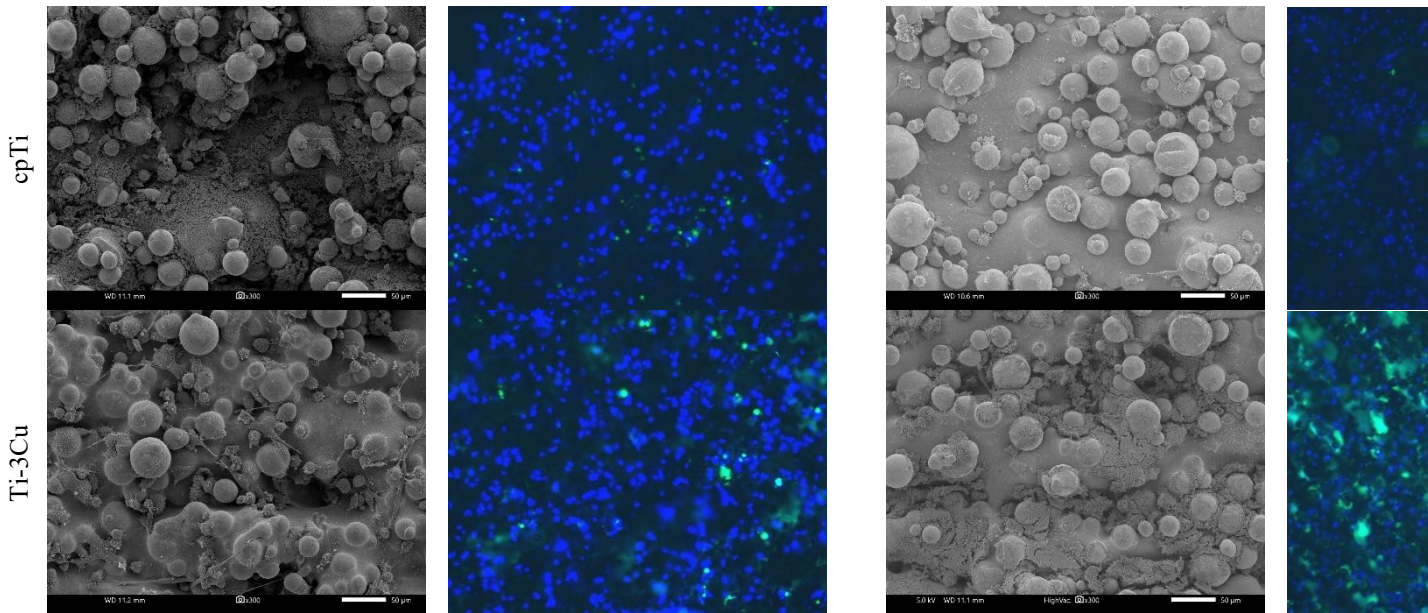


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability (cont.)

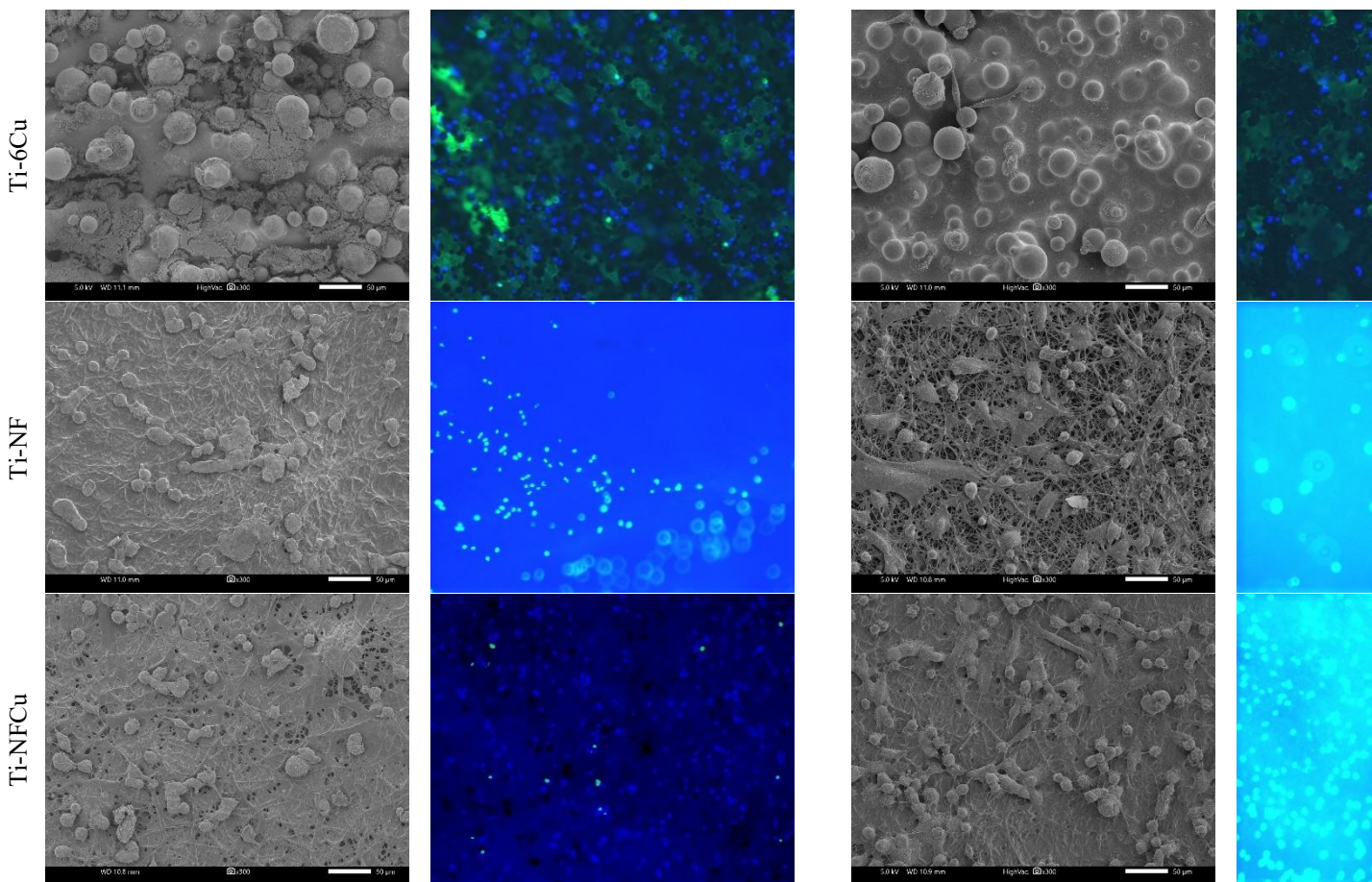


Figure 6.16: Fluorescence images (10 $\times$ magnification) and SEM micrographs (scale = 50 μ m) for cell viability of MDA-MB-231 cells (cont.) and **Error! Reference source not found.** for the C2C12 and MDA-MB-231, respectively. Each individual tile consisted of an overlay of images attained from the DAPI (blue fluorescence), GFP (green fluorescence) and TRANS channels. Consequently, the green fluorescence represented the dead cells and the remaining blue fluorescence represented the live cells.

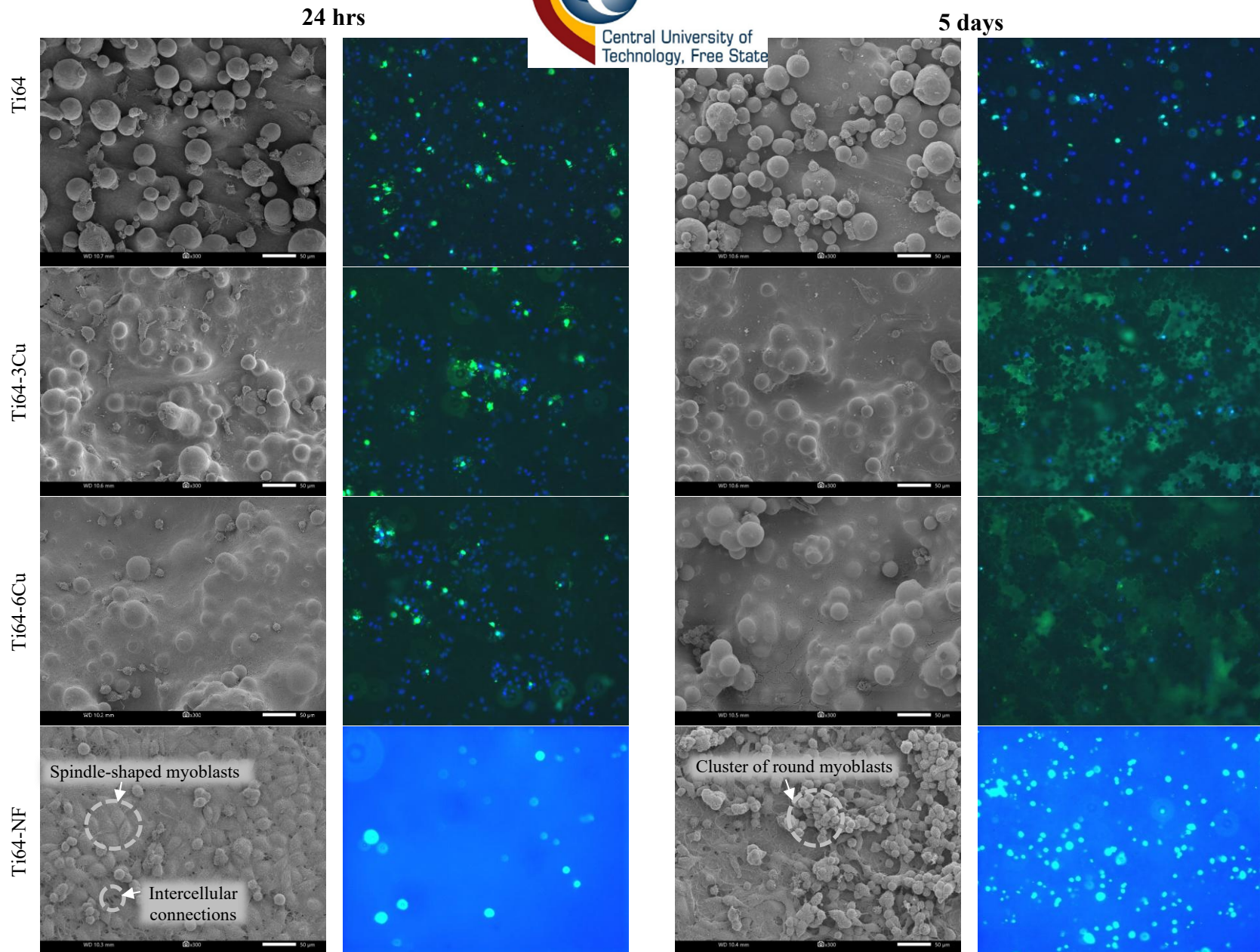


Figure 6.15: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 μm) for cell viability of C2C12 cells

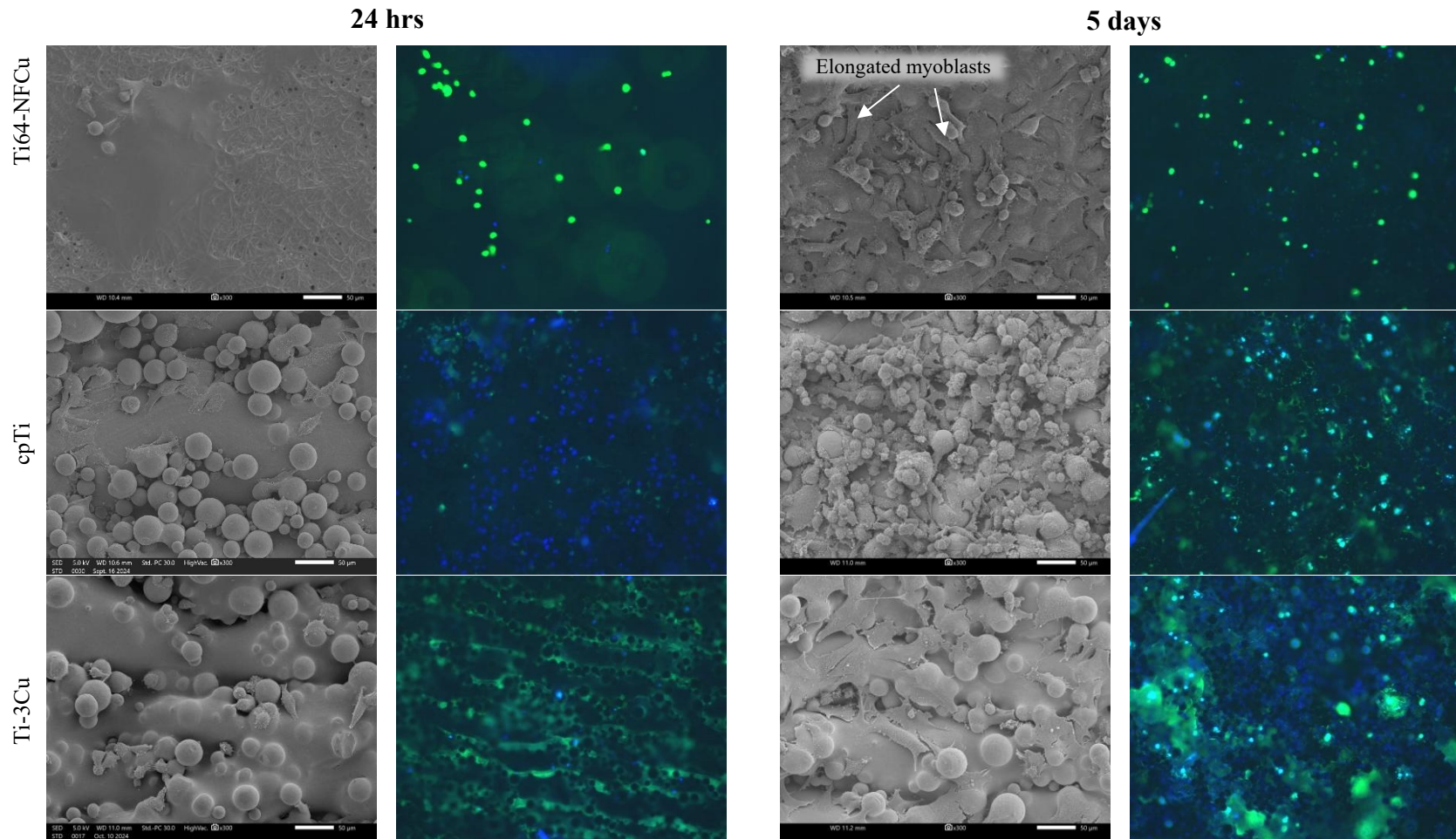


Figure 6.15: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 μm) for cell viability of C2C12 cells (cont.)

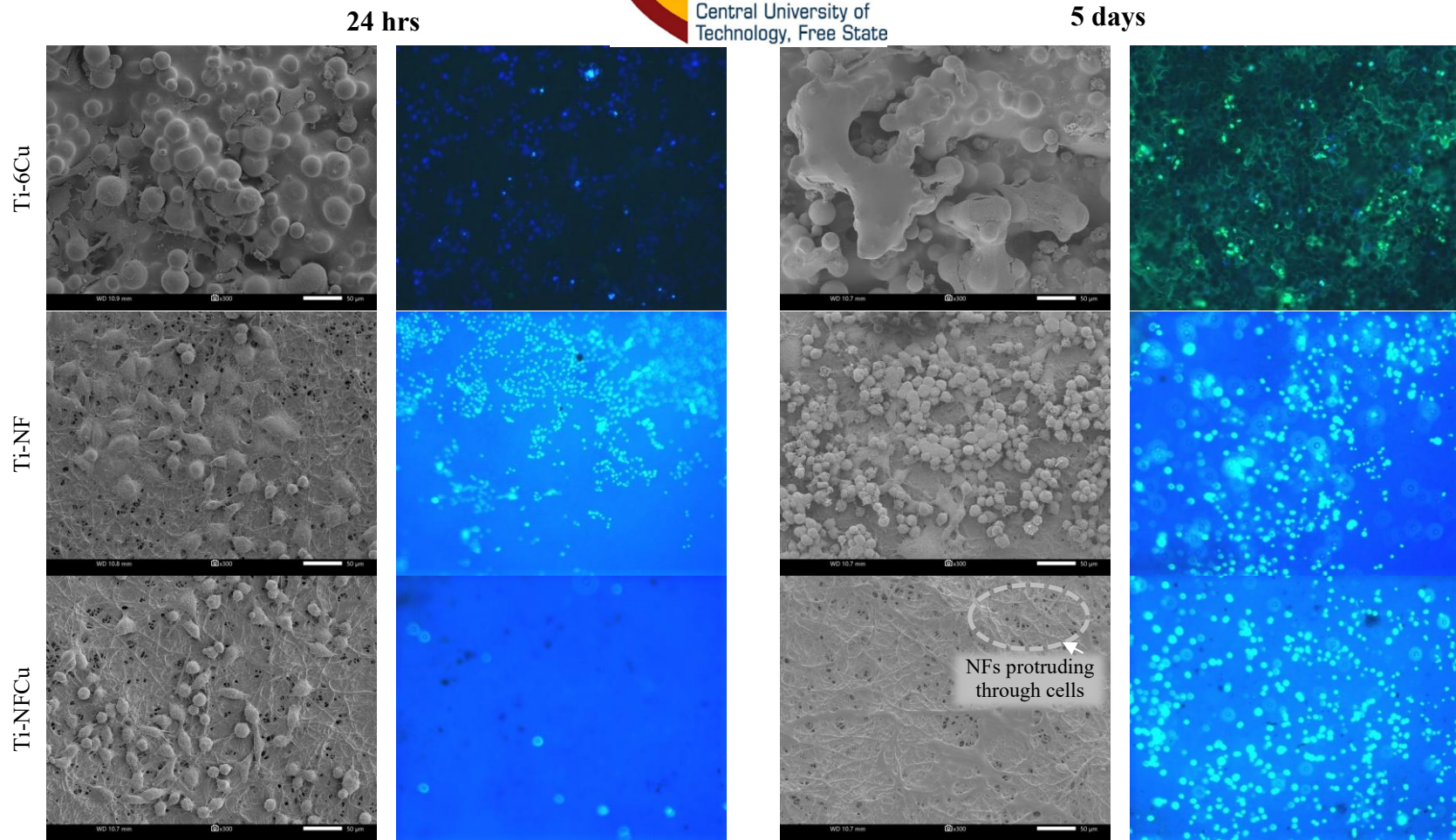


Figure 6.15: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 μm) for cell viability of C2C12 cells (cont.)

