MESOTHERAPY EPOREX TREATMENTS ON POST-OPERATIVE MAMMARY REDUCTION SCARRING

By:

TRACEY MAUD BOSHOFF

Dissertation submitted in fulfilment of the requirement for the degree

MAGISTER TECHNOLOGIAE: SOMATOLOGY

In the

DEPARTMENT OF HEALTH SCIENCES
FACULTY OF HEALTH AND ENVIRONMENTAL SCIENCES

At the

CENTRAL UNIVERSITY OF TECHNOLOGY, FREE STATE

2016

Supervisors: Dr. L. Botes
D. Tech (Biomedical Technology) (CUT)

Mrs. M. Vosloo
M. Tech (Somatology) (CUT)

DECLARATION OF INDEPENDENT WORK

I, TRACEY MAUD BOSHOFF (student number) hereby, declare that this research project submitted for the **Magister Technologiae: Somatology** degree in the **Faculty of Health and Environmental Sciences** at the **Central University of Technology, Free State,** is my own independent work and complies with the code of Academic integrity, as well as other relevant policies, procedures, rules and regulations and has not previously been submitted to any institution by myself or any other person in the fulfilment of the requirements for the attainment of any qualification.

SIGNATURE OF STUDENT

June 2016

DATE

ACKNOWLEDGEMENT

JESUS, *the name above all names, thank you for the strength, energy and peace that surpassed all understanding controlling my thoughts and feelings* (*Philippians 4:6-7*).

Furthermore, I would like to thank the following individuals whom contributed to this research study:

Dr. L. Botes, your wisdom and faith in me with every step of my dissertation is an appreciation I can't begin to explain. Thank you for your consistent professional support, inspiration and continuous contributions towards my research study.

Mrs. M. Vosloo, your support, time, positive assistance and for going beyond in your contribution to my research study, helping me create perfection.

Dr. P. Coetzer and the medical practice, for all their positive support, assistance and continued belief in my research study.

My **second assessor,** for being available, assessing to the best of her ability and for her added smile during each assessment.

All 30 **participants,** my study would never have started or ended without your dedication and commitment to the treatments. You have all made this study a great success.

Chris Boshoff, my amazing husband. Thank you for your motivation, understanding, love and seeing me through my lowest moments.

My sons **Christopher, Keanen and Brenden Boshoff** you never understood the process but your hugs and kisses on the odd moments, gave me new energy. You are all a blessing in my life. I love you all.

My **Mom, Dad, Wesley and Elaine Burmeister,** you were my secured support, always providing positive words and continuous prayers.

Leonie and Jurie Boshoff, thank you for your support with my children, you were my strength and my added encouragement when I felt I could not keep going.

My **Colleagues** you were my strong hold that kept me rational, I would not have made it without your messages and constant encouragement. **Guida van der Westhuizen** and **Juanita Jonker** your shoulders never missed a tear, thank you for positive words and being a blessing in my life.

Mrs. L. Potgieter for ensuring accurate statistics and for consistent patience and explanations and **Mr. S. van der Merwe,** for his assistance.

Mrs. S. Rossouw for your outstanding contribution towards my study and most of all your time.

Last but not least, the **Central University of Technology** for the opportunity to pursue such a study and for continued financial support.

EXECUTIVE SUMMARY

Background

Enlarged breasts are a major concern for women all over the world impacting them both physically and emotionally. Mammary reduction is a well-known surgical procedure but not without complications. Post-operative scarring continues to be a reality and a lingering concern for women and also a reason why women sometimes opt not to go for surgery. Women are continually seeking for new treatment modalities to improve scar appearance post-operatively. Mesotherapy Eporex treatments with active ingredients is well established for treating cellulite, wrinkles, muscle spasms and to firm skin, but lacks scientific evidence whether it could be effective to treat post-operative scarring. Therefore, the aim of the research study was to assess if Mesotherapy Eporex treatment with active ingredients could effectively reduce scar appearance after bilateral mammary reduction surgery.

Methods

A randomized, experimental design was used to assess the Mesotherapy Eporex treatment with active ingredients in thirty (30) female participants receiving mammary reduction surgery. Demographic- [age (years), ethnicity (W/B/C/A)] anthropometric- [weight (kg), height (cm) and Body Mass Index (BMI)] and clinical data [bra size (cup A-DD), ptosis (cm), concomitant medication, and amount of breast tissue removed (g)] were recorded from the patients' medical record. The Wise pattern with inferior pedicle surgical technique (inverted T) was used on all patients.

The Mesotherapy Eporex treatment with active ingredients (2ml *Asian Centella,* 5ml 20% Vitamin C and 5ml Hyaluronic Acid) commenced six (6) weeks after surgery. Thirty (30) participants were randomly divided into two (2) groups of 15 (fifteen) participants each. Group one (1) Mesotherapy Eporex treatment was performed on the right breast (experimental breast) and the left breast served as the control breast (not receiving

treatment) and vice versa for group two (2). Each participant received eight (8) Mesotherapy Eporex treatments on the experimental breast three (3) days apart. Scar appearance was assessed with the Vancouver Scar Scale (VSS) by the researcher, independent assessor (prior to treatment 1, 3, 5, 7 and after treatment 8) and plastic surgeon [prior to treatment one (1) and after treatment eight (8)]. The plastic surgeon and independent assessor was blinded and did not know which breast was treated.

Results and discussion

No statistical significant differences were observed for demographic, anthropometric and clinical data indicating that the sample population was comparable. No difference in healing ability was observed between the right experimental breast and left experimental breast (p=0.3288). Both, the independent assessor (-0.567) and plastic surgeon (-1.700) reported improvements in scar appearance when comparing the baseline VSS results with the endpoint VSS results after applying the Mesotherapy Eporex treatment (p=0.0002). Inter-observer reliability remains a problem due to the subjective nature of the VSS. The internal consistency of the VSS was lower than acceptable (Cronbach's alpha 0.4355). Emphasis was placed on the results of the independent assessor because assessments were done throughout the treatment period. The independent assessor reported a maximum of 11% or minimum of 0.6% improvement in scar appearance in the experimental group and a statistical significant improvement for scar pliability (95% CI: -3.88 ; -0.87) and height (95% CI: -2.58 ; -0.11). Improvement in scar appearance can be attributed to the Mesotherapy Eporex treatment with active ingredients (reduced inflammation due to deeper product penetration, promote collagen synthesis, restore tissue firmness, increase skin elasticity, decrease collagen stiffness and act as free radical scavengers).

The assessor (95% CI: -2.10; -0.16), ptosis (95% CI; 0.03; 0.33), and amount of right-sided breast tissue (95% CI: -0.01; -0.00) removed influenced the effectiveness of the Mesotherapy Eporex treatments (p=<0.05). The VSS is a subjective assessment tool explaining the influence of the assessor on the effectiveness of the treatment. Ptosis is an indication of the amount of breast tissue to be surgically removed and the more breast tissue removed the greater the chance of more aggressive scarring post-operatively.

Conclusion

In spite of the subjectivity and imprecision of the measurement system used, the effectiveness of the Mesotherapy Eporex treatment with active ingredients is not in question. There was a relative change in scar appearance over time in favour of the breast receiving Mesotherapy treatment at that time.

KEY WORDS: Female breast, Mammary reduction surgery, Healing, Scarring, Mesotherapy Eporex treatment, Vitamin C, Hyaluronic acid, *Asian Centella*.

TABLE OF CONTENTS

LIST OF APPENDICES 128

LIST OF FIGURES

CHAPTER 2 **PAGE**

APPENDICES — PAGE

LIST OF TABLES

CHAPTER 2 — PAGE

ABBREVIATIONS AND SYMBOLS

-	Not applicable
%	Percentage
○	Degree Celsius
Ass	Assessment
AS	Atrophic Scarring
BMI	Body Mass Index
CB/Con	Control Breast
CI	Confidence Interval
cm	Centimetre
Da	Dalton (unit of mass used for molecular weight)
DNA	Deoxyribonucleic acid
EB/Exp	Experimental Breast
FDA	Food and Drug Administration
g	Gram
GCP	Good Clinical Practice
HA	Hyaluronic Acid
HTS	Hypertrophic Scarring
kg	Kilograms
KS	Keloid Scarring
L	Left
m^2	Square Meter
MHz	Mega Hertz
ml	Millilitres

mm	Millimetre
MR	Mammary reduction
MSS	Manchester Scar Scale
n	Sample Size
nm	Newton Meters
O_2	Oxygen
OLS	Ordinary least squares
POSAS	Patient and Observer Scar Assessment Scale
QU	Quadrant
R	Right
RCOOT	Relative change observed overtime
SC	Stratum Corneum
SD	Striae Distensae
UK	United Kingdom
V	Volt
VSS	Vancouver Scar Scale
WeS	Wound evaluation Scale
Yrs	Years

CHAPTER 1

INTRODUCTION

1.1 Background

The visual appearance of female breasts differs considerably between individuals. These differences are associated with the amount of adipose tissue found below the breast tissue and the surrounding lobules. Women presenting with mammary hypertrophy, gigantomastia (extremely large breasts) and ptosis (nipple sagging) usually suffer psychological symptoms like stress, depression and feelings of shyness or embarrassment (Saariniemi *et al*., 2009), as well as physical discomfort including persistent neck, upper back and shoulder pain (Fernandes *et al*., 2007; Setälä *et al*., 2009; Sofianos *et al*., 2015). The goal of mammary reduction surgery is to improve breast weight-related symptoms while achieving acceptable breast shape and symmetry.

Mammary reduction surgery is the treatment method of choice for enlarged breasts and is one of the top 10 elective surgeries performed on both male and female patients worldwide. In 2010, 4645 adolescent girls underwent breast reduction surgery to treat macromastia or juvenile breast hyperthrophy (Crerand and Magee, 2013). In 2011 mammary reduction was one of the top ten reconstructive procedures in the UK (Draelos and Pugliese, 2011).The frequency of this surgery is observed in all racial groups, although it is predominant in African-Americans (Plastic Surgery Statistics Report, 2011).

However, mammary reduction surgery is not without complications or risks. Complications associated with mammary reduction surgery may include partial and superficial wound dishiscence, infection and sebsequent scarring (Setälä *et al*., 2009; Saleem and John, 2013). From an aesthetic perspective, post-operative scarring is most probably one of the major concerns for female patients receiving mammary reduction surgery (Monstrey *et al*., 2014; Purohit, 2008). According to Abu-Nab and Grunfeld (2007) scarring can be a major source of dissatisfaction after aesthetic breast surgery.

Besides surgical technique, numerous pre-operative risk fators can contribute to post-operative complications (Brown *et al*., 2012, Saleem and John, 2013; Pierpont *et al*., 2014). Age, obesity, the amount of breast tissue removed usually indicated by the degree of ptosis are variables considered by the plastic surgeon before opting to perform breast reduction surgery due to the impact on post-operative complications (Setälä *et al*., 2009; Ellsworth *et al*., 2009; Saleem and John, 2013; Hannson *et al*., 2014; Srinivasaiah *et al*., 2014).

To address patient dissatisfaction with regard to post-operative scarring several treatment modalities have been introduced namely; massages, silicone gels or plasters, creams/gels, peels, pressure garments, steroid injections, radiotherapy and camouflage makeup (Monstrey *et al*., 2014; Garg *et al*., 2014). Fortunate, for us as technology improves, new treatment techniques are developed.

Mesotherapy was introduced by a French physician, Dr. Michel Pistor, in 1952. A new modification of mesotherapy by which both ionised and neutral drugs can be transported into the dermis and subcutaneous tissue is known as no needle Mesotherapy (Sivagnanam, 2010). The Mesotherapy Eporex machine is used for both medicinal and aesthetic treatments and promotes the absorption of medicines, vitamins, minerals and amino acids. The treatment modality encourages the active gel to penetrate more deeply into the epidermis, dermis and hypodermis. Deeper active ingredient penetration is accomplished by applying a dual wavelength laser light and then four (4) components; electroporation, active current, hydrophoresis and cryophoresis (Latha and Vandana, 2011; Konda and Thappa, 2013). Although the application of the Mesotherapy Eporex treatment is well established for cellulite, wrinkles, muscle spasms and firming of skin (Konda and Thappa, 2013); its effectiveness to reduce the appearance of surgical scarring still needs to be scientifically investigated.

In this study Mesotherapy Eporex with active ingredients was selected as treatment method to reduce scar appearance after bilateral mammary reduction surgery by forcing active ingredients into the skin. The Mesotherapy Eporex treatment with active ingredients was applied over a period of one (1) month, with two (2) sessions weekly for a total of eight (8) treatments. The purpose of the active ingredients was to supply the skin with antioxidants and moisture, to aid in wound healing, promote formation of mature collagen fibres and to act as anti-inflammatory agents in an attempt to improve scar appearance both in texture

and colour. During and after the application of the Mesotherapy Eporex treatments the Vancouver scar scale (subjective assessment) was the scar rating scale used by the researcher (not blinded), independent assessor (blinded) and plastic surgeon (blinded) to assess scar appearance. The VSS assess four (4) skin characteristics namely pliability, vascularity, pigmentation and scar height.

1.2 Aim and objectives

1.2.1 Aim

The aim of the research study was to assess if Mesotherapy Eporex treatment with active ingredients could effectively reduce scar appearance after bilateral mammary reduction surgery.

1.2.2 Objectives

The objectives of the study were:

- To determine the effectiveness of the Mesotherapy Eporex treatment with active ingredients by comparing the scar appearance of the experimental breast (Mesotherapy Eporex treatment with active ingredients) with the control breast (no Mesotherapy Eporex treatment) using the VSS.

- To determine the percentage of improvement of scar appearance after applying the Mesotherapy treatment for each of the four (4) categories included in the VSS by subtracting the endpoint measurements [after treatment eight (8] from the baseline measurements [prior to treatment one (1)].

- To determine if scar appearance (wound healing) differ between the right and left breast by randomizing the application of the Mesotherapy Eporex treatment between the right and left breast.

- To determine if the amount of breast tissue removed, ptosis, age, and BMI influenced the effectiveness of the Mesotherapy Eporex treatment while accounting

for a possible difference between the assessors (plastic surgeon and independent assessor).

1.3 Dissertation Outline

Chapter 1 outlines the research problem concerning scar appearance after bilateral mammary reduction surgery, Mesotherapy Eporex treatment with active ingredients as a treatment option, and rationale for performing the research study.

Chapter 2 reviews the literature pertinent to the understanding of the anatomy and physiology of the female breast, developmental changes, bilateral mammary reduction surgery, complications associated with bilateral mammary reduction surgery with special emphasis on scar formation, treatment modalities used to reduce scar formation and assessment tools used to evaluate scarring. Chapter 2 also formed the basis for the research design outlined in Chapter 3.

Chapter 3 outlines the research design, data collection, special investigations, statistical analysis and ethical considerations applied in the research study to produce scientific data.

Chapter 4 presents results, starting with demographic, anthropometric and clinical data followed by VSS assessment results of scar appearance. The percentage of improvement for each skin characteristic included in the VSS after Mesotherapy Eporex treatment was evaluated as well as factors that might have an impact on the effectiveness of the treatment.

Chapter 5 is a discussion of the results.

Chapter 6 concludes the study with an account of the implications of the results obtained including the limitations of the study and possible recommendations for future studies.

Chapter 7 lists the references cited in the study.

Chapter 8 includes all the appendices comprising ethical clearance documents, Mesotherapy Eporex instruction manual, and photographs of surgical wound healing before and after Mesotherapy Eporex therapy.

CHAPTER 2

LITERATURE REVIEW

2.1 The mammary integumentary system

Human skin is composed of three heterogeneous layers: epidermis (outer layer), dermis (medial layer) and the hypodermis (inner layer) (Kolarsick *et al*., 2011;Sutradhar and Miller, 2013). The epidermal layer forms a protection barrier against injury, contamination and moisture loss. The epidermal layer is made up of epithelial cells that are continually displaced towards the surface to be shed, encouraging cell regeneration. Structures, such as hair follicles, sebaceous and sweat glands, start in the dermis and extend through to the epidermal surface. These structures assist with the bodies" heat and cold regulation without interrupting the epidermo-dermal junction. The second layer, known as the dermis, is a network of collagen and elastin fibres supplied by blood capillaries, which supports the epidermis (Kolarsick *et al*., 2011; McLafferty *et al*., 2012). The epidermal appendages found in the dermis provide strength to the skin (Verhaegen *et al*., 2012). Lying below the dermis is the hypodermis/subcutaneous fatty tissue layer which is comprised of nerve fibres, sensory organs, blood vessels, hair follicles and lymphatic glands (Sutradhar and Miller, 2013). Any damage to the dermal and hypodermal layers could result in skin stiffness. The stiffness is a result of collagen and elastic fibres getting orientated in the direction of stress, possibly interrupting the nerve and blood supply of the surgical area (Gauglitz *et al*., 2011; Son and Harijan, 2014).

Skin thickness varies across the human body ranging from thin skin on the eyelids to thick skin on the hands and feet. The thicker the dermis the higher the volume of collagen and elastin found in that specific area. The thickness of breast skin tissue ranges from 0.83mm to 2.35mm (Sutradhar and Miller, 2013). According to a Sutradhar and Miller (2013) skin in the medial breast region was thicker than skin in the lateral region of the breast, however no difference in thickness was found between the superior and inferior regions of the breast dermal layers. Due to the thicker skin on the medial side of the breast, compared to the lateral side, the elastic properties are higher which could contribute to an increased possibility for developing abnormal scarring in that region.

2.2 Anatomy and physiology of the female breast

In the female breast, dimension and mass can vary substantially between individuals (Gefen and Dilmoney, 2007). Anatomic variation can occur in volume, width, length, projections, shape, and positions on the chest wall (Avsar *et al*., 2010).

The breast is composed of glandular (secretory) and adipose (fatty) tissue supported by a loose framework of fibrous connective tissue called Cooper"s ligaments. The glandular tissue contains 15–20 lobes with lobules that contain 10-100 alveoli, each approximately 0.12mm in diameter, situated beneath the adipose tissue. Each breast lobe is considered to be a single entity (Kopans, 2007; Hassiotou and Geddes, 2013). Adipose fat is interspersed between and above the lobules providing support and contributing to the rounded contouring of the breasts.

The glandular tissue is drained by a ductal system that stores and transports milk to the nipple during lactation. The ductal system found in the breast is comprised of numerous small ductules that drain to the alveoli. The ductules merge to culminate in one main duct that dilates slightly to form a lactiferous sinus (2.0–4.5mm) (Hammond and Loffredo, 2012). The main duct then narrows at the „waist" before it passes through the nipple and connects at the centre of the nipple. The nipple is surrounded by a dark pigmented area known as the areola (Jesinger, 2014).The areola is composed of longitudinal and horizontal smooth muscle fibres adhering to the nipple base and is often aligned with the nipple ducts (Hassiotou and Geddes, 2013). The areola has oil glands which extend through to the surface of the skin to provide lubrication during breastfeeding (Jesinger, 2014).

The vascular anatomy of the breast includes the auxiliary artery, internal thoracic and intercostal arteries (Ellis and Mahadevan, 2013). The internal thoracic artery accounts for 60% of the total blood supply to the breast. This thoracic artery supplies rich oxygenated blood to the breasts supporting various reduction techniques without the risk of nipple and areolar (loose connective tissue) necrosis (Gabriel, 2011). The motion/bounce of the female breast is the result of the elasticity of connective tissue fibres present within the breast tissue itself (Hassiotou and Geddes, 2013).

2.3 Breast developmental stages

Breast development is split into the following stages: foetal growth, pubertal expansion, pregnancy and post-menopausal (Geddes, 2007). The time course of breast development initiates in the foundation phase, with mammary buds developing during embryonic life, it continues with minimal growth during infancy, and is followed by a rapid growth phase at puberty (Hassiotou and Geddes, 2013).

When a female reaches puberty, hormones including oestrogen, progesterone, prolactin, insulin, thyroxine and growth hormones, are produced in moderation and may lead to the development of enlarged breast tissue (Javed and Lteif, 2013). The rapid breast growth is driven by the ovulation cycle and the body"s pursuit to establish a regular menstrual cycle. The sudden increase in breast size can be attributed to an increase of adipose tissue within the mammary gland (Hassiotou and Geddes, 2013). When breast volume increases beyond physical comfort, it is not uncommon for women to become self-conscious about their breasts, leading to social withdrawal, and thus hindering the development of important social skills (Saariniemi *et al*., 2009) Furthermore, women with large breasts prior to pregnancy experience further breast tissue enlargement due to the release of pregnancy hormones (Hassiotou and Geddes, 2013).

2.3.1 Factors influencing mammary gland development

Various factors can influence mammary gland development such as age and BMI, hormonal changes, pregnancy and menopause (Mansel *et al*., 2009; Wade *et al*., 2010; Brown *et al*., 2012; Wolfswinkel *et al*., 2013). Women presenting with macromastia (breast hypertrophy) during menopause, due to hormonal changes, also experience weight gain. It is common for the breasts to enlarge as the weight of a patient increases (Hammond, 2009). A patient"s body mass index (BMI) is of great importance when considering a patient for breast reduction surgery. If the patient has a BMI of >35kg/m^2, the patient is encouraged to lose weight and maintain the loss for six (6) months to one (1) year before mammary reduction surgery is performed. This approach ensures that unexpected weight changes do not influence the post-operative size and shape of the breast (Hammond, 2009; Solorzano and McCartney, 2010).

During the pregnancy and lactation cycle the mammary gland undergoes complete remodelling, maturating into a functional milk secretory organ (Hassiotou and Geddes, 2013). After pregnancy, women usually experience an increase in breast size due to breastfeeding leading to the development of post-lactation ptosis (drooping of the nipple) (Purohit, 2008; Rinker *et al*., 2008). Therefore, women are more likely to consider mammary reduction surgery, due to enlarged breasts, after pregnancy. Once the pregnancy and lactation stage is complete the mammary alveolar cells clear, encouraging the breasts to regress into non-functional organs until the next pregnancy and lactation stage. However, if a subsequent pregnancy does not occur, the glandular epithelium regresses and adjacent connective tissue is gradually replaced by fat. This process continues into menopause (Hassiotou and Geddes, 2013).

Post-menopausal mammary changes are associated with ovarian functional decay and are characterized by the reduction of glandular breast tissue and an increase in the surrounding adipose tissue (Hassiotou and Geddes, 2013; Savolainen-Peltonen *et al*., 2014). Post-menopausal breasts may be heavy and large, leading to physical breast abnormalities (hypertrophy, gigantomastia and ptosis). Women who experience secondary enlargement, due to fatty infiltration of their breasts after menopause, usually require the removal of more than 1800g of adipose tissue (per breast) during mammary reduction surgery (Purohit, 2008).

2.4 History of mammary reduction surgery

Mammary reduction surgery was developed by Theodor Billroth from 1869 to 1876 followed by Alfred Pousson in 1897. Billroth and Pousson believed that removing the entire breast rather than performing a reduction was the procedure of choice (Purohit, 2008). However, during the late 19th century, the “natural breast” concept was introduced by Galliard to salvage part of the breast tissue, and thus mammary reduction went from reconstructive surgery to aesthetic surgery (Shiffman, 2010). In 1928, Biesenbergers performed breast reduction surgery on patients by separating skin from the lateral half of the breast gland and transporting the nipple to markings made in line with the inframammary crease. Biesenbergers‟ mammary reduction technique is still the procedure of choice to create proportionate, youthful-looking breasts with minimal scarring (Saleem and John, 2013). In 1956 Dr RJ Wise used Biesenbergers mammary reduction technique which included:

a) The separation of the skin from the mammary gland.
b) Resection of the lateral half of the mammary gland.
c) The transposition of the nipple on the retained gland (Purohit, 2008).

However, Wise modified the technique through the development of improved excision techniques, hence the Wise pattern technique (Figure 1.2). Robbins (1977), Courtiss (1977) and Goldwyn (1977) contributed to the development of the inferior pedicle technique, which aimed to reduce visible scarring (Purohit, 2008; Shiffman, 2010).

2.4.1 Applications for mammary reduction surgery

Mammary reduction (MR) surgery is a common cosmetic and oncological surgical procedure not only restricted to bringing down the size of the breast proportionate to the build of the individual, but also to overcome the discomfort caused by massive, ill-shaped and hanging breasts. Through the years MR surgery has evolved from mere reduction of breast mass to the enhancement of aesthetic appeal producing minimum scar load (Saleem and John, 2013; Sofianos *et al*., 2015).

Breast size are reduced by surgically removing excess glandular, skin and adipose tissue in order to improve the patient‟s psychological, aesthetical and physical wellbeing (Crerand and Magee, 2013).

Mammary reduction surgery aims to modify the breasts size to be in proportion to the patient‟s body structure, be symmetrical, and retain the breasts‟ rounded and natural shape with excellent nipple projection, without losing blood supply to the nipple. Therefore, the goal of mammary reduction surgery is once-off surgery, with the preservation of breast function and minimal scarring (Copcu, 2009; Saleem and John, 2013).

