

**DEVELOPMENT OF A SMALL- TO MEDIUM-SIZED RESORBABLE, BONE-  
REGENERATING SCAFFOLD USING ADDITIVE MANUFACTURING**

**by**

**MORAKANE GLORIA MOLETSANE**

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Promoter: Prof W B du Preez, PhD, Pr Sci Nat

Co-promoter: Prof D J de Beer, DTech

Co-promoter: Dr Shathani Nkhwa, PhD

BLOEMFONTEIN, SOUTH AFRICA

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## DECLARATION OF INDEPENDENT WORK

I, MORAKANE MOLETSANE, identity number \_\_\_\_\_ and student number \_\_\_\_\_, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree Doctor of Engineering in Mechanical Engineering, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules, and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

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**25/06/2026**

**DATE**



## ABSTRACT

Lithography-based ceramic is a recent additive manufacturing technology for scaffolds with complex geometry and internal structure. This technology opens the possibility of manufacturing custom-made biodegradable bone regeneration scaffolds. All the specimens used in this investigation were made of hydroxyapatite LithaBone HA480 from Lithoz GmbH. This material is an improvement from the previous material HA400 from Lithoz. The manufacturing of HA480 specimens was done on a CeraFab S65 machine with a layer thickness of 25  $\mu\text{m}$  at Lithoz in Vienna, Austria. The other specimens and implant demonstrator were manufactured at the Centre for Rapid Prototyping and Manufacturing (CRPM), South Africa, using the same material and process parameters. The aim of the study was to create a customisable, resorbable three-dimensional porous bone scaffold with controllable degradation and bone regeneration rates by additive manufacturing. Selection of a suitable lattice structure for the study was done through comparing Maxwell's criteria, porosity and mechanical properties between diamond, rhombic and diagonal lattice structures. The diamond lattice structure was selected because of its Maxwell number indicating a stretch-dominated structure, interconnected pores, and good mechanical properties. The calcium to phosphorus ratio of the HA480 was found to be  $1.64 \pm 0.21$  which was comparable to that of standard hydroxyapatite. Microhardness testing was performed on the solid HA480. Solid HA480 exhibited similar micro-hardness on surfaces parallel ( $556 \pm 25$  HV) and perpendicular ( $559 \pm 27$  HV) to the build direction.

To determine the degradation ability of the material, HA480 diamond lattice structure specimens were immersed in simulated body fluid (SBF) for 1, 7, and 28 days. Biodegradation weight loss results indicated that after 28 days the scaffold degraded by 8.11%. Further investigation regarding biodegradation was done by using scanning electron microscopy (SEM) which revealed evidence of material loss for Day 7 with decreased size of the struts. Surface analysis in the SEM on specimens at Day 28 revealed bone-like apatite, which confirmed that HA480 possessed enhanced bioactivity.

Compression tests were performed according to the standard ISO 13175-3:2012. A set of ten HA480 diamond lattice structure specimens were compressed until failure to determine the compressive strength for dry, as well as hydrated specimens immersed in SBF for 1 and 28 days. The dry condition compressive strength was 10.7 MPa, while for the hydrated specimens, the compressive strength was 7.59 MPa after 1 day and 3.04 MPa after 28 days. The modulus of elasticity for HA480 was  $0.5 \pm 0.4$  GPa for the dry specimens which was higher than for cancellous bone. For the hydrated specimens after 1 day and 28 days of biodegradation the modulus of elasticity was  $0.4 \pm 0.1$  GPa and  $0.6 \pm 0.02$  GPa, respectively.

Specimens of HA480 were sintered and after 28 days were analysed by Fourier transform infrared spectroscopy. The results were comparable to those of standard hydroxyapatite. The surface roughness of HA480 was measured as  $R_a = 0.60 \pm 0.05$   $\mu\text{m}$  which was appropriate for cell attachment. Solid HA480 specimens were seeded with human osteoblast cell line hFOB 1.19 for determining the material's

biocompatibility and cell attachment properties. The fluorescence level results were lower on Day 1, but cell proliferation increased on Day 3, while fluorescence decreased on Days 5 and 7. Optimal cell attachment was observed from Day 1 to Day 7.

An implant demonstrator with a diamond lattice structure was designed and printed in HA480 material. The accuracy of the LCM and the biomedical and mechanical properties of the implant demonstrated that the LCM technology can be used to manufacture custom-designed scaffolds for bone regeneration.

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## **DEDICATION**

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## LIST OF PUBLICATIONS EMANATING FROM THIS STUDY

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## LIST OF ABBREVIATIONS

|                      |                                                                 |
|----------------------|-----------------------------------------------------------------|
| 1D                   | One-dimension                                                   |
| 2D                   | Two-dimensions                                                  |
| 3D                   | Three-dimensions                                                |
| AM                   | Additive Manufacturing                                          |
| ASTM                 | American Society for Testing and Materials                      |
| BCP                  | Biphasic Calcium Phosphate                                      |
| BHA                  | Bovine Hydroxyapatite                                           |
| BMPs                 | Bone Morphogenetic Proteins                                     |
| BTE                  | Bone Tissue engineering                                         |
| CA/P                 | Calcium and Phosphorous ratio                                   |
| CAD                  | Computer Aided Design                                           |
| CAE                  | Computer-Aided Engineering                                      |
| CT                   | Computed Tomography                                             |
| DLP                  | Digital Light Processing                                        |
| DMEM/F-12<br>Mixture | Dulbecco's Modified Eagle Medium (DMEM) and Ham's F-12 Nutrient |
| EOS                  | Electro Optical Systems GmbH                                    |
| FDA                  | Food and Drug Administration                                    |
| FDM                  | Fused Deposition Modelling                                      |
| FEA                  | Finite Element Analysis                                         |
| FTIR                 | Fourier Transform Infrared spectroscopy                         |
| HA                   | Hydroxyapatite                                                  |
| HA480                | LithaBone 480                                                   |
| HA400                | LithaBone 400                                                   |
| hFOB 1.19            | Human Foetal Osteoblastic 1.19                                  |

|              |                                         |
|--------------|-----------------------------------------|
| IGF          | Insulin-like growth factor              |
| LCM          | Lithography-based ceramic manufacturing |
| LPBF         | Laser power bed fusion                  |
| MSC          | Mesenchymal stem cells                  |
| n.s          | Non-significant                         |
| PAM          | Polyacrylamide                          |
| PBS          | Phosphate-buffered saline               |
| PCL          | Polycaprolactone                        |
| PDGF         | Platelet-derived growth factor          |
| pH           | Potential of Hydrogen                   |
| PLA          | Polylactic Acid                         |
| PVA          | Polyvinyl alcohol                       |
| SBF          | Simulated Body Fluid                    |
| SEM          | Scanning Electron Microscopy            |
| SLA          | Stereolithography                       |
| SLS          | Selective Laser Sintering               |
| STL          | Standard Tessellation Language          |
| TGF- $\beta$ | Transforming Growth Factor- $\beta$     |
| TPMS         | Triply Periodic Minimal Surface         |
| USD          | United States Dollar                    |
| XRD          | X-ray Diffraction                       |
| $\beta$ -TCP | $\beta$ -Tricalcium phosphate           |

## CHAPTER 1: INTRODUCTION

### 1.1 Background

In 2024, the global maxillofacial implant market was valued at USD 42.9 billion and is projected to reach USD 55 billion by 2031 due to population growth [1]. This is a forecasted annual market growth rate of 3.61%, as indicated in Figure 1-1. The high market demand presents a huge opportunity to develop innovative solutions and materials.

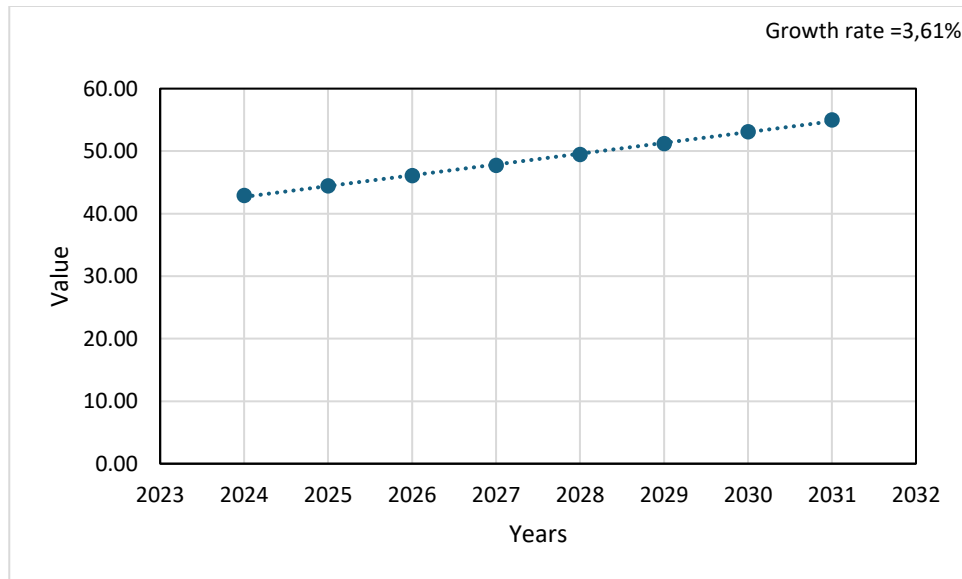


Figure 1-1: Forecast of the global maxillofacial implant market in billion USD for the year 2024 to 2031 [1].

In 2023, South African facial implants generated a revenue of USD 31.5 million. The market is expected to grow by 6.5% to reach USD 49.1 million in 2030 [2]. In 2023, chin and mandibular implants represented the primary revenue-generating product segments. There is local demand for mandibular bone regeneration implants, and the market continues to grow.

In 2019, there were 10.7 million cases of maxillofacial bone fractures reported globally [3]. Maxillofacial fracture mainly involves the fracture of the upper face, upper midface, lower midface, and lower face bones, as displayed in Figure 1-2 [4][5].

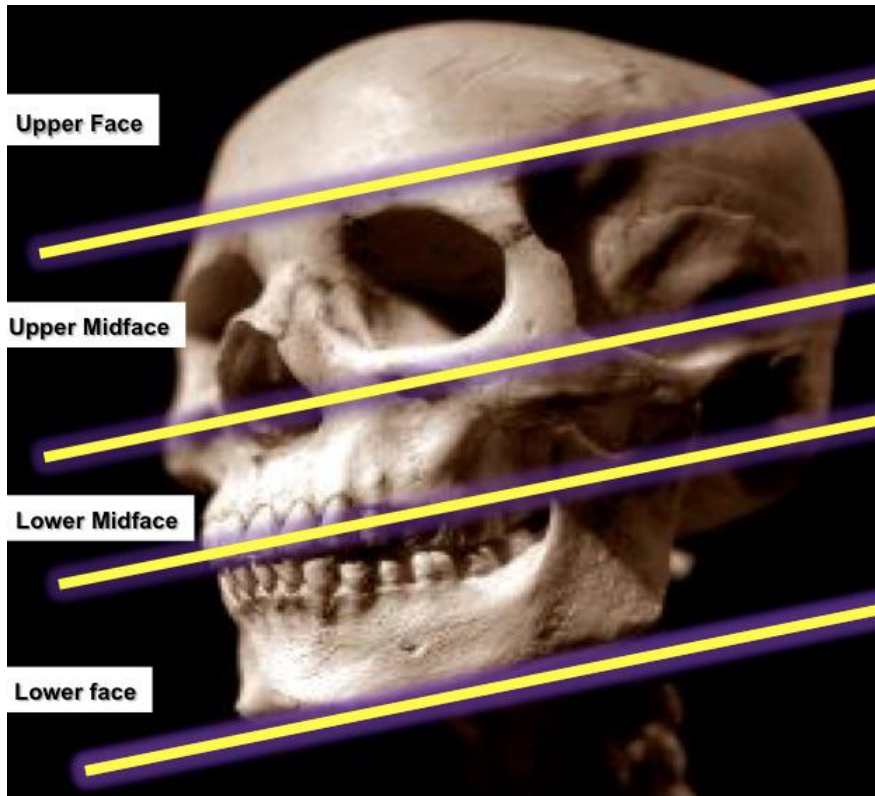


Figure 1-2: Human anatomy of bone structure for maxillofacial [4].

The most common fractures in the lower midface and lower face involve the mandible or maxilla. Figure 1-3a) illustrates that the mandible human anatomy consists of different sections, and one of them is the alveolar process (or ridge). This part holds the teeth in place and creates stability for the teeth [6]. These ridges are found in both maxilla and mandible, and they are made up of the cortical and trabecular bone, as shown in Figure 1-3b) [7]. Cortical bone is the strong outer layer composed of Haversian bone and compacted bone lamella [8]. Trabecular bone is found in the centre of the cortical bone. It is a spongy bone with different pore size [7][8][9].

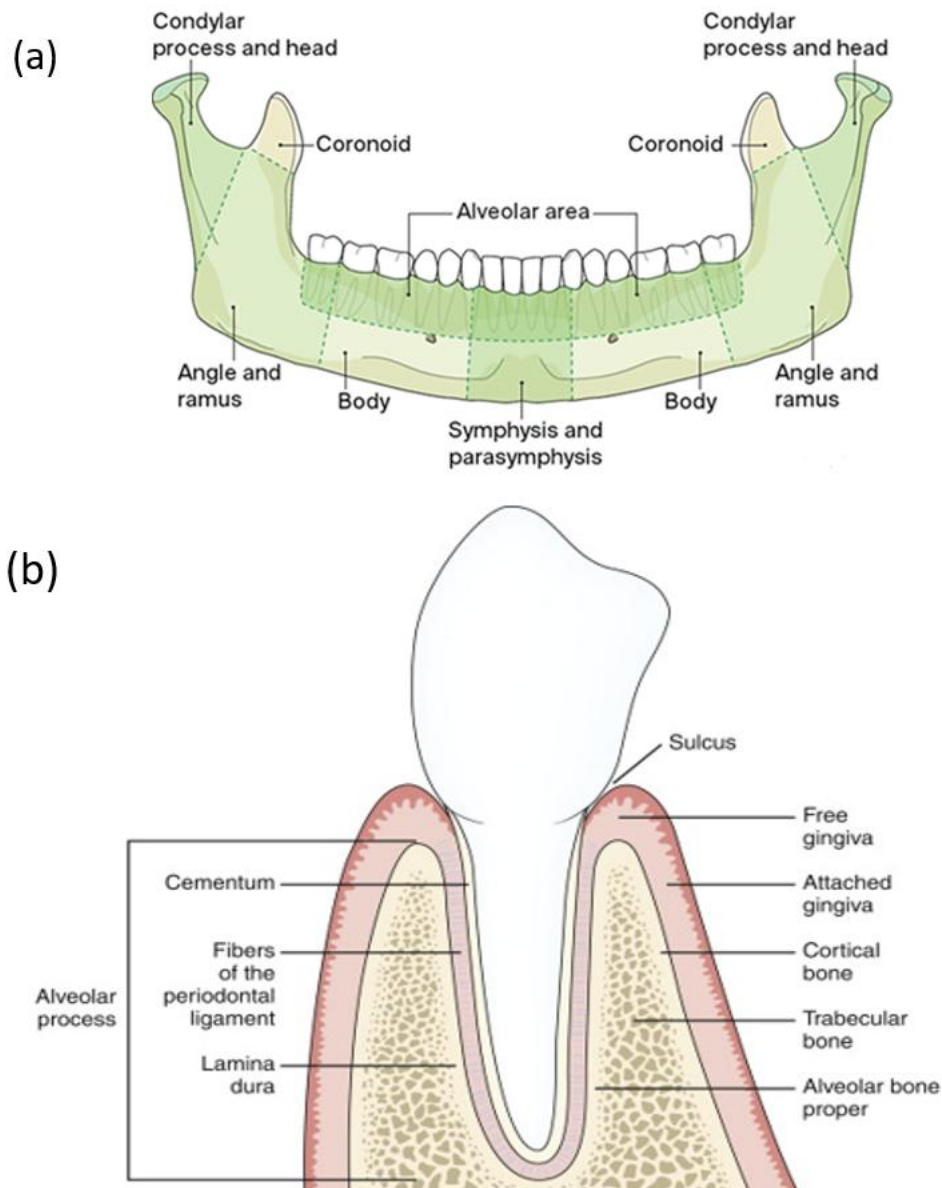


Figure 1-3: (a) Human mandible anatomy (b) anatomical component of tooth, the supporting alveolar bone and their interface[6][7].

The alveolar ridge can fracture due to tooth extraction, tumour removal, poor oral healthcare, or accidents [10]. This resorption occurs due to a lack of mechanical stimulation on the alveolar ridge caused by missing teeth and gum disease. Such resorption can affect a patient's well-being, impacting speech, especially if there are no teeth, and it complicates implant placement. It is estimated that alveolar resorption



reaches 50% after three months of tooth loss. Poor oral care can lead to diseases that ultimately cause a patient to lose their teeth [11][12].

The 2020 WHO report indicates that oral diseases and maxillo-mandibular fractures are some of the burden diseases in South Africa [13]. The oral health diseases, excluding accidental oral fracture, as classified by WHO in the 2019 report on oral diseases in South Africa are shown in Figure 1-4. In this report untreated caries of deciduous teeth in children aged 1-9 is described as the most common oral disease, as well as untreated caries of permanent teeth in individuals aged five and older (27.9 %) [14][15]. From the age of 15 years, periodontal disease starts with inflammation of the gums and alveolar resorption. Edentulism disease, which is a complete loss of teeth by extraction or aging, is the lowest-ranked oral disease [13][16].

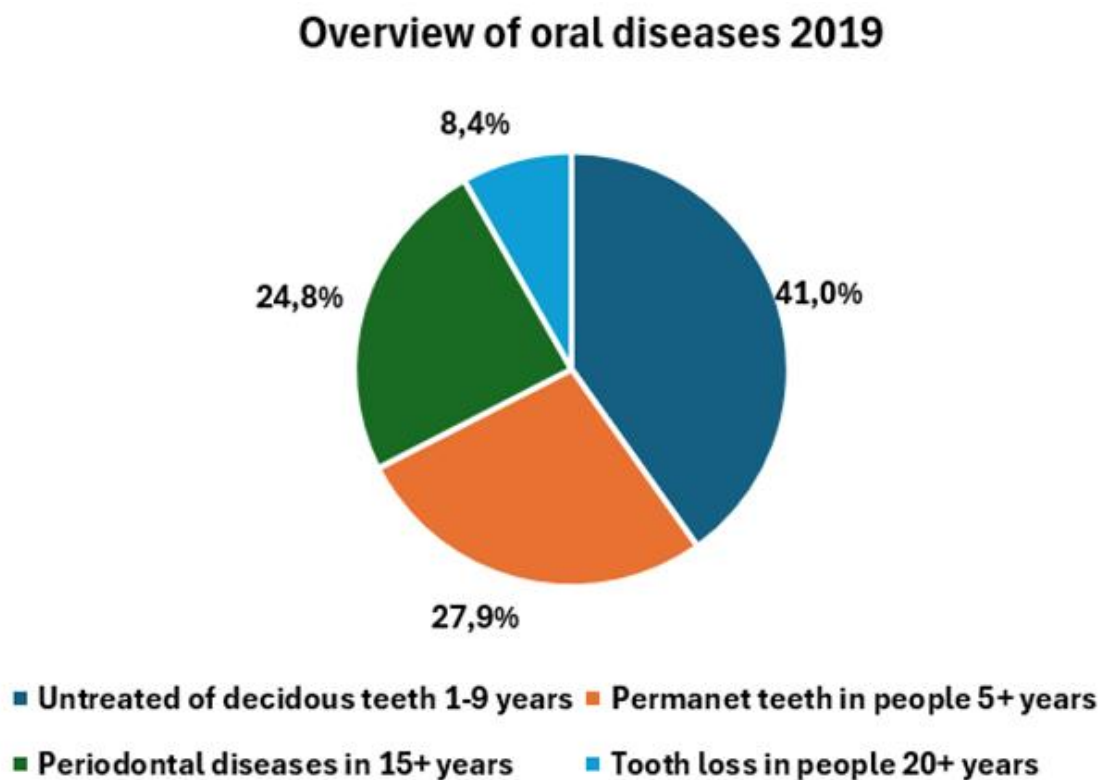


Figure 1-4: Overview of 2019 oral diseases in South Africa [13].

Figure 1-5 illustrates the progression of periodontal diseases, which originate from gingival inflammation and are characterized by subsequent loss of bone support between

teeth [17]. If not treated, these conditions can lead to rapid resorption of alveolar bone as indicated in Figure 1-5 [18]. The oral diseases may be caused by the high intake of sugar, lack of access to healthcare facilities, use of tobacco, genetic inheritance, and high alcohol consumption [13][19]. Genetics influence oral health for example through tooth development, saliva, oral microbes and enamel structure. Alterations in genes predispose teeth decay by compromising strength of the hard tissue despite good hygiene [14].

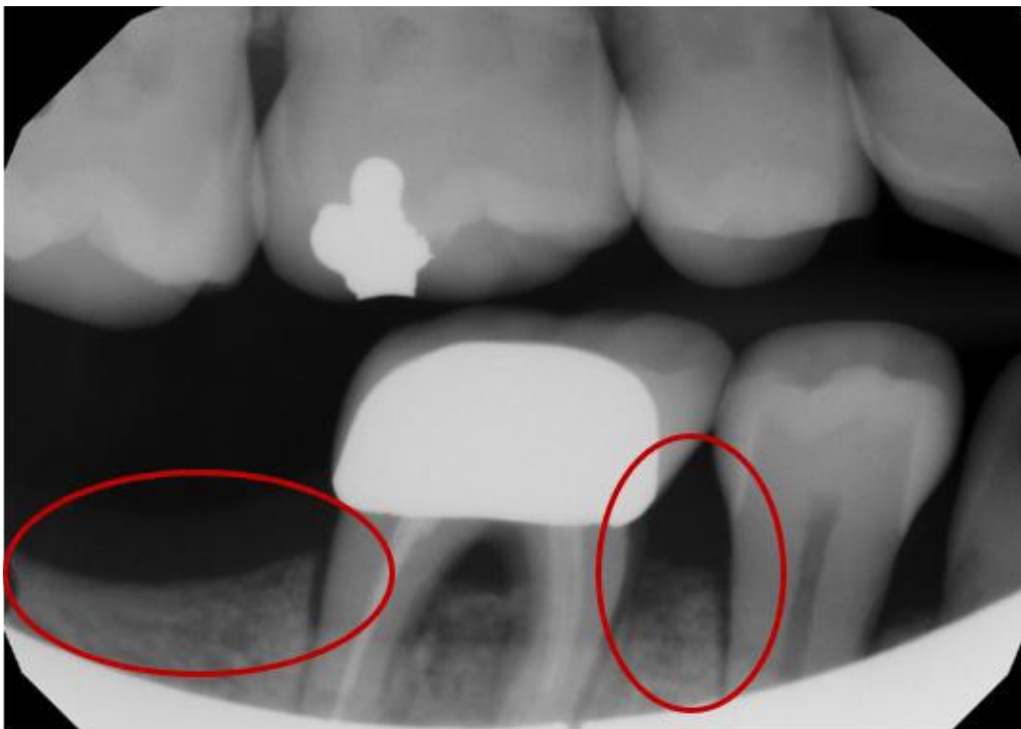


Figure 1-5: Mandibular alveolar ridge with periodontitis disease with horizontal bone loss at the red circle. Light regions are dental plaques [18].

Current external solutions for the alveolar ridge are fixing with plates or wire, synthetic bone substitutes, bone grafting and growth factor substitutes. Growth factor substitute consists of human protein to stimulate cell proliferation such as bone morphogenetic proteins (BMPs) [20][21]. Figure 1-6 presents examples of solutions for alveolar bone augmentation, some are clinically proven and restore the patient's defect, with drawbacks. For instance, autografts, classified as reliable for bone regeneration, are often used for bone regeneration for complete healing of alveolar and future insertion of

implants [3][9][22]. Current research is focusing on improving the synthetic bone substitutes compared to natural bone grafts due to their availability and the unnecessary need for a donor.

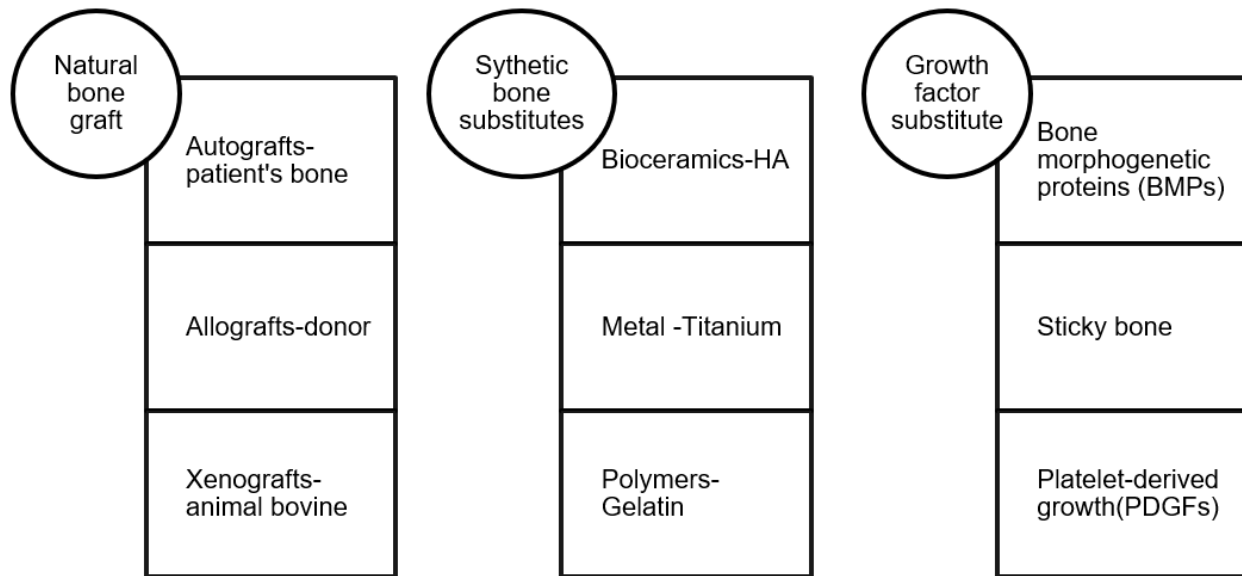


Figure 1-6:Current alveolar ridge bone regeneration augmentation options [23].

Synthetic bone substitutes such as calcium phosphates are made for bone tissue scaffolds which are conventionally manufactured [21][24].The conventional manufacturing of bone scaffolds includes freeze drying, gas foaming, phase separation, and electro-spinning [25][26]. Even though these conventionally manufactured scaffolds show evidence of bone regeneration in clinical trials and animal trials, they have limitations including control over scaffold geometry and pore sizes, pore interconnectivity, and internal channel construction [27][28].

The design and manufacturing limitations of synthetic grafts and conventionally manufactured scaffolds were broadened for bone tissue engineering scaffolds using Additive Manufacturing (AM). According to ASTM F2792-12a, AM is a process where powder or liquid material is joined together layer by layer to form a complete three-dimensional (3D) part [29]. AM has been introduced to manufacture custom-made bone

regenerating scaffolds to control the detailed geometry of the scaffold according to a patient's defect. The 3D porous bone tissue engineering scaffold is temporarily implanted in a bone defect to restore the bone functionality. As a temporary template, it should have the shape and morphology of the lost bone. The scaffold should degrade as new bone fills the defect [30][31][32]. Bioceramics have been successfully used in synthetic bone grafts, demonstrating bioactivity upon implantation. Bioceramics like hydroxyapatite (HA) is a potential material for alveolar ridge scaffolds as it has already been used in bone regenerating conventionally manufactured scaffolds and animal trials [33]. Due to its similarity to the bone and forming part of the inorganic component of the human bone, it has bioactivity to cells when implanted in the defect for bone regeneration. Advantages of HA for bone regeneration include: its calcium-to-phosphorus ratio (Ca/P), which is close to the value of natural bone, crystallinity, and slow resorption rate that can allow bone to regenerate [34][35]. As a ceramic, the drawbacks for HA are its brittleness and it is mostly used for coating on material to improve bioactivity of bone implants or low loading stress and slow degradation which can cause health issues if the bone has completed healing and the material is still remaining [36][37].

The slow degradation can be controlled by the design and use of AM which can address every required design detail, including the internal structures, pore size, and geometry [38]. Due to the high complexity of alveolar fracture and small defects, AM is a potential technique to manufacture a controllable customised bone-regeneration scaffold [25][26].

However, as this a new technology, definitive conclusions regarding the use of alveolar ridge manufacturing for small-sized bone scaffolds have yet to be established. Several international projects are investigating suitable design methods, manufacturing process and materials for bone regeneration [26][38][39]. AM technologies like lithography-based ceramic manufacturing (LCM) still have challenges, including the manufacturing processes of ceramics, shrinkage, and the brittleness of ceramics, which can lead to cracks during sintering and failure of the scaffold [40][41]. Figure 1-7 shows the shrinkage of HA after sintering.

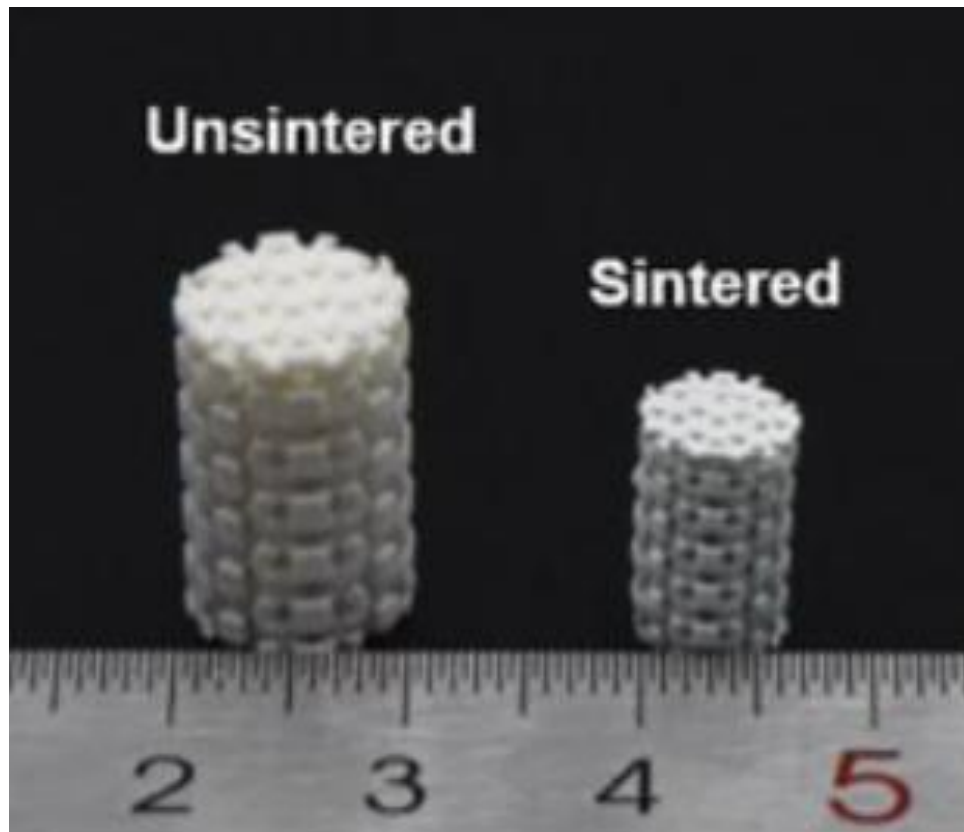


Figure 1-7: Photograph of HA shrinkage of LCM manufactured before and after sintering [42]

The shrinkage of HA can affect the final pore size of the scaffold or result in clogged pores. The pores are essential for bone regeneration, movement of cells, metabolic waste and ionic waste from material degradation of the scaffold [43]. The pore-size of the scaffold of 10-100  $\mu\text{m}$  are micropores suitable for proteins and ion exchange [44]. Pores that are bigger than 100  $\mu\text{m}$  allow the movement of cells and waste are called macropores [44] [45][46]. The pore size of the scaffold needs to be at least 100  $\mu\text{m}$ –400  $\mu\text{m}$  for bone regeneration, but small enough to establish a surface area for efficient attachment of many cells to the surface of scaffolds. Findings from Alabort *et al.*, [47] and Seehanam *et al.*, [48] demonstrated the scaffold of pore size 500  $\mu\text{m}$  had evidence of bone regeneration and good mechanical properties. Therefore, there is a wide range of pore sizes that are suitable for bone regeneration alveolar scaffolds. However, there is no optimal pore size that have been established as scaffold requirements varies [43][45]. Use of AM technology with combinations of pore sizes that mimic bone morphology can

create a promising bone-regenerating scaffold, as bone porosity and interconnectivity of pores also influence the tissue ingrowth [49][50].

## 1.2 Problem statement

Current additively manufactured bone regenerating scaffolds are built with precise shape, size, porosity, and pore distribution [43]. However, the AM biodegradable scaffolds still do not meet the standards required for bone regenerations. Properties, such as material, material biocompatibility, machine process, pore size and post processing, influence the quality of the bone scaffold [51]. There are no clear conclusions on designing the controllable scaffold pores for bone regeneration. Bone is composed of cortical bone, which is the less dense outer layer, and trabecular bone, which has various sizes of interconnected pores [52]. As bone has a combination of pore sizes it would be ideal for the scaffold to also have a combination of pore sizes. Abbasi et al.,[53] has shown that a combination of pore sizes that mimic natural bone would stimulate bone regeneration. Therefore, AM broadened the design space for complex porous scaffold because of its ability to manufacture complex structures.

The demand for small to medium-sized customised bone defect scaffolds has risen due to increased human life expectancy [54]. Modulus of elasticity for biomaterial bone scaffolds should match that of the bone; mismatch can cause the growing bone to be resorbed due to a high modulus of elasticity of the scaffold causing stress shielding [55]. The scaffold must allow bone regeneration simultaneously with controllable degradation of the scaffold material, without losing structure for bone cells to attach [56][57]. Generally, no material matches all the essential requirements of the bone regenerating scaffold [58]. There is a crucial need for improved biomaterials to have the required mechanical strength with timed bio-resorption and bone regeneration rate for complete bone healing. Hence, the use of hydroxyapatite is promising to be manufactured by LCM. However, there are limited methodology standards on using ceramics AM technology for small-sized bone regeneration scaffolds.

### **1.3 Aim and Objectives of the Study**

The aim of the study was to create a customisable, resorbable three-dimensional (3D) porous bone scaffold with controllable degradation and bone regeneration rates using AM. This was achieved with the following objectives:

- To select an FDA-approved biomaterial that has the potential to be used in available additive manufacturing systems.
- To investigate appropriate scaffold lattice structures, built by using AM, that mimic natural bone structure and properties.
- To determine factors influencing controllable degradation and bone regeneration rates of an additively manufactured scaffold.
- To demonstrate and validate the effectiveness of the additively manufactured scaffold for customised alveolar ridge reconstruction.

The success of the study could result in a proven procedure for additive manufacturing of bone scaffolds with controllable bone regeneration and degradation rates. The application demonstrated by the customised alveolar ridge reconstruction of the defect indicated in Figure 1-8: CT image of the patient's alveolar ridge bone defect. Figure 1-8.

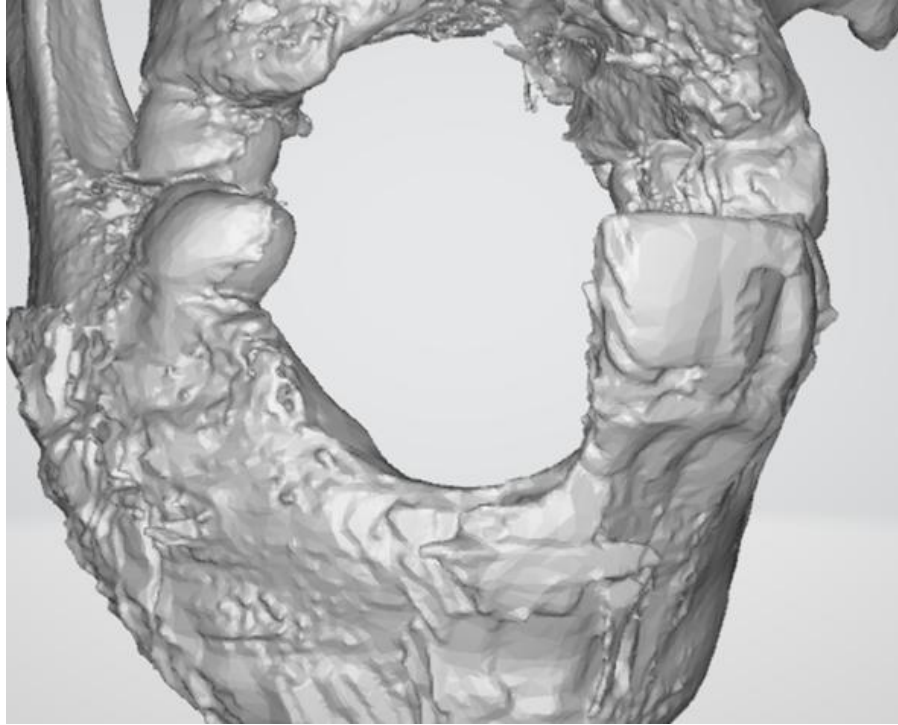


Figure 1-8: CT image of the patient's alveolar ridge bone defect.

#### **1.4 Limitations of the Study**

The project concentrated on developing a small-sized alveolar ridge scaffold implant demonstrator that could stimulate bone growth. The manufacturing process of the implant demonstrator was only focused on additive manufacturing of a bone scaffold. Non-resorbable materials were not considered in this study. The experiments supporting the study were *in vitro*. The focus of the study excluded other areas that were beyond the objectives, among them implant demonstrators handling strain, and the fluid flow in the scaffold.

#### **1.5 Thesis Outline**

Chapter 1 introduces the study, which consists of the background, aim, objectives, unique contribution, limitations of the study and thesis outline. Chapter 2 presents a comprehensive literature review and background knowledge on bone, bone tissue engineering scaffolds, various types of additive manufacturing, biomaterials, additive



manufacturing for bioresorbable materials, and the selection of lattice structures. In Chapter 3 the methods and analytical procedures used to reach the objectives are described. The preparation of HA 480 slurry is explained in this chapter. Machines used for this research including the lithography based-ceramic manufacturing (LCM) printing machine, X-Ray diffraction (XRD), SEM, and micro-computed tomography (micro-CT) analysis are discussed in detail. In Chapter 4, the results of the printability of HA480 by using LCM and the characterization of the different lattice structures for bone regeneration are presented. The impact of the lattice design, surface roughness, printability of the scaffolds, mechanical properties, and surface morphology is discussed. In Chapter 5 the material degradation results as well as the mechanical properties following the degradation are presented and discussed. This chapter also contains a discussion on the response of human osteoblasts cells (hFOB 1.19) to the HA480 material, which was utilized to assess the cell growth and adhesion. The application of AM was demonstrated by a prototype small-sized alveolar ridge scaffold in Chapter 6. Chapter 7 presents the study's conclusions and offers recommendations for future research.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Bone anatomy and composition

Bone is a tissue that provides structure in the human body [59]. It provides mechanical support to joints and ligaments. Bones as tissues are dynamic, constantly renewing and repairing by bone remodelling. Bone protects vital organs including the brain, heart, and bone marrow [60]. Bone has two types of structures, which are cortical and trabecular bone. These two bone types are occasionally located in the same bone, for example, in a long bone. Cortical bone is the dense bone that forms 80% of the human skeletal. Trabecular is the inner part of the bone, which is more porous for bone cell movement and often contains bone marrow [59][61][62].

Bone consists of an organic and inorganic phase, mineral, and water content, which can differ with age and health conditions [36]. The bone composition consists of 60-70% inorganic material, primarily crystalline hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Organic phase of bone is approximately 30-35% of its mass, consists predominantly of Type I collagen fibers, which are primary structural protein of the organic component and bone cells [37]. The remaining composition of the bone has about 10% water [36][37]. Bone has a complex hierarchy that includes the microstructure, sub-microstructure, nanostructure, and sub-nanostructure as shown in Figure 2-1. The macrostructure image shows a section of the whole bone of a human being. At the mesoscale, the bone tissue is made of two types of bone: cortical and trabecular bone. In long bones like the femur, cortical bone is the dense outer part of the bone with a porosity of about 6%, whereas the trabecular bone is the porous part of the bone, which is mainly found inside the cortical bone [63][64]. These two bone types normally co-exist so the cortical bone provides the structural integrity of the bone and stability while the trabecular bone absorbs impact forces depending on different bone locations and functions [65].

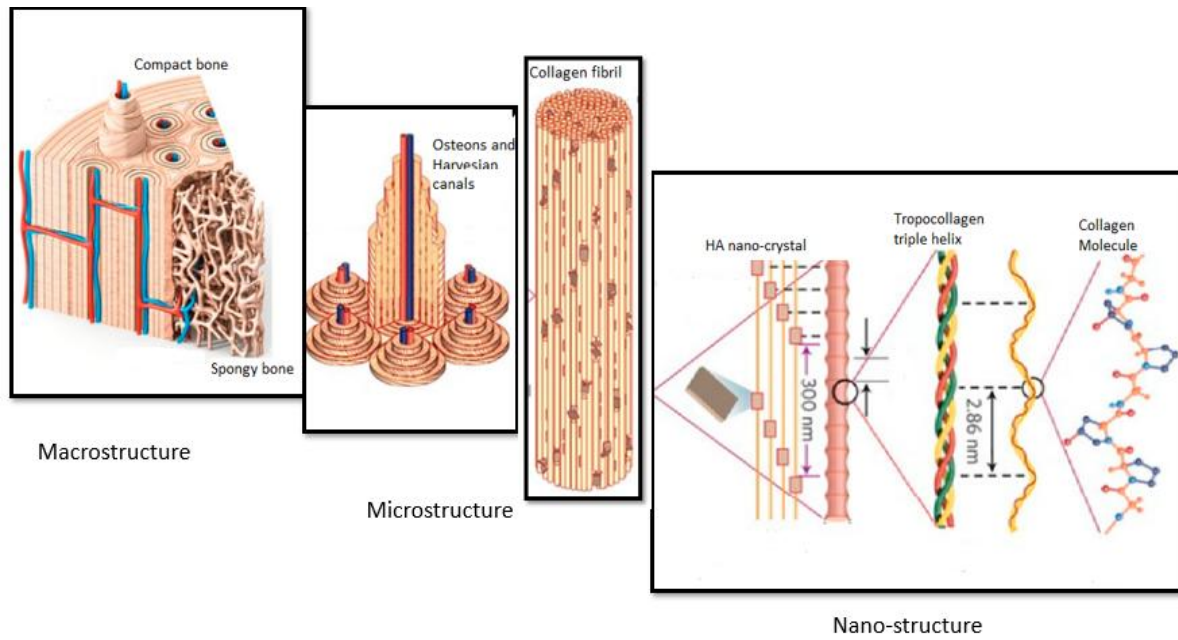


Figure 2-1: Hierarchical structural organisation of a bone [66].

### 2.1.2 Bone cells

Bone consists of cells that have different functions: osteoblasts, osteocytes, and osteoclasts [60]. Osteoblast cells are derived from mesenchymal stem cells (MSC), which form and mineralize bone [59]. Osteoblast cells are mainly cuboidal cells and are flattened when resting, as shown in Figure 2-2.

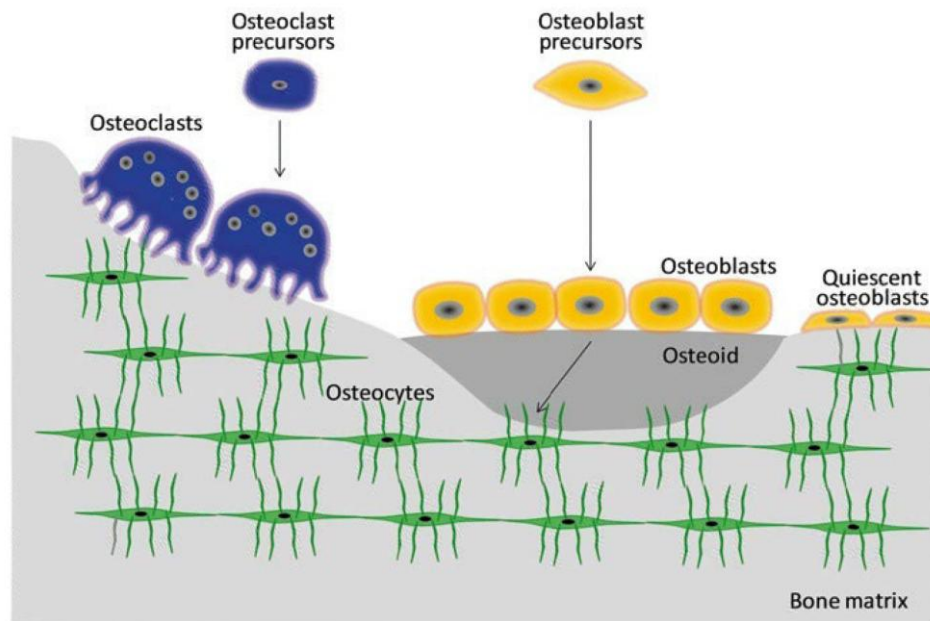


Figure 2-2: Demonstration of different types of human bone cells [67].

Osteoblast cells constitute 4-6% of the residential bone cells and are cells that remain in the bone and work with osteoclasts in the resorption of the bone during human life [60]. The organic bone matrix contains living cells, collagen (90% type 1), proteoglycans, and matrix proteins. The organic bone matrix is mineralized by the discharge of calcium phosphate crystals and hydroxyapatite to produce the hardness and strength of the bone [67]. Inside the mineralised bone matrix, BMPs, insulin-like growth factor (IGF), and transforming growth factor- $\beta$  (TGF- $\beta$ ) are stored. They are proteins that control bone growth, remodelling and regenerations [60][63].

Osteoclasts are large, acidophilic cells with 2 to 100 nuclei that receive signals from osteoblasts and osteocytes for bone resorption [60][68]. Osteoclasts are complex compared to other cells. They have a finger-like structure that assists the cells with movement during resorption, as shown in Figure 2-2. The irregular osteoclast is called ruffled border, which is the sealing zone, ensuring the attachment to the bone surface. In human's osteoclast life expectancy varies from ten days to six weeks, depending on the location and the need [69][68].

Osteocytes are the most abundant and long-living bone cells with a life span of about 10 to 25 years [67]. In a four-stage process, the osteocytes are formed: osteoid, pre-osteocyte, young osteocyte, and mature osteocyte. Osteocyte morphology depends on their function, s bone maintenance, detecting the load applied on the bone and responding, and during remodelling, it gives a signal for bone breakdown and building [8].

### 2.1.3 Bone fracture healing

Bone healing process is divided into four stages: hematoma formation, fibrocartilaginous callus formation, proliferation or bony callus formation, and bone remodelling [23], [70]. In some cases, these processes can overlap, as shown in Figure 2-3 [71].

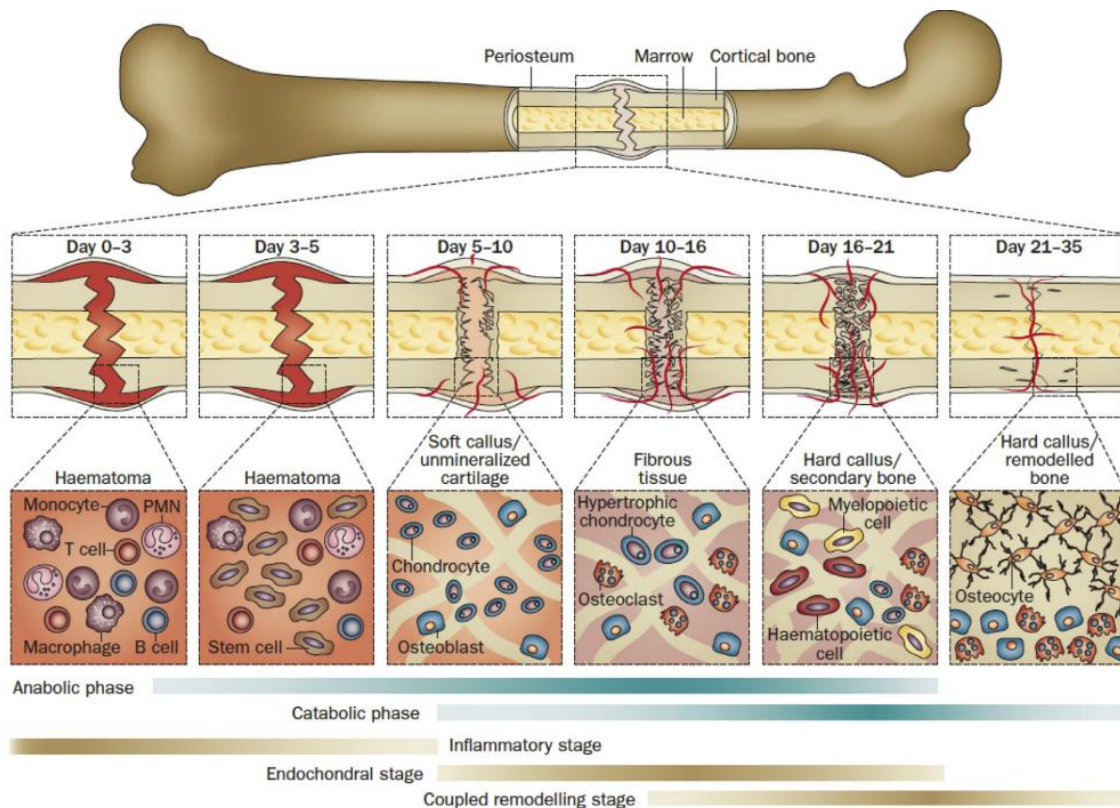


Figure 2-3: Typical demonstration of human bone healing after fracture [72].