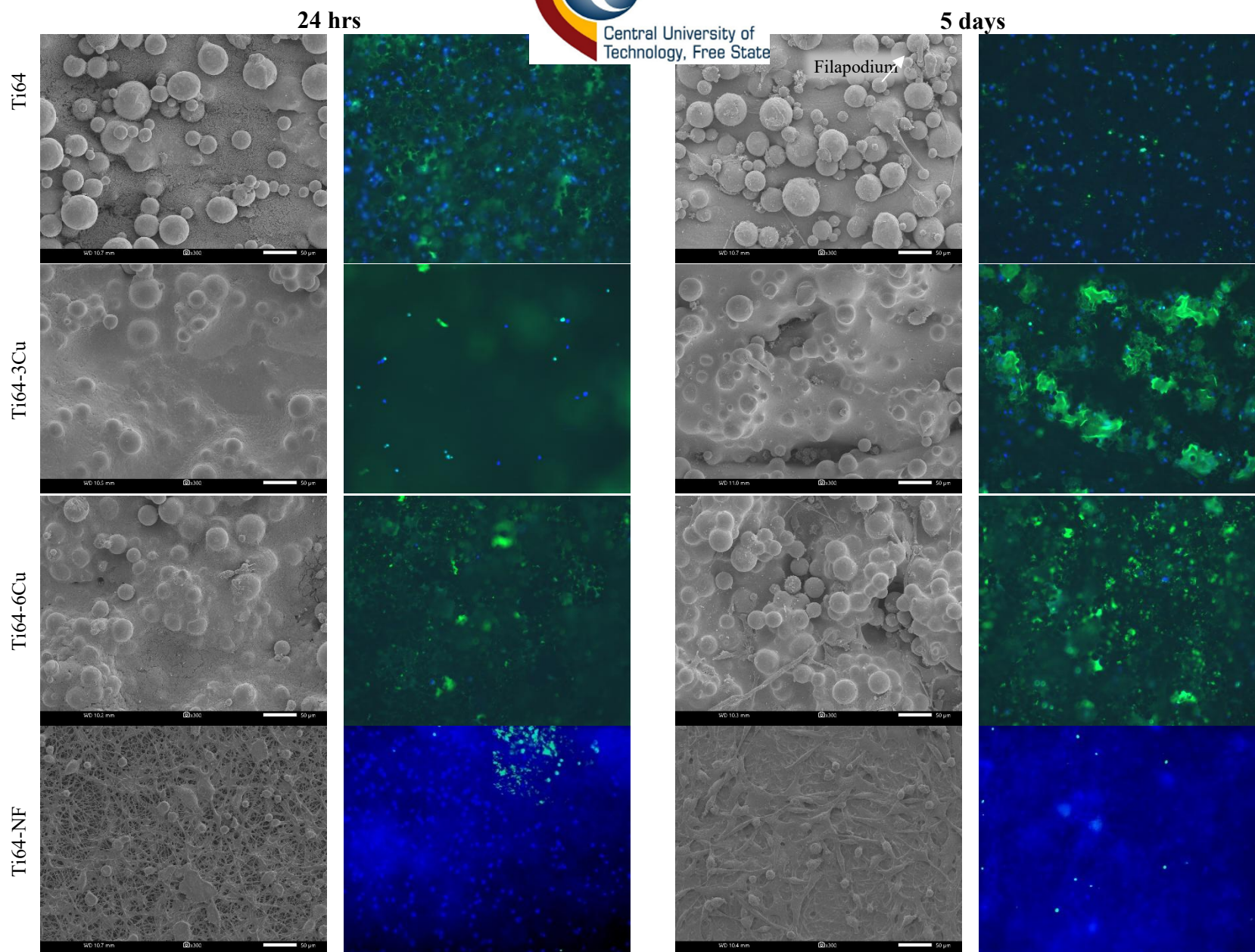


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 μm) for cell viability of MDA-MB-231 cells

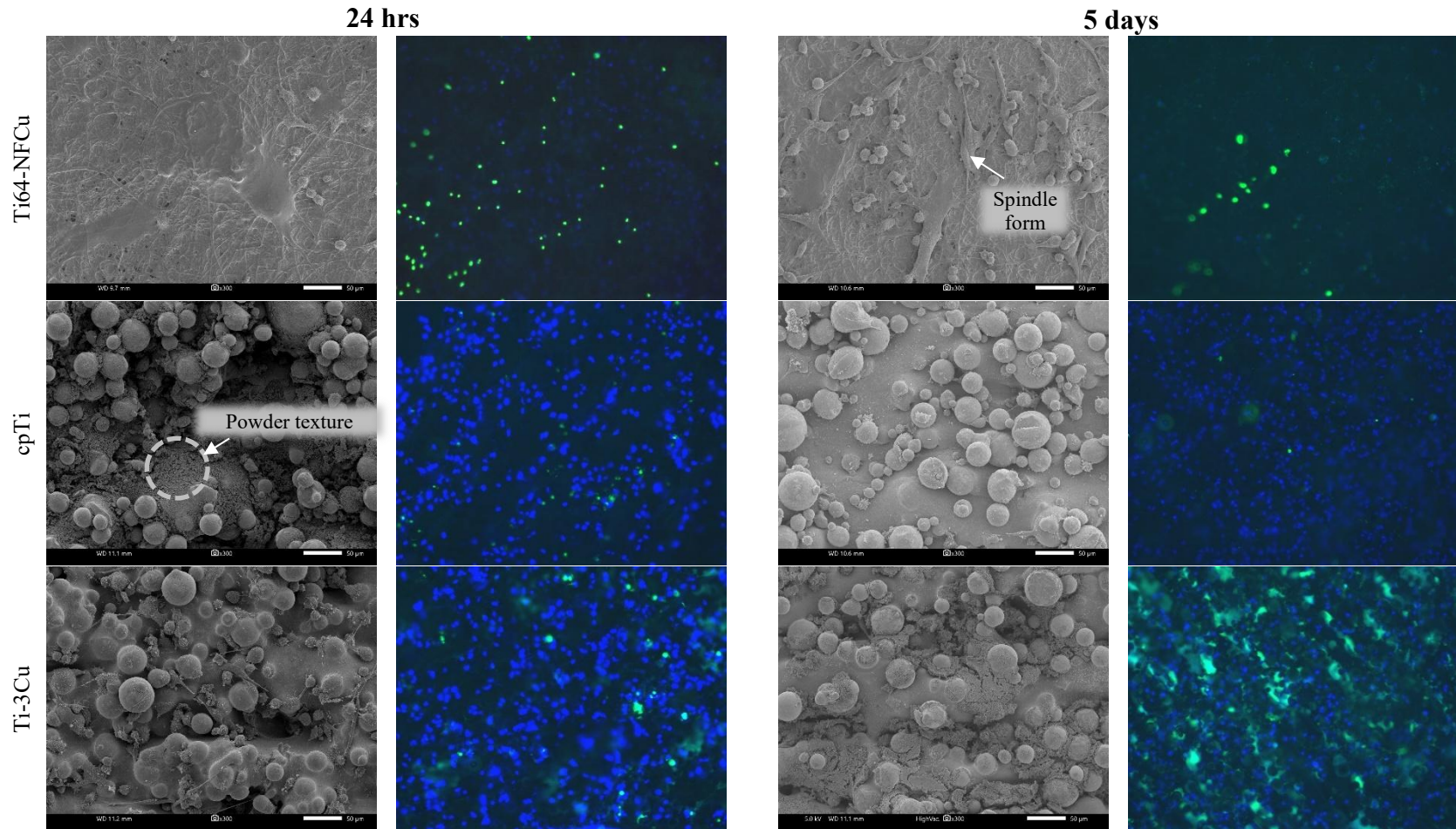


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 μm) for cell viability of MDA-MB-231 cells (cont.)

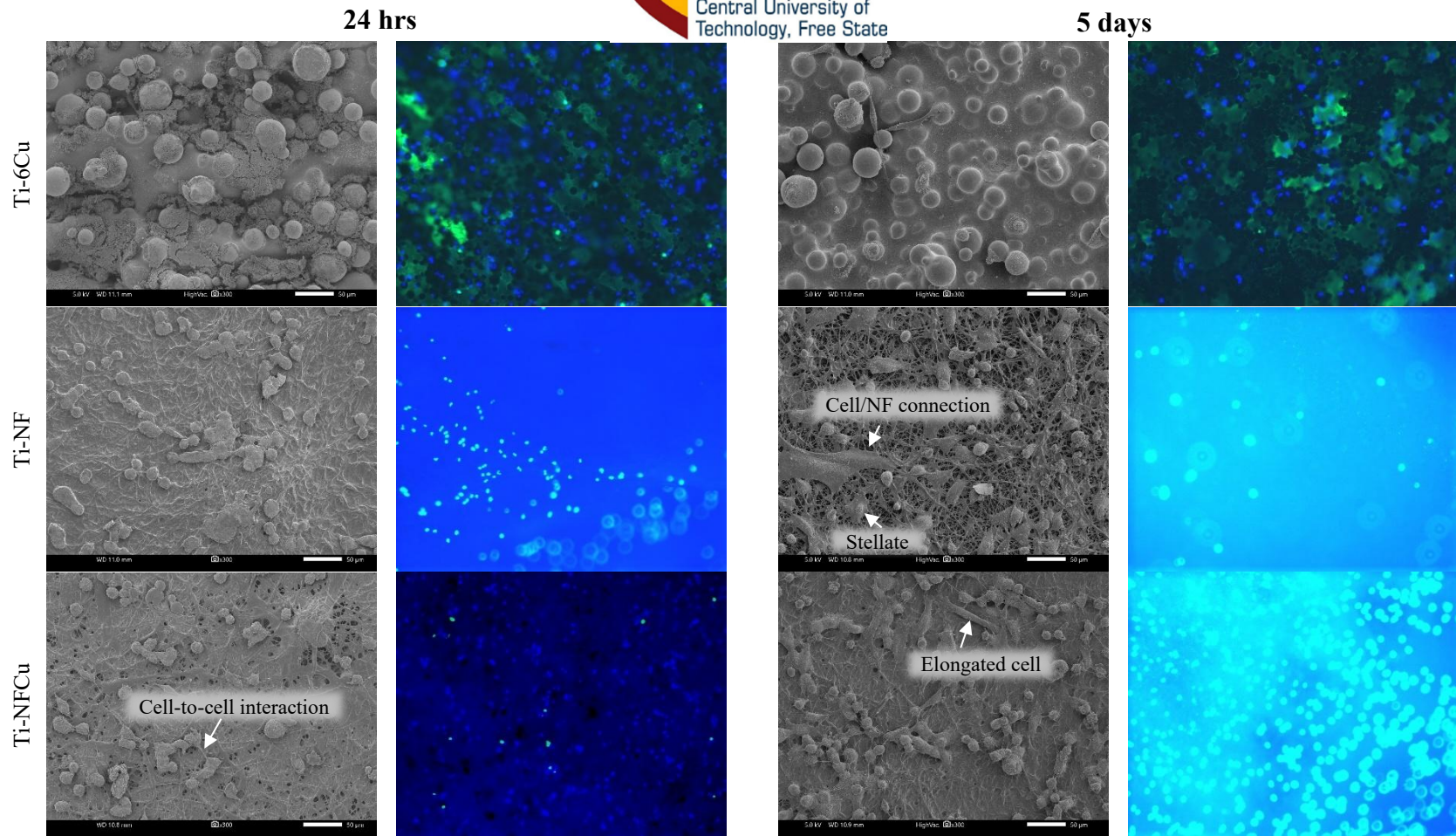


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability of MDA-MB-231 cells (cont.)

For some NF coatings, images illustrating the required blue/green fluorescence could not be obtained, and the images appeared to show overexposure to light(

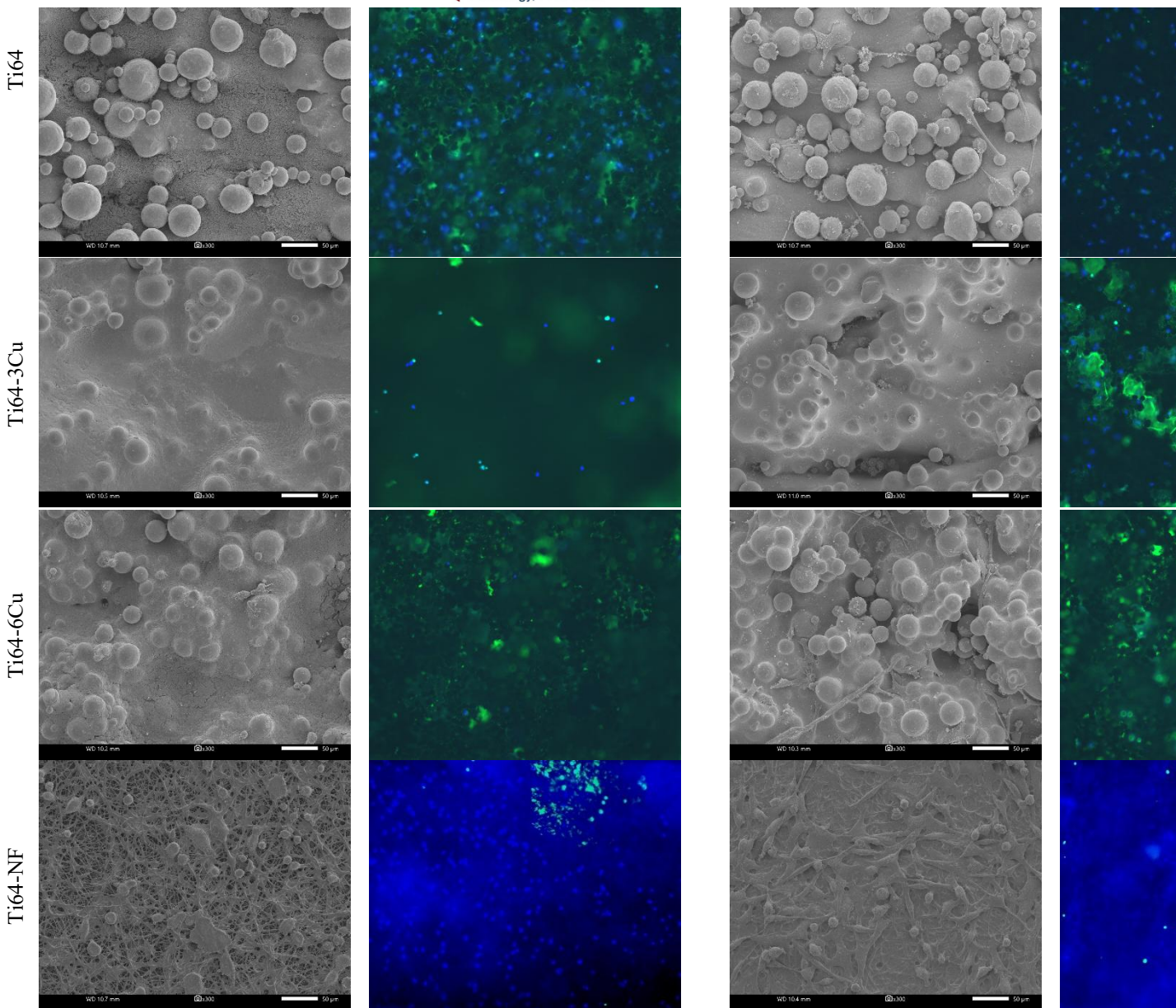
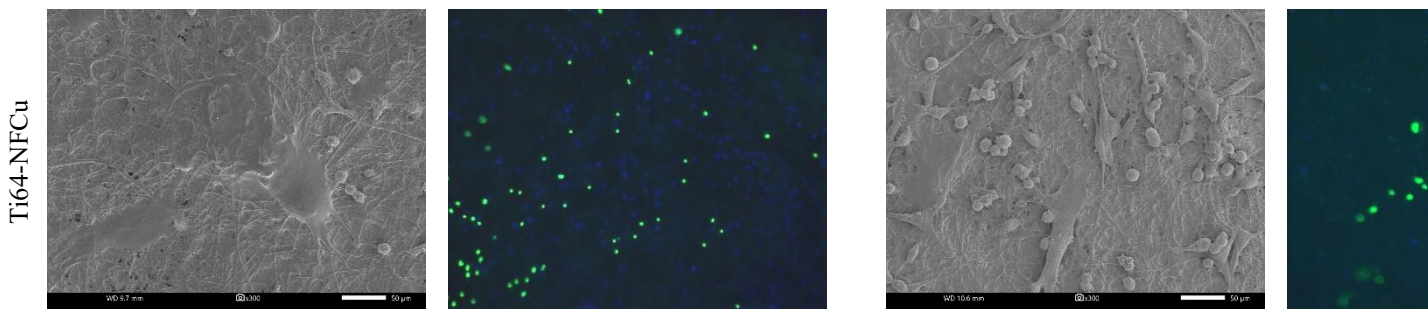


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability



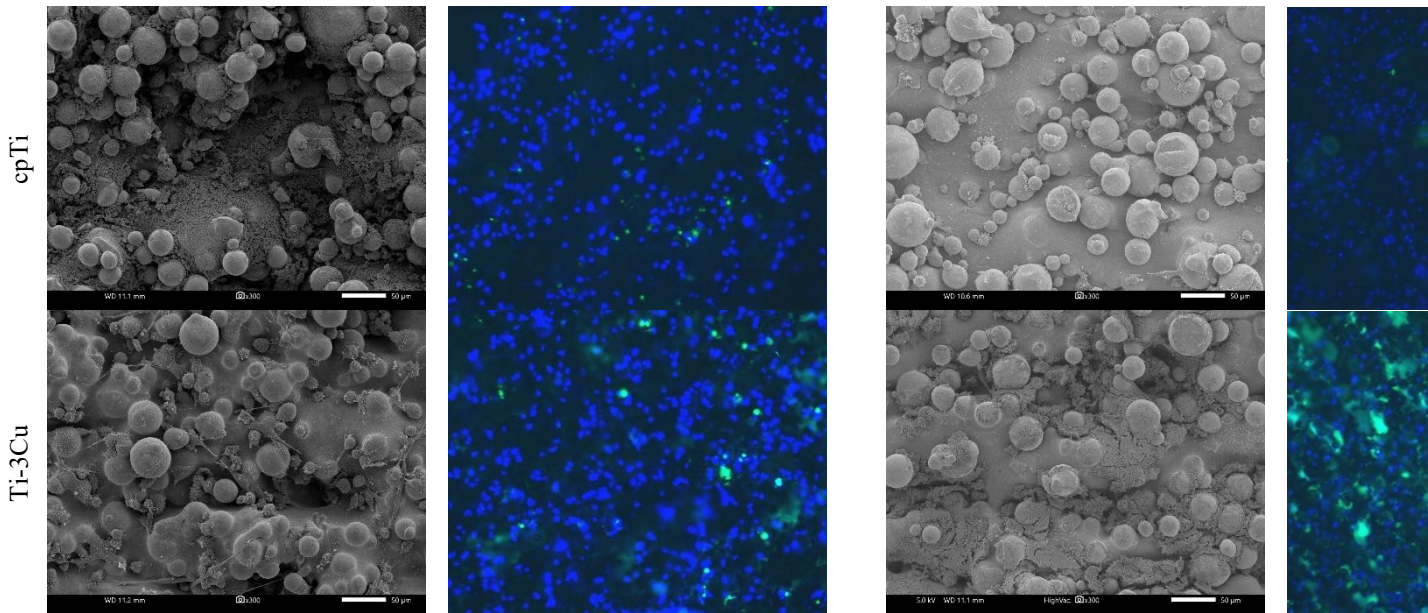


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 μm) for cell viability (cont.)