The most frequent causes associated with requests for mammary reduction surgery are medical, namely increased breast size, weight, discomfort or gravitational force (Karaaslan *et al*., 2013). These causes are usually associated with complications such as backache (cervical and upper thoracic back discomfort), neck pain causing headaches, skin irritation/rashes, poor posture, shortness of breath (dyspnoea), shoulder grooves and the inability to exercise (Table 2.1) (Fernandes *et al*., 2007; Setälä *et al*., 2009; Sofianos *et al*.,

2015; Strong and Hall-Findlay, 2015). Breast reduction surgery can also result in physiological benefits for the patients which include improvements in self-esteem and depression (Cerand and Magree, 2013).

Table 2.1 Complications associated with mammary hypertrophy

Complication	Description
Back and neck pain	Discomfort and pain in the back muscles are the result of the surrounding muscles (upper back and chest) not being strong enough to support the weight of the breasts. Without the support of the surrounding muscles, the shoulders automatically roll forward causing stress on the back and neck, as well as compression on the thoracic vertebrae"s and thereby exaggerating a natural thoracic lordosis. This poor posture creates a snow ball effect, since the larger the breasts, the more pressure is exerted on the vertebrae, often leading to compressed nerve fibers causing discomfort, pain and headaches (Fernandes *et al*., 2007;Karaaslan *et al*., 2015).
Skin irritation/rashes	Skin irritations and rashes are caused by trapped moisture and body heat generated underneath and in-between the breasts. The moisture can encourage fungal infection development resulting in personal hygiene concerns (Mistiaen and Van Halm-Walters, 2010; Wolfswinkel *et al*., 2013).
Poor posture	Poor posture is associated with the excess weight of the breasts and the poor strength of the surrounding muscles (Lapid *et al*., 2013; Sahin *et al*., 2013).
Dyspnea (shortness of breath)	The large amount of breast tissue may exert pressure on the thoracic cavity resulting in shortness of breath (Nafae *et al*., 2013).
Shoulder grooving	Patients tend to wear smaller sized bras due to a lack of knowledge regarding proper bra fitment, for example, a patient requiring an F size bra chooses to wear a DD sized bra. Thinly sized shoulder straps pull down into the shoulders resulting in wheals on the shoulders (Radosa *et al*., 2013; Coltman *et al*., 2015).
Inability to exercise	The larger the breasts, the more difficult it is to exercise. The inability to exercise may potentially encourage inactivity and obesity (Scurr *et al*., 2016). Thus, the muscles are weaker and more problems are experienced with posture and back/neck pain.

2.4.2 Mammary reduction surgery

Numerous operative techniques are available for mammary reduction surgery and the choice of technique depends on the surgeon"s preference and estimated resection volume (Nelson *et al*., 2008; Wong *et al*., 2014). To qualify for breast reduction surgery the minimum amount of breast tissue that has to be removed is 500g or more per breast (depending on health insurance plans and countries). The estimated volume of breast tissue to be removed can be determined using the Schnur scale, which utilizes the body surface area, determined by height and weight (Wolfswinkel *et al*., 2013). Other methods used by surgeon"s to estimate the amount of breast tissue remove during surgery includes; water displacement and the Grossman-Roudner device, mammograms, ultrasound, magnetic resonance imaging, three-dimensional computed tomography and three-dimensional photography. Furthermore, anthropometric measurements have also been used to estimate resection amounts (Murray *et al*., 2008; Kececi and Sir, 2014).

Sternal notch-to-nipple distance (ptosis) is one way to assist plastic surgeons preoperatively, to estimate the amount of breast tissue removed during surgery (Hernanz *et al*., 2014; Ikander *et al*., 2014). Breast ptosis was originally categorized by Regnault (1976) and is classified based on the position of the nipple relative to the inframammary fold and the breast base in the standing position (Liu, 2009). These objective measurements are categorized according to grade 0-3. Grade 0 signifies no ptosis, in which the nipple lies above the inframammary fold. Grade I is known as mild ptosis that occurs when the nipple is at the same level or up to 1cm below the inframammary fold. Grade II (moderate) ptosis refers to when the nipple falls 1 to 3cm below the inframammary fold. Grade III (severe) ptosis becomes apparent when the nipple falls 3cm below the inframammary fold (Liu, 2009; Andrades and Prado., 2007).

The patients" requirements, ptosis degree, breast size, skin and gland quality, including the surgeons experience and preferences are taken into consideration when deciding on the appropriate pedicle technique for the nipple areola in reduction mammaplasty (Murray *et al*., 2008; Castro *et al*., 2013). Grade 1 ptosis would be best to utilize a superior based pedicle. Moderate to severe ptosis requires greater elevation of the nipple-areola complex and larger amounts of skin need to be excised. Severe breast hypertrophy and a type III

ptosis, a safer pedicle is recommended (inverted T resection with an inferior pedicle) in spite of a longer scar (Andrades and Prado. 2007).

Table 2.2 Selecting the breast reduction technique based on hypertrophy and ptosis degree (Andrades and Prado, 2007)

		PTOSIS		
		Mild	Moderate	Severe
HYPERTROPHY	Mild (< 500g)	Shorter Scars Upper pole based pedicles		
	Moderate (500g-1000g)			
	Severe (>1000g)			Longer Scars Lower pole based pedicles

2.4.3 Surgical procedures/techniques used during mammary reduction surgery

Mammary reduction surgery entails two technical procedures, namely resection and the placement of the pedicle to retain the nipple areola (Purohit, 2008; Saleem and John, 2013). A skin resection pattern is the removal of skin, whereas a pedicle placement refers to a technique developed to ensure continuous blood supply to the nipple by maintaining its connection to the underlying breast tissue. An inferior pedicle is mostly associated with an inverted T skin resection whereas a superior pedicle is associated with a vertical skin resection (Purohit, 2008; Wong *et al*., 2014).

2.4.3.1 Placement of pedicles

Skin resection patterns can be combined with most pedicles (Purohit, 2008; Wong *et al*., 2014). Pedicle techniques include the inferior, superior, central, lateral and lastly the medial pedicle technique (Figure 2.1). Pedicles situated around the breast, are named according to their position on the breast, and are used to maintain blood supply to the nipple areola complex (Andrades and Prado, 2008). According to Saleem and John (2013), the inferior

pedicle technique is reproducible across a range of breast sizes with varying ptosis. Utilizing this technique allows easy access to the different breast quadrants, precision in shaping of the breast, and the retention of parenchyma, including the skin envelope (Saleem and John, 2013).

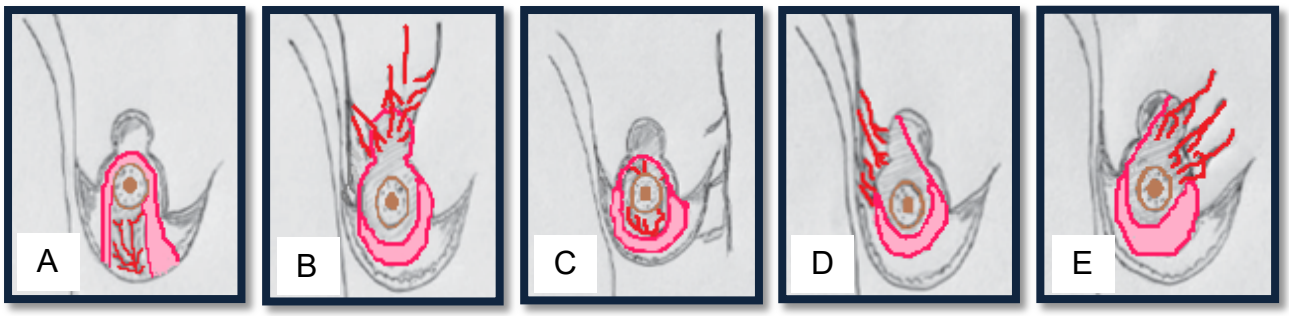


Figure 2.1 **Blood supply of pedicles** *(A) blood supply of the inferior pedicle (B) blood supply of the superior pedicle (C) blood supply of the central pedicle (D) blood supply of the lateral pedicle (E) blood supply of the medial pedicle* (Purohit, 2008)

2.4.3.2 Skin resection patterns

Skin resection patterns include the following techniques: inverted T resection (Wise pattern), vertical resection, circumareolar resection and lateral skin resection (Purohit, 2008; Wong *et al*., 2014).

Plastic surgeons seem to prefer the Wise pattern (inverted T resection) approach with an inferior pedicle technique (69%), also referred to as a keyhole pattern (Chopra *et al*., 2013). The Wise pattern technique removes glandular tissue and excess skin, and includes nipple transportation (Purohit, 2008). The Wise pattern (inverted T resection) with an inferior pedicle technique facilitates wide access to the breast parenchyma, and is thus effective for a variety of breast reduction sizes, including unusually large breasts and patients with excess skin due to substantial weight loss (Cutress *et al*., 2013; Wong *et al*., 2014). In addition, this technique yields predictable results, including the preservation of nipple sensitivity (Chopra *et al*., 2013).

In this research study, emphasis will be placed on the Wise pattern with an inferior pedicle technique for this is the surgical methods of choice used by the plastic surgeon when performing bilateral mammary reduction surgery.

2.4.4 The Wise pattern with inferior pedicle surgical technique used for mammary reduction surgery

2.4.4.1 Marking the skin resection pattern

Enlarged breasts tend to sag as one ages often causing breasts to sag past the inframammary fold, adding to nipple ptosis. The ideal anatomical nipple height is 21cm from the sternum-bone notch to the nipple (Khan and Bayat, 2008). Therefore, a plastic surgeon makes measured markings on a patient‟s breasts before surgery commences. The markings determines the amount of skin and breast tissue to be resected as well as breast asymmetry (Barbosa *et al*., 2010).

Prior to mammary reduction surgery the patient is placed in an upright position and the suprasternal notch and midclavicular points are marked. A vertical line is drawn between the suprasternal point and the xiphoid. The proposed nipple level is marked according to the inframammary crease using the finger method; referred to as point A (Figure 2.2) (Moio and Schonauer, 2015). Secondly, the vertical limb angles are marked by placing the thumb and index finger 6.5cm equally adjacent to the nipple in a pinching motion to create marking B (lateral) and C (medial), which will assist in determining the volume of resection required. The medial (E) and lateral (D) limits of resection are marked on the breast by displacing the gland medially and laterally in the opposite directions (Figure 2.2) (Purohit, 2008). The breast is elevated, the inframammary crease is marked and the point where the inframammary crease joins the breast is marked F. Markings of point C to E, and B to D are then connected with straight lines. The medial and lateral lines should be adjusted so that they are equidistant from the breast meridian and marking F to ensure equal distance between the nipple height and inframammary crease (Cutress *et al*., 2013).

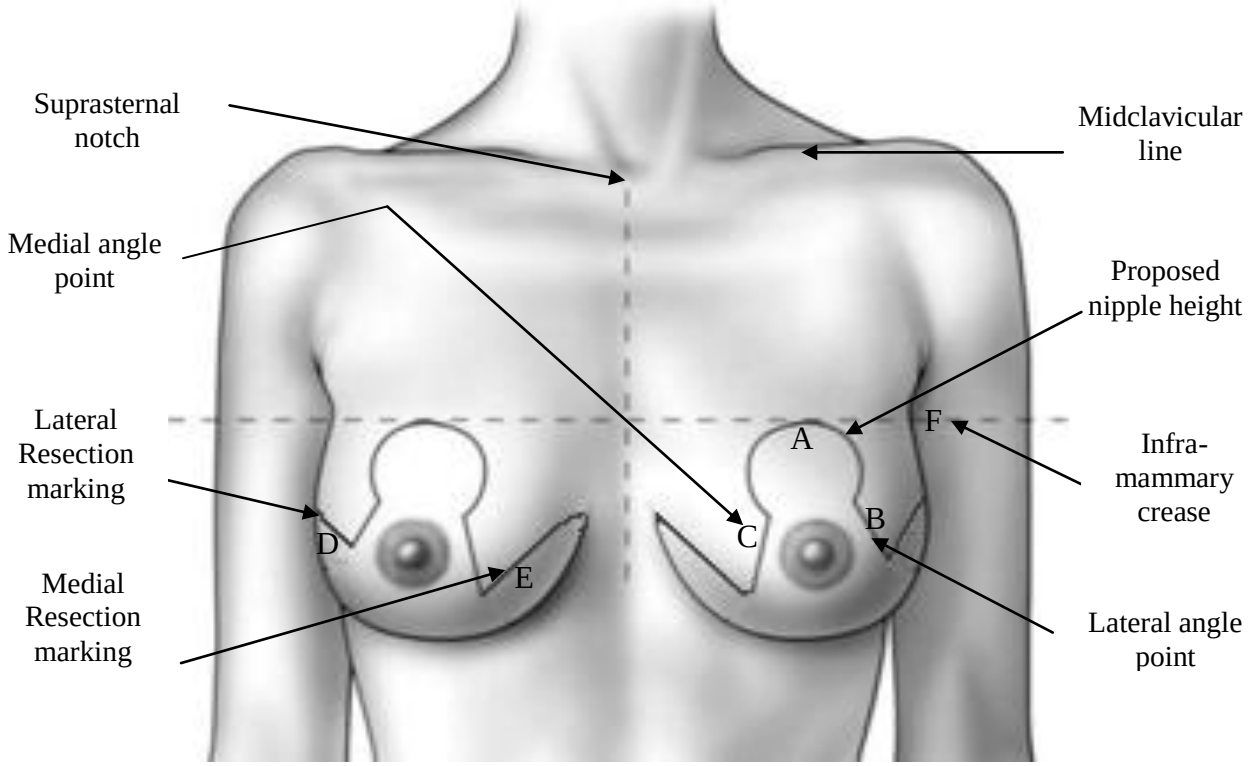


Figure 2.2 Markings of the inferior skin resection pattern (Jatoi *et al*., 2006)

2.4.4.2 Inverted T resection technique with inferior pedicle

The term inferior pedicle refers to an inverted U-shaped incision based at the inframammary fold, moving up and around the nipple area. The pedicle is 6-8cm wide, is centred on the breast meridian, and extends for about 2cm above the nipple areola complex. The areola is stretched and by using a cookie cutter, a marking is made around the areola. The marking is about 3.5 to 4.5cm in diameter and is incised into the dermis. The inferior pedicle skin is de-epithelialized (Figure 2.3 and 2.4 A). Incisions are then made on the lateral and medial sides of the breast. The superior flaps are thinned out to achieve the natural breast shape (Figure 2.4 B). The Wise pattern with inferior pedicle surgical technique (inverted T) is used to keep the nipple “alive”, keeping it connected to the arterial and venous blood supply, thus maintaining circulation and sensation (Purohit, 2008; Khondoker and Ahmed, 2012).

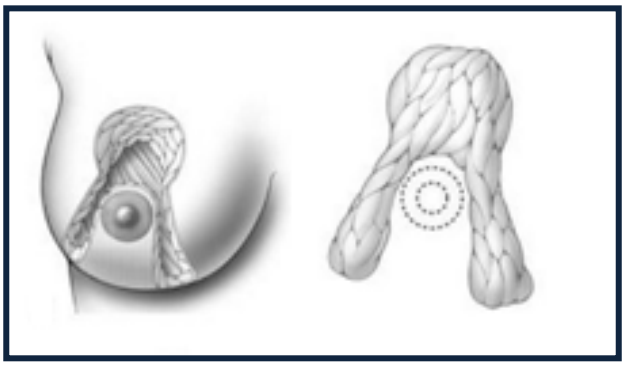


Figure 2.3 **De-epithelialized skin of the inferior pedicle operative technique** (Jatoi *et al*., 2006)

The superior flaps (Figure 2.4 B, points B and C) are pulled towards each other and one suture is inserted to ensure an adequate opening for the „new" nipple areola complex (Figure 2.4 C). Once the nipple areola complex is in position it is sutured, following the vertical line down to the inframammary fold, using continuous intra-dermal sutures and sterri-strips, creating an inverted T (Figure 2.5C) (Purohit, 2008; DeGeorge *et al*., 2013).

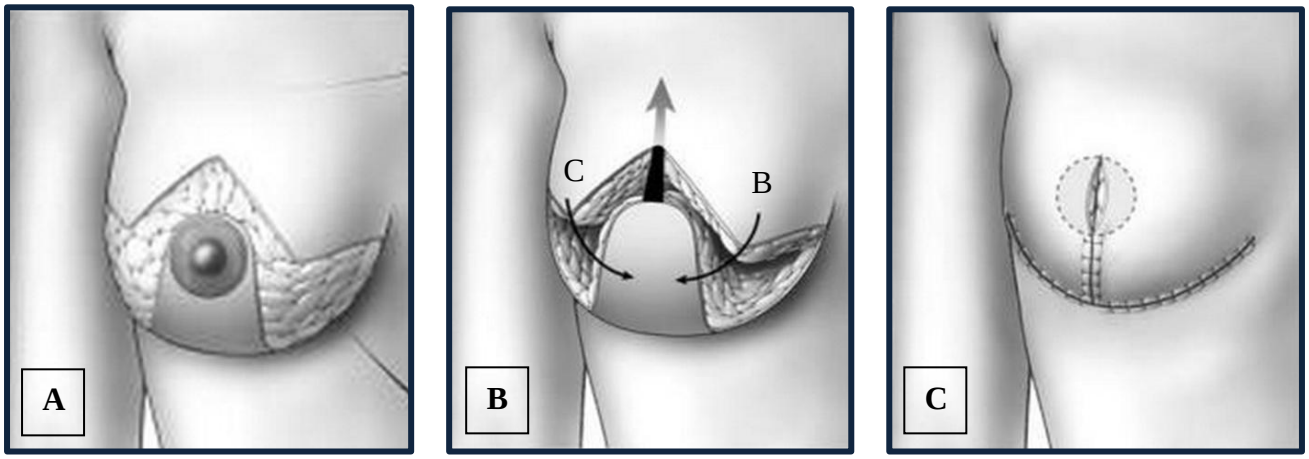


Figure 2.4 **Wise pattern with inferior pedicle operative technique** (A) De-epithelialized skin (B) B and C pulled together (C) One surture to ensure adequate opening for nipple areola complex (Jatoi *et al*., 2006)

2.4.4.3 Advantages of the Wise pattern inferior pedicle technique

The Wise pattern inferior pedicle technique can be used for all breast sizes and for varying degrees of ptosis (Saleem and John, 2013). The technique ensures sufficient blood supply to the nipple areola complex, promoting good circulation and retains both the fine sensation in the area and the possibility of breast-feeding after mammary reduction surgery (Wong *et al*., 2014).

2.4.4.4 Disadvantages of the Wise pattern inferior pedicle technique

The Wise pattern inferior pedicle technique may result in “dog ears” being created during the suturing process. “Dog ears” are prominent lateral bulges on both ends of the transverse scar (Weissman *et al*., 2014; Hansen, 2016). Other disadvantages associated with the technique are, a hypo-pigmented patch of the nipple, delayed healing and webbing (no cleavage/breast separation) of the pre-sternal region of the breast. The webbing of the pre-sternal region includes damage to the peripheral nervous system, intercostal nerves and the thoracic spinal nerves. The nerve damage leads to a loss of sensation in the breast area (Saleem and John, 2013). Hypertrophic and keloid scarring are both associated with mammary reduction surgery (thus the Wise pattern with an inferior pedicle technique may result in a long unsightly scar running along the inverted incision) (Hansen, 2016).

2.5 Complications associated with mammary reduction surgery

Complications and risks associated with mammary reduction surgery include, bleeding, in conjunction with or without, hematoma formation, breast sensitivity accompanied by breast numbness, delayed wound healing, breast asymmetry, hyper-pigmentation, fat and tissue necrosis, infection and scarring irregularities (Baker *et al*., 2009; Saleem and John, 2013; Weissman *et al*., 2014). The possibility of a bacterial infection at the incision site of the breast exists, and such an infection could contribute to swelling, redness, pus discharge and pain (Purohit, 2008; Nezhadhoseini *et al*., 2015).

2.5.1 Bleeding with or without the formation of a hematoma

Bleeding during surgery increases the risk of a patient receiving a blood transfusion whereas bleeding after surgery causes the breast to swell, turn purple/blue and slow down the healing process (Hardy *et al*., 2008). Swelling due to bleeding usually occurs within the first 24 hours after surgery and often requires a second surgery to drain and stop the bleeding in the breast. Extensive swelling prevents blood perfusion to the skin causing oxygen (O_2) delivery to the skin to be compromised. The lack of O_2 may result in complications such as infection, localized death of skin cells (necrosis) and wound separation (Pierpont *et al*., 2014).

Excessive bleeding from broken blood vessels can disrupt wound healing causing the formation of a hematoma (which is a form of bruising). Hematomas are identified by the acute asymmetry of the breast, presenting with pain, and can occur up to nine (9) days post-operatively (Seth and Kim, 2010). Once a hematoma is detected it is important to remove it as the hematoma separates healthy tissue from the blood supply, creating a medium for bacterial growth (Saleem and John, 2013).

2.5.2 Loss of sensation/sensitivity and numbness

Loss of sensation and numbness in the breast area are experienced by many patients after mammary reduction surgery (von Sperling *et al*., 2011). The loss of sensation could be permanent, especially in the areola area, as this is where the nerves converge. However, normal sensation (dysaesthesia) in the nipple and the surrounding tissue could return within a few months or up to a year after the surgery. The sensation may return once the nervous system has healed the synapses connections between the nerve endings (Saleem and John, 2013).

2.5.3 Infection and wound healing complications

Infection and poor wound healing can occur at the junction of the two scar lines that join the medial and lateral flaps with the inframammary fold (inverted T). This complication is more frequent when the flaps are excessively thinned (Saleem and John, 2013). Poor wound healing due to compromised blood supply frequently occurs in patients with an elevated BMI (>25kg/m^2), diabetes and/or myocardial injury (Setälä *et al*., 2009). Although patients are scrubbed with an antimicrobial solution prior to the surgical procedure to prevent bacterial infections, infections still occur. If infection occurs due to compromised blood supply at the injured site, it can contribute to excessive scar formation (Garvey *et al*., 2006).

2.5.4 Breast asymmetry

Breast symmetry is considered important for both emotional and physical self-acceptance and for spousal acceptance (Khondoker and Ahmed, 2012). Surgical procedures where large amounts of breast tissue are removed are usually accompanied by the risk of breast distortion (one breast being higher than the other), as well as a height difference in the

positioning of the nipples (Garcia, 2016). Patients with extremely large breasts are made aware of the risk of asymmetrical breasts after surgery and the possibility of a second operation in an attempt to restore breast symmetry. However, the number of surgical interventions increases the likelihood of more severe scarring (Purohit, 2008).

2.5.5 Hypo-pigmentation

Hypo-pigmentation can be seen as a surgical complication linked to the operative technique used by the plastic surgeon (DeGeorge *et al*., 2013). After surgery or trauma to a patient"s mammary gland, inflammation can cause the melanin (skin pigment) production to be reduced or damaged causing hypo-pigmentation (Figure 2.10) (Vachiramon and Thadanipon, 2011).

2.5.6 Fat and tissue necrosis

Fat necrosis usually results from surgical trauma and is recognized as a sterile inflammatory process in which fat filled macrophages are surrounded by interstitial infiltration of plasma cells (Tan *et al*., 2006) . Necrosis can present as single or multiple smooth, round firm nodules/masses associated with pain, erythema, inflammation and skin thickening. The destruction of the intra-cellular framework of the breast causes extensive cell death, resulting in necrosis. Nipple necrosis is a dreaded complication (Handel and Yegiyants, 2016) emanating from the decreased vascularity of either the skin flaps or the pedicle in which the nipple areola complex is based. Nipple necrosis can be avoided by ensuring that the pedicle of choice has been incised in a pyramidal shape (Saleem and John, 2013). It is important to note that all the complications mentioned above can contribute to, or worsen, scarring after mammary reduction surgery.

2.6 Scar formation

Scarring is classified as either normal or abnormal, depending on the patient"s lifestyle, medical history, healing ability, genetics, incision depth, length and location of the surgery performed (Hess, 2011; Son and Harijan, 2014). Scarring is the body"s natural healing mechanism, creating a “plaster” at the site of injury. Surgery or any physical trauma to the skin could result in scar formation. Some scars heal quickly while others may take many

years to heal (Kaartinen *et al*., 2011). Most mammary reduction patients can expected scarring after surgery (Monstrey *et al*., 2014). Scarring can occur in all ethnic groups, presenting more often in African Americans, Hispanic and Asian ethnic groups than in Caucasian skin (Bian *et al*., 2013;). In some cultures scars are seen as being symbolic of heroism, for others the symbolism is religious, and for many patients scars are a reminder of hostile events (Oultram, 2009).

2.6.1 Normal versus abnormal scarring

Different types of scars can develop from an injury, and the scars are classified as normal or abnormal. Normal scarring is the result of an uncomplicated healing process and presents as a flat, unrecognizable scar in contrast to the surrounding skin. Injuries penetrating only the epidermis will heal without a resulting scar. Healing of a normal scar usually takes 24 hours once the basal cell layer in injured healthy skin begin to proliferate, re-creating an intact epidermal layer (Hill and Pickart, 2009). Abnormal scarring develops when the healing process of a wound does not follow the normal process. An inflamed wound could take longer to heal due to an extended inflammatory phase. Abnormal scarring includes atrophic, hypertrophic and keloid scarring (Son and Harijan, 2014; Gozali *et al*., 2015). It is attributed to delayed epithelisation in areas of high tension for example the sternal regions, deltoid and other areas of movement (Penn *et al*., 2012).