The healing process occurs immediately after the fracture occurs by blood clot formation [72]. These clots immediately form a pathway for healing, which involves an increase in vasculisation and cell migration, causing inflammation [72]. In fracture healing, the phase

or fibrocartilaginous callus formation, in this stage of fracture healing, platelets are stimulated by the platelet-derived growth factor (PDGF), and the transforming growth factor- $\beta$  (TGF- $\beta$ ), to trigger an inflammatory response in which the bone remodelling process occurs for complete bone healing [73]. Bone remodelling of the formed woven bone is replaced by lamellar bone, which can take months or even years for full restoration and function of the bone [73][74]. Bone is being repaired constantly and renewed by the process of bone remodelling. This can happen over 10 years in a human adult's life.

## **2.2 Maxillofacial region**

This study focused on the lower face of the maxillofacial region, specifically the mandible bone. The mandible is the strong and movable part of the skull, as shown in Figure 2-4 [12]. As the study aimed to determine a small-sized scaffold for bone regeneration, the focus was not be on mandible load-bearing. The attention was on the alveolar process of the mandible.



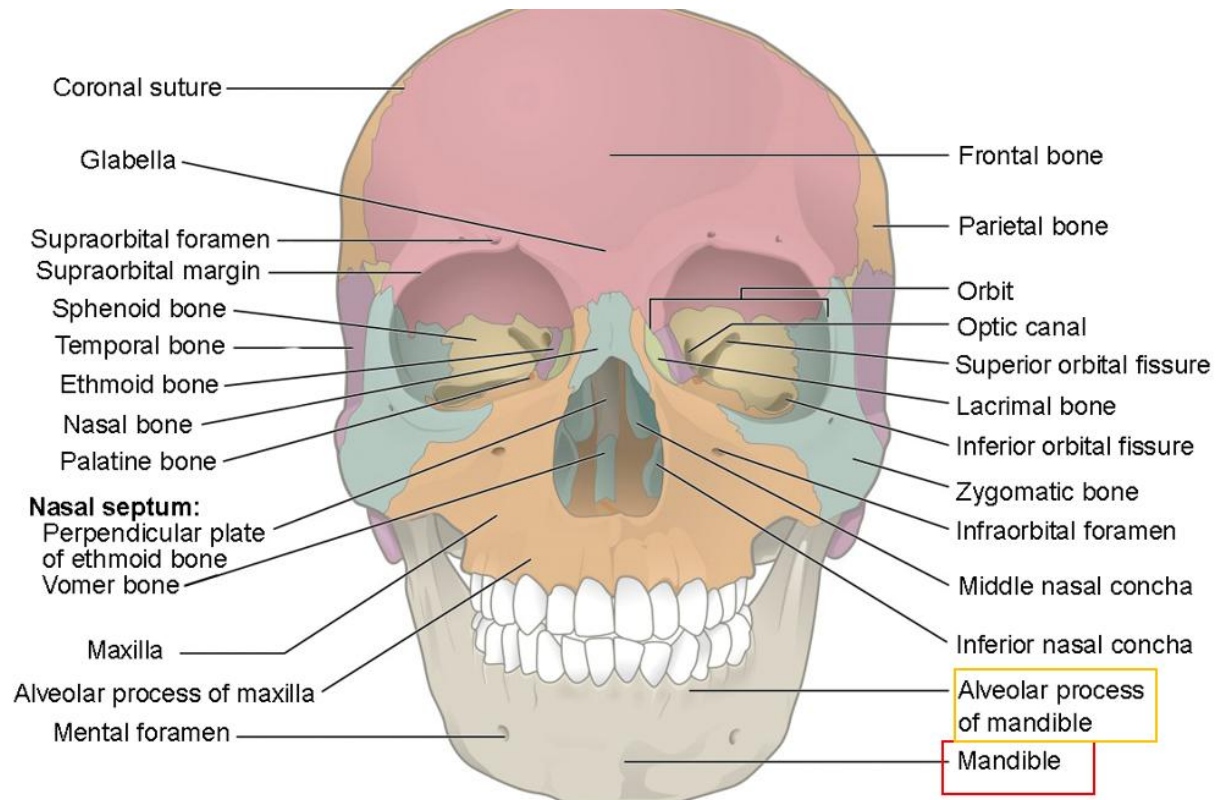


Figure 2-4: Anterior view of maxillofacial region indicating mandible and alveolar process of mandible [12].

The alveolar process is a complex bone located above the lower dense part of the mandible. This bone is attached to the tooth through periodontal ligament (PDL) connectivity tissues [7]. The cross-section of alveolar bone includes alveolar bone proper supporting the teeth. The alveolar support is made up of the cortical bone and trabecular bone as indicated in Figure 2-5. Alveolar bone is a critical part of the jaw because it is a supporting structure for the teeth. It is a supporting bone that distributes the chewing forces to the surrounding structures of the jaw [11].

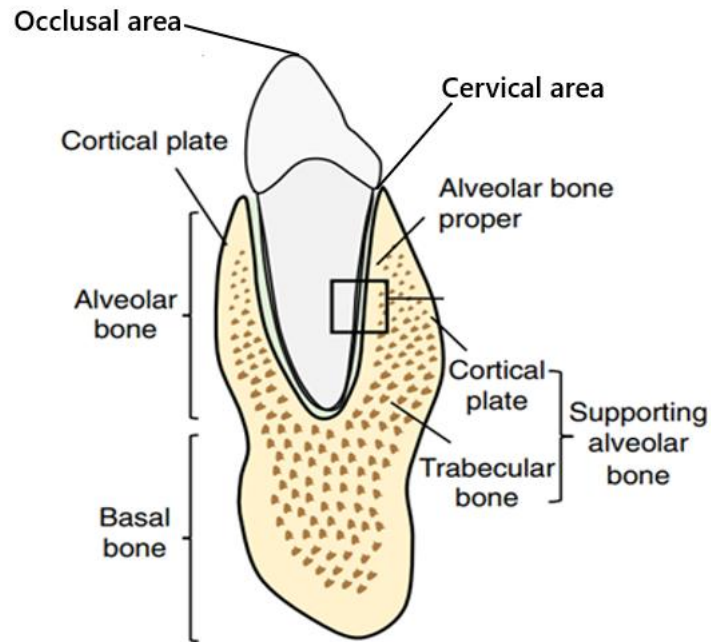


Figure 2-5: Schematic cross-section mandible with alveolar bone [12].

In the cross-section Figure 2-5, two types of bone are shown, namely cortical and trabecular, which make up the main human bones [12]. The trabecular, also known as cancellous bone, is found inside the cortical bone. It is less dense than the cortical bone [66]. With trauma or teeth extraction, the alveolar process can experience loss of bone because of high resorption through bone marrow cells of the jaw, which generates more osteoclasts compared to long bones [75][76]. The alveolar bone loss can lead to a horizontal or vertical defect, which can affect eating or speech of the patient if the condition remains untreated [77]. Therefore, augmentation of the alveolar process must be done as soon as possible to prevent more bone loss.

### 2.2.1 Alveolar ridge augmentation

Alveolar ridge augmentation is an oral procedure performed on patients who have lost teeth due to extraction [76]. This is to improve the quantity and quality of the bone that supports a dental implant. The reason for augmentation is that after tooth extraction the alveolar bone around the empty socket experiences resorption as there is no mechanical stimuli for bone to regenerate [78]. The Seibert method of classification categorises



alveolar ridge defects as follows: Class I and Class II, as well as Class III, which is a combination of both [79]. Each class has its proposed augmentation shown in Table 2-1.

Table 2-1: Alveolar ridge defect classification according to Seibert [10][79][80].

| <b>Seibert's Classification</b> | <b>Definition</b>                                                                                                                                                                                                          | <b>Treatment</b>                                                                                                                        |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| <b>Class I</b>                  | Horizontal defect – loss of tissue width with normal ridge height.<br><br>The loss affects the buccal aspects which is the surface of gum facing cheeks and lingual aspects surface is facing the tongue of alveolar ridge | Soft tissue interproximal graft technique.<br><br>Interproximal is the process where the gum between teeth is treated to reduce damage. |
| <b>Class II</b>                 | Vertical treatment – loss of tissue height with normal ridge width.<br><br>The loss affects the occlusal and cervical area of the alveolar in Figure 2-5.                                                                  | Alveolar augmentations: through bone grafts or allografts                                                                               |
| <b>Class III</b>                | Combination of horizontal and vertical bone loss.                                                                                                                                                                          | Alveolar augmentations: through bone grafts or allografts                                                                               |

In terms of loss of teeth, the vertical height of the defect is classified according to Luhr categorising [81]. The height is measured from the base of the mandible. The classification is Class I which is mild and is 16-20 mm, Class II is 11-15 mm, which is moderate and Class III  $\leq 10$ mm from the same base of the mandible, as shown in Figure 2-6 [82]. This classification on the severity of the defect and together with the Seibert class, the appropriate augmentation can be used.

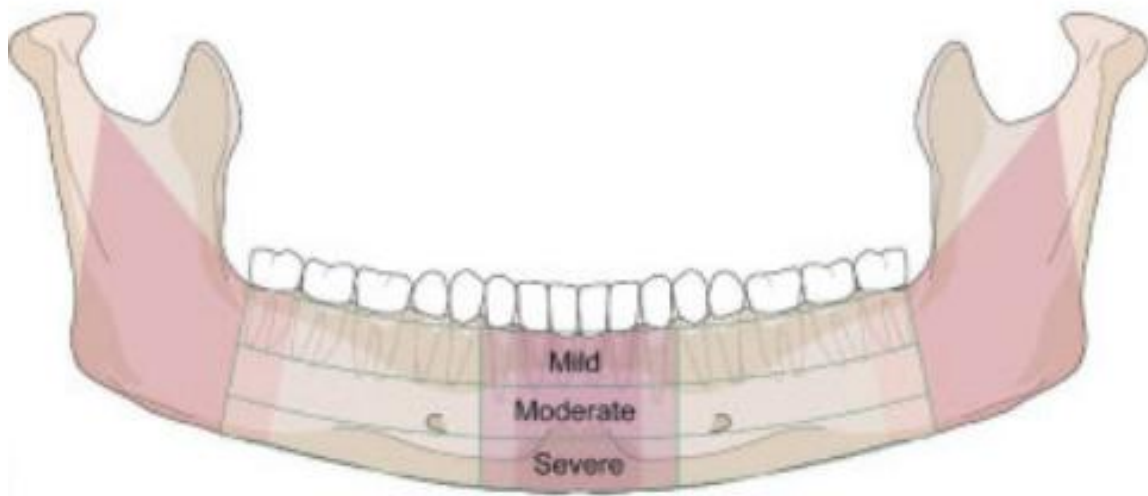


Figure 2-6: Luhr classification of bone loss measured by height from the lower part of the mandible [82].

Defect resorption can be either vertical or horizontal [83]. As shown Figure 2-7 extraction of the lower left canine and first premolar results in a vertical alveolar ridge defect [78].



Figure 2-7: Vertical alveolar ridge after extraction of left canine and first premolar teeth [78].

In some cases, the tooth is lost because of gum diseases or accidents or tumour removals which cause the alveolar ridge to deteriorate [78]. Resorbable small bone scaffolds are in high demand. Defects in the maxillofacial region are complex because the area includes bone and cartilage, nerves and blood vessels, adjacent skin, and skin lining and gums, as well as teeth and sensory organs [84].

### **2.2.2 Current alveolar augmentation treatment for Class II and III**

#### **Seibert's classification**

There have been successful cases of alveolar augmentation using autografts which are reliable because of their osteoinductivity and osteogenesis in than other types of grafts [85]. The autografts are harvested from the patient's own body. Tibia is commonly used for small to medium jaw reconstruction [86]. Bone harvested from patient generally contribute to successful autografts with less risk of infection. Complications can still be experienced by the patient due to limited supply, additional surgery, donor site mobility and extended recovery. Allografts and xenografts are harvested from an external donor

[87][88]. They offer support and may trigger immune response or tissue rejection. Synthetic grafts are a bone substitutes made from a natural or synthetic material designed to achieve bone regeneration [89]. In Table 2-2 the commercially available bone grafts are listed,

Table 2-2 Bone regeneration grafts.

| <b>Types of bone grafts</b>          | <b>Description</b>                                                                                                                                                                                                | <b>Examples</b>                                           | <b>Reference</b> |
|--------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|------------------|
| <b>Autograft</b>                     | The bone is harvested from the patient's own body, like the chin or hip.<br><br>High biocompatibility.<br><br>Post-surgery pain and the cost of surgery may affect the patient.                                   | -                                                         | [85][86]         |
| <b>Allograft</b>                     | The bone is harvested from another patient.<br><br>High risk of infection and implant rejection.                                                                                                                  | Bio4® Viable Bone Matrix (Stryker)                        | [87]             |
| <b>Xenograft</b>                     | The bone is harvested from an animal, for example, a pig.<br><br>Xenograft are easy to access.<br><br>Graft from different species may cause immune reaction has different degradation rate                       | Porcine-Derived Xenografts<br>OsteoBiol® (Tecnoss, Italy) | [88]             |
| <b>Alloplasts (Synthetic grafts)</b> | Synthetic substitutes are designed and manufactured to meet the biological and mechanical performance of lost bone.<br><br>They reduce infection and are created from biocompatible material like hydroxyapatite. | PerOssalR (Osartis,Munster, Germany)                      | [89]             |

Currently, there are food and drug administration (FDA) approved synthetic bone substitutes like PerOssal made of hydroxyapatite, which is biodegradable during

osteogenesis [89]. The FDA rhBMP-2 is Infuse™ Bone Graft, and MasterGraftR (Medtronic) consists of tricalcium phosphates [90]. Synthetic bone grafts on the market are bioresorbable with intergranular microporosity, biocompatible and bioactive, promoting cell proliferation [89]. The process of inserting graft material stimulates bone regeneration and integration, which is beneficial as it prevents further bone loss. Bone implants can be implanted to restore the patient's dignity. Ideally, a tissue-engineered scaffold for maxillofacial implantation would succeed if it were tailored such that it mimics the macro and micro-environment [91].

The current bone grafts available on the market for bone regeneration are at high cost, not specifically tailored for the patient's defect, and have limited availability in Africa [92]. The 2021 WHO NCD Country Capacity Survey (NCD CCS) reported on national procedures for detection, management and rehabilitation of oral health diseases in public hospitals as shown in Figure 2-8 [19]. In developed places like Europe (EUR) and Region of the Americas (AMR) it was possible to reach up to 50% of patients in need of oral services. However, Africa Region (AFR) it was over 40% of the countries that offered these services only for urgent treatment and oral pain relief. It also appears that the low-income patients are mostly assisted when urgent treatment is needed [19]. Therefore, there is a need in improving the existing oral solutions in Africa to be accessible to the majority of countries.

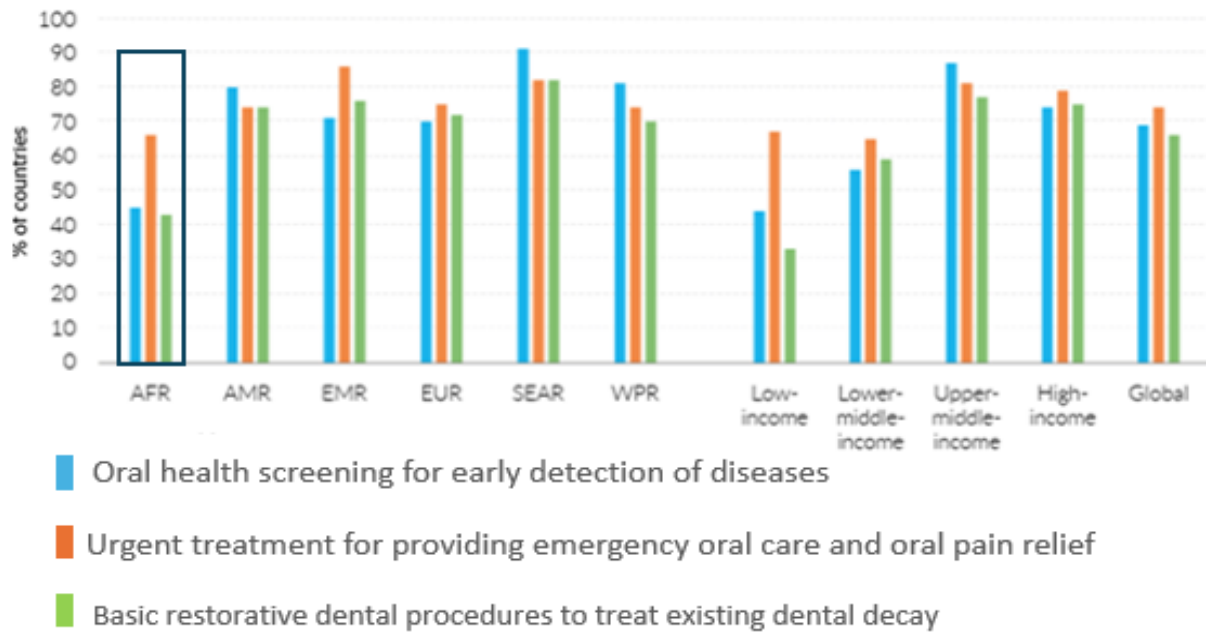


Figure 2-8: Oral health care by continent and country, with Africa having the lowest service. Abbreviations: Africa Region (AFR), Region of the Americas (AMR), Eastern Mediterranean Region (EMR), Europe (EUR), South- East Asia region (SEAR) and Western Pacific Region (WPR) ([19].

## 2.3 Bone tissue engineering requirements

### 2.3.1 Properties required for a bone tissue engineering scaffold

Different methods have been applied to repair and regenerate damaged bone tissue in the human body [89]. The limitations of bone grafts have given potential for bone tissue engineering. Bone tissue engineering is the use of engineering and biological principles in the development of bone substitutes that are intended to restore bone functionality [93]. This approach involves creating a 3D porous structure with mechanical and biological properties similar to those of bone [94]. The ideal function of such a scaffold is to act as a temporary non-cellular structure that stimulates cell regeneration until the bone defect is completely regenerated [26]. There are specific properties that biomaterial must

include, such as controllable biodegradability and biocompatibility, as indicated in Table 2--3.

Table 2--3 Requirements of bone tissue engineering.

| <b>Bone tissue engineering requirements</b>        | <b>Solution</b>                                                                                                                                                        |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>3D structure and interconnectivity of pores</b> | <p>Scaffolds must have interconnected pores for mass transportation.</p> <p>The pores must allow cell migration and vascularization [95][96].</p>                      |
| <b>Mechanical Properties</b>                       | <p>Mechanical properties must mimic strength of bone throughout the degradation process until new bone is formed [66].</p>                                             |
| <b>Biocompatibility</b>                            | <p>Material used for the scaffold should not release toxins during degradation.</p> <p>Scaffold should allow cell proliferation and adhesion for bone growth [26].</p> |
| <b>Bioactivity</b>                                 | <p>Ability to create a stable interaction with the bone [97].</p>                                                                                                      |
| <b>Biodegradable</b>                               | <p>Controllable degradation rate and bone regeneration [40].</p>                                                                                                       |
| <b>Custom designed</b>                             | <p>Scaffold design must fit to the defect of the bone [98].</p>                                                                                                        |
| <b>Manufacturing</b>                               | <p>Cost-effectiveness of the technology and reduction of material waste.</p> <p>Handling and sterilisation of the scaffold.</p> <p>Repeatability and quality [99].</p> |

One of the requirements for biomaterial degradation for a bone regenerating scaffold is the pore size of the scaffold [44]. The pore size can be divided into three categories, which are mesopores with diameter less than 100 nm, micropores with a diameter less than 100µm and macropores greater than 100 µm [44][100]. All these three types of pores are important for the functionality of the scaffold. Structures with micropores have a large surface area for ion exchange and bone protein absorption, whereas macropores, assist in nutrient and waste migration in the scaffold [101]. Owing to the large surface area, structures with micropores stimulate ion exchange and provide superior cell attachment compared to macropores [101]. Drawbacks, including restricted cell infiltration and waste movement in micropores, should be noted when considering microporous structures. Ideal bone scaffold must have micropores and macropores to mimic the bone. The optimum pore size for vascularization and bone growth between 50 -400 µm [95][102]. The pores size of a bone scaffold should exceed 10 µm, which is larger than the size of osteoblasts, to enable vascularization and facilitate cell and fluid movement into the implant [44]. Osteoblasts, the bone-forming cells, range from 10–50 µm in size. Bone attachment and adequate vascularization were observed on magnesium scaffolds with macropores of 250-400 µm [103]. Human osteoblast cells were able to adhere to the scaffold and migrate in the interconnected HA macropores of size 100-300 µm with pore wall thickness of 50 µm [57].

An increase of pore size can reduce the mechanical strength of the bone scaffold [104]. However, SLA-produced HA bone scaffolds with 300 – 600 µm pores was able to promote cell growth and maintain good mechanical strength even with bigger pore sizes [56][105]. In AM titanium-based implants, the different pores have specific roles: the pores ranging from 100-350 µm promote bone ingrowth, and those with 350-1000 µm pore size with high permeability reduce the modulus of elasticity of titanium to match that of bone more closely [57]. Many variables that affect the behaviour of the pores in implants. Therefore, it is not easy task to determine the ultimate pore size [106]. Variables such as the type of material, manufacturing process and the area of implantation all play a role [58][107]. The  $\beta$ -TCP scaffold investigated by El-Rashidy, J. *et al.*, [108] were additively manufactured in the same conditions but with different pore sizes: 300-400 µm, 400-500 µm, 500-600 µm and 600-700 µm. Scaffolds with large pores created less fibrous tissue compared to



300-400  $\mu\text{m}$  pores [108]. This large pore size cell growth differs from the HA scaffold which was able to promote bone growth at 600  $\mu\text{m}$ . Bone scaffolds are typically designed with a lattice structure to control cell porosity and interconnectivity for optimal permeability.

The scaffold must mimic the complexity, orientation, and interconnectivity of bone pores to permit fluid flow, as shown in Figure 2-9.

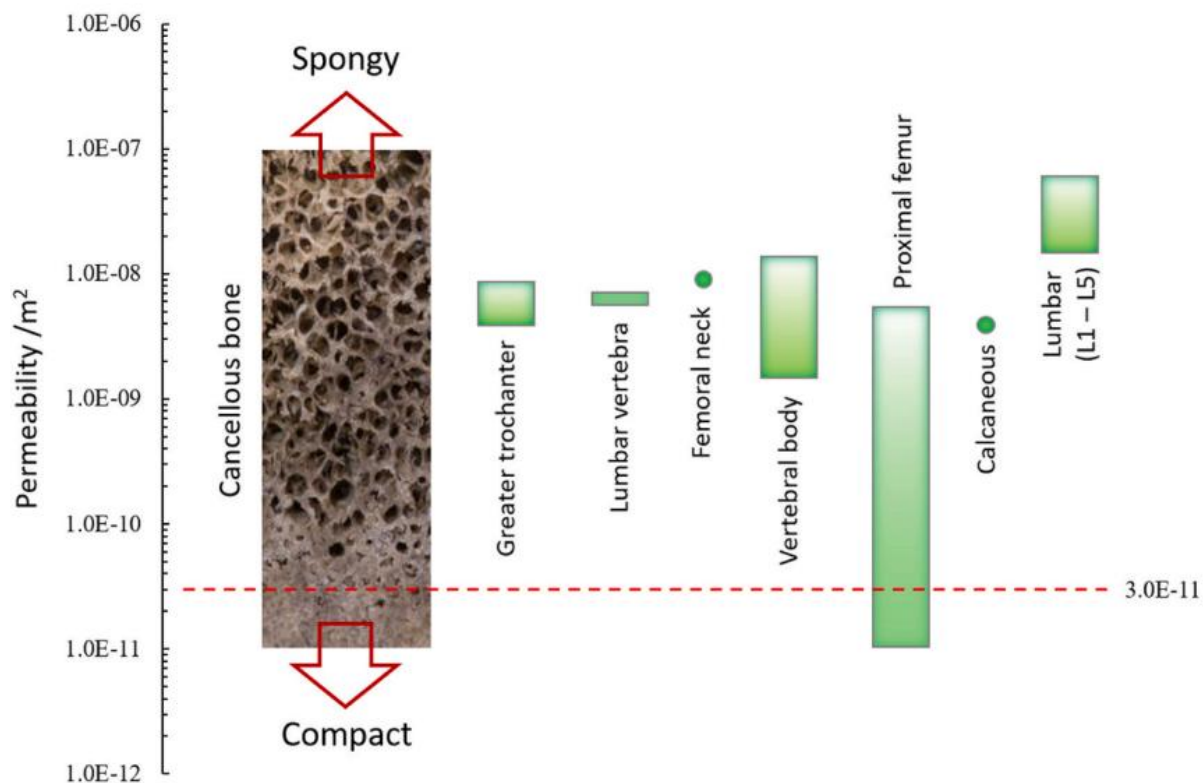


Figure 2-9: Typical bone cross-section showing permeability range [99].

Permeability is necessary for the biological function of the scaffold, as it involves effective porosity with open pores that facilitate fluid flow [109]. When designing ceramics for bone implants, having more than 85% porosity can weaken the mechanical properties [110].

### 2.3.2 Lattice structure

A cellular structure is ideal for scaffolds because it allows flexible design, interconnected pores, and customizable pore size for optimal permeability [109]. A lattice or cellular structure is a 3D network made up of interconnected struts. A cellular structure for bone scaffolds was proposed by Ashby [111]. These structures can be foams, honeycombs, and lattices. Foams are randomly generated, and the cells have random walls. Depending on the application required, specific structures can be selected as shown in Figure 2-10.

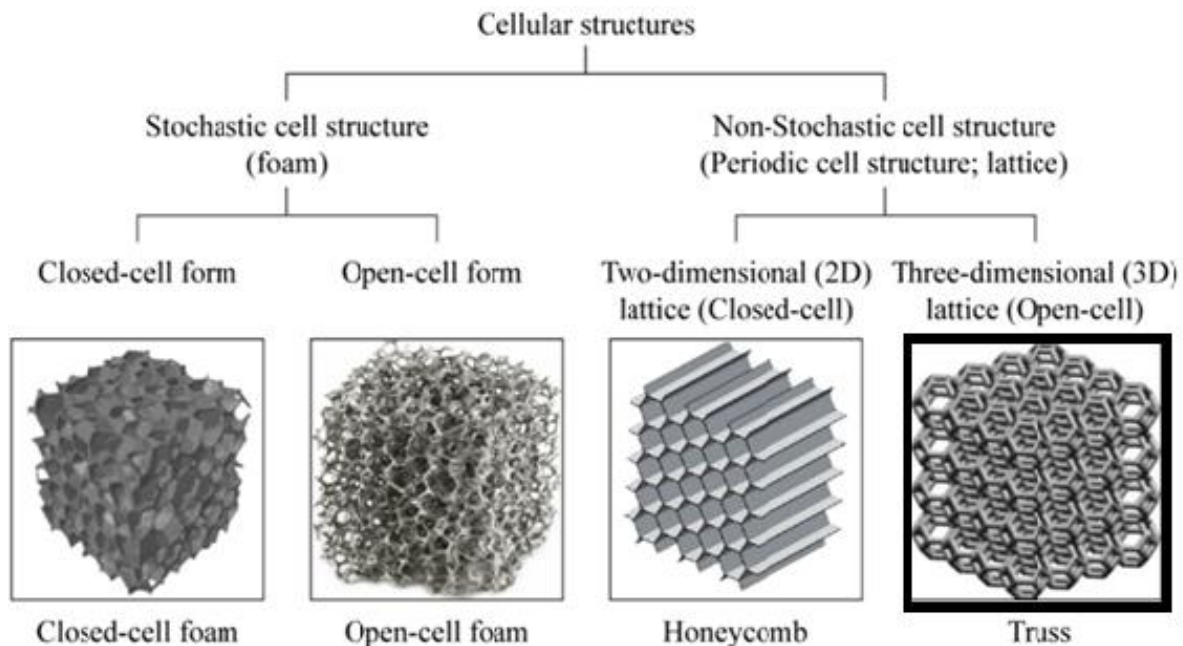


Figure 2-10: Various cellular lattice structures[113].

The lattice structure is a porous 3D structure composed of repeated interconnected cells, struts and nodes as shown in Figure 2-11[114]. Lattice structures consist of two categories, uniform and non-uniform. A uniform lattice structure refers to a repeatedly arranged porous structure in which each cell has the same shape and size as its unit cell, whereas in a non-uniform lattice structure the cells have different topological shapes and geometric sizes that are repeated.

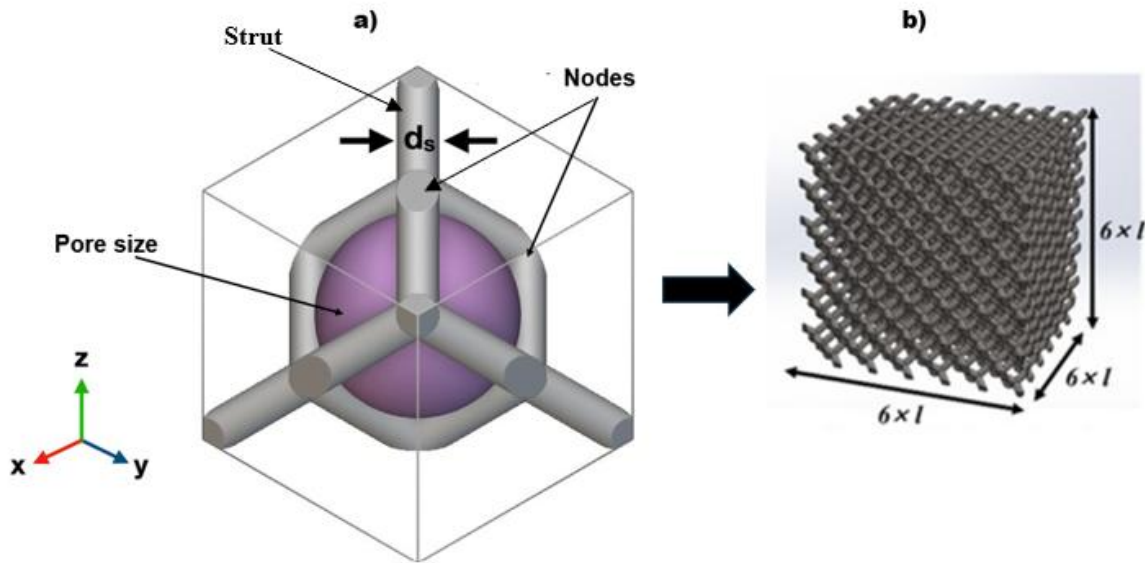


Figure 2-11: Diamond lattice structure a) diamond unit cell b) whole diamond lattice structure [115][116].

At the unit cell level, the lattice structure can be manipulated by changing the connectivity, cell size, or strut dimensions to the desired properties, particularly fluid permeability and bone cell adhesion [115][117]. The strut of the lattice structure is easy to design and modify. One challenge of the strut-based lattice is its sharp angles, which have high stress concentrations that can lead to premature failure [117].

Unit cells can be designed using methods like primitive cell-based approaches, an implicit surface-based method, where a mathematical equation is used, or topology optimization [114]. Diamond unit cell with sharp angles in Figure 2-12 a) is modified to reduce stress concentration and the modification affects the entire lattice structure, which also can improve the cell attachment [117][119]. In Figure 2-12 b) the parameters size of the cell (C), diameter of the strut (D), and optimized radius between the struts (R) accurately control the pore size and interconnected network of the whole lattice structure [118].

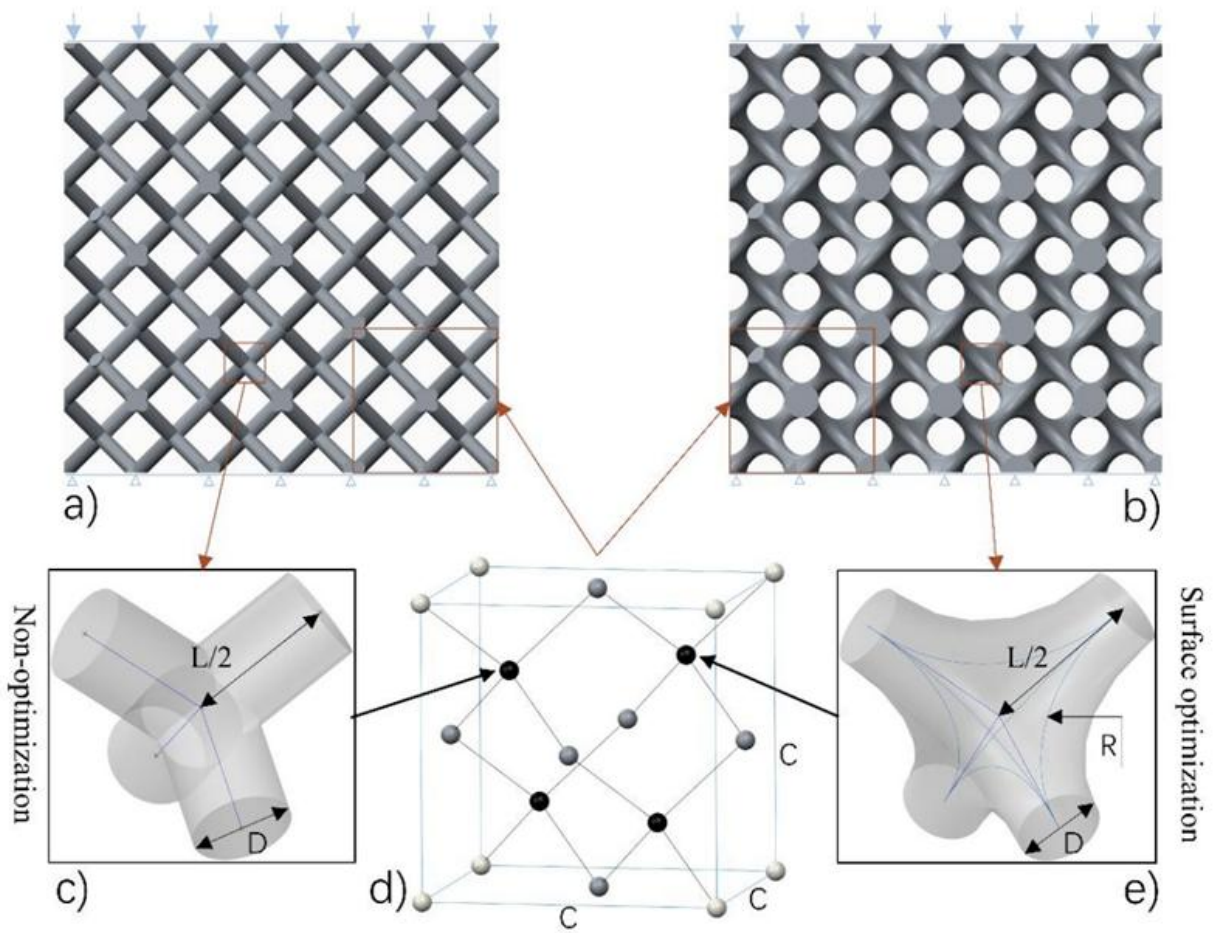


Figure 2-12: Modified diamond lattice structure (a) without optimisation, (b) a sample with surface optimization at the nodes (  $D=0.6$ ,  $R=1.6\text{mm}$ ) (c) a node without optimisation, (d) cell in the diamond cellular structure of  $3 \times 3 \times 3 \text{ mm}$  and (e) a node with the optimised surface [117][118].

If the length of the lattice strut is  $L$  in Equation 2.1 and the size of the cell is  $C$ , then:

$$L = \frac{\sqrt{3}}{4} C \quad (2.1)$$

### 2.3.3 Design of lattice structures

The design of a lattice structure is affected by the principles shown in Figure 2-13. The dominant factors to consider in 3D lattice design are the material of the lattice, the design,

and the relative density. Extensive work provided guidance on lattice structure design [114].

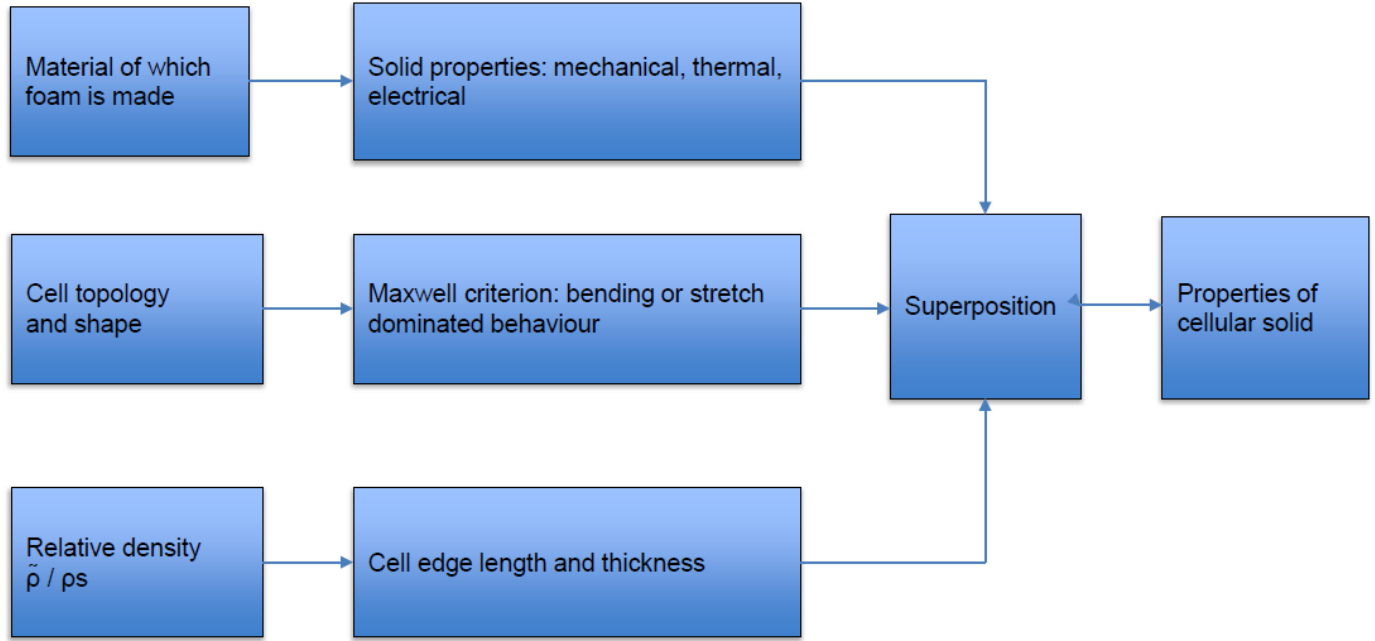


Figure 2-13: Design variables of lattice structures [114].

Based on their calculations, Ashby *et al.*, [123] proposed the relationships between the mechanical properties and the relative density of the porous structure of metal foams given in Equation 2.2 [111]. However, these equations are also applied to other materials like polymers and ceramics. Equation 2.3 is used to calculate volume.

$$\frac{E}{E_s} = \alpha \left( \frac{\rho}{\rho_s} \right)^n \quad (2.2)$$

$$V_1 = \frac{64\sqrt{3}}{9} L^3 \quad (2.3)$$

$$\frac{G}{G_s} = \frac{3}{8} \alpha \left( \frac{\rho}{\rho_s} \right)^n \quad (2.4)$$

$$\sigma_{\rho l} = (0.25 \rightarrow 0.35) \sigma_{y, s} \left( \frac{\rho}{\rho_s} \right)^m \quad (2.5)$$

Where  $\rho$  is the density of the lattice structure and  $\rho_s$  is the density of the solid material,  $E$  and  $E_s$  are the Young's modulus of the porous material and that of the solid material, respectively. In Equation 2.4, the shear modulus  $G$  and  $G_s$  are the shear modulus of the porous structure and that of the solid material, respectively. The plateau stress in Equation 2.5 denoted by  $\sigma_{pl}$  is the stress at which the porous structure starts to collapse on itself, while  $\sigma_{pl,s}$  is the yield stress of the solid material. Symbols  $\alpha$ ,  $m$  and  $n$ , are constants varying from 0.1 to 4, 1.5 to 2, and 1.8 to 2.2, respectively [123][124].

Equation 2.6 provides the formula for circular strut cross-sections within a single cell. The relative density is defined as the ratio of foam density to the density of the solid material, or it can be the volume ratio of the material to the solid material, given by Equation 2.7.

$$V_{st} - c = 4\pi d^2 L \quad (2.6)$$

$$\rho = \frac{V_{st} - c}{V_1} \quad (2.7)$$

The diamond lattice structure modulus of elasticity is similar to that of the cell. For calculating the modulus of the cell, Equation 2.8 is used, where  $E_s$  is the modulus of the matrix material and  $\rho$  is the apparent density of the cell.

$$\frac{E_p}{E_s} = \frac{\rho^2}{3.85 + 1.41\rho} \quad (2.8)$$

### 2.3.4 Maxwell stability of lattice unit cell

The deformation and failure of lattice structures at the unit cell level have been classified according to Maxwell stability criteria [125]. This depends on the number of struts and nodes in one unit cell. The Maxwell (M) criteria, Equation 2.9, determines whether the unit cell is bending or stretching during compression loading. The bending-dominated

deformation is occurring when the value of  $M$  is less than zero after calculation ( $M < 0$ ) and the struts bend under external load [111][126]. In cases where the joints are rigidly constrained, the Maxwell stability is classified as stretch-dominated ( $M > 0$ ) under deformation. Stretch-dominated applies to high stiffness structures. For example, in Figure 2-14 (a) the pin-point frame, when loaded in compression, transitions from a stretch-dominated to a bending-dominated structure. In Figure 2-14(b) the triangular frame becomes stiff during compression, the transverse strut carries tension, and it demonstrates the stretch-dominated structure [117]. The calculation of the Maxwell stability criteria is given in Equation 2.9. Where  $b$  is the number of struts and  $j$  the number of nodes.

$$M = b - 3j + 6 \quad (2.9)$$

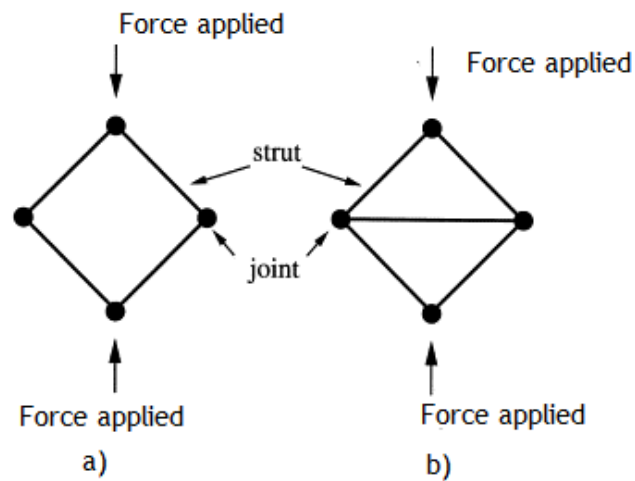


Figure 2-14: Maxwell stability demonstration under compression forces a) bending-dominated b) stretch-dominated [111][117][126].

Figure 2-15 presents a stress-strain graph which illustrates Maxwell stability [127]. The bending-dominated stress-strain graph has low yield strength in comparison with the



stretch-dominated one. However, it has a longer plateau slope. The bending-dominated structure is good for large deformation and can absorb energy under large deformation at a lower stress level [105].

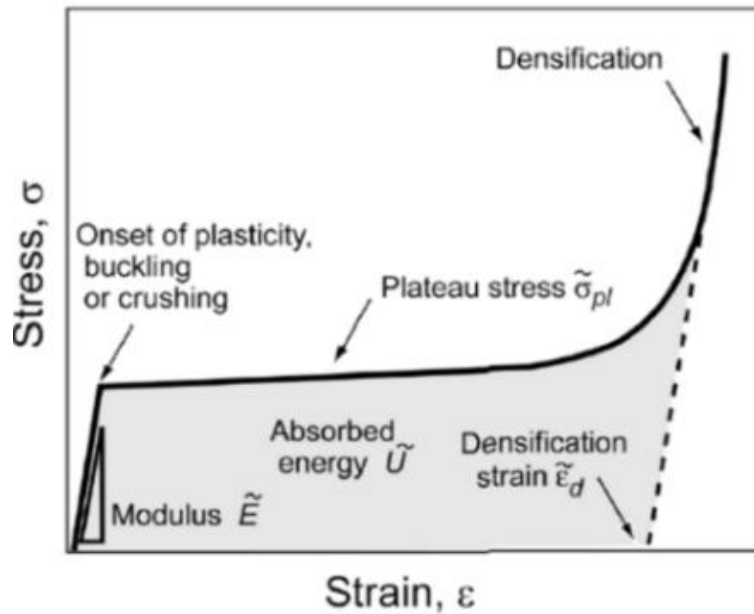


Figure 2-15: Bending-dominated lattice structure stress-strain graph [127].

In Figure 2-16 the stretch-dominated stress-strain graph has a high modulus of elasticity and yield strength [127]. The Maxwell stability from the stress-strain graph demonstrates that it is suitable for high strength and stiffness requirements.



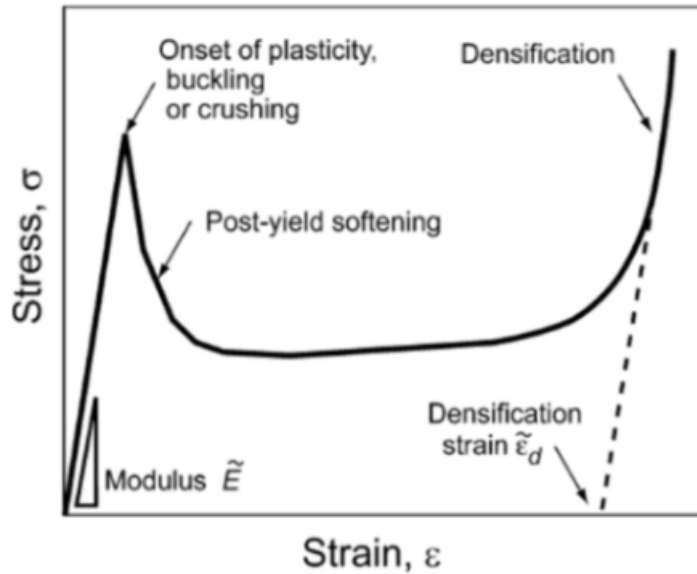


Figure 2-16: Stretch-dominated lattice structure stress-strain graph [127].

## 2.4 Finite element analysis of a lattice structure

Finite element analysis (FEA) is a computational method used to simulate how a structure will react to forces, heat, and other physical variables. In FEA, a discretisation process is applied to a CAD model. This involves forming an array of elements, nodes, coordinates, connectivity, element types, displacement boundary conditions, and distributed loads using a pre-processor program [128][129][130]. Figure 2-17 shows examples of one-dimensional (1D), two-dimensional (2D), and three-dimensional elements used for different types of models.

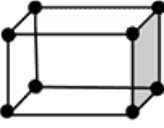
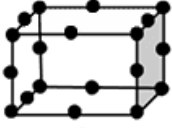
|                                | Element Name                  | Element Shape                                                                                                                                                            |                                                                                                                                                                            |
|--------------------------------|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                |                               | First Order                                                                                                                                                              | Second Order                                                                                                                                                               |
| 1D Elements<br>Line Element    | Spring, Damper<br>Beam, Truss |                                                                                        |                                                                                         |
| 2D Elements<br>Surface Element | Shell, Plane2D                | <br> | <br> |
| 3D Elements<br>Volume element  | Hexahedral                    |                                                                                        |                                                                                         |
|                                | Tetrahedral                   |                                                                                        |                                                                                         |

Figure 2-17: Examples of types of FEA elements used for different structures [128]  
[129][130].

The finite element mesh used in the discretisation of the lattice geometry is important as it affects the accuracy of the simulation of the model. For a complex geometry particularly a lattice structure, the 3D element used is the quadratic tetrahedron [128].

#### 2.4.1 Stiffness equation of the spring elements

In FEA, the stiffness of elements is calculated to evaluate the displacement of the unit cell from the degree of freedom. Therefore, the stiffness  $k$  is calculated for each element with Equation 2.10.

$$k = \frac{AE}{L} \quad (2.10)$$

Where,  $A$  is the area,  $E$  is the elastic modulus of the bar, and  $L$  is the length. For example, a simple bar with two nodes is shown in Figure 2-18. The forces  $F_1$  and  $F_2$  acting on one element are given by Equations 2.11 and 2.12 where  $u_1$  and  $u_2$  are the nodal displacements.