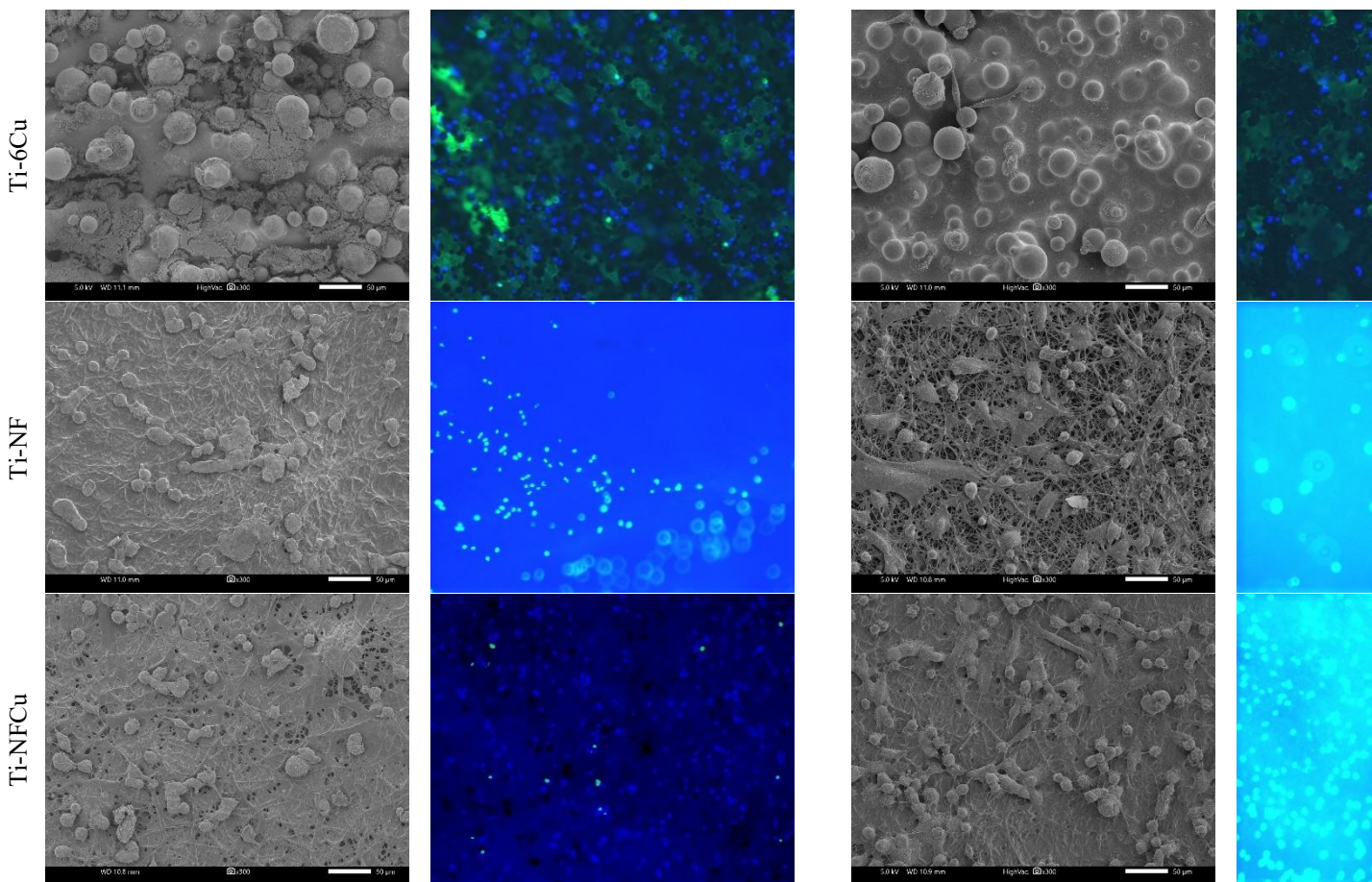


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 μm) for cell viability of MDA-MB-231 cells (cont.) and **Error! Reference source not found.**). This may be attributed to the use of wide-field fluorescence microscopy with multiple fluorescence planes. Wide-field fluorescence microscopy may be affected by background fluorescence due to the non-localised excitation and light-scattering-induced crosstalk. The background leads to poor contrast and the loss of optical sectioning capability required for multi-plane imaging (Shi, et al., 2019).

For the C2C12, the SEM and fluorescence images for the control sample Ti64 were similar at 24 hrs and 5 days of culture, with minimal cell adhesion (especially after 5 days) observed in the SEM images, and the fluorescence images showed live cells predominated over dead cells in both periods. These observations support the known properties of Ti surfaces of biocompatibility (dominance of live cells) and bioinertness (poor cell adhesion). Liu et al. (2011) showed that C2C12 cells readily attached to and proliferated on Ti6Al4V substrates, becoming confluent by day 4 of culture. For this study, the fluorescence images of Ti64-xCu ($x = 0, 3, 6$ wt.%) were similar at the 24-hr culture period, with more blue than green fluorescence, indicating more live cells than dead. At the 24-hr incubation period and for Ti64-3Cu and Ti64-6Cu, SEM showed that C2C12 myoblasts had attached onto the solid surface and metal powder particles, and adopted a flat spindle shape, particularly for Ti64-3Cu, evidencing the likely formation of the extracellular matrix (ECM) and resulting in cell adhesion (Feller, et al., 2015). The elongated shapes were not apparent on the surface of Ti64-6Cu; instead, the myoblasts present retained their globular shape. A sheet-like membrane layer had formed on the Ti64-3Cu and Ti64-6Cu surfaces, covering the entire solidified surface. This membrane appeared denser after 5 days of culture, and on the Ti64-6Cu, with some cracks in the layer covering it. The fluorescence images after 5 days showed a green fog over the surfaces of Ti64-3Cu and Ti64-6Cu, with evenly distributed blue-stained nuclei. This phenomenon was not observed in the control sample and may therefore be attributed to Cu^{2+} ion-induced cell toxicity after 5 days.

The control sample cpTi showed strong C2C12 cell colonisation, as shown in the SEM in

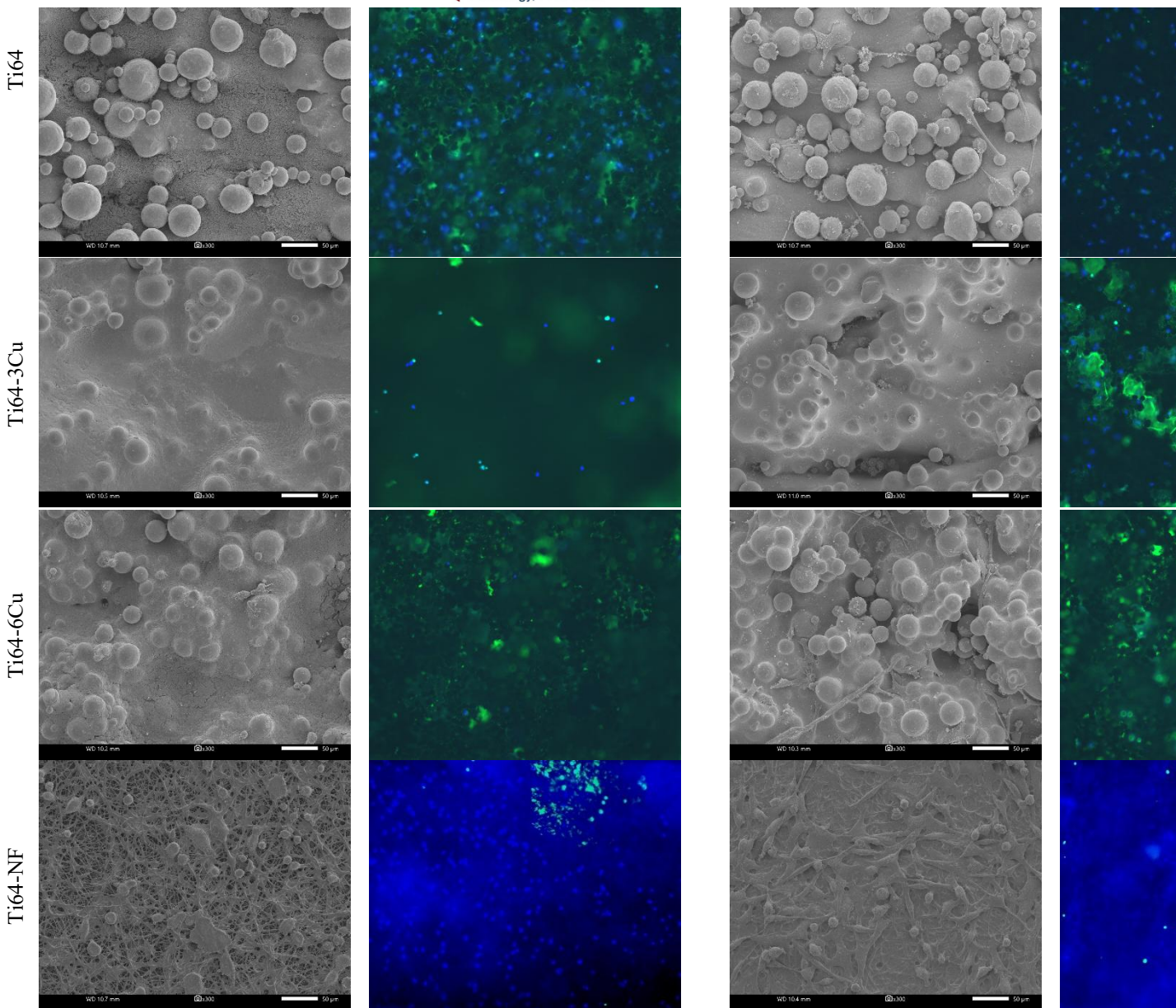
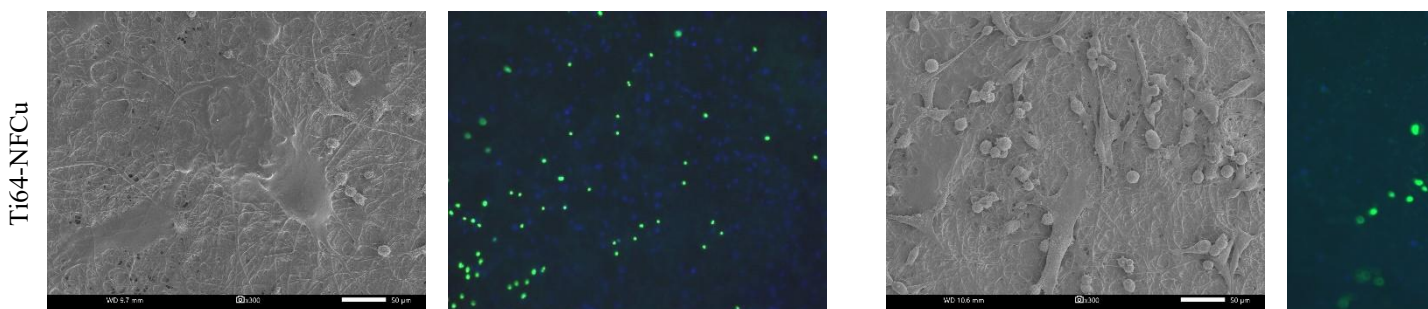


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability



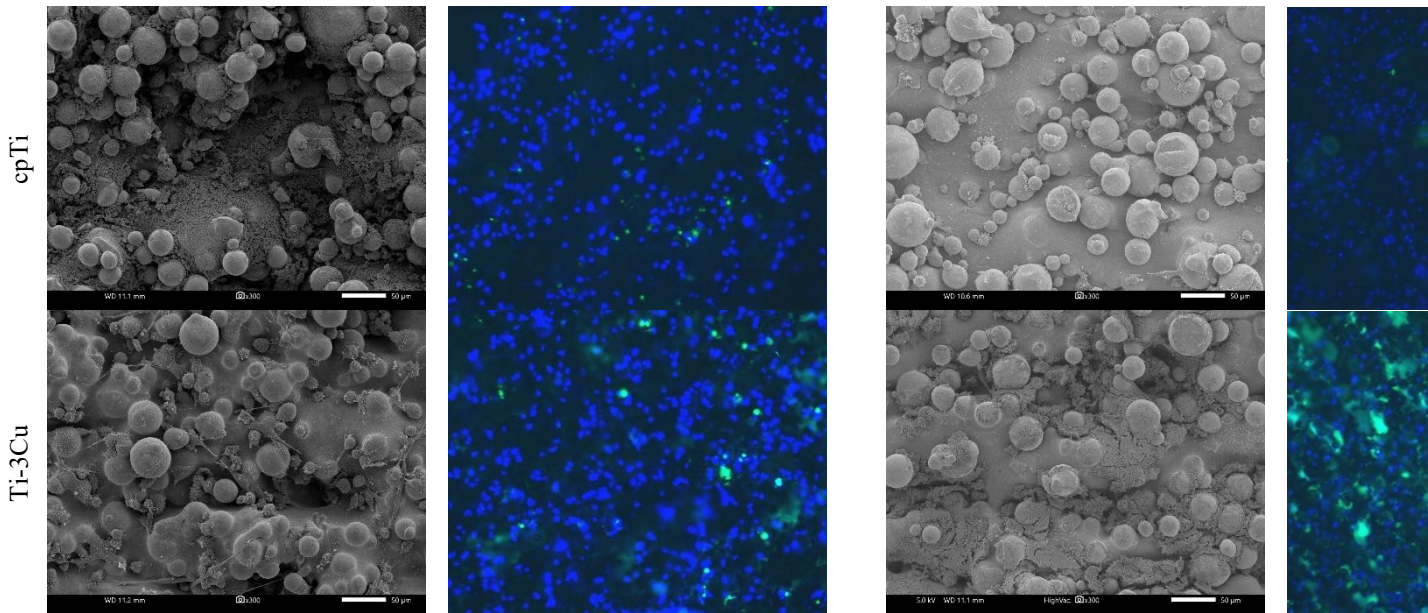


Figure 6.16: Fluorescence images (10× magnification) and SEM micrographs (scale = 50 µm) for cell viability (cont.)

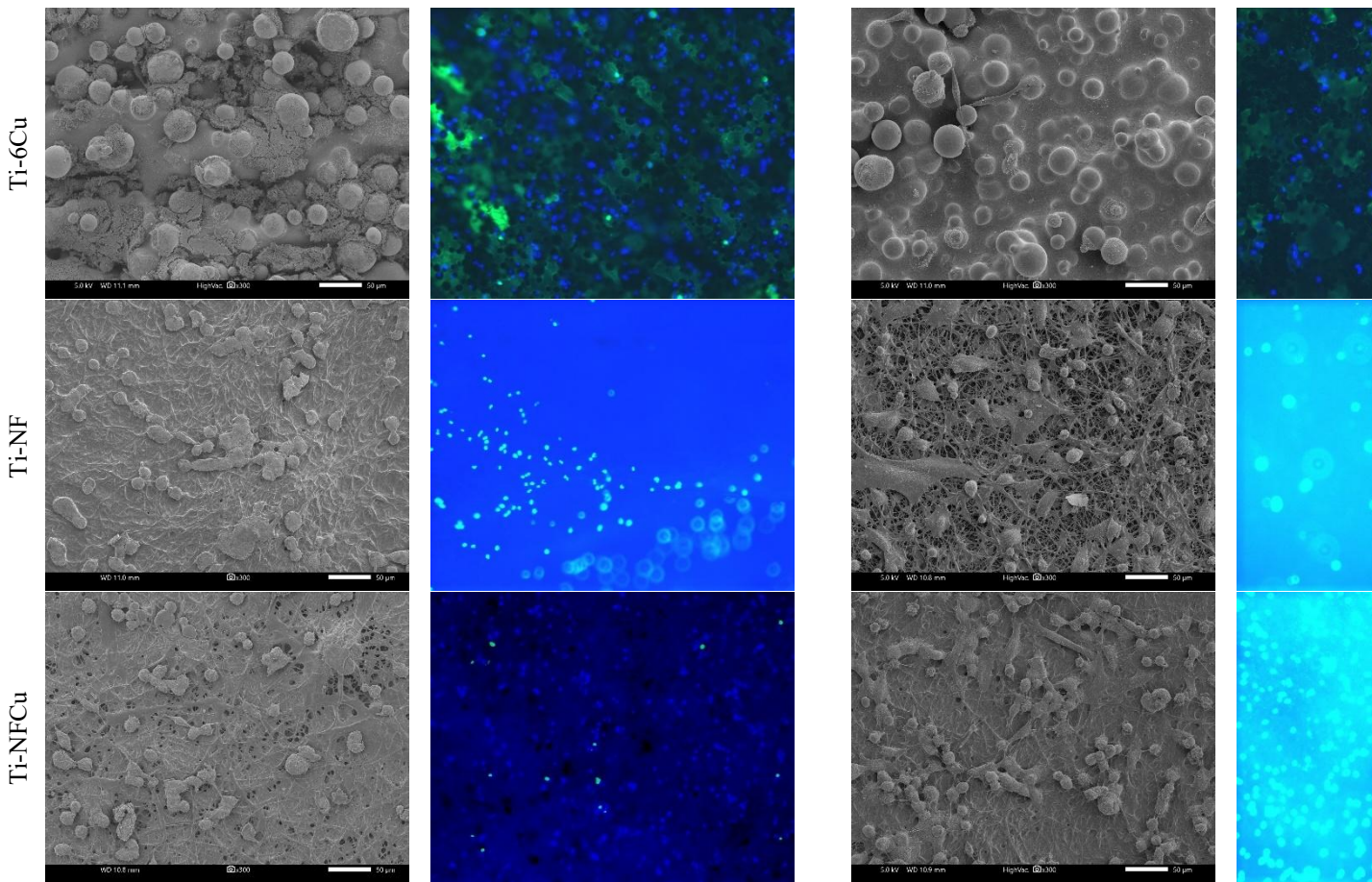


Figure 6.16: Fluorescence images (10 $\times$ magnification) and SEM micrographs (scale = 50 μ m) for cell viability of MDA-MB-231 cells (cont.). After 24 hr, mononucleated myoblast adhesion and subsequent likely ECM formation were evident, as well as possible differentiation and proliferation of myoblasts. Cell viability is also demonstrated by the dominant blue fluorescence. After 5 days, the cpTi surfaces appeared almost completely colonised by adherent, differentiated C2C12 myoblasts. The differentiation rate may have been so rapid that the large volume of cells was competing for space, leading to cell death as seen in the fluorescence image. Civantos et al. (2020) observed that C2C12 cells attached to cpTi substrates within 24 hrs, and cell density increased over time, up to 72 hrs. During this study, the SEM of Ti-3Cu after 24 hrs showed some myoblast adhesion, but mostly exhibited coverage by the sheet of membrane, and the fluorescence micrographs showed a green fluorescence hue consistent with the membrane orientation. After 5 days, the myoblasts had adhered and differentiated onto the membrane covering the Ti-3Cu surface. Clear cell-to-cell connections were visible with the formation of flat, widely spread myoblasts. The fluorescence image also showed blue cell nuclei illuminated on a green background. For the Ti-6Cu, cell adhesion had been initiated, and differentiation was observed within 24 hrs of culture, as shown in the SEM image and supported by the blue nuclear coverage of the surface. After 5 days, however, green fluorescence dominated, and the SEM revealed the formation of a membrane sheet with cracks and a large mass of cells, which may be presumed dead due to the extensive green fluorescence. Therefore, similar to the Ti64 specimens, the Cu content of the alloyed cpTi may be attributed to the cytotoxicity of the C2C12 cell.