Scars, in the early wound healing stages, are classified as being red, swollen and sensitive, and over time, as the redness dissipates exposing the true colour of the scar, as darkened (hyper-pigmentation) or lightened (hypo-pigmentation) (Chadwick *et al*., 2012). Scar tissue has reduced hydration (amount of moisture) and less elasticity than the surrounding skin and is identified by its colour, texture and contours (Monstrey *et al*., 2014). Damage to the skin encourages the skin"s protection mechanism to respond by thickening and stimulating elastin and excess collagen production, causing abnormal scarring (Hill and Pickart, 2009).

2.6.2 The pathophysiology of scarring

A wound is a disturbance of normal tissue that occurs from either physical or pathological processes (Hill and Pickart, 2009), but luckily with damage comes repair. Healing is a process by which the damaged tissue repairs itself to join the surrounding healthy tissue of

the body through three phases known as the inflammatory, proliferative and remodelling phases (Figure 2.5) (Reinke and Sorg, 2012).

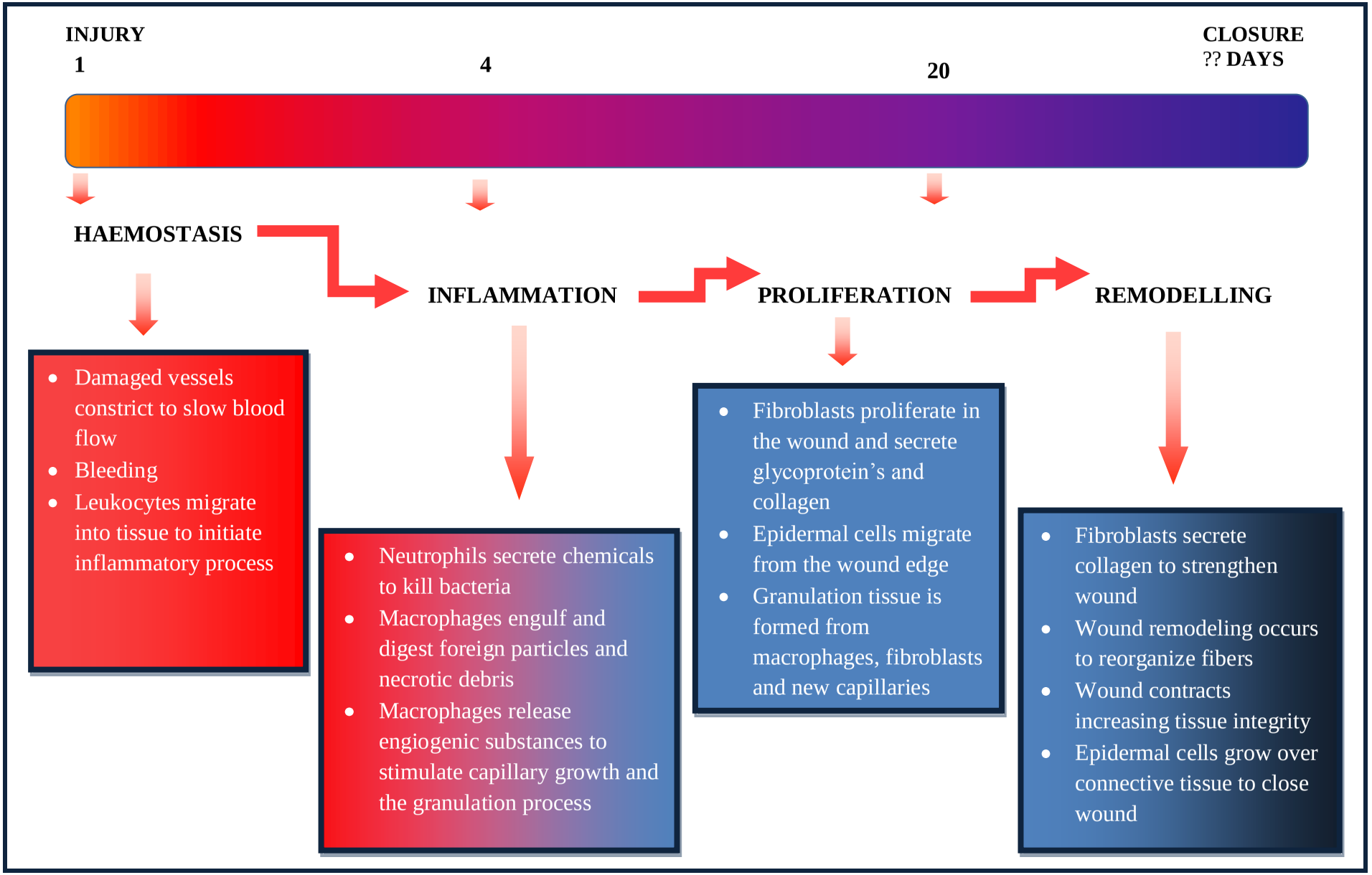


Figure 2.5 **Schematic representation of the phases of wound healing** (Young and McNaught, 2011)

In order to understand the inflammatory, proliferative and remodelling phases, a basic knowledge regarding the skin layers, their function and the effect they have on a wound, is required (Table 2.3).

Table 2.3 Skin structure, function and effects on wound healing (Stein and Küchler, 2013)

SKIN STRUCTURE AND FUNCTION		
LAYER	**FUNCTION**	**EFFECTS ON WOUND**
EPIDERMIS		
Epithelial cells – arise from basal cell layer, shed as flattened nuclear squamous	• Barrier to injury, contamination and moisture loss	• Allows infection, water loss, and tissue desiccation
Melanocytes – neural crest cells producing melanin	• Protect against UV light; responsible for skin pigmentation and tanning	• Patchy alteration in skin color
DERMIS		
Collagen – protein, a major constituent	• Strength and support	• Strength of repair depends on amount and quality of collagen, Altered appearance
Elastin – protein	• Elasticity	• Reduced amount in scar tissue which is inelastic
Nerves	• Detects pain, temperature, touch, position, vibration • Provide information on environment, protection	• Nerve damage causes loss of sensation leading to increased susceptibility to injury
Capillaries – dense network supplied from hypodermis	• Provide supply of nutrients and oxygen and remove waste products	• Form major component of granulation tissue
EPIDERMAL APPENDAGES		
Hair follicles – lined by epidermal cells	• Produce hair • Insulation/thermoregulation increase sensitivity of skin especially to light touch	• Unaffected by superficial damage. Destroyed by full thickness damage, leading to hair loss. Source of epidermal cells in partial thickness wounds
Sweat glands	• Produce sweat • Thermoregulation	• Unaffected by superficial damage. Destroyed by full thickness damage, leading to localized loss of sweat production
Sebaceous glands	• Produce sebum • Maintains hair and skin condition and pH • Antimicrobial	• Unaffected by superficial damage. • Destroyed by full thickness damage, leading to dry, fissured scar
HYPODERMIS OR SUBCUTANEOUS TISSUE		
Fat – soft mobile layer	• Insulates, stores energy, cushions	• Contour defects
Connective tissue – contains nerve and blood supply	• Attaches skin to underlying tissue supports • Divides tissue into compartments	• Tethering of skin • Shearing interrupts nerve and blood supply

2.6.3 The inflammatory phase

Healing starts with inflammation at the site of injury which is characterized by redness, pain and swelling from constricted lacerated vessels (LeBert and Huttenlocher, 2014). Inflammation takes place within the first three (3) days (0-3 days) at the injured wound site, to prevent infection (Brown, 2015). An injury to the tissue is followed by gradual haemostasis. During haemostasis, the microcirculation creates thrombocytes (platelets) and fibrinogen (blood-clotting protein) to start the formation of a haemostatic plug in the area to prevent further bleeding and encourage healing. Once the haemostatic plug has formed, the next process addresses the removal of dead tissue from the wound in order to prevent infection (Velnar *et al*., 2009).

The process of dead tissue removal is assisted by phagocytic cells which are produced by the inflammatory cells. Within 24 hours and continuing for the first five (5) days after injury, neutrophils (rich in fibrin) enter the area to assist in killing local bacteria and removing dead tissue (Young and McNaught, 2011). Leucocytes increase within 24 hours of the inflammatory phase and, together with lymphocytes, are critical for the removal of necrotic tissue (Franz *et al*., 2007). Macrophages are released and are responsible for scavenging debris, secreting growth factors (chemokine"s and cytokines) (Figure 2.6) and for initiating the transition from the inflammatory phase to the proliferative phase (Velnar *et al*., 2009).

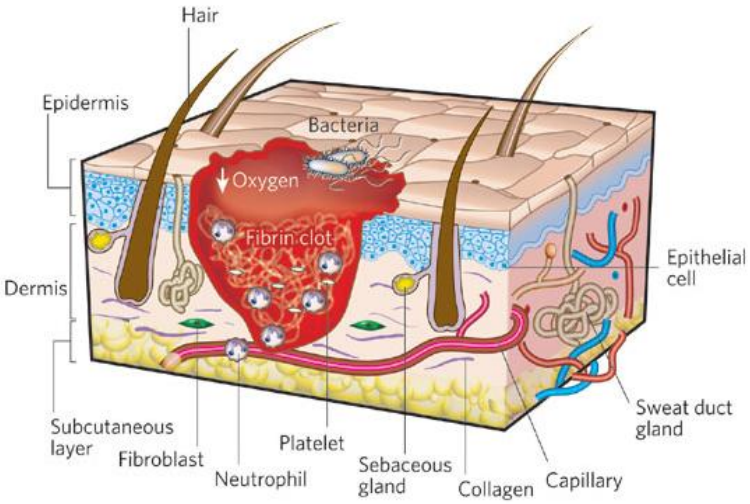


Figure 2.6 The inflammatory phase of wound healing (Gurtner *et al*., 2008)

2.6.4 Proliferative phase

The proliferative phase starts at day 4 or 5 (Son and Harijan, 2014), of the healing process, and continues to day 21. During this phase, the haemostatic plug is replaced by granulation tissue (macrophages, fibroblasts and endothelial cells). Both type I and type III collagen are associated with the proliferative phase. Type I collagen is the most abundant type of collagen found in the normal dermis. However, during the proliferative phase of healing fibroblast cells multiply, encouraging collagen type III to be laid down, creating a structural framework for endothelial cells. Type III collagen is quickly produced by young fibroblasts before the tougher type I collagen is synthesized (Van den Broek *et al*., 2012). The restoration of the epithelium is of importance for tissue integrity as it re-strengthens the skin. Keratinocytes found in the follicles of skin and below the scab, migrate across the granulation tissue causing a separation of the scab from underlying tissue. During the migration of the keratinocytes, plasminogen activators release plasmin to assist in dissolving the scab. As the keratinocytes continue to migrate, new epithelial cells are formed, completing the healing process of the damaged tissue (Figure 2.7). If the proliferative phase of cell death does not start, hypertrophic and keloid scars can develop (Velnar *et al*., 2009).

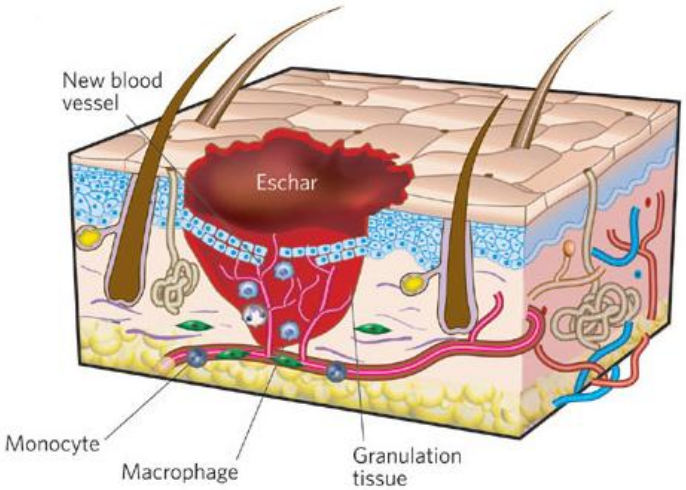


Figure 2.7 The proliferative phase of wound healing (Gurtner *et al*., 2008)

2.6.5 Remodelling phase

The remodelling phase starts around day 21 and continues to day 365. Wound remodelling occurs once the proliferative phase comes to an end by means of programmed cell death. Type III collagen formed during the proliferative phase degenerates and is replaced with stronger type I collagen, which accounts for 10-20% of the collagen in the dermis of adults (Hill and Pickart, 2009). In an unwounded dermis the collagen fibre bundles are arranged in a basket-weave pattern whereas in an acute wound the matured collagen fibres are oriented in overlapping arrays parallel to the wound surface and usually along lines of maximum tension, thereby contributing to scar appearance (Figure 2.8) (Velnar *et al*., 2009).

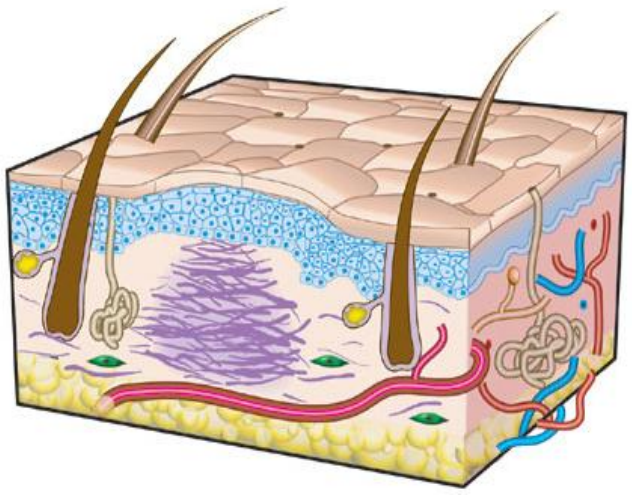


Figure 2.8 The remodelling phase of wound healing (Gurtner *et al*., 2008)

2.6.6 Different types of scar formations

Depending on the extent of wound injury and genetic factors, various scars can develop during wound healing for instance atrophic, hypertrophic and keloid scars. Atrophic, hypertrophic and keloid scars are most often associated with acne (Gozali *et al*., 2015) while) hypertrophic and keloid scars are usually seen after breast reduction surgery (Monstrey *et al*., 2014). All ethnic groups are prone to keloid and hypertrophic scarring; however, it is most frequently seen in African Americans, Hispanics and Asians; possibly due to collagen type III production and melanocyte stimulating hormone anomalies (Juckett and Hartman-Adams, 2009; Hunasgi *et al*., 2013).

2.6.6.1 Atrophic scars

Atrophic scars (AS) are typically rounded, flat or inverted and present in patients that have suffered from acne or chickenpox, leaving a pit-like appearance (Levy and Zeichner, 2012). Endogenous factors including involuntary stretching due to surgery or excessive tension on the wound edges, could lead to dermal dehiscence, which can result in the development of striaedistensae (SD). SD is formed after excessive dermal stretching and presents with a scar like appearance, showing hypo-pigmentation and dermal fibrosis, due to a prolonged inflammatory phase (Figure 2.10) (Ud-Din and Bayat, 2014). Increased melanisation has been noted to be more severe in African American women compared to Caucasian women (Ud-Din and Bayat, 2014). Treatments for AS include chemicals peels, micro-dermabrasion, laser therapy and surgery (Levy and Zeichner, 2012). A treatment specifically mentioned for SD is treatment with the Mesotherapy Eporex machine. The machine is used in combination with active ingredients, such as Vitamin C which is an antioxidant and assists in healing, skin elasticity and promotes fibroblast stimulation (Vedamurthy, 2007).

2.6.6.2 Hypertrophic scars

Hypertrophic scarring (HTS) presents as a red, warm, hard, pruritic (itchy), painful/tender and lumpy scar due to uneven collagen production and it is raised above the skin level but does not exceed the original lesion‟s boundary (Figure 2.9) (Ud-Din and Bayat, 2014). HTS is mostly caused by surgical incisions and these scars usually appear within one (1) month of injury, but can continue to develop over a few years (Juckett and Hartman-Adams, 2009). After surgery, the human body heals itself by producing collagen which is deposited in the skin to provide protection. In the case of hypertrophic scarring the collagen accumulation is arranged in whorls and nodules rather than a basket-weave pattern resulting in a hypertrophic appearance (Huang *et al*., 2013,). HTS gradually matures taking anything from 12-18 months to form (Donovan *et al*., 2016).

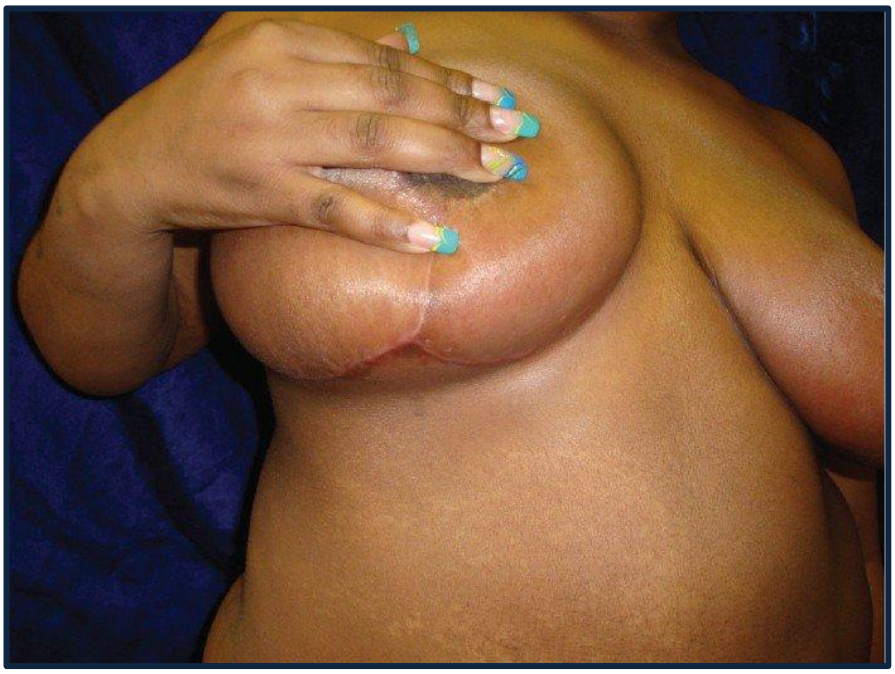


Figure 2.9 **Hypertrophic breast scarring at the inframammary crease** (Chopra *et al*., 2014)

2.6.6.3 Keloid scars

A keloid scar conforms to a hypertrophic scar except that it extends beyond the natural architecture of the injured area (original lesion"s boundary) (Hunasgi *et al*., 2013). A keloid scar is red in colour, raised in height, large in shape and reflects a shiny appearance (Figure 2.10) (Monstrey *et al*., 2014). The pathophysiology of keloid scarring is not fully understood, and Van der Veer *et al*. (2009) suggested that during the proliferation phase, fibroblasts, which form connective tissue, develop larger quantities of collagen fibres than is considered normal. This type of scarring does not subside, rather it continues to mature over time as collagen synthesis in keloids is 20 times greater than in normal unscarred skin (Shen *et al*., 2015). Keloid scarring is difficult to treat and due to its unsightly exterior, patients may resort to surgical interventions to improve the visual appearance of the scar (Hill and Pickart, 2009).

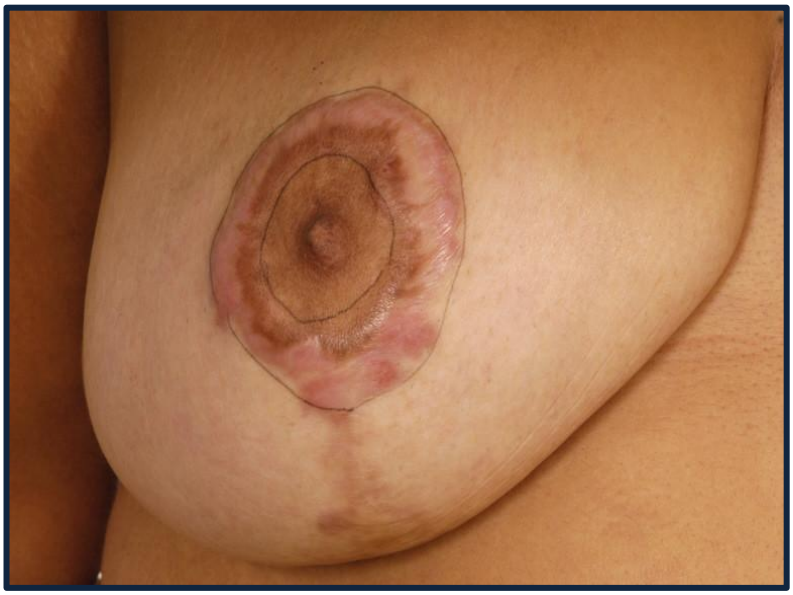


Figure 2.10 Keloid scarring at the nipple areola (Davalia *et al*., 2008)

2.6.7 Factors delaying wound healing that can contribute to scar formation

Various factors have an impact on wound healing and the amount of scar tissue formed after mammary reduction surgery. These factors are associated with improved or poor wound healing. In general, these factors include genetics, medical history (for example medication, diabetes, post-surgical care and disease or illness), physical stress, active lifestyle and ethnicity (Hill and Pickart, 2009; Huang *et al*., 2013).

2.6.7.1 Genetics

Genetics and aging have an effect on scar formation (Shermak *et al*., 2011; Thompson *et al*., 2013). A genetic factor could be, for example, being born with a suppressed immune system which in turn affects the inflammatory phase of healing, making it difficult for the body to heal at the required rate, thus resulting in keloid scarring (Brown *et al*., 2008; Shih and Bayat, 2010).

Wound healing may occur in older patients, mainly due to co-morbidities and inadequate nutritional intake, altered hormonal responses, poor hydration and compromised immune, circulatory, and respiratory systems which increase the risk of skin breakdown causing a delay in wound healing (Hess, 2011).

2.6.7.2 Medical history

A. Disease or illness

Chronic diseases that cause immune suppression such as diabetes, coronary artery disease, peripheral vascular disease, cancer to name a few can negatively affect wound healing (Hess, 2011). The impaired vascular flow causes poor tissue oxygenation and decreases epithelialisation, thereby prolonging healing which could encourage abnormal scar formation (Guo and Dipietro, 2010).

B. Smoking

Smoking is a preventable lifestyle factor that affects both the rate and quality of wound healing, and significantly increases the risk of postoperative, wound-related complications (Ahn *et al.*, 2008; Cope, 2014). Smoking could have an adverse effect on healing as it causes vasoconstriction of the blood vessels resulting in low oxygen levels (Sørensen *et al*., 2009). Carbon monoxide, found in the blood of smokers, preferentially binds to haemoglobin, reducing the delivery of O_2 to the wound. The lower O_2 levels cause a delay in the inflammatory phase by diminishing the function of neutrophils and macrophages, resulting in poor wound healing (Deliaert *et al*., 2012). In cosmetic surgery outcomes in smokers appear to be worse, and plastic surgeons and reconstructive surgeons are often reluctant to perform cosmetic surgeries on individuals who refuse to quit smoking (Guo and Dipietro, 2010).

C. Post-surgical care and active lifestyle

Post-surgical care is of great importance as this can determine the speed of wound healing (Anderson and Hamm, 2012). Vigorous treatments or resuming physical activity too early can stimulate inflammation and oedema, prolonging the inflammatory phase, and can result in wound disruption. An inflamed wound results in increased collagen deposition. By being gentle during the early stages of healing, rehabilitation can progress faster at later stages of healing (Gantwerker and Hom, 2012). However, being active is also of great importance as this improves blood circulation; increases O_2 delivery, assists in lymph removal and supports the body"s response to emotional and physical stress (Guo and Dipietro, 2010).

Because physical activity has a positive influence on immunity, being physically active before/after surgery within limits could encourage wound healing.

D. Physical stress

Stress has an impact on overall health and social behaviour. Stress lowers the strength of the immune system and since the immune system plays a vital role in wound healing, high stress levels result in delayed wound healing (Godbout and Glaser, 2006; Vileikyte, 2007). In addition to the direct influences of anxiety and depression on immunity, stress also affects an individual"s daily habits. Daily stress can contribute to poor sleeping patterns, inadequate nutrition, lower levels of exercise and possible susceptibility to alcohol or drug abuse (Guo and Dipietro, 2010).

E. Nutrition and hydration

Nutrition and hydration are the construction blocks for tissue repair, therefore a patient"s diet should contain proteins, carbohydrates, fat and vitamins to promote wound healing (Arnold and Barbul, 2006; Hill and Pickart, 2009). Vitamin C is a vitamin essential for wound healing and should be supplemented on a daily basis. Vitamin C provides tensile strength to newly formed collagen and is required for proper immune function (Collins, 2013). Vitamin E maintains and stabilizes cellular membrane integrity and has anti-inflammatory properties (Guo and Dipietro, 2010).

F. Medication

Medications can affect the healing process; negatively or positively. Common medications known to compromise the healing process include chemotherapeutic drugs, glucocorticoid steroids and non-steroidal anti-inflammatory drugs. These drugs have a tendency to destroy the cell structures that are required for cell reproduction (Guo and Dipietro, 2010; Wigston *et al*., 2013). Medications can also affect the body by lowering the immune system which in turn results in poor wound healing (Kidd *et al*., 2016).