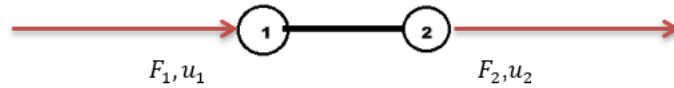


Figure 2-18: Demonstration of single-element stiffness with force and displacement.

$$F_1 = k_1(u_1 - u_2) \quad (2.11)$$

$$F_2 = k_1(-u_1 + u_2) \quad (2.12)$$

Therefore, the matrix of  $F_1$  and  $F_2$  related to displacement is shown in Equation 13.

$$\begin{Bmatrix} F_1 \\ F_2 \end{Bmatrix} = \begin{bmatrix} k & -k \\ -k & k \end{bmatrix} \begin{Bmatrix} u_1 \\ u_2 \end{Bmatrix} \quad (2.13)$$

In FEA, to satisfy the analysis conditions, the sum of applied forces and moments must be equal to zero [130]. This behaviour follows Hooke's law, which is in Equation 2.14. Equation 2.15 is the stiffness of one element. In structures that are composed of more than one element for example trusses, Equation 12.16 and 2.17 are used where  $e$  is the stiffness index and  $N$  the number of elements.

$$[F] = -[k]u \quad (2.14)$$

$$[F] = [k^e]u \quad (2.15)$$

$$[K] = \sum_{e=1}^N k^e \quad (2.16)$$

$$[F] = \sum_{e=1}^N \{f^e\} \quad (2.17)$$

## 2.5 Biomaterials for bone repair

Biomaterials selected for bone scaffolds should have the Food and Drug Administration (FDA) approval and meet the required mechanical properties of the scaffold [131]. The statistics for 2023 to 2033 show a steady growth in the biomaterials market for orthopaedic, dental, and cosmetic medical implants. Orthopaedic implants lead with 35% of the market share. Metallic implants have the largest share of the global market for orthopaedic implants and will continue to grow. The growth of innovation in polymers and ceramics with the use of AM is driving the market share of these materials as shown in Figure 2-19 [1][3].

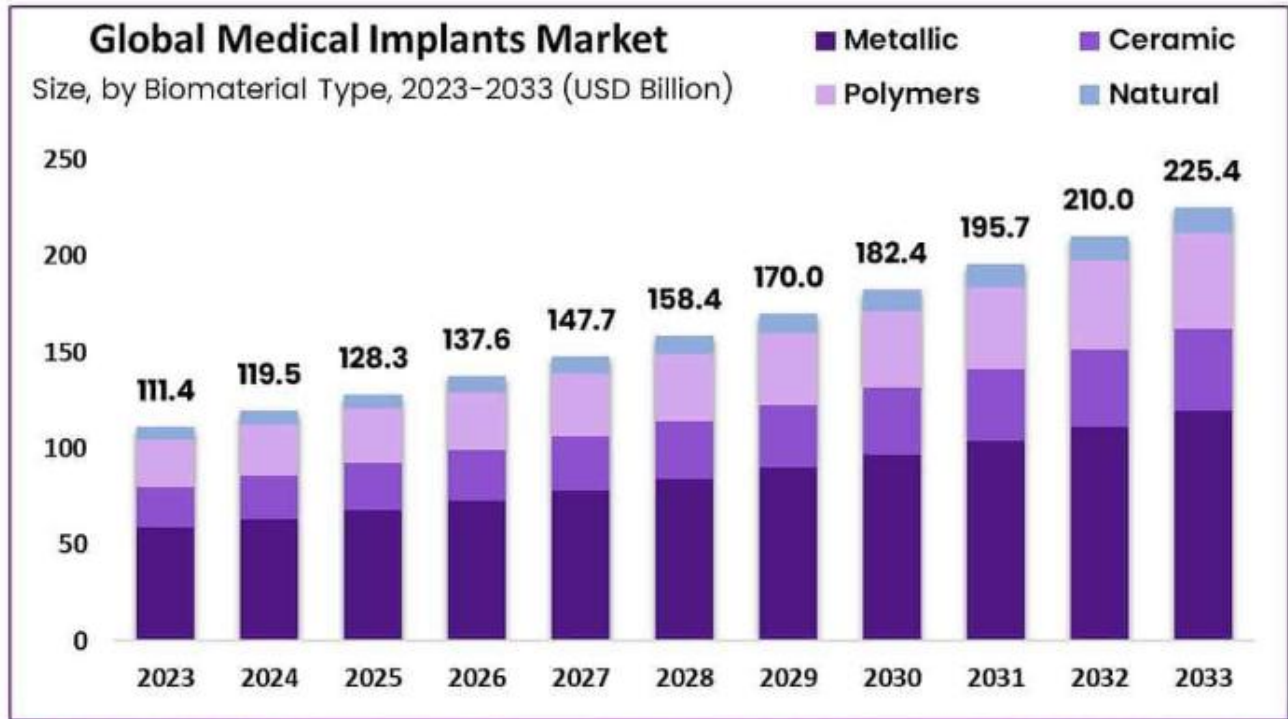


Figure 2-19: Global prediction of biomaterial market growth for the period 2023 to 2033 [1].

Calcium phosphates (CaP) is the family of minerals containing calcium cations ( $\text{Ca}^{2+}$ ) and metaphosphate ( $\text{PO}_3^-$ ) orthophosphate ( $\text{PO}_4^{3-}$ ) pyrophosphate ( $\text{P}_2\text{O}_7^{4-}$ ) anions and also hydroxide ( $\text{OH}^-$ ) ions or hydrogen ( $\text{H}^+$ ) ions [132]. Calcium phosphates are often used in a bone graft, bone regeneration, and dentistry in the form of coating of metals, paste, cements, and scaffolds [28]. The CaPs are primary salts present in human bone and tooth enamel. The CaPs have been used for bone regeneration because of their good biodegradation qualities.

Biodegradation is the process caused by the action of living micro-organisms or cells when a material breaks down into its simpler components to reduce the complexity of a chemical molecule or wear away by erosion [36][133]. Degradation is essential in the bone scaffold to allow the bone to regenerate while the material resorbs in the body without secreting any toxins [134]. Degradation of calcium phosphate depends on its calcium and phosphorus (Ca/P) ratio also, its solubility depends on its composition, crystallinity, grain size, and porosity [23][132]. Calcium phosphates are mainly distinguished by their Ca/P ratio, ranging from 0.5 to 2. Materials with Ca/P ratio  $<1$  is not

suitable for biological implantation because of their high solubility [36]. The high solubility does not give the bone defect enough time to regenerate fully. Therefore, highly soluble calcium phosphates are limited to being used in defect filling and bone cavities, for example, monocalcium phosphate monohydrate. The phosphates with a Ca/P ratio above 1.5 are characterized by their slow release and good biodegradation when implanted in bone defects, facilitating complete bone healing. These calcium phosphates include  $\alpha$ -tricalcium phosphate and hydroxyapatite [135].

For bone regeneration, the highly recommended phosphates with CA/P ratio of 1.5 to 1.67 are apatites for example hydroxyapatite [136][137]. In Table 2-, the composition of hydroxyapatite is compared to the composition of bone, enamel and dentine [138]. This similarity makes the material widely preferred for dentistry and orthopaedics scaffolds.

Table 2-: Composition of hydroxyapatite in comparison with the composition of bone, enamel and dentine [139].

| Composition, wt%    | Hydroxyapatite wt% | Bone wt% | Enamel wt% | Dentine wt% |
|---------------------|--------------------|----------|------------|-------------|
| Calcium             | 39.6               | 34.8     | 36.5       | 35.1        |
| Phosphorous         | 18.5               | 15.2     | 17.1       | 16.9        |
| Ca/P ratio          | 1.67               | 1.71     | 1.63       | 1.61        |
| Total inorganic (%) | 100                | 65       | 97         | 70          |
| Total organic (%)   | —                  | 25       | 1.5        | 20          |
| Water (%)           | —                  | 10       | 1.5        | 10          |

### 2.5.1 Hydroxyapatite for bone regeneration

Hydroxyapatite (HA) is a bioceramic material with chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  [140]. Compared with other phosphates it has unique biocompatibility, bioactivity, and is osteoconductive [37][141]. As a result, the material is normally applied in non-load-bearing implants, such as the middle ear, metal implant coating and fillings of teeth

implants [142]. Hydroxyapatite's mechanical strength is affected by powder particle size, morphology (dense or porous), grain size distribution, sintering process, and surface morphology defects [143].

The chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  includes calcium, phosphate ions, and hydrogen bonds. Phosphate ions ( $\text{PO}_4$ ) moieties are the largest as they build the unit cell, determining the structure as shown in Figure 2-20. In the unit cell of HA, the phosphate ions are positioned such that the nine atoms of oxygen from  $\text{PO}_4^{3-}$  surround four Ca ions parallel to the hexagonal axis. The remaining six Ca atoms are encircled by the remaining six O atoms of the  $\text{PO}_4^{3-}$  moieties [88][144][145].

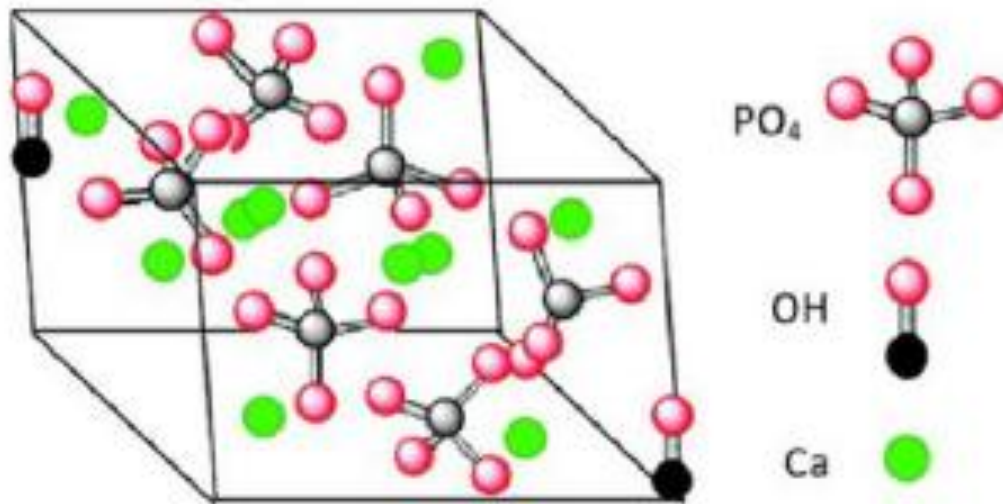


Figure 2-20: Hydroxyapatite crystal unit structure [145].

Table 2-4 shows the properties of hydroxyapatite for biomedical applications, for example good biocompatibility, high bioactivity, low biodegradation that allows time for bone regeneration, and a modulus of elasticity comparable to bone [139][143]. The mechanical properties of HA are listed for dense and porous forms, as well as its biological behaviour.

Table 2-4: Hydroxyapatite properties [139][146].

| Property                  | Values                        | Comment                       |
|---------------------------|-------------------------------|-------------------------------|
| Binding energy            | -280.6 eV                     | -                             |
| Kohan-Sham gap            | -5.4 eV                       | -                             |
| Dielectric constant       | 7.40-10.47                    | -                             |
| Thermal conductivity      | 0.013 W/( cm .K)              | -                             |
| Relative density          | 95% -99.% %                   | -                             |
| Decomposition temperature | >1000 °C                      | -                             |
| Melting point             | 1614°C                        | -                             |
| Theoretical density       | 3.156 g/cm <sup>3</sup>       | For dense HA                  |
| Tensile strength          | 38- 300 MPa<br>3 MPa          | For dense HA<br>For porous HA |
| Compressive strength      | 120-900 MPa<br>2- 100 MPa     | For dense HA<br>For porous HA |
| Bending strength          | 38-250 MPa<br>2- 11 MPa       | For dense HA<br>For porous HA |
| Young's elastic modulus   | 35-120 GPa                    | For dense HA                  |
| Fracture toughness        | 0.7 -1.2 MPa.m <sup>1/2</sup> | Decreases with porosity       |
| Fracture energy           | 2.3 -20 J/m <sup>2</sup>      | Behaves like a ceramic        |
| Vickers hardness          | 3-7 GPa                       | For dense HA                  |
| Poisson's ratio           | 0.27                          | For synthetic HA (bone ~0.3)  |
| Fracture energy           | 2.3-20J/m <sup>2</sup>        | -                             |
| Fracture toughness        | 0.7-1.2 MPa m <sup>1/2</sup>  | Decreases with porosity       |
| Fracture hardness         | 3-7 GPa                       | Dense HA                      |
| Biocompatability          | High                          | High                          |
| Biodegradation            | Low                           | Low                           |
| Bioactivity               | J\High                        | J\High                        |
| Osteoconduction           | High                          | High                          |
| Osteoinduction            | Nil                           | Nil                           |



## 2.5.2 LithaBone HA480

LithaBone HA480 is a hydroxyapatite material developed by Lithoz GmbH for LCM of biomedical implants. The material consists of hydroxyapatite powder that complies with the surgical implant standard ASTM F1185 [147]. LithaBone HA480 is an improvement over the previously used LithaBone HA400 material, produced by the same company. The process of producing the slurry is the same for HA400 and HA480, but an enhancement was made to the binder for HA480 [148]. The supplier of the raw materials did not disclose the powder size used nor any improvements in binder properties. The binder is designed to evaporate easily at high temperatures to prevent surface cracks after sintering, as previously experienced with LithaBone 400 [148]. Table 2-5 shows the material properties of HA480 powder and the physical and mechanical properties of sintered HA480.

Table 2-5: HA480 properties for powder and sintered samples [149].

| Property                                      | Value                                  |
|-----------------------------------------------|----------------------------------------|
| <b>Powder</b>                                 |                                        |
| Purity [%]                                    | ≥ 95                                   |
| Heavy metal content [ppm]                     | Max. 50                                |
| Complies with ASTM F1185                      | Yes                                    |
| <b>Sintered samples</b>                       |                                        |
| Theoretical density [g/cm <sup>3</sup> ]      | 3.16                                   |
| Relative density [%]                          | 95 - 98                                |
| Three-point bending strength (as fired) [MPa] | 154                                    |
| Max. wall thickness (sintered)[mm]            | 10                                     |
| Characteristic strength (3PB, as fired) [MPa] | 129                                    |
| Weibull distribution                          | 13                                     |
| Biocompatibility                              | Not cytotoxic according to ISO 10993-5 |

The process of creating the slurry commences by using the commercially available HA ( $[\text{Ca}_{10}(\text{PO}_4)_6](\text{OH})_2$ ) powder characterized by Lithoz GmbH [150]. The HA powders are dried for about 24h at 120°C and added in three steps to the separately prepared organic matrix containing solvent, reactive monomers, and a photo initiator (usually less than 1 wt%) [151]. At each step, a precise amount of powder was added to the matrix to create a homogeneous slurry, as shown in Figure 2-21. The slurry was mixed for 30 s at 1800 rpm and then for 30 s at 2750 rpm using a Speed Mixer<sup>TM</sup> DAC 400.1 FVZ (Hauschild, Germany). When all the powder had been added to the binder the slurry underwent a dispersion process with milling beads for 3 hours. A rheological additive was added to the ready-for-use slurry to bring stability, and it was stored at a constant temperature of 5° [152].

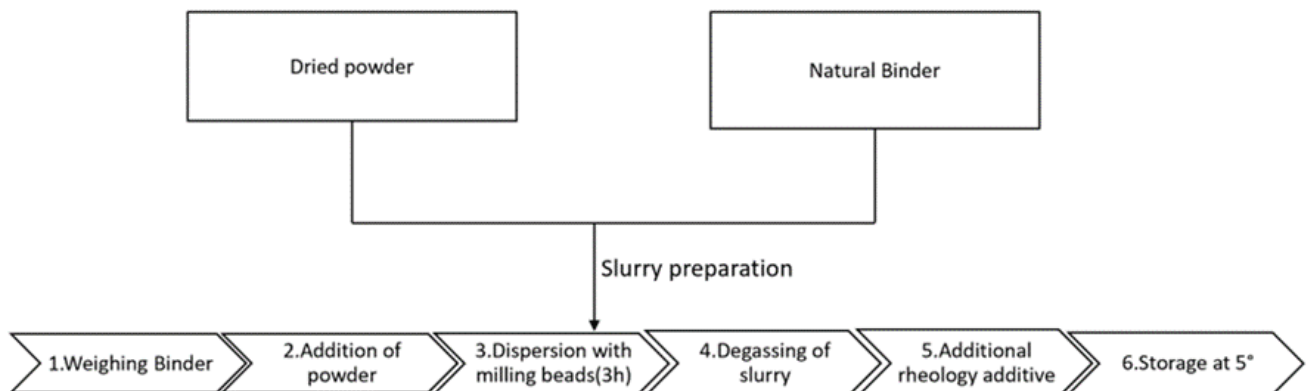


Figure 2-21: Systematic diagram of HA480 slurry preparation [152].

## 2.6 Conventional techniques for scaffold fabrication

Conventional techniques for bone regeneration scaffold fabrication, for example gas foaming, particle leaching/solvent casting, and phase separation, have shown increasing improvement in recent years [99]. Conventional fabrication of tissue engineering scaffolds has been clinically tested and has shown evidence of bone regeneration. These methods, while unable to precisely control pore architecture, remain popular because of their reliability and cost-effectiveness [153]. Solvent casting is an adaptable technique for

producing polymer composites and porous ceramics. It allows the improvement of the dispersion of particulates compared to polymer melts, enhancing material properties [153][154]. A salt-polymer composite is formed by adding an insoluble salt to a solution and subsequently evaporating the solvent. This composite is then submerged in water to leach out the salt, yielding a highly porous structure [26]. The choice of solvent is crucial as it impacts particle self-assembly where material components rearrange into ordered structure, with solvent parameters like dielectric constant and chemical affinity influencing stabilization mechanisms [144].

Gas foaming is a promising technique for fabricating porous scaffolds for bone regeneration[145]. This method utilizes CO<sub>2</sub> or other gases to create interconnected porous structures in various biomaterials, including polycaprolactone (PCL) [154], gelatin where large pores were evident with lower tensile strength [155], and chitosan, agarose and hydroxyapatite composites [156]. In some instances, the gas foaming technique can be combined with other methods, like freeze-drying, to enhance scaffold properties like macropores [157]. Gas foaming allows for the tailoring of scaffold porosity, mechanical strength, and bioactivity [158]. Recent innovations include *in situ* gas foaming using magnesium particles and supercritical CO<sub>2</sub> foaming to form pores near the surface of the scaffold using gas [159]. The scaffolds demonstrate improved cell adhesion, proliferation, and osteogenic differentiation promoting new bone formation [157][160]. *In vivo* studies have shown promising results for bone regeneration using gas-foamed scaffolds, demonstrating their potential for clinical applications in bone tissue engineering [161]. Limitation on design in conventional manufacturing, interconnection of pores, and lack of customised scaffolds leaves an opportunity to use AM. The SEM-SE image in Figure 2-22 displays the good interconnection of the scaffold and how it mimics trabecular bone.

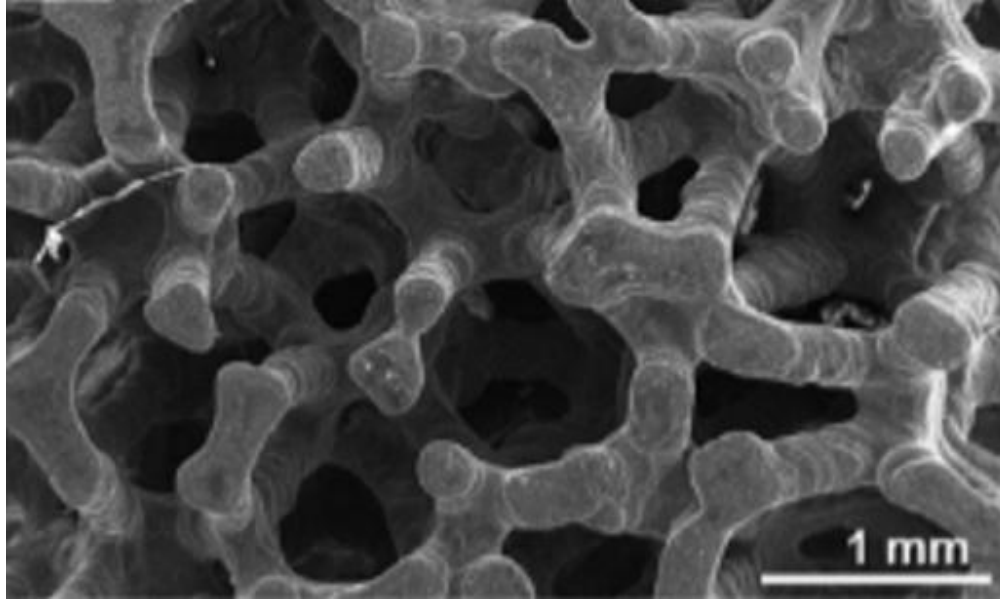


Figure 2-22: SEM-SE image of the LCM HA showing the interconnected porous structure [162].

Table 2-6. compares (AM) with conventional manufacturing processes for bone regeneration scaffolds, highlighting differences in design, material efficiency, and structural characteristics.

Table 2-6: Comparison between AM and conventional manufacturing of bone regeneration scaffolds

| Parameter                                          | Additive manufacturing                                                 | Conventional manufacturing                                     |
|----------------------------------------------------|------------------------------------------------------------------------|----------------------------------------------------------------|
| <b>Manufacturing</b>                               | Layer by layer of the material based on a 3D model [93][163].          | Includes freeze drying, solvent casting and gas foaming [146]. |
| <b>Digital design reproducibility and accuracy</b> | High reproducibility. Accuracy [164][165].                             | Moderate reproducibility [166].                                |
| <b>Customisation and personalisation</b>           | Manufacturing complex structures. Internal channels for implants[165]. | Limited for complex design [146].                              |
| <b>Porosity</b>                                    | Tailored pores [41].                                                   | Pores depending on the manufacturing process [157].            |
| <b>Biological</b>                                  | Pores are tailored for expected biological behaviour [153].            | Pores are not optimised for biological activities [166].       |
| <b>Cost</b>                                        | Higher cost [168].                                                     | Lower cost [168].                                              |
| <b>Limitations</b>                                 | Non-load bearing defects [169].                                        | For simple bone defects [146].                                 |

## 2.7 Additive manufacturing of a hydroxyapatite bone scaffold

The AM is the process of joining powder or liquid material layer by layer from 3D CAD model data to form 3D parts with high complexity, as defined in ASTM standard ISO/ASTM 52900:2021 [170]. The AM techniques can create 3D-printed bone scaffolds that mimic natural human bone, providing a growth-directing structure for cell migration and proliferation.

In the pre-process of AM, the scaffold has to be designed according to the patient's defects and specifications. The pre-process AM has two stages; firstly, it uses computer-

aided design software to draw a 3D model. The second process, computed tomography (CT) scan provides a detailed image of the patient [39]. The information of the defect is then reverse-engineered to form a scaffold that is filled with pores that are chosen [39][171]. Both processes are used for AM and follow the same procedure of going through the slicing software and converting to the STL file, which is the format for most AM machines. For bone regeneration, biomedical AM manufacturing processes for hydroxyapatite, share similar steps.

However, limitations exist such as low slurry viscosity, high melting point of HA, low thermal conductivity, and brittleness which makes it challenging to manufacture and meet medical standards [39][94]. The manufacturing of hydroxyapatite using AM technology is divided into two types: single and multi-step. Single-step AM can produce complex shapes directly; no sacrificial binder is used. This process is called Laser powder bed fusion (LPBF) and Direct Energy Deposition (DED). The multi-step AM beginning with printing of the complex shape using LCM technology, and is followed by post-processing including debinding and sintering treatment of the green body as shown in Figure 2-23 [137].

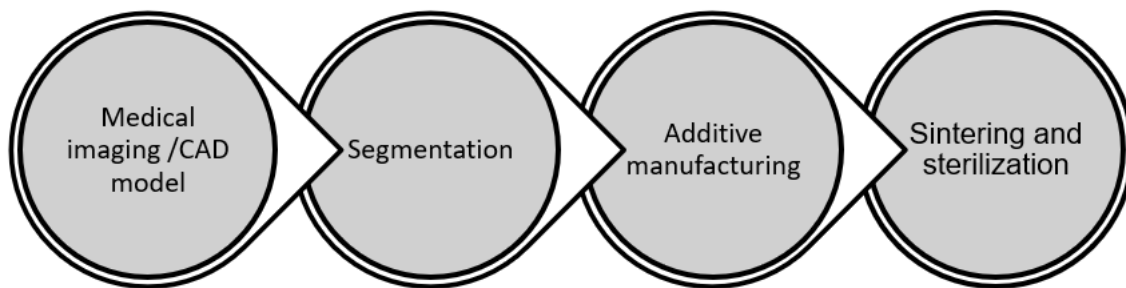


Figure 2-23: AM multistage steps for the processing of hydroxyapatite [171].

The process parameters for HA480 on CeraFab S65 to manufacture biodegradable bone scaffolds shown in Table 2-7.

Table 2-7: Process parameters for fabricating HA480 in the CeraFab S65 [149].

| Parameter                                     | Standard value | Influencing parameters       |
|-----------------------------------------------|----------------|------------------------------|
| Layer thickness [ $\mu\text{m}$ ]             | 25             | Resolution vs printing speed |
| Support structure thickness [ $\mu\text{m}$ ] | 300            | Part mass & support design   |
| <b>Geometry corrections</b>                   |                |                              |
| Contour offset [ $\mu\text{m}$ ]              | 0              | Tolerances                   |
| Pixel alignment                               | -              | -                            |
| Lateral XY shrinking compensation             | 1.25           | -                            |
| Build direction [Z] shrinking compensation    | 1.36           | -                            |
| Z curing depth compensation                   | -              | Lateral channels /holes      |
| Z curing depth compensation layers            | -              | Layer thickness & DLP energy |
| <b>Dispenser</b>                              |                |                              |
| Dispensation correction                       | 1.2            | Surface /volume ratio        |
| Dispensation correction support               | 1.3            | Surface /volume ratio        |
| <b>Coating</b>                                |                |                              |
| Angle of rotation start (rotations)           | 720            | Cured area                   |
| Angle of rotation general (rotations)         | 360            | Cured area                   |
| Rotation speed [ $^{\circ}/\text{s}$ ]        | 100            | Design & orientation         |
| <b>Contact</b>                                |                |                              |
| Settling time start [s]                       | 30             | Cured area                   |
| Settling time general [s]                     | 10             | Cured area                   |
| Tilt up speed start [ $^{\circ}/\text{s}$ ]   | 9              | Design & orientation         |
| Tilt up speed general [ $^{\circ}/\text{s}$ ] | 30             | Design & orientation         |

The green body samples from the machine go through a stage of thermal process to remove the binder and inorganic materials [171]. The first post-processing step is preconditioning according to the Lithoz guide shown in Table 2-8.

Table 2-8: Preconditioning cycle of HA480 samples in a furnace [149].

| Heating time [h] | Heating rate [K/min] | Temperature [°C] | Dwell time [h] |
|------------------|----------------------|------------------|----------------|
|                  |                      | 25               |                |
| 16.67            | 0.05                 | 75               | 6              |
| 6.67             | 0.025                | 85               | 6              |
| 6.67             | 0.025                | 95               | 8              |
| 3.33             | 0.025                | 100              | 8              |
| 7.58             | 0.011                | 105              | 6              |
| 7.58             | 0.011                | 110              | 6              |
| 7.58             | 0.011                | 115              | 6              |
| 7.58             | 0.011                | 120              | 20             |
| 3.96             | -0.4                 | 25               |                |

After pre-conditioning, the temperature of the furnace is reduced to 20 °C [149]. As shown in Table 2-9 the temperature is gradually increased at a certain heating rate until the sintering temperature is reached. HA480 is sintered at 1300 °C for 2 hours.



Table 2-9: Sintering procedure of HA480 [149].

| Heating time [h] | Heating rate<br>[K/min] | Temperature<br>[°C] | Dwell time<br>[h] |
|------------------|-------------------------|---------------------|-------------------|
| -                | -                       | 25                  | -                 |
| 3.17             | 0.5                     | 120                 | 5.8               |
| 3.33             | 0.2                     | 160                 | 10.5              |
| 1.67             | 0.2                     | 180                 | 7                 |
| 3.33             | 0.2                     | 220                 | 7                 |
| 8.33             | 0.16                    | 300                 | 7                 |
| 2.6              | 0.16                    | 325                 | 6                 |
| 10.94            | 0.16                    | 430                 | 6                 |
| 7.29             | 0.16                    | 500                 | 0                 |
| 3.81             | 1.75                    | 900                 | 2                 |
| 13.3             | 0.5                     | 1300                | 2                 |
| 1.25             | -2                      | 1150                | 2                 |
| 2.08             | -2                      | 900                 | 2                 |
| 3.65             | -4                      | 25                  | -                 |

Common multistage processes for hydroxyapatite include different techniques in AM of namely stereolithography (SLA) [110], digital light processing (DLP) [173], fused deposition modelling (FDM), selective laser sintering (SLS) [174], and binder-based 3D [175][176]. In Table 2-10, examples of previous work done on hydroxyapatite scaffolds produced by additive manufacturing techniques are listed. The mechanical properties shown in Table 2-10 were found to be comparable with those of cancellous bone. Also, the biocompatibility and cell attachments on the scaffold indicated that the material can be used for bone regeneration [173][177]. Manufacturing of HA scaffolds has been successfully done by different AM techniques. However, there are still no specific

standards on which pores are to be used for bone scaffolds, nor on AM machine limitations for small parts.

Table 2-10: Literature on HA manufactured by different AM technologies.

| HA studies                                                                                                                                                                | Outcomes                                                                                                                                                                                                                                                                                                                                    | References |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>Mechanical properties of HA on different AM technology</b>                                                                                                             |                                                                                                                                                                                                                                                                                                                                             |            |
| <b>Material: Hydroxyapatite</b><br><br><b>Manufacturing process: slurry HA was printed through 3D gel printing.</b>                                                       | <p>The samples experience shrinkage after sintering (width <math>17.8 \pm 0.2</math> and height <math>16.2 \pm 0.2\%</math>).</p> <p>The mechanical properties of printed HA exceeded the cancellous bone compressive strength of <math>16.77 \pm 0.38</math> MPa and elastic modulus <math>492 \pm 11</math> MPa.</p>                      | [40]       |
| <b>Material: Bovine hydroxyapatite (BHA) – Glycerine</b><br><br><b>Manufacturing process: Homemade 3D printer</b>                                                         | <p>The mechanical properties of printed BHA were comparable with the commercial HA and human bone.</p> <p>The highest value of compressive strength was <math>5.71 \pm 0.42</math> MPa.</p>                                                                                                                                                 | [176]      |
| <b>Material: Hydroxyapatite (LithaBone HA480 slurry)</b><br><br><b>Manufacturing process: LCM</b>                                                                         | <p>For printed samples some of the small pores (<math>160</math> <math>\mu\text{m}</math>) appeared clogged during the manufacturing process and they were not used for experiments.</p> <p>Different HA480 lattices were tested.</p> <p>Compressive strength in the range from <math>100</math> MPa to <math>1.3</math> GPa was found.</p> | [177]      |
| <b>Material: Hydroxyapatite LithaBone HA 480 (HA) and LithaCon 3Y 280 for parts made of zirconia (<math>\text{ZrO}_2</math>)</b><br><br><b>Manufacturing process: LCM</b> | <p>An HA480 inner ring and <math>\text{ZrO}_2</math> outer ring were sintered together at a temperature of <math>1300^\circ\text{C}</math>. A successful sintered joint implant was manufactured using both materials.</p>                                                                                                                  | [178]      |

| HA studies                                                                                                                                                                                                                 | Outcomes                                                                                                                                                                                                                                                                                                             | References |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>Material: LithaBone HA480</b><br><br><b>Manufacturing process: LCM</b>                                                                                                                                                  | Diamond lattice structure HA480<br>compressive strength: 5.4 MPa                                                                                                                                                                                                                                                     | [179]      |
| <b>Evidence of cell growth on AM manufactured properties of HA on different AM technology</b>                                                                                                                              |                                                                                                                                                                                                                                                                                                                      |            |
| <b>Material: HA and polymer binders: polyvinylpyrrolidone (PVP), polyacrylamide (PAM), and polyvinyl alcohol (PVA).</b><br><br><b>Manufacturing process: 3D printer with constant process parameters for all materials</b> | The compressive strength was similar for all scaffolds.<br>Young's modulus increased as:<br>PVA/HA>PAM/HA> PVP/HA. PVA/HA                                                                                                                                                                                            | [180]      |
| <b>Material: HA powder (12µm)</b><br><br><b>Manufacturing process: DLP</b>                                                                                                                                                 | The HA bone scaffold had pores (300µm - 600µm) suitable for cell attachment.<br>The compressive strength of the scaffold was 15.25 MPa, and the modulus of elasticity was 0.97 GPa.<br>Microhardness was 270 HV at 94.9% density. DLP printed scaffolds showed biological properties suitable for bone regeneration. | [181]      |
| <b>Material: HA with binder mixed with 37.84 vol% resin and 62.16 vol% HDDA</b><br><br><b>Manufacturing process: SLA</b>                                                                                                   | HA scaffolds with different lattice design were tested for compressive strength. Lattices were frame, circular, and octahedral. The octahedral had the highest compressive strength of 5.6 MPa.<br>Cell attachment was evident on the HA disks.                                                                      | [182]      |

| HA studies                                                                                           | Outcomes                                                                                                                                                            | References |
|------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
|                                                                                                      | On the lattice cell proliferation was assessed over 7 days. The pores with acute angles supported differentiation more effectively than the square and round pores. |            |
| <b>Material HA:</b><br><b>Manufacturing process: DLP</b>                                             | Pores ranging from 100 to 800 $\mu\text{m}$ (80% porosity) yielded compressive strength of 1.60 MPa and modulus of elasticity 513 MPa.                              | [110]      |
| <b>Material: HA slurry LithaBone</b><br><b>Manufacturing process: LCM (CeraFab 7500 system)</b>      | High osteoconductivity for HA LithaBone 400 based scaffold was found. Osteoclastic resorption was evident with the formation of osteoclasts and resorption pits.    | [98]       |
| <b>Material: HA slurry LithaBone</b><br><b>Manufacturing process: LCM (CeraFab 7500 system)</b>      | Diamond HA structure demonstrated higher bone ingrowth than lattice and primitive structures.                                                                       | [148]      |
| <b>Material: Filament composite of PLA and HA or PLA and BG</b><br><b>Manufacturing process: FDM</b> | PLA plus BG showed outstanding results from water and protein absorption, cell adhesion and osteoinductive differentiation when compared to PLA with HA             | [183]      |
| <b>Material : HA slurry LithaBone HA 400</b><br><b>Manufacturing process: DLP</b>                    | HA 400 LithaBone samples were biocompatible and suitable for osteogenic applications. Cells were attached over 35 days.                                             | [184]      |

| HA studies                                                                                   | Outcomes                                                                                                                                                                                                                                                                             | References |
|----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>Material :HA slurry LithaBone HA 400</b><br><b>Manufacturing process: LCM CeraFab7500</b> | Lattice structure and adaptive minimal surface (ADMS) scaffolds were assessed <i>in vivo</i> on calvaria defects of New Zealand white rabbits. Osteoconductivity of both the microarchitecture of the lattice and ADMS was effective for both noncritical and critical size defects. | [185]      |
| <b>Material: HA slurry LithaBone HA 400</b><br><b>Manufacturing process: LCM</b>             | LCM for triply minimal surface scaffolds successfully printed accurate pore sizes ranging from 350 to 800µm. No impurities were observed.                                                                                                                                            | [41]       |

The clinical management of bone defects remains an ongoing challenge in dental and orthopaedic surgery. Conventionally manufactured bone scaffolds have been on the market with limitations regarding customised scaffolds. Commercialised hydroxyapatite bone scaffolds are listed in Table 2-12.

Table 2-11: Clinical use of commercially available hydroxyapatite scaffolds manufactured using AM.

| Commercial product                                                | Clinical application                                                                        | References |
|-------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------|
| <b>MyBone Custom® Implant (MBCI)</b>                              | Maxillofacial reconstruction<br><br>Continuous osteointegration observed after a year.      | [165]      |
| <b>Customized 3D printed nanohydroxyapatite bone block grafts</b> | Horizontal alveolar ridge new bone formation.                                               | [164]      |
| <b>D-printed polycaprolactone/hydroxyapatite implants</b>         | Craniomaxillofacial defects                                                                 | [186]      |
| <b>3D Printed HA Implant</b>                                      | Temporal hollowing reconstruction.<br><br>Post-surgery the implant was securely positioned. | [169]      |

## 2.8 Conclusion

Alveolar ridge augmentation has previously been repaired with the use of bone grafts to promote bone regeneration. This process provides limited design flexibility for bone grafts, such as pore connectivity and patient custom-made grafts. Bone tissue engineering through additive manufacturing and resorbable materials is directing opportunity to address these gaps. Biodegradable material such as HA is suitable due to its biocompatibility and slow degradation that can give enough time to allow bone to regenerate. The combination of HA and AM have recently been researched. However, there remain problems of shrinkage, brittleness of the material, and process parameter limitations. An ideal scaffold that satisfies all requirements for bone tissue engineering scaffolds is still needed. AM scaffolds still face challenges such as controlled degradation, optimal pore size for bone regeneration and balance between porosity and mechanical

strength. HA as a slow degrading bio-ceramic affects the relationship between biodegradation and bone regeneration, which must still be established. The design of bone scaffolds using lattice structures influence degradation of HA, good mechanical properties and cell movement inside the scaffold.

In this literature review the bone anatomy was discussed to understand the cells constituting bone. This provided clarity on how they behave and attach, which is important for the study on designing bone scaffolds. Alveolar ridge bone defects and their classification were listed. The classification of alveolar ridge defects offers essential guidance to identify the size of defect and potential design concepts of bone scaffold to be applied. Conventional and additive manufacturing were discussed and compared to each other. Drawbacks such as pore design limitation led to the selection of additive manufacturing as an effective technology. Bone tissue engineering as a recent technology for creating bone scaffolds was explored. Important properties, including porosity, biomaterials, and manufacturing processes, were discussed.

It was found that the pore size of the bone scaffold had to be more than 100  $\mu\text{m}$  to allow cell ingrowth and biological waste removal. In previous studies lattice structure were commonly used because of the ability to change pore size. An example of how a diamond lattice structure was modified was discussed. Lattice structures are manufacturable using AM because of the technology's ability to manufacture complex structures. In comparison with conventional manufacturing AM can produce custom-made bone scaffolds and repeatability is possible. Examples of HA additive manufactured scaffolds were given with the related biological and mechanical behaviour. This demonstrated that the LCM technology could be successfully used for this application. The literature revealed the key properties required for creating bone regenerating scaffolds, which includes bone pore sizes, bone composition, selection of lattice structure, bioceramic materials, manufacturing processes, clinically available bone scaffolds, and biological and mechanical properties of bone scaffold. This information created a baseline for this study.

## CHAPTER 3

### METHODOLOGY

#### 3.1 Material - LithaBone HA480

LithaBone HA 480 was used for all the specimens that were produced by LCM.

#### 3.2 Design and fabrication for AM

Designing ceramics for 3D printing involves consideration of machine parameters and the shrinkage after sintering. Since the ceramic slurry is a composite of ceramic powder and polymer or organic binder, the shrinkage factor is taken into account during preparation for printing [96][100].

#### 3.3 Selection of lattice structure

To find a suitable lattice for HA480, different lattice structures were proposed. Strut-based lattices were selected in Figure 3-1. The lattice selected based on their potential for LCM and sintering parameters of HA480. The design guideline and constraints from the supplier were considered in particular overhangs and wall thickness [188]. To achieve a suitable lattice structure, the strut type cell topology was classified according to the Maxwell number  $M$  before printing of the samples. The number of nodes and struts from each cell diamond, rhombic, and diagonal were derived, and the Maxwell stability was calculated.

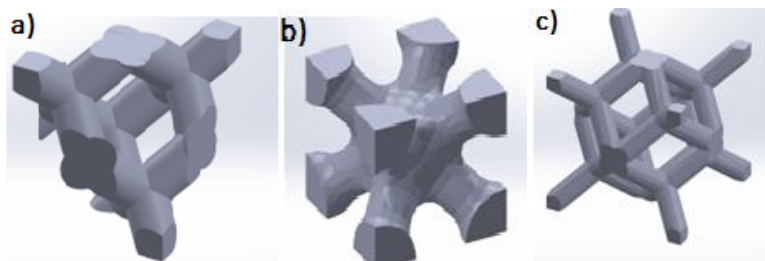


Figure 3-1: Strut-based lattice cells: a) diamond, b) diagonal, and c) rhombic dodecahedron



### 3.4 Fabrication of HA480 samples using AM technology

All samples were manufactured using CerabFab System S65 at Lithoz. CerabFab S65 in Figure 3-2 this is a state-of-the-art technology for the additive manufacturing of ceramics through digital lighting process-based LCM.

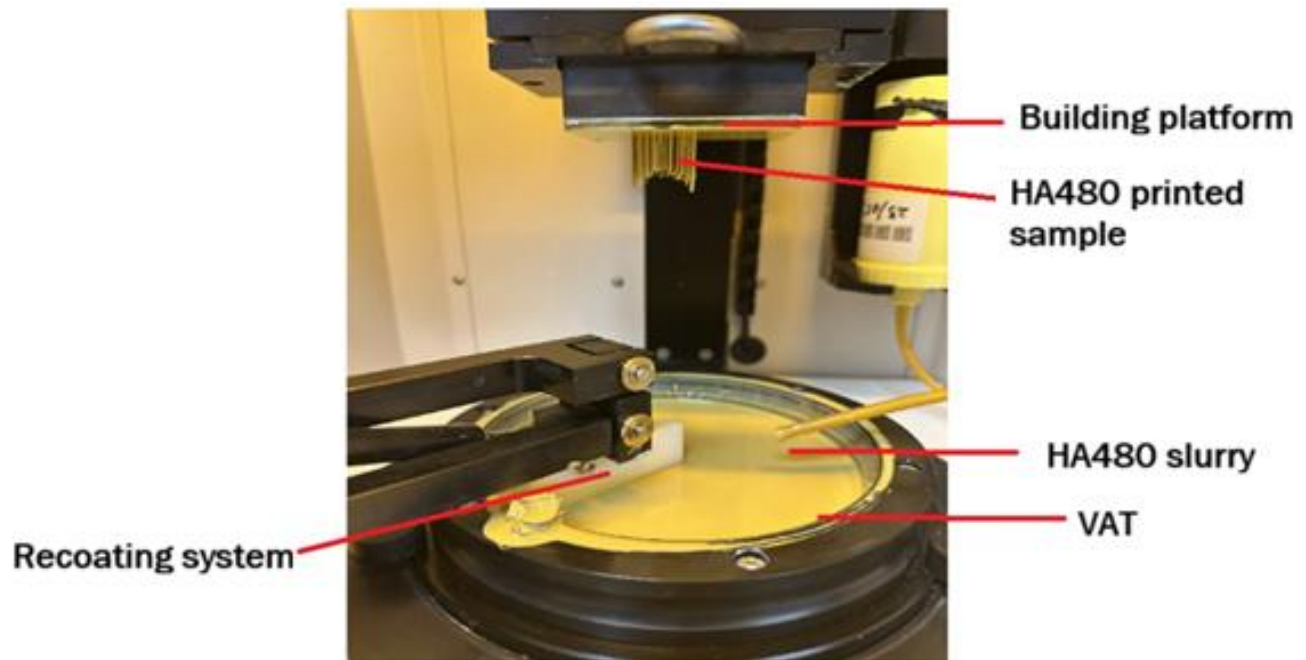


Figure 3-2: Photograph of CeraFab 65 main components [184].

The preparation of printing started where AM CAD models were transferred to standard tessellation language (STL format) and the files were then imported into 3Data Expert® software from company Deskartes (Espoo, Finland). The software was used for slicing the 3D model, translating the model into an individual layer, and generating the necessary support structures. The sliced 3D model was then loaded into the data preparation software of the CeraFab System S65, where the orientation of the part on the building platform and printing parameters were selected.

In contrast to the other AM technologies, CerabFab S65 the build platform is placed upside-down above the VAT, moving along the Z-axis during the manufacturing process. The build platform was dipped in the rotating vat, which contains the ceramic slurry. A thin slurry layer was applied to a transparent vat and building platform, filling a small

space. A recoating system applied a fresh layer before curing. Photosensitive chemicals were cured by selective light exposure, photopolymerizing monomers.

The model was printed layer by layer by the ceramic slurry being automatically dispensed in the rotating vat. The bottom of the vat was made of transparent glass through which the light source can illuminate the suspension from below the vat. The process continued layer by layer until a 3D part is completed [184].

### **3.4.1 Post-processing of HA480**

The as-built HA480 specimens' "green bodies", which consist of a cross-linked organic binder system and hydroxyapatite particles, were then prepared for post-processing. The support structures were removed with a scalpel. Excess uncured slurry was removed from the green body specimens, and were cleansed before transferred to thermal post-processing. The cleansing step was carried out using a direct stream of compressed air from an airbrush pistol and by using the commercial cleaning medium LithaSol 20. Cleaned specimens were placed in a furnace for the preconditioning process, with the temperature gradually increasing to 120 °C as shown in [171].

### **3.5 Density measurement of HA480**

The true density of the material was measured using an Anton Paar Ultrapyc 3000. This machine measured the HA480 diamond lattice structure sample. In the presence of the porous material, the helium gas used for the machines filled the voids of the lattice to get the true density. The small shell was filled to the two-thirds with 20 diamond lattice structures with a total weight of 1.95 g. The helium pressure was 19 psi. The measurement of the density was repeated for three cycles.

### **3.6 Shrinkage measurement**

The shrinkage of the material was determined by comparing the dimensions of the sintered samples in Figure 3-3 with those of the CAD model.

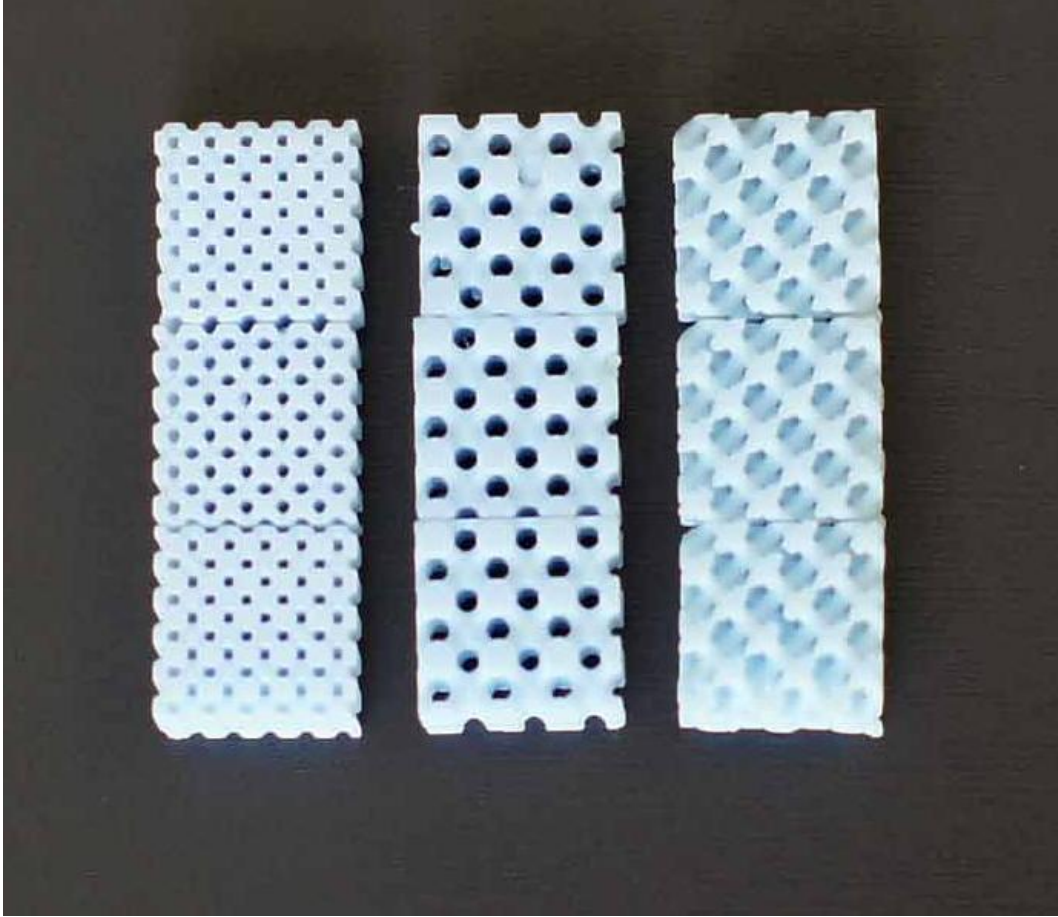


Figure 3-3: Printed HA480 lattice structures.

The length, width and thickness were measured with a micrometer. Four specimens for each lattice structure were measured and the average was recorded. The shrinkages in the Z direction ( $r_{sz}$ ) and in the XY plane ( $r_{sr}$ ) were compared. Linear shrinkage was calculated by using Equations 3.1 and 3.2 [189].

$$r(sz) = \frac{H - Hs}{H} \quad (3.1)$$

$$r(sr) = \frac{D - Ds}{D} \quad (3.2)$$

Where  $H$  is the measurement of the CAD model and  $H_s$  the measurement of the sintered specimen in the Z direction. The symbol  $D$  is the dimension of the CAD model and  $D_s$  the measurement of the sintered specimen  $h$  in the XY direction.