Viewing SEM micrographs of the coated samples, after 24 hrs of culture, myoblasts had colonised the surfaces of the NF-coated samples. On the surfaces of Ti64-NF, Ti-NF and Ti-NFCu after 24 hrs, initiation of cell adhesion was visible. In contrast, numerous adherent cells exhibited a spindle-shaped morphology, which is characteristic of mononucleated myoblasts and indicative of stable adhesion to the substrate (Jo, et al., 2020; Dos Santos, et al., 2023). The myoblasts had elongated and established links with the NFs. A high cell density could be observed on Ti64-NFCu at 24 hrs and 5 days post-seeding, with the development of a membrane layer that covered a large portion of the NF scaffold after 24 hrs and at the 5-day incubation period, multiple layers of myoblasts, some seemingly differentiated, had formed, such that the NFs were fully colonised and not visible. The C2C12 cells on Ti64-NF and Ti-NF after 5 days displayed various morphologies. Some cells

formed clusters of globular-shaped myoblasts in addition to those that had elongated and possibly differentiated. These clusters seemed to have formed on elongated myoblasts and had initiated the formation of additional layers of elongated myoblasts. This indicates that the NFs promoted cell attachment, differentiation, and proliferation after 5 days of culture. The cell density and phenotype on Ti-NFCu after 5 days were similar to those of Ti64-NFCu after 24 hrs, with the formation of pseudopodia-like elongations along NFs orientation. Song et al. (2016) observed a similar structure in C2C12 cells cultured on PCL-based NFs fabricated by electrospinning, with typical myoblast-like cells. Some NFs in this study were found to protrude from beneath the NFs, suggesting cell migration through the NF pores. Pacilio et al. (2023) fabricated poly(L-lactide-co- ϵ -caprolactone) (PLCL) NFs coated with morphogenesis. The authors cultured C2C12 cells on the NFs and demonstrated the biocompatibility of the PLCL scaffold through cell viability, proliferation, and morphogenesis. Furthermore, their study proved cell–cell adhesions amongst myoblasts and myotube formation on the NFs. Jo et al. (2020) made similar observations after culturing C2C12 myoblasts on PU-based NFs.

Common phenotypes of viable MDA-MB-231 cells include elongated, globular-shaped, spindle-like, epithelial and stellate (primarily adopted during invasion) (**Error! Reference source not found.**) (Zipperstein, et al., 2015; Franchi, et al., 2020). The Ti64-xCu ($x = 0, 3, 6$ wt.%) did not exhibit these morphologies 24 hrs post-seeding. The control Ti64 sample displayed powdery texture particles covering the surface, and the fluorescence image showed a distribution of green masses and blue-stained nuclei. Basso et al. (2013) identified flake structures that detached from the substrate as dead cells. The Ti64-3Cu and Ti64-6Cu SEM images showed the formation of a membrane-like layer, as observed with C2C12, after 24 hrs. The fluorescence image showed minimal live/dead staining for Ti64-3Cu and predominantly green illuminance. Low cell density was visible on the Ti64 surface after 5 days, with cell migration and elongations into filopodia, and the fluorescence showed widespread blue illumination and a few green spots. Conversely, the Ti64-3Cu and Ti64-6Cu showed mostly green fluorescence across their surfaces, with Ti64-3Cu showing more blue nuclei than Ti64-6Cu after 5 days of culture, while the membrane layer covering the surfaces appeared in the SEM images without specific cell morphology. Thus, as with the C2C12, apoptosis of MDA-MB-231 cells by Cu^{2+} ions was conceivable for the LPBF Ti64-3Cu and Ti64-6Cu specimens. Shirai et al. (2009) observed in vitro toxicity of the Ti-5%Cu alloy,

and their *in vivo* model showed improved biocompatibility, with Ti-1%Cu alloy pins demonstrating good osteoconductive properties. Conversely, the authors observed that osteoid formation was diminished in the Ti-5% Cu alloy, and thus, bone conduction was not possible on it.

At the 24-hr incubation period, the cpTi control and Ti-6Cu samples developed powdery textures after seeding with MDA-MB-231 cells. At the same time, Ti-3Cu presented several globular cells and a few stellate and spindle shapes with filopodia extensions. These morphologies seemed to be covered with microvilli and some microvesicles. Blue fluorescence nuclei dominated the surfaces of cpTi, Ti-3Cu and Ti-6Cu. Green fluorescence was barely visible on the cpTi and Ti-3Cu surface, but was evident on Ti-6Cu. Thus, Cu content seems to affect cell viability after 24 hrs. Likewise, after 5 days, green deposits were pronounced on Ti-3Cu and Ti-6Cu, appearing as a powdery texture and a membrane-like sheet, respectively, in the SEM images. Blue nuclei were also prominent after 5 days on Ti-3Cu and Ti-6Cu. The control surface was populated with blue-illuminated spots after 5 days, while the green was sparse. The powdered texture was not observed on cpTi. Cell viability was higher on the control than on the Cu alloy surfaces.

All NF-coated surfaces (Ti64-NF, Ti64-NFCu, Ti-NF, and Ti-NFCu) were densely populated by MDA-MB-231 cells at both 24 hrs and 5 days post-seeding, with possible deposition of the ECM. Although cell extensions such as filopodia may be challenging to distinguish from polymer fibres, cell-to-NF and cell-to-cell connections were formed, resulting in multilayered cell structures after 24 hrs and 5 days. Ti64-NF after 24 hrs exhibited a lower cell density, with isolated globular, elongated, and flat stellate phenotypes, and still retained the original NF matrix. Epithelial-like cells or epithelial-mesenchymal transitioned cells were observed on the Ti64-NF after 5 days, and appeared to be forming a membrane of flattened cells that had migrated through the NF openings. MDA-MB-231 cells on Ti64-NFCu after 24 hrs and 5 days appeared flattened and well spread. After 5 days, spherical, stellate and flattened polygonal phenotypes with lamellapodia and filopodia were forming on the flat, spread-out cells.

The Ti-NF seemed to have created an evenly distributed monolayer of flattened cells onto which round-to-flat changing cells had adhered after 24 hrs. After 5 days, the cell colonisation on the Ti-

NF resembled that of Ti64-NF at 24 hrs. Ti-NFCu displayed a similar cell population at 24 hrs and 5 days, but with greater coverage after 5 days. As with the other surface, monolayer membrane formation occurred, along with globular, spindle, elongated, and stellate phenotypes. The ability of MDA-MB-231 cells to form various phenotypes with microvilli and microvesicle surface features, and extensions, such as lamellipodia and filopodia, suggests that the substrate supported cell adhesion and survival (that is, the Ti substrate was bioactive). Transmission electron microscopy by Lin et al. (2003) revealed the presence of additional cytoplasmic organelles, such as rough endoplasmic reticulum and mitochondria, in more flattened, well-spread cells. Moreover, a rounded morphology had a smaller surface area and was more easily detachable from the substratum than the flat, protracted morphology. A round morphology is typical of a suspension phase (Cavo, et al., 2018). Balachander et al. (2015) reported cell spreading and increased cell mass after culturing MDA-MB-231 cells on PCL. Similarly, Rabionet et al. (2017) and Sivakumar et al. (2022) utilised DAPI/actin fluorescent staining and SEM to demonstrate that MDA-MB-231 cells adhered and proliferated on PCL matrices.

Overall, the topographical features of the control and in situ alloyed surfaces did not influence the cellular response, as cell morphologies formed on the round partially melted particles and on the solidified molten pool were similar. A linear relationship between cell viability and Cu concentration was not established. The cpTi control promotes cell adhesion and seemingly differentiation and proliferation better than the Ti64 control. This is also supported by the EDS values (Table 6.3 Table 6.4). The EDS mapping was conducted to indicate the presence of organic biological matter and differentiate it from the metal surfaces and polymer composite components. The EDS results showed that cpTi after 5 days contained sodium (Na) and phosphorus (P) – which are elements found in the ECM (Soles, et al., 2023) – when cultured with C2C12 and contained sodium when cultured with MDA-MB-231 after 24 hrs. These elements were not detected on the Ti64 surface after seeding with C2C12 and MDA-MB-231 cells for 24 hrs and 5 days. The carbon content detected on cpTi was notably higher than on Ti64 for C2C12 and MDA-MB-231 after 5 days and 24 hrs, respectively, and only slightly lower than on Ti64 after 24 hrs and 5 days, respectively, indicating a higher content of organic matter on cpTi than on Ti64.

Table 6.3: EDS elemental mapping of samples seeded with C2C12 (in wt.%)

Element	24 hrs							5 days						
	Ti	Al	Cu	C	O	Na	P	Ti	Al	Cu	C	O	Na	P
Ti64	71.7	3.1		14.5	10.7			77.9	3.4		10.4	8.3		
Ti64-3Cu	54.8	3.6	13.5	15.9	12.3			68.3	2.8	4.7	15.2	9.0		
Ti64-6Cu	49.7	2.3	13.6	23.0	11.4			55.7	2.6	9.6	19.9	12.3		
Ti64-NF	0.3			70.6	29.1			0.4			70.6	29.0		
Ti64-NFCu	0.4		1.5	69.8	28.3			0.4		0.4	70.3	28.8	0.2	
cpTi	81.7			11.3	7.0			39.5			41.2	18.8	0.1	0.3
Ti-3Cu	41.2		5.0	36.3	17.5			50.5		10.2	27.3	12.0		
Ti-6Cu	57.2		11.5	20.4	10.8			51.1		5.5	29.3	14.1		
Ti-NF				71.4	28.6			0.2			71.0	28.7	0.1	
Ti-NFCu	0.2			71.7	28.1			0.2			71.9	27.9		

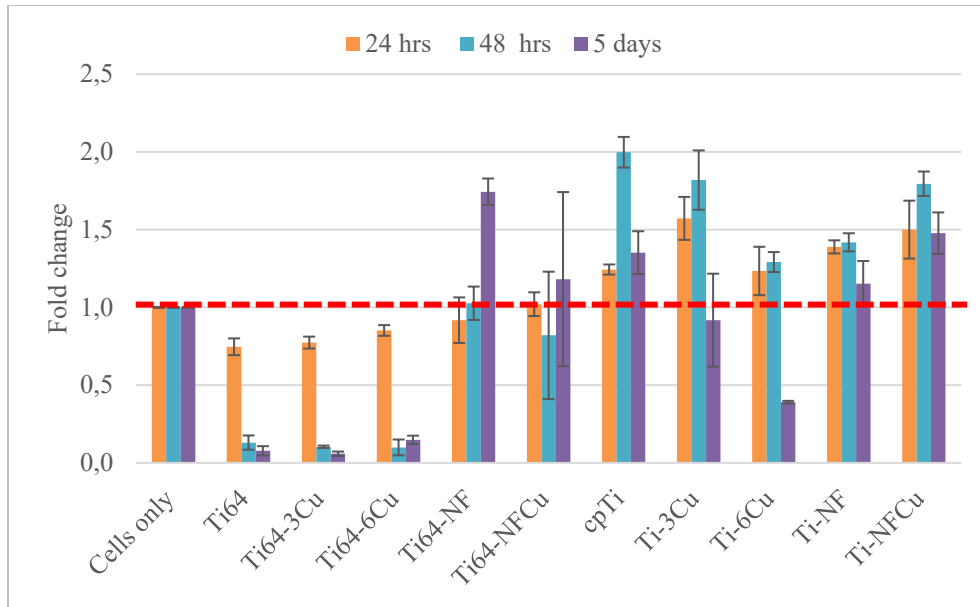
Table 6.4: EDS elemental mapping of samples seeded with MDA-MB-231 (in wt.%)

Element	24 hrs							5 days						
	Ti	Al	Cu	C	O	Na	P	Ti	Al	Cu	C	O	Na	P
Ti64	64.5	2.7		18.5	14.3			71.6	2.9		15.1	10.4		
Ti64-3Cu	74.8	3.3	9.0	6.5	6.4			69.1		9.5	14.5	6.9		
Ti64-6Cu	42.3	1.7	6.3	30.8	19.0			56.4	2.5	13.6	19.1	8.5		
Ti64-NF	0.5			71.9	27.6			0.3			71.3	28.4		
Ti64-NFCu			2.6	69.5	27.9			0.3		2.6	68.8	28.1	0.2	
cpTi	59.6			24.1	16.1	0.2		82.8			9.6	7.6		
Ti-3Cu	65.1		7.6	17.5	9.8			50.4		6.0	27.6	16.1		
Ti-6Cu	44.4		6.4	31.0	18.3			62.0		18.3	12.8	7.0		
Ti-NF	0.1			71.9	27.9	0.1		0.2			72.5	27.3		
Ti-NFCu	0.2			71.9	27.9			0.2			72.9	26.8		

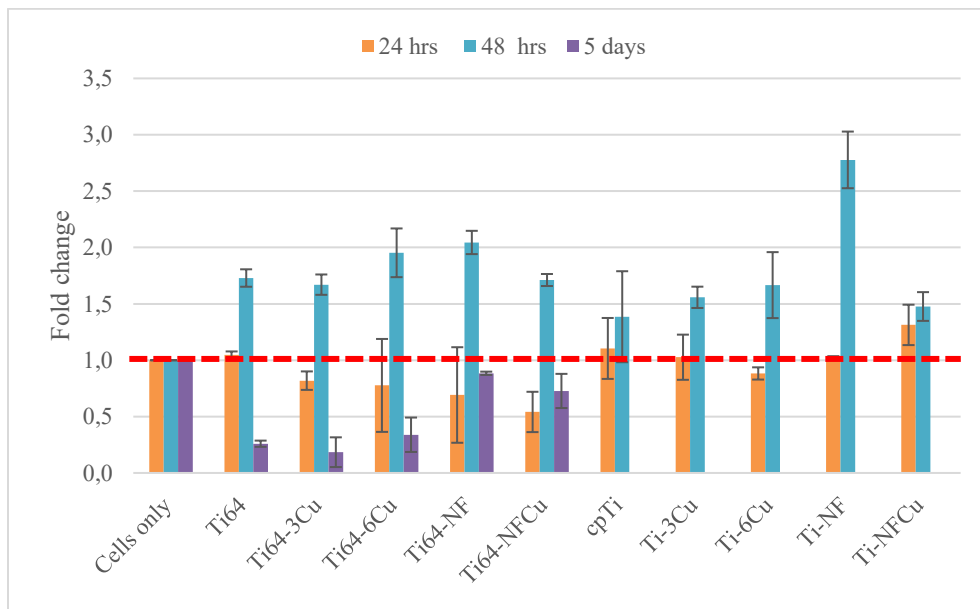
From the SEM and fluorescence micrographs, it is evident that the NF-coated samples enhanced cell motility more effectively than the bare control and Cu-alloyed samples. The EDS detected Na on Ti64-NFCu cultured with C2C12 and MDA-MB-231 for 5 days and on Ti-NF after 5 days and 24 hrs, respectively. Sodium was not detected in any of the Cu-alloyed samples. From the SEM micrographs and EDS values, it may be deduced that the NF-coated specimens promoted cell bioactivity more efficiently than the bare Ti samples. Saito et al. (2014) seeded C2C12 cells with cpTi for 3, 48, and 96 hrs and observed cell proliferation at all the time points, with the highest proliferation at 96 hrs post-seeding. The authors also studied mineral induction for 5 days and established that C2C12 cells differentiated into the osteoblast phenotype on cpTi discs, with a calcium content of 0.13 mg/cm^2 at 12 days following mineral induction. Representative examples of the EDS spectra for the elemental mapping are presented in Appendices B and C for C2C12 and MDA-MB-231, respectively.

6.3.3.1. Resazurin assay

Figure 6.17 shows the RFU fold change values obtained from the resazurin assay to establish cell viability. The %RFU values are shown in Appendix D. The values from the well containing only the cell suspension were used as a reference and normalised to 1 (that is, 100% viability). The results for the cpTi samples tested for viability with MDA-MB-231 at the 5-day interval are not included due to experimental error. The resazurin results matched those of the cell culture and showed that cpTi displayed an overall higher cell viability than Ti64, and the samples coated with NFs exhibited an overall higher cell viability than the bare specimens.



(i)



(ii)

Figure 6.17: Relative fluorescence units for cell viability assay of (i) C2C12 (iii) MDA-MB-231 cell lines (red dashed line is the reference line)

For C2C12, the Ti64- x Cu ($x = 0, 3, 6$ wt.%) samples showed a fold change below 1, with excessive reduction at 48 hrs and 5 days. The Ti64-NF and Ti64-NFCu displayed lower fold changes at 24

and 48 hrs, respectively, which increased at 5 days post-seeding in both samples. Decreased cell viability was observed for Ti-3Cu and Ti-6Cu after 5 days of culture, whereas in Ti-NF and Ti-NFCu, there was a fold change above 1 at all three incubation periods, although it decreased and remained above 1 after 5 days. The fold changes of C2C12 cells were highest at 48 hrs for the specimens of Ti- x Cu ($x = 0, 3, 6$ wt.%), Ti-NF and Ti-NFCu. Similarly, the fold changes in the MDA-MB-231 cells were highest and well above the reference level after 48 hrs of culture across the board. MDA-MB-231 viability was critically lower after 5 days for the Ti64- x Cu ($x = 0, 3, 6$ wt.%) samples and was lower than that after 24 hrs. At the 5-day incubation period, the fold change in MDA-MB-231 for Ti64-NF and Ti64-NFCu was below 1 but higher than at 24 hrs. No correlation was observed between cell viability and Cu content in C2C12 and MDA-MB-231 cells. Nevertheless, there seemed to be less cytotoxicity exhibited by the NFs when compared to the Cu-alloyed Ti64 and cpTi specimens for both C2C12 and MDA-MB-231 after 5 days. Thus, it may be postulated that the NFs promoted biocompatibility more effectively and had a better-controlled Cu ion release rate at extended periods.