G. Ethnicity

When comparing the structure of white skin to dark skin, in general dark skin has a thick, compact dermis with prominent and numerous fibre fragments which could complicate the healing process. Keloid scarring is more prominent in black skin due to higher levels of compacted collagen bundles (Czerkasij, 2013). Melanocyte-stimulating hormone anomalies, familial predisposition, with autosomal dominant and recessive genetic variants is recognized as possible causes for why darkly pigmented skin carries a 15- to 20-fold increased risk for keloid formation (Kose and Waseem, 2008; Juckett *et al*., 2009; Ahuja *et al*., 2015).

2.6.7.3 Amount of breast tissue removed

The chance of skin loss and delayed wound healing increases as the quantity of resection increases (Andrades and Prado, 2007). Hypertrophic breast with moderate to severe ptosis require greater elevation of the nipple-areola complex and larger amounts of skin excised. This could result in closure of the surgical site to be tight or excessively thinned flaps increasing chances of wound breakdown. This usually occurs at the junction of two scar lines and in this case the Wise pattern junction of the inverted T (Saleem and John, 2013).

Overweight and obese women usually have large breasts, which logically explain the connection between large resections and local complications (Murray *et al*., 2008; Setälä *et al*., 2009). Ayadin *et al*. (2016) found a strong correlation between breast resection weight and the risk of complications to surgery such as delayed wound healing, wound dehiscence, nipple/areola necrosis, haematoma, sarcoma, fat necrosis, hypertrophic scarring and infections. In order to prevent delayed wound healing and dehiscence in patients with high BMI, antibiotic coverage for a period of five (5) days could be regarded as useful (Saleem and John, 2013).

Ptosis is another method to determine the amount of breast tissue removed during surgery. The higher the grade of ptosis the more breast tissue is being removed leading to longer scars with the disproportionate skin and excessive dog ears (Andrades and Prado, 2007; Shiffman, 2009).

2.6.8 The assessment of scar tissue formation post-mammary reduction surgery

A deep wound could potentially lead to excessive scar formation, whereas a superficial wound should heal without leaving a scar. According to Brusselaer *et al*. (2010) several scar scales have been developed over the last 30 years of which the Vancouver Scar Scale (VSS) and the Patient and Observer Scar Assessment Scale (POSAS) are the most widely used (Brusselaers *et al*., 2010). An instrument used to measure any health status, in this case the amount of scar hypertrophy or activity, is required to include four (key) features, namely validity, reliability, consistency and feasibility (Duncan *et al*. 2006). To date, no ideal objective measurement tool has been developed or identified to assess scar tissue formation post mammary reduction surgery. However, the Vancouver Scar Scale (VSS) remains the most frequently used scar rating scale by doctors when assessing linear surgical scars, burn scars and skin grafts (Fearmonti *et al*., 2010; Ferriero *et al*., 2015). The VSS scale was used in this research study to evaluate scar formation.

2.6.8.1 Vancouver Scar Scale (VSS)

The VSS (Table 2.5) was first described by Sullivan in 1990 as a tool for the monitoring of changes in scar quality over time (Gankande *et al*., 2013). The VSS assessment evaluates the treatment response by evaluating scar vascularity, pigmentation, pliability and the height of a scar (Fearmonti *et al*., 2010; Wei *et al*., 2015). Truong *et al*. (2005) tested the VSS scale in the assessment of linear scars after breast cancer surgery. They concluded that the VSS scale is valid and reliable when assessing linear scars, although the level of reliability in their study was lower than what is usually considered acceptable (r=0.64). Despite its poor reliability, subjective assessment is still considered the gold standard in scar assessment (Kaartinen *et al*., 2011).

A. Vascularity

Vascularity of a scar is related to the stage of wound healing. Highly active scars have a greater blood supply leading to red and raised scarring whereas mature scars have fewer and smaller capillaries leading to a paler and flatter scar surface (Perry *et al*., 2010; Wei *et al*., 2015). Scar vascularity is divided into normal colour (old scar), which is in turn the exact same colour as the healthy skin surrounding the scar, and pink, red and purple (in the case of a recent scar). The colour of a scar is assessed by visual inspection Hallam *et al*., 2013).

B. Pigmentation

Pigmentation is tested by blanching the skin to remove any blood supply to the area (Levy and Emer, 2012). Pigmentation refers to the number of melanocytes, bile and carotene pigments found in the scar. Normal pigmentation implies the same skin colour surrounding a scar. Hyper-pigmentation is the increase of melanocytes that creates darkening of the scar in contrast to the adjacent skin, whereas hypo-pigmentation is a decrease in melanocytes creating a lighter appearance of the scar opposed to the adjacent skin (Chadwick *et al*., 2012; Czerkasij, 2013).

C. Pliability

The pliability of a scar is measured by the normal movement, glide and stretch of the skin. The term pliability is highly subjective and describes suppleness, texture, skin tone and skin hardness (Ferriero *et al*., 2015). The skin"s firmness and extensibility reflects both the morphological and physiological properties of a scar, revealing the equilibrium between the intra- and intermolecular cross-links of collagen, elastin and reticulin fibres embedded in the dermis. Range of motion is the simplest method for measuring the functional effect of the skin"s pliability (Morien *et al*., 2008).

D. Height

Changes in the height of a scar could reflect the maturity of a scar and also the relation to the overabundance of collagen deposition (Gauglitz *et al*., 2011). Scar height is defined as the elevation of the scar raised above the surrounding tissue (Thompson *et al*., 2015). Rulers are used to measure the external size of a scar, for example the length, width, height and elevation (Ferriero *et al*., 2015).

The VSS has zero (0) to five (5) possible scores for each characteristic being assessed (Table 2.5). The scores are achieved by comparing each characteristic with the adjacent healthy skin. The scale is scored on a range of 0-13, where 0 reflects normal skin healing and 13; poor skin healing (Kaartinen *et al*., 2011). The emphases of the subjective measurements are on the appearance and cosmetic aspects of scarring. The scoring should depend on the severity of the scar area being assessed (Gankande *et al*., 2013).

Table 2.4 Vancouver Scar Scale (VSS) (Kaartinen *et al*., 2011)

PARAMETER	DESCRIPTOR	POINTS
Vascularity	Normal	0
	Pink	1
	Red	2
	Purple	3
Pigmentation	Normal	0
	Hypo-pigmentation	1
	Hyper-pigmentation	2
Pliability	Normal	0
	Supple	1
	Yielding	2
	Firm	3
	Ropes	4
	Contracture	5
Height	Flat	0
	<2mm	1
	2-5 mm	2
	>5 mm	3
Total score		**/13**

2.6.9 Treatment modalities for post-operative scar formation

The treatment of scar tissue formation is not a spontaneous decision as scars respond differently to various treatment techniques, and usually more than one scar treatment is required. A scar treatment technique cannot guarantee the removal of scar tissue without any visible marks remaining. It is possible to improve the visual appearance of a scar and surrounding skin through various methods of surgical and non-surgical treatments (Levy and Zeichner, 2012). These methods aim to reduce inflammation, increase hydration and assist in collagen synthesis (Rabello *et al*., 2014).

2.6.9.1 Re-surgical and radiation treatment

Re-surgery can be used to treat keloid scarring by changing the appearance of the scar shape by altering its position (Rabello *et al*., 2014). The process is risky as it can trigger a

larger formation of keloid scarring in the same area. New scars could take up to 2 years to mature (Ogawa and Chin, 2008).

Surgery and radiation can also be used in conjunction with one another to treat keloid scars. Radiotherapy is commonly used to suppress the overgrowth of scar tissue (Shen *et al*., 2015). Low doses of superficial radiation can be applied to the operation site from the first day after surgery, aiming to alter the fibroblast formation and increase the breakdown of collagen production (Velnar *et al*., 2009).

2.6.9.2 Non-surgical treatments

Numerous non-surgical treatment modalities can be used against scar formation. These treatment options include message, steroid injections, laser, silicone gel or sheets, cryotherapy, peels, gels and creams, onion extracts, interferon etc. to name just a few (Velnar *et al.*, 2009; Monstrey *et al*., 2014).

Di-Ud and Payat (2014) stated that tissue healing could be enhanced through electrode stimulation and, more recently, electrode stimulation in the treatment of abnormal raised dermal scarring, alleviating the symptoms of pain, pruritus, and inflammation.

A modern technique used to treat a variety of clinical conditions (cellulite, wrinkles/aging, acne and muscle aches) is non-invasive Mesotherapy Eporex (Figure 2.13). The treatment encompasses the use of modulated electrical wave lengths and ionized ingredients that are propelled into the skin at varying depths (Konda and Thappa, 2013). For the purpose of this research project emphasis will be placed on the effectiveness of Mesotherapy Eporex treatments on scar appearance.

2.7 Isophoresis via the Mesotherapy Eporex machine

The skin is the largest, most important organ of the human body covering 2m² of surface area and is 1-2mm thick (Panzade and Puranik, 2012). The skin has three (3) layers namely the epidermis, dermis and hypodermis. Transdermal systems have been developed over the years for the purpose of delivering ionic and non-ionic agents into the different skin layers at variable depths (Herwadkar and Banga, 2012). The Mesotherapy Eporex machine

uses the new innovative isophoresis technique which is a transdermal delivery system, bringing about the synergy of known techniques such as iontophoresis, ionophoresis and electroporation (Figure 2.11) (Vedamurthy, 2007).

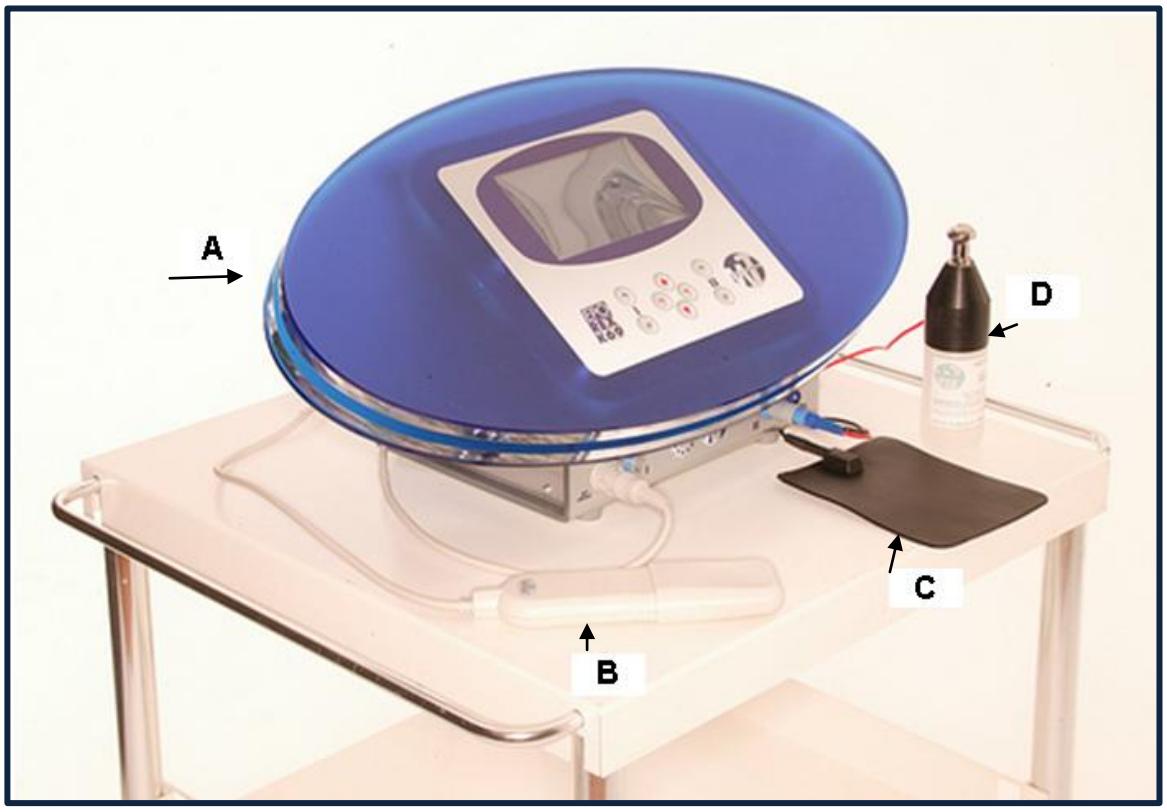


Figure 2.11 Mesotherapy Eporex machine *(A)* Mesotherapy Eporex *control board (B) Ultrasonic spatula (C) Electrical pad (D)* Mesotherapy Eporex *apparatus (hand piece) with active ingredients*

Iontophoresis gained popularity at the beginning of the 20th century. Leduc introduced this method for administering pharmacological agents into the skin in the 1900's , 2008). The iontophoresis technique utilizes a continuous direct electrical current (galvanic), to repel charged molecules across a cellular membrane. Iontophoresis increases blood and lymphatic circulation as well as cellular metabolism (Rawat *et al*., 2008)

Iontophoresis makes use of two (2) electrodes to complete an electrical circuit; one of the electrodes is referred to as the active electrode and the other as the indifferent electrode. The electrodes, in conjunction with a pulsed direct current, cause an acidic or alkaline chemical reaction to occur at the electrode site. Depending on the polarity an acidic effect is produced under the anode (positive pole) causing vasoconstriction of blood vessels, an

astringent effect on the pores, tissue firmness and soothing of nerve endings. The cathode (negative pole) on the other hand produces an alkaline effect causing vasodilatation, relaxation of pores, tissue softness and stimulation of nerve endings (Dhote *et al*., 2012; Panzade and Puranik, 2012).

The ionophoresis current is similar to the iontophoresis current, encouraging movement of charged ions in an electrical field, with positively charged ions moving towards the cathode and negatively charge ions to the anode. Ionophoresis uses a direct current without modulated waves (Dhote *et al*., 2012). Due to the fixed frequency used by ionophoresis, there is limited ingredient penetration which may lead to chemical burns at the treatment site due to prolonged skin exposure. Other disadvantaged of ionophoresis includes difficulty in stabilizing the therapeutic agent in the application vehicle and the complexity of the drug release system (Krueger *et al*., 2014).

Electroporation uses high voltage pulses for short durations (Ma *et al*., 2014; Ita, 2016). Electroporation is best known as a physical transfection method where the skin cells are exposed to a brief electrical pulse, thereby opening the pores in the cell membrane, allowing micro-molecules to enter the cell (Santra and Tseng, 2013).

Isophoresis uses a synergy of the 3 penetration methods (Figure 2.12) expanding the scope of transdermal delivery (Dhote *et al*., 2012). The electrical pulses created by the Mesotherapy Eporex machine induce a structural re-arrangement of the semi-permeable cell membranes encouraging the increased uptake of macro and micro molecules. Isophoresis uses a low frequency modulated (non-continuous) alternating current, which is a pulse, released with a low frequency elasto-modulated profile with an intensity that can be adjusted on the system by the operator. The low frequency modulated alternating current allows hydro-soluble molecules to enter the extra-cellular compartments of the skin during the “on” phase. During the “off” phase no current flows through the electrode, preventing the tissues from absorbing too much energy thus avoiding possible thermal damage [Mesotherapy Eporex K69 User Manual (Appendix A1)].

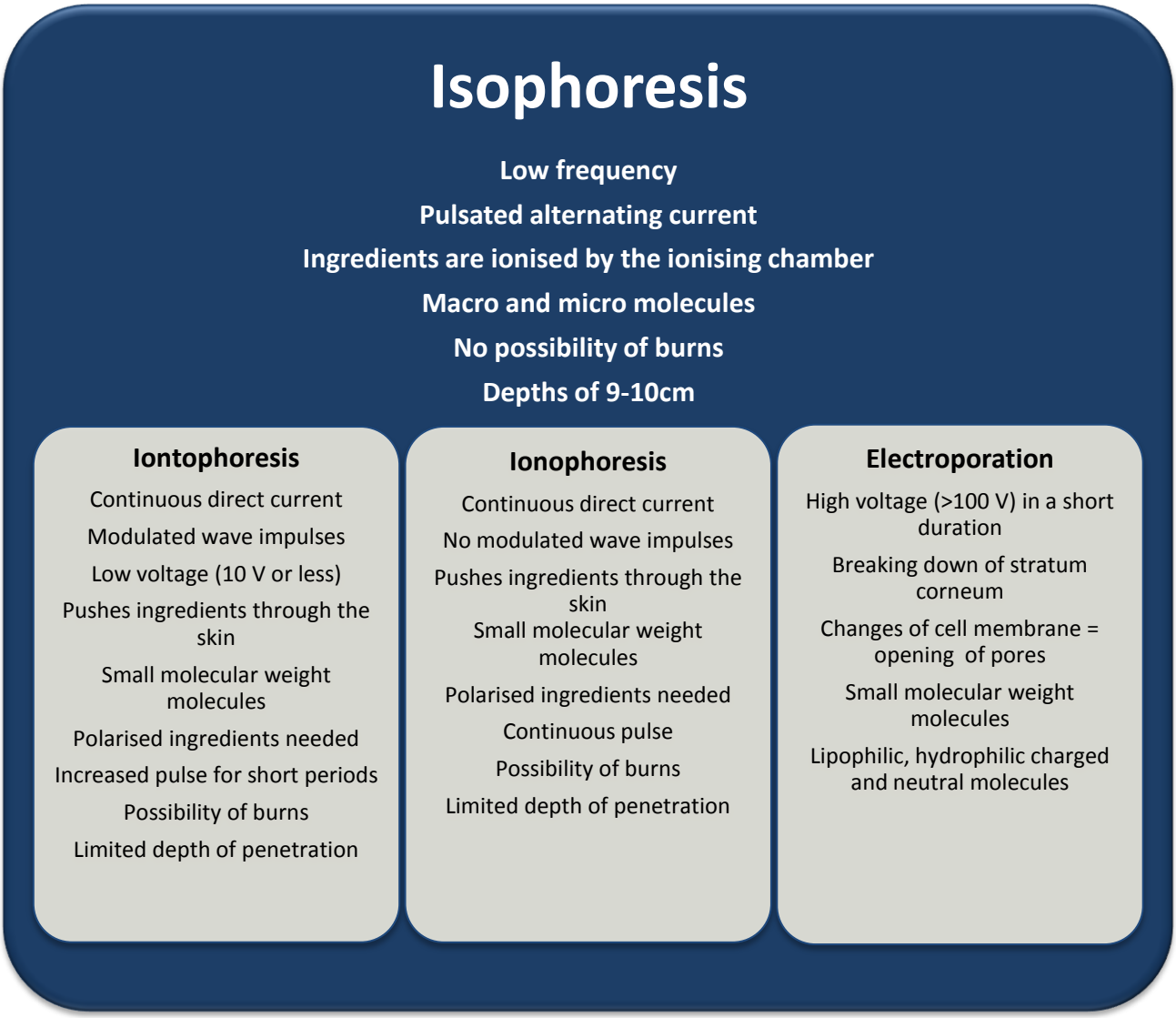


Figure 2.12 Combination of three (3) different currents, forming isophoresis

The Mesotherapy Eporex machine differs from other electrical currents and machines with respect to the wave modularity it uses and the presence of an ionization chamber (Figure 2.14). The isophoresis transmission can be summarized into three (3) processes. Firstly, the low frequency modulated alternating current ionises the molecules in the chamber, secondly ingredients are propelled to different depths of the skin and lastly electroporation occurs via osmosis. The ionization chamber prepares active ingredients by ionizing them and, in combination with an electrical current, up to 90% of the active ingredients can be delivered to depths of 0.1cm to 10cm in the treatment area (Goldman and Hexsel, 2010; Mammucari *et al*., 2011).

2.7.1 Ultrasound for exfoliation of corneocytes and improved skin tone

In conjunction with the Mesotherapy Eporex treatment, an ultrasonic spatula is used for skin exfoliation, healing and improved product penetration. The ultrasonic spatula is held at a 45° angle allowing ultrasonic waves to cover a large surface, exploiting the principle of cavitation (transfer of liquid molecules to vapour) (Figure 3.2). Cavitation encourages the removal of corneocytes and improves blood circulation in the area. The application of low-frequency ultrasound enhances the stratum corneum"s (SC) permeability for a variety of water soluble ingredients. The ultrasound prepares the skin for the transdermal delivery of active ingredients prior to the main treatment by promoting the exfoliation of 20-50% of corneocytes (D"Angelo, 2009; Tekchandani, 2016).

After the completion of the main treatment the ultrasound spatula can be used to encourage healing and assist in the deeper penetration of ingredients utilized during a treatment. The ultrasound spatula is held at a 45° angle facing upward with the flat side on the area of treatment (Figure 3.4). The ultrasound waves created by the ultrasound spatula cause vibrations in the molecules which create heat and further encourage cellular stimulation and deeper product penetration (MacGregor and Tanzi, 2013).

2.7.2 Intracellular absorption/transdermal delivery

One of the skin's primary functions is that of protection. The skin is covered with a natural barrier, preventing exogenous substances such as molecules or ions from entering the body. The route of penetration for active ingredients into the skin is to cross the stratum corneum and pass through the epidermis (Panzade and Puranik, 2012), to reach the dermis (middle layer of the skin) and subcutaneous tissue. The channels (trans-cellular route) responsible for water transport into the cells, allow for the transport of substances such as water, salts and molecules through the cellular membrane via chemical impulses. The chemical impulses induce the flow of active ingredients from cell to cell (osmosis), resulting in a macroscopic distribution (Sharma *et al*., 2015). The electrical impulses created by the current, allow for a temporarily confused cellular membrane - a vibration allowing the ingredients to pass through the cell layers (Figure 2.13). Once the electrical current is removed, the cells return to normal and the penetrated ingredients are retained within the cell (Rawat *et al*., 2008).

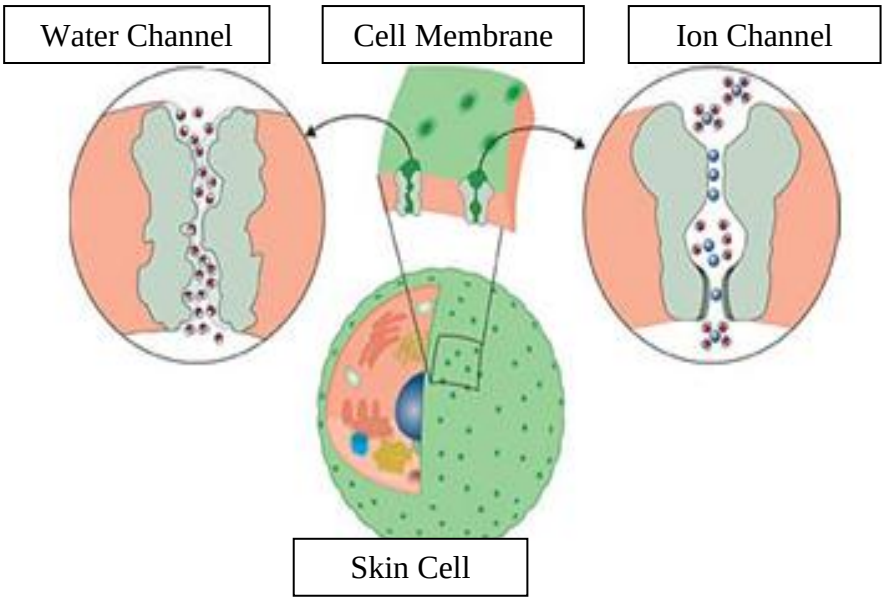


Figure 2.13 Intracellular penetration through the skin barrier by hydrophilic keratinocytes (Tosti and De Padova., 2007).

2.7.3 The Mesotherapy Eporex hand piece

The Mesotherapy Eporex hand piece (Figure 2.14) has an internal stainless steel ionization chamber, responsible for the electrical preparation of molecules. A plastic bottle, containing clear conductive gel and active ingredients chosen for the treatment, is shaken to combine the ingredients and connected to the hand piece. The active gel is squeezed into the chamber ensuring constant contact with the ionization chamber and thus allowing ionization of the ingredients through the elasto-modulated current. The application of the front stainless steel roller to the skin creates a constant and graduated transmission of active ingredients into the skin. The continuous transmission provides optimal depth transmission, creating electro-pores and encouraging osmosis (transferring of ingredients between cells) [Tosti and De Padova., 2007; Mesotherapy Eporex K69 User Manual (Appendix A1)].

Figure 2.14 Mesotherapy Eporex hand piece and the ionization chamber

Electro-pores are channels used to transport active ingredients at a specific phase of electrical induction (on phase) and can preserve the active ingredients until the correct depth in the skin is reached to induce the diffusion of high weighted molecular structures (Konda and Thappa, 2013).

Electrolytes (therapeutic ingredients from herbs, plants, flowers and fruits) contain both acids and salts which increase electrical conductivity. With the assistance of iontophoresis the skin‟s resistance is broken down and a chemical change occurs within the skin. When electrolytes are dissolved in a water base, the electrolytes partly split and form ions carrying a negative (cation) or positive (anion) charge. When these electrolytes come into contact with the direct current, movement between the ions occurs, encouraging like charges to repel and opposite charges to attract (electrical principle) (Dhote *et al*., 2012 Bhatia and Banga, 2014). In the human body the tissue fluids possess electrolytic properties, thus allowing a current to pass through the skin and propel ingredients to the deeper layers of the skin (Panzade and Puranik, 2012).