### 3.7 XRD analysis

The instrument used for XRD of the specimens was a Panalytical Empyrean X-ray Diffractometer equipped, with an anode consisting of Cu and a W cathode (filament) with an X Celerator detector<sup>68</sup>. Samples of HA480 of 28 days biodegradation and plain HA 480 samples were scanned between  $3.5^\circ$  and  $70^\circ$   $2\theta$  at tube settings of 45 kV and 40 mA. A Data Collector v. 3.0c was used for data collection and Highscore v. 3.0e with the ICDD PDF-2 database was used to identify the phase.

### 3.8 FTIR analysis

HA480 samples of sintered and 28 days biodegradation to identify phases for structural information by FTIR spectral analysis. The samples were analysed with the Thermo-Nicolet 6700 FTIR machine, 4000 – to  $500\text{ cm}^{-1}$ . HA480 samples were crushed and mixed with KBr, at a ratio of 1:10. The mixture of KBr and crushed HA480 was recorded for 16 scans, with resolution of  $4\text{ cm}^{-1}$ .

### 3.9 CT analysis

A solid rectangular block and a diamond lattice in Figure 3-4 cube manufactured from HA480, were sent for CT analysis at Stellenbosch University. The HA480 solid rectangular block was analysed for porosity and lattice structure cube for the measurement of struts and nodes. A CT scanned Nikon XT H 225 system was used with parameters as follows: 60kV, 100  $\mu\text{A}$ , and 5.89  $\mu\text{m}$  voxel size. Foam analyses were done with VGSTUDIO MAX 2024.1.



Figure 3-4: Printed HA480 sample diamond lattice structurer before CT scan.

### 3.10 Scanning Electron Microscopy (SEM)

General microstructure and pore morphology of the sintered lattice structures were analysed using a JSM-6610 Scanning Electron Microscope (SEM) in the Department of Geology of the University of the Free State. The mode to study the image was Secondary Electron images (SE). The chemical analysis and elemental mapping of the specimens were conducted with an energy dispersive X-ray spectroscopy (EDS) system. Sintered specimens were carbon-coated for the SEM-EDX analyses.

However, for cell culture and biodegradation, a scaffold was mounted on aluminium pin stubs using double-sided carbon tape and coated with iridium (for conductivity) using a Leica EM ACE600 high vacuum sputter coater (from Vienna, Austria). The microstructural and morphological analyses of the specimens were performed in a JEOL JSM-7800F



Extreme-resolution Analytical Field Emission SEM (from Tokyo, Japan) equipped with an Oxford Instruments X-Max 80 EDS detector, using Aztec software

### 3.11 Microhardness testing of HA480

Microhardness testing of HA480 was done firstly by preparing the surface. The samples were mounted in a clear resin on a Struers Citopress-15 (Struers, Denmark) mounting machine. They were then polished and ground in Tegramin-30 (Struers, Denmark) ground and polishing machine. The polished samples in the clear resin were mounted on a microhardness testing machine FM-700 Digital Vickers Microhardness Tester from Future-Tech Corporation.

One solid HA480 sample was tested by applying a pyramid-shaped diamond indenter. Indentations were made with a load of 300 g applied for 10 seconds. Forty indentations in three rows were made on the test specimens shown in Figure 3-5. Microhardness was tested both on the surface of the sample's height and width [173].

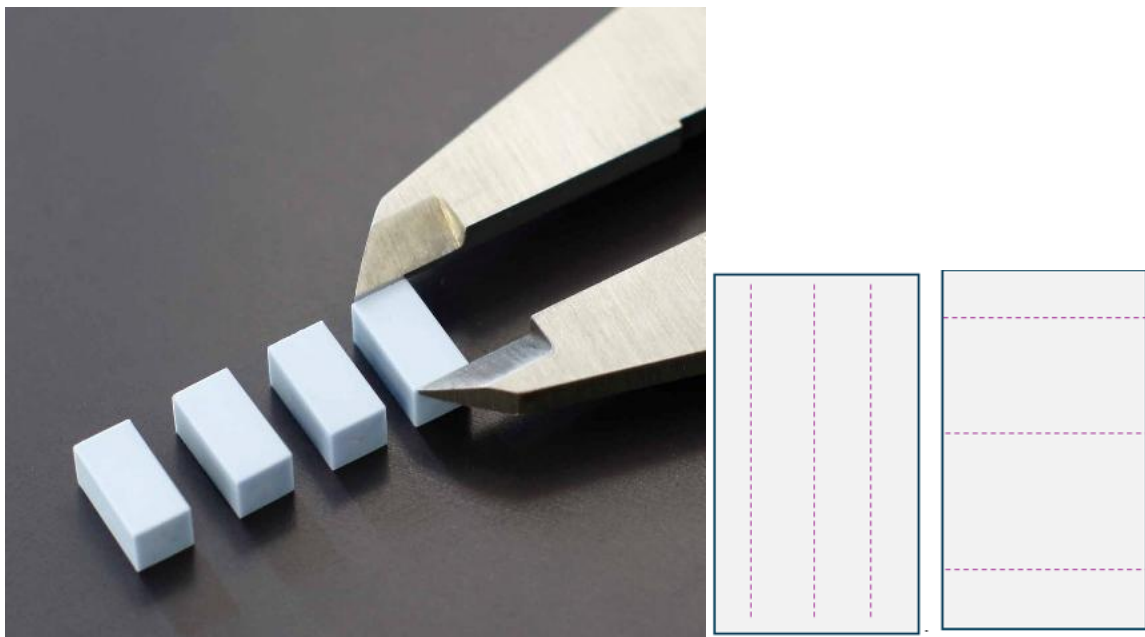


Figure 3-5: HA480 solid specimens for microhardness testing, and diagram showing the position of the indentations.

### **3.12 Compression testing**

#### **3.12.1 Compression test for different lattice structures**

Compression specimens were designed and compressed according to ISO 13175-3:2012(E) standards [190]. The standard requires the cube samples for compression to be 10 x 10 x 10 mm. However, due to the small building platform of the machine and the high cost of the material, the samples were reduced in size. The sample size for testing was 5 x 5 x 3 mm. Lattices were selected according to their Maxwell stability, printability and if high sintering temperature does not cause cracks. Three different lattice designs were compressed on an MTS Criterion Model 43 electric testing machine at the Centre of Rapid Prototyping (CRPM). For each set of lattice design samples three cubes were compressed at a speed of 1mm/min until failure. The MTS Criterion Model 43 had high load cells of 30 kN, which were less sensitive to the size of the compression specimens.

#### **3.12.2 Compression test for diamond lattice structure**

A diamond lattice was selected to be the lattice of interest in this study. HA480 diamond lattice cube (5 x 5 x 5 mm) specimens were mechanically compressed using an MTS E43 machine with a strain rate of 1mm per minute until failure. The machine load cells were 4kN. Ten specimens for each set of diamond lattice structures were compressed for dry and hydrated according to the standard ISO 13175-3:2012 [190]. Hydrated samples were immersed in simulated body fluid for 1 day at 37°C. The other HA480 specimens that went through 28 days of biodegradation in the SBF solution as in Section 3.14 were compressed at the same conditions [190].

### **3.13 Swelling profile of the scaffolds**

Samples of LCM HA480 diamond lattice structure cubes, measuring 5 x 5 x 5 mm, manufactured at CRPM were evaluated to calculate the swelling ratio. The dry weight of the samples was measured ( $W_0$ ). The dry samples were immersed in a phosphate-buffered saline (PBS) solution at pH = 7.4 in an oven at 37°C for a specified period. Three

samples were tested at each time point: 5 minutes, 60 minutes, and 24 hours. The samples were immersed, removed from the solution, and excess PBS was wiped with filter papers. The swollen samples were weighed ( $W_t$ ). Data were collected for each measurement, and the swelling ratio was calculated using Equation 3.3 [191]:

$$\text{Swelling ratio}(\%) = \frac{W_t - W_o}{W_o} \quad (3.3)$$

### 3.14 Biodegradation of HA480

The biodegradation of the HA 480 was evaluated by calculating the weight loss ratio with Equation 3.4. The diamond lattice cubes of 5 x 5 x 5 mm were tested. Lattice dry samples were immersed in the simulated body fluid (SBF) prepared by BioAssaix laboratory at the Department of Biochemistry and Microbiology, at Nelson Mandela University (South Africa). The SBF was prepared according to the protocol by Kokubo and Takadama [97]. Before dry samples were immersed in the SBF they were weighed ( $W_0$ ). The samples were then placed in the 50 mm diameter falcon tubes as demonstrated in Figure 3-6 for 1, 7, and 28 days. The samples were then taken out of the SBF and rinsed with distilled water for ten seconds. Samples were then dried in an oven of 50°C for 24 hours and weighed  $W_1$ . Biodegradation was evaluated by percentage weight loss, SEM, EDX, and X-ray diffraction (XRD) analysis.

$$\%Weight\ Loss = \frac{W_0 - W_1}{W_0} \times 100 \quad (3.4)$$



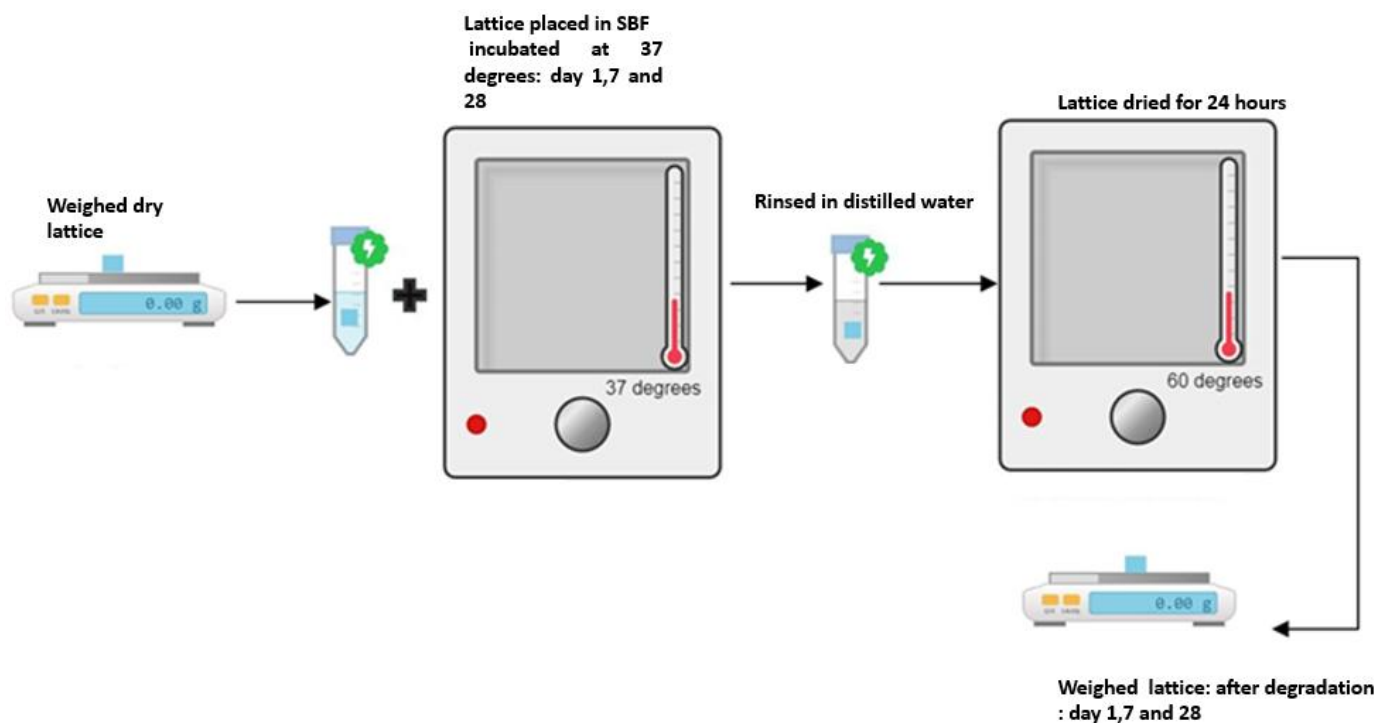


Figure 3-6: Schematic overview of the biodegradation experimental procedure.

### 3.15 Cell culture study

For analysis of material biocompatibility, cell proliferation, and cell attachment, non-porous disc HA480 scaffolds with 12 mm diameter and 1.9 mm in thickness were used. Instead of using a complicated lattice structure, which might be difficult to analyse cell attachment on them, the non-porous disc HA480 scaffold was used to analyse the cell attachment in Figure 3-7. Since it was the same material, the expectation is that similar results should also be obtained in a lattice structure. Non-powdered gloves were used to handle the samples to a falcon tube. Falcon tubes were then placed in the autoclave in the machine for sterilization 35-40 minutes cycle. Further sterilisation was performed to reduce contamination as cells are sensitive to contamination. The scaffolds were immersed in ethanol for 10 seconds.



Figure 3-7: HA480 non-porous disc scaffold for cell culture.

### 3.15.1 Cell line maintenance

Human foetal osteoblasts (hFOB 1.19) cells were purchased from Cellonex (South Africa). Cells were routinely maintained as per supplier instructions: they were maintained in 10 cm culture dishes in complete medium (DMEM/F-12, 10% FBS, G418) and incubated at 30°C in a humidified atmosphere with 5% CO<sub>2</sub>. Reagents used are listed in Table 3-1.

Table 3-1 Reagents used.

| Reagents                                                                              | Suppliers                                   |
|---------------------------------------------------------------------------------------|---------------------------------------------|
| DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) without phenol red | Cellonex, South Africa                      |
| Foetal Bovine Serum (FBS)                                                             | Cellonex, South Africa                      |
| G418 and PBS with and without $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ were              | Thermo Fisher Scientific (Waltham, MA, USA) |
| CellTiter Blue <sup>®</sup>                                                           | Promega (Madison, WI, USA)                  |
| Bis-benzamide H 33342 trihydrochloride (Hoechst)                                      | Sigma-Aldrich                               |
| Propidium iodide (PI)                                                                 | Sigma-Aldrich                               |

The quantity of resorufin produced is proportional to the number of viable cells and was quantified using a microplate fluorometer equipped with a 560 nm excitation/590 nm emission filter set.

The following steps were followed for standard and growth curve determination:

- hFOB 1.19 cells were cultured until they matured to 80% coverage.
- Cells were then seeded in 96 well plates at a density of  $4 \times 10^3$  and  $6 \times 10^3$  cells/well in 100  $\mu\text{L}$  aliquots and left overnight to attach and recover.
- On days 1, 3, 5 and 7, CellTiter Blue<sup>®</sup> was added at 20  $\mu\text{L}$  per 100  $\mu\text{L}$  medium and incubated for 4 hours.
- Fluorescence was measured at excitation/emission wavelengths of 560nm using a BioTek<sup>®</sup> Synergy Hx multimode plate reader (Winooski, VT, USA).

### **3.15.2 Proliferation analysis on HA480 cell culture samples using CellTiter Blue®**

HA480 cell culture samples were placed in a 24-well plate using a sterilised tweezer. Cells were seeded onto scaffolds at a density of 35 000 cells per well (volume: 1 mL) and allowed to attach and recover overnight. HA480 cell culture samples were removed from the seeded plate and placed into a new 24-well plate.

The following method was performed on Days 1, 3, 5 and 7:

- CellTiter Blue® (20 µL per 100 µL medium) was added.
- Incubated for a minimum of 4 hours.
- 200 µL CellTiter Blue® was transferred to a new 96-well plate.
- Fluorescence was measured using a 560 nm excitation filter set.

### **3.15.3 Preparation of cell attached HA480 samples for SEM analysis**

HA480 cell culture samples were prepared for cell attachment and SEM morphology studies. Cell-cultured samples were removed and transferred to a new 24-well plate. The scaffolds were then fixed using 4% formaldehyde overnight for microscopy. The scaffolds were then washed with PBS and dehydrated using graded ethanol-water solutions (30%, 50%, 70%, 90%, and twice at 100%) and kept in a fume hood to dry at room temperature. Sample preparation and examination using SEM were performed as described in Section 3.10.

## **3.16 Finite Element Analysis of HA480 diamond lattice structure**

The nTopology design flowchart of a diamond lattice for FEA is shown in Figure 3-8.

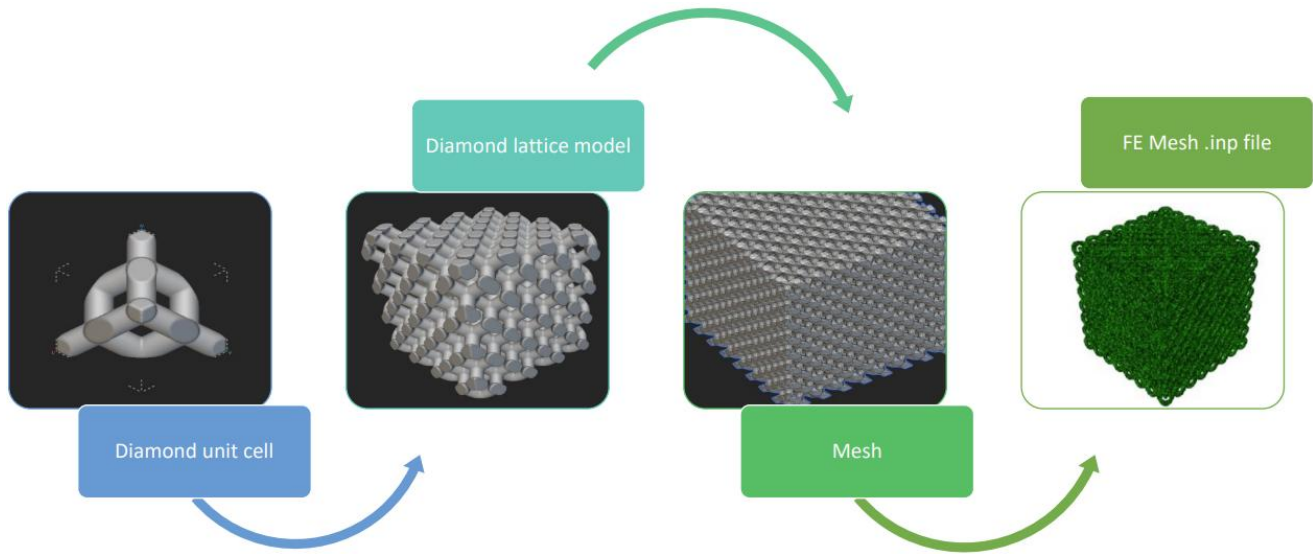


Figure 3-8: nTopology design flowchart of a diamond lattice for FEA

The diamond cell was imported into the nTopology Implicit model of the 5 x 5 x 5 mm cube to form a 3D model. The dimensions of the model were the same as the specimen used in the experimental compression testing.

The mesh applied for the diamond lattice structure was a quadratic tetrahedral element mesh. Thereafter the meshed lattice model was prepared for ABAQUS simulation software as FE Mesh .inp file.

Figure 3-9 shows the flowchart of the process followed for FEA analysis of the diamond lattice structure.

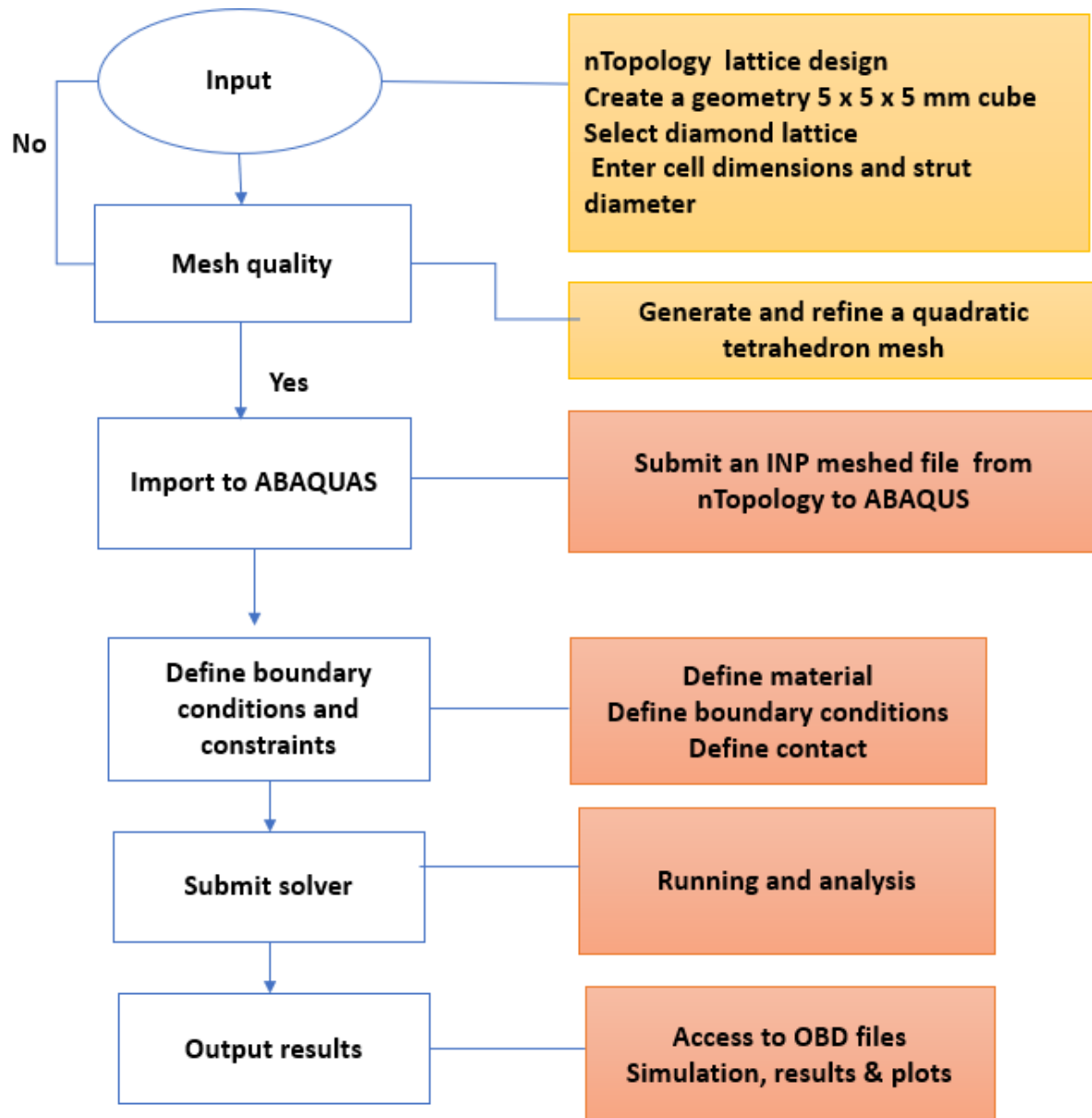


Figure 3-9: Flowchart of the process followed for FEA analysis of the HA480 diamond lattice structure.

The process input started with designing of the 3D lattice structure similar to the structure of the specimen used in the experimental testing. The meshed .inp file was imported to ABAQUS and the HA480 material properties were defined: density of  $3030 \text{ kg/m}^3$  and a theoretical Poisson's ratio of 0.3 for HA. The lattice was then set up with displacement

boundary conditions that represented the condition of the experimental compression testing.

The file was submitted to ABAQUS solver for simulation. The resultant output were the load and displacement. These were then used to derive stress from dividing the load by the surface area. The strain was derived by dividing the displacement by the height of the lattice model. The stress-strain curve was generated and the modulus of elasticity was calculated by determining the gradient of the stress-strain curve.

### 3.16.1 Set up for FEA analysis

Figure 3-10, shows the conditions during the set up of the model in ABAQUS. These conditions were the same as the conditions used in the experimental testing.

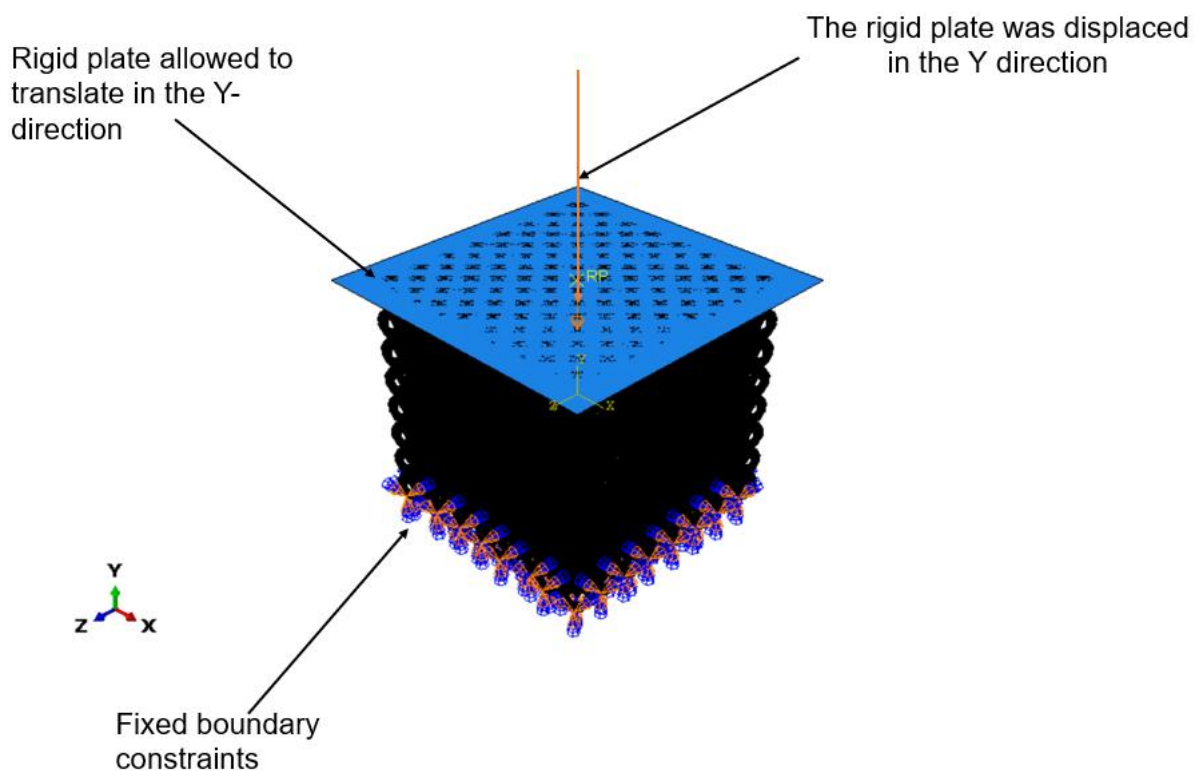


Figure 3-10: Constraints and displacement on the FEA model.



To simulate the compression behaviour of the lattice the diamond lattice was placed under a rigid plate which was constrained on the upper surface of the lattice. The lower surface of the lattice was constrained based on the boundary conditions used during the experimental testing with the condition  $ENCASATER U1=U2=U3=UR1=UR2=UR3=0$ . The movement of the top plate was conducted by applying a displacement in the Y direction while all other directions were restricted and kept to zero ( $U1=U2=U3=UR1=UR2=UR3$ ). The displacement value applied was the same as that of the experimental testing. The material parameters used in the computational model were based on the experimental mechanical testing parameters. The material property input for the model was the same as the experimental mechanical parameters of porous HA480, with a density of  $3030 \text{ kg/m}^3$ . The theoretical Poisson's ratio of 0.3 for HA was used [192].

### 3.17 Statistical analysis

Statistical analysis was conducted using Social Science Statistics software. One-way ANOVA was employed to assess the significance between samples and specimen groups, with a significance level set at  $p < 0.05$  for all tests (\*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ ) (n.s: not significant).



## CHAPTER 4:

### RESULTS AND DISCUSSION: SCAFFOLD LATTICE STRUCTURES, BUILT BY ADDITIVE MANUFACTURING, THAT MIMIC NATURAL BONE STRUCTURE AND PROPERTIES

*Part of the results presented in this chapter are from the published work: Moletsane, M.; Du Preez, W.; De Beer, D. and Nkhwa, S.. Characterisation of bone-regenerating scaffolds produced through lithography-based ceramic manufacturing. S. Afr. J. Ind. Eng. 2024, vol. 34(4), pp. 47-58. ISSN 2224-7890. [http://dx. doi. org/10.7166/34-4-2984](http://dx.doi.org/10.7166/34-4-2984).*

#### 4.1 INTRODUCTION

To achieve the desired porosity of the scaffold, it was designed with a lattice structure, that is, a porous, 3D structure composed of repetitive interconnected cells, struts, and nodes [160].

This chapter presents the selection of a lattice structure by considering the Maxwell criterion, the printability, and the potential to be used for bone regeneration. Diamond, diagonal, and rhombic lattice structure cubes were manufactured using the AM technology described in Chapter 3.3 by Lithoz. The material used for all the specimens was hydroxyapatite HA480.

#### 4.2 Optimization of the unit cell

A lattice structure is a repetition of a unit cell. The selection of a proper unit cell for bone regeneration is important, as it can also allow cells to attach and proliferate. Optimisation of the unit cell for the lattice structure is important to know how the whole lattice will behave. The scaffold stability was optimised by calculating the Maxwell value for all three unit cells shown in Figure 4-1. The results showed that the diamond lattice structure was a bending-dominated unit cell, because the Maxwell number was less than zero in Table 4-1 [117]. As diamond unit cell is a bending-dominated and can be used to absorb energy. Table 4-1 shows the diagonal and rhombic dodecahedron unit cells had  $M > 0$ , which are

stretch-dominated structures. The lattice work by Park *et al.*, [117], which included the diamond and rhombic dodecahedrons, differed as all the lattices were bending-dominated. The number of struts for the rhombic structure is 48, which matched the optimisation results obtained by Park *et al* [117]. However, their Maxwell number indicated stretch-dominated characteristics that differed from the results caused by their increased numbers of nodes [117]. The bending-dominated lattices deform consistently compared to the stretch-dominated ones, even though the stretch was high stiffness and strength [111]. The current study focused on achieving controllable material degradation. It is ideal to use a lattice structure that deforms consistently [99][172]

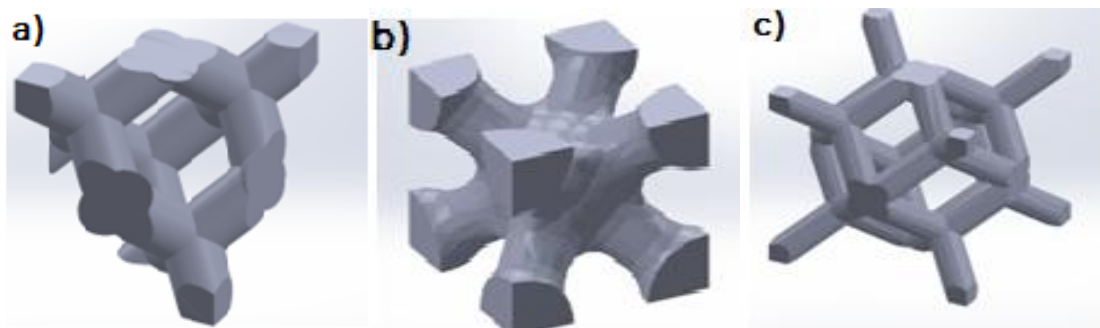


Figure 4-1: CAD designs for lattice unit cell structure: a) diamond, b) diagonal, and c) rhombic dodecahedron [117]

Table 4-1 shows the results from Equation 2.9 used to determine whether the cell unit was bending- or stretch-dominated. Maxwell ( $M$ ) value was recorded accordingly. When  $M < 0$ , the cell is bending-dominated; when Maxwell  $M > 0$ , it was stretch-dominated.

Table 4-1: Maxwell stability of unit cells

|                         | <b>Diamond</b> | <b>Diagonal</b> | <b>Rhombic<br/>dodecahedron</b> |
|-------------------------|----------------|-----------------|---------------------------------|
| <b>Number of nodes</b>  | 7              | 1               | 12                              |
| <b>Number of struts</b> | 9              | 8               | 48                              |
| <b>Maxwell value</b>    | -6             | 11              | 18                              |

The diamond lattice was a bending-dominated structure with a Maxwell value of negative 6 that is suitable for energy absorption and damage tolerance. Results for diagonal and rhombic dodecahedron show stretch-dominated structures with positive Maxwell values which are mechanically efficient.

### 4.3 HA480 lattice structure shrinkage

The CAD model of all three lattices used the same cube measuring 5 x 5 x 3 mm. During the LCM fabrication preparation, the material shrinkage compensation was set up to maintain accuracy of pores with various pre-set diameters. Table 4-2 shows that there was overcompensation for shrinkage, as evidenced by the negative shrinkage values.

Table 4-2: Shrinkage percentage calculations of different lattice structures HA480

|                      | Length (mm)                       | Width (mm)                        | Thickness (mm)                     |
|----------------------|-----------------------------------|-----------------------------------|------------------------------------|
| <b>Diamond</b>       |                                   |                                   |                                    |
| CAD                  | 5                                 | 5                                 | 3                                  |
| Printed and sintered | $5.38 \pm 0.1$                    | $5.39 \pm 0.1$                    | 3.1                                |
| <b>Shrinkage %</b>   | <b><math>-7.6 \pm 0.6</math></b>  | <b><math>-7.8 \pm 0.2</math></b>  | <b>0</b>                           |
| <b>Diagonal</b>      |                                   |                                   |                                    |
|                      | Length (mm)                       | Width (mm)                        | Thickness (mm)                     |
| CAD                  | 5                                 | 5                                 | 3                                  |
| Printed and sintered | $5.4 \pm 0.1$                     | $5.39 \pm 0.1$                    | $3.4 \pm 0.1$                      |
| <b>Shrinkage %</b>   | <b><math>-8.1 \pm 0.1</math></b>  | <b><math>-7.8 \pm 0.2</math></b>  | <b><math>-13.33 \pm 0.3</math></b> |
| <b>Rhombic</b>       |                                   |                                   |                                    |
|                      | Length (mm)                       | Width (mm)                        | Thickness (mm)                     |
| CAD                  | 5                                 | 5                                 | 3                                  |
| Printed and sintered | $5.43 \pm 0.04$                   | $5.41 \pm 0.03$                   | $3.43 \pm 0.02$                    |
| <b>Shrinkage %</b>   | <b><math>-8.5 \pm 0.83</math></b> | <b><math>-8.3 \pm 0.64</math></b> | <b><math>-7.6 \pm 0.54</math></b>  |

The three lattice structures, diamond, diagonals, and rhombic, have negative actual sintered shrinkage percentage errors, with an overcompensation of less than 10%. Overcompensation ensures that the pores of the lattice are not reduced in size, which

could affect cell migration and waste movement if the scaffold is implanted into the body [147]. The post-processing of hydroxyapatite, involves experiencing shrinkage during the debinding and sintering of the samples. The binder evaporates during the debinding process, and the HA particles fuse during the subsequent sintering process [166].

#### 4.4 Structural integrity lattice

The results in Figure 4-2 indicated the samples' pores were clear of all impurities. This proved that the precision of the CeraFab System S65 was adequate for printing complicated lattices without slurry clogging the pores. Figure 4-2 a) and c) shows that the diagonal and rhombic structures had many dead-end channels instead of an open network like the diamond lattice [125][193].

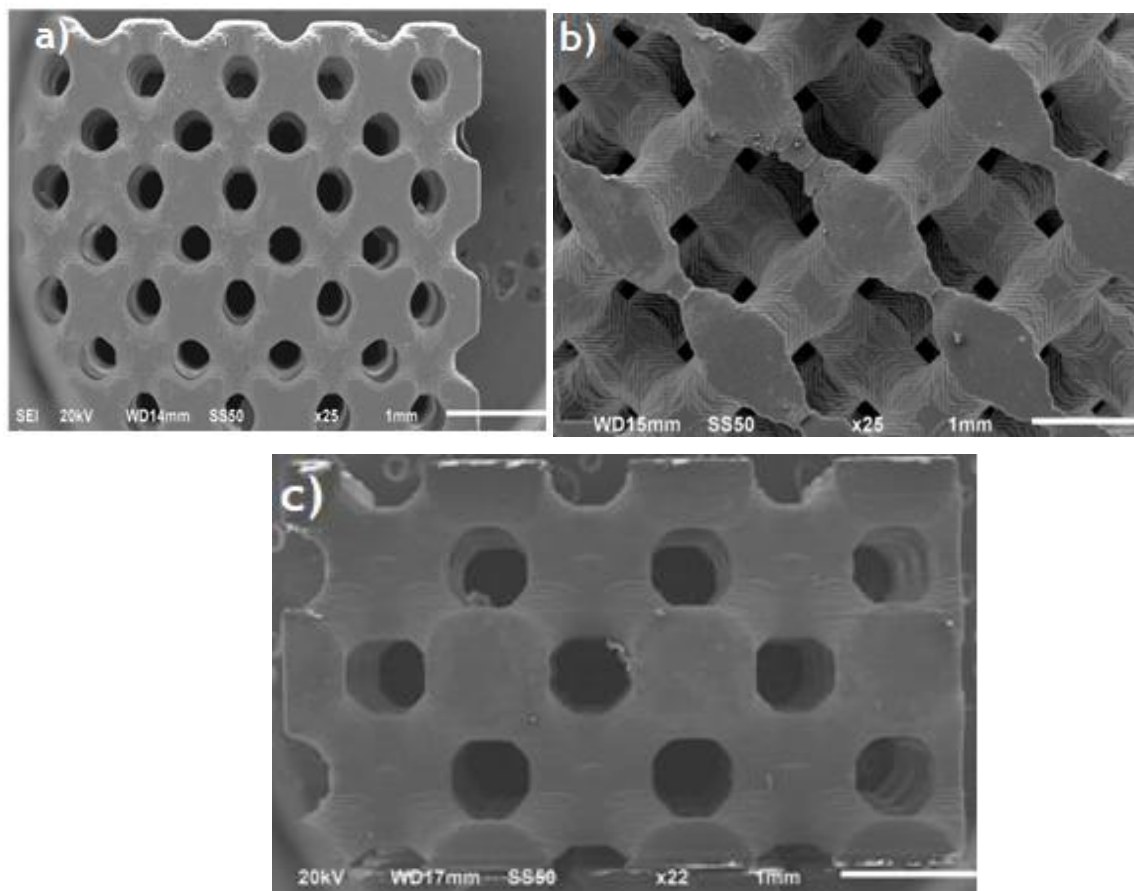


Figure 4-2: SEM analysis of HA480 lattice structure a) rhombic b) diamond and c) diagonal

## 4.5 Physical and chemical properties of the lattices

### 4.5.1 Lattice structure defect on the first layers

In the diamond lattice structure shown in Figure 4-3, the first layers were attached to the printing platform were cracked, and other sections were broken. In comparison, the top or side view layers were not affected.

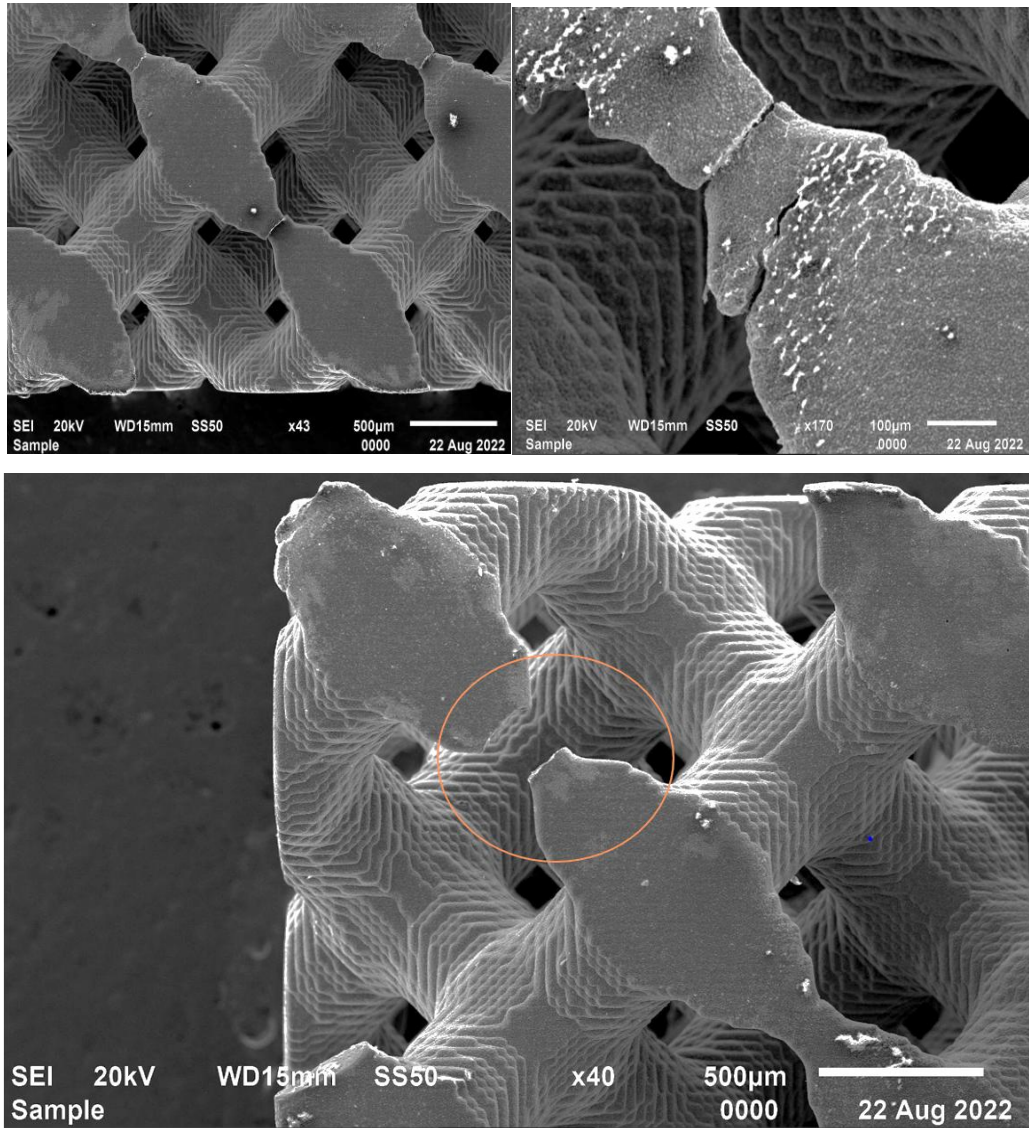


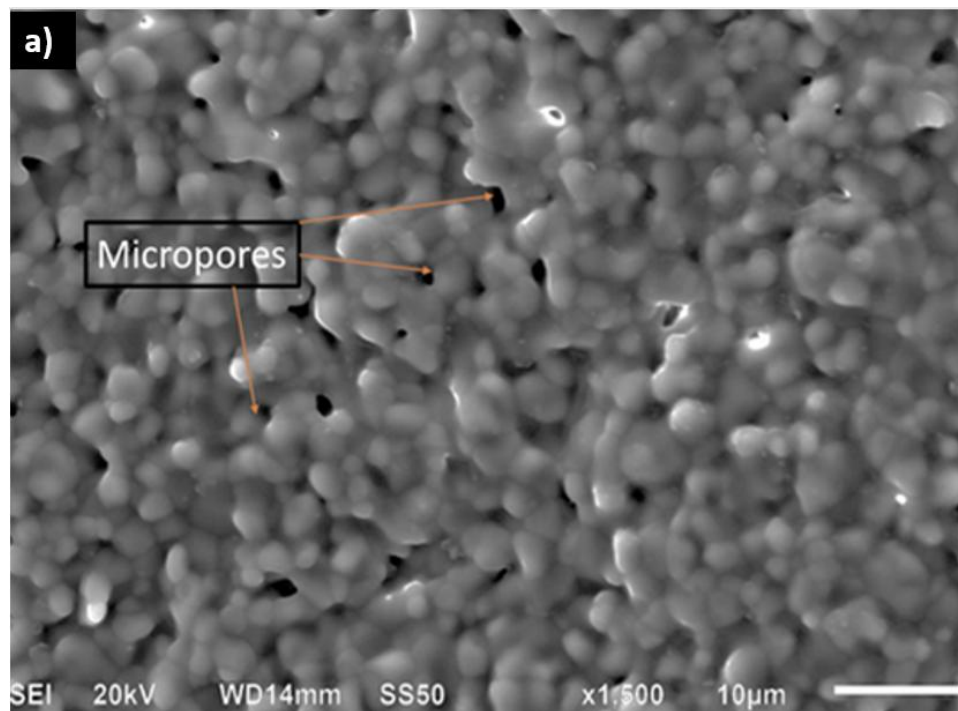
Figure 4-3: SEM-SE images of the first-layer overhang cracks in the diamond lattice. The broken overhang is circled in orange.



Removal of the samples from the building platform probably caused the breakages. Kang *et al.*, [194] found that the first print layers of lattice structures had chipped edges of their removal from the building platform, which could initiate crack growth, resulting in premature scaffold failure[194][182]. Printing supports are recommended for such lattice structures to avoid the structure itself being damaged [195].

#### 4.5.2 Additive manufacturing layers

SEM-SE showed that the stacked layers of HA480 formed a complete 3D part, as shown in Figure 4-4.



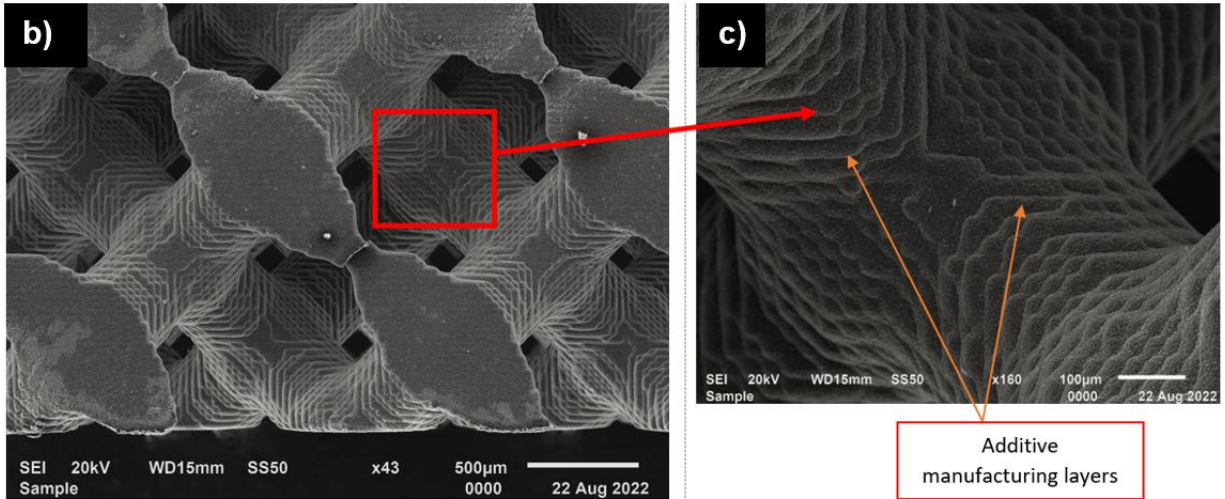


Figure 4-4: SEM-SE images of a) micropores, b) macropores and c) HA480 LCM layers.

All the printed 3D lattice structures showed successful additive manufacturing. From the SEM results, macropores were evident in all the lattice structures, as illustrated in Figure 4-4b). Macropores are essential for fluid permeability [196]. During debinding, the evaporation of binder leads to the formation of micropores, resulting in a large surface area that is conducive for ion exchange, as shown in Figure 4-4c).

#### 4.5.3 HA480 composition

The EDX spectra of the different HA480 lattice structures is shown in Figure 4-5, Figure 4-6 and Figure 4-7. The spectra confirm the presence of Ca, O, and P, the constituents of the biocompatible calcium phosphate phase.



Log full scale counts: 251969

22628K15(33)\_pt2

Cursor: 4.500 keV  
17 Counts

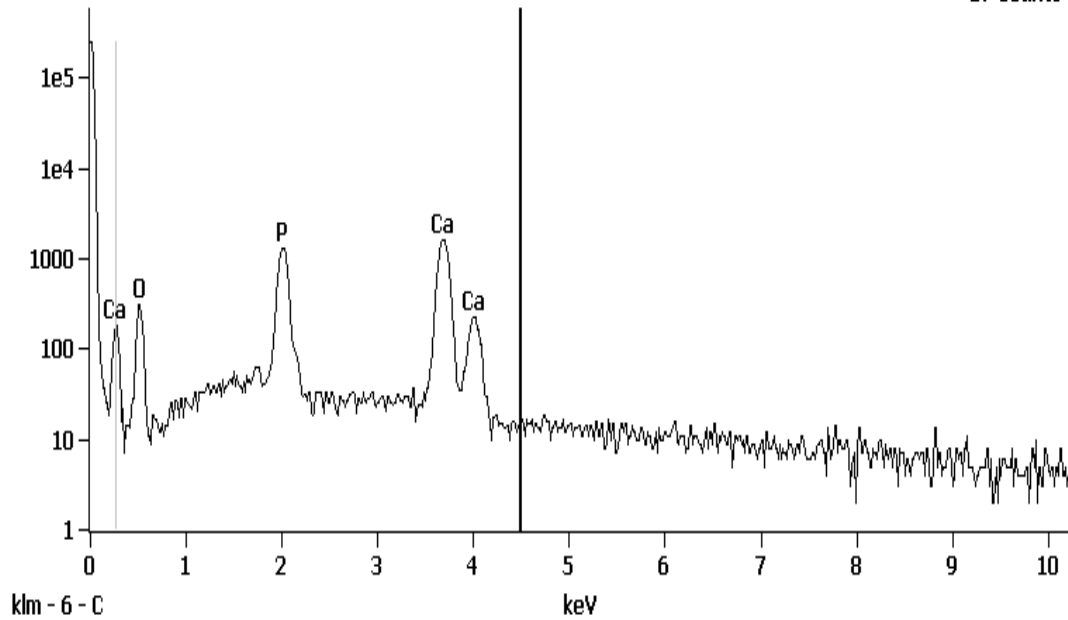


Figure 4-5: EDX composition spectrum of diamond lattice structure.