In the same manner that Cu is able to induce bacterial cell death through mechanisms of enhancing ROS production and promoting cytoplasmic leakage, causing the cell membrane to rupture, it has also been shown that Cu is capable of using these mechanisms to induce cellular and mitochondrial toxicity of mammalian cells (Modica-Napolitano, et al., 2019). Liu et al. (2016) demonstrated that Cu nanoclusters are capable of inducing cytotoxicity and apoptosis in C2C12 cells by generating ROS. Ren et al. (2014) used a Cell Counting Kit-8 (CCK-8) to evaluate the cytotoxicity of the alloy Ti6Al4V-3Cu against vigorously grown L929 cells and found no significant difference in cell viability between Ti6Al4V and Ti6Al4V-3Cu alloys during the tested periods of 2, 4 and 7 days. The cells exuded a normal and pleasant state in the extract of Ti6Al4V-3Cu alloy. Das et al. (2018) investigated the cell viability of mouse fibroblast seed on PCL nanofibrous coating using the resazurin assay and observed that cell viability increased with time.

6.4. Summary

The current chapter presents an investigation which scrutinised the dual functionality of functionalised Ti6Al4V ELI and cpTi samples. The samples had been previously functionalised by alloying with Cu particles and electrospinning with PCL/PU and PCL/PU/CuO NFs to introduce antibacterial properties and promote bioactivity without inducing cytotoxicity. Antimicrobial analysis was conducted using the agar diffusion assay and the plate count method. The agar diffusion assay did not show a bactericidal effect of the test specimen. However, the plate count method showed a reduction in bacterial growth associated with Cu/CuO content, particularly against Gram-positive strains. Even among Gram-negative bacteria, the few instances of antibacterial activity were related to a Cu/CuO-containing specimen. Thus, the antibacterial property of Cu was demonstrated and was more pronounced in samples produced using cpTi than in those produced using Ti64, and in NF-coated samples than in bare samples. The superior functionality of NF-coated samples was also demonstrated during cell culture experiments. Cytotoxicity of the C2C12 and MDA-MB-231 cells by the Cu-alloyed Ti64 and cpTi sample was apparent. The NF-coated samples promoted adhesion, differentiation, and proliferation in C2C12 and MDA-MB-231 cells more efficiently and maintained cell viability over extended periods. Coating Ti implant surfaces with NF impregnated with Cu/CuO antibacterial agent has proven to be a viable option for attaining multifunctional biomedical implants.

References

- Abdal-hay, A., Agour, M., Kim, Y. -K., Lee, M. -H., Hassan, M. K., El-Ainin, H. A., . . . Ivanovski, S. (2019). Magnesium-particle/polyurethane composite layer coating on titanium surfaces for orthopedic applications. *European Polymer Journal*, 112, 555–568. doi:10.1016/j.eurpolymj.2018.10.012
- Al Mustafa, M., Agis, H., Müller, H. D., Watzek, G., & Gruber, R. (2015). In vitro adhesion of fibroblastic cells to titanium alloy discs treated with sodium hydroxide. *Clinical Oral Implants Research*, 26(1), 15–19. doi:10.1111/clr.12294
- Al-Khateeb, A., Al-Hassani, E. S., & Jabur, A. R. (2023). Metallic implant surface activation through electrospinning coating of nanocomposite fiber for bone regeneration. *International Journal of Biomaterials*, 2023, Article ID 1332814. doi:10.1155/2023/1332814
- Arciola, C. R., Campoccia, D., & Montanaro, L. (2018). Implant infections: adhesion, biofilm formation and immune evasion. *Nature Reviews Microbiology*, 16(7), 397–409. doi:10.1038/s41579-018-0019-y
- Balachander, G. M., Balaji, S. A., Rangarajan, A., & Chatterjee, K. (2015). Enhanced metastatic potential in a 3D tissue scaffold toward a comprehensive in vitro model for breast cancer metastasis. *ACS Applied Materials & Interfaces*, 7(50), 27810–27822. doi:10.1021/acsami.5b09064
- Basso, F. G., Pansani, T. N., de Oliveira, C. F., Turrioni, A. P., Soares, D. G., Hebling, J., & Costa, C. A. (2013). Cytotoxic effects of zoledronic acid on human epithelial cells and gingival fibroblasts. *Brazilian Dental Journal*, 24(6), 551-558. doi:10.1590/0103-6440201302229
- Bassous, N. J., Jones, C. L., & Webster, T. J. (2019). 3-D printed Ti-6Al-4V scaffolds for supporting osteoblast and restricting bacterial functions without using drugs: Predictive equations and experiments. *Acta Biomaterialia*, 96, 662–673. doi:10.1016/j.actbio.2019.06.055
- Bayot, M. L., & Bragg, B. N. (2024). *Antimicrobial Susceptibility Testing*. Retrieved February 23, 2025, from <https://www.ncbi.nlm.nih.gov/books/NBK539714/>
- Bhaskar, P., Bosworth, L. A., Wong, R., O'brien, M. A., Kriel, H., Smit, E., . . . Cartmell, S. H. (2017). Cell response to sterilized electrospun poly(E-caprolactone) scaffolds to aid tendon

- regeneration in vivo. *Journal of Biomedical Materials Research Part A*, 105A, 389–397. doi:10.1002/jbm.a.35911
- Buyck, J. M., Luyckx, C., Muccioli, G. G., Krause, K. M., Nichols, W. W., Tulkens, P. M., & Van Bambeke, F. (2017). Pharmacodynamics of ceftazidime/avibactam against extracellular and intracellular forms of *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy*, 72(5), 1400–1409. doi:10.1093/jac/dkw587
- Cavo, M., Caria, M., Pulsoni, I., Beltrame, F., Fato, M., & Scaglione, S. (2018). A new cell-laden 3D Alginate-Matrigel hydrogel resembles human breast cancer cell malignant morphology, spread and invasion capability observed “in vivo”. 8, . *Scientific Reports*, 8, 5333. doi:10.1038/s41598-023-40680-x
- Centers for Disease Control and Prevention (CDC). (2023). *Steam Sterilization*. Retrieved July 2024, 23, from <https://www.cdc.gov/infection-control/hcp/disinfection-sterilization/steam-sterilization.html>
- Cheng, L., Lei, L., & Guo, S. (2010). In vitro and in vivo evaluation of praziquantel loaded implants based on PEG/PCL blends. *International Journal of Pharmaceutics*, 387, 129–138. doi:10.1016/j.ijpharm.2009.12.010
- Chouhan, V. (2016). Qivas therapy for treatment of implant associated MDR bacterial infection. *Journal of Orthopaedic Case Reports*, 6(2), 69–71. doi:10.13107/jocr.2250-0685.440
- Civantos, A., Giner, M., Trueba, P., Lascano, S., Montoya-García, M. -J., Arévalo, C., . . . Torres, Y. (2020). In vitro bone cell behavior on porous titanium samples: Influence of porosity by loose sintering and space holder techniques. *Metals*, 10(5), 696. doi:10.3390/met10050696
- Creative Bioarray. (2025). *Resazurin Cell Viability Assay* . Retrieved May 26, 2025, from <https://www.creative-bioarray.com/support/resazurin-cell-viability-assay.htm>
- Culture Collections. (2025). *Cell line profile MDA-MB-231*. Retrieved May 24, 2025, from <https://www.culturecollections.org.uk/products/cell-cultures/ecacc-cell-line-profiles/mda-mb-231/>
- Das, S., Gurav, S., Soni, V., Ingle, A., Mohanty, B. S., Chaudhari, P., . . . Bellare, J. R. (2018). Osteogenic nanofibrous coated titanium implant results in enhanced osseointegration: In vivo preliminary study in a rabbit model. *Tissue Engineering and Regenerative Medicine*, 15(2), 231–247. doi:10.1007/s13770-017-0106-6

- David, A., Scott, L. E., Da Silva, P., Mayne, E., & Stevens, W. S. (2023). Storage of *Mycobacterium tuberculosis* culture isolates in Microbank™ beads at a South African laboratory. *African Journal of Laboratory Medicine*, 12(1), 2172. doi:10.4102/ajlm.v12i1.2172
- Dhaliwal, J. S., Abd Rahman, N. A., Ming, L. C., Dhaliwal, S. K., Knights, J., & Albuquerque Junior, R. F. (2021). Microbial biofilm decontamination on dental implant surfaces: A mini review. *Frontiers in Cellular and Infection Microbiology*, 11, 736186. doi:10.3389/fcimb.2021.736186
- Domańska, I. M., Oledzka, E., & Sobczak, M. (2020). Sterilization process of polyester based anticancer-drug delivery systems. *International Journal of Pharmaceutics*, 587, 119663. doi:10.1016/j.ijpharm.2020.119663
- Dos Santos, A. E., Cotta, T., Santos, J. P., Camargos, J. S., do Carmo, A. C., Alcântara, E. G., . . . Jorge, E. C. (2023). Bioactive cellulose acetate nanofibre loaded with annatto support skeletal muscle cell attachment and proliferation. *Frontiers in Bioengineering and Biotechnology*, 11, 1116917. doi:10.3389/fbioe.2023.1116917
- Drugs.com. (2025). *What are the strongest antibiotics available?* . Retrieved May 16, 2025, from <https://www.drugs.com/medical-answers/what-strongest-antibiotics-available-3579737/>
- Dubreuil, L., Jehl, F., Cattoen, C., Bonnet, R., Bru, J. P., Caron, F., . . . Weber, P. (2020). Improvement of a disk diffusion method for antibiotic susceptibility testing of anaerobic bacteria. French recommendations revisited for 2020. *Anaerobe*, 64, 102213. doi:10.1016/j.anaerobe.2020
- Duque, M., Bonnin, R., & Dortet, L. (2024). Evaluation of the French novel disc diffusion-based algorithm for the phenotypic screening of carbapenemase-producing Enterobacterales. *Clinical Microbiology and Infection*, 30(3), 397.e1-397.e4. doi:10.1016/j.cmi.2023.12.00
- ElectrospinTech. (2022). *Electrospun fibers Sterilization* . Retrieved May 16, 2025, from <https://electrospintech.com/fibersterilization.html>
- Epoke, J., Igumbor, E. O., Asuquo, A. E., & Tichawowona, C. (2003). Locally prepared antibiotic sensitivity discs: A substitute for imported commercial discs. *Global Journal of Pure and Applied Sciences*, 9(4), 453-456. doi:10.4314/gjpas.v9i4.16051

- Feller, L., Jadwat, Y., Khammissa, R. A., Meyerov, R., Schechter, I., & Lemmer, J. (2015). Cellular responses evoked by different surface characteristics of intraosseous titanium implants. *BioMed Research International*, 2015, 171945. doi:10.1155/2015/171945
- Fowler, L., Engqvist, H., & Öhman-Mägi, C. (2019). Effect of copper ion concentration on bacteria and cells. *Materials (Basel)*, 12(22), 3798. doi:10.3390/ma12223798
- Franchi, M., Piperigkou, Z., Karamanos, K. A., Franchi, L., & Masola, V. (2020). Extracellular matrix-mediated breast cancer cells morphological alterations, invasiveness, and microvesicles/exosomes release. *Cells*, 9(9), 2031. doi:10.3390/cells9092031
- Ghasemian Lemraski, E., Jahangirian, H., Dashti, M., Khajehali, E., Sharafinia, S., Rafiee-Moghaddam, R., & Webster, T. J. (2021). Antimicrobial double-layer wound dressing based on chitosan/polyvinyl alcohol/copper: In vitro and in vivo assessment. *International Journal of Nanomedicine*, 16, 223-235. doi:10.2147/IJN.S266692
- Guo, M., Wang, M., Deng, H., Zhang, X., & Wang, Z. Y. (2013). A novel anticancer agent Brousoflavonol B downregulates estrogen receptor (ER)- α 36 expression and inhibits growth of ER-negative breast cancer MDA-MB-231 cells. *European Journal of Pharmacology*, 714(1-3), 56-64. doi:10.1016/j.ejphar.2013.05.047
- Guo, S., Lu, Y., Wu, S., Liu, L., He, M., Zhao, C., . . . Lin, J. (2017). Preliminary study on the corrosion resistance, antibacterial activity and cytotoxicity of selective-laser-melted Ti6Al4V-xCu alloys. *Materials Science and Engineering: C*, 72(2017), 631-640. doi:10.1016/j.msec.2016.11.126
- Guo, Y., Cen, Z., Zou, Y., Fang, X., Li, T., Wang, J., . . . Liu, C. (2012). Whole-genome sequence of *Klebsiella pneumonia* strain LCT-KP214. *Journal of Bacteriology*, 194(12), 3281. doi:10.1128/JB.00531-12
- Haider, A., Kwak, S., Gupta, K. C., & Kang, I. -K. (2015). Antibacterial activity and cytocompatibility of PLGA/CuO hybrid nanofibre scaffolds prepared by electrospinning. *Journal of Nanomaterials*, 2015, Article ID 832762. doi:10.1155/2015/832762
- Haq, I. U., Khan, T. A., & Krukiewicz, K. (2024). Etiology, pathology, and host-impaired immunity in medical implant-associated infections. *Journal of Infection and Public Health*, 17(2), 189-203. doi:10.1016/j.jiph.2023.11.024

- Holy, C. E., Cheng, C., Davies, J. E., & Shoichet, M. S. (2001). Optimizing the sterilization of PLGA scaffolds for use in tissue engineering. *Biomaterials*, 22(1), 25-31. doi:10.1016/S0142-9612(00)00136-8
- Hu, J., Li, H., Wang, X., Yang, L., Chen, M., Wang, R., . . . Zhang, E. (2020). Effect of ultrasonic micro-arc oxidation on the antibacterial properties and cell biocompatibility of Ti-Cu alloy for biomedical application. *Materials Science and Engineering: C*, 115, 110921. doi:10.1016/j.msec.2020.110921
- Jang, M. B., Scheffold, J., Røst, L. M., Cheon, H., & Bruheim, P. (2022). Serum-free cultures of C2C12 cells show different muscle phenotypes which can be estimated by metabolic profiling. *Scientific Reports*, 12, 827. doi:10.1038/s41598-022-04804-z
- Jin, X., Gao, L., Liu, E., Yu, F., Shu, X., & Wang, H. (2015). Microstructure, corrosion and tribological and antibacterial properties of Ti-Cu coated stainless steel. *Journal of the Mechanical Behavior of Biomedical Materials*, 50, 23-32. doi:10.1016/j.jmbbm.2015.06.004
- Jo, S. B., Erdenebileg, U., Dashnyam, K., Jin, G. Z., Cha, J. R., El-Fiqi, A., . . . Kim, H. W. (2020). Nano-graphene oxide/polyurethane nanofibres: Mechanically flexible and myogenic stimulating matrix for skeletal tissue engineering. *Journal of Tissue Engineering*, 11, 1–10. doi:10.1177/2041731419900424
- Jones, R. N., Barry, A. L., Fuchs, P. C., & Thornsberry, C. (1986). Disk diffusion susceptibility testing of two macrolide antimicrobial agents: Revised interpretive criteria for erythromycin and preliminary guidelines for roxithromycin (RU 965). *Journal of Clinical Microbiology*, 24(2), 233–239. doi:10.1128/jcm.24.2.233-239.1986
- Joshi, A., Iyer, V., Blasubramaniau, U., Kagal, A., & Bharadwaj, R. (2008). Comparison of efficacy of three commercially available antibiotic discs. *Indian Journal of Medical Microbiology*, 26(2), 160-162. doi:10.1016/S0255-0857(21)01935-6
- Ju, J., Zan, R., Shen, Z., Wang, C., Peng, P., Wang, J., . . . Yang, T. (2023). Remarkable bioactivity, bio-tribological, antibacterial, and anti-corrosion properties in a Ti-6Al-4V-xCu alloy by laser powder bed fusion for superior biomedical implant applications. *Chemical Engineering Journal*, 471, 144656. doi:10.1016/j.cej.2023.144656

- Kaczmarek, A., Wrońska, A. K., Sobich, J., & Boguś, M. I. (2025). The multifunctional role of IFN- γ in *Galleria mellonella* (Lepidoptera) immunocompetent cells. *Cytokine*, 185, 156804. doi:10.1016/j.cyto.2024.156804
- Katayama, T., Chigi, Y., & Okamura, D. (2023). The ensured proliferative capacity of myoblast in serum-reduced conditions with Methyl- β -cyclodextrin. *Frontiers in Cell and Developmental Biology*, 11, 1193634. doi:10.3389/fcell.2023.1193634
- Khan, H. A., Alghamdi, A. A., Rajendra Prasad, N., Alrokayan, S. H., Almansour, B. S., & Hatamilah, A. A. (2023). The role of mitochondrial dysfunction in cytotoxic effects of *Solanum nigrum* water extract on MCF-7 and MDA-MB-231 breast cancer cells. *Frontiers in Bioscience-Landmark*, 28(8), 180. doi:10.31083/j.fbl2808180
- Khatua, S., Simal-Gandara, J., & Acharya, K. (2022). Understanding immune-modulatory efficacy in vitro. *Chemico-Biological Interactions*, 352, 109776. doi:10.1016/j.cbi.2021.109776
- Kiran, A. S., Kizhakeyil, A., Ramalingam, R., Verma, N. K., Lakshminarayanan, R., Kumar, T. S., . . . Ramakrishna, S. (2019). Drug loaded electrospun polymer/ceramic composite nanofibrous coatings on titanium for implant related infections. *Ceramics International*, 45(15), 18710-18720. doi:10.1016/j.ceramint.2019.06.097
- Krakhmalev, P., Yadroitsev, I., Yadroitsava, I., & de Smidt, O. (2017). Functionalization of biomedical Ti6Al4V via in situ alloying by Cu during laser powder bed fusion manufacturing. *Materials*, 10, 1154. doi:10.3390/ma10101154
- Lee, Y., Bae, M. -H., Lee, H., Kim, M., & Lee, K. (2025). Evaluation of the disk diffusion test for bacteroides fragilis group clinical isolates. *Annals of Laboratory Medicine*, 45(1), 70-76. doi:10.3343/alm.2024.0159
- Lei, J., Yao, G., Sun, Z., Wang, B., Yu, C., & Zheng, S. (2019). Fabrication of a novel antibacterial TPU nanofibre membrane containing Cu-loaded zeolite and its antibacterial activity toward *Escherichia coli*. *Journal of Material Science*, 54, 11682–11693. doi:10.1007/s10853-019-03727-x
- Li, K., Li, P., Yang, J. -J., Deng, C., Zhang, L. -C., Li, W., . . . Zhou, S. (2025). Study on the corrosion, tribocorrosion, biocompatibility, antibacterial performances of Ti6Al4C-Cu alloy produced by laser powder bed fusion. doi:10.2139/ssrn.5153427
- Li, Z., Hu, H., Zhou, J., Wang, Q., Zhang, L., Shen, X., . . . Jiang, Y. (2024). Enhancement in mechanical properties, antimicrobial properties and osseointegration ability of porous