2.7.4 Active ingredients used in conjunction with Mesotherapy Eporex treatments

Numerous active ingredients can be utilized with the Mesotherapy Eporex machine to treat scar appearance. These active ingredients include *Asian Centella*, Hyaluronic Acid, Vitamins A, B, C, organic silica, poly nucleotides-DNA, soybean extract, polyphenols and mineral salts (Latha and Vandana, 2011; Konda and Thappa, 2013) . However, the suppliers (Mesoskin Therapy Worldwide, Orlando, Florida) of the Mesotherapy Eporex ingredients recommended the use of three (3) active ingredients for the treatment of scarring (namely *Asian Centella*, Hyaluronic Acid and Vitamin C). Based on this information *Asian Centella*, Hyaluronic Acid and Vitamin C were used in conjunction with the Mesotherapy Eporex machine for the treatment of scarring in this research study.

2.7.4.1 Asian Centella (*Centella asiatica (L.)*)

Asian Centella is an aromatic herb found in abundance on moist, sandy/clay, damp fields in India. This herb is a faintly aromatic, stoloniderous, perennial creeper which attains a height up to 15cm (Tripathi *et al*., 2015). The chemical constituents of the *Centella* plant have an important role in both medicinal and nutraceutical applications (Seevaratnam *et al*., 2012; Bylka *et al*., 2013).

Asian Centella has positive attributes which include:

Figure 2.15 Positive attributes of *Asian Centella*

Asian Centella is a water soluble ingredient aiding in wound healing by stimulating type I collagen and by increasing antioxidant levels (Song *et al*., 2012). Oral and topical administration of *Asian Centella* used from week six (6) to eight (8) may increase wound

strength. *Asian Centella* extract contains asiaticosides and madecassosides (purified molecule of *Asian Centella*) which have a stimulating action, through collagen and elastin biosynthesis in the dermal fibroblasts and vein walls, thus improving the tone and elasticity of the skin, aiding in early treatment of keloid formation and they exhibit anti-inflammatory properties (Song *et al*., 2012; Somboonwong *et al*., 2013).

2.7.4.2 Hyaluronic Acid (HA)

HA is a natural compound found in the human body. However, with age the quantities of HA deteriorate dramatically which makes the skin susceptible to wrinkles, dehydration and infection (Papakonstantinou *et al*., 2012). HA is hygroscopic and increases the permeability of the skin by hydrating the stratum corneum as it binds with water molecules found in between the intercellular spaces of tissues, thus allowing the receptors found in HA to be distributed in the skin tissue facilitating the localization of HA (Yang *et al*., 2012). HA is a linear polysaccharide with more than 50% of the body"s HA found in skin tissue. The application of iontophoresis has been limited to the delivery of small molecules with a charge, whereas ionophoresis disrupts the lipid structure of the stratum corneum to enhance the skin"s permeability allowing deeper product penetration (Konda and Thappa, 2013).

In conjunction with ionophoresis, HA absorbs an additional 500 water molecules, swelling in size and allowing the transport of essential nutrients from the blood stream to the skin cells, contributing to dermis renewal and removal of harmful compounds. HA aids in wound healing (Konda and Thappa, 2013), through the stimulation of agents required for collagen synthesis, as HA can enhance the proliferation of fibroblasts through receptors on the cell membrane. HA also acts as a humectant and protects against free-radical scavengers (Papakonstantinou *et al*., 2012; Yang *et al*., 2012).

HA is likely to play a multifaceted role during the stages of healing. A variety of cell functions are essential for tissue repair and this may be the result of the HA rich network. These functions include facilitation of cell migration into the provisional wound matrix, cell proliferation and organization of the granulation tissue matrix (Necas *et al*., 2008;Xu *et al*., 2012). HA was observed to enhance cellular infiltration (Necas *et al*., 2008). Wound tissue in the early inflammatory phase of wound repair is abundant in HA thus an increased

synthesis of HA plays a crucial role in the skin wound healing process. These benefits contribute to smoothing of the skin and improving the appearance of scars (Yang *et al*., 2012).

2.7.4.3 Vitamin C (Ascorbic Acid)

Vitamin C is an essential nutrient for human beings and is a water soluble antioxidant that can be delivered topically or transdermally (Telang, 2013). Vitamin C acts as a co-factor for hydroxylation of the proline and lysine residues of pro-collagen and thereby promotes the formation of the triple-helical conformation of mature collagen fibres (Michels and Frei, 2013). Vitamin C assists in wound healing by decreasing oxidative stress; causing skin to remain radiant and bright. Vitamin C also acts as a natural antihistamine contributing to the release of histamine, therefore aiding in wound healing (Lima *et al*., 2009).

Vitamin C is found in high concentrations in immune cells, and is consumed quickly by the body during infections. It is not certain how with the immune system; it has been hypothesized to modulate the activities of phagocytes, the production of cytokines and lymphocytes, and the number of cell adhesion molecules in monocytes encouraging faster healing phases (Velnar *et al.,* 2009; Guo and DiPietro, 2010; Paiva *et al*., 2013).

Topical and Mesotherapy intradermal applications of Vitamin C concentrations vary from 5% - 30%, the higher the percentage of Vitamin C concentration the lower its absorption rate in the skin, rendering the antioxidant effect ineffective. A study conducted by Michels and Frei (2013) explains that a 20% Vitamin C concentration has the highest absorption rate in the dermal layers, helping to stimulate the formation of the epidermal barrier and re-establish the SC. Thus the application of 20% Vitamin C would allow maximal antioxidant absorption (Telang, 2013). After being delivered into the skin, Vitamin C is stabilized and remains in the tissue for a period of four (4) days (Michels and Frei, 2013). Thus, the re-application of Vitamin C could possibly enhance the healing process during all three (3) healing phases.

2.7.5 Indications and advantages of the Mesotherapy Eporex treatment

The Mesotherapy Eporex treatment is non-invasive, painless and provides a range of benefits which include the alleviation of inflammatory conditions, skin conditions, muscular aches and pain relief (Latha and Vandana, 2011; Konda and Thappa, 2013). The treatment enhances ingredient penetration with a strict control of transdermal penetration rates. Ingredients used during this treatment aid in healing, collagen stimulation, increased moisture levels, improved skin elasticity and texture. The main advantages of no needle Mesotherapy is that it is completely painless, no bruising, erythema or swelling, materials can penetrate to deeper levels, immediate rapid response and it is cost effective and larger areas can be treated in comparison with injection Mesotherapy (Konda and Thappa, 2013; Bagwe and Sarawade, 2014).

2.7.6 Contra-indications and disadvantages of the Mesotherapy Eporex treatment

Contra-indications for Mesotherapy Eporex (non-invasive) treatment is based on contra-indications applicable to the application of electrical currents and includes; pregnancy, breast feeding, open wounds, cardiac pacemakers, allergy to active ingredients (Kopitar *et al*., 2012). As the Mesotherapy Eporex treatment is time consuming, it could be considered a disadvantage to both the participant and therapist.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The objectives identified for this research study were four-fold. The first objective was to determine the effectiveness of the Mesotherapy Eporex treatments with active ingredients by comparing the scar appearance of the experimental breast to that of the control breast. Secondly, to determine the percentage of improvement of scar appearance after applying the Mesotherapy treatment for each of the four (4) categories included in the VSS. Thirdly to investigate whether scar appearance (wound healing) differs between the right and left breast by randomizing the application of the Mesotherapy Eporex treatment between the right and left breast. Lastly, to investigate the possible influence that the variables ptosis, breast tissue removed, BMI and age have on scar appearance, thus Mesotherapy Eporex treatment effectiveness after receiving bilateral mammary reduction surgery.

3.2 Study design

A randomized, experimental study was used to assess the effectiveness of the Mesotherapy Eporex therapy with active ingredients.

3.3 Study location

The research study was conducted at the medical practice of a private plastic surgeon situated at a private hospital in Bloemfontein, Free State, South Africa.

3.4 Study population and sampling

A research population is also known as a well-defined collection of individuals known to have similar characteristics. All the individuals within a certain population usually have a common, binding characteristic or trait. Therefore, female participants that received bilateral mammary reduction surgery that met the inclusion criteria were recruited to participate in

the study to ensure a homologous population. All participants were recruited from a single plastic surgery practice to ensure standardized surgical procedures and post-operative care.

3.4.1 Sample size

Thirty female participants (n=30) that received bilateral mammary reduction surgery were recruited for the research study. The 30 participants were randomly divided into 2 (two) groups of 15 participants each (P1-P30).

3.4.2 Sample selection

A biostatistician randomized the 30 participants into equal groups consisting of 15 participants each in which the Mesotherapy Eporex treatments were performed on the right breast (experimental breast) and the left breast served as the control breast (not receiving Mesotherapy Eporex treatments). Simultaneously, in the other 15 participants, the Mesotherapy Eporex treatments were performed on the left breast (experimental breast) and the right breast served as the control breast (not receiving Mesotherapy Eporex treatments). This was done to evaluate if healing differs between the right and the left breast.

3.4.3 In- and exclusion criteria

To address the objectives of the study, the selection criteria for the patients were based on two main variables namely bilateral mammary reduction surgery and Mesotherapy Eporex treatment. The specific requirements for the inclusion and exclusion of patients in this study are outlined in the inclusion and exclusion criteria.

3.4.3.1 Inclusion criteria

Participants were included in the study if they met the following criteria:

i. Female participants from the age of 20 years.
ii. Participants that qualified to received bilateral mammary reduction surgery.
iii. The Wise pattern approach with inferior pedicle surgical technique.

iv. Female participants willing to avail themselves for Mesotherapy Eporex treatment six (6) weeks after receiving bilateral mammary reduction surgery.
v. Female participants of all ethnic groups.
vi. Participants fully literate in English.

3.4.3.2 Exclusion criteria

The following participants were excluded from the study:

- Male participants.
- Contra-indications include chronic diseases (diabetes, HIV, auto-immune diseases and epilepsy), pregnancy and cardiac pace makers.

  Patients with the chronic diseases mentioned above were excluded because chronic diseases can interfere with the process of wound healing. HIV patients were excluded because these patients suffer from severe forms of common skin diseases (seborrheic dermatitis and psoriasis) which can increase skin sensitivity (Halder *et al*., 2012). The application of an electrical current via the Mesotherapy Eporex machine on already sensitive skin will increase patient discomfort. Patients with cardiac pacemakers and pregnancy were excluded because the application of electrical current (Mesotherapy Eporex) can interfere with cardiac pacemakers and can be harmful to the unborn child (Rukari and Alhat, 2014).

3.5 Conceptual framework for data collection

The data was collected according to the conceptual framework summarized in figure 3.1.

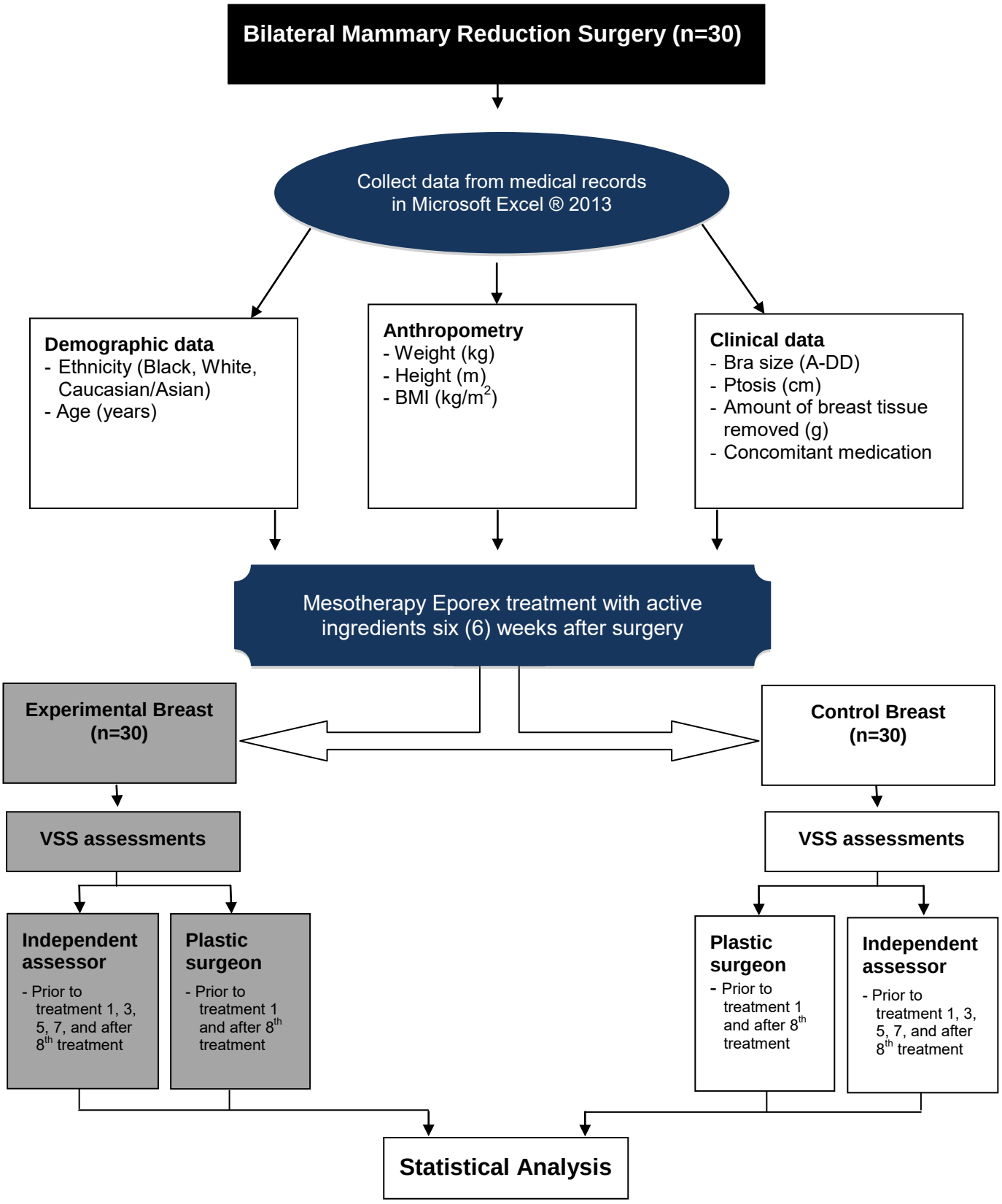


Figure 3.1 Conceptual framework for data collection

3.6 Data collection

Demographic, anthropometric and clinical data were extracted from patients' medical records supplied by the plastic surgeon. Six (6) weeks post-operatively the Mesotherapy Eporex treatment commenced and scar appearance was evaluated using the VSS. A client information card was used to record the demographic, anthropometric and clinical data of each participant (Appendix B).

3.6.1 Demographic, anthropometric and clinical data

Demographic data [ethnicity (Black/White/Caucasian/Asian) and age (years)], anthropometric data [weight (kg), length (m) and body mass index (BMI-kg/m^2)] and clinical data [bra size (cup A-DD), ptosis (cm), amount of breast tissue (g) removed from each breast during surgery, and concomitant medication] were recorded for each participant prior to the commencement of the Mesotherapy Eporex treatment.

BMI (kg/m^2) was calculated according to the following formula and interpreted according to Table 3.1.

(Corbin *et al*., 2016)

Table 3.1 Rating scale for body mass index (BMI) (Corbin *et al*., 2016)

Classification	BMI kg/m^2
Low	less than 17
Normal (good fitness zone)	17-24
Overweight	25-30
Obese (high risk)	over 30

[BMI = Body Mass Index; kg = kilogram; m^2 = meter square]

Breast ptosis was determined by measuring the distance from the supra sterna notch down to the nipple in cm. If this measurement exceeds 21cm then a patient is presenting with breast ptosis (Khan and Bayat, 2008). The grade of breast ptosis was determined using the Regnault classification system (Castro *et al*., 2013) (Table 3.2). The grade of ptosis is

determined by the proximal measurement from the nipple to the inframammary crease measured in cm. Plastic surgeons use the grade of ptosis as estimation for the amount of skin resection and breast tissue to be removed during surgery.

Table 3.2 Regnault classification system for breast ptosis (1976)

Grade 1 (Minor ptosis)	The nipple is at or up to 1cm below the inframammary crease.
Grade 2 (Moderate ptosis)	The nipple is at 1cm up to 3cm below the inframammary crease.
Grade 3 (Major ptosis)	The nipple ptosis is more than 3cm below the inframammary crease.

3.6.2 Wise pattern with inferior pedicle surgical technique

The surgical procedure was standardized and only the Wise pattern approach with an inferior Pedicle was used on all patients that received bilateral mammary reduction surgery. The Wise pattern approach with an inferior pedicle is also the surgical method of choice used by the plastic surgeon when performing bilateral mammary reduction surgery. The surgical technique is summarized in Appendix C.

3.6.3 Mesotherapy Eporex treatment

The aim of the research study was to determine the effectiveness of Mesotherapy Eporex treatment using specific active ingredients on linear scars produced by bilateral mammary reduction surgery. The Mesotherapy Eporex treatment commenced six (6) weeks after surgery to allow sufficient wound healing to take place and to reduce skin sensitivity.

Each participant received eight (8) treatments lasting about thirty (30) minutes each using the Mesotherapy Eporex machine (serial number I472OT3787, Bester Medical Aesthetics, Cape Town, South Africa) for the introduction of active ingredients into the incision site. The treatments were scheduled three (3) days apart to allow for skin sensitivity to subside.
In group one (1) (n=15), the right breast was treated with the Mesotherapy Eporex machine (experimental breast) and the left breast did not receive any treatment (control breast). In group two (2) (n=15), the left breast served as the experimental breast and the right breast served as the control breast.

The Mesotherapy Eporex treatment was performed by making use of three (3) active ingredients and a water based gel:

- 2ml *Asian Centella* ampoule (Mesotherapy Eporex worldwide, BE corporation, Bodi junction, Australia),
- 5ml 20% Vitamin C ampoule (Mesotherapy Eporex worldwide, BE corporation, Bodi junction, Australia),
- 5ml Hyaluronic Acid ampoule (Mesotherapy Eporex worldwide, BE corporation, Bodi junction, Australia) and
- 60ml clear water based conductive gel (Bester medical aesthetics, Cape Town, South Africa).

The active ingredients provided beneficial effects such as skin rejuvenation, hydration, strengthening of capillary walls, stimulation of collagen and elastin, anti-inflammatory properties and decrease myofibroblast production.

3.6.3.1 Mesotherapy Eporex treatment protocol

Each Mesotherapy Eporex treatment procedure was divided into three (3) steps:

- **Step 1:** Exfoliation using the ultrasonic spatula with the water based ultra-peel.
- **Step 2:** Scar treatment using the Mesotherapy Eporex machine with active ingredients set on the stretch mark programme.
- **Step 3:** Enhanced product penetration using the ultrasonic spatula.

Step 1

Pre-Treatment– Exfoliation

The first step of the treatment procedure focused on desquamation of the treatment area.

- Each participant's treatment commenced with a pre-treatment using the ultrasonic spatula.
- A water based ultra-peel solution (Bester Medical Aesthetics, Cape Town, South Africa), containing lactic acid and denatured alcohol, was applied to the scar by means of a facial brush.

- The derma-purification program was selected and pre-set for a maximum of three (3) minutes. The ultrasound spatula was placed facing downward at a 45° angle (Figure 3.2) on the scar. Movements were made in an upward motion, thus allowing the spread of ultrasonic waves to create a high pressure peeling, removing skin sebum and lifting of keratinized cells from the scar surface, including a 1cm radius surrounding the scar tissue.
- Due to the high liquid concentration of the water based ultra-peel solution, no residue was left on the skin after the exfoliation process. However, warm water with gauze was used to cleanse the skin further.

Figure 3.2 **Correct use of the ultrasonic spatula designed for exfoliation** [Mesotherapy Eporex Manual, 2007 (Appendix A2)]

Step 2

Main Treatment – Active ingredients

The second step of the treatment procedure focused on the penetration of the active ingredients into the skin.

The active gel for the main treatment was prepared as follows:
A 2ml *Asian Centella* ampoule, 5ml 20% Vitamin C ampoule and 5ml Hyaluronic acid ampoule were pre-mixed with 60ml clear conductive gel in a clear plastic bottle.

- The main treatment commenced by placing an electrical pad, covered with a sponge and saturated with tap water under the participant's scapula to complete the electrical current.

- The Mesotherapy Eporex ball applicator (Bester Medical Aesthetics, Cape Town, South Africa) was connected to the stainless steel hand piece.
- A plastic bottle dispenser, containing the active gel, was connected to the Mesotherapy Eporex stainless steel hand piece (Bester Medical Aesthetics, Cape Town, South Africa). Through the application of pressure on the plastic bottle, the active gel was released into the stainless steel chamber of the hand piece where ionization of the ingredients occurred.
- The researcher applied a small amount of active gel onto the scar via the ball applicator without any current.
- On the Mesotherapy Eporex machine, the stretch mark program was selected and treatment time was set at 14 minutes.
- According to the participant's comfort threshold, the intensity of the treatment was determined, starting at zero (0) and increasing the intensity until the participant was comfortable with the sensation felt on the treatment area.
- With each treatment thereafter, the treatment intensity started at the previously recorded intensity. An increase in intensity was allowed with each treatment received.
- Continuous criss-cross movements were performed over the scar, lateral to medial, medial to lateral and around the nipple area (Figure 3.3).
- Continuous application of the active gel via the ball applicator during the criss-cross movements ensured that the skin remained moisturised.
- The active gel was left on the skin in order to complete step 3.

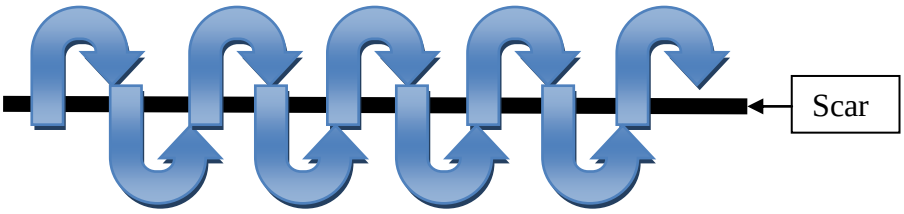


Figure 3.3 Criss-cross action of the Mesotherapy Eporex ball applicator

Step 3

Post Treatment – Enhancing product penetration

The third step of the procedure focused on enhanced penetration of the active gel and supported the healing process by using an ultrasonic spatula.

- The skin reactivation tone-up program was selected and pre-set for a maximum of 14 minutes.
- An electrical wrist strap, saturated with tap water, was placed around the participant's wrist to complete the electrical current.
- The ultrasonic spatula was placed (facing upwards) at a relatively flat 30° angle (Figure 3.4), moving over the scar tissue, including up to 1cm surrounding the scar tissue, using a slow up and down motion to encourage further penetration of the active gel into the scar tissue.

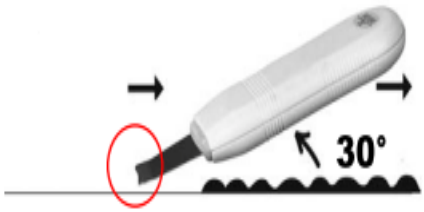


Figure 3.4 Correct use of the ultrasonic spatula designed for the skin reactivation tone-up settings [Mesotherapy Eporex Manual, 2007 (Appendix A2)]

- The treatment was completed with the removal of the excess active gel from the treatment area, with warm water and gauze.
- The skin was left clean. No creams or gels were applied to the surgical scar upon completion of the Mesotherapy Eporex treatment.

3.6.4 Assessment of scar appearance using Vancouver Scar Scale (VSS)

3.6.4.1 Data collectors

The Mesotherapy Eporex treatment on the 30 participating women that received bilateral mammary reduction surgery commenced six (6) weeks after surgery. The researcher, plastic surgeon and independent assessor (laser therapist), each assessed both of the participants' breasts by evaluating the linear scars produced by bilateral mammary reduction surgery using the VSS (Appendix D). All the assessments post-operatively were individually recorded by the researcher, plastic surgeon and independent assessor on a pre-set assessment table (Appendix E).

3.6.4.2 Biasness

Biasness was addressed by keeping both the plastic surgeon and independent assessor blind to which breast was receiving the Mesotherapy Eporex treatment. Both breasts were evaluated using the VSS before the Mesotherapy Eporex treatment with active ingredients commenced (baseline) and then before the 3rd, 5th, 7th and after the 8th treatment. The researcher applied the treatment and was not blinded to the breast receiving treatment. Although the researchers' results was not taken into consideration when conclusions were made, results of the relative change observed over time of the VSS measurement for the researcher is displayed in Chapter 4 to show how the researcher's results compared with the plastic surgeon and independent assessor.

It is important to note that the researcher's results were also not taken into account when any recommendations were made about the effectiveness of Mesotherapy Eporex treatment with active ingredients on scar appearance after bilateral mammary reduction surgery because biasness could not be excluded.