Log full scale counts: 143176

22628K15(28)\_pt1

Cursor: 5.411 keV  
2 Counts

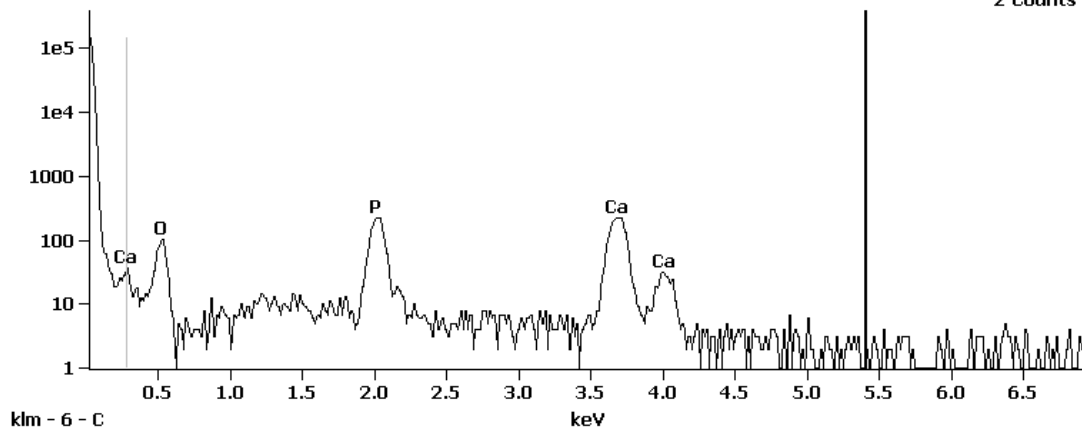


Figure 4-6: EDX composition spectrum of diagonal lattice structure.

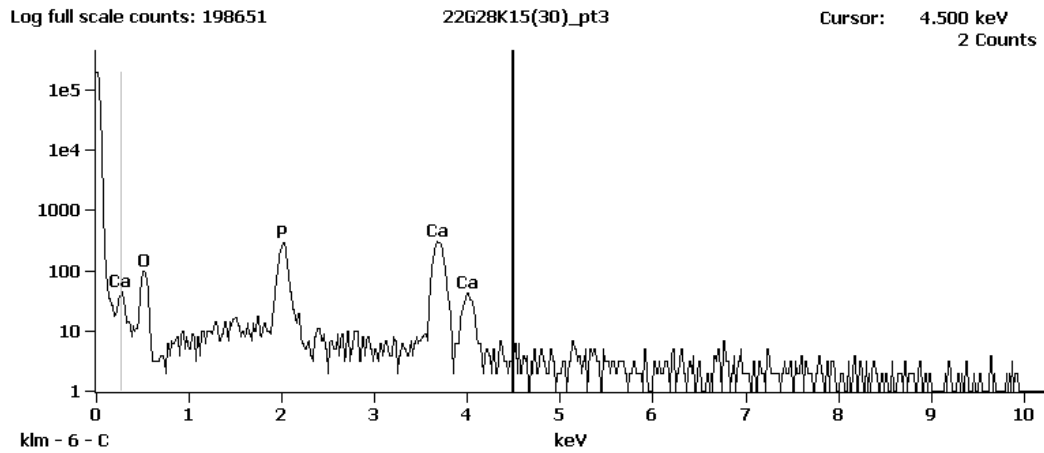


Figure 4-7: EDX composition spectrum of rhombic lattice structure.

From the EDX results given in Table 4-3, the average Ca/P wt.% ratio of the HA480 was calculated.

Table 4-3: HA480 element analysis from EDX.

| Lattice design  | O          | P         | Ca       | Ca/P           |
|-----------------|------------|-----------|----------|----------------|
| <b>Rhombic</b>  | 60.0±1.35  | 14.1±0.2  | 25.1±0.3 | 1.8            |
|                 | 60.5±2     | 14.2±0.2  | 25.2±0.3 | 2              |
|                 | 60.5±0.9   | 18.5±0.2  | 24.8±0.4 | 1              |
|                 | 60.90±1.33 | 14.4 ±0.2 | 24.4±0.3 | 2              |
| <b>Average</b>  |            |           |          | <b>1.6±0.2</b> |
| <b>Diagonal</b> | 60.0±1.4   | 14.1±0.2  | 25.1±0.3 | 2              |
|                 | 60.5±2     | 14.2±0.2  | 25.2±0.3 | 1              |
|                 | 60.5±0.9   | 18.5±0.2  | 24.8±0.4 | 1.8            |
|                 | 60.9±1.3   | 14.4 ±0.2 | 24.4±0.3 | 1.7            |
| <b>Average</b>  |            |           |          | <b>1.6±0.2</b> |
| <b>Diamond</b>  | 60.2±2.5   | 17.9±0.5  | 24.8±1   | 1              |
|                 | 61.4±3.1   | 15.3±0.4  | 23.3±0.6 | 2              |
|                 | 60.6±3.7   | 14.2±0.34 | 25.2±0.6 | 2              |
|                 | 60.4±3.8   | 13.9±0.4  | 24.5±0.6 | 2              |
| <b>Average</b>  |            |           |          | <b>1.6±0.2</b> |

The average for lattice structures  $1.6 \pm 0.2$ , which is close to the theoretical Ca/P ratio of hydroxyapatite of 1.67 [197]. The HA480 lattice structure was analysed by EDX to verify that the Ca/P ratio was the same as the standard HA [198]. The EDX results of the LCM HA480 at various locations on the specimen indicated the calcium phosphate elements suitable for bone regeneration [139].

## 4.6 FTIR results for HA480

The FTIR spectra of the crushed HAA480 of the green body and the sintered specimen were recorded in the range from 4000 - 500  $\text{cm}^{-1}$  and are shown in Figure 4-8.

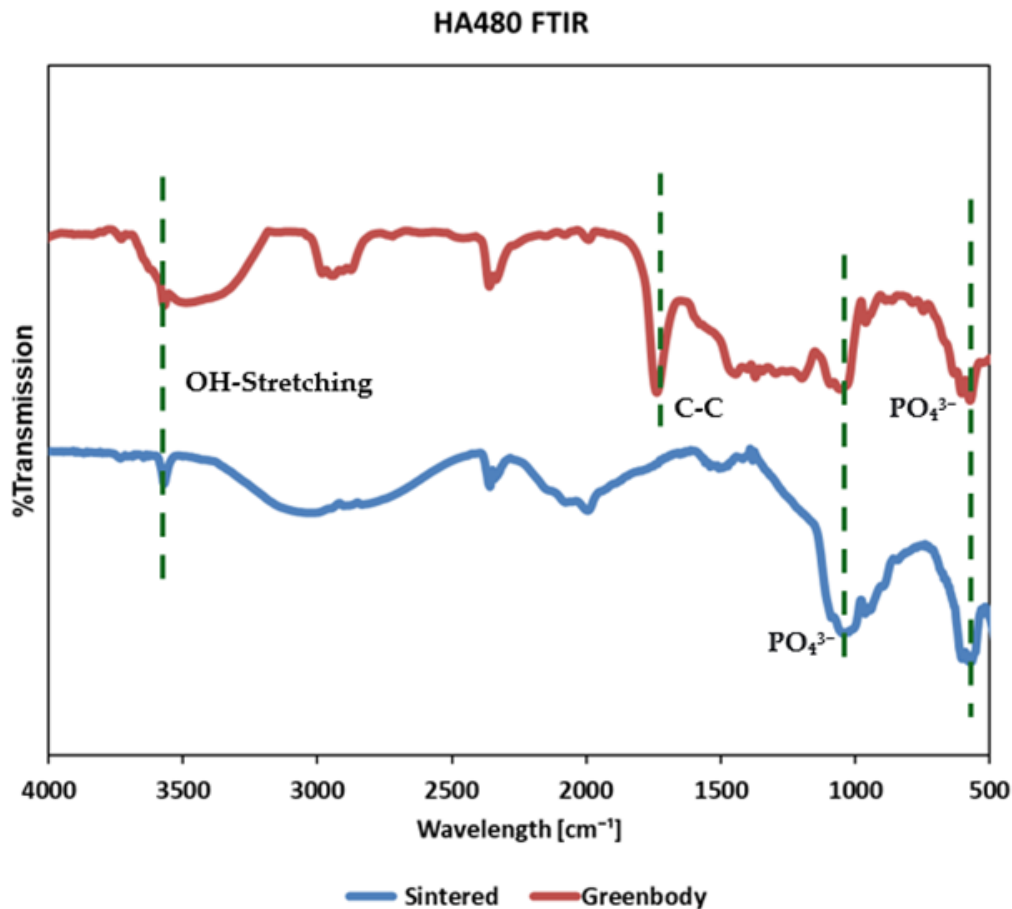


Figure 4-8: FTIR spectra of HA480 sintered (blue) and green body (red).

This test was conducted to assess whether residual binder remained in the HA480 following the sintering process and to determine the comparability of the HA480 with the HA specification. The HA480 spectra indicated 3569.7  $\text{cm}^{-1}$  for sintered and 3567.7  $\text{cm}^{-1}$  for green body, which demonstrated the hydroxyapatite OH stretching behaviour. A sharp peak around 1043.3  $\text{cm}^{-1}$  is caused by stretching vibrations of the phosphate ( $\text{PO}_4^{3-}$ ) [199]. The absorption stage for both the green body and sintered HA480 was evident at

570.8  $\text{cm}^{-1}$  and 565  $\text{cm}^{-1}$ , respectively. The absorption was associated with HA, being a P-O bending vibration. The absorption peak at 1735.6  $\text{cm}^{-1}$  in the green body indicated crosslinking [200]. The difference in the shape of the red graph (green body) and blue graph (sintered) indicated that no binder was left in the HA480 after sintering. The binder was completely removed leaving HA480 sintered part. The peaks for HA480 were comparable to those in other studies on HA printed scaffolds in Table 4-4 [104][194].

Table 4-4: HA480 FTIR results peaks in comparison with published values.

| Sample                     | OH stretching ( $\text{cm}^{-1}$ ) | ( $\text{PO}_4^{3-}$ ) stretching vibration ( $\text{cm}^{-1}$ ) | C-H stretching ( $\text{cm}^{-1}$ ) | $\text{PO}_4^{3-}$ bending vibration ( $\text{cm}^{-1}$ ) |
|----------------------------|------------------------------------|------------------------------------------------------------------|-------------------------------------|-----------------------------------------------------------|
| Current work               |                                    |                                                                  |                                     |                                                           |
| HA480 green body           | 3567.7                             | 1043.3                                                           | 2873                                | 570.8                                                     |
| HA480 sintered (1300°C)    | 3569.7                             | 1043.3                                                           | Not detected                        | 565                                                       |
| Tripathi and Basu [104]    |                                    |                                                                  |                                     |                                                           |
| HA sintered (1200°C)       | 3568                               | 985                                                              | Not reported                        | 1030 and 565                                              |
| Kang <i>et al.</i> , [194] |                                    |                                                                  |                                     |                                                           |
| HA green body              | Not reported                       | 1041                                                             | 2862                                | 570                                                       |
| HA sintered (1250 °C)      | ~3568                              | 1041                                                             | Not reported                        | 570                                                       |

## 4.7 Mechanical and physical properties of the lattice structure

### 4.7.1 Microhardness

Microhardness was tested on both vertical and horizontal directions. From the Vickers microhardness results for H480, the horizontal average results were  $556 \pm 25$  HV, and

the vertical results were  $559 \pm 27$  HV. The results were similar in both directions. The values were within the standard range (300-700 HV) of the microhardness of hydroxyapatite as compared in Table 4-5 [139][144]. The hardness of ceramics depends on porosity and grain size and porosity increases, the hardness decreases [172].

Table 4-5: HA480 hardness results in comparison with published values.

| Material                     | Average Vickers Hardness (HV) | Reference    |
|------------------------------|-------------------------------|--------------|
| <b>HA480 results</b>         |                               |              |
| HA480 horizontal results     | $556 \pm 25$                  | Current work |
| HA480 vertical results       | $559 \pm 27$                  | Current work |
| <b>Literature references</b> |                               |              |
| HA dense                     | 300 - 700                     | [144]        |
| AM produced HA               | 160 – 281                     | [202]        |

The rectangular sample with exact dimensions was evaluated by micro-CT analysis. Figure 4-9 shows, the dense rectangular HA480 had porosity in the centre of the sample. These elongated pores are a result of the release of gas during debinding [136].

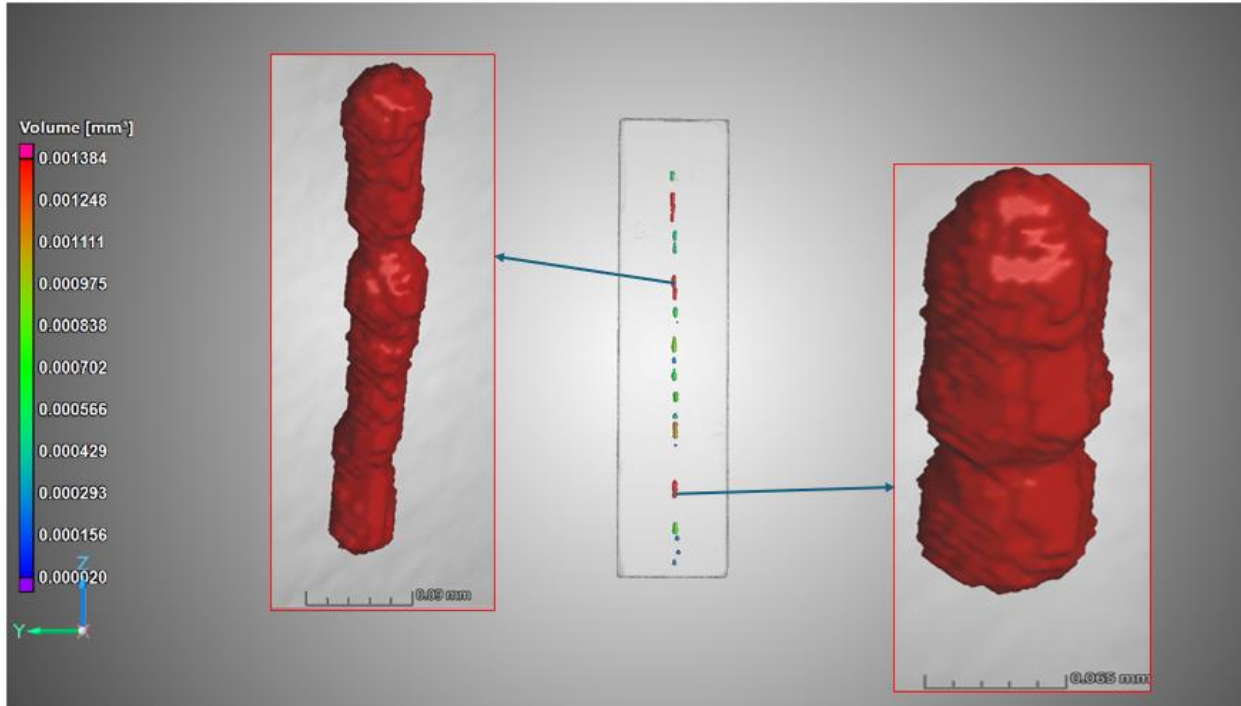


Figure 4-9: Micro-CT results of the hardness test specimen showing enlarged views of the porosity.

#### 4.7.2 Compressive strength of different HA480 lattice's structure

Three specimens of each HA480 lattice structure design were compressed until failure at the CRPM. The findings are shown in Figure 4-10.

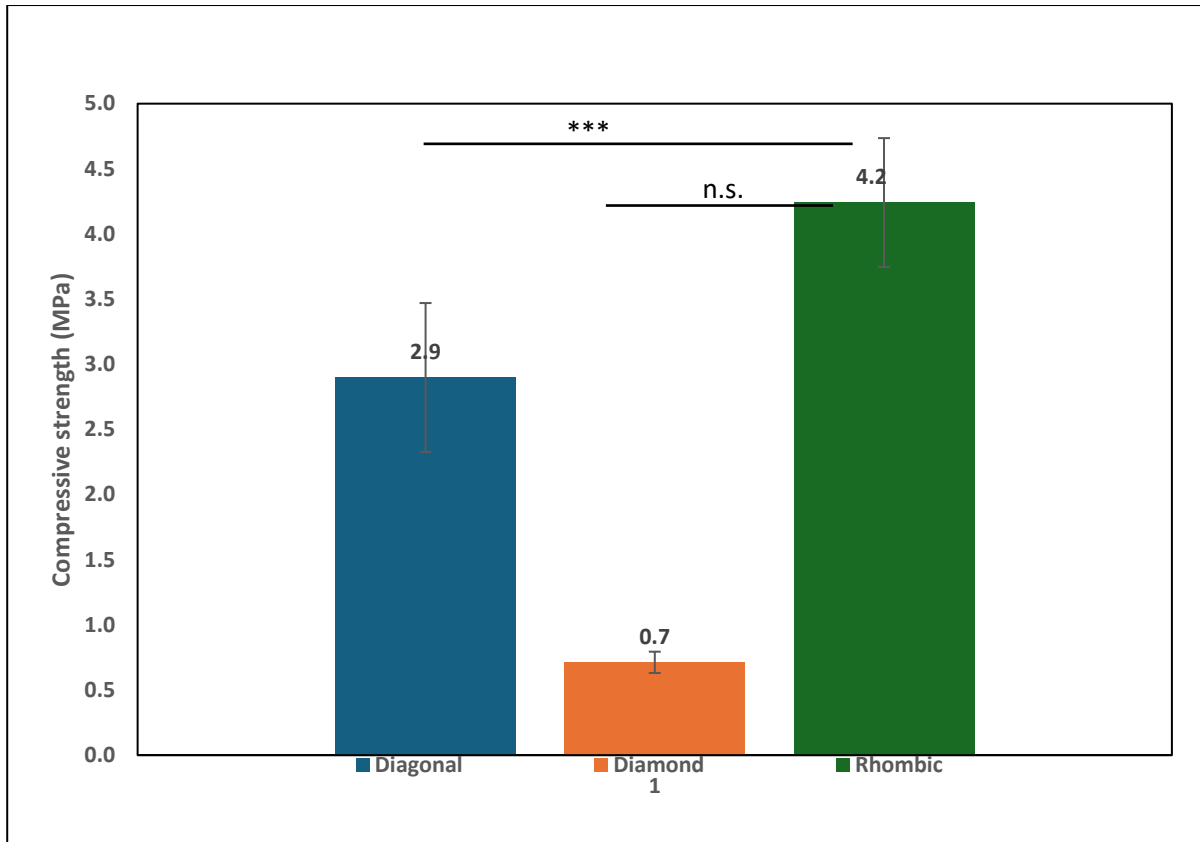


Figure 4-10: Compressive strength of HA480 with different lattice (n=3) tested at CRPM with Statistically significant differences between different data are indicated as \*\*\* ( $p < 0.001$ ), and n.s ( $p \geq 0.05$ ).

Three lattice design specimens of HA480, were compressed until failure on load cells of 30 kN. The diagonal lattice structure HA480 compressive strength was  $2.9 \pm 0.3$  MPa. The rhombic dodecahedron lattice had a higher compressive strength of  $4.2 \pm 0.5$  MPa, which had no significant impact on diamond compressive strength of  $0.7 \pm 0.1$  MPa ( $p = 1$ ). As stretch-dominated structures, the rhombic and diagonal lattices had higher strength. While diamond had lower compression strength, it demonstrated bending-dominated Maxwell stability. All three samples were highly significantly different ( $p < 0.0001$ ) to each other. The diagonal and rhombic dodecahedron lattice structures exceeded the minimum strength of cancellous bone. The LCM HA480 compressive strength was within the acceptable range compared to ( $5.6 \pm 0.85$ ) to porous HA LCM printed bone scaffolds investigated by Kang *et al.* [194].



However, in comparison with HA480 studied with different lattice structures by D'Andrea *et al.* [192], the minimum strength obtained was for the diamond lattice, with the value of  $8.3 \pm 1.7$  MPa. This significant difference in comparison to the current results was due to the compression machine used initial testing at CRPM which is for testing higher strength specimens. Additionally, the current specimens, although they were manufactured with the same process parameters as D'Andrea *et al.* [192], had different holding time of 1 hour. Further investigation of mechanical testing was conducted on a small-scale compression machine with a 4 kN load-cell at North-West University.

#### **4.7.3 Mechanical properties of the diamond structure**

Specimens were tested on a small-scale compression testing machine with a load-cell of 4 kN at NWU, as explained in Section 3.12. The diamond lattice specimens were compressed until failure for both dry conditions and hydrated conditions incubated in the simulated body fluids (SBF) and the compression strength and modulus of elasticity were calculated. Figure 4-11 displays the average compressive strength of the diamond lattice specimens.

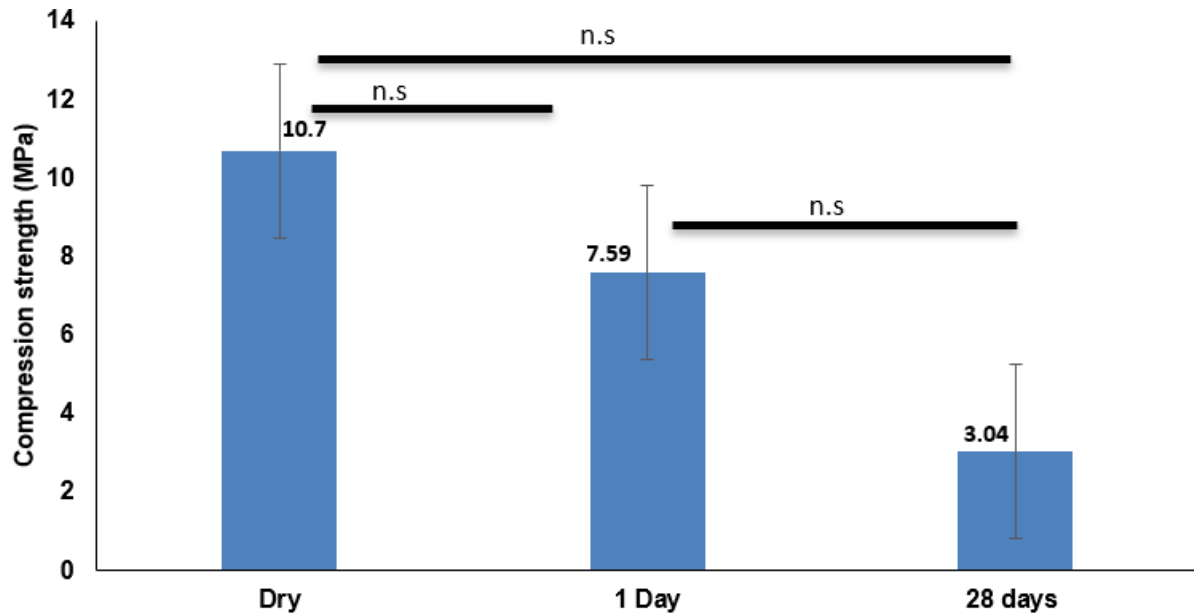


Figure 4-11: Compressive strength of HA480 diamond lattice structure (n=10) before and after immersion in the SBF. Statistically significant differences between different data are indicated as n.s ( $p \geq 0.05$ ).

The hydrated samples that were immersed in SBF for 1 day had a compressive strength of  $7.59 \pm 4.1$  MPa. This good mechanical strength of the scaffold indicated that the material should be able to withstand the attachment and movement of cells that would be moving into the scaffold in the body. The compressive strength of the sample after immersion in the SBF for 28 days of biodegradation was  $3.04 \pm 0.2$  MPa due to material degrading in the solution. This was a considerable loss of strength when compared to the dry samples and was the result of the HA480 having degraded with 8.11% mass loss. The compressive strength of the dry sample is comparable with the diamond lattice structure of HA480 of  $8.3 \pm 1.7$  MPa [180]. Liu *et al.*, [173] reported the compressive strength of an HA scaffold of 15.25 MPa, which is higher than that of HA480.

The modulus of elasticity of the diamond lattice specimens is presented in Figure 4-12. The dry diamond lattice structure had a modulus of elasticity value of  $0.5 \pm 0.4$  GPa.

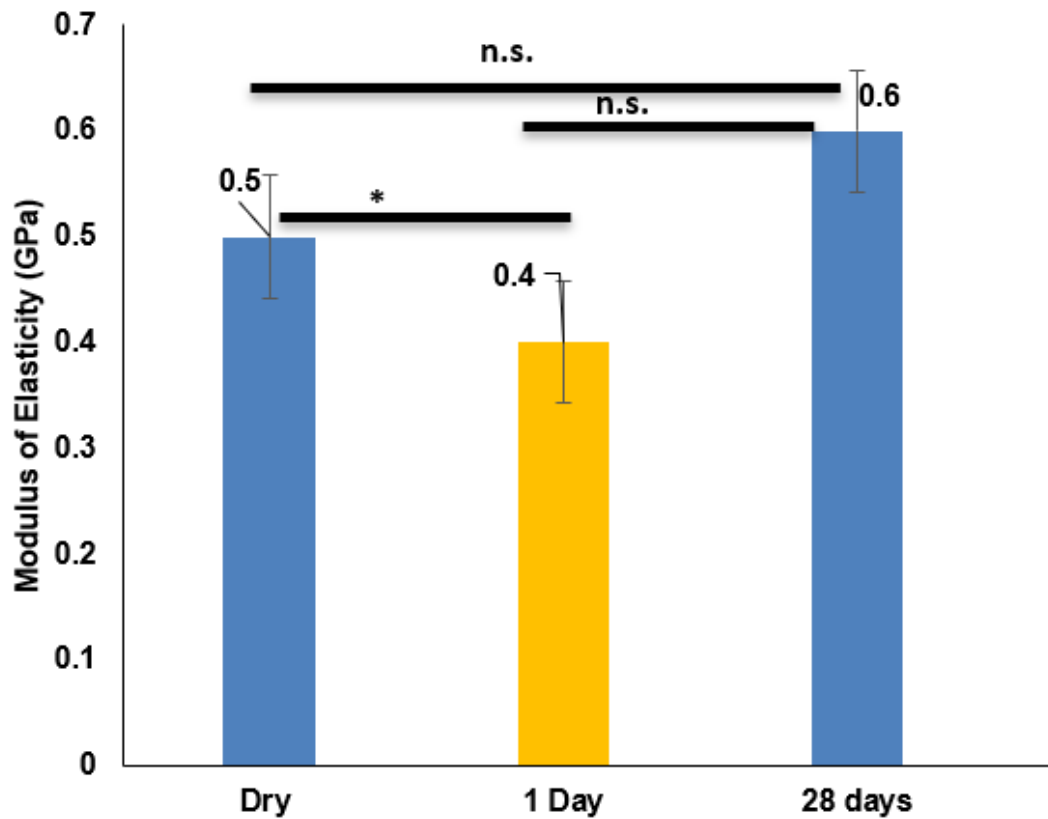


Figure 4-12: Modulus of elasticity of HA480 diamond lattice structure (n=10) before and after immersion in the SBF. Statistically significant differences between different data are indicated as \* ( $p < 0.05$ ) and n.s. ( $p \geq 0.05$ ).

The modulus of elasticity of the samples after 28 days in SBF was  $0.6 \pm 0.2$  GPa, surpassing the values of both the dry and 1-day hydrated specimens. The results are comparable with HA480 scaffolds immersed in SBF for 2 weeks, with a modulus of  $513 \pm 290$  MPa [162]. Modulus of elasticity of dry HA400 diamond lattice triply periodic minimal surface (TPMS) was previously found to be 320 MPa, which is lower than that of the dry diamond lattice of HA480 in this study [179]. Liu *et al.*, [172] found that the HA printed scaffolds had a modulus of elasticity is 0.97 GPa which was higher than the HA480 value of  $0.6 \pm 0.2$  GPa. The modulus of elasticity of HA480 is within the range of 0.1 GPa to 1.678 GPa for cancellous bone [203]. The fracture surfaces of the diamond HA480 after compression are displayed in Figure 4-13.

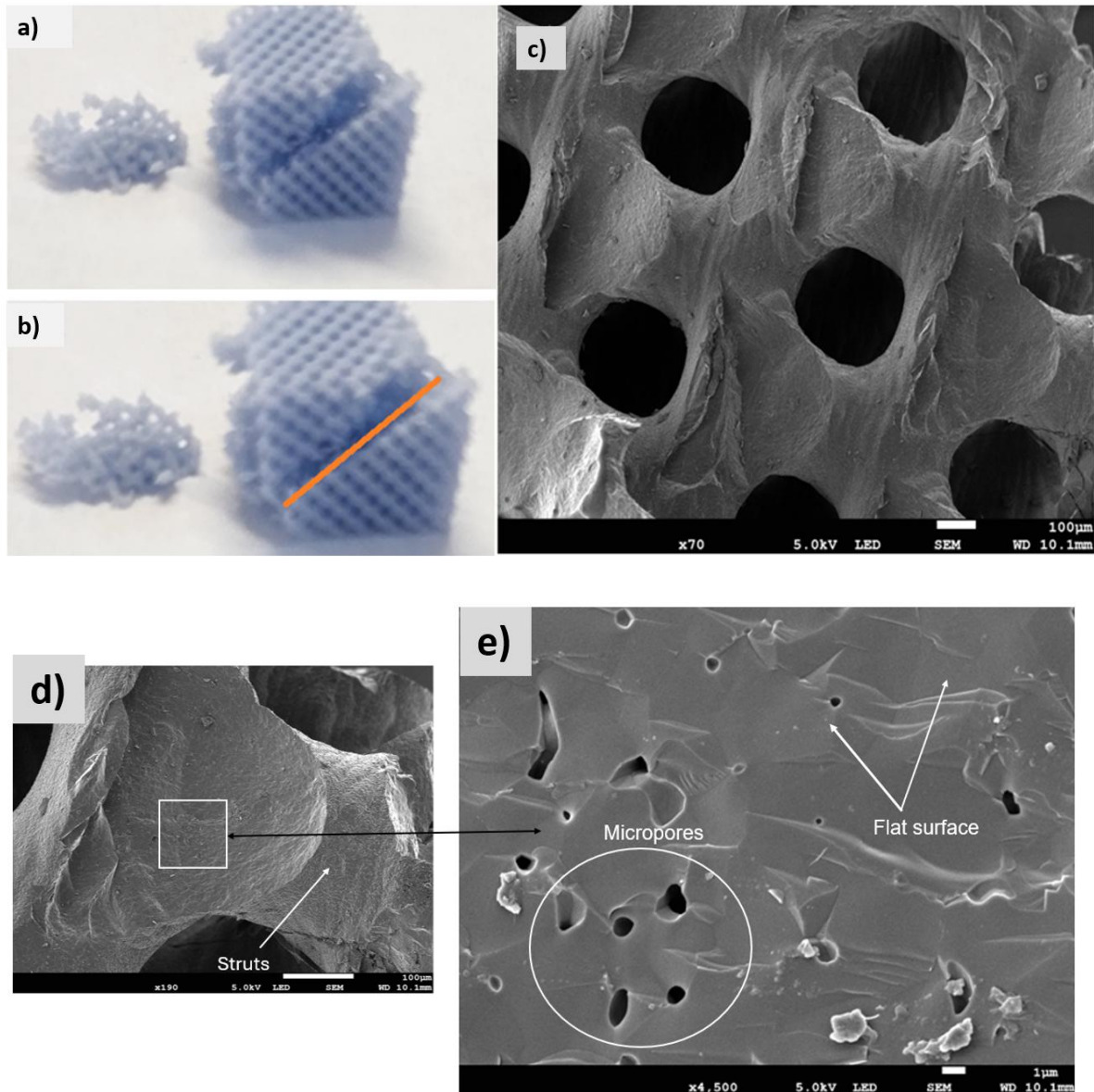


Figure 4-13: HA480 fracture analysis after compression: a) compressed HA480 diamond lattice, b) shear band indicated by orange line, c) SEM-SE image of the fracture surface at low magnification, d) SEM-SE image of the fracture at the node of lattice and e) SEM-SE image of node shows a region of micropores at high magnification.

It was observed that the specimen experienced shear stress at an angle of  $45^\circ$  as evident in Figure 4-13 a) and b). In this specimen, the stress concentration did not depend on the

struts only because failure occurred on nodes also. Mostly, the shear band formed at a  $45^\circ$  angle to the direction of the applied force. The shear plane orientation of  $45^\circ$  is comparable with the diamond lattice cylinder compressed by D'Andrea *et al*, [180]. The behaviour of the HA480 shear band is in agreement with the compressed diamond lattice structure TiAl6V, which was a bending-dominated [125].

Figure 4-13, c), d), and e), show SEM-SE images of the fracture surface of the specimen. The image in Figure 4-13c) shows that the fracture occurred at the nodes of the lattice structure. The fractured node displayed flat surfaces with micropores. These micropores are beneficial for cell growth. This is favourable for material degradation and cell development. The flat fracture surface confirmed that HA480, being ceramic, is a brittle material. These results showed that stress concentration did not depend solely on the strut geometry.

#### 4.8 Comparison of HA480 Diamond lattice structure and alveolar ridge.

The alveolar ridge is made up of cortical and trabecular bone as shown in Figure 4-14 [7].

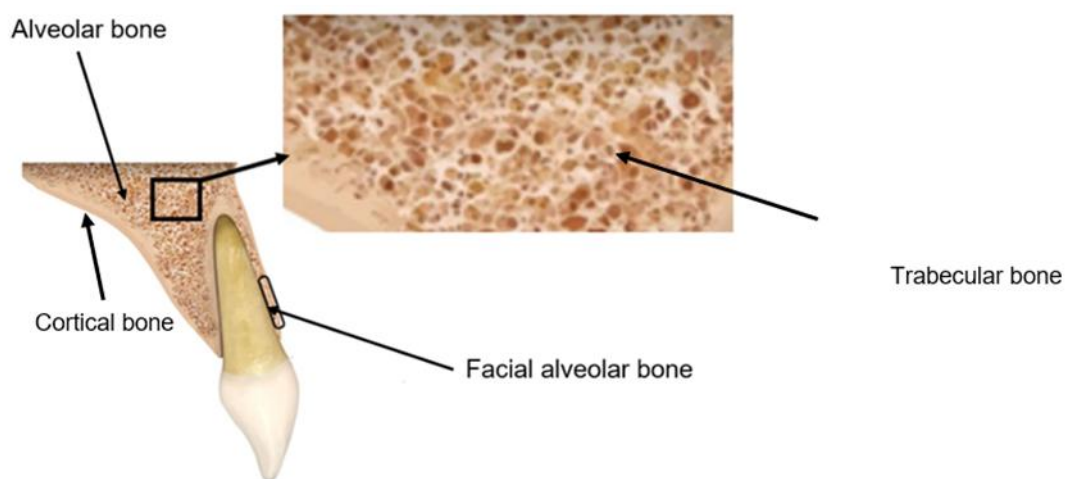


Figure 4-14: Alveolar ridge internal structure [204].

The design of the bone scaffold was based on the trabecular bone geometry and mechanical properties. The bone scaffold was for non-load bearing application. The

HA480 diamond lattice structure pore distribution was obtained through CT analysis shown in Figure 4-15.

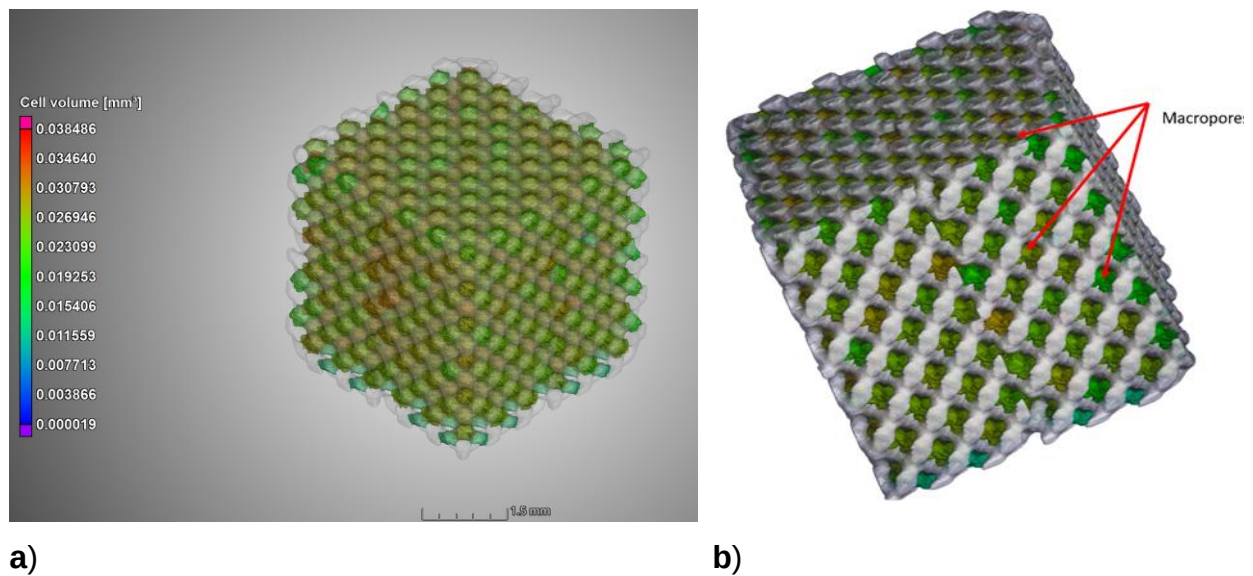


Figure 4-15: 3D illustration of HA480 lattice structure (a) pore size volume distribution  
(b) lattice cube pore network and connectivity

The combination of pore size in the diamond lattice structure ranged between 0.03-0.372 mm represented in Figure 4-15 (b). The pores were comparable to macropores in the trabecular bone of range from 0.3 to 0.6 mm and cortical bone of 0.01-0.05 mm for biological fluid movement [205]. Micropores were found in the struts and nodes of the lattice as indicated in the SEM-SE image of a compression fracture surface in Figure 4-16.



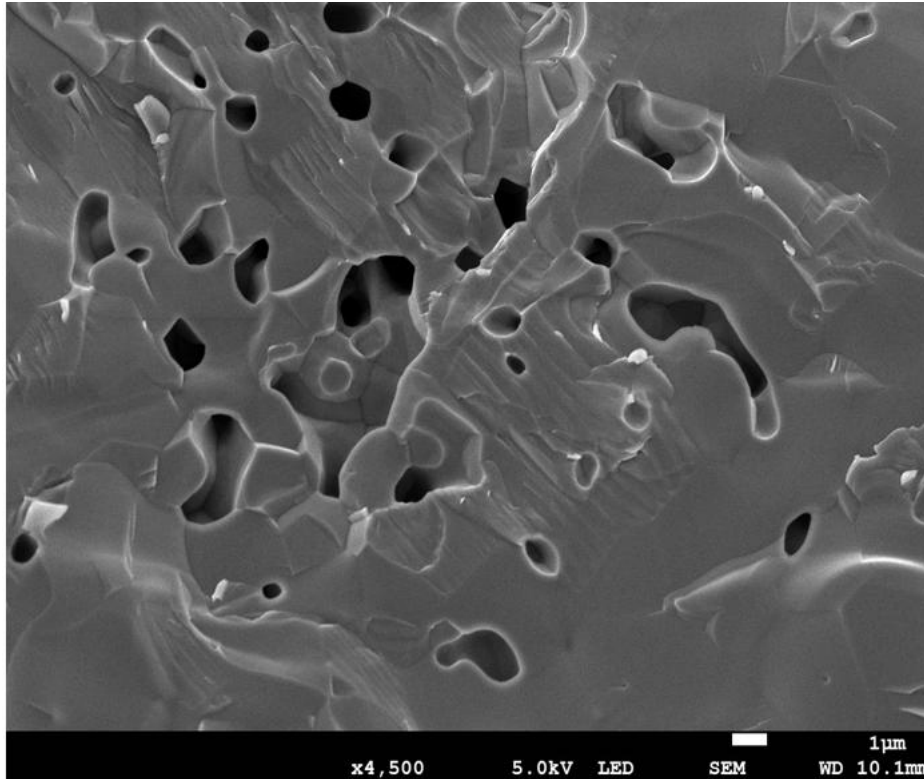


Figure 4-16: SEM-SE compression fracture surface of HA480 showing micropores.

Micropores are beneficial for cell growth and are comparable with the required micropores for bone regeneration structures [44]. The HA480 macro- and micropores were comparable to trabecular bone of alveolar ridge.

The HA480 diamond lattice structure had a modulus of elasticity value of  $0.5 \pm 0.4$  GPa and compressive strength of  $10.7 \pm 1.14$  MPa. These mechanical properties were above the minimum values of trabecular bone which has compressive strength of 2-12 MPa and modulus of elasticity of 1 to 22.3 GPa [206][205]. However, the HA480 values were smaller than the strength of cortical bone of 100-230 MPa and modulus of elasticity of 7-30 GPa [206][205]. The porosity of the HA480 diamond lattice structure mimicked the complexity of trabecular bone pores and interconnectivity which would provide ability of scaffold for permeability. The HA480 modulus of elasticity corresponded with that of trabecular bone potential for the osseointegration when implanted [207].

## 4.9 FEA analysis results

The FEA results obtained with the ABAQUS software are displayed in Figure 4-17.

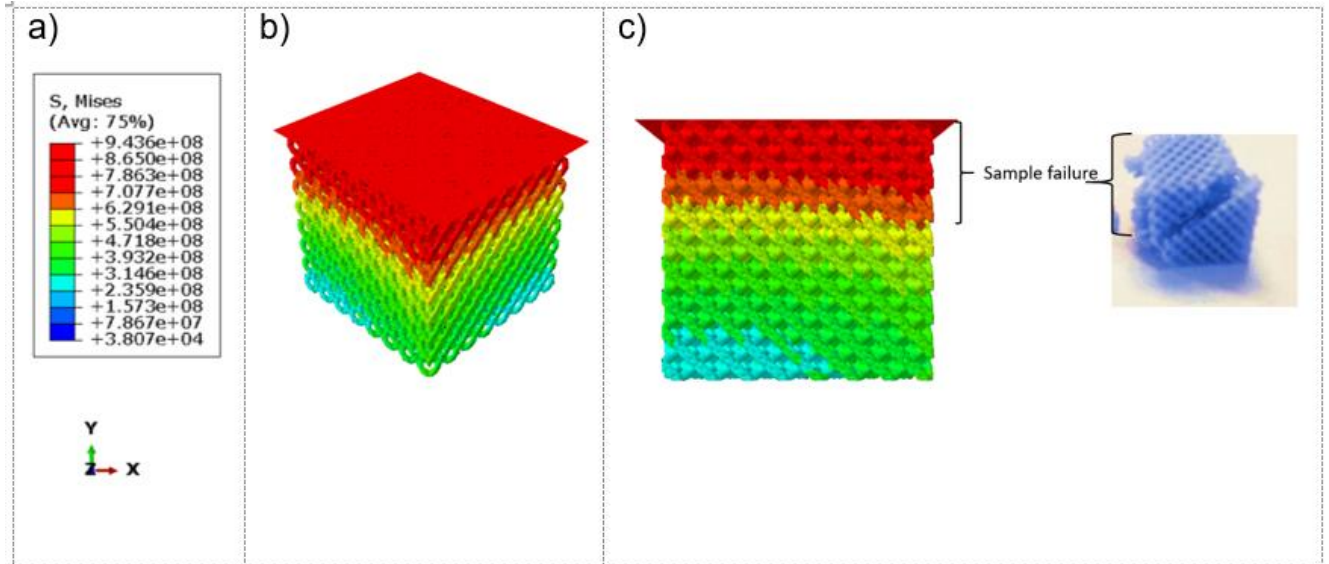


Figure 4-17: a) Von Mises stress graph plot b) stress distribution on the FEA model and sample failure for FEA and experiment of the HA480 lattice.

The von Mises stress distribution of the HA480 diamond lattice is shown in Figure 4-17. Stress concentrations were observed in expected locations, like lattice nodes. Maximum stresses indicated by the red colour were found near the point of rigid plate loading in Figure 4-17b). The area near the contact place experience high force concentration and leading to high stress intensification. The finite element sample failure agreed with the experimental fracture displayed in Figure 4-17c). The differences caused by the experiment being done between two metal plates and related friction were not accounted for in the FEA.

### 4.9.1 Experimental validation of the FEA

From the FEA and experimental results, load vs displacement curves were plotted as shown in Figure 4-18.



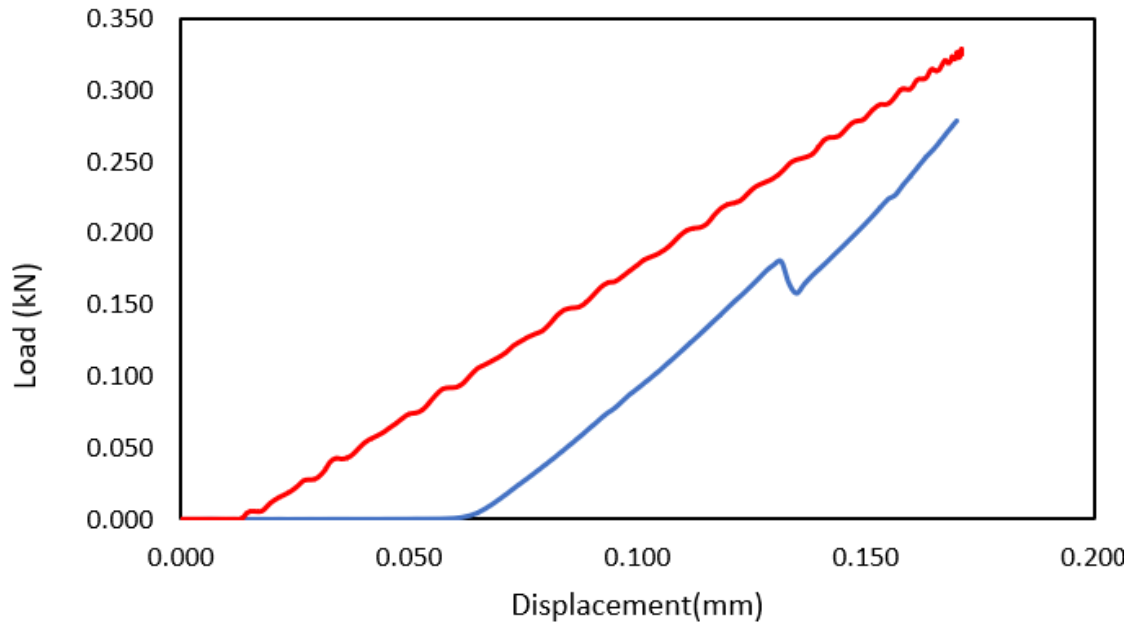


Figure 4-18: Comparison of load vs displacement of HA480 between the experimental (blue) and ABAQUS simulated (red) curves

A fairly good correlation of the curve of the simulated model with the experimental curve was observed. Strain was derived by dividing the displacement by the height of the lattice model. The stress-strain curves were generated and the Modulus of Elasticity was calculated by obtaining the gradient between two points of the stress and strain curve.

Table 4-6 compares the calculated elastic modulus and peak of the stress experimental tests and the FEA data.

Table 4-6: Comparison between the mechanical properties of HA480 obtained by experimental compression and numerical simulation.

|                              | Modulus of elasticity [GPa] | Maximum strength [MPa] |
|------------------------------|-----------------------------|------------------------|
| <b>Experimental</b>          | 0.5±0.4                     | 10.6±1.14              |
| <b>FEA</b>                   | 0.6±0.1                     | 13.2±0.13              |
| <b>Percentage difference</b> | 20%                         | 20%                    |

Validation of the finite element model was established by comparison with experimental data. The computational model results of a modulus of elasticity of 0.6 GPa, differed by

20% from the experimentally derived value of  $0.5 \pm 0.4$  GPa. The maximum compressive strength, indicative of ultimate load-bearing capacity, is 20% difference between FEA and experimental results. These differences demonstrate the FEA model's effectiveness for simulating material behaviour. The variation within such percentage is familiar in literature for experimental measurement versus FEA. D'Andrea *et al.*, [208] found an octet lattice had variations of 42% and 47.2%, respectively, for young's modulus and compressive strength between as analysed and measured experimentally. The differences were influenced by strut waviness and oversizing [208]. Wang *et al.*, [209] reported that a triply periodic minimal surface shell lattice had similar difference of 17.24% for the young modulus. The experimental difference was influenced by surface roughness [209]. There is still a need to include geometric irregularities accurately in computational models such as strut waviness, internal pores, surface roughness and thickness variation that were not included in the FEA analysis.