- TC4-5Cu alloys prepared via additive manufacturing. *Journal of Materials Research and Technology*, 29, 3011-3023. doi:10.1016/j.jmrt.2024.02.007
- Liang, X. D., Zhang, X. G., Wang, W. Q., Liang, J. W., Zhao, X. H., Liu, M. Q., . . . Zhang, Z. H. (2025). Tribological, corrosion and antibacterial behaviors of Ti-xCu alloy prepared by laser powder bed fusion. *Journal of Materials Research and Technology*, 35, 5242-5259. doi:10.1016/j.jmrt.2025.02.183
- Lin, K. L., Tsai, P. C., Hsieh, C. Y., Chang, L. S., & Lin, S. R. (2011). Antimetastatic effect and mechanism of ovatodiolide in MDA-MB-231 human breast cancer cells. *Chemico-Biological Interactions*, 194(2-3), 148–158. doi:10.1016/j.cbi.2011.10.002
- Lin, V. C., Jin, R., Tan, P. H., Aw, S. E., Woon, C. T., & Bay, B. H. (2003). Progesterone induces cellular differentiation in MDA-MB-231 breast cancer cells transfected with progesterone receptor complementary DNA. *The American Journal of Pathology*, 162(6), 1781-1787. doi:10.1016/S0002-9440(10)64313-1
- Liu, H., Liu, R., Ullah, I., Zhang, S., Sun, Z., Ren, L., & Yang, K. (2020). Rough surface of copper-bearing titanium alloy with multifunctions of osteogenic ability and antibacterial activity. *Journal of Materials Science & Technology*, 48, 130-139. doi:10.1016/j.jmst.2019.12.019
- Liu, P., Smits, J., Ayers, D. C., & Song, J. (2011). Surface mineralization of Ti6Al4V substrates with calcium apatites for the retention and local delivery of recombinant human bone morphogenetic protein-2. *Acta biomaterialia*, 7(9), 3488–3495. doi:10.1016/j.actbio.2011.05.025
- Liu, Y., Liang, J., Wang, Q., He, Y., & Chen, Y. (2016). Copper nanoclusters trigger muscle cell apoptosis and atrophy in vitro and in vivo. *Journal of Applied Toxicology*, 36, 454–463. doi:10.1002/jat.3263
- Lu, Y., Cai, W. -j., Ren, Z., & Han, P. (2022). The role of staphylococcal biofilm on the surface of implants in orthopedic infection. *Microorganisms*, 10(1909), 10. doi:10.3390/microorganisms10101909
- Mahmoudi, P., Akbarpour, M. R., Lakeh, H. B., Jing, F., Hadidi, M. R., & Akhavan, B. (2022). Antibacterial Ti-Cu implants: A critical review on mechanisms of action. *Materials Today Bio*, 17, 100447. doi:10.1016/j.mtbio.2022.100447
- Manakhov, A. M., Sitnikova, N. A., Tsygankova, A. R., Alekseev, A. Y., Adamenko, L. S., Permyakova, E., . . . Solovieva, A. O. (2021). Electrospun biodegradable nanofibres coated

- homogenously by Cu magnetron sputtering exhibit fast ion release. Computational and experimental study. *Membranes* , 11(12), 965.
- Manfredi, D., & Calignano, F. (2017). Laser powder bed fusion of aluminum, titanium and nickel based alloys: Materials and design investigations. *2017 International Conference on Electromagnetics in Advanced Applications (ICEAA)*. Verona: 2017 International Conference on Electromagnetics in Advanced Applications (ICEAA). doi:10.1109/ICEAA.2017.8065546
- Manhas, R., Rathore, A., Havelikar, U., Mahajan, S., Gandhi, S. G., & Mahapa, A. (2024). Uncovering the potentiality of quinazoline derivatives against *Pseudomonas aeruginosa* with antimicrobial synergy and SAR analysis. *The Journal of Antibiotics*.
- Modica-Napolitano, J. S., Bharath, L. P., Hanlon, A. L., & Hurley, L. D. (2019). The anticancer agent elesclomol has direct effects on mitochondrial bioenergetic function in isolated mammalian mitochondria. *Biomolecules*, 9(8), 298. doi:10.3390/biom9080298
- Muñoz-Escobar, A., Ruíz-Baltazar, Á. d., & Reyes-López, S. Y. (2019). Novel route of synthesis of PCL-CuONPs composites with antimicrobial properties. *Dose-Response*, 17(3), 1-11. doi:10.1177/1559325819869502
- Naderi, N., Griffin, M., Malins, E., Becer, R., Mosahebi, A., Whitaker, I. S., & Seifalian, A. M. (2016). Slow chlorine releasing compounds: A viable sterilisation method for bioabsorbable nanocomposite biomaterials. *Journal of Biomaterials Applications*, 30(7), 1114-1124. doi:10.1177/0885328215613666
- Ng, H. P., Nandwana, P., Devaraj, A., Semblanet, M., Nag, S., Nakashima, P. N., . . . Banerjee, R. (2015). Conjugated precipitation of twin-related α and Ti₂Cu phases in a Ti–25V–3Cu alloy. *Acta Materialia*, 84, 457-471. doi:10.1016/j.actamat.2014.10.053
- Niioka, H., Asatani, S., Yoshimura, A., Ohigashi, H., Tagawa, S., & Miyak, J. (2018). Classification of C2C12 cells at differentiation by convolutional neural network of deep learning using phase contrast images. *Human Cell*, 31(1), 87–93. doi:10.1007/s13577-017-0191-9
- Omni Calculator. (2024). *Cell Dilution Calculator*. Retrieved July 10, 2024, from <https://www.omnicalculator.com/biology/cell-dilution>
- Pacilio, S., Costa, R., Papa, V., Rodia, M. T., Gotti, C., Pagnotta, G., . . . Focarete, M. L. (2023). Electrospun Poly(L-lactide-co- ϵ -caprolactone) scaffold potentiates C2C12 myoblast

- bioactivity and acts as a stimulus for cell commitment in skeletal muscle myogenesis. *Bioengineering*, 10(2), 239. doi:10.3390/bioengineering10020239
- Prinzi, A., & Rohde, R. (2023). *The Role of Bacterial Biofilms in Antimicrobial Resistance*. Retrieved November 15, 2024, from <https://asm.org/articles/2023/march/the-role-of-bacterial-biofilms-in-antimicrobial-re>
- Pro-lab Diagnostic. (2011). *Microbank™ Worldwide Performance Portfolio*. Retrieved December 3, 2023, from <https://www.pro-lab-direct.com/v/vspfiles/microbank/microbank-wwp-portfolio.pdf>
- Pye, A. D., Lockhart, D. E., Dawson, M. P., Murray, C. A., & Smith, A. J. (2009). A review of dental implants and infection. *Journal of Hospital Infection*, 72(2), 104-110. doi:10.1016/j.jhin.2009.02.010
- Rabionet, M., Yeste, M., Puig, T., & Ciurana, J. (2017). Electrospinning PCL scaffolds manufacture for three-dimensional breast cancer cell culture. *Polymer*, 9(8), 328. doi:10.3390/polym9080328
- Rampurawala, A., & Patil, A. (2021). Surface Modification of Titanium Orthodontic Implants. In D. Gabrić, & M. Vuletić (Eds.), *Current Concepts in Dental Implantology* (pp. 1-30). London: IntechOpen. doi:10.5772/intechopen.100038
- rapidmicrobiology.com. (2010). 2010. Retrieved March 24, 2025, from <https://www.rapidmicrobiology.com/archived-news?id=1041h5>
- Raquid, J. P., Prieto, J. C., Arciaga, D. M., Corpuz, R. H., Llorente, S. M., Mabbayad, J. M., . . . Paraoan, C. E. (2018). Role of *Pseudomonas aeruginosa* biofilm in the development of antibiotic resistance in *Escherichia coli* S M Llorente, J. *Asian Journal of Biological and Life Sciences*, 7(2), 40-46. doi:10.5530/AJBLS.2018.7.2
- Rediguieri, C. F., Sassonia, R. C., Dua, K., Kikuchi, I. S., & de Jesus Andreoli Pinto, T. (2016). Impact of sterilization methods on electrospun scaffolds for tissue engineering. *European Polymer Journal*, 82, 181-195. doi:10.1016/j.eurpolymj.2016.07.016
- Ren, J., Ma, Z., Li, M., Zhang, Y., Liu, W., Liao, Z., & Yang, K. (2014). Antibacterial properties of Ti-6Al-4V-xCu alloys. *Journal of Materials Science & Technology*, 30(7), 699-705.
- Ribeiro, M., Monteiro, F. J., & Ferraz, M. P. (2012). Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. *Biomatter*, 2(4), 176-194. doi:10.4161/biom.22905

- Rodríguez-Tobías, H., Morales, G., & Grande, D. (2019). Comprehensive review on electrospinning techniques as versatile approaches toward antimicrobial biopolymeric composite fibers. *Materials Science and Engineering: C*, 101, 306-322. doi:10.1016/j.msec.2019.03.099
- Rogers, W. R. (2005). *Sterilisation of Polymer Healthcare Products*. Shrewsbury: Rapra Technology.
- Ryan, B. M., Dougherty, T. J., Beaulieu, D., Chuang, J., Dougherty, B. A., & Barrett, J. F. (2001). Efflux in bacteria: what do we really know about it? *Expert Opinion on Investigational Drugs*, 10(8), 1409-1422. doi:10.1517/13543784.10.8.1409
- Saito, M., Karakida, T., Yamamoto, R., Nagano, T., Yamakoshi, Y., Hayakawa, T., . . . Gomi, K. (2014). Differentiation potential of osteoblast from cultured C2C12 cells on zirconia disk. *Dental Materials Journal*, 33(2), 275–283. doi:10.4012/dmj.2013-321
- Sanchez Armengol, E., & Laffleur, F. (2021). The progress on sulfhydryl modified polymers with regard to synthesis, characterization and mucoadhesion. *International Journal of Pharmaceutics*, 592, 120016. doi:10.1016/j.ijpharm.2020.120016
- Sarwar, M. N., Ali, H. G., Ullah, S., Yamashita, K., Shahbaz, A., Nisar, U., . . . Kim, I. -S. (2022). Electrospun PVA/CuONPs/bitter gourd nanofibres with improved cytocompatibility and antibacterial properties: Application as antibacterial wound dressing. *Polymers*, 14(7), 1361. doi:10.3390/polym14071361
- Shearer, H., Ellis, M. J., Perera, S. P., & Chaudhuri, J. B. (2006). Effects of common sterilization methods on the structure and properties of poly(D,L lactic-co-glycolic acid) scaffolds. *Tissue Engineering*, 12(10), 2717-2727. doi:10.1089/ten.2006.12.2717
- Shi, R., Jin, C., Xie, H., Zhang, Y., Li, X., Dai, Q., & Kong, L. (2019). Multi-plane, wide-field fluorescent microscopy for biodynamic imaging in vivo. *Biomedical Optics Express*, 10(12), 6625–6635. doi:10.1364/BOE.10.006625
- Shirai, T., Tsuchiya, H., Shimizu, T., Ohtani, K., Zen, Y., & Tomita, K. (2009). Prevention of pin tract infection with titanium-copper alloys. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 91B(1), 373-380. doi:10.1002/jbm.b.31412
- Sivakumar, S., Schmid, R., Wieland, A., Strissel, P. L., Strick, R., Fischer, L., . . . Kengelbach-Weigand, A. (2022). Role of fiber thickness and surface treatment of electrospun

- polycaprolactone matrices on the growth of different breast cancer-associated cells. *Advanced Materials Interfaces*, 9, 2101808. doi:10.1002/admi.202101808
- Soles, A., Selimovic, A., Sbrocco, K., Ghannoum, F., Hamel, K., Moncada, E. L., . . . Cvetanovic, M. (2023). Extracellular matrix regulation in physiology and in brain disease. *International Journal of Molecular Sciences*, 24(8), 7049. doi:10.3390/ijms24087049
- Song, J., Gao, H., Zhu, G., Cao, X., Shi, X., & Wang, Y. (2016). The construction of three-dimensional composite fibrous macrostructures with nanotextures for biomedical applications. *Biofabrication*, 8(3), 035009. doi:10.1088/1758-5090/8/3/035009
- Tanner, A., Maiden, M. F., Lee, K., Shulman, L. B., & Weber, H. P. (1997). Dental implant infections. *Clinical Infectious Diseases, Suppl 2*, S213-S217. doi:10.1086/516243
- Tarannum, N., Khatoon, S., & Dzantiev, B. B. (2020). Perspective and application of molecular imprinting approach for antibiotic detection in food and environmental samples: A critical review. *Food Control*, 118, 107381. doi:10.1016/j.foodcont.2020.107381
- Tenover, F. C. (2009). Antibiotic Susceptibility Testing. In M. Schaechter (Ed.), *Encyclopedia of Microbiology* (3rd ed., pp. 67-77). Cambridge: Academic Press. doi:10.1016/B978-012373944-5.00239-X
- U.S. Centers for Disease Control and Prevention (CDC). (2024). *Antimicrobial Resistance Threats in the United States, 2021-2022*. Retrieved November 11, 2024, from <https://www.cdc.gov/antimicrobial-resistance/data-research/threats/update-2022.html>
- Ukwuoma, C. O., Okolie, N., & Nwosu, D. C. (2014). Effect of paper quality of locally made antibiotic discs on antibiotic sensitivity of some clinical bacterial isolates. *Asian Journal of Phytomedicine and Clinical Research*, 2(1), 11-21.
- Wang, S., Zhang, B., Li, T., Li, Z., & Fu, J. (2020). Soil vanadium(V)-reducing related bacteria drive community response to vanadium pollution from a smelting plant over multiple gradients. *Environment International*, 138, 105630. doi:10.1016/j.envint.2020.105630
- Wang, X., Dong, H., Liu, J., Qin, G., Chen, D., & Zhang, E. (2019). In vivo antibacterial property of Ti-Cu sintered alloy implant. *Materials Science and Engineering: C*, 100, 38-47. doi:10.1016/j.msec.2019.02.084
- Washington, J. A., & Wilson, W. R. (1985). Erythromycin: a microbial and clinical perspective after 30 years of clinical use (First of Two Parts). *Mayo Clinic Proceedings*, 60(3), 189–203. doi:10.1016/s0025-6196(12)60219-5

- Wei, Z., Shan, Z., & Shaikh, Z. A. (2018). Epithelial-mesenchymal transition in breast epithelial cells treated with cadmium and the role of Snail. *Toxicology and Applied Pharmacology*, 344, 46–55. doi:10.1016/j.taap.2018.02.022
- World Health Organization (WHO). (2024). *WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance*. Retrieved November 9, 2024, from <https://www.who.int/publications/i/item/9789240093461>
- Wu, J. -H., Chen, K. -K., Chao, C. -Y., Chang, Y. -H., & Du, J. -K. (2020). Effect of Ti2Cu precipitation on antibacterial property of Ti-5Cu alloy. *Materials Science and Engineering: C*, 108, 110433. doi:10.1016/j.msec.2019.110433
- Xin, C., Wang, N., Chen, Y., He, B., Zhao, Q., Chen, L., . . . Yang, X. (2022). Biological corrosion behaviour and antibacterial properties of Ti-Cu alloy with different Ti2Cu morphologies for dental applications. *Materials & Design*, 215, 110540. doi:10.1016/j.matdes.2022.110540
- Xu, C., Wang, Q., Feng, X., & Bo, Y. (2014). Effect of evodiagenine mediates photocytotoxicity on human breast cancer cells MDA-MB-231 through inhibition of PI3K/AKT/mTOR and activation of p38 pathways. *Fitoterapia*, 99, 292-299. doi:10.1016/j.fitote.2014.10.010
- Yoganarasimha, S., Trahan, W. R., Best, A. M., Bowlin, G. L., Kitten, T. O., Moon, P. C., & Madurantakam, P. A. (2014). Peracetic acid: A practical agent for sterilizing heat-labile polymeric tissue-engineering scaffolds. *Tissue Engineering Part C: Methods*, 20(9), 714-723. doi:10.1089/ten.tec.2013.0624
- Zhang, E., Li, F., Wang, H., Liu, J., Wang, C., Li, M., & Yang, K. (2013). A new antibacterial titanium–copper sintered alloy: preparation and antibacterial property. *Materials Science and Engineering C*, 33, 4280-4287.
- Zhang, E., Wang, X., Chen, M., & Hou, B. (2016). Effect of the existing form of Cu element on the mechanical properties, bio-corrosion and antibacterial properties of Ti-Cu alloys for biomedical application. *Materials Science and Engineering: C*, 69, 1210-1221. doi:10.1016/j.msec.2016.08.033
- Zhao, Y., Huang, Q., Bai, F., Luo, M., & Tang, J. (2023). Characterization of Staphylococcus aureus and methicillin-resistant S. aureus from yak. *Food Safety and Health*, 1(1), 82–92. doi:10.1002/fsh3.12011

- Zhu, L., Elguindi, J., Rensing, C., & Ravishankar, S. (2012). Antimicrobial activity of different copper alloy surfaces against copper resistant and sensitive *Salmonella enterica*. *Food Microbiology*, 30, 303-310. doi:10.1016/j.fm.2011.12.001
- Ziperstein, M. J., Guzman, A., & Kaufman, L. J. (2015). Breast cancer cell line aggregate morphology does not predict invasive capacity. *PLoS One*, 10(9), e0139523. doi:10.1371/journal.pone.0139523

CHAPTER 7 CONCLUDING REMARKS AND RECOMMENDATIONS

7.1. Conclusion

Additive manufacturing (AM), or three-dimensional (3D) printing, technology has evolved over the decades, and the application of AM techniques, such as laser powder bed fusion (LPBF), continues to make great strides in various industries. In the medical sector, AM has multifaceted contributions, including improving human life (of patients and medical practitioners) and unburdening the economy and the environment. Research on the development of novel smart materials with multiple functionalities is invaluable for sustaining the benefits of AM as the industrial revolution advances. Titanium and its alloys are favoured biomaterials because of their outstanding properties. However, complications related to metallic implants persist, with the leading ones being infection and lack of implant stability. The nature of Ti, which lacks antibacterial properties and is bioinert, does not contribute to alleviating implantation complications and may even exacerbate them. Copper (Cu) is widely used for its antibacterial properties, and polymer nanofibres (NFs) are promising as bioactive coatings for metal implants. The current research aimed to improve the clinical functionality of Ti and its alloys, particularly Ti6Al4V, manufactured via LPBF, by leveraging a synergy of technologies.

The LPBF EOSINT M 280 machinery and its operations were comprehensively described. The employed powders, namely Ti6Al4V ELI (also referred to as Ti6Al4V or Ti64 in this chapter), cpTi and Cu, were characterised and found to meet the morphological and elemental requirements of LPBF. Ti6Al4V ELI and cpTi were in situ alloyed with 3 and 6 wt.% Cu. Through the process of single track analysis, a series of hierarchical steps was followed to optimise the production of Ti6Al4V-xCu and Ti-xCu ($x = 3, 6$ wt.%) and cpTi using LPBF. Optimisation commenced with the analysis of the single track to optimise laser power and scanning speed. The double layers were then studied to optimise the hatch distance. The three-dimensional (3D) samples were built, and porosity and microstructure were inspected to determine the final optimal process parameters. Therefore, it was illustrated that LPBF is proficient at manufacturing implant material from elemental powders, in situ alloyed to produce a novel material.