3.6.4.3 Vancouver Scar Scale measurements

Each experimental breast was subjected to eight (8) Mesotherapy Eporex treatments with active ingredients. The researcher and independent assessor performed scar assessment measurements using the VSS on all participants before the 1st (baseline), 3rd, 5th, 7th and after the 8th treatment. The plastic surgeon only assessed the participants before the 1st (baseline) and after the 8th Mesotherapy Eporex treatment. Figure 3.5 summarize the assessment schedule followed during the treatment period. The VSS measurements were only done before the 1st (baseline), 3rd, 5th, 7th and after the 8th treatment because it was deemed to provide sufficient data to observe a trend to establish whether Mesotherapy Eporex treatment was effective in reducing scar appearance after bilateral mammary reduction surgery.

Although assessments were done throughout the course of the Mesotherapy Eporex treatments the assessment prior to treatment one (1) and the assessment after treatment eight (8) were statistically the most important measurements.

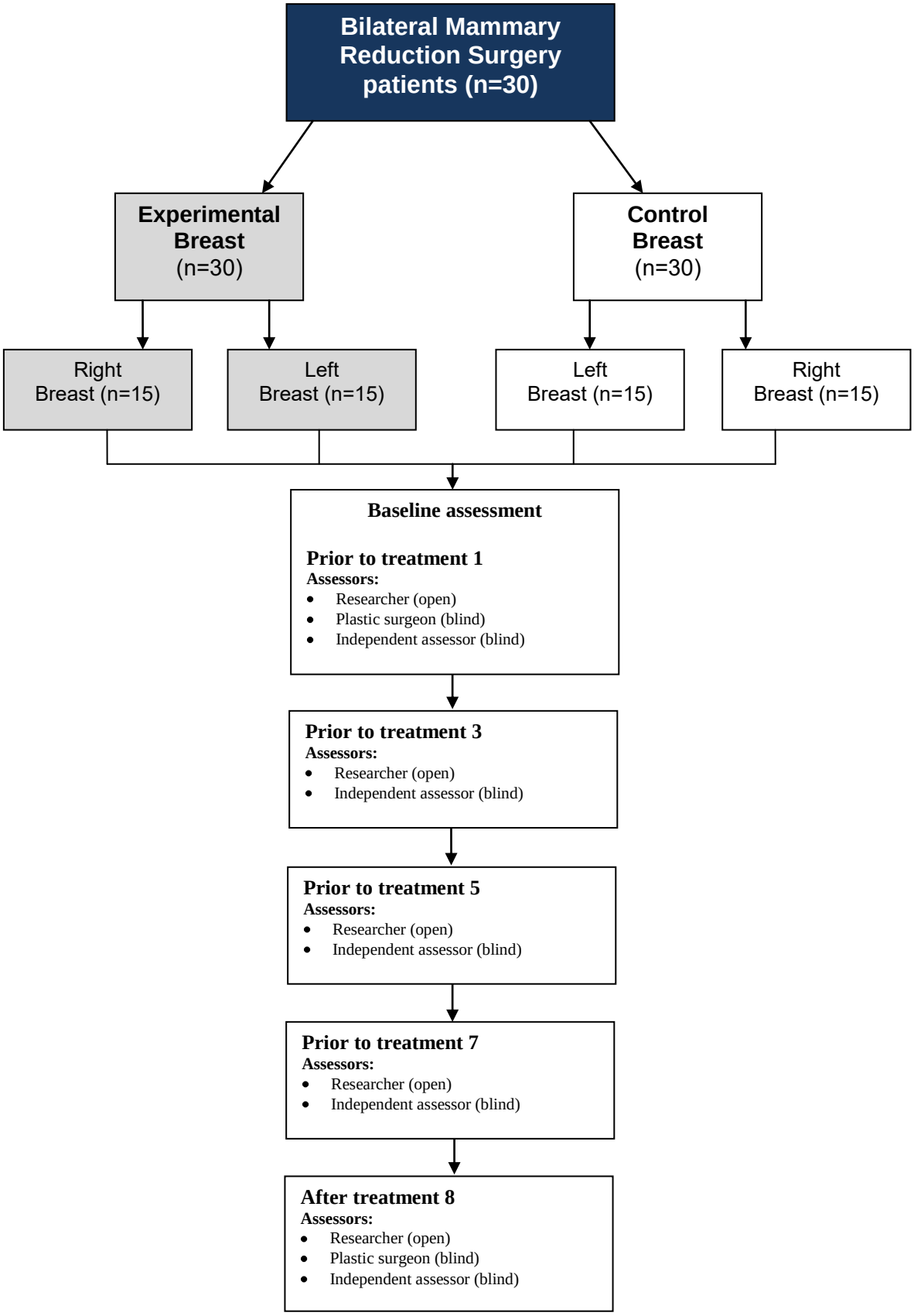


Figure 3.5 **VSS assessment schedule prior to and after Mesotherapy Eporex treatments**

[Open – Researcher aware of which breast was receiving the experimental treatments, Blind – plastic surgeon and independent assessor blinded to which breast was receiving the treatment]

The VSS has four (4) categories with possible scores of zero (0) to between two (2) and five (5) depending on the characteristic being assessed (vascularity 0-3, pigmentation 0-2, pliability 0-5 and height 0-3). Scores are allocated by comparing each characteristic with the adjacent healthy skin. The scale is scored on a range of 0-13, where zero (0) reflects normal skin healing and 13, poor skin healing. Thus, the lower the VSS score, the better the scar appearance (Table 3.3).

Table 3.3 Vancouver Scar Scale (VSS) (Kaartinen *et al*., 2011)

PARAMETER	DESCRIPTOR	POINTS/SCORES
Vascularity	Normal	0
	Pink	1
	Red	2
	Purple	3
Pigmentation	Normal	0
	Hypo-pigmentation	1
	Hyper-pigmentation	2
Pliability	Normal	0
	Supple	1
	Yielding	2
	Firm	3
	Ropes	4
	Contracture	5
Height	Flat	0
	<2mm	1
	2-5 mm	2
	>5 mm	3
TOTAL SCORE		**/13**

For the purpose of this research study the vascularity of the surgical scars (pink, red and purple) was supported by utilising a colour index (Table 3.4). The colour index chart was provided to each assessor as a guideline for the assessment of the scar vascularity to ensure that the assessors' perception of the colours did not vary greatly.

Table 3.4 Colour index assessing vascularity using the Vancouver Scar Scale

Pink	Red	Purple
		
Pink refers to a light shade of red	Red refers to a colour or pigment; the chromatic colour resembling the hue of blood.	An intermediate colour between red and blue, however more red than blue.

**The amount of redness assessed in scar tissue is dependent on the presence of blood vessels in the area, which is tested by how quickly the skin turns red after blanching (pressure applied to the scar using the pointer finger and then releasing allowing blood flow to return to the area)* (Hallam *et al*., 2013).

Scar height was measured from the natural skin surface to the elevated point of the scar with a ruler. The same ruler was used by all three (3) assessors.

A) Validation of Vancouver Scar Scale

Scar assessment is part of the evaluation process of patients requiring rehabilitation after surgery. The efficacy of these assessments is based on the use of validated tools, important to ensure reliable outcome measurements. Literature on the VSS focuses predominantly on burn scars, but has also been validated for rating post-surgical scars (Truong *et al*., 2005; Ferriero *et al*., 2010; Kaya *et al*., 2015). In scientific scar studies, subjective assessment with scar rating scales, the VSS in particular, remains the most well-known and mostly used (Idriss and Maibach, 2009; Kaartinen *et al*., 2011; Nguyen *et al*., 2015). Although it has been reported that subjective assessment is unreliable, it has been considered the gold standard in scar assessment. The VSS scale has an acceptable internal consistency of 0.79 (Cronbach's alpha) and 0.66 inter-observer reliability (Spearman's correlation coefficients) for overall scores ($p < 0.001$) (Truong *et al*., 2005; Ferriero *et al*., 2010).

The VSS is also the assessment tool of choice used by the plastic surgeon that formed part of the research team in this study to assess post-operative scarring in women receiving bilateral mammary reduction surgery.

3.6.5 Breast photography

Photographic images were taken to visually compare the control breasts to the experimental breasts prior to the first, and after the last, treatment. Note that these photographs are not regarded as a scientific result but only added for illustration purposes (Appendix F).

3.7 Statistical analysis

On completion of all eight (8) Mesotherapy treatments the demographic-, anthropometric-, clinical data and results generated from the VSS were captured in Microsoft ® Excel ® 2013 (Microsoft Corporation, Redmond, USA) for data cleaning. All numerical data such as age, weight, height, ptosis, amount of breast tissue removed were checked for validity using the following formulae:

=*IF* (data is outside normal range) *OR* (cell contains sting data), *check data validity*

The data were then exported to R (R Core Team, 2014) for analysis. The data was further summarised and transformed using R scripts. Numeric and graphical data summaries were provided as a starting point. The data was summarised using means, percentages and frequencies for categorical data and medians and percentiles for continuous data. Significant differences were noted at $p < 0.05$. This is equivalent to a one (1) in 20 rate of false discovery and is the standard in clinical trials. A p<5% indicates that the observed statistic (from the data) is unlikely to occur (less than 5% chance) if the assumption of no improvement after treatment was true. It thus provides evidence against the assumption that the treatment is not effective.

For the VSS assessments the data measurement scale was given as less is better, and this was maintained through all transformations. The first transformation was that the baseline [prior to treatment one (1)] was subtracted from the endpoint [after treatment eight (8)]. This gave a measurement of the change observed over time. The second transformation was that the control breast measurements were subtracted from the experimental breast measurements. This gave relative change observed over time, relative to the baseline

value. This relative change observed over time (RCOOT) is what was analysed going forward using a paired t-test. We will refer to this as the treatment effect from here on.

The difference in scar appearance between the right and the left breast was determined by generating p-values from the Two Sample version of Hotelling's T^2 test.

The assumption was that there is no treatment effect. The natural, commonly used statistical test for this is the t-test. However, there is a dependence structure present in the data caused by multiple observations belonging to the same patient effect. To account for this the multivariate equivalent test, known as Hotelling's T^2 test was used.

After we have established whether the Mesotherapy treatment was effective, independent variables (plastic surgeon relative to independent assessor, age, ptosis, BMI and amount of breast tissue removed) that could play a role in the effectiveness of the treatment (dependent variable) were investigated, while accounting for the difference between the two (2) assessors (plastic surgeon and independent assessor). A linear model was fitted using the Ordinary Least Squares (OLS) methodology taking into account the factors (plastic surgeon relative to independent assessor, age, ptosis, BMI, amount of breast tissue removed) that might have an impact on the treatment effectiveness.

The internal consistency of the VSS was tested by means of Cronbach's alpha and the inter-observer reliability of the VSS was tested by means of the interclass correlation coefficient.

3.8 Ethical aspects and good clinical practice

3.8.1 Ethical clearance

Ethical clearance (ECUFS NR 185/2012) was obtained from the University of the Free State's Ethics Committee before the study commenced (Appendix G).

Upon completion of the study the researcher availed herself to treat the control breast in exactly the same manner as the experimental breast.

3.8.2 Good Clinical Practice (GCP)/Quality assurance

All clinical work conducted during this research project was subjected to the Good Clinical Practice guidelines. The Declaration of Helsinki's basic principle number three (3), states that research should be conducted only by scientifically qualified people and under the supervision of adequately qualified personnel involved (South African Good Clinical Practice guidelines, 2006 ; World Medical Association Declaration of Helsinki, 2013). Fundamentally GCP requires oversight of the local ethics committee, verification of the investigator's qualifications, a study protocol, informed consent and essential documentation needed to undertake the study, monitoring, submission of reports and maintenance of records. By applying the GCP guidelines in this research study, public assurance is provided that the rights, safety and wellbeing of the participants are protected and that the research data are credible.

3.8.3 Confidentiality

Participants were assigned a study identification number between P1–P30 to ensure confidentiality at all times and non-disclosure of personal details.

At no time during the research study was any of the participants' identification or information made known to any individual(s) that were not part of the research team. All the information regarding this study is kept confidential and kept in secured storage for a period of five (5) years.

CHAPTER 4

RESULTS

4.1 Introduction

This chapter presents the demographic, anthropometric and clinical results recorded for the 30 women receiving bilateral mammary reduction surgery that participated in the research study. This is followed by the results obtained from the VSS measurements (plastic surgeon and independent assessor) determining the effectiveness of the Mesotherapy Eporex treatments. The percentage of scar appearance for each of the four (4) categories included in the VSS was calculated for both the blinded assessors and for the independent assessor only. The independent assessor, ptosis, age, amount of breast tissue removed and BMI were investigated as independent variables that might impact the effectiveness of the Mesotherapy Eporex treatment. The chapter concludes with the validity and reliability results of the VSS.

4.2 Demographic, anthropometric and clinical data

4.2.1 Demographic data

The mean age of the 30 participating women was 42 years; the youngest being 21 years and the oldest, 73 years of age. Advanced age is known to affect the wound healing process, but every participant acted as her own control. Age was considered only as an independent variable to assess whether age would be a factor that could influence the effectiveness of the Mesotherapy Eporex treatment.

Most (n=17) of the participants were African Americans (57%) whereas 12 were Caucasian (40) and only 1 participant was Asian (3%).

4.2.2 Anthropometric data

The 30 participants had a mean weight and height of 77.2kg and 1.62m, respectively, and the mean BMI for all participants was 23.9kg/m^2 ranging between 18kg/m^2 and 30 kg/m^2 (Table 4.1).

Table 4.1 Anthropometric data

Variable (Unit)	25th percentile Minimum	75th percentile Maximum	Standard deviation	Mean	Minimum	Maximum
Height (m)	1.58	1.65	0.06	1.62	1.52	1.75
Weight (kg)	70.5	87.3	10.8	77.2	57.0	100.0
BMI (kg/m^2)	22.0	26.7	3.3	23.9	18.0	30.0

[BMI = Body Mass Index; m = meter; kg = kilogram; m^2 = meter square]

Sixty three percent (63.0%) of the participants presented with a normal BMI and the remaining 37.0% of participants were classified as overweight according to their BMI (Corbin *et al*., 2016). None (0%) of the participants that received breast reduction surgery presented with a low (good fitness zone) or obese (high risk) BMI (Figure 4.1).

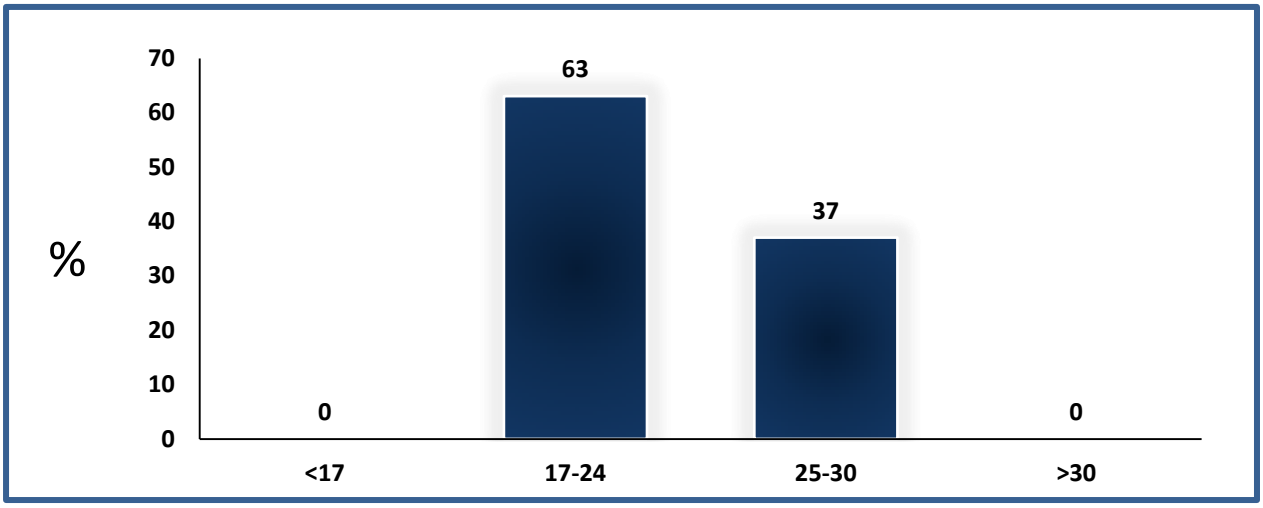


Figure 4.1 Participants (n=30) BMI rating scale classification (<17 = low; 17-24 = normal; 25-30 = overweight and >30 = obese) (Corbin *et al*., 2016)

4.2.3 Clinical data

4.2.3.1 Concomitant Medication

Prescription and over-the-counter drugs and supplements taken by the participants along with the study intervention were recorded as required by regulatory bodies. This information was only collected as a history item and at no time during the study were participants requested to discontinue any of the medication taken due to their participation in the study. Because every participant acted as her own control the influence of medication on the scar appearance after the Mesotherapy Eporex treatment was not investigated in this study. Twelve (40.0%) of the participants took concomitant medication which continued during the study if need be. Eight (26.6%) of the participants took multivitamins and five (5) participants (16.6%) took anti-depressants (Table 4.2).

Table 4.2 Concomitant medication

Variable	Yes n (%)	No n (%)
Concomitant medication	**12 (40.0)**	**18 (60.0)**
Multivitamins	8 (26.6)	22 (73.4)
Anti-depressants	5 (16.6)	25 (83.4)
Hypertension medication	4 (13.4)	26 (86.6)
Iron	4 (13.4)	26 (86.6)
Asthma inhaler	3 (10.0)	27 (90.0)
Magnesium	3 (10.0)	27 (90.0)
Vitamin C	2 (6.6)	28 (93.4)
Primrose oil	2 (6.6)	28 (93.4)
Aloe salt	1 (3.4)	29 (96.6)
Calcium	1 (3.4)	29 (96.6)
Altroxin	1 (3.4)	29 (96.6)
Controloc	1 (3.4)	29 (96.6)
Pro-biotic	1 (3.4)	29 (96.6)
Coxflam	1 (3.4)	29 (96.6)
Equidin	1 (3.4)	29 (96.6)
Cholesterol	1 (3.4)	29 (96.6)
Omega 3's	1 (3.4)	29 (96.6)

4.2.3.2 Bra size

Bra size was recorded by the plastic surgeon prior to surgery, six (6) (20.0%) of the participants wore a 34D bra size, five (5) (16.7%) a 38DD, and a further five (5) (16.7%) a 44DD (Table 4.3). The average BMI for the participants with a 34D bra size was 20.6 kg/m^2, for 38DD; 22.1kg/m^2 and for 44DD; 27.4kg/m^2. The bigger bra sizes were reported for the overweight participants as can be seen from the BMI values. Obesity is a known indication for increased breast size and post-operative complications after breast reduction surgery. None (0%) of the participants presented as obese according to their BMI classification. Therefore, obesity was not the determining factor for increased breast size in this study population. Besides obesity an increase in breast size can also be attributed to genetics and the result of hormones produced during the participants different life stages.

Table 4.3 Bra sizes of mammary reduction participants prior to surgery (n=30)

Bra size	n (%)
34C	2 (6.6)
34D	6 (20.0)
36C	2 (6.6)
36E	1 (3.4)
38C	2 (6.6)
38D	2 (6.6)
38DD	5 (16.7)
40DD	1 (3.3)
42D	1 (3.3)
42DD	3 (10.0)
44DD	5 (16.7)

4.2.3.3 Breast ptosis

If the distance measured from the supra sterna notch down to the nipple exceeds 21cm then a patient is regarded as having breast ptosis (Khan and Bayat, 2008). Breast ptosis was measured prior to surgery and a median of 32.0cm was recorded for all the participants. The minimum ptosis was 24.0cm and the maximum was 45.0cm (Table 4.4). Therefore all the participants (100%) in this study presented with breast ptosis.

Table 4.4 Breast ptosis of participants prior to surgery (n=30)

Ptosis (cm)	
Median	32.0
Standard deviation	5.0
25th Percentile	30.0
75th Percentile	36.5
Minimum ptosis	24.0
Maximum ptosis	45.0

It is also important to know the degree of ptosis because it provides an indication to the plastic surgeon of the amount of breast tissue to remove during surgery and it is also one of the most objective ways to decide what surgical technique (besides surgeon preference) is suitable for each individual because ptosis can be clinically measured (Andrades and Prado, 2008). The grade of ptosis was determined using the Regnault classification system (1976) (Castro *et al*., 2013). To determine the grade of ptosis, a proximal measurement from the nipple to the inframammary crease was measured in cm. Ninety seven percent (97%) of the participants presented with Grade 3 (major ptosis) meaning that the nipple is more than 3cm below the inframammary crease (Table 4.5).

Table 4.5 Degree of ptosis according to the Regnault classification system (1976)

Variable	Grade 1 (Minor ptosis)	Grade 2 (Moderate ptosis)	Grade 3 (Major ptosis)
Degree of ptosis (n=30)	0 (0%)	1(3%)	29 (97%)

4.2.3.4 Amount of breast tissue removed

The weight of breast tissue removed from each breast was measured intra-operatively. The median weight of breast tissue removed from the right breast during mammary reduction surgery was 674.0g and from the left breast, 673.5g. The minimum weight of breast tissue removed from both breasts combined during surgery was 317.0g and the maximum combined weight of breast tissue removed was 5761.0g (Table 4.6).

Table 4.6 Breast tissue removed during mammary reduction surgery (n=30)

Breast Tissue Removal (g)	Right Breast (g)	Left Breast (g)	Total (R+L)
Median	674.0g	673.5g	1347.5g
Standard deviation	737.8g	634.0g	1371.7g
25th Percentile	503.7g	457.2g	961.0g
75th Percentile	1038.0g	1021.2g	2059.2g
Minimum breast tissue removed	121.0g	196.0g	317.0g
Maximum breast tissue removed	3273.0g	2488.0g	5761.0g

[g = gram; R = right; L = left]

4.2.4 Vancouver Scar Scale measurements

4.2.4.1 Experimental breast versus control breast

Only the experimental breast was treated with the Mesotherapy Eporex machine. Both breasts were assessed by the independent assessor (before the 1st, 3rd, 5th, 7th and after the 8th treatment) and plastic surgeon (before the 1st and after treatment 8).

Keeping in mind that less is better the mean VSS score of the plastic surgeon and independent assessor improved when the mean endpoint VSS score [after treatment eight (8)] was compared with the mean baseline [prior to treatment one (1)] VSS score for both the experimental and control breast (Table 4.7).

Table 4.7 Mean VSS assessment results for plastic surgeon and independent assessor

Assessment:	Plastic Surgeon		Independent Assessor	
	Exp Breast	Con Breast	Exp Breast	Con Breast
1st visit assessment	**6.2**	**6.2**	**5.1**	**5.3**
3rd visit assessment	**-**	**-**	**4.2**	**4.7**
5th visit assessment	**-**	**-**	**3.6**	**4.0**
7th visit assessment	**-**	**-**	**2.7**	**3.7**
After 8th visit assessment	**2.6**	**4.3**	**2.7**	**3.5**

[Exp = experimental; Con = control; - =no assessment done during that time interval]

The scar characteristics of the experimental breast (▲) appear to improve more with treatment compared to the control breast (■) (not receiving treatment) for both the plastic surgeon and independent assessor (Figure 4.2A and B). The mean VSS score of the independent assessor showed an improvement from baseline to treatment seven (7) reaching a plateau at treatment eight (8) for the experimental breast. Whereas, the control breast showed a gradual improvement from baseline to end point (Figure 4.2B). Figure 4.2B showed that the improvement in scar appearance was the greatest between the mean baseline [prior to treatment one (1)] VSS score and the mean 3rd visit assessment VSS score for the independent assessor for both the experimental (5.13 vs. 4.23) and control breast (5.3 vs. 4.73).