#### 4.10 Density of HA480

The results of determining the true density of HA480 are presented in Table 4-7. The actual density was automatically calculated by the machine as indicated in Section 3.4

Table 4-7: Density results of HA480 repeated for three cycles

| Run (cycle)    | Volume (cm <sup>3</sup> ) | Density (g/cm <sup>3</sup> ) | Temperature (°C) |
|----------------|---------------------------|------------------------------|------------------|
| 1              | 0.6                       | 3.02                         | 27.5             |
| 2              | 0.6                       | 3.04                         | 27.5             |
| 3              | 0.6                       | 3.03                         | 27.7             |
| <b>Average</b> | <b>0.6</b>                | <b>3.04± 0.01</b>            | <b>27.6±0.1</b>  |

The volume and temperature of each cycle were constant, and the calculated true density by the machine for each cycle was the same at 0.6 g/cm<sup>3</sup> and 27.6° respectively. The average true density of the HA480 is  $3.04 \pm 0.01$  g/cm<sup>3</sup>, with a percentage variance of 0.2% from the average of the three cycles. LithaBone HA480 material used standard FDA-approved HA powder particles to form the material slurry. Qing *et al.*, [118] indicated that

the density of hydroxyapatite powder was  $3.16 \text{ g/cm}^3$ . The true density of HA480 corresponds with the density of standard HA, which is biocompatible.

#### 4.11 Discussion

In this chapter, the aim was to select a lattice that mimicked the bone structure. HA480 material satisfied the expected hydroxyapatite characteristics, specifically the CA/P ratio, surface roughness of LCM parts that could promote cell attachment, and hardness values in Table 4-5. The HA480 printability was accurate, and adding a shrinkage factor in the design before manufacturing compensated for shrinkage, ensuring the pores maintained good integrity [147]. It was demonstrated that the LCM process could be successfully used to build all three types of lattice structures.

The compressive strength of HA480 for the diagonal and rhombic lattices was higher compared to the diamond lattice structure Figure 4-10. The unit cell of the diagonal, with a thicker node, may delay the degradation of the material, while the rhombic has more struts per cell, requiring more material to manufacture a 3D-printed lattice cube. However, since the study focuses on a small-sized scaffold that allows fluid permeability, the diamond structure was chosen for the remaining experiments. Diamond was a bending-dominated structure that deforms gradually compared to a stretch-dominated structure Table 4-1.

#### 4.12 Conclusion

The compressive strength and modulus of elasticity of the HA480 diamond lattice structure were above the minimum of cancellous bone. The LCM produced diamond lattice can be used for bone regeneration for no load applications. The Ca/P ratio of the LCM HA480 demonstrated that the material can be used for bone regeneration, because it was almost the same as the recommended Ca/P ratio for hydroxyapatite. The CT scanned specimen indicated heterogeneous pores sizes that are recommended for bone regeneration. Validation of FEA gave a variance of results of 20 % which is due to LCM irregularities, pores, surface roughness and the FEA model's geometric difference from the experimental specimens.

## CHAPTER 5

### RESULTS AND DISCUSSION: FACTORS THAT INFLUENCED CONTROLLABLE DEGRADATION AND BONE REGENERATION RATES OF AN ADDITIVELY MANUFACTURED SCAFFOLD

#### 5.1 Introduction

The biodegradability of bone scaffold depends on its physical and biological properties. These factors were evaluated for HA480: physical properties include material composition, scaffold porosity, surface area and geometry, Ca/P ratio, and crystallinity. Biological properties include hydrophilicity, pH environment, and cellular interaction. As discussed in, a diamond lattice structure was selected as the most appropriate for the scaffold researched in this study. Consequently, the biodegradation of this structure was analysed, focusing on the factors that affected biodegradation. The selected diamond lattice scaffold was *in vitro* tested for the formation of surface apatite and cell attachment, results indicated that the material could be used for bone regeneration. The biodegradation rate and mechanical strength of the material after degradation were analysed. The work based on this chapter included the diamond lattice structure and cell culture on an HA480 solid circular disk.

#### 5.2 Diamond lattice Structure dimensions

Figure 5-1 shows a CT image of the diamond lattice cube before degradation. The struts were about 0.2 mm thick, while the maximum thickness of the nodes was 0.35 mm. The larger nodes were expected to withstand the mechanical pressure applied, while the strut size allowed the scaffold to have a suitable pore size to allow cell attachment and enough permeability for nutrients and cells.

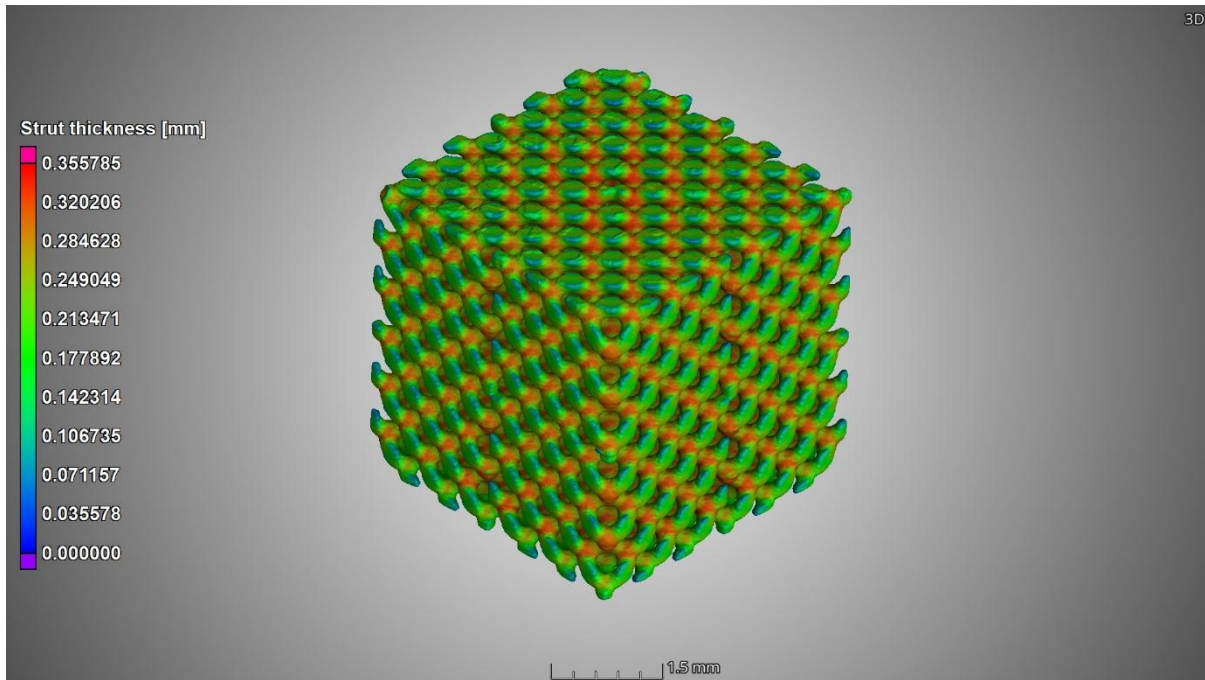


Figure 5-1: CT image of HA 480, diamond lattice with green colour represents the struts and red the nodes.

### 5.3 Swelling profile of the scaffold

The diamond lattice immersed in PBS and incubated in an oven at 37°C was weighed and the swelling was calculated according to the process in Section 3.12. The diamond lattice swelling profile calculated during the incubation is presented in Figure 5-2.

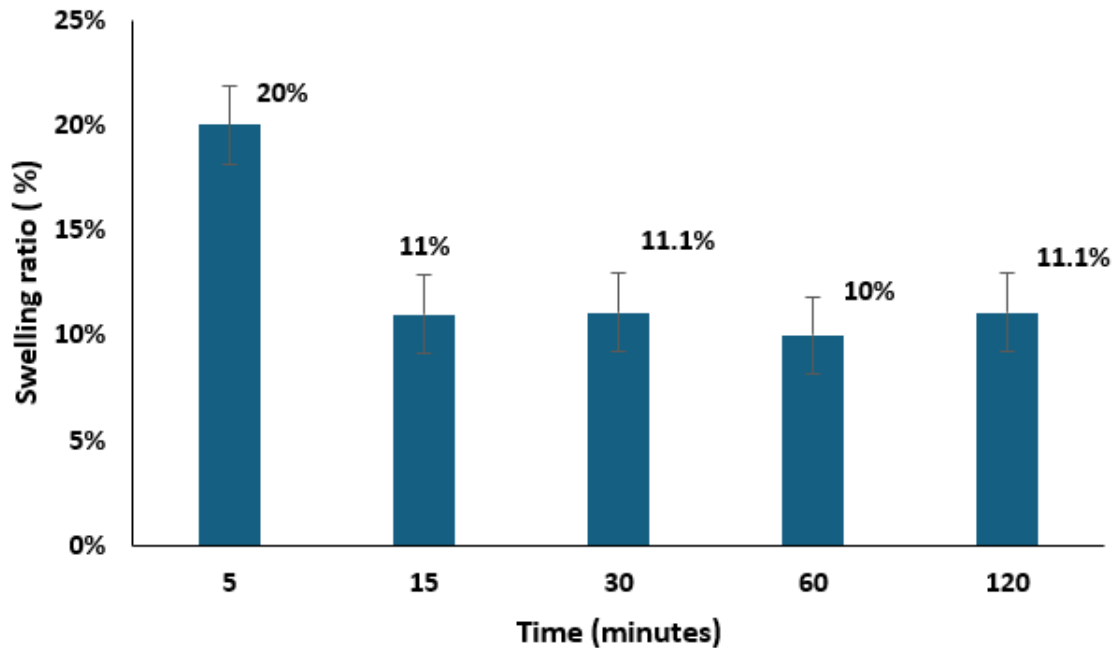


Figure 5-2: Swelling profile of HA480 diamond lattice structure (n=3) in PBS.

The rapid PBS uptake of 20% occurred within the first 5 minutes. In 15 minutes, the swelling ratio reduced to 11 %. The decrease in swelling ratio remained constant until 120 minutes. The rapid swelling of the HA480 in the study of Ergul *et al.*, [210] of composite hydrogels of 15wt % HA added to CH/PVA were comparable [210]. The comparison was in terms of behaviour, not values, where the rapid absorption of PBS in the hydrogels and constant swelling over time were observed because of the high HA in the hydrogels [210] [211]. As HA480 is a ceramic, the values for swelling ratio values were not comparable with composite materials or hydrogels of HA with polymers [191]. Since the HA480 diamond lattice was intended for biological conditions in the human body, the swelling rate needed to be determined. The swelling rate indicates how fast the material can absorb biological fluids and expand. The diamond structure was able to absorb the PBS because of its porosity.

## 5.4 Biodegradation of HA480

### 5.4.1 Biodegradation weight loss

The weight loss ratio of the HA480 diamond lattice was calculated using the method in Section 3.13. The experiment on weight loss was done using the protocol of Kokubo [97]. The average calculated weight loss is displayed in Figure 5-3.

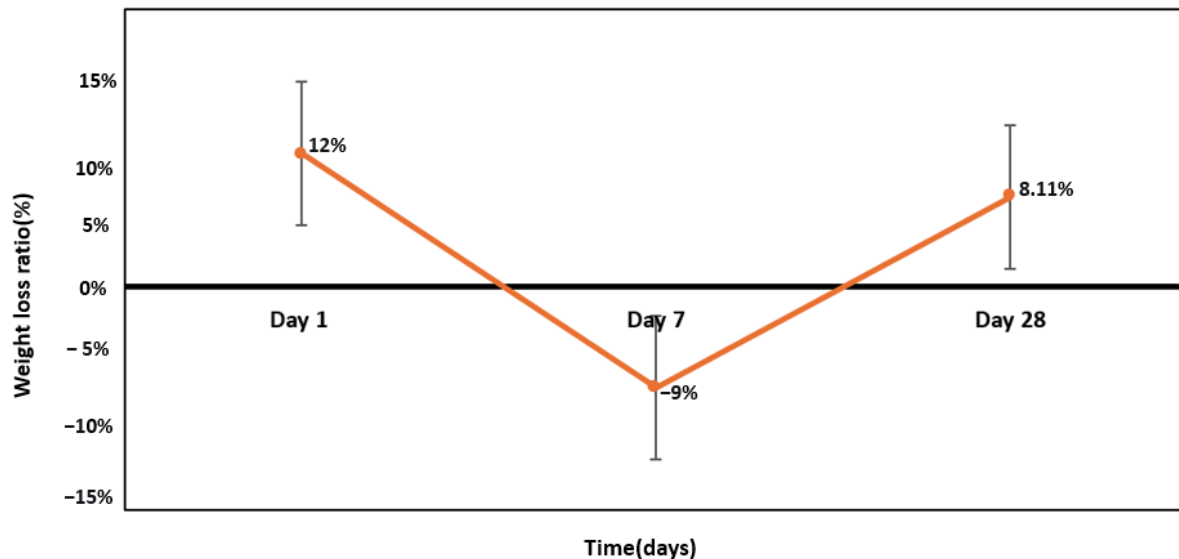


Figure 5-3: Weight loss of HA480 diamond lattice specimens (n=3) in SBF.

The mass of the scaffold increased instead of decreased. This was due to ion exchange and the precipitation of calcium and phosphorus ions from the SBF solution onto the surface of HA480 [164]. The experiment was comparable to a composite scaffold of bovine cancellous HA, whereby at day 7, the weight loss decreased but did not become negative. After 14 days, it increased again due to the leaching of  $\text{Ca}^{2+}$  ions [165]. In comparison with the HA bio-printed by Shao *et al.* [40], the biodegradation was 10.38% after 5 weeks of degradation [40]. The weight loss indicated material degradation. Its porous structure, HA480 achieved biodegradation after 28 days.

#### **5.4.2 SEM-SE evaluation of HA480 diamond lattice after degradation**

SEM-SE images of the biodegradation effect on the HA480 surface are shown with the related EDX spectra for 1, 7 and 28 days in Figure 5-5, Figure 5-7 and Figure 5-9.



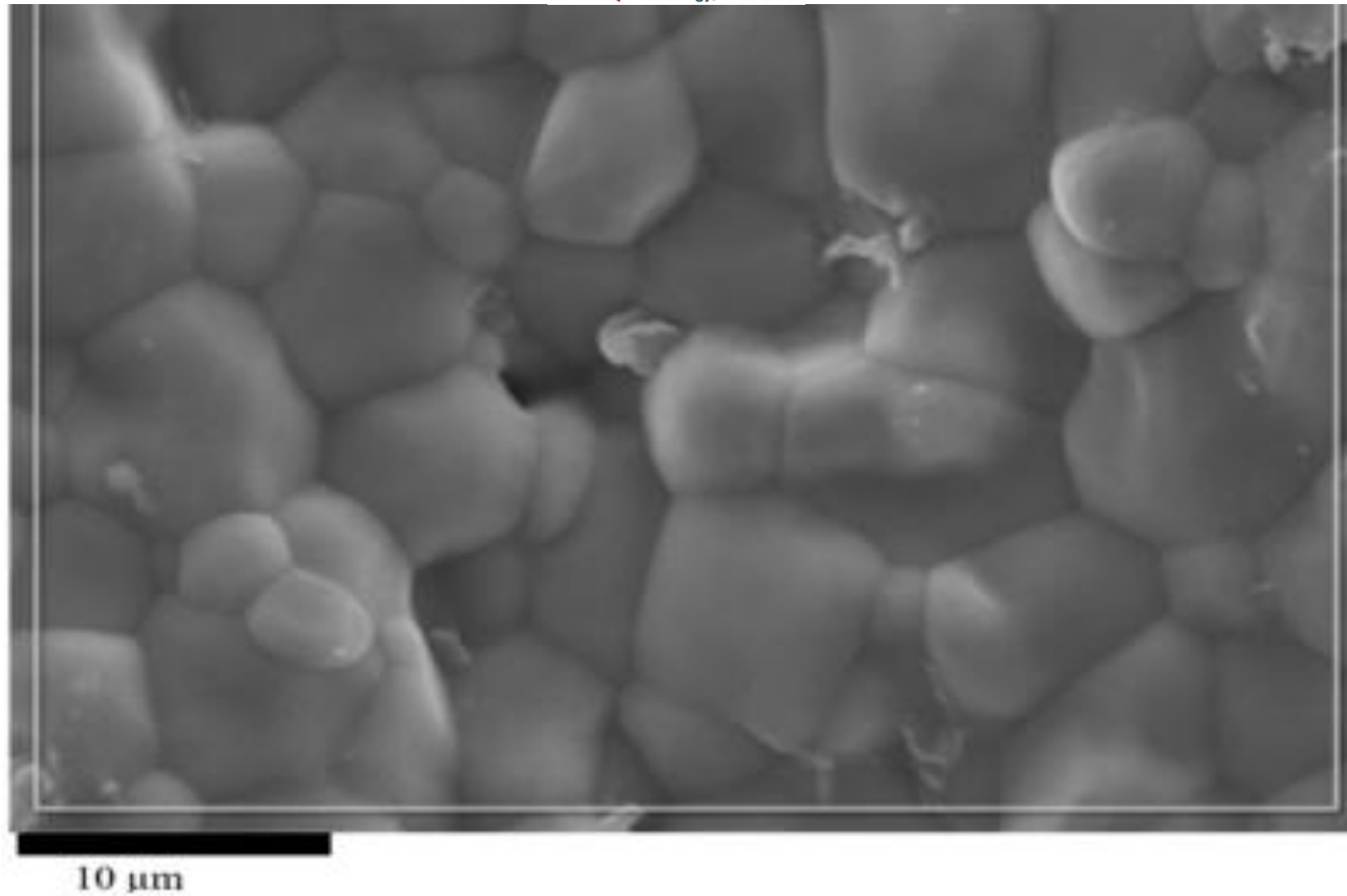


Figure 5-4: Day 1 HA480 in SBF SEM-SE image.

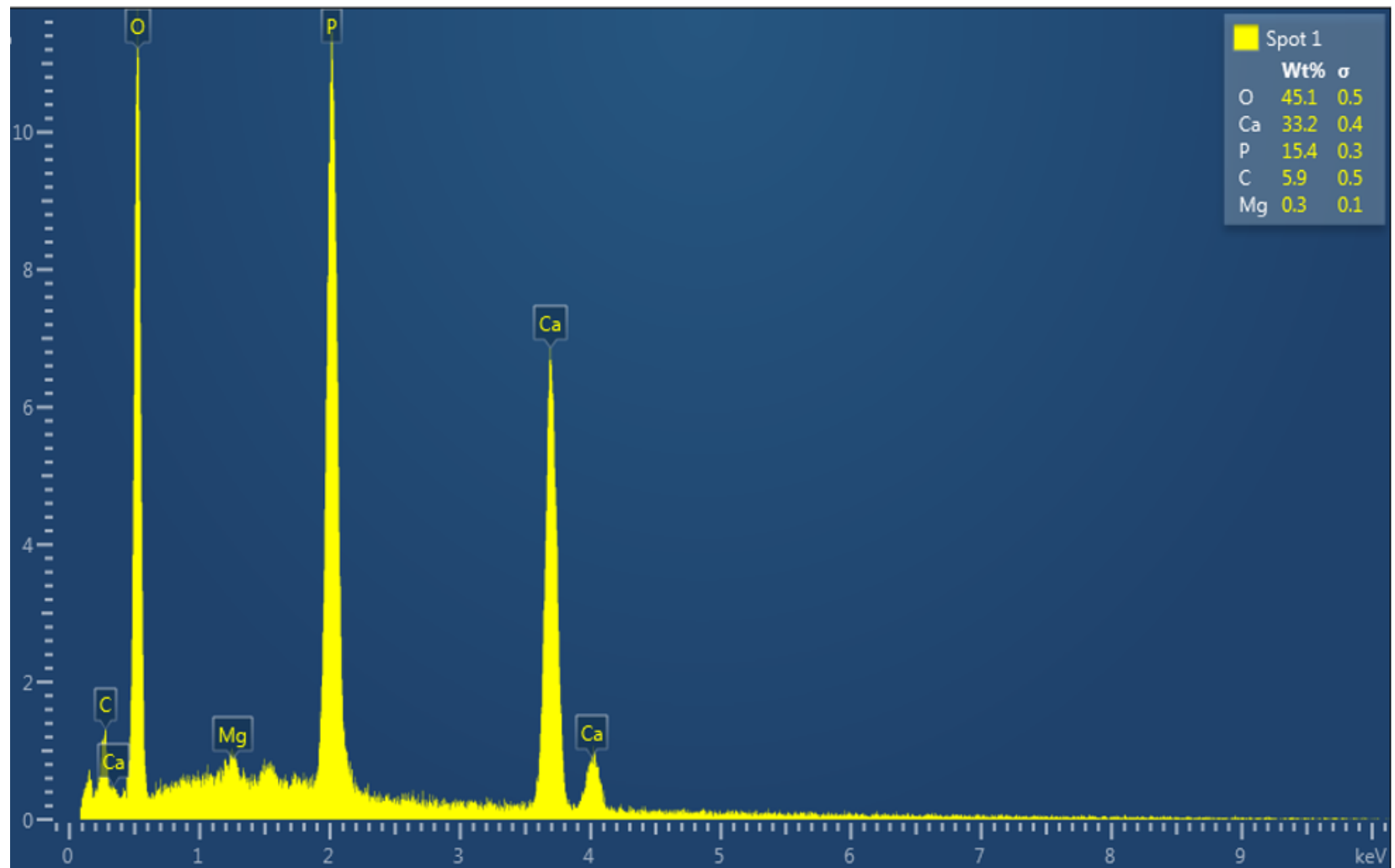
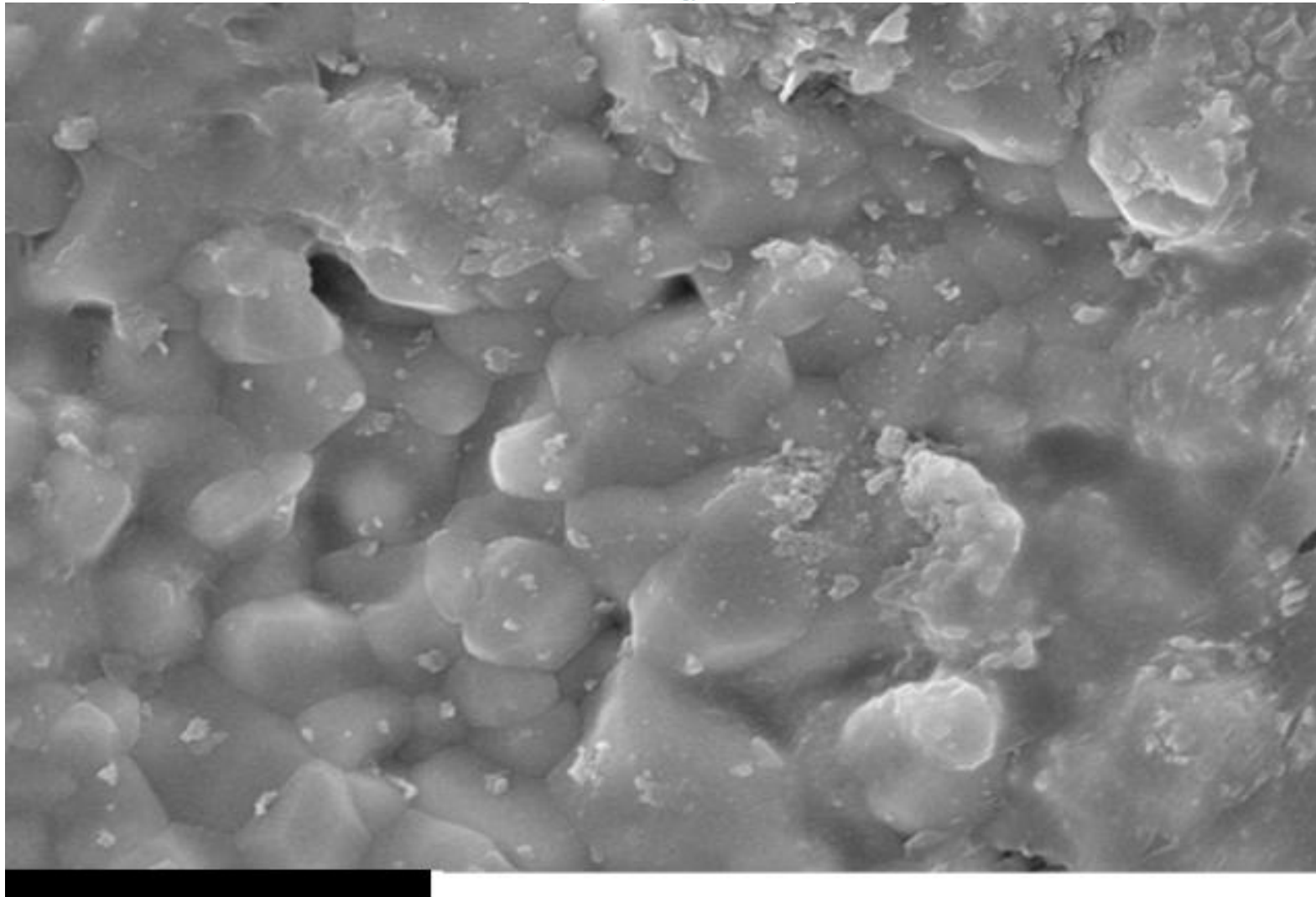


Figure 5-5: Day 1 in SBF EDX spectrum.



10  $\mu\text{m}$

Figure 5-6: Day 7 HA480 in SBF SEM-SE image.

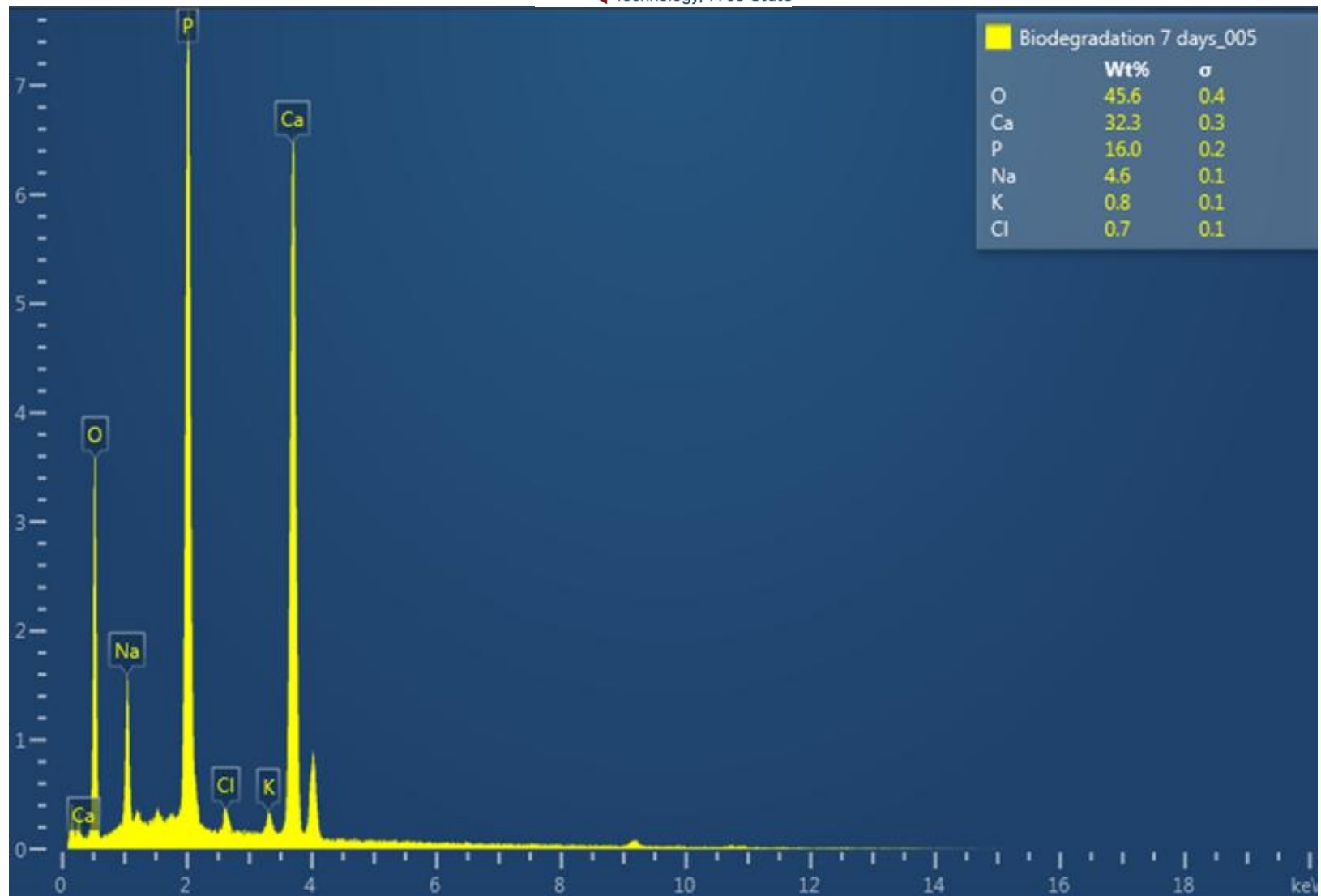


Figure 5-7: Day 7 HA480 in SBF EDX spectrum.

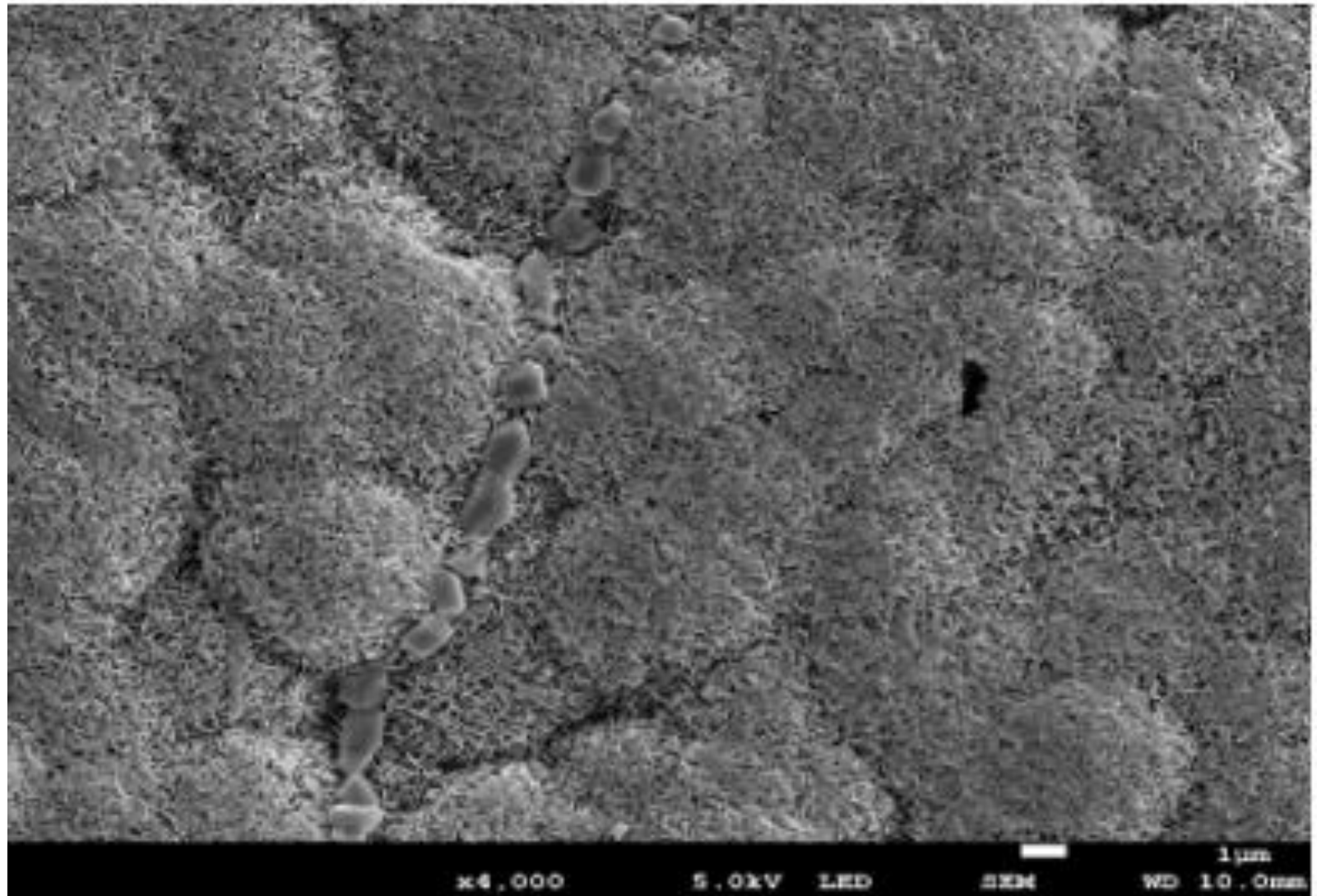


Figure 5-8: Day 28 HA480 in SBF EDX spectrum.

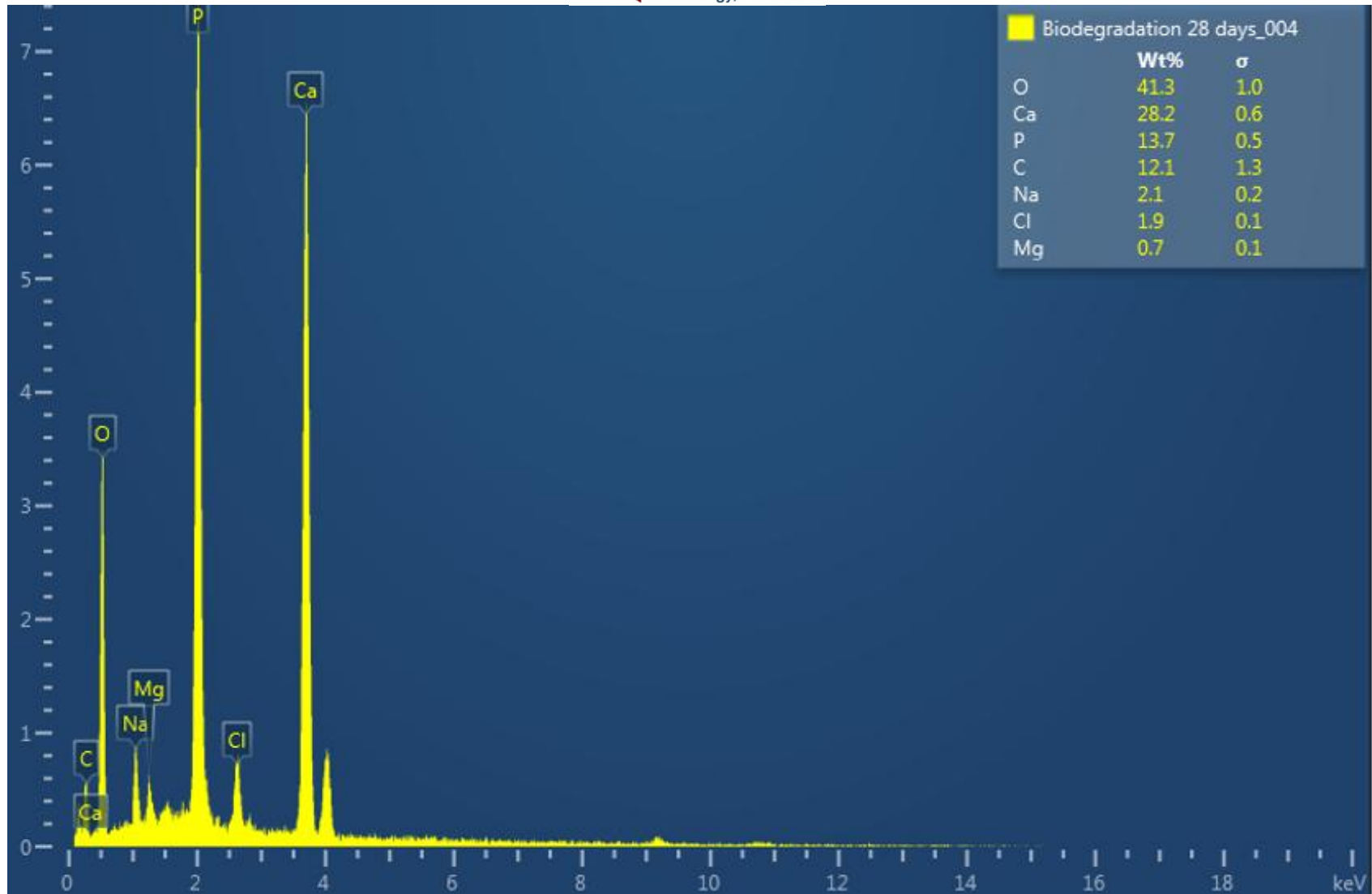


Figure 5-9: Day 28 HA480 in SBF EDX spectrum.



The surface morphology for samples submerged in the SBF were analysed to determine if there had been changes apart from mass degradation and if apatite formed on the HA480 surfaces. An apatite layer provides the bonding surface of the scaffold and the host tissue as explained in the SBF protocol by Kokubo *et al.* [97]. In Figure 5-5, the SEM-SE micrograph and EDX spectrum, respectively, of the diamond lattice are presented after 1 day in the SBF. The grain boundaries were visible, showing a mixture of small and larger grains and calcium, oxygen, and phosphorus were detected. After day 7, the grain boundaries were not well defined in Figure 5-7 but appeared to be covered by an amorphous layer that could be the result of the HA480 dissolving in the SBF as a result of pH and ion exchange. In the red square, there are small crystalline substances on the surface. After 7 days, the EDX spectrum in Figure 5-7 b) showed additional elements (chlorine, potassium, and sodium), which were precipitated from the SBF solution. After 28 days, as shown in Figure 5-9 a) flakes were visible on the surface. The grain boundaries were again better defined after 28 days, with the formation of a thin amorphous surface layer, which could be bone-like apatite, as seen in Figure 5-9 a). The 28 days SEM-SE image results were comparable to the immersed in SBF protocol, and that layer was identified as the formation of apatite [97].

### **5.5 Calcium to phosphorus ratio for HA480 in SBF after biodegradation test**

As shown in Figure 5-10, the calculated Ca/P ratio from EDX results for HA480 after samples immersed in SBF with maximum of three spots per HA480 sample were measured on the surface layers for Day 1, 7 and 28. Ca/P ratio results for HA480 after having been soaked for 1 day in SBF was higher than the rest, with a value of  $2.2 \pm 0.9$ . The more days the samples were in the SBF, the more the Ca/P ratio declined, reaching the value  $2 \pm 0.5$  after 28 days. The Ca/P ratio of the HA480 diamond lattice in the SBF indicated an ion exchange between the SBF solution and the HA480. The value of the Ca/P ratio of pure HA was 1.67 [197].

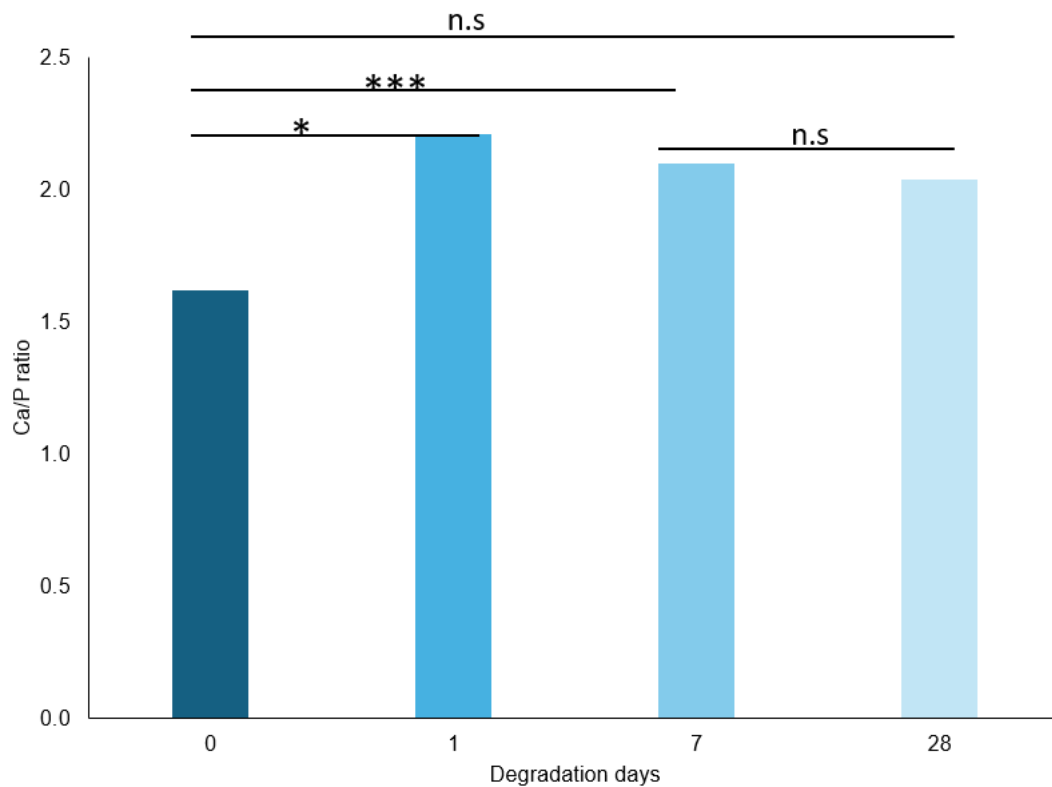


Figure 5-10: Ca/P ratio, determined through EDS, of HA480 lattice structure (n=3) after submersion in SBF for different time. Statistically significant differences between different data are indicated as \* ( $p < 0.05$ ), \*\*\* ( $p < 0.001$ ), and n.s ( $p \geq 0.05$ ).

## 5.6 The grain size of HA480 after biodegradation

The grain size was measured from the SEM images using the ImageJ software. The results of grain size in Figure 5-11 indicated that after 1 day of degradation, the HA480 samples had an average grain size of  $3.37 \pm 0.85 \mu\text{m}$ . After 7 days of degradation, there was a decrease in grain size to  $2.74 \pm 0.87 \mu\text{m}$ . On day 28 the grain size had increased to  $4.26 \pm 0.81 \mu\text{m}$ . It is known that the finer the microstructure, the higher the mechanical strength [49]. The grain size of the HA480 sintered at  $1300^\circ\text{C}$  in this study  $2.39 \pm 0.9$  was comparable to a published study on HA400 sintered at the same temperature ( $1300^\circ$ ), with grain size of  $3 \mu\text{m}$  [212]. The HA480 biodegradation grain results are comparable to  $\beta$ -TCP fabricated via LCM sintered at  $1200^\circ\text{C}$  with a grain size of  $2 \mu\text{m}$  [213]. Laser-sintered ceramics had finer grain sizes of less than  $1 \mu\text{m}$  [214]. Material with a large grain size is typically expected to have lower compressive



strength than that having a small grain size. HA480 28 days with a large grain size had lower compressive strength.

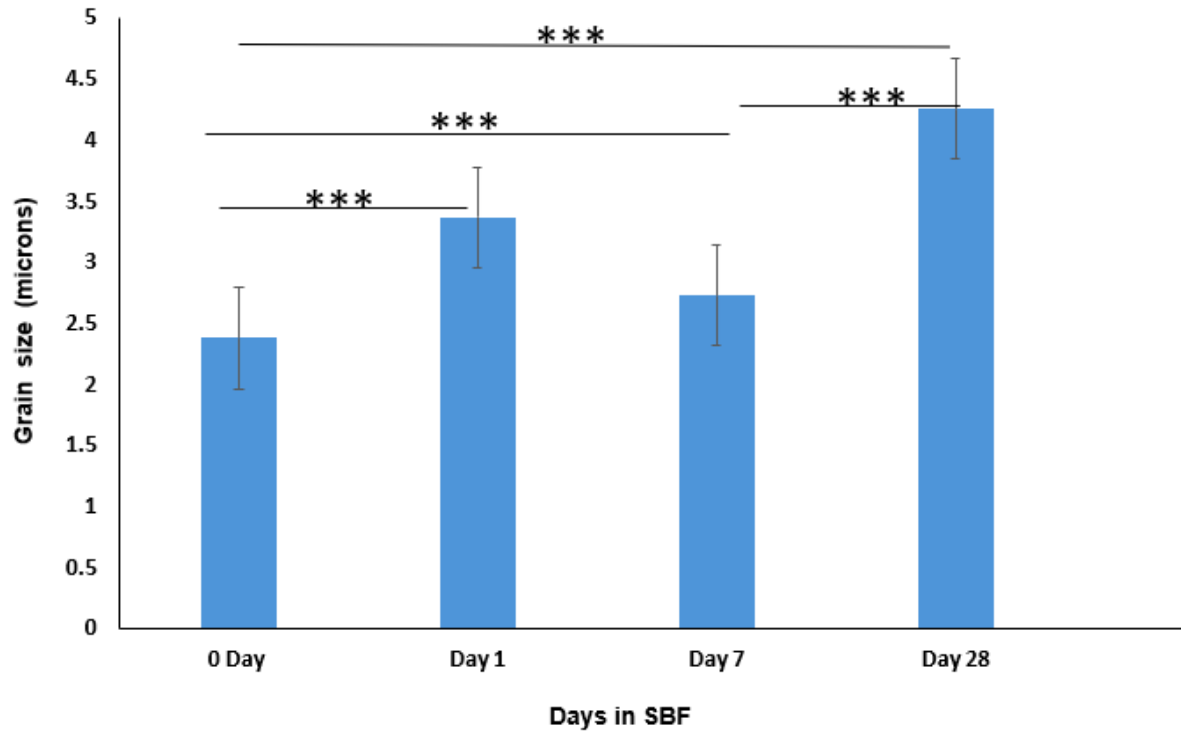


Figure 5-11: Average grain size of HA480 diamond lattice structure (n=3) after submersion in SBF for different days. Statistically significant differences between different data are indicated as \*\*\* ( $p < 0.001$ ).

## 5.7 XRD of HA480 before and after 28 days of biodegradation

Figure 5-12, XRD of HA480 before and after degradation is shown.

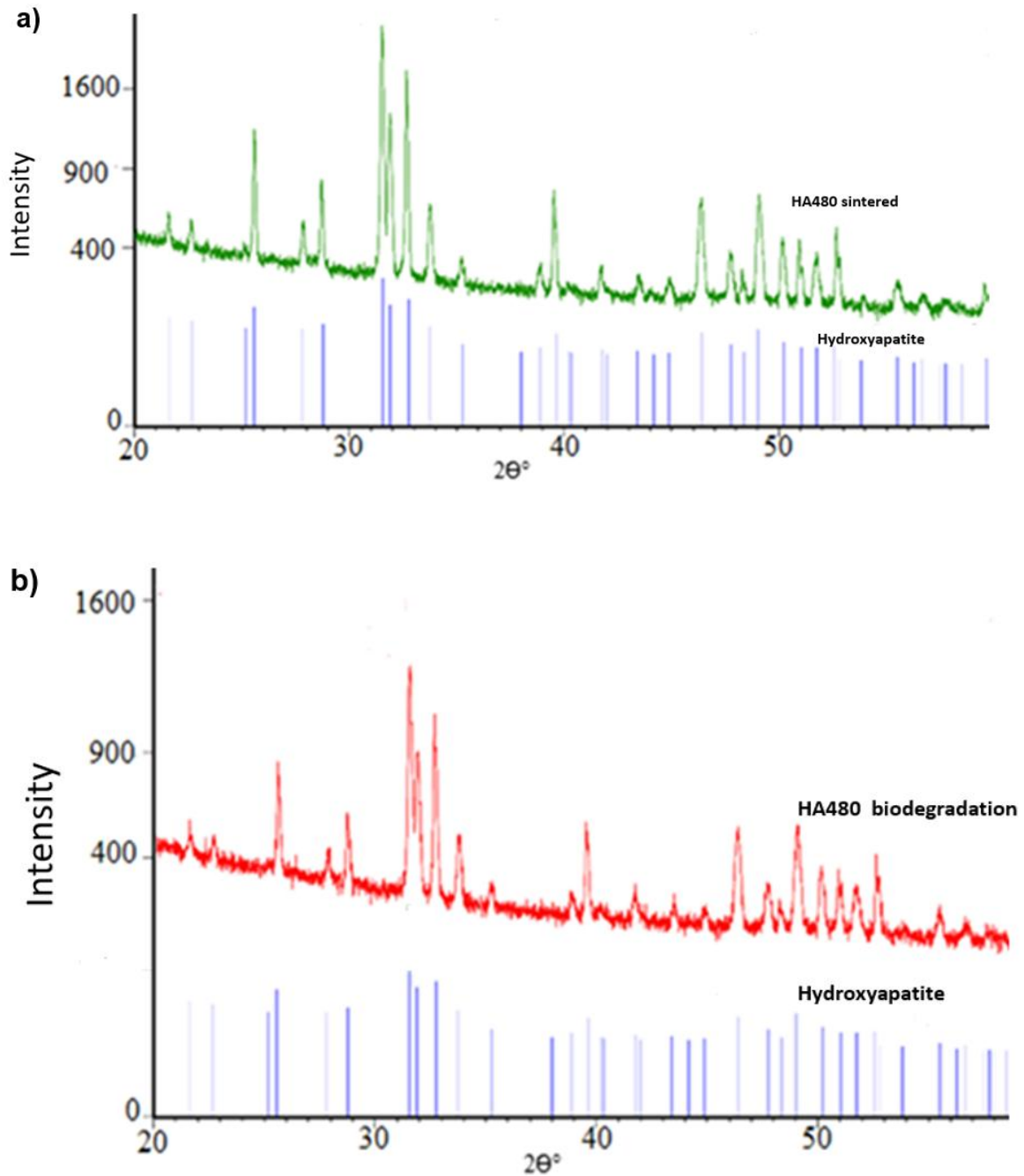


Figure 5-12: XRD results a) HA480 sintered and hydroxyapatite graph b) HA480 28 days after biodegradation and hydroxyapatite graph.