Characteristics and mechanical properties of the optimised alloy samples (that is, Ti6Al4V- x Cu and Ti- x Cu ($x = 3, 6$ wt.%) and cpTi) were determined. The XRD patterns showed that the in situ alloying with Cu particles was accomplished. Surface roughness testing indicated that Cu particles increased the roughness of Ti6Al4V but decreased that of cpTi. Copper increased the microhardness of Ti6Al4V and cpTi. The tensile test demonstrated that the addition of Cu increased the strength and retained elastic modulus, and decreased the ductility (% elongation) of Ti6Al4V and cpTi. Fractography results implied possible improved ductility with additional Cu. The literature proposed heat treatment as a strategy to improve the mechanical behaviour of specimens.

Three-dimensional samples of Ti6Al4V- x Cu and Ti- x Cu ($x = 3, 6$ wt.%) and cpTi were produced from these powder compositions using the optimised parameters. Ti6Al4V ELI samples were manufactured using previously optimised parameters. To coat the surfaces of as-built Ti6Al4V and cpTi with polymer NFs, the electrospinning technology was employed. Various polymer composites and adhesives were explored by testing primarily the adhesion strength of the NF scaffold to the Ti surface to establish the most suitable polymer composition. The compositions that satisfied the adhesion properties were poly(ϵ -caprolactone)/polyurethane (PCL/PU, 3:2 (w/w)) and poly(ϵ -caprolactone)/polyurethane/copper oxide nanoparticles (PCL/PU/CuO NPs, 3:2:0.3 (w/w/w)). Electrospinning was applied to coat the surfaces of Ti6Al4V and cpTi with PCL/PU and PCL/PU/CuO nanofibres with a solution of PU as a primer to promote adhesion at the NF-metal interface. The NFs were then characterised and tested for adhesive strength. The results revealed that the electrospinning technique was effective for coating the Ti surface with nanoscale fibres, and X-ray diffraction (XRD) showed that the CuO NPs were successfully immobilised within the NF matrix.

The ultimate purpose of modifying Ti and its alloys is to achieve functionality in the physiological environment. As such, in vitro analyses were conducted to explore the antibacterial and biocompatibility functionality of in situ Cu-alloyed Ti specimens, namely Ti6Al4V- x Cu and Ti- x Cu ($x = 3, 6$ wt.%) and NF-coated Ti specimens, namely Ti6Al4V-NF, Ti6Al4V-NFCu, Ti-NF

and Ti-NFCu. For these samples, the NF represents PCL/PU, and NFCu denotes PCL/PU/CuO. Unalloyed and bare Ti6Al4V and cpTi were included as controls. Antimicrobial analysis was conducted using the agar diffusion assay and the plate count method. The bacteria used were common isolates from infected dental and orthopaedic implants. They included Gram-positive *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and ATCC 43300, and *Staphylococcus epidermidis* (*S. epidermidis*), and Gram-negative *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Pseudomonas aeruginosa* (*P. aeruginosa*). The agar diffusion assay did not show bactericidal activity of the samples, including those containing Cu/CuO, against the bacterial strains. The plate count methods showed distinct antibacterial properties of the samples, particularly against Gram-positive bacteria. The antibacterial effect was strongly associated with Cu/CuO-containing specimens and was more pronounced in the NF-coated samples. Biocompatibility was observed in a few instances in which enumerated counts were higher than those of the original bacterial suspension, especially for *E. coli*.

C2C12 and MDA-MB-231 cell lines were used to evaluate the biocompatibility and cytotoxicity of the samples. Scanning electron microscopy (SEM) with an energy-dispersive X-ray spectrometer (EDS) and live/dead fluorescence imaging were used to assess the bioactivity of the metal and polymeric surfaces. Cytotoxicity was evaluated using the resazurin cell viability assay. Cytotoxicity was observed among the Cu-alloyed samples. Compared with the control samples, the toxicity against C2C12 and MDA-MB-231 cells was attributed to the Cu content. Cell death in bacterial cells and cell lines was attributed to Cu-induced production of toxic levels of reactive oxygen species (ROS). The results from SEM, EDS, live/dead staining, and cell viability assays showed that the NF-coated samples promoted Ti surface bioactivity more effectively than the bare samples for both C2C12 and MDA-MB-231 cells. Furthermore, NFs are applied to the surface of LPBF Ti and thus do not affect its signature mechanical properties. Therefore, coating Ti implants with PCL/PU and PCL/PU/CuO may be a feasible approach to achieving functional implants and improving implantation success.

The envisaged contributions from the experimental investigations are as follows:

- » Expand the production of biomedical implants from novel metallic feedstock using additive manufacturing techniques such as LPBF and provide the procedure for optimising the novel powder compositions to obtain highly dense objects.
- » Illustrate the application of traditional methods to determine the mechanical properties of the novel metallic devices with the intent of promoting the mechanical functioning of the devices in biomedical applications.
- » Activate the bioactivity of the innately inert surfaces of metal implants by coating them with sufficiently adherent nanofibre scaffolds. The nanostructure of the organic scaffold mimics the extracellular matrix (ECM) components of the human body and is thus envisaged to mimic the function of ECM in the corporeal environment. The implantation period may be extended by alleviating challenges associated with implant stability.
- » Demonstrate the application of the nanofibre scaffolds from novel biocompatible polymer compositions using a simple, yet sophisticated, and well-researched electrospinning technology.
- » Successfully incorporate metal/metal oxide-based antibacterial agents in the novel metal and/or polymer compositions towards the reduction of the infection rate associated with medical implants.
- » Provide research protocols for the validation of the functionality of metal alloys alloyed with antibacterial agents and/or coated with polymer nanofibres with or without imbuing the nanofibres with antibacterial elements. The protocols include using traditional methods adapted to test the novel materials under a physiological environment, and allow for additional adaptation for future research.

7.2. Verification of research objectives

The research objectives that motivated the present project are listed below, along with the suggested solutions derived from the intensive numerical and experimental work.

1. To determine the process parameters for in situ alloying of Ti6Al4V-xCu and Ti-xCu ($x = 3$ and 6 wt.%) using LPBF technology.

The description of the in situ alloy process of Ti6Al4V-xCu and Ti-xCu ($x = 3$ and 6 wt.%) is detailed in Chapter 3. The systematic, hierarchical approach, commonly used to optimise the processing parameter of LPBF technology for cpTi, Ti6Al4V-xCu and Ti-xCu ($x = 3$ and 6 wt.%) is also described in Chapter 3. The chapter concludes with the listing of the achieved optimum process parameters that produced highly dense 3D objects.

2. To print Ti6Al4V-xCu, cpTi and Ti-xCu ($x = 3, 6$ wt.%) test samples via LPBF for mechanical and biological analyses.

The LPBF fusion optimum process parameters achieved in Chapter 1 were used to successfully fabricate dense 3D specimens with the required geometry for each experiment, as discussed in Chapters 4 (determination of mechanical properties), 5 (electrospinning coating with polymer NFs), and 6 (antibacterial and tissue culture evaluations).

3. To investigate the mechanical properties of uncoated Ti6Al4V, Ti6Al4V-xCu, cpTi and Ti-xCu ($x = 3$ and 6 wt.%) specimens.

Chapter 4 describes the determination of the mechanical properties of elemental cpTi and in situ alloyed Ti6Al4V-xCu and Ti-xCu ($x = 3$ and 6 wt.%). The mechanical properties were determined for the Vickers microhardness test and the tensile test. The results showed that Cu increased the microhardness and strength of the Ti6Al4V and cpTi, and reduced their ductility.

4. To use the electrospinning apparatus for coating Ti6Al4V and cpTi specimens with polycaprolactone (PCL) and polyurethane (PU) NFs and PCL/PU NFs doped with copper oxide nanoparticles (CuO NPs) and determine the adhesive strength of the NF coatings.

As extensively discussed in Chapter 5, LPBF Ti6Al4V ELI and cpTi specimens were coated with PCL/PU and PCL/PU/CuO NPs nanofibers using an electrospinning machine configured for a simple solution electrospinning technique. PU solution was applied as a primer. A direct pull-off adhesion tester was used to determine the adhesion strength of the NF scaffold onto the Ti64 and

cpTi. The results showed that the adhesion strength values were comparable to those reported in the literature. Moreover, the NF coating remained firmly adhered to the substrate throughout subsequent experiments.

5. To employ biological assays in establishing the antibacterial properties and human tissue interactions of Ti6Al4V-xCu and Ti-xCu ($x = 3$ and 6 wt.%) and NF-coated cpTi and Ti6Al4V specimens.

Chapter 6 provides a detailed description of the biological assays used to determine antibacterial properties, biocompatibility, and cytotoxicity. Bacterial strains commonly associated with orthopaedic and dental implants (namely, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *S. epidermidis*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*) were used. It was established that the NF-coated samples showed potential antibacterial activity, whereas the Cu-alloyed samples did not exhibit convincing antimicrobial efficacy. The NF-coated samples also showed better biocompatibility with the studied cell lines (C2C12 and MDA-MB-231) and lower toxicity than the Cu-alloyed specimens.

7.3. Recommendations for future research

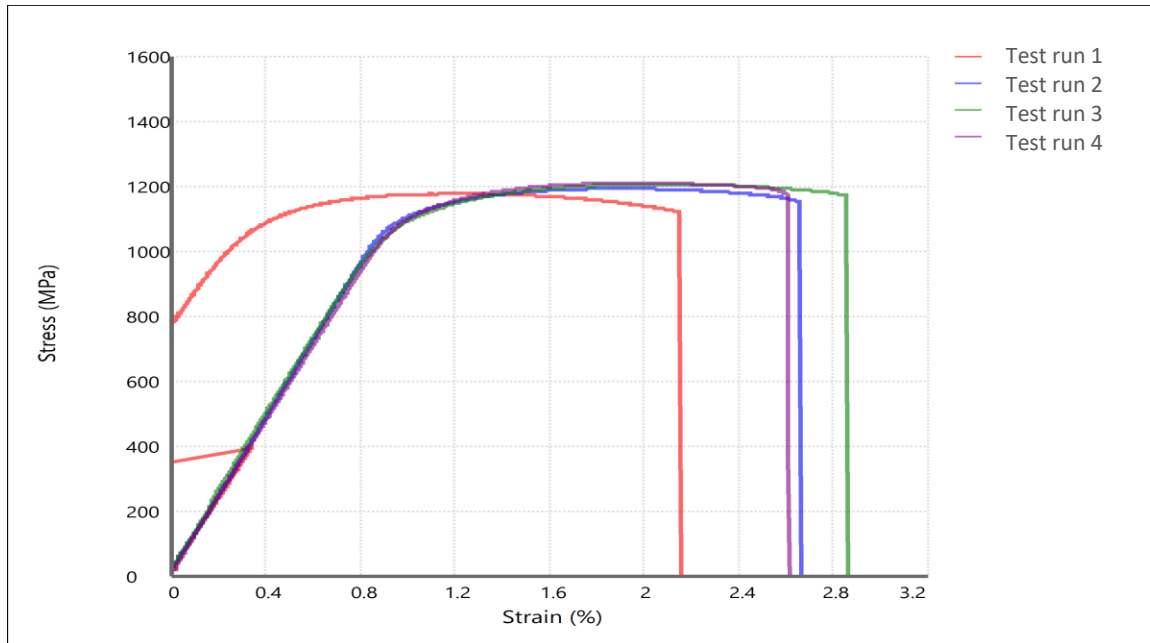
Through research interventions, AM has reached great heights and achieved significant development in the essential yet delicate field of medicine. Therefore, it is through research that more advanced developments may be achieved toward the success of implant surgeries and the subsequent relief of economic burdens and improved human well-being. Recommendations for future research are as follows:

- » The samples produced using the LPBF should be surface-treated using methods such as polishing and acid etching to achieve improved and predictable surface topographies. Surface treatments should also be applied to vary surface roughness and to study the interactions of NF coating, bacteria, and cell lines with the various roughness levels.
- » The use of Cu nanoparticles as an alloy element may enhance the bactericidal effect of Ti metal alloys without inducing undesirable cell toxicity.

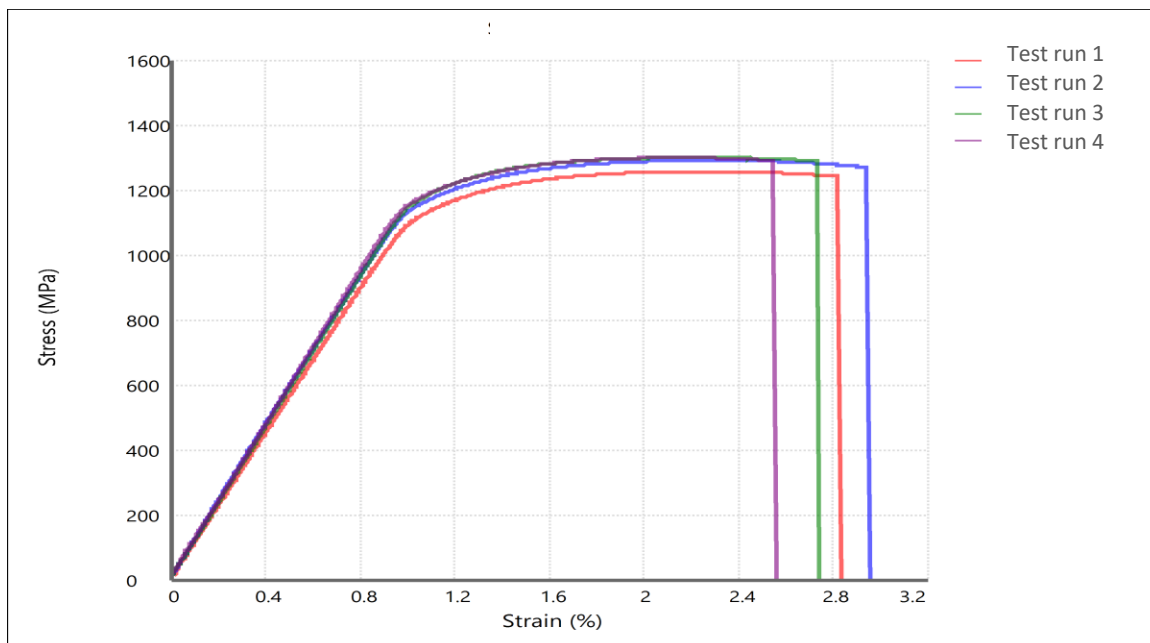
- » In situ manufactured CuO nanoparticles should be characterised to observe morphology, measure particle diameter, and determine chemical composition.
- » Supplementary experiments may provide additional insights into the mechanisms of interactions at the implant-NF-cell and implant-cell interfaces. These experiments include fatigue, compressive testing, wettability, degradation rates, ion release rates, the pullout test, density and corrosion rates in conditions that mimic the physiological environment.
- » Degradation, corrosion, and ion release rates should be investigated over extended periods in solutions that emulate natural human blood plasma and secretions, such as simulated body fluid (SBF) and artificial saliva, to ensure the continued performance and reliability of an implant in vivo. The investigations should be carried out in dynamic environments and with multi-directional, cyclical loading to simulate regular body movements, such as locomotion or limb movements.
- » Anaerobic bacteria such as *Streptococcus mutans*, *Porphyromonas gingivalis* and *Prevotella intermedia* are common inhabitants on dental implants. Biocidal strategies against these bacteria would prove beneficial.
- » Stem cells and/or cell lines that differentiate into osteoblasts, such as mesenchymal stem cells (MSCs), may be included in cell culture studies to assess the ability of specimens to achieve osseointegration.
- » In vivo investigations using animal models to establish the response of the corporal environment, especially the PCL/PU and PCL/PU/CuO coated metal implant materials, towards clinical applicability.
- » Simulation and machine learning models should be used to assist in predicting a broad range of possible outcomes and to better direct experimental work before undertaking extensive in vitro and invasive in vivo methodology.