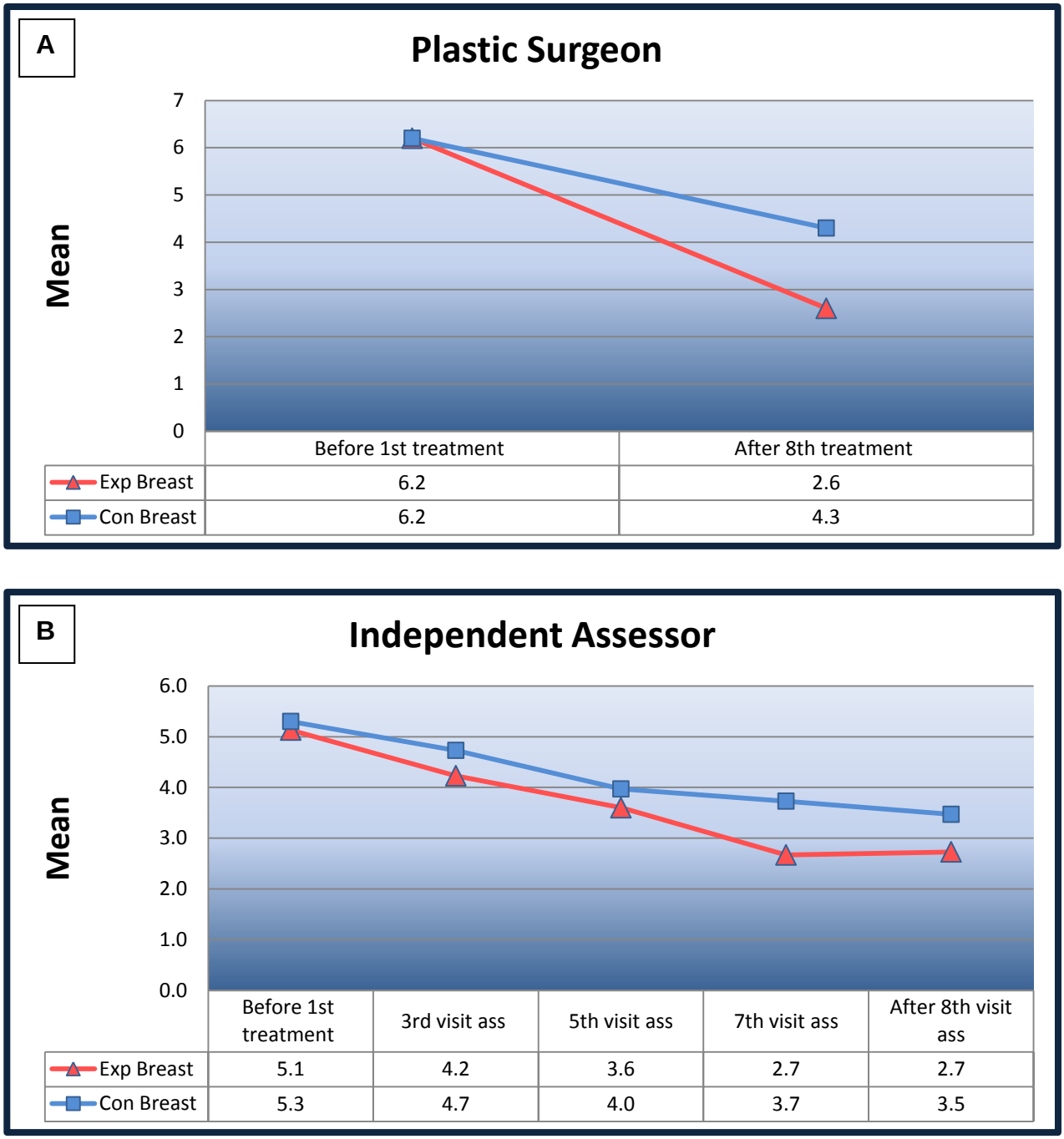


Figure 4.2 **Mean VSS for the experimental and control breast over time (n=30)** [*(A-B) – mean individual VSS assessments by assessor*

4.2.4.2 Effectiveness of Mesotherapy Eporex treatment using VSS measurements

The data measurement scale was given as less is better, and this was maintained through all transformations. The first transformation was that the starting measurements were subtracted from the ending measurements. This gave a measure of the change observed over time. The second transformation was that the control measurements were subtracted from the experimental measurements. This gave relative change observed over time, relative to the baseline value [prior to visit one (1)]. This relative change observed over time (RCOOT) is what was analyzed going forward using a paired t-test. We will refer to this as the treatment effect from here on.

The assumption was that there is no treatment effect. The natural, commonly used statistical test for this is the t-test. However, there is a dependence structure present in the data caused by multiple observations belonging to the same patient effect. To account for this the multivariate equivalent test, known as Hotelling's test was used.

When considering the experimental phase, which was the primary focus of the experiment, then there were 30 complete participant observations. All three assessors (plastic surgeon, independent assessor and researcher's) VSS measurements indicated an improvement for the treatment breast, relative to the control breast, over time (Table 4.8). When the baseline to endpoint change in the experimental breast was compared to the baseline to endpoint change in the control breast, the plastic surgeon VSS measurements indicated the highest level of improvement, an average difference of -1.700, indicating better healing or less scarring for the experimental breast compared to the natural healing of the control breast. Because the researcher was not blinded to the breast receiving treatment the researcher's results will be excluded from all the statistical analysis going forward.

The p-value for the blinded assessors (plastic surgeon and independent assessor) was 0.0002. According to this value it is safe to conclude that the treatment is

effective, based on this sample ($p<0.05$). Therefore, if the results were extended to other populations we are 95% certain that the same treatment effect will be obtained.

Table 4.8 Relative change observed over time for all 3 assessors (n=30)

Independent assessor (p-value)	Plastic surgeon (p-value)	Researcher (p-value)
-0.567	-1.700	-1.433

The results were further broken down for interest sake. If only the plastic surgeons' assessments were considered then again a highly significant p-value of 0.0002 was obtained, if only the assessments of the independent assessor was considered then the p-value was 0.0842. This was close to being significant but not conclusive. When using a non-inferiority methodology a p-value of <0.1 is considered statistically significant.

A comparison was made between the plastic surgeon and independent assessor in terms of correlation, variability and average values. There was no evidence of correlation (p-value=0.3705) between the results of the independent assessor and plastic surgeon. With regard to variability there was no difference (p-value=0.1950) and in terms of average values, there was a borderline no difference (p-value=0.0501) between the plastic surgeon and independent assessor. The VSS is a subjective measurement tool and is known for its inter-observer variability, and it is no surprise that there is no evidence of correlation between the assessments made by the plastic surgeon and independent assessor. Although there was a difference with regard to the VSS measurements between the plastic surgeon and independent assessor, both indicated an improvement in scar appearance (VSS measurements) after the completion of the Mesotherapy Eporex treatment.

Due to the evidence of no correlation of the results between the independent assessor and plastic surgeon and because the independent assessor performed assessments throughout the treatment process only the independent assessors results was further statistically analyzed.

The total scores (per participant) for the control and experimental groups were expressed as percentages and the change from baseline was calculated. The scores

reported for visit one (1) were defined as the baseline value and the last visit (visit eight (8) was the endpoint value. The changes were therefore calculated as visit 8 - visit 1. These changes were subjected to a t-test and the 95% confidence interval (CI) was calculated for the total VSS score. The individual values for change from baseline for the control and experimental breast are available in Table 4.9.

Table 4.9 Change from baseline (%) for the control and experimental groups per participant

Subject	Change_Control	Change_Experimental
p1	-7.69	-30.77
p2	7.69	-30.77
p3	0.00	-15.39
p4	-7.69	-15.39
p5	-46.15	-30.77
p6	0.00	-15.39
p7	-7.69	-7.69
p8	23.08	0.00
p9	0.00	-15.39
p10	-23.08	-38.46
p11	-30.77	-30.77
p12	-15.39	-15.39
p13	-15.39	-15.39
p14	-23.08	-23.08
p15	-15.39	-23.08
p16	0.00	-15.39
p17	-23.08	-23.08
p18	-38.46	-38.46
p19	-7.69	-7.69
p20	-7.69	-23.08
p21	-15.39	-23.08
p22	-7.69	-23.08
p23	-30.77	-30.77
p24	-23.08	-23.08
p25	-61.54	-23.08
p26	-7.69	-7.69
p27	-7.69	0.00
p28	-7.69	-15.39
p29	-7.69	-15.39
p30	-7.69	-7.69

[Change_control = change from baseline for control group/per participant; Change_Exprimental = change from baseline for experimental group/per participant]

Descriptive statistics for the change from baseline for the experimental and control breast are depicted in Table 4.10.

Table 4.10 Descriptive statistics: change from baseline

Variable	Min	1st Qu	Median	Mean	3rd Qu	Max
Change_Control	-61.538	-23.077	-7.692	-13.333	-7.692	23.077
Change_Experimental	-38.462	-23.077	-19.231	-18.974	-15.385	7.692

[Change_control = change from baseline for control group; Change_Experimental = change from baseline for experimental group; Min = minimum; 1st Qu = 1st quadrant; 3rd Qu = 3rd quadrant; Max = maximum]

The 95% CI for the change in baseline for the experimental and control group is -10.70 to -0.58 (Table 4.11). Therefore, in this trial, the change from baseline in the experimental group is at most 11% more and at least 0.6% more than in the control group, with an average of -5.64% (Table 4.11). Statistical significance is confirmed by the fact that the 95% confidence interval exclude zero. Thus taking the individual baselines in consideration, the endpoint score (visit 8) in the experimental group is overtly lower than the endpoint score of the control group (Table 4.11).

Table 4.11 Statistical analysis result: change from baseline

Variable	t	df	95% confidence interval	Sample estimates: mean difference
Change_Experimental and Change_ Control	-2.2817	29	-10.70 ; -0.58	-5.64

[Change_Experimental and Change_Control = change from baseline for experimental group and change from baseline for control group]

4.2.4.3 VSS scores: vascularity, pigmentation, pliability and height

The total VSS score is divided into four (4) categories assessing vascularity, pigmentation, pliability and height. Table 4.12 summarizes the mean results of the experimental and control breast recorded by the two (2) blinded assessors (plastic surgeon and independent assessor) for each of the four (4) categories included in the VSS.

Table 4.12 Mean VSS score categories: experimental versus control breasts (n=30)

Assessment:	Plastic Surgeon		Independent Assessor	
	Exp Breast	Con Breast	Exp Breast	Con Breast
1st visit assessment	6.2	6.2	5.1	5.3
Vascularity	1.4	1.4	1.4	1.2
Pigmentation	1.4	1.3	1.3	1.3
Pliability	2.7	2.7	1.7	1.8
Scar Height	0.8	0.8	0.8	0.9
3rd visit assessment	-	-	4.2	4.7
Vascularity	-	-	1.1	1.2
Pigmentation	-	-	1.2	1.2
Pliability	-	-	1.3	1.5
Scar Height	-	-	0.6	0.8
5th visit assessment	-	-	3.6	4.0
Vascularity	-	-	0.9	1.0
Pigmentation	-	-	1.2	1.2
Pliability	-	-	1.0	1.2
Scar Height	-	-	0.5	0.6
7th visit assessment	-	-	2.7	3.7
Vascularity	-	-	0.7	0.9
Pigmentation	-	-	1.0	1.1
Pliability	-	-	0.6	1.2
Scar Height	-	-	0.3	0.5
After 8th visit assessment	2.6	4.3	2.7	3.5
Vascularity	0,5	0,8	0.7	0.8
Pigmentation	0.9	1.3	0.9	1.0
Pliability	1.0	1.7	0.8	1.1
Scar Height	0.2	0.5	0.3	0.5

[Exp = experimental; Con = control; - = no assessment done during that time interval]

A Line (scatter graph with only points, no lines) was created to illustrate the vascularity, pigmentation, pliability and scar height improvements for both the control and experimental breast by comparing the baseline levels with the results obtained after the 8th treatment for the plastic surgeon and independent assessor combined (Figure 4.3). Although an improvement can be seen in all four (4) of the categories included in the VSS, scar pliability decreased the most when comparing the baseline assessment with assessment eight (8) in both the experimental and control breast (Figure 4.3).

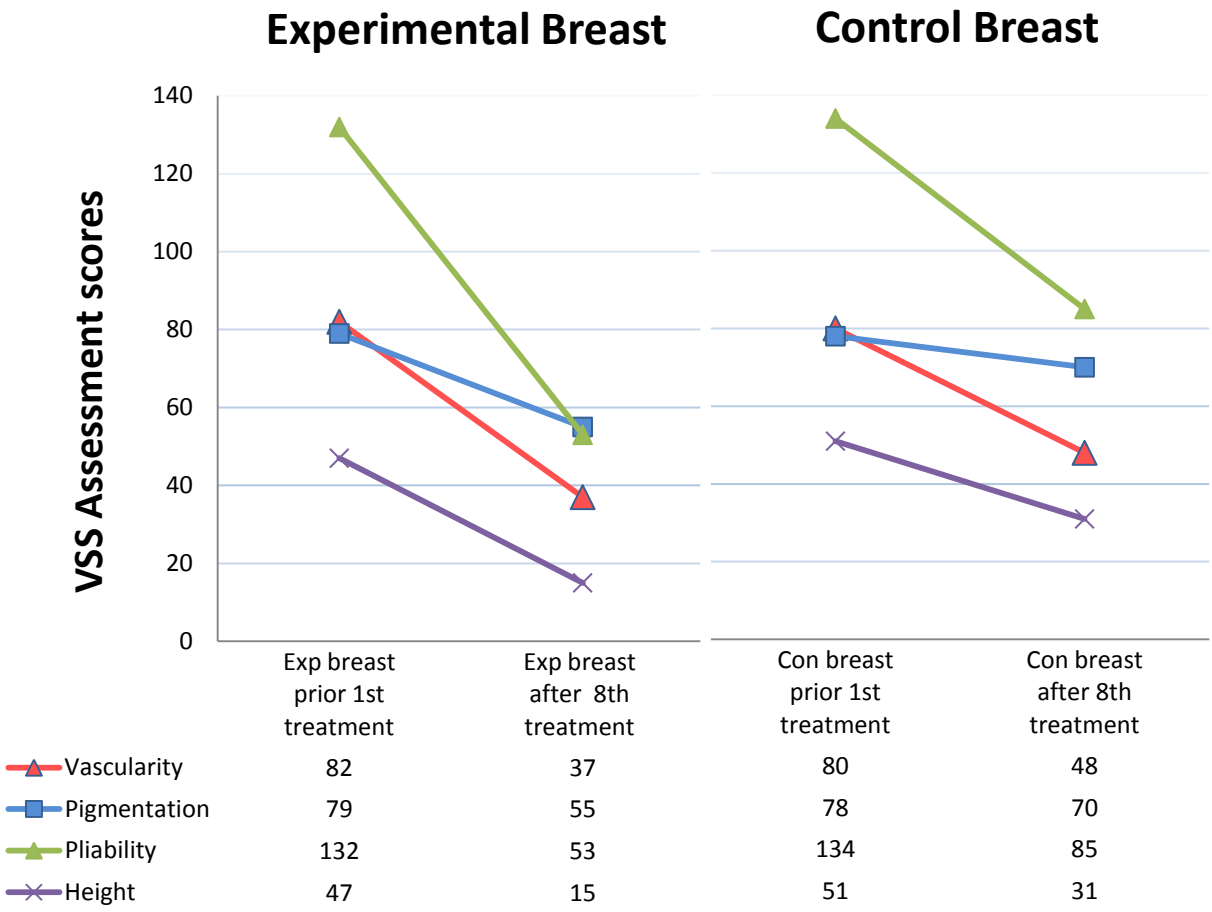


Figure 4.3 **VSS assessment of scar characteristics for experimental and control breast (n=30)** **[commencement of treatment = 6 weeks post-operative]**

A mean percentage of improvement of the control and experimental breast for each category of the VSS was calculated using the results of the plastic surgeon and independent assessor (both blinded) (Figure 4.4A). The percentage of improvement for all four (4) categories of the VSS was also calculated by including just the results of the independent assessor (Figure 4.4B). The mean percentages for each of the

four (4) VSS categories were calculated by subtracting the mean results from treatment eight (8) from the mean baseline results.

Statistical method calculating mean percentage of improvement for both assessors (example for vascularity):

- 1st treatment = [Sum of vascularity score per participant (plastic surgeon) + sum of vascularity score per participant (independent assessor) / 2 = mean vascularity score per participant]
- Sum of mean vascularity score per participant / 30 = mean vascularity score
- 8th treatment = [Sum of vascularity score per participant (plastic surgeon) + sum of vascularity score per participant (independent assessor) / 2 = mean vascularity score per participant]
- Sum of mean vascularity score per participant / 30 = mean vascularity score
- Mean 8th treatment vascularity score – mean 1st treatment vascularity score 100/1 = - value (the negative value is ignored because less is better).

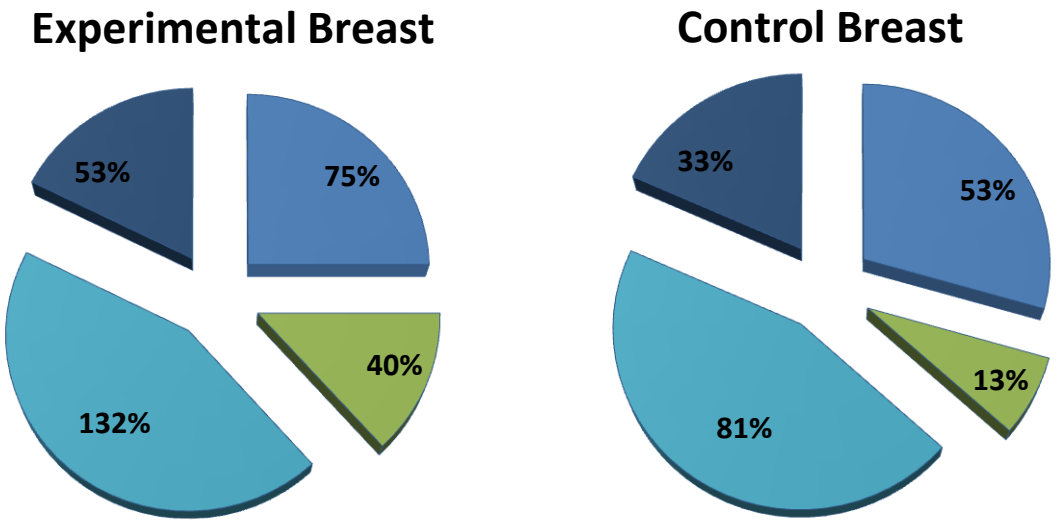
Statistical method calculating mean percentage of improvement for independent assessor (example for vascularity):

- 1st treatment = (sum of vascularity score per participant / 30 = mean vascularity score)
- 8th treatment = (sum of vascularity score per participant / 30 = mean vascularity score)
- Mean 8th treatment vascularity score – mean 1st treatment vascularity score 100/1 = - value (the negative value is ignored because less is better).

The percentage of improvement for pliability was the highest for both the control and experimental breast, 81% and 132%, respectively (plastic surgeon and independent assessor's results combined) (Figure 4.4A). Pliability also improved the most when only the result of the independent assessor was taken into account (90% experimental breast and 67% control breast) (Figure 4.4B). Besides pliability the percentage of improvement seen in the other three (3) categories (vascularity,

pigmentation and scar height) was also higher in the experimental breast than in the control breast for plastic surgeon and independent assessors results combined and for only the results of the independent assessor.

A - **Assessors VSS combined results**



B - **Independent VSS results**

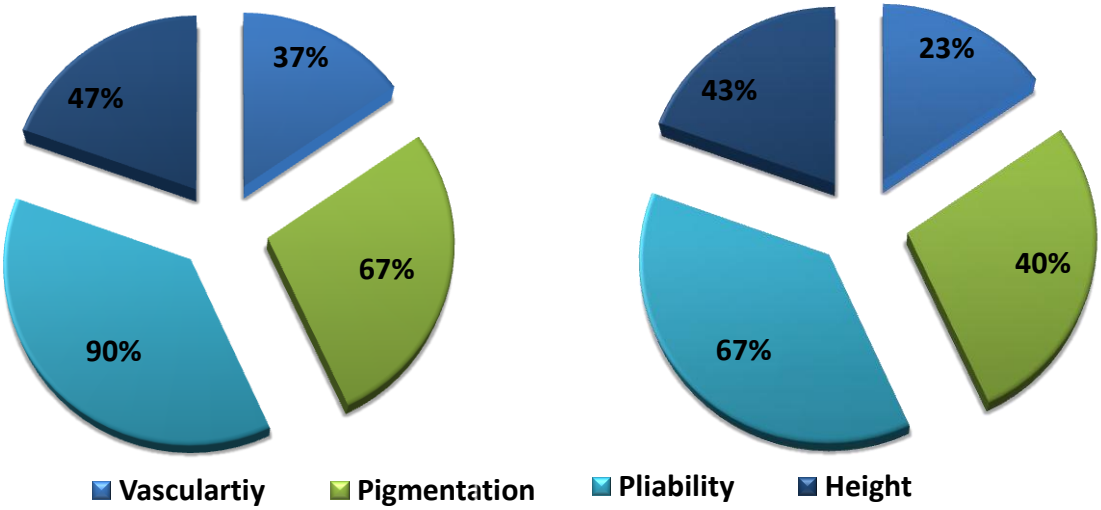


Figure 4.4 **Mean percentage of improvement of each VSS category: experimental and control breast (n=30)**

[A) Mean percentage of improvement of VSS results for plastic surgeon and independent assessor combined B) Mean percentage of improvement of independent assessor's VSS results]

Again, due to the evidence of no correlation between the results of the independent assessor and plastic surgeon and because the independent assessor performed assessments throughout the treatment process only the independent assessor's results was statistically analyzed using the following transformations and analyses. The individual baseline scores (visit 1) and mean follow-up scores (visits 3, 5, 7 and 8) were transformed to percentages by dividing the scores by 13 (total of the scores over the four (4) parameters) and expressing these scores as percentages. The individual scores are depicted in Table 4.13.

Table 4.13 Individual baseline and follow-up scores (vascularity, pigmentation, pliability, height) expressed as percentages for control and experimental breast

	Individual baseline (visit 1) scores transformed to percentages								Mean follow-up (visits 3, 5, 7 and 8) scores transformed to percentages							
Subject	BVas_C	BVas_E	BPig_C	BPig_E	BPli_C	BPli_E	BHeig_C	BHeig_E	MVas_C	MVas_E	MPig_C	MPig_E	MPli_C	MPli_E	MHeig_C	MHeig_E
p1	0.00	7.69	7.69	7.69	30.77	30.77	7.69	7.69	9.62	3.85	7.69	7.69	19.23	11.54	3.85	1.92
p2	15.39	15.39	0.00	0.00	23.08	7.69	7.69	15.39	15.39	3.85	9.62	0.00	15.39	7.69	7.69	0.00
p3	7.69	7.69	7.69	7.69	7.69	23.08	7.69	23.08	7.69	5.77	7.69	5.77	7.69	7.69	7.69	7.69
p4	7.69	15.39	7.69	7.69	7.69	7.69	7.69	7.69	7.69	9.62	5.77	1.92	3.85	1.92	0.00	0.00
p5	15.39	15.39	15.39	15.39	23.08	23.08	15.39	0.00	11.54	9.62	11.54	9.62	13.46	9.62	7.69	5.77
p6	0.00	0.00	15.39	15.39	7.69	23.08	0.00	0.00	7.69	3.85	15.39	15.39	7.69	7.69	1.92	1.92
p7	7.69	7.69	7.69	7.69	7.69	7.69	0.00	0.00	7.69	7.69	7.69	7.69	3.85	3.85	0.00	0.00
p8	7.69	7.69	7.69	7.69	7.69	7.69	0.00	0.00	7.69	7.69	7.69	7.69	9.62	7.69	5.77	1.92
p9	7.69	7.69	7.69	7.69	15.39	15.39	7.69	7.69	3.85	7.69	3.85	7.69	15.39	7.69	7.69	0.00
p10	15.39	15.39	7.69	7.69	15.39	15.39	7.69	7.69	5.77	3.85	7.69	5.77	9.62	5.77	0.00	0.00
p11	7.69	7.69	7.69	7.69	7.69	7.69	7.69	7.69	5.13	3.85	3.85	3.85	3.85	3.85	3.85	0.00
p12	15.39	15.39	7.69	7.69	15.39	7.69	15.39	7.69	7.69	7.69	7.69	7.69	15.39	7.69	9.62	3.85
p13	15.39	15.39	7.69	7.69	7.69	7.69	7.69	7.69	9.62	13.46	7.69	9.62	11.54	11.54	11.54	11.54
p14	7.69	7.69	7.69	7.69	7.69	7.69	0.00	0.00	1.92	1.92	1.92	1.92	1.92	1.92	1.92	0.00
p15	7.69	7.69	7.69	7.69	30.77	30.77	7.69	0.00	7.69	7.69	11.54	11.54	26.92	21.15	3.85	3.85
p16	7.69	7.69	7.69	7.69	15.39	15.39	15.39	15.39	11.54	7.69	9.62	7.69	17.31	7.69	11.54	7.69
p17	7.69	7.69	7.69	7.69	7.69	15.39	7.69	15.39	3.85	7.69	3.85	5.77	5.77	7.69	3.85	9.62
p18	23.08	23.08	15.39	15.39	7.69	7.69	7.69	7.69	5.77	5.77	11.54	11.54	3.85	3.85	7.69	7.69
p19	23.08	23.08	7.69	7.69	15.39	15.39	0.00	0.00	9.62	7.69	7.69	7.69	17.31	11.54	9.62	5.77
p20	7.69	7.69	15.39	15.39	23.08	23.08	15.39	15.39	7.69	7.69	15.39	15.39	23.08	13.46	13.46	9.62
p21	7.69	7.69	7.69	7.69	15.39	7.69	7.69	7.69	1.92	0.00	7.69	7.69	9.62	1.92	7.69	1.92
p22	7.69	7.69	7.69	7.69	15.39	15.39	7.69	7.69	7.69	5.77	7.69	5.77	9.62	7.69	5.77	0.00
p23	7.69	7.69	15.39	15.39	15.39	15.39	7.69	7.69	3.85	7.69	7.69	7.69	5.77	7.69	0.00	3.85
p24	7.69	7.69	7.69	7.69	15.39	7.69	7.69	7.69	7.69	7.69	7.69	5.77	1.92	1.92	1.92	1.92
p25	23.08	23.08	15.39	15.39	15.39	7.69	15.39	7.69	7.69	7.69	5.77	5.77	3.85	9.62	3.85	5.77
p26	7.69	7.69	7.69	7.69	7.69	7.69	0.00	0.00	7.69	7.69	7.69	7.69	1.92	1.92	0.00	0.00
p27	7.69	7.69	15.39	15.39	7.69	7.69	7.69	7.69	7.69	7.69	15.39	15.39	7.69	9.62	3.85	3.85
p28	7.69	7.69	15.39	15.39	7.69	7.69	0.00	7.69	7.69	7.69	15.39	15.39	0.00	1.92	0.00	0.00
p29	7.69	7.69	15.39	15.39	7.69	7.69	0.00	7.69	7.69	7.69	15.39	15.39	1.92	1.92	0.00	3.85
p30	7.69	7.69	7.69	7.69	7.69	7.69	7.69	7.69	7.69	7.69	7.69	7.69	11.54	9.62	5.77	7.69

[p = participant; BVas_C = Baseline Vascularity Control; BVas_E = Baseline Vascularity Experimental; BPig_C = Baseline Pigmentation Control; BPig_E = Baseline Pigmentation Experimental; BPli_C = Baseline Pliability Control; BPli_E = Baseline Pliability Experimental; BHeig_C = Baseline Height Control; BHeig_E = Baseline Height Experimental; MVas_C = Mean Vascularity Control; MVas_E = Mean Vascularity Experimental; MPig_C = Mean Pigmentation Control; MPig_E = Mean Pigmentation Experimental; MPli_C = Mean Pliability Control; MPli_E = Mean Pliability Experimental; MHeig_C = Mean Height Control; MHeig_E = Mean Height Experimental]

The baseline scores (per category) for the control and experimental groups were subjected to a t-test, testing for the difference in means for paired data. Ninety five percent (95%) confidence intervals were calculated and reported (Table 4.14). No statistically significant differences were reported for any of the four (4) categories (vascularity, pliability, pigmentation and height) included in the VSS. This is confirmed by the fact that the 95% confidence intervals (CIs) include zero.

Table 4.14 Baseline values (per category) for the control and experimental group

Variable	t	df	95% confidence interval	Sample estimates: mean difference
Baseline vascularity (experimental) and baseline vascularity (control)	1.44	29	-0.22 ; 1.24	0.51
Baseline pigmentation (experimental) and baseline pigmentation (control)	Not calculated	29	Not calculated (due to equal means and variance)	0.00
Baseline pliability (experimental and baseline pliability (control)	-0.24	29	-2.45 ; 1.94	-0.26
Baseline scar height (experimental) and baseline scar height (control)	0.25	29	-1.81 ; 2.32	0.26

Then, the mean follow-up (visit 3, 5, 7, and 8) scores (per category) for the control and experimental groups were subjected to a t-test, testing for the difference in means for paired data. Ninety five percent (95%) confidence intervals were also calculated. Statistically significant differences were seen for the parameters pliability (CI: -3.88; -0.87) and scar height (CI: -2.58; -0.11) as is confirmed by the fact that the 95% confidence interval exclude zero (Table 4.15). Therefore, in this trial, pliability improved at most 4% more and at least 0.9% more in the experimental group than in the control group, with an average of -2.37% (Table 4.11). Scar height improved at most 3% more and at least 0.1% more in the experimental group than in the control group, with an average of -1.35% (Table 4.11).

Table 4.15 Mean follow-up VSS score (per category) for control and experimental group

Variable	t	df	95% confidence interval	Sample estimates: mean difference
Mean vascularity (experimental) and mean vascularity (control)	-1.23	29	-1.82 ; 0.45	-0.68
Mean pigmentation (experimental) and mean pigmentation (control)	-1.43	29	-1.40 ; 0.25	-0.58
Mean pliability (experimental) and mean pliability (control)	-3.22	29	-3.88 ; -0.87	-2.37
Mean scar height (experimental) and mean scar height (control)	-2.22	29	-2.58 ; -0.11	-1.35

The individual mean follow-up (visit 3, 5, 7, and 8) scores (per category) transformed to percentages for the control and experimental groups are presented in Figure 4.5-4.8. Because less is better the majority of the individuals showed lower percentage values for the experimental breast compared to the control breast for all four (4) VSS categories.

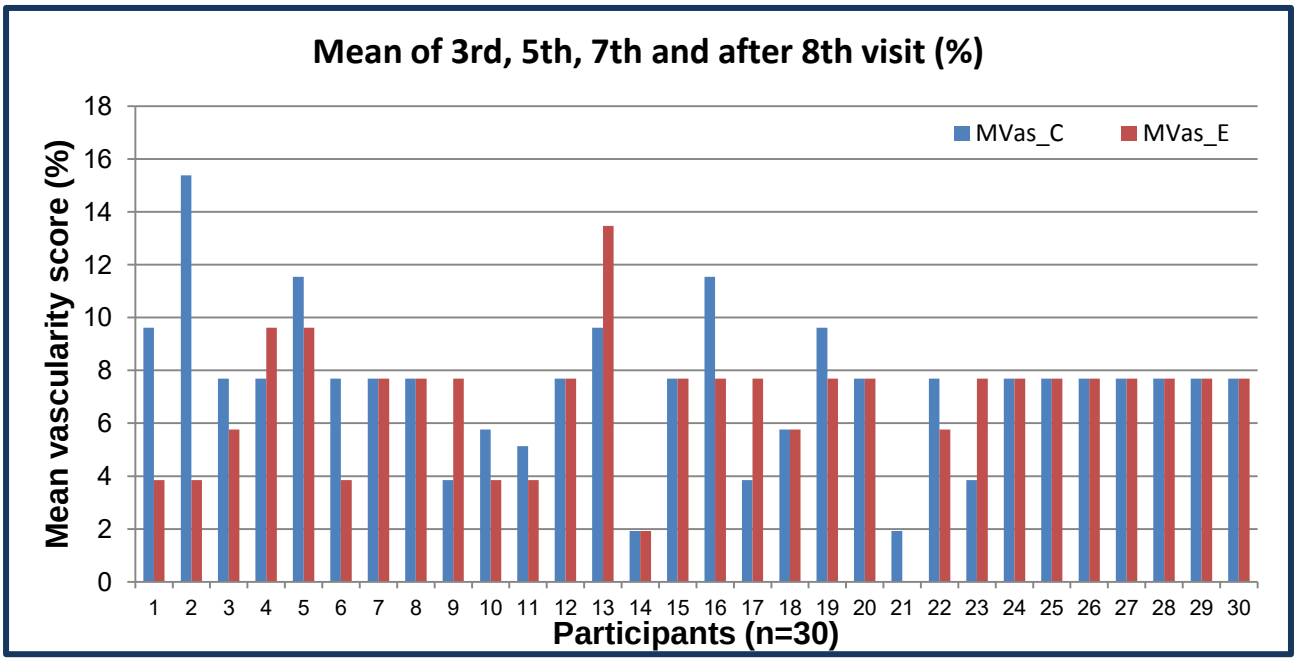


Figure 4.5 **Mean vascularity follow-up scores (3rd, 5th, 7th and after 8th visit) for the control and experimental groups** (if no bar is displayed for either the control or experimental breast it means that the value = 0)
[MVas_C = Mean Vascularity Control; MVas_E = Mean Vascularity Experimental]

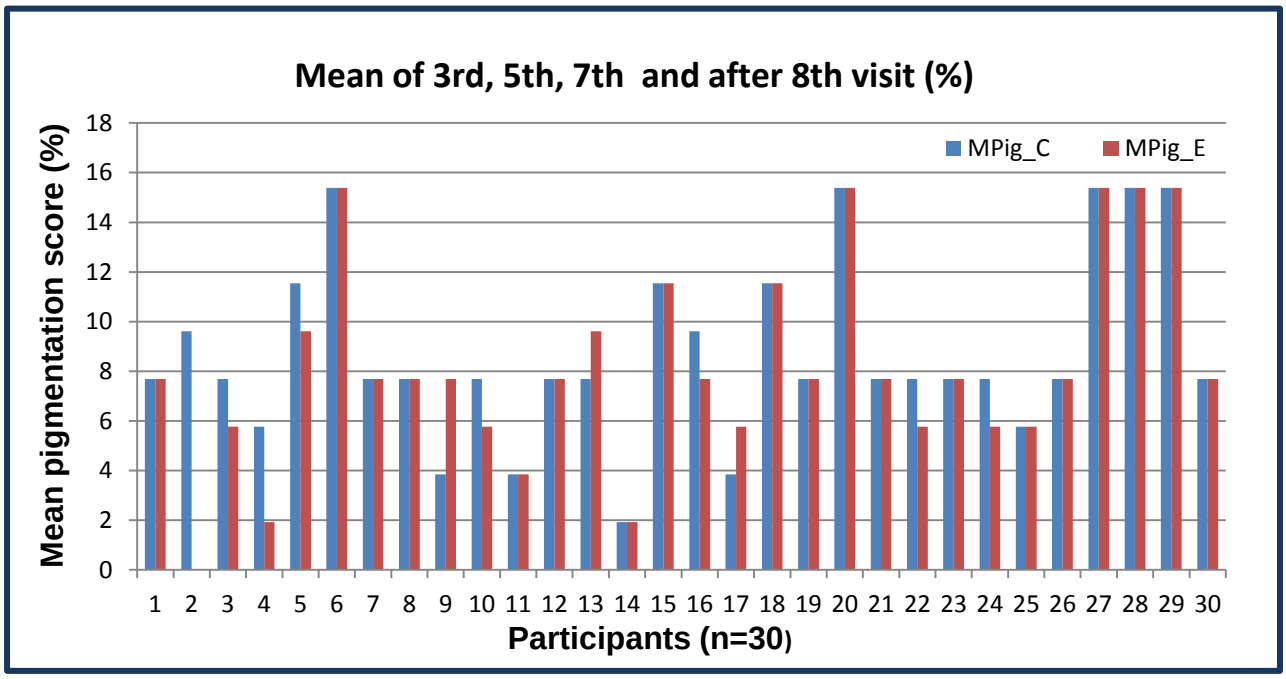


Figure 4.6 **Mean pigmentation follow-up scores (3rd, 5th, 7th and after 8th visit) for the control and experimental groups** (if no bar is displayed for either the control or experimental breast it means that the value = 0)
[MPig_C = Mean Pigmentation Control; MPig_E = Mean Pigmentation Experimental]

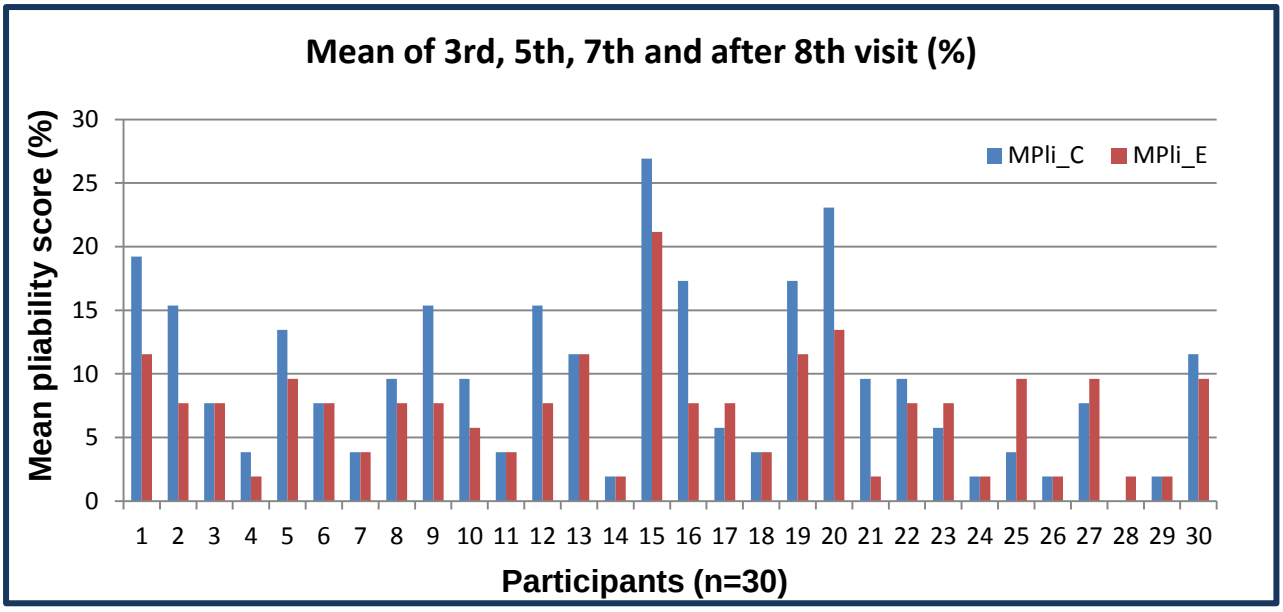


Figure 4.7 **Mean pliability follow-up scores (3rd, 5th, 7th and after 8th visit) for the control and experimental groups** (if no bar is displayed for either the control or experimental breast it means that the value = 0)
[MPli_C = Mean Pliability Control; MPli_E = Mean Pliability Experimental]

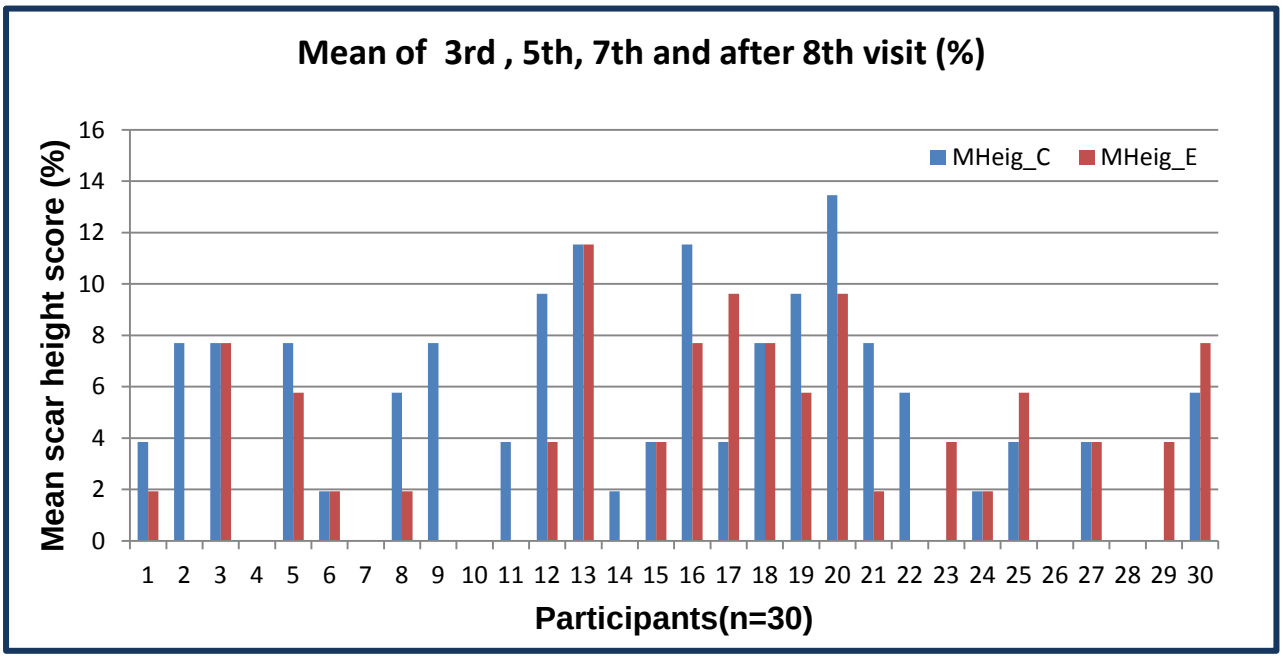


Figure 4.8 **Mean scar height follow-up scores (3rd, 5th, 7th and after 8th visit) for the control and experimental groups** (if no bar is displayed for either the control or experimental breast it means that the value = 0)
[MHeig_C = Mean scar Height Control; MHeig_E = Mean scar Height Experimental]

4.2.5 Scar appearance of the right breast versus the left breast

The appearances of the mammary reduction scars were compared between the right and left breast to determine if there is a difference between the healing ability of the left and the right breast. No statistical evidence was observed in the relative healing

between the right and left experimental breasts (p=0.3288). The p-values were determined from the Two Sample version of Hotelling's T^2 test.

4.2.6 Factors impacting the effectiveness of Mesotherapy Eporex treatment

After establishing that the treatment was effective, it was important to investigate which factors played a role in the effectiveness of the Mesotherapy Eporex treatment, while accounting for a possible difference between the assessors (plastic surgeon and independent assessor). This information can be used to advise a patient in advance of the treatment and what level of treatment effect to expect.

It is important to note that the dependent variable was not healing but treatment effectiveness, i.e. how much more healing occurred under treatment than under control. First, a complete linear model was fitted, taking into account many factors (plastic surgeon relative to the independent assessor, age, ptosis, breast tissue removed from right breast, breast tissue removed from left breast and BMI) that might, hypothetically, have an impact on the treatment effectiveness (Table 4.16).

Table 4.16 Factors impacting the effectiveness of Mesotherapy Eporex treatment

Independent Variable	Estimate	Error	t-stat	p-value	Significance
Intercept	-5.233	2.550	-2.052	0.045	*
Plastic surgeon (relative to independent assessor)	-1.133	0.490	-2.313	0.025	*
Age	-0.006	0.022	-0.257	0.798	
Ptosis	0.203	0.084	2.405	0.020	*
Breast tissue removed right	-0.002	0.001	-2.129	0.038	*
Breast tissue removed left	0.002	0.001	1.543	0.129	
BMI	-0.059	0.093	-0.636	0.527	
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1 Residual standard error: 1.897 on 53 degrees of freedom Multiple R-squared: 0.2335, Adjusted R-squared: 0.1467 F-statistic: 2.691 on 6 and 53 DF, p-value: 0.02354					

Statistical significant differences were observed for the intercept (p=0.045), plastic surgeon relative to the independent assessor (p=0.025), ptosis (p=0.020) and the amount of breast tissue removed from the right breast (p=0.038) (Table 4.16). The intercept was the treatment effectiveness indicated by the independent assessor for a patient with 0 age, ptosis, breast tissue removed and BMI (kg/m^2). Remember that the data measurement scale was given as less is better. The independent assessor was considered the baseline, and then the plastic surgeon observed significantly greater treatment effectiveness (estimate = -1.133), after adjusting for the other factors. Therefore, on average the plastic surgeon observed 1.133 more treatment effectiveness (improvement in scar appearance) relative to the independent assessor (Table 4.16). The treatment is less effective the higher the ptosis value (estimate = 0.203). Interesting results were obtained for the amount of breast tissue removed from the right breast because the more breast tissue removed from the right breast the more the treatment effectiveness (estimate = -0.002). A possible reason could be that the patients receiving right treatment first may have randomly had more right (or left) breast tissue removed in general, leading to bias. The model as a whole is significant, indicating that the given factors do explain variation in treatment effectiveness (23% to be precise) (Table 4.16).

Table 4.17 appears to be the best model for factors impacting the effectiveness of the Mesotherapy Eporex treatment since it does not include the confounding effect of the highly insignificant variables. There is little difference between this model (Table 4.17) and the model provided in Table 4.16.

Table 4.17 Significant factors impacting the effectiveness of Mesotherapy Eporex treatment

Independent Variable	Estimate	Error	t-stat	p-value	95% CI
Intercept	-6.117	2.219	-2.757	0.008	-10.56 ; -1.67
Plastic surgeon (relative to independent assessor)	-1.133	0.483	-2.344	0.023	-2.10 ; -0.16
Ptosis	0.180	0.077	2.324	0.024	0.03 ; 0.33
Breast tissue removed right	-0.003	0.001	-2.288	0.026	-0.01 ; -0.00
Breast tissue removed left	0.002	0.001	1.705	0.094	-0.00 ; 0.01
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 Residual standard error: 1.872 on 55 degrees of freedom Multiple R-squared: 0.2255, Adjusted R-squared: 0.1692 F-statistic: 4.004 on 4 and 55 DF, p-value: 0.006384					

After excluding the highly insignificant factors from the model the p-values were; intercept (p=0.008), plastic surgeon relative to the independent assessor (p=0.023), ptosis (p=0.024) and breast tissue removed from the right breast (p=0.026) (Table 4.17). The intercept was the treatment effectiveness indicated by the independent assessor for a patient with 0 ptosis and breast tissue removed. According to the CI in Table 4.17 the plastic surgeon observed at most 2% more and at least 0.1% more improvement in scar appearance, after adjusting for the other factors. The higher the ptosis value the less the treatment effectiveness. The CI showed that ptosis caused at most a 0.03% and at least a 0.16% decrease in scar appearance (treatment effectiveness). The CI for the amount of breast tissue removed right showed at most a 0.01% and at least a 0.00% more improvement in scar appearance (Table 4.17). This percentage of improvement or decline is very low.

Again, the model as a whole is significant, indicating that the given factors do explain variation in treatment effectiveness (23% to be precise) (Table 4.17).

4.2.7 Reliability of the VSS measurement tool

The internal consistency of the VSS was tested by means of Cronbach's alpha and the inter-observer reliability of the VSS was tested by means of the interclass correlation

coefficient. An interclass correlation coefficient of one (1) indicates that measurements carried out by different numbers of observers produce the same results. The inter-observer reliability was tested when two (2) observers (plastic surgeon and independent assessor) assessed the scar areas and for a single observer (independent assessor). The internal consistency of the VSS appeared to be lower than acceptable (Cronbach's alpha, 0.4355). The corresponding interclass correlation coefficient value for the VSS was 0.2888 with a 95% confidence interval of -0.069 to 0.583.

CHAPTER 5

DISCUSSION

5.1 Introduction

After cosmetic breast surgery the final breast appearance is the most important result that ultimately determines patient satisfaction. In patients with mammary hypertrophy, (extremely large breasts) and ptosis (nipple sagging) a decrease in psychological and physical discomfort are contributing factors why patients opt for mammary reduction surgery (Setälä *et al*., 2009). The operative procedure has evolved from a mere reduction of breast mass to enhanced aesthetic appeal with a minimum scar load (Saleem and John, 2013; Crerand and Magee, 2013). However, post-operative scarring can be a major source of dissatisfaction after aesthetic breast surgery (Monstrey *et al*., 2014).

In most mammary reduction surgery cases, normal scarring is the result of an uncomplicated healing process. However, abnormal scarring develops when the healing process of a wound does not follow the body's expected healing phases; known as the inflammatory, proliferative and remodelling phases. The healing process follows a specific time sequence with temporary overlapping; and abnormal scarring can occur due to prolonging of the inflammatory and proliferative phases (Reinke and Sorg, 2012). The majority of non-healing wounds fail to progress through these phases of wound repair and remain in a chronic inflammatory state which ultimately leads to abnormal wound repair and the development of hypertrophic or keloid scars (Velnar *et al*., 2009; Reinke and Sorg, 2012).

To promote wound healing and reduce scarring, several treatment methods have been introduced, for example, invasive Mesotherapy (injection), lasers, compression therapy, cryotherapy, corticosteroids, topical silicone gel, radiation and Bleomycin (Gauglitz, 2013; Son and Harijan, 2014). A new non-invasive technique namely Mesotherapy with active ingredients applied by the Mesotherapy Eporex machine was used in this study to evaluate whether the treatment could improve the scar appearance six (6) weeks after bilateral mammary reduction surgery. The Mesotherapy Eporex machine assists in the transdermal

delivery of active ingredients (with varying molecular weight) via an electrical pulse (isophoresis) which stimulates a structural re-arrangement of the skin's semi-permeable cell membranes. The process of structural re-arrangement is necessary to allow water soluble ingredients to penetrate the skin's protective sebum layer found in the upper epidermis (Fabbrocini *et al*., 2007). The Mesotherapy Eporex technique by which both ionised and neutral drugs can be transported into the dermis and subcutaneous tissue are mainly used for the treatment of cellulite, skin rejuvenation, hyper-pigmentation, reduction of wrinkles, pores and skin lifting/tightening (Konda and Thappa, 2014).

Therefore, the aim of the research study was to evaluate the effectiveness of Mesotherapy Eporex treatments on scar appearance in participants receiving bilateral mammary reduction surgery. Each participant received eight (8) treatments and scar appearance was evaluated using the VSS prior to the 1st, 3rd, 5th, 7th and after the 8th treatment.

5.2 Demographic, anthropometric and clinical data

The mean age of the 30 participants was 42 years with a mean weight and height of 77.2kg and 1.62cm, respectively. Each participant served as her own control, as healing in each individual occurs differently.

Only thirty seven percent (37.0%) of the participants were classified as being overweight (25-30kg/m^2) and none of the participants in this study were classified as obese (over 30.0kg/m^2) according to the BMI (kg/m^2) rating scale (Corbin *et al*., 2016) (Figure 4.1). The BMI results corresponded with literature because mammary reduction surgeries are not recommended for obese patients (Brown *et al*., 2012). In the case of obese patients the plastic surgeon would request the patient to lose weight and to keep it off for a period of six (6) months to one (1) year prior to mammary reduction surgery to prevent and limit