The highest peaks, detected at  $2\theta$  between  $31.6^\circ$  and  $32.8^\circ$ , were the same as those of pure hydroxyapatite [200]. HA480 were comparable to the powders and sintered scaffold of HA480 found by D'Andrea et. al. [180]. The presence of an apatite layer was confirmed by the EDX in Figure 5-9 spectrum obtained from biodegradation

specimens after 28 days. Kobubo's *et al.*, [97] protocol specifies that SEM analysis of 28-day samples should be complemented with XRD to confirm the presence of apatite.

## 5.8 Surface roughness of HA480 lattice

The surface roughness of the HA480 cell culture samples was measured for both the green body and in the sintered state, and the results are displayed in Figure 5-13. The surface roughness of the cell culture samples had to be tested as they were evaluated for the cell attachment behaviour of HA480.

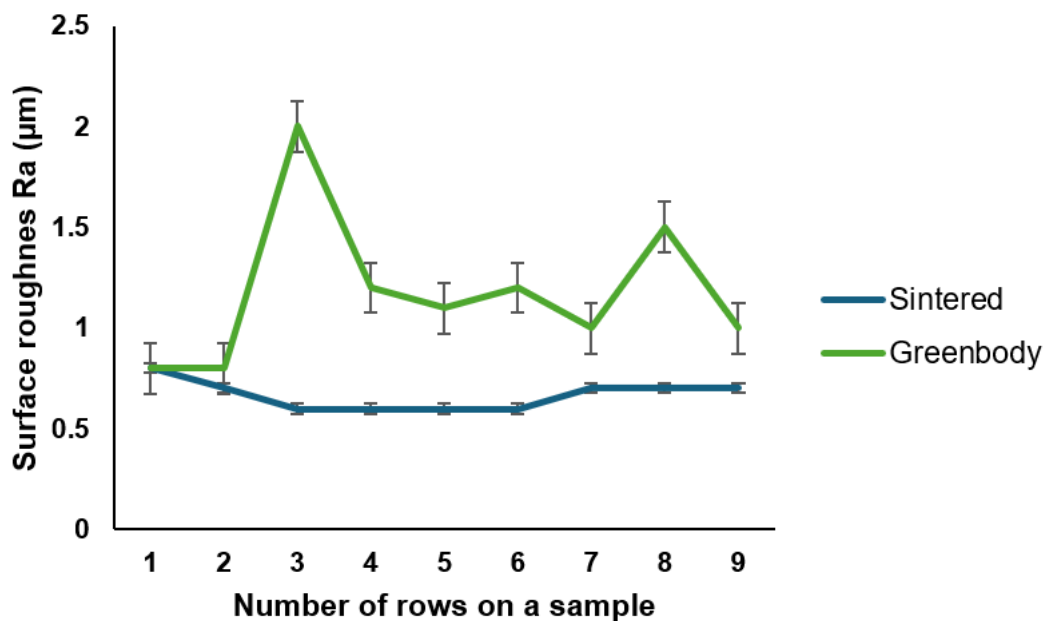


Figure 5-13: Surface roughness for the HA480 green body and the sintered specimen.

From the values in Figure 5-13, the average was calculated, and the sintered sample had an average roughness of  $Ra = 0.60 \pm 0.05 \mu\text{m}$ . This Ra value was similar to that of the re-crushed and sintered HA ( $0.77 \mu\text{m}$ ) by Pramanik *et al.* [142]. The green body's surface roughness was calculated to be  $Ra = 1.20 \pm 0.38 \mu\text{m}$ . The higher Ra values for the unsintered green parts were expected because the samples were not yet fully dense. During debinding, the binder was evaporated, and during sintering of the HA480 particles, the final full density was achieved. The surface roughness required for cell adhesion of osteoblasts ranges from  $0.2$  to  $8 \mu\text{m}$  [169]. Surface roughness helps cells attach and proliferate on the scaffold, creating a bond between the implant

and surrounding tissue [169]. The presence of appropriate surface roughness on sintered HA480 suggests that cell adhesion and proliferation could be enhanced [142].

## 5.9 Cell proliferation on HA480 scaffolds

Surface roughness was measured  $R_a = 0.60 \pm 0.05 \mu\text{m}$  with possibility of cell adherence to HA480 surface, a cell culture study was conducted. The proliferation of hFOB 1.19 cells seeded on 3-D printed specimens was analysed by using the CellTiter Blue<sup>®</sup> viability assay over 7 days, as described in Section 3.15.

Standard and cell growth curves were constructed and the results are shown in Figure 5-14.

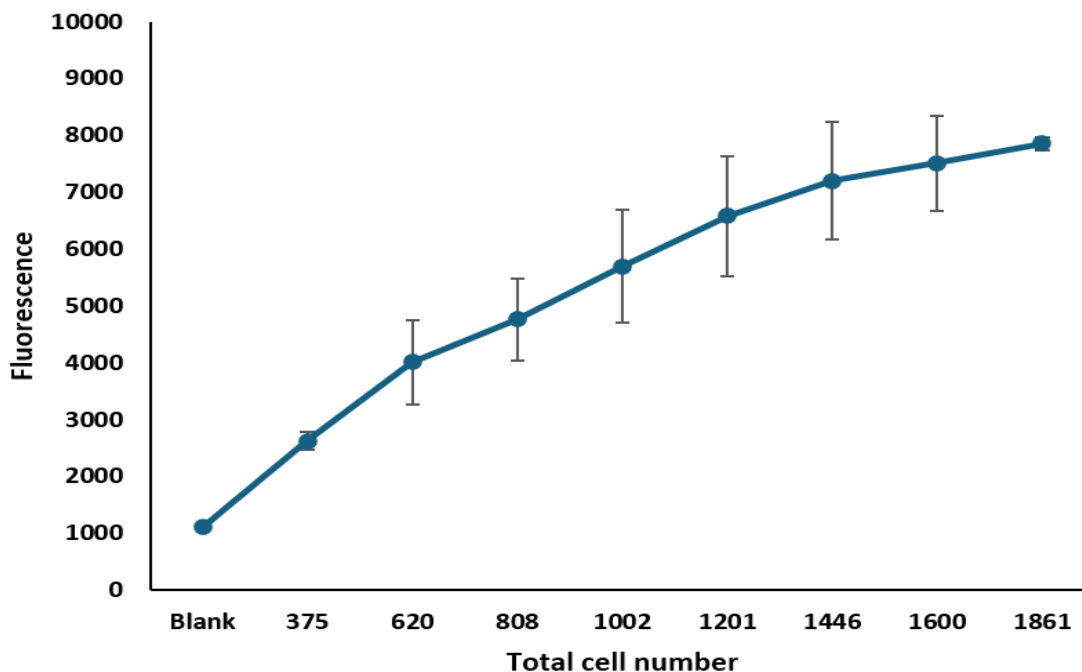


Figure 5-14: Standard curve of total cell number relative to the fluorescence intensity of CellTiter-Blue. Error bars indicate standard deviation of quadruplicate values obtained from a single experiment.

The results indicate a rising curve between the total number of cells and increase in the fluorescence intensity. The consistency of hFOB cells shows that CellTiter-Blue can be used for cell culture studies. Therefore, the assay provides an opportunity for HA480 cell seeding.

The proliferation results of hFOB 1.19 cells, which were flood-seeded on an HA480 non-porous disc cell culture specimen, and the control of hFOB cells without the HA480 are illustrated in Figure 5-15.

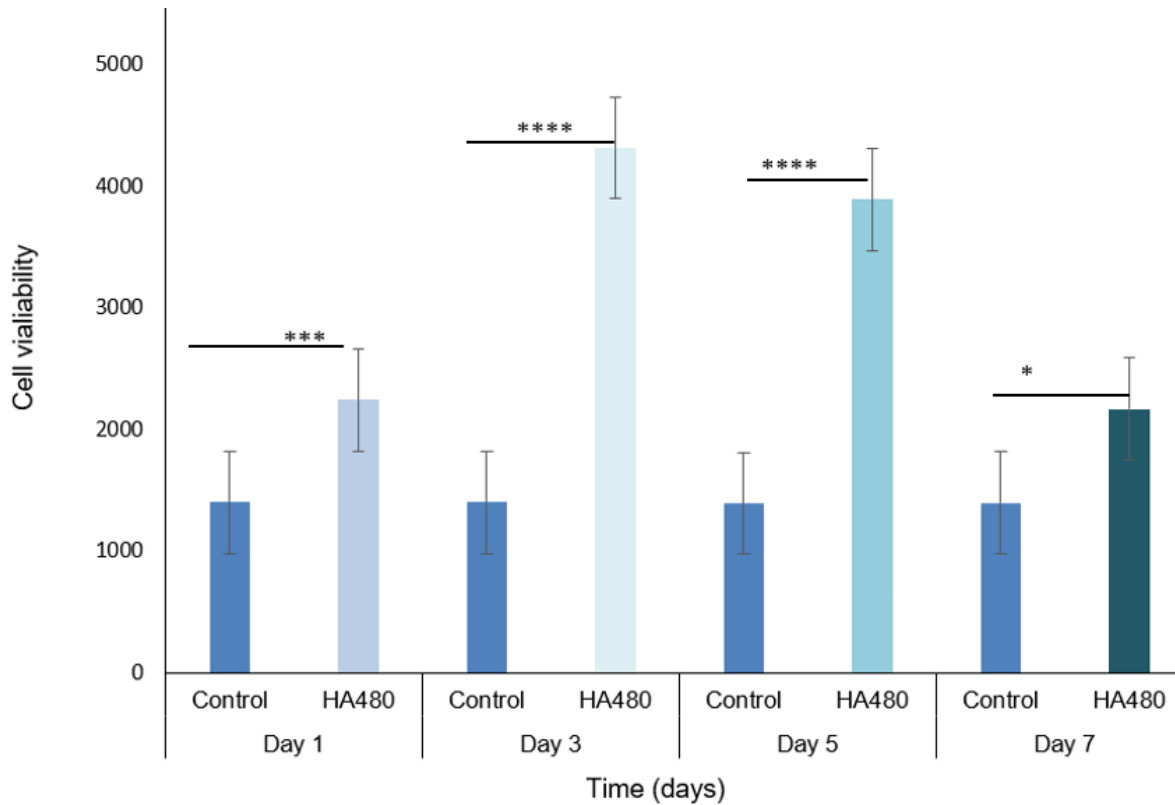


Figure 5-15: Proliferation of hFOB 1.19 cells on non-porous disc HA480 specimens over 7 days. Results indicate the average of duplicate values determined during a single experiment. Statistically significant differences between different data are indicated as \* ( $p < 0.05$ ), and \*\*\* ( $p < 0.001$ ).

As illustrated in hFOB 1.19 cells on HA480 specimens, the fluorescence was lower than expected at day 1 in comparison to the standard and cell growth curves. In Figure 5-15 hFOB cells showed very highly significant cell viability compared to the hFOB in the control plate ( $p < 0.001$ ) for day 1. An increase in cell proliferation was observed with very highly significant proliferation, in contrast to control cells on day 3. On day 5, there a decrease in fluorescence, indicating a significant reduction in cell growth on the surface of HA480. Compared to proliferation, the cell viability in HA480 was highly significant when contrasted with the control cells. Cells on HA480 scaffold reduced in multiplying and demonstrated significant difference with the control cells. Cell viability

on HA480 across Days 1, 3, 5, and 7 showed that hFOB cell proliferation was statistically significant ( $p < 0.035$ ). The results for HA480 on Day 1 and 3 were comparable with hFOB. 1.19 osteoblast proliferation on ceramic zirconia [215]. The results differed from the results for an HA400 lattice scaffold seeded with hBMSCs in a previous study, which showed a significant increase in scaffold strength over 21 days [216]. However, the ability for the cell to proliferate for 7 days is in support of the ISO 10993-5 standard requirement for HA480 to be biocompatible.

### **5.10 Cell attachment on the HA480 scaffold**

To evaluate the biocompatibility of the material, the hFOB cells were seeded on HA480 specimens for different numbers of days. For the Day 1 specimen, the SEM images in Figure 5-16 showed that the cells were stuck together on the surface of the specimen and had not yet spread across the entire surface of the HA480. The SEM images in Figure 5-17 of the day 3 specimen showed that, at lower SEM magnification, the cells were scattered and there was an increase in their number compared to Day 1. The cells were breaking away to fill the surface of the scaffold, and it was evident that they were starting to flatten and attach to the scaffold. From the SEM images in Figure 5-18, it is clear that on Day 5, the cells had flattened and were more attached to the surface of the scaffold, with evidence of scaffold resorption. The SEM images in Figure 5-19 of the Day 7 specimen indicated that the size of the cells on the surface of the specimen appeared to decrease and was needed to observe the few remaining cells and cell attachment showed finger-like projections on the microvilli, as seen in Figure 5-19. This illustrates the attachment of hFOB cells to the scaffold surface, even though the proliferation had decreased by Day 7. The cells were attached to the surface of the scaffold, reaching confluency. The cell's ability to reach Day 7 proved that the material did not produce cytotoxicity. In the HA sample study by Kang *et al.* [194], cell attachment and elongation were observed on the surface, which suggested that HA480 has the potential to be used for bone regeneration scaffolds. The HA480 results for Day 1 and 3 were comparable with hFOB 1.19 osteoblast proliferation on ceramic zirconia [215]. The results differ from an HA400 lattice scaffold seeded with hBMSCs, which showed a significant increase on the scaffold for 21 days [216] Future work could involve observing the attachment of hFOB cells to the HA480 lattice

structure. Future work could involve observing the attachment of hFOB cells to the HA480 lattice structure.

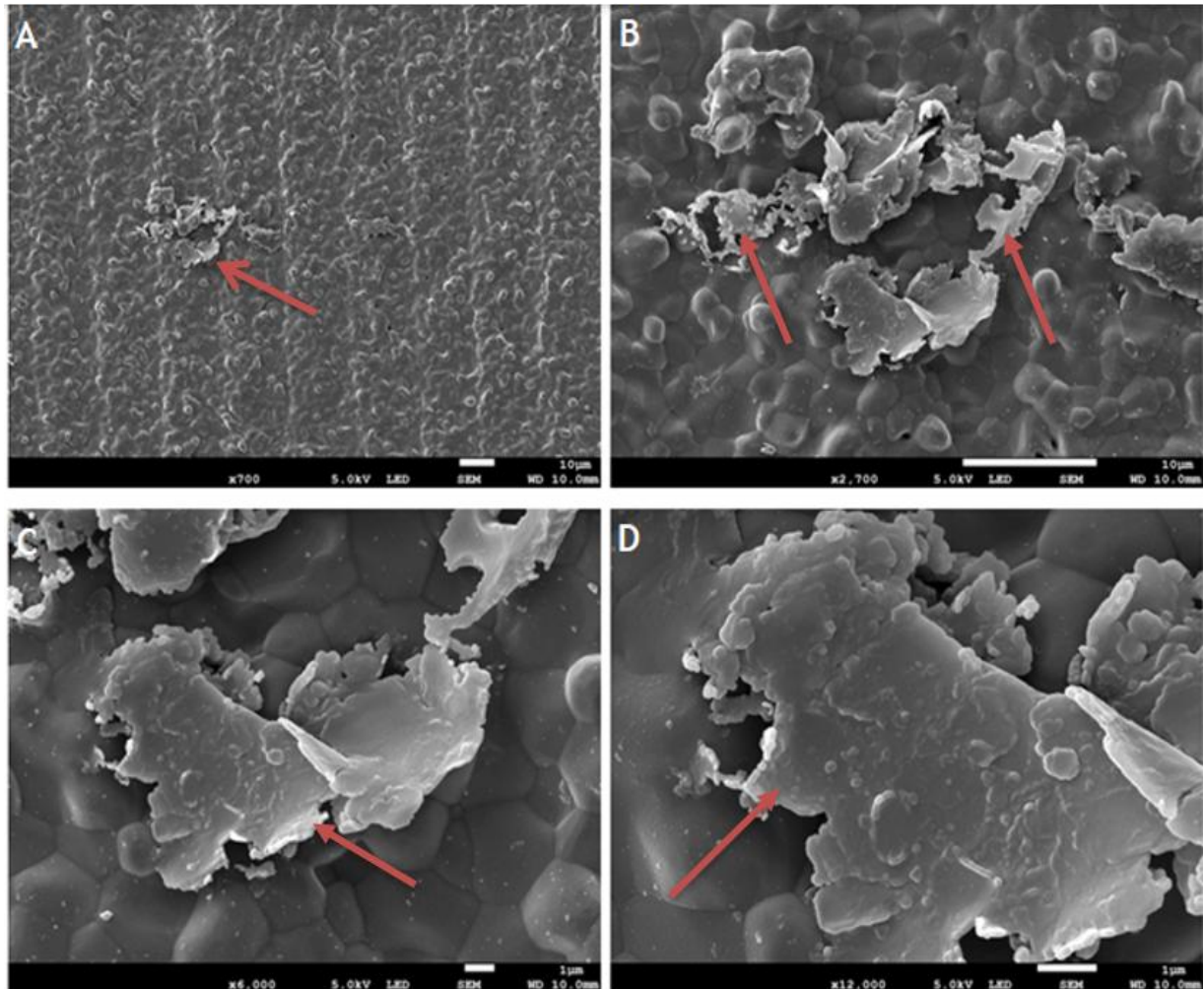


Figure 5-16: SEM-SE images of cell attachment on HA480 on day 1, with the red arrows indicating the cell from low to high magnification.



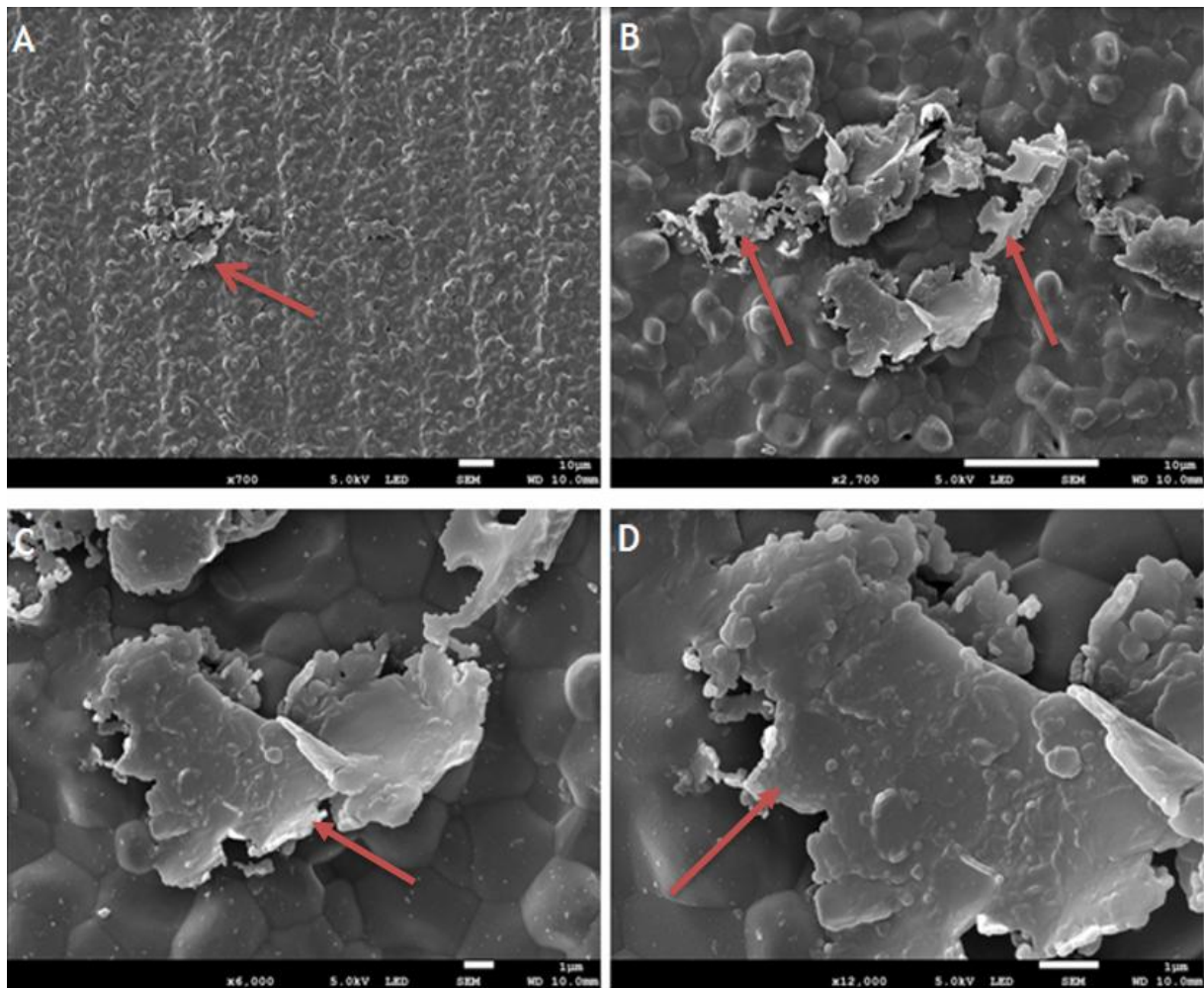


Figure 5-17:SEM-SE images of cell attachment on HA480 after 3 days, with the red arrows indicating the cell from low to high magnification



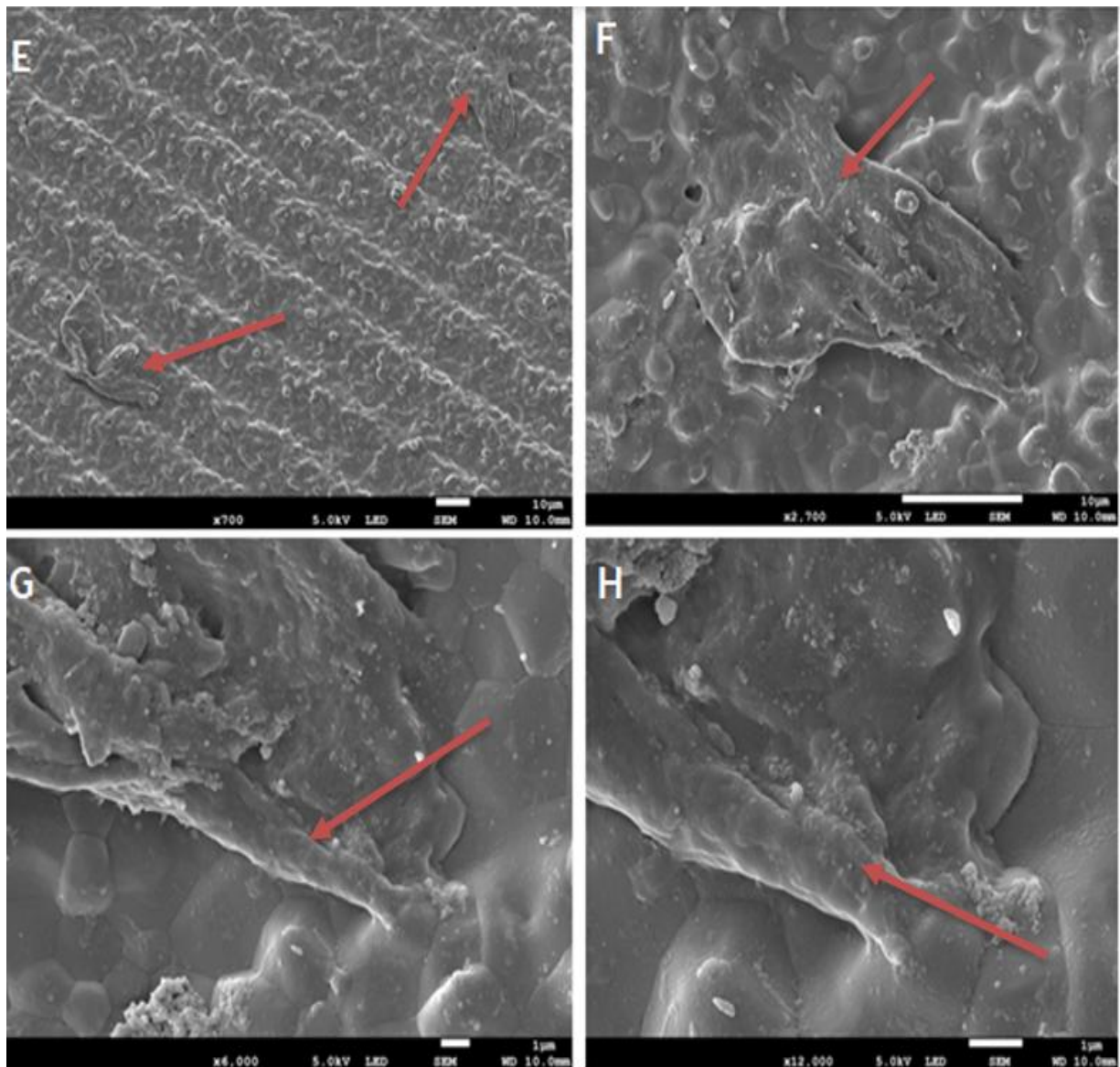


Figure 5-18: SEM-SE images of cell attachment on HA480 after 5 days, with the red arrows indicating the cell from low to high magnification

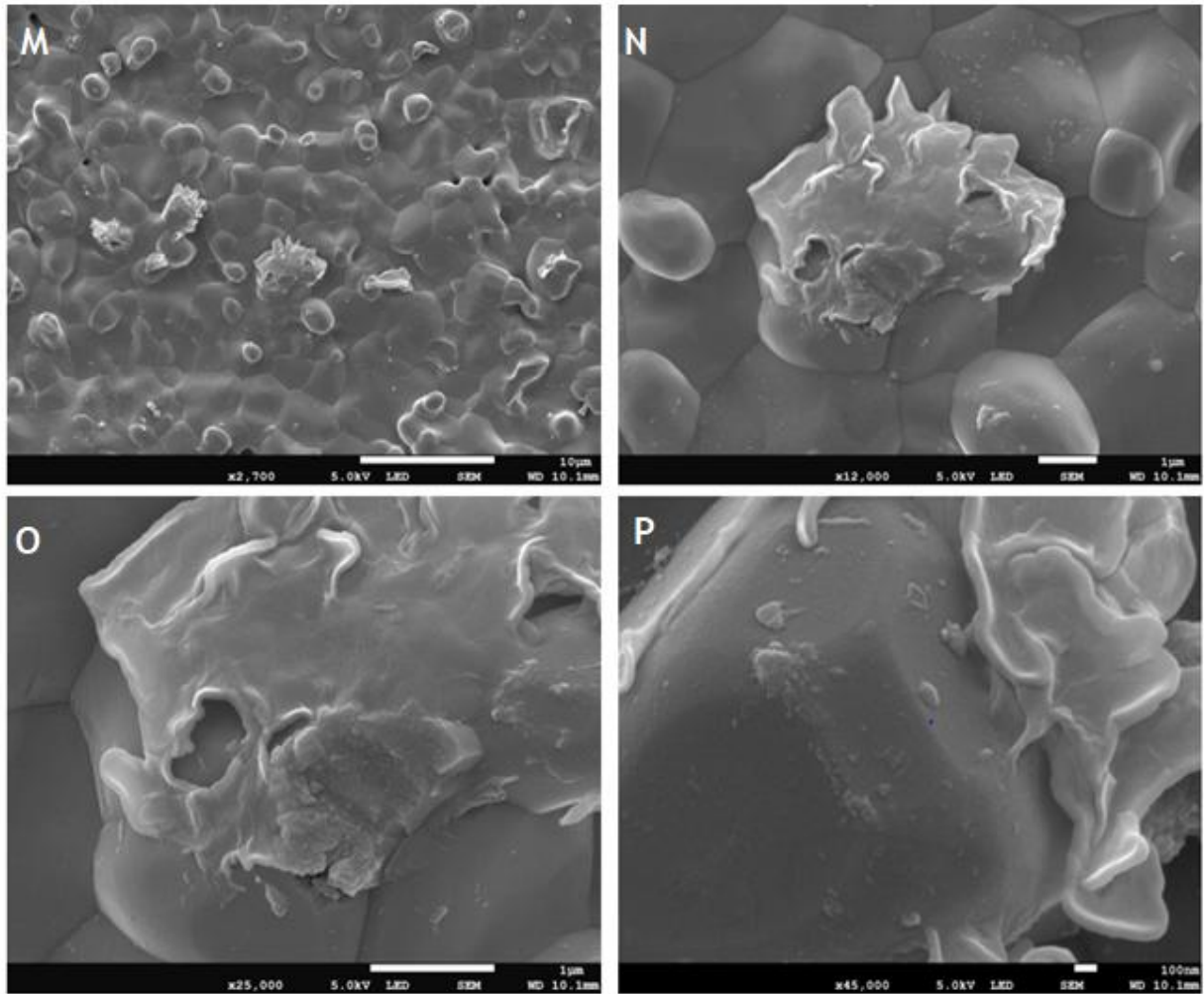


Figure 5-19: SEM-SE images of cell attachment on HA480 after 7 days at very high magnification.

### 5.11 CA/P ratio during cell culture

EDX results Ca/P ratio of HA480 in cell culture media for Day 1 to 7 are reported in Figure 5-20.

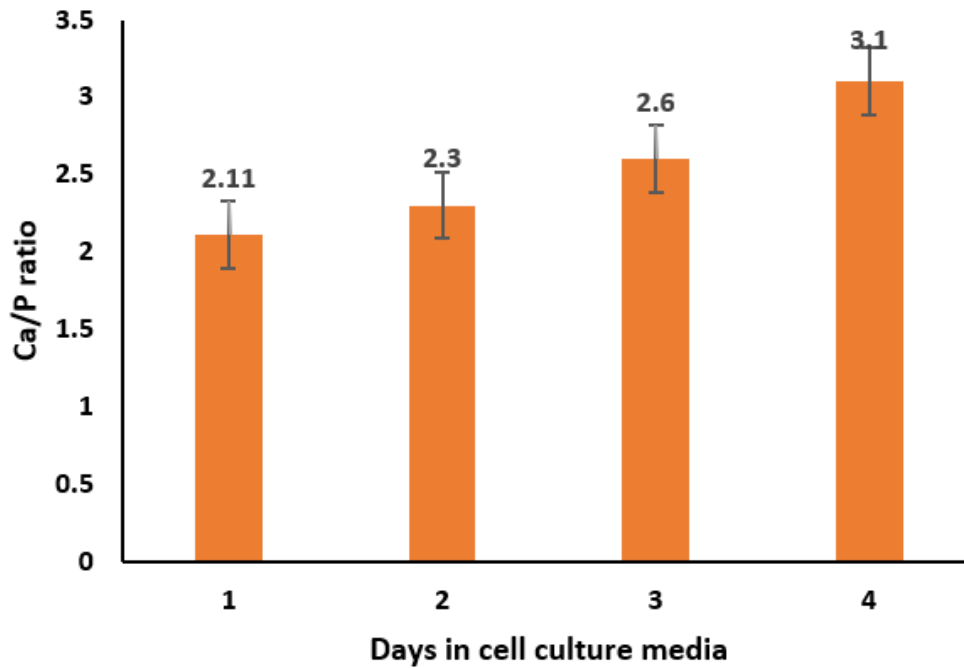


Figure 5-20: Ca/P ratio of HA480 in cell culture media.

Day 1 the Ca 35.6 wt% and 15.4 wt% and the calculate average Ca/P ratio was  $2.11 \pm 0.09$ . Scaffold in the cell culture media for the first 3 days had the Ca/P ratio of HA480 higher than of standard HA 1.67 [218]. Throughout the cell culture the increase observed is on calcium and the phosphorus remains 15 wt%. The increase of Ca is increase due to surface reaction of HA480 with biological fluids [187].

### 5.12 Potential of HA480 for bone scaffold applications

With HA480 being a new material, the purpose of the study was to evaluate whether the material could degrade and promote to bone regeneration in the human body. HA480 diamond lattice structure was successfully tested for biodegradation behaviour. The degradation of the material was favourable, and the structure's integrity was maintained throughout the 28 days of testing. The CT analysis of the HA480 diamond lattice had struts a thickness of approximately 0.2 mm and an average pore size of 300  $\mu\text{m}$ . Pore size would allow cells to grow and migrate into the scaffold. Determination of the Ca/P ratio confirmed that HA480 was a slow-degrading material. The formation of apatite on the surfaces of HA480 indicated bioactivity. Therefore, further research was conducted on the biocompatibility of HA480. HA480 cell culture

sample were seeded with hFOB cells, resulting in increased cell proliferation. This ability for the cell to proliferate on the scaffold indicated that the material was biocompatible, and on Day 7, the cells on the HA480 material proliferated are than the control cells. As indicated by the SBF Figure 5-7, the apatite formation could influence cell proliferation, indicating that the HA480 is bioactive. The cell attachment analysis showed that hFOB 1.9 osteoblast cells adhered to the surfaces of HA480 in Figure 5-18 and Figure 5-19. The obtained results proved that HA480 was biocompatible and could be used for a bone regeneration scaffold.

### **5.13 Conclusion**

An important required property of a bone scaffold, which is degradation, was evaluated with the formation of the apatite on the surface of HA480 scaffold. A cell culture study was performed with HA480 specimens seeded with hFOB 1.9 osteoblast cells. This resulted in increased cell proliferation. The cell attachment analysis showed that hFOB 1.9 osteoblast cells adhered to the surfaces of HA480. The obtained results proved that HA480 was biocompatible and could be used for a bone regeneration scaffold. It can be concluded that the HA480 diamond lattice structure has the potential to be used for small- to medium-sized biodegradable bone regenerating scaffolds.

## CHAPTER 6

### ADDITIVE MANUFACTURING OF A RESORBABLE BONE REGENERATION SCAFFOLD DEMONSTRATOR

#### 6.1 Introduction

This chapter discusses the application of the results of this study to develop a small bone-regeneration implant demonstrator for an alveolar ridge defect, produced in HA480 by LCM at CRPM.

#### 6.2 Methodology

##### 6.2.1 Design of the alveolar ridge implant

An alveolar ridge implant was designed for a 34-year-old male patient who suffered a gunshot wound that had removed part of his alveolar bone. A CT scan performed on the patient's face provided the images shown in Figure 6-1.

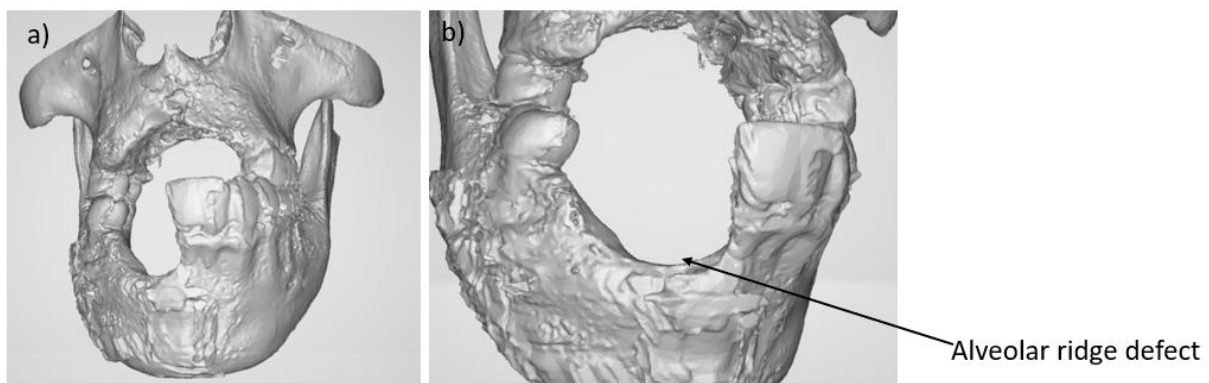


Figure 6-1: CT scan of a patient's alveolar ridge a) front view and b) right side view.

The patient had lost part of the alveolar bone and teeth. Due to the patient's age and the nature of the defect, the alveolar ridge could not regenerate itself and was classified as a combination vertical and horizontal defect. This work was done in consultation with Prof. Cules van den Heever, the Clinical Adviser to CRPM, and the

CRPM team. The implant was reverse engineered by using Magics software to provide a scaffold that would fill the missing part of the alveolar ridge, as shown Figure 6-2.

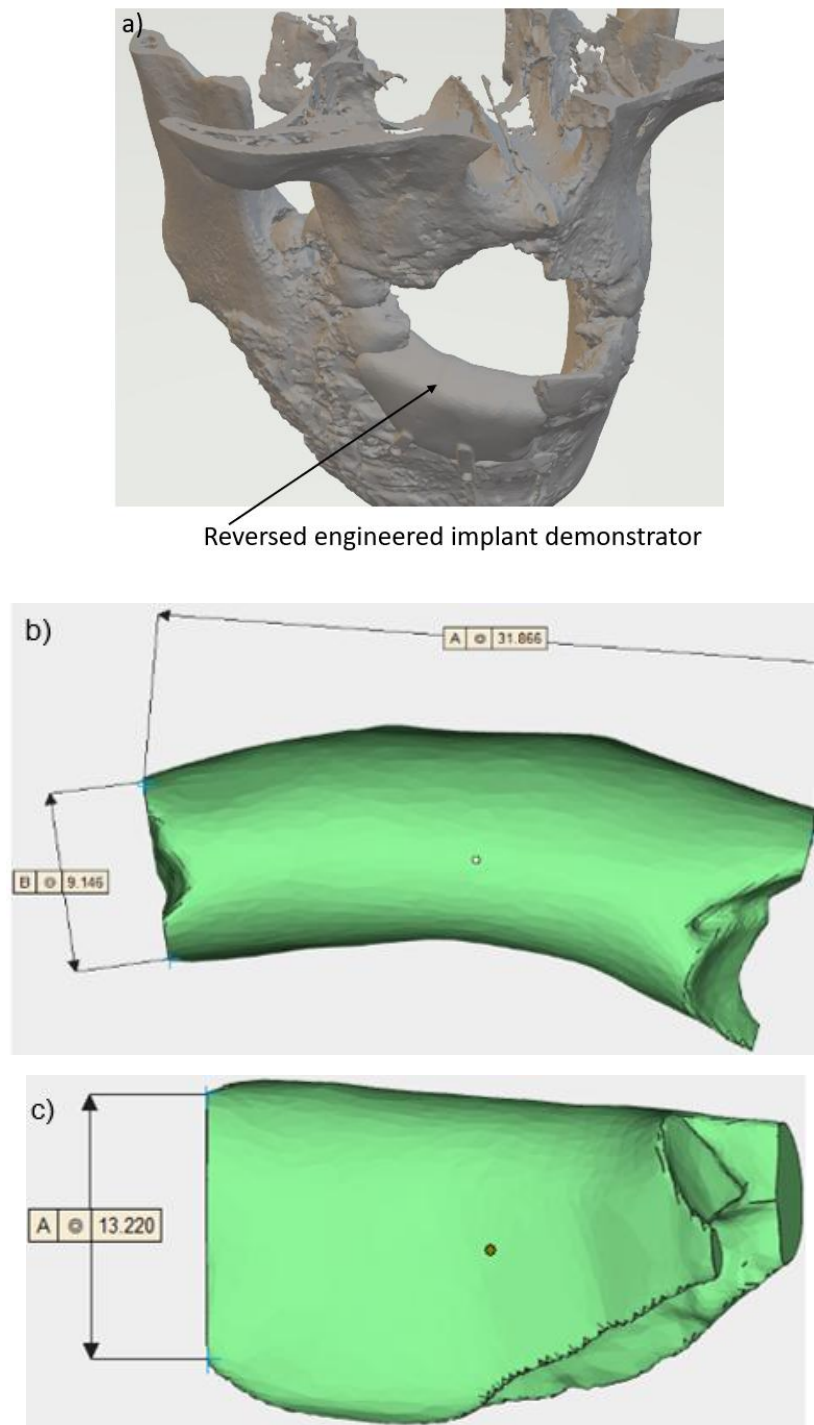


Figure 6-2: a) Reverse-engineered implant demonstrator on the alveolar bone defect, b) top view of implant demonstrator with dimensions in (millimeters) and c) back view of implant demonstrator.



Based on the current study, it was decided to design the implant demonstrator with a diamond lattice structure, shown in Figure 6-3.

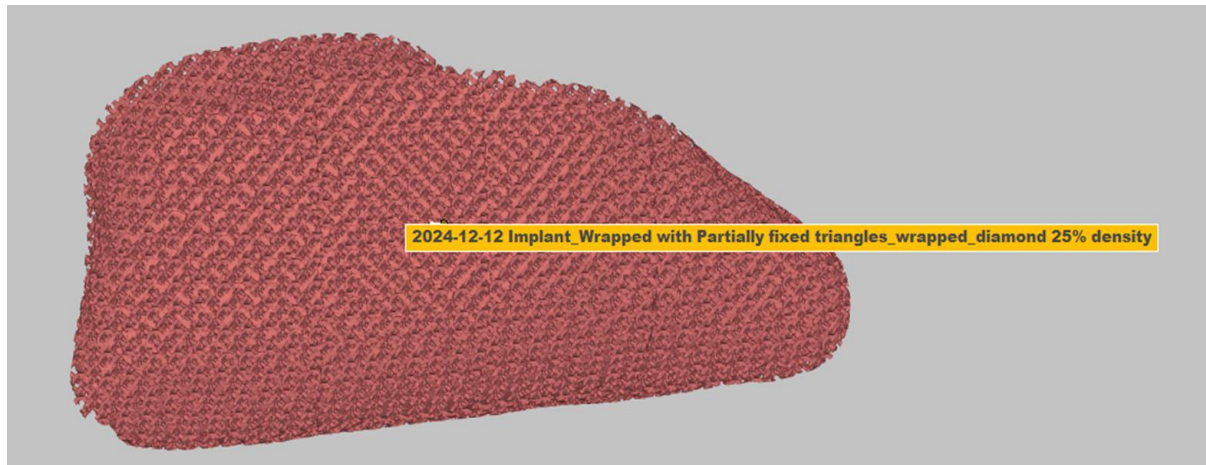


Figure 6-3: Implant demonstrator filled with diamond lattice structure.

In Figure 6-4 the STL model of the implant with the diamond lattice structure was placed in the defect in the CT model to evaluate the fitting. The holes for the mini plate screws for attachment are also shown.

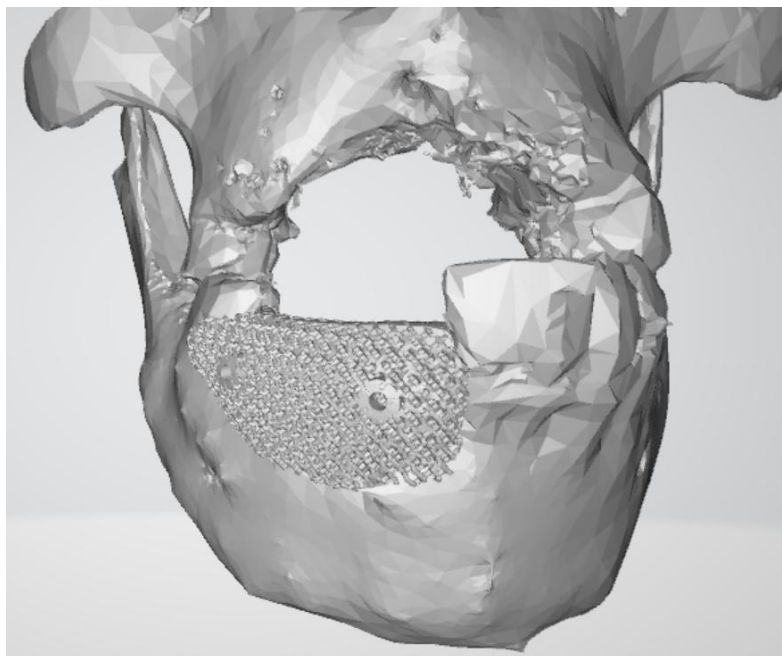


Figure 6-4: Reverse engineered implant model with diamond lattice structure placed in the defect in the CT model.

### 6.2.2 Additive manufacturing of the alveolar ridge implant

Figure 6-5 illustrates how the design of the alveolar ridge implant was prepared for the LCM process, with the inclusion of the support structure.

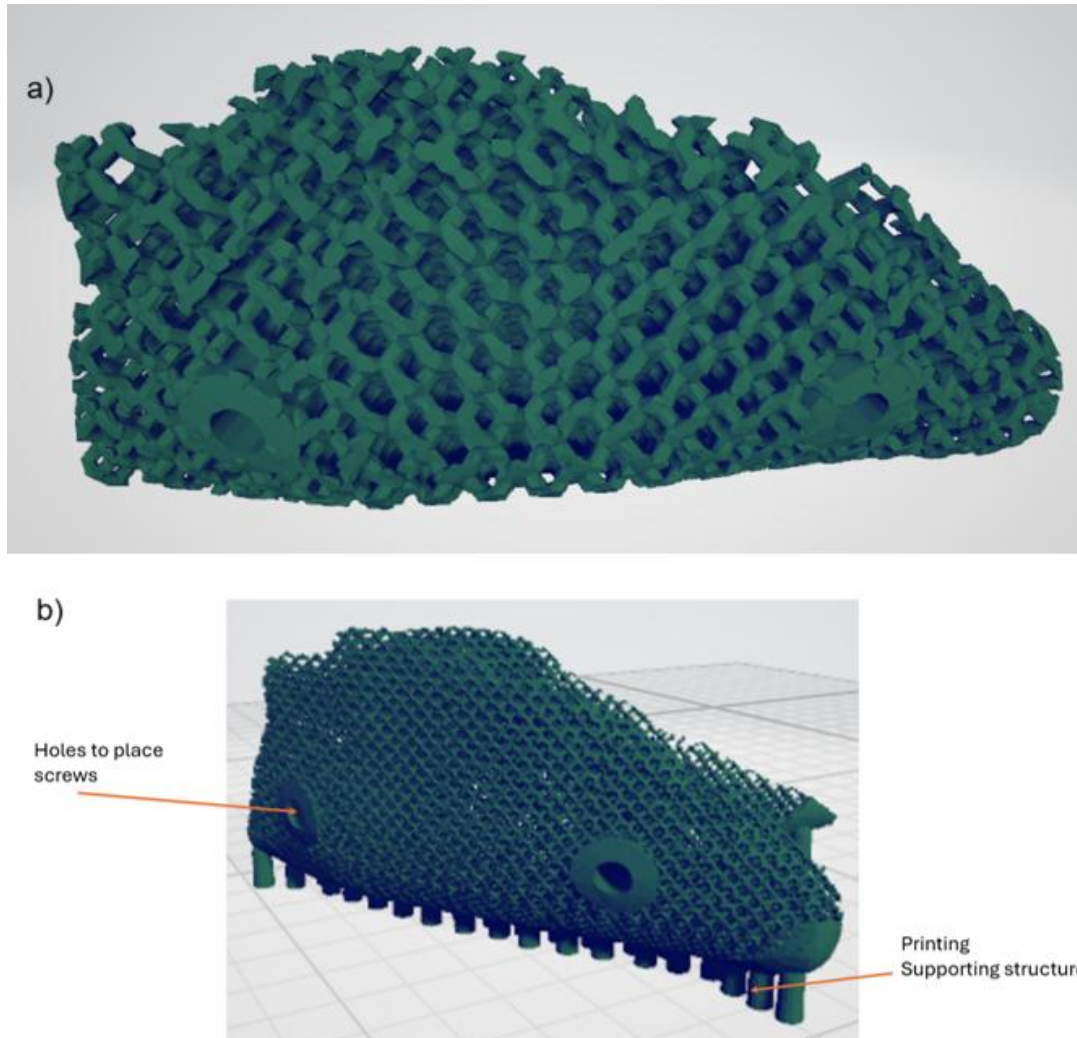


Figure 6-5: a) Design of the alveolar ridge implant consisting of a diamond lattice b) support structures added for LCM printing.

HA480 material was used for building the implant with the diamond lattice by LCM at CRPM. The process parameters were the same in this study to build the test specimens. After completion of the LCM process, the green body of the alveolar ridge implant had the post-processing heat treatment which consisted of debinding and sintering steps.



### 6.2.3 Additive manufacturing CT model of the patient

The CT model of the patient with the defect was manufactured using the AM technology of Selective Laser Sintering (SLS). Polyamide-12 Grade PA 2200 powder was used in an EOS P396 machine for building the CT model.

### 6.3 Results

The alveolar ridge diamond lattice implant was successfully built by using LCM. Figure 6-6 shows the as-built green body of the scaffold, still attached to the build platform by the support structures. In Figure 6-7 the sintered alveolar ridge implant is shown with supports removed.

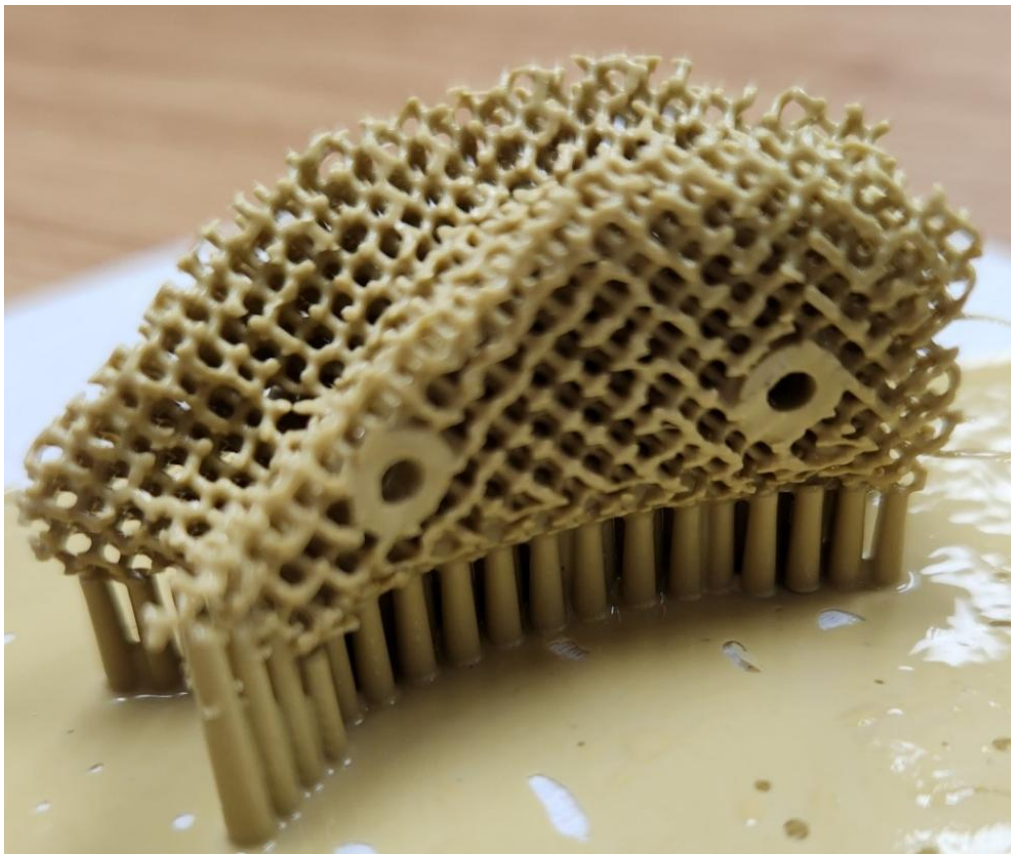


Figure 6-6: As-built green body of the HA480 implant with support structures.



Figure 6-7: Sintered alveolar ridge implant.

Figure 6-8 illustrates the fitting of HA480 LCM manufactured implant in the polyamide prototype with the alveolar defect.

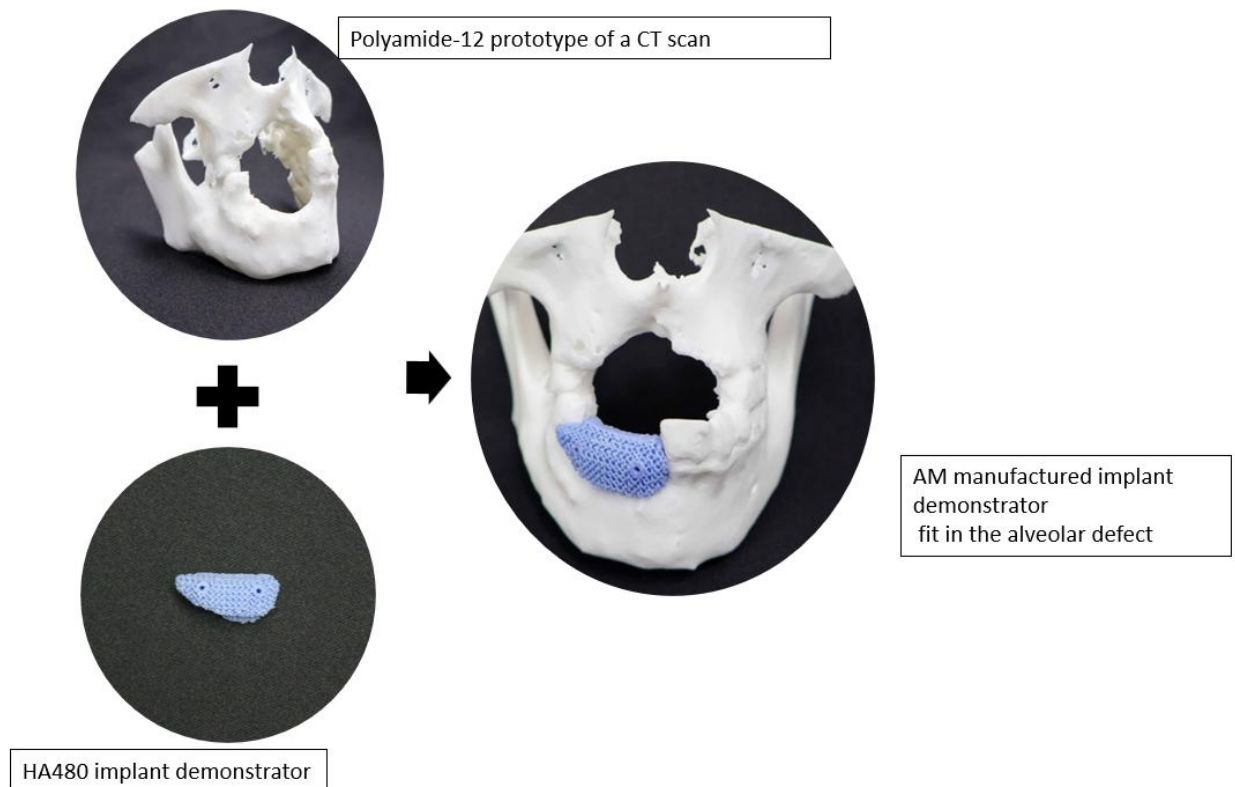


Figure 6-8: Demonstration of the fitting of the LCM customised alveolar ridge implant in the model of the patient's mandible.

## 6.4 Discussion

There are biodegradable bioceramic scaffolds in the market, such as cerament, osteoset resorbable beads, ostim graft and perOssal® scaffolds, which are FDA approved [215][202]. These bone scaffold have good degradation rate and osteoconduction and are osteoinductive. However, they are not custom-made for patients, their shapes are blocks, cylinders, wedges or granules and are moulded during surgery. The implant demonstrator produced in this study was specific for the patient. The biological, mechanical and degradation behaviour of the implant demonstrator are expected to be the same as those of the test specimens tested in this study. The successful manufacturing of the implant demonstrator shows the effectiveness of AM technology for the manufacturing of small- to medium-sized biodegradable bone scaffolds.

## CHAPTER 7

### CONCLUSIONS AND RECOMMENDATIONS

#### 7.1 Conclusions

The first objective was to select the FDA approved material, and LithaBone 480 from Lithoz was selected. The HA480 powder particle material used, is FDA approved HA and the binder used is classified information by the company. The EDX of HA480 was found  $1.64 \pm 0.21$  comparable to the HA. Therefore, HA480 can be used for slow degradation scaffolds.

FTIR graphs of HA480 indicated that the peaks were comparable to those of standard HA. No trace of binder material was detected. The diamond lattice structure was selected among diagonal and rhombic structures using Maxwell criteria selection and considering biological material movement.

The objective of the work reported in chapter 5 was to determine the factors that influence controllable degradation and bone regeneration rates of an additively manufactured scaffold.

The HA480 diamond lattice structure was analysed for swelling rate which displayed that the lattice within the lattice in 5 minutes it was able to absorb the water. In the biological world meaning it will absorb fluids withing 5 minutes of implantation because of its porous structure. The HA480 solid structure only absorbed water after 24 hours which confirmed that the lattice structure was efficient in comparison with a solid scaffold.

The biodegradation test for the diamond lattice was done for 1, 7 and 28 days. At Day 1 there was a high mass loss of 12% biodegradation of the lattice structure in the SBF. On Day 7 there was an increase of mass which was probably caused by surface ion exchange between the SBF and the HA480. The increase of mass resulted in a weight loss of 9%. On Day 28 the degradation the weight loss was 8.11%. In SEM-SE images for Day 28 evidence was found of a flake-like structure similar from the SBF protocol. This provided confirmation that HA480 has the potential for bioactivity when implanted in the body.

The grain size of a plain HA480 was measured as  $2.378\ \mu\text{m}$  with compressive strength  $10.7\pm 1.4\ \text{MPa}$ . After 28 days of biodegradation the grain size increased to  $4.258\ \mu\text{m}$  with decreased compressive strength of  $3.04\pm 0.01\ \text{MPa}$ . The CA/P ratio of the HA480 was measured as 1.61 through degradation the CA/P ratio increased for the days it was in the SBF. On Day 28 the CA/P ratio was recorded as 2 due to ion exchange of material and biological solution.

Surface roughness value was  $R_a = 0.60\pm 0.05\ \mu\text{m}$  suitable for osteoblast cells attachment and there was no need to modify the surface. The hFOB1.19 osteoblast cells were seeded on solid disk scaffold HA480 to analyse cell attachment and growth. Cell proliferation from Day 1, 3, 5 was evident and after Day 7 the proliferation declined. The HA480 demonstrated biocompatibility as cells proliferated. The hFOB1.19 osteoblast attached on the surface of HA480 and change morphology until Day 7.

Through addressing, the objectives in of the study. Proof of concept was done creating a custom designed bone scaffold of an alveolar bone. The defect was replaced with bone scaffold of HA480 diamond lattice structure. HA480 small-medium sized bone regenerating scaffold was successfully manufactured and displayed on the nylon printed model of the defect.

## 7.2 Recommendations for future work

- Since LithaBone HA480 is a new material, further *in vitro* biodegradation tests should be done in different media, for example saliva.
- Further work should include in vitro evaluation of HA480 bone scaffold such as different medium for swelling ratio, live/dead fluorescence imaging, ion release profiling, gene expression analysis, mineralisation and pH influence on cells gamma sterilisation prior to in vivo studies. Incorporating confocal imaging to analyse cell infiltration would provide more comprehensive results on material biocompatibility.
- Future work should include macrophage-based inflammatory assays to complement the current fundings on material biocompatibility.
- Given the guidance provided in this study, further research on the design of porous, small, non-load-bearing scaffolds should be conducted.



- Future work should also look on the reinforcement of biopolymers for mechanical improvement such as modulus of elasticity of HA480.
- Future work should focus extended cell culture timeline to day 28 to relate with the biodegradation study.
- To reduce material costs and waste in future research, the flow chart in Figure 7-1 should be applied. The flow starts with analysing the shape of the defect and reverse-engineering the scaffold. This should be done to evaluate which pore size and lattice design can be printed in the implant. This is recommended because some lattice structures can be manufactured with simple geometries, however, when applied to complex bone defect geometries slurry curing can be difficult due to strut and pore design.

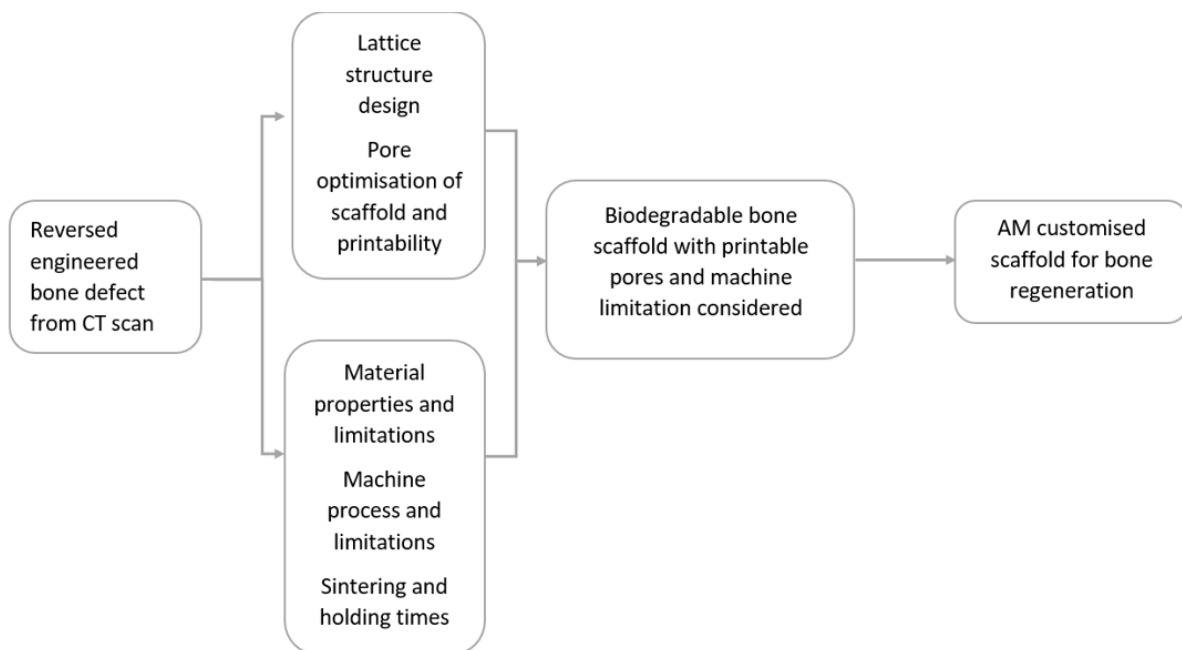


Figure 7-1: Proposed systematic steps for fabrication of LCM customised bone regeneration

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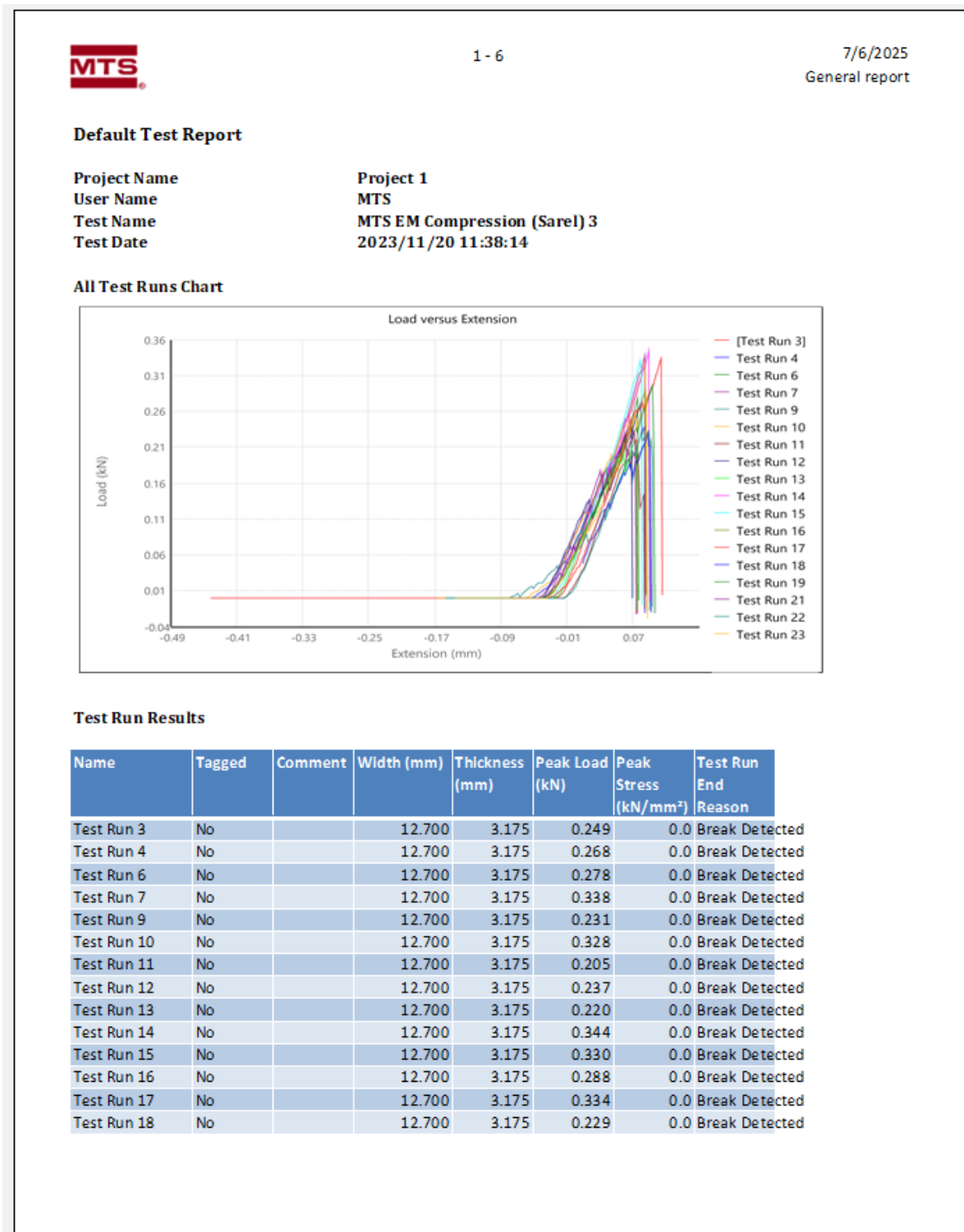
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## **APPENDIX**

## Appendix A: Results from compression testing on a dry ha480 diamond lattice structure







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|             |            |  |        |       |       |       |                |
|-------------|------------|--|--------|-------|-------|-------|----------------|
| Test Run 19 | No         |  | 12.700 | 3.175 | 0.296 | 0.0   | Break Detected |
| Test Run 21 | No         |  | 12.700 | 3.175 | 0.232 | 0.0   | Break Detected |
| Test Run 22 | No         |  | 12.700 | 3.175 | 0.234 | 0.0   | Break Detected |
| Test Run 23 | No         |  | 12.700 | 3.175 | 0.278 | 0.0   | Break Detected |
|             | Mean       |  | 12.700 | 3.175 | 0.273 | 0.01  |                |
|             | Standard D |  | 0.000  | 0.000 | 0.046 | 0.001 |                |

#### Test Run Comments

| Specimen    | Tagged | Specimen Comment |
|-------------|--------|------------------|
| Test Run 3  | No     |                  |
| Test Run 4  | No     |                  |
| Test Run 6  | No     |                  |
| Test Run 7  | No     |                  |
| Test Run 9  | No     |                  |
| Test Run 10 | No     |                  |
| Test Run 11 | No     |                  |
| Test Run 12 | No     |                  |
| Test Run 13 | No     |                  |
| Test Run 14 | No     |                  |
| Test Run 15 | No     |                  |
| Test Run 16 | No     |                  |
| Test Run 17 | No     |                  |
| Test Run 18 | No     |                  |
| Test Run 19 | No     |                  |
| Test Run 21 | No     |                  |
| Test Run 22 | No     |                  |
| Test Run 23 | No     |                  |

#### Test Results

| Specimen    | Tagged | Yield Segment Length (%) | Stress Limit High (kN/mm <sup>2</sup> ) | Load End Point (kN) | Strain Point 06 (mm/mm) | Information 2 | Chord Modulus Strain Point 1 (mm/mm) | Information 10 |
|-------------|--------|--------------------------|-----------------------------------------|---------------------|-------------------------|---------------|--------------------------------------|----------------|
| Test Run 3  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 4  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 6  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 7  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 9  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 10 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 11 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |



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|             |    |     |     |   |       |  |       |  |
|-------------|----|-----|-----|---|-------|--|-------|--|
| Test Run 12 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 13 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 14 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 15 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 16 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 17 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 18 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 19 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 21 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 22 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |
| Test Run 23 | No | 2.0 | 0.2 | 4 | 0.050 |  | 0.020 |  |

| Specimen    | Information 3 | Load Limit High (kN) | Information 7 | Secant Modulus Elongation Point 1 (mm) | Header 1 | Information 9 | Time Limit (sec) | Information 6 |
|-------------|---------------|----------------------|---------------|----------------------------------------|----------|---------------|------------------|---------------|
| Test Run 3  |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 4  |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 6  |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 7  |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 9  |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 10 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 11 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 12 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 13 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 14 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 15 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 16 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 17 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 18 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 19 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 21 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 22 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 23 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |

| Specimen   | Strain Limit High (mm/mm) | Chord Modulus Strain Point 2 (mm/mm) | Chord Modulus Elongation Point 1 (mm) | Strain Point 03 (mm/mm) | Information 5 | Test ID | Test Rate (mm/min) | Secant Modulus Strain Point 2 (mm/mm) |
|------------|---------------------------|--------------------------------------|---------------------------------------|-------------------------|---------------|---------|--------------------|---------------------------------------|
| Test Run 3 | 10                        | 0.050                                | 0.254                                 | 0.050                   |               |         | 1.00               | 0.050                                 |
| Test Run 4 | 10                        | 0.050                                | 0.254                                 | 0.050                   |               |         | 1.00               | 0.050                                 |
| Test Run 6 | 10                        | 0.050                                | 0.254                                 | 0.050                   |               |         | 1.00               | 0.050                                 |



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|             |    |       |       |       |  |  |      |       |
|-------------|----|-------|-------|-------|--|--|------|-------|
| Test Run 7  | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 9  | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 10 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 11 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 12 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 13 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 14 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 15 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 16 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 17 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 18 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 19 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 21 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 22 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |
| Test Run 23 | 10 | 0.050 | 0.254 | 0.050 |  |  | 1.00 | 0.050 |

| Specimen    | Strain Point 05 (mm/mm) | Strain End Point (mm/mm) | Header 3 | Information 8 | Break Threshold (kN) | Information 1 | Information 4 | Strain Point 01 (mm/mm) |
|-------------|-------------------------|--------------------------|----------|---------------|----------------------|---------------|---------------|-------------------------|
| Test Run 3  | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 4  | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 6  | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 7  | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 9  | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 10 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 11 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 12 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 13 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 14 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 15 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 16 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 17 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 18 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 19 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 21 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 22 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |
| Test Run 23 | 0.050                   | 0.100                    |          |               | 0.002                |               |               | 0.050                   |

| Specimen | Platen Separation (mm) | Yield Offset (mm/mm) | Preload (kN) | Slope Segment Length (%) | Outer Loop Rate (Hz) | Strain Limit Low (mm/mm) | Strain Point 07 (mm/mm) | Secant Modulus Strain Point 1 (mm/mm) |
|----------|------------------------|----------------------|--------------|--------------------------|----------------------|--------------------------|-------------------------|---------------------------------------|
|          |                        |                      |              |                          |                      |                          |                         |                                       |



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|             |        |       |       |        |     |     |       |       |
|-------------|--------|-------|-------|--------|-----|-----|-------|-------|
| Test Run 3  | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 4  | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 6  | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 7  | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 9  | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 10 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 11 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 12 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 13 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 14 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 15 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 16 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 17 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 18 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 19 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 21 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 22 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 23 | 50.800 | 0.002 | 0.044 | 20.000 | 100 | -10 | 0.050 | 0.050 |

| Specimen    | Extension<br>Limit Low<br>(mm) | Break<br>Marker<br>Elongatio<br>n (mm) | Load Limit<br>Low (kN) | Break<br>Drop (%) | Memo | Stress<br>Limit Low<br>(kN/mm <sup>2</sup> ) | Break<br>Sensitivity<br>(%) | Data<br>Acquisitio<br>n Rate<br>(Hz) |
|-------------|--------------------------------|----------------------------------------|------------------------|-------------------|------|----------------------------------------------|-----------------------------|--------------------------------------|
| Test Run 3  | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 4  | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 6  | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 7  | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 9  | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 10 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 11 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 12 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 13 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 14 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 15 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 16 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 17 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 18 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 19 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 21 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 22 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |
| Test Run 23 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 10.0                                 |



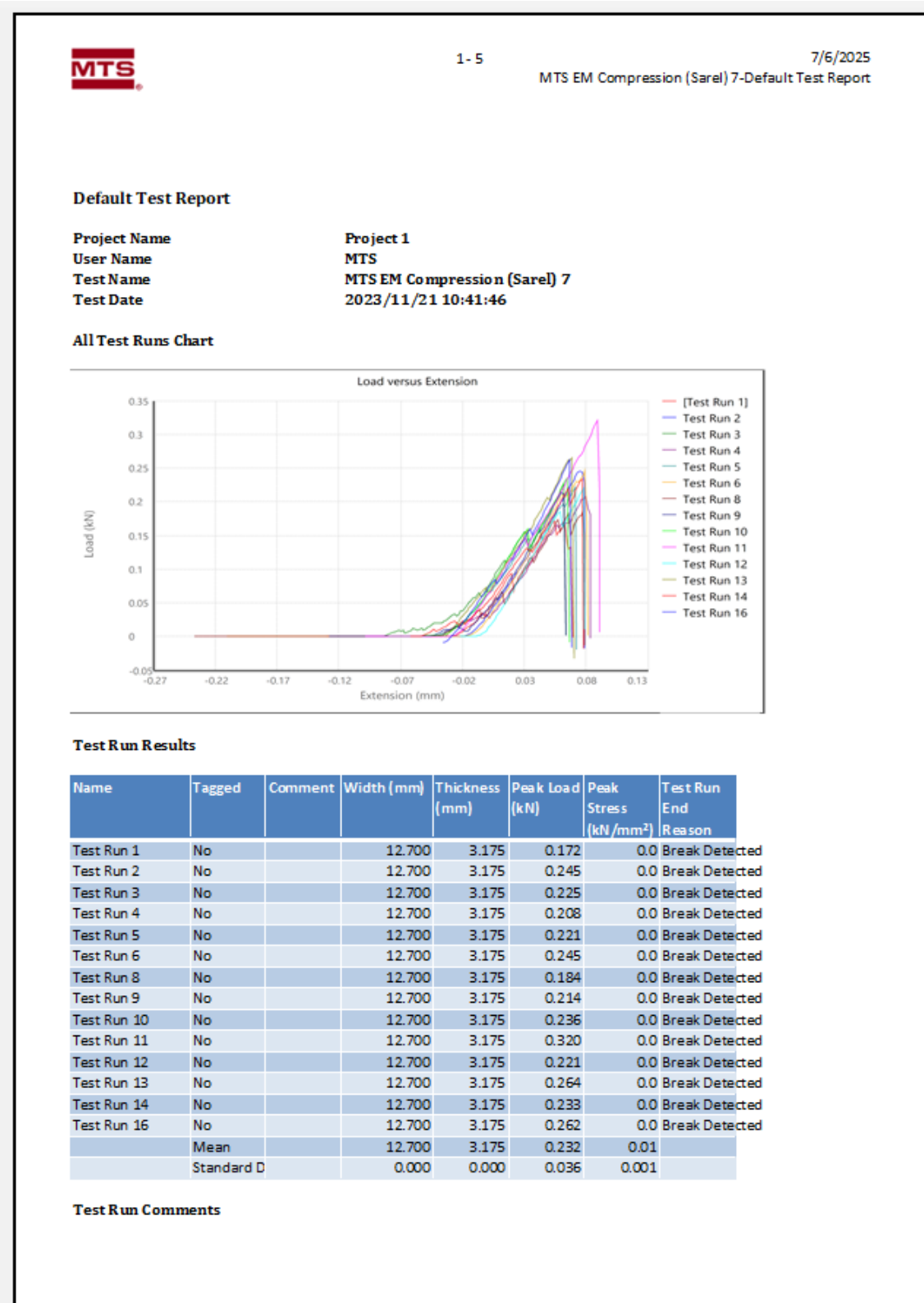
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| Specimen    | Yield Angle (rad) | Preload Speed (mm/s) | Strain Point 08 (mm/mm) | Slack Preload (kN) | Chord Modulus Elongation Point 2 (mm) | Strain Point 02 (mm/mm) | Strain Point 04 (mm/mm) | Extension Limit High (mm) |
|-------------|-------------------|----------------------|-------------------------|--------------------|---------------------------------------|-------------------------|-------------------------|---------------------------|
| Test Run 3  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 4  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 6  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 7  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 9  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 10 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 11 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 12 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 13 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 14 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 15 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 16 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 17 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 18 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 19 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 21 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 22 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 23 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |

| Specimen    | Header 2 |
|-------------|----------|
| Test Run 3  |          |
| Test Run 4  |          |
| Test Run 6  |          |
| Test Run 7  |          |
| Test Run 9  |          |
| Test Run 10 |          |
| Test Run 11 |          |
| Test Run 12 |          |
| Test Run 13 |          |
| Test Run 14 |          |
| Test Run 15 |          |
| Test Run 16 |          |
| Test Run 17 |          |
| Test Run 18 |          |
| Test Run 19 |          |
| Test Run 21 |          |
| Test Run 22 |          |
| Test Run 23 |          |

## Appendix B : Compression test results of HA480 diamond lattice structure after 1 day of biodegradation in SBF





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MTS EM Compression (Sarel) 7-Default Test Report

| Specimen    | Tagged | Specimen Comment |
|-------------|--------|------------------|
| Test Run 1  | No     |                  |
| Test Run 2  | No     |                  |
| Test Run 3  | No     |                  |
| Test Run 4  | No     |                  |
| Test Run 5  | No     |                  |
| Test Run 6  | No     |                  |
| Test Run 8  | No     |                  |
| Test Run 9  | No     |                  |
| Test Run 10 | No     |                  |
| Test Run 11 | No     |                  |
| Test Run 12 | No     |                  |
| Test Run 13 | No     |                  |
| Test Run 14 | No     |                  |
| Test Run 16 | No     |                  |

#### Test Results

| Specimen    | Tagged | Yield Segment Length (%) | Stress Limit High (kN/mm <sup>2</sup> ) | Load End Point (kN) | Strain Point 06 (mm/mm) | Information 2 | Chord Modulus Strain Point 1 (mm/mm) | Information 10 |
|-------------|--------|--------------------------|-----------------------------------------|---------------------|-------------------------|---------------|--------------------------------------|----------------|
| Test Run 1  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 2  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 3  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 4  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 5  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 6  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 8  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 9  | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 10 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 11 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 12 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 13 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 14 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 16 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |

| Specimen   | Information 3 | Load Limit High (kN) | Information 7 | Secant Modulus Elongation Point 1 (mm) | Header 1 | Information 9 | Time Limit (sec) | Information 6 |
|------------|---------------|----------------------|---------------|----------------------------------------|----------|---------------|------------------|---------------|
| Test Run 1 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 2 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 3 |               | 22                   |               | 0.254                                  |          |               | 10000            |               |





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|             |  |    |  |       |  |  |       |  |
|-------------|--|----|--|-------|--|--|-------|--|
| Test Run 4  |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 5  |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 6  |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 8  |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 9  |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 10 |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 11 |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 12 |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 13 |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 14 |  | 22 |  | 0.254 |  |  | 10000 |  |
| Test Run 16 |  | 22 |  | 0.254 |  |  | 10000 |  |

| Specimen    | Strain<br>Limit High<br>(mm/mm) | Chord<br>Modulus<br>Strain<br>Point 2<br>(mm/mm) | Chord<br>Modulus<br>Elongation<br>Point 1 (mm) | Strain<br>Point 03<br>(mm/mm) | Information 5 | Test ID | Test Rate<br>(mm/min) | Secant<br>Modulus<br>Strain<br>Point 2<br>(mm/mm) |
|-------------|---------------------------------|--------------------------------------------------|------------------------------------------------|-------------------------------|---------------|---------|-----------------------|---------------------------------------------------|
| Test Run 1  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 2  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 3  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 4  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 5  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 6  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 8  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 9  | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 10 | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 11 | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 12 | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 13 | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 14 | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |
| Test Run 16 | 10                              | 0.050                                            | 0.254                                          | 0.050                         |               |         | 1.00                  | 0.050                                             |

| Specimen    | Strain<br>Point 05<br>(mm/mm) | Strain End<br>Point<br>(mm/mm) | Header 3 | Information 8 | Break<br>Threshold<br>(kN) | Information 1 | Information 4 | Strain<br>Point 01<br>(mm/mm) |
|-------------|-------------------------------|--------------------------------|----------|---------------|----------------------------|---------------|---------------|-------------------------------|
| Test Run 1  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 2  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 3  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 4  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 5  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 6  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 8  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 9  | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 10 | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 11 | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |
| Test Run 12 | 0.050                         | 0.100                          |          |               | 0.002                      |               |               | 0.050                         |



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|             |       |       |  |  |       |  |  |       |
|-------------|-------|-------|--|--|-------|--|--|-------|
| Test Run 13 | 0.050 | 0.100 |  |  | 0.002 |  |  | 0.050 |
| Test Run 14 | 0.050 | 0.100 |  |  | 0.002 |  |  | 0.050 |
| Test Run 16 | 0.050 | 0.100 |  |  | 0.002 |  |  | 0.050 |

| Specimen    | Platen Separation (mm) | Yield Offset (mm/mm) | Preload (kN) | Slope Segment Length (%) | Outer Loop Rate (Hz) | Strain Limit Low (mm/mm) | Strain Point 07 (mm/mm) | Secant Modulus Strain Point 1 (mm/mm) |
|-------------|------------------------|----------------------|--------------|--------------------------|----------------------|--------------------------|-------------------------|---------------------------------------|
| Test Run 1  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 2  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 3  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 4  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 5  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 6  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 8  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 9  | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 10 | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 11 | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 12 | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 13 | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 14 | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |
| Test Run 16 | 50.800                 | 0.002                | 0.044        | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |

| Specimen    | Extension Limit Low (mm) | Break Marker Elongation (mm) | Load Limit Low (kN) | Break Drop (%) | Memo | Stress Limit Low (kN/mm <sup>2</sup> ) | Break Sensitivity (%) | Data Acquisition Rate (Hz) |
|-------------|--------------------------|------------------------------|---------------------|----------------|------|----------------------------------------|-----------------------|----------------------------|
| Test Run 1  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 2  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 3  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 4  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 5  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 6  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 8  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 9  | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 10 | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 11 | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 12 | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 13 | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 14 | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |
| Test Run 16 | -508                     | 2.540                        | -4                  | 50.0           |      | -0.2                                   | 90                    | 10.0                       |



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MTS EM Compression (Sarel) 7-Default Test Report

| Specimen    | Yield Angle (rad) | Preload Speed (mm/s) | Strain Point 08 (mm/mm) | Slack Preload (kN) | Chord Modulus Elongation Point 2 (mm) | Strain Point 02 (mm/mm) | Strain Point 04 (mm/mm) | Extension Limit High (mm) |
|-------------|-------------------|----------------------|-------------------------|--------------------|---------------------------------------|-------------------------|-------------------------|---------------------------|
| Test Run 1  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 2  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 3  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 4  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 5  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 6  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 8  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 9  | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 10 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 11 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 12 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 13 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 14 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |
| Test Run 16 | 0.000             | 0.042                | 0.050                   | 0.0004             | 12.700                                | 0.050                   | 0.050                   | 508                       |

| Specimen    | Header 2 |
|-------------|----------|
| Test Run 1  |          |
| Test Run 2  |          |
| Test Run 3  |          |
| Test Run 4  |          |
| Test Run 5  |          |
| Test Run 6  |          |
| Test Run 8  |          |
| Test Run 9  |          |
| Test Run 10 |          |
| Test Run 11 |          |
| Test Run 12 |          |
| Test Run 13 |          |
| Test Run 14 |          |
| Test Run 16 |          |

## Appendix C: Compression test results of HA480 diamond lattice structure after 28 days of biodegradation in the SBF.





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Ankia Compression Test 25-Default Test Report

| Specimen   | Tagged | Yield Segment Length (%) | Stress Limit High (kN/mm <sup>2</sup> ) | Load End Point (kN) | Strain Point 06 (mm/mm) | Information 2 | Chord Modulus Strain Point 1 (mm/mm) | Information 10 |
|------------|--------|--------------------------|-----------------------------------------|---------------------|-------------------------|---------------|--------------------------------------|----------------|
| Test Run 3 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 4 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |
| Test Run 5 | No     | 2.0                      | 0.2                                     | 4                   | 0.050                   |               | 0.020                                |                |

| Specimen   | Information 3 | Load Limit High (kN) | Information 7 | Secant Modulus Elongation Point 1 (mm) | Header 1 | Information 9 | Time Limit (sec) | Information 6 |
|------------|---------------|----------------------|---------------|----------------------------------------|----------|---------------|------------------|---------------|
| Test Run 3 |               | 20                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 4 |               | 20                   |               | 0.254                                  |          |               | 10000            |               |
| Test Run 5 |               | 20                   |               | 0.254                                  |          |               | 10000            |               |

| Specimen   | Strain Limit High (mm/mm) | Chord Modulus Strain Point 2 (mm/mm) | Chord Modulus Elongation Point 1 (mm) | Strain Point 03 (mm/mm) | Information 5 | Test ID | Test Rate (mm/min) | Secant Modulus Strain Point 2 (mm/mm) |
|------------|---------------------------|--------------------------------------|---------------------------------------|-------------------------|---------------|---------|--------------------|---------------------------------------|
| Test Run 3 | 10                        | 0.050                                | 0.254                                 | 0.050                   |               |         | 1.00               | 0.050                                 |
| Test Run 4 | 10                        | 0.050                                | 0.254                                 | 0.050                   |               |         | 1.00               | 0.050                                 |
| Test Run 5 | 10                        | 0.050                                | 0.254                                 | 0.050                   |               |         | 1.00               | 0.050                                 |

| Specimen   | Strain Point 05 (mm/mm) | Strain End Point (%) | Header 3 | Information 8 | Break Threshold (kN) | Information 1 | Information 4 | Strain Point 01 (mm/mm) |
|------------|-------------------------|----------------------|----------|---------------|----------------------|---------------|---------------|-------------------------|
| Test Run 3 | 0.050                   | 50.000               |          |               | 0.002                |               |               | 0.050                   |
| Test Run 4 | 0.050                   | 50.000               |          |               | 0.002                |               |               | 0.050                   |
| Test Run 5 | 0.050                   | 50.000               |          |               | 0.002                |               |               | 0.050                   |

| Specimen   | Platen Separation (mm) | Yield Offset (mm/mm) | Preload (N) | Slope Segment Length (%) | Outer Loop Rate (Hz) | Strain Limit Low (mm/mm) | Strain Point 07 (mm/mm) | Secant Modulus Strain Point 1 (mm/mm) |
|------------|------------------------|----------------------|-------------|--------------------------|----------------------|--------------------------|-------------------------|---------------------------------------|
| Test Run 3 | 25.500                 | 0.002                | 0.500       | 20.000                   | 100                  | -10                      | 0.050                   | 0.050                                 |



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Ankia Compression Test 25-Default Test Report

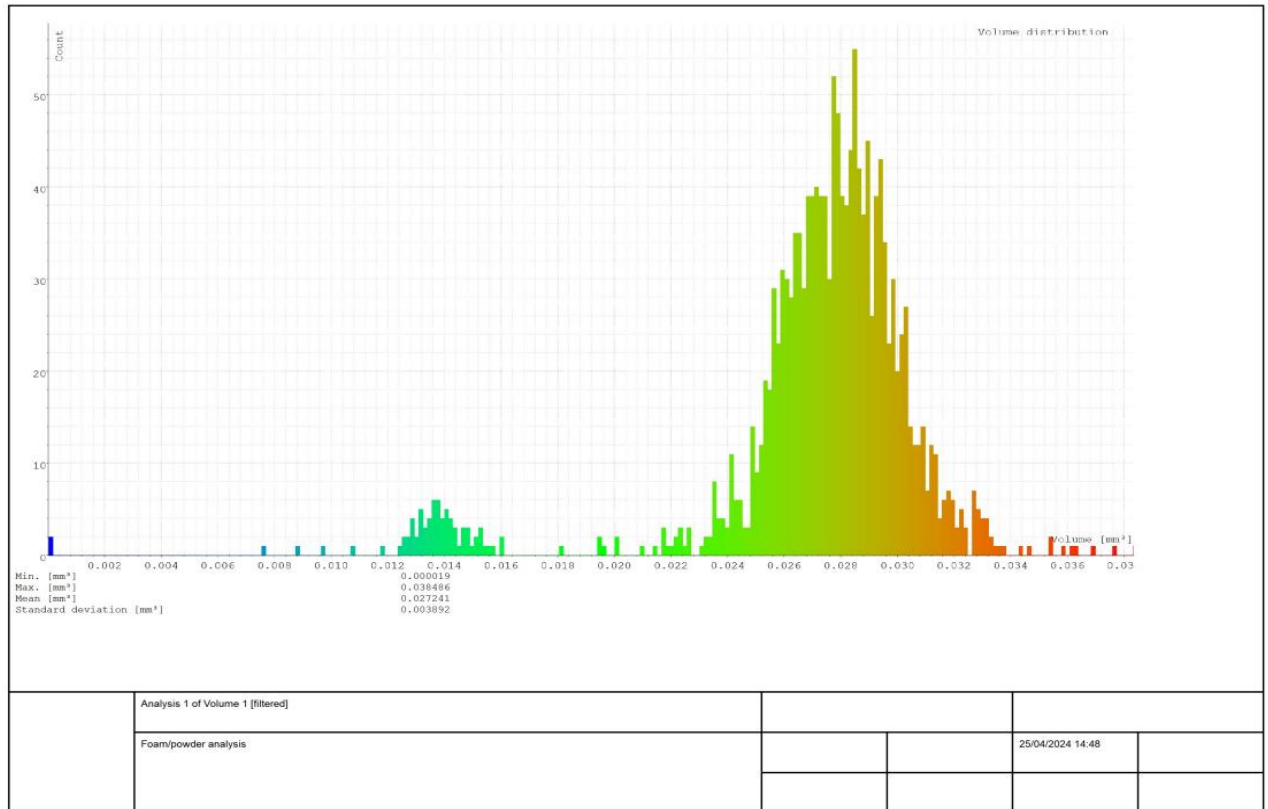
|            |        |       |       |        |     |     |       |       |
|------------|--------|-------|-------|--------|-----|-----|-------|-------|
| Test Run 4 | 25.500 | 0.002 | 0.500 | 20.000 | 100 | -10 | 0.050 | 0.050 |
| Test Run 5 | 25.500 | 0.002 | 0.500 | 20.000 | 100 | -10 | 0.050 | 0.050 |

| Specimen   | Extension<br>Limit Low<br>(mm) | Break<br>Marker<br>Elongatio<br>n (mm) | Load Limit<br>Low (kN) | Break<br>Drop (%) | Memo | Stress<br>Limit Low<br>(kN/mm <sup>2</sup> ) | Break<br>Sensitivity<br>(%) | Data<br>Acquisitio<br>n Rate<br>(Hz) |
|------------|--------------------------------|----------------------------------------|------------------------|-------------------|------|----------------------------------------------|-----------------------------|--------------------------------------|
| Test Run 3 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 5.0                                  |
| Test Run 4 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 5.0                                  |
| Test Run 5 | -508                           | 2.540                                  | -4                     | 50.0              |      | -0.2                                         | 90                          | 5.0                                  |

| Specimen   | Yield<br>Angle<br>(rad) | Preload<br>Speed<br>(mm/min) | Strain Point<br>08<br>(mm/mm) | Slack<br>Preload<br>(kN) | Chord<br>Modulus<br>Elongatio<br>n Point 2<br>(mm) | Strain<br>Point 02<br>(mm/mm) | Strain<br>Point 04<br>(mm/mm) | Extension<br>Limit High<br>(mm) |
|------------|-------------------------|------------------------------|-------------------------------|--------------------------|----------------------------------------------------|-------------------------------|-------------------------------|---------------------------------|
| Test Run 3 | 0.000                   | 3.000                        | 0.050                         | 0.0004                   | 12.700                                             | 0.050                         | 0.050                         | 508                             |
| Test Run 4 | 0.000                   | 3.000                        | 0.050                         | 0.0004                   | 12.700                                             | 0.050                         | 0.050                         | 508                             |
| Test Run 5 | 0.000                   | 3.000                        | 0.050                         | 0.0004                   | 12.700                                             | 0.050                         | 0.050                         | 508                             |

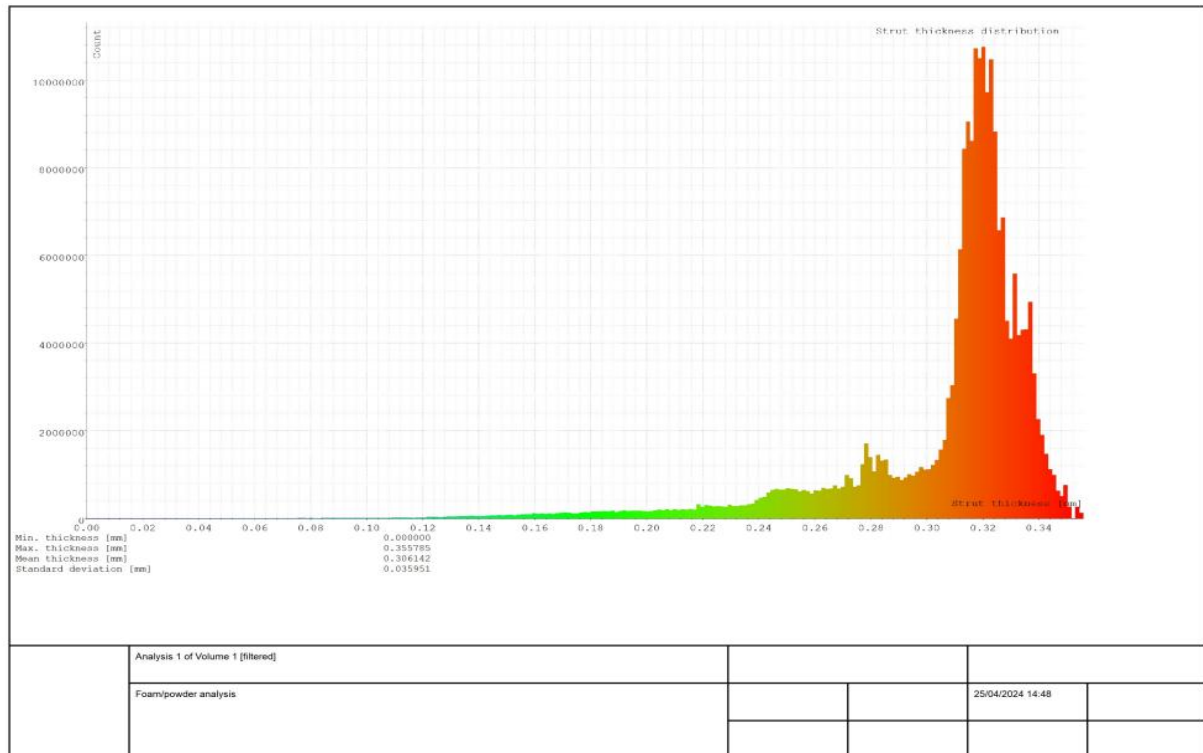
| Specimen   | Header 2 |
|------------|----------|
| Test Run 3 |          |
| Test Run 4 |          |
| Test Run 5 |          |

## Appendix D: CT scan analysis for HA480 diamond lattice volume distribution





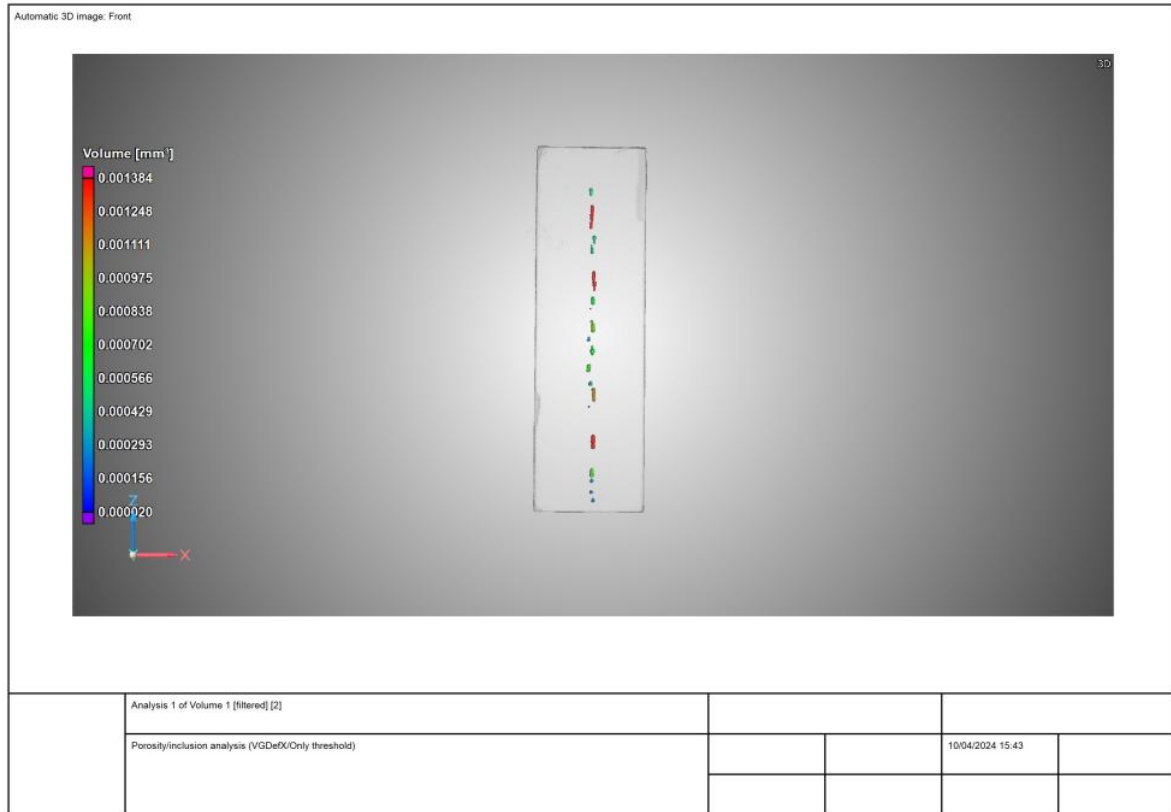
## Appendix E: CT scan analysis for diamond lattice strut thickness distribution



## Appendix F: CT scan analysis for solid HA480



## Appendix G: CT porosity analysis for solid HA480 perpendicular surface view



## Appendix H: Solid CT porosity analysis for solid HA480 top view