APPENDIX A: Stress versus strain curves of LPBF cpTi and Ti64 alloys

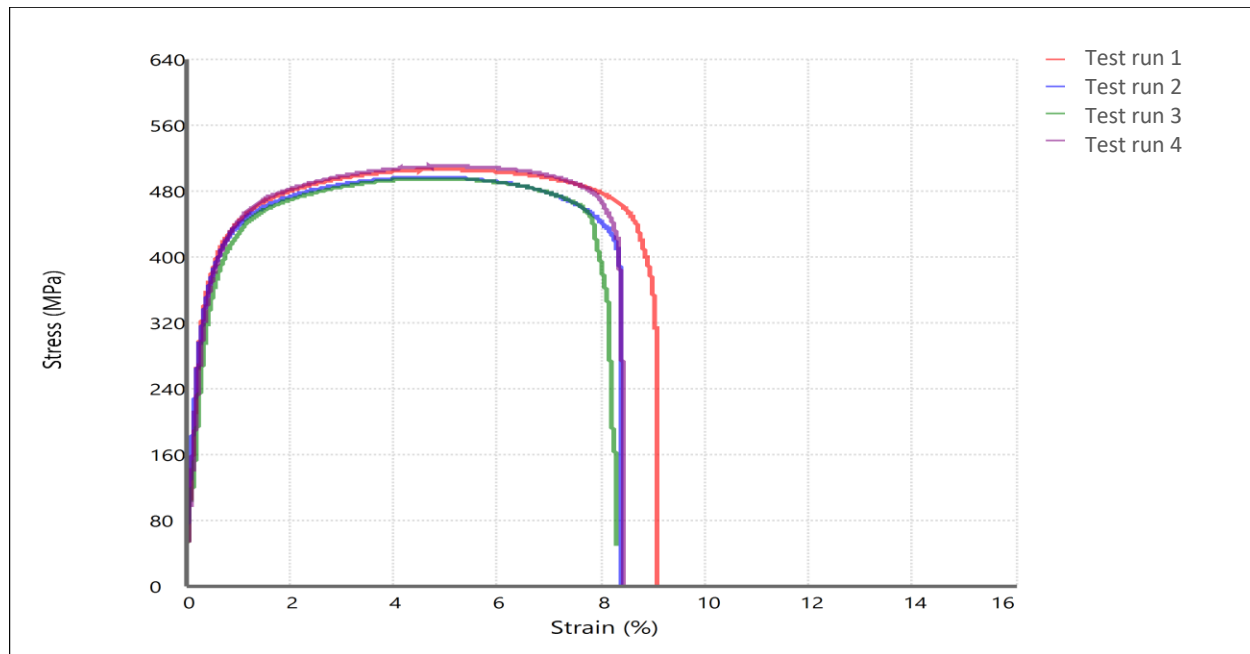
(a) Ti64-3Cu



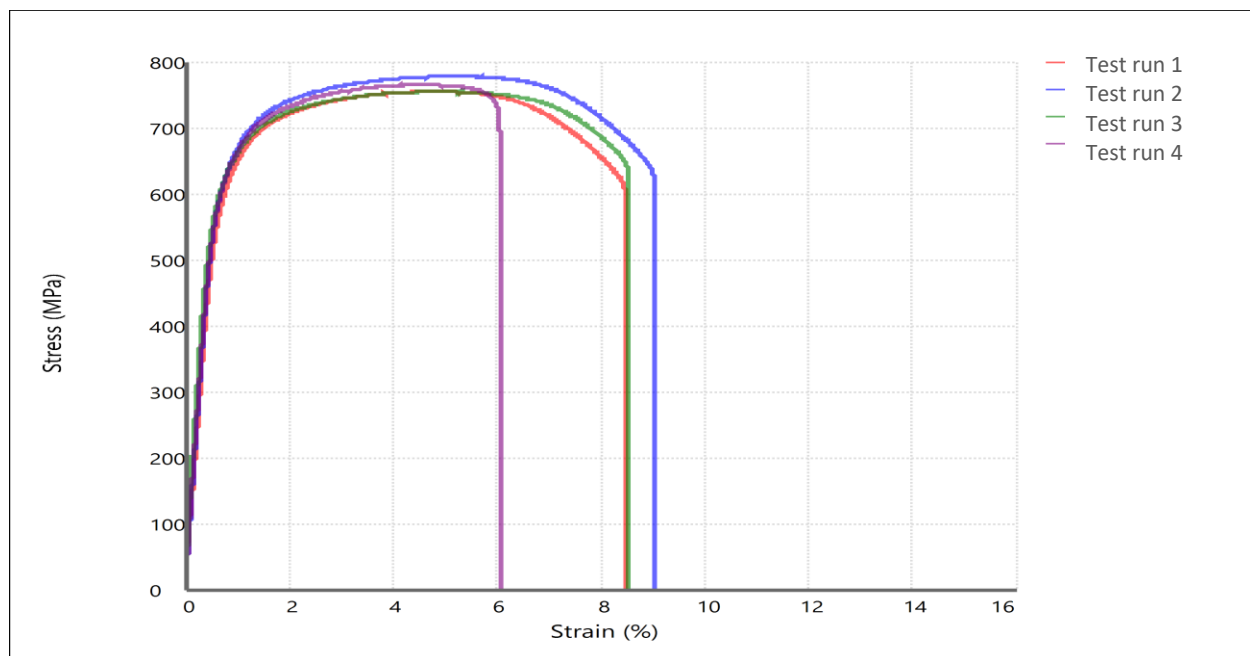
(b) Ti64-6Cu



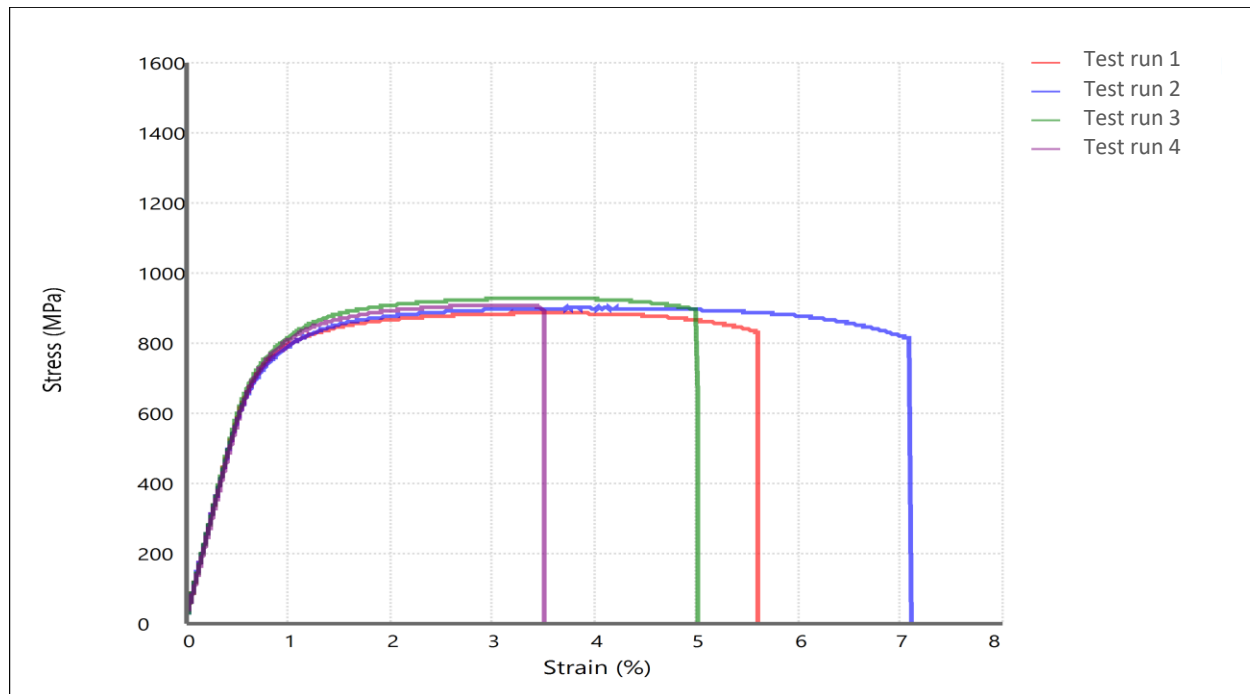
(c) cpTi



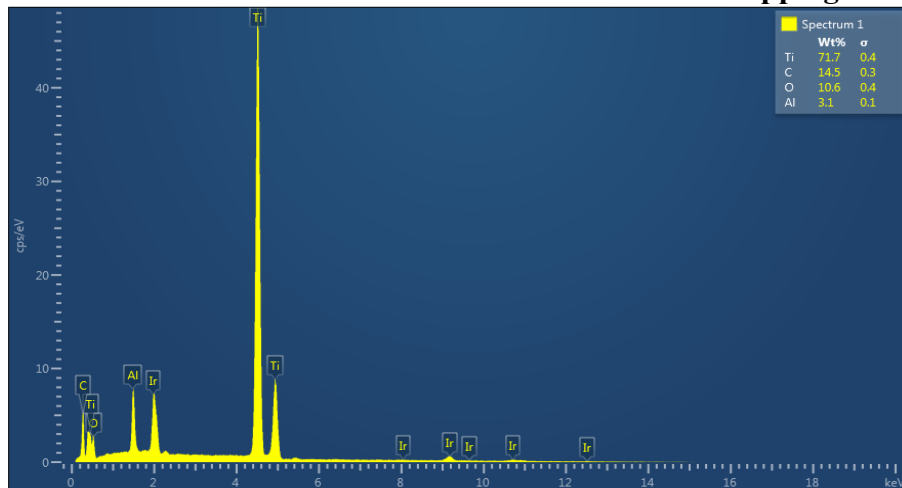
(d) Ti-3Cu



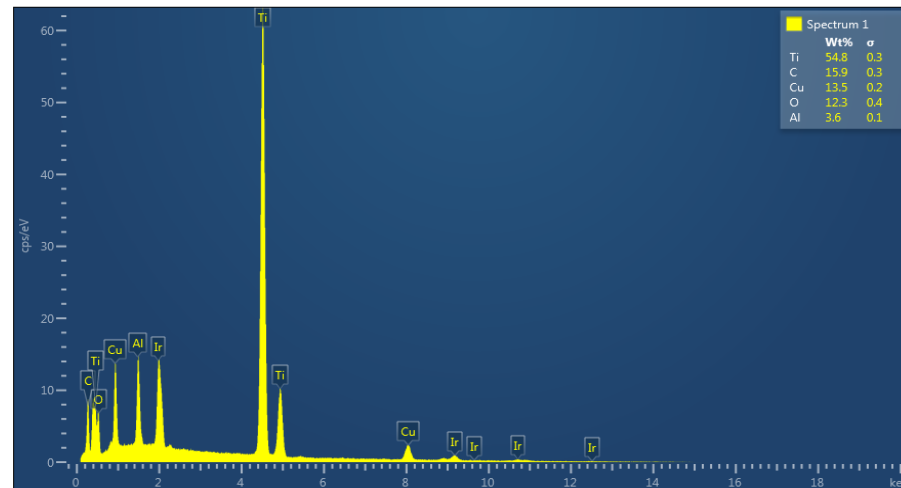
(e) Ti-6Cu



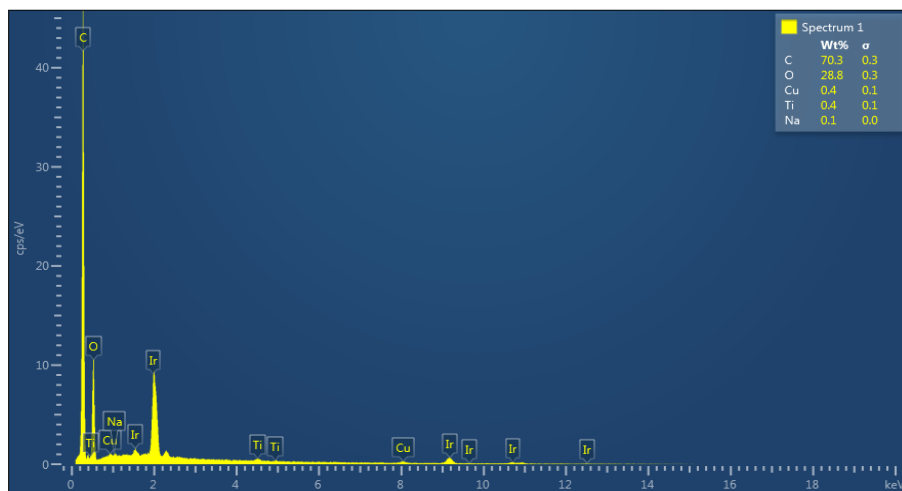
APPENDIX B: Illustrations of EDS elemental mapping for C2C12



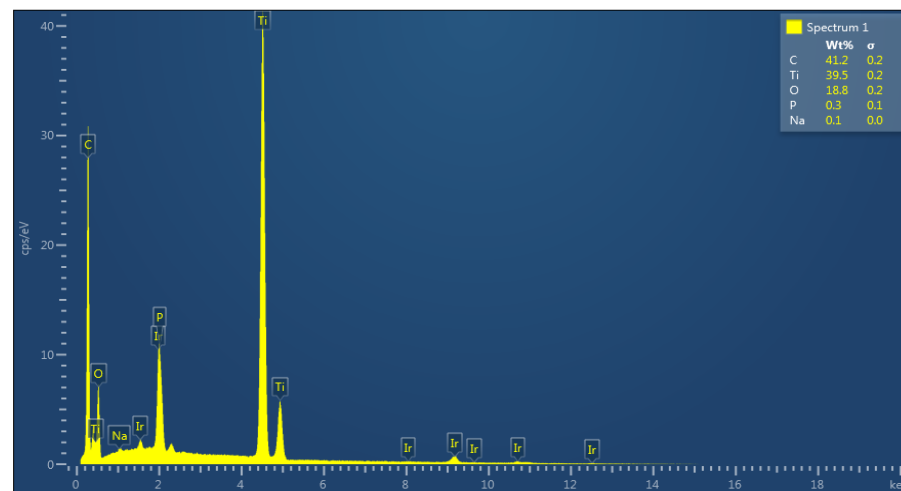
Ti64, 24 hrs



Ti64-3Cu, 24 hrs

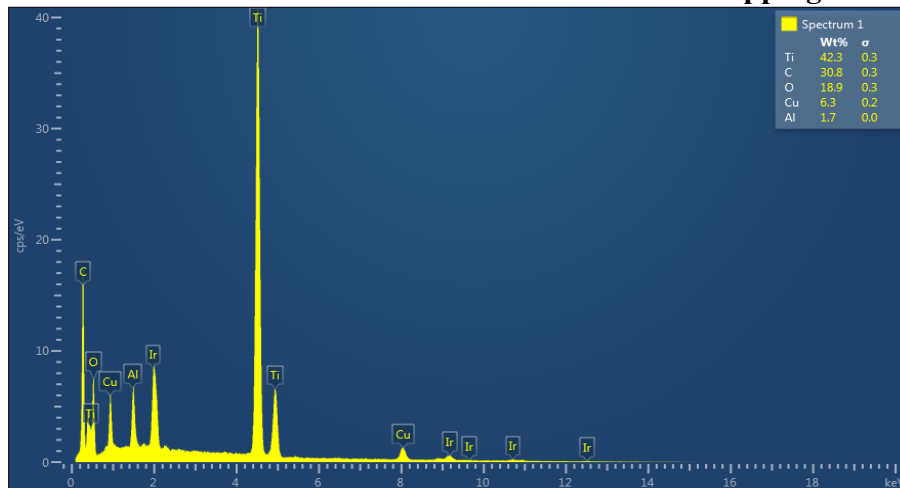


Ti64-NFCu, 5 days

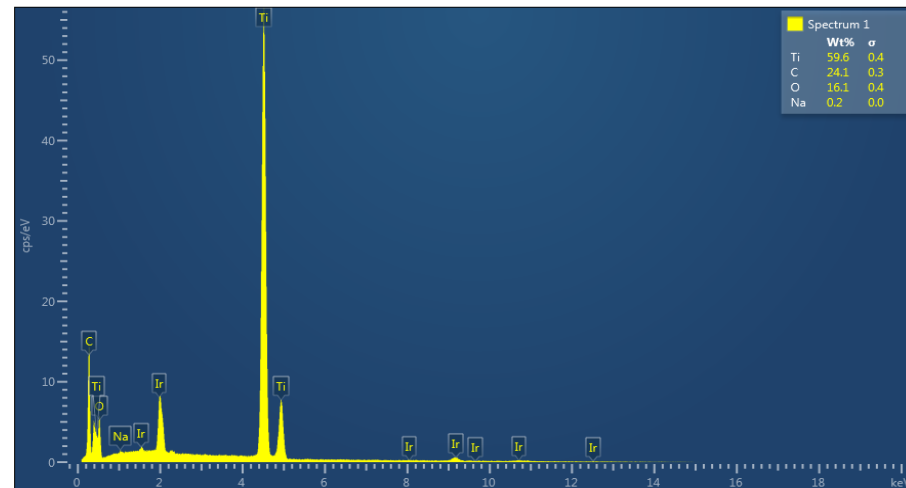


cpTi, 5 days

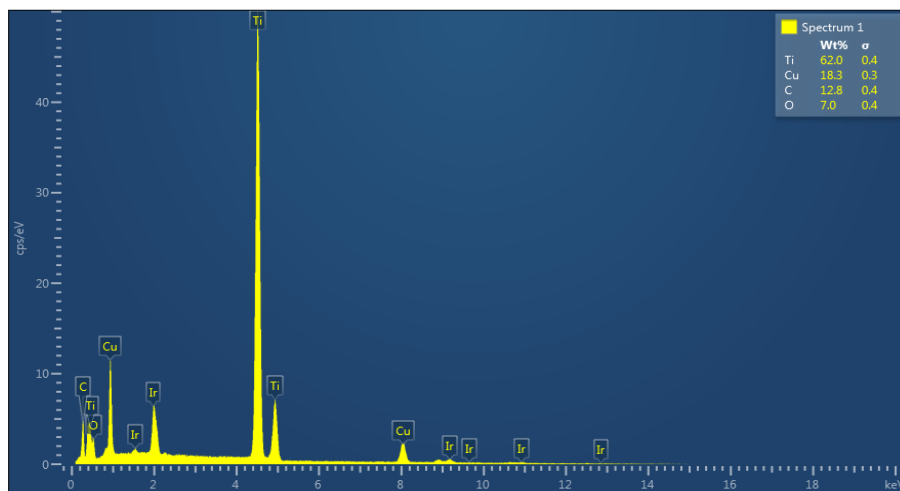
APPENDIX C: Illustrations of EDS elemental mapping for MDA-MB-231



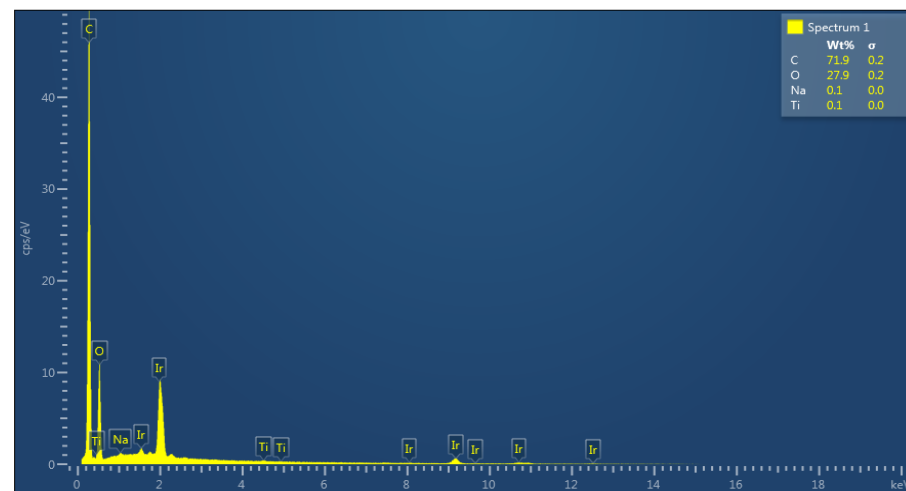
Ti64-6Cu, 24 hrs



cpTi, 24 hrs



Ti-6Cu, 5 days



Ti-NF, 24 hrs

APPENDIX D: Average relative fluorescence units (RFU) from resazurin assay

	C2C12 (RFU)			MDA-MB-231 (RFU)		
Duration	24 hrs	48 hrs	5 days	24 hrs	48 hrs	5 days
Cells only	430.71 ± 66.43	275.43 ± 110.51	764.67 ± 69.88	99.35 ± 37.25	142.33 ± 33.26	301.43 ± 39.77
Ti64	321.67 ± 23.25	35.95 ± 12.65	59.30 ± 22.90	104.20 ± 2.90	246.10 ± 11.00	78.20 ± 8.30
Ti64-3Cu	333.32 ± 16.50	28.50 ± 2.10	45.05 ± 10.65	81.35 ± 8.15	237.75 ± 12.85	55.55 ± 39.75
Ti64-6Cu	366.87 ± 14.85	27.45 ± 14.05	112.80 ± 21.00	77.15 ± 40.95	277.95 ± 30.75	102.20 ± 46.00
Ti64-NF	395.42 ± 63.10	282.80 ± 29.60	1333.75 ± 65.15	68.70 ± 42.10	291.00 ± 14.70	266.50 ± 4.40
Ti64-NFCu	439.67 ± 32.95	225.90 ± 112.90	903.50 ± 428.60	50.56 ± 14.56	243.60 ± 7.60	219.30 ± 45.70
cpTi	535.70 ± 14.00	550.35 ± 27.15	1034.10 ± 105.30	109.75 ± 26.85	197.25 ± 57.45	
Ti-3Cu	677.40 ± 59.50	501.00 ± 52.60	702.00 ± 228.60	102.00 ± 19.90	221.75 ± 13.45	
Ti-6Cu	531.65 ± 67.05	355.80 ± 17.70	298.65 ± 6.45	87.70 ± 5.40	237.15 ± 41.65	
Ti-NF	598.40 ± 18.20	390.80 ± 16.00	881.35 ± 111.65	102.55 ± 0.05	395.25 ± 35.75	
Ti-NFCu	646.25 ± 80.25	494.55 ± 21.65	1130.10 ± 101.90	130.50 ± 17.80	210.15 ± 18.15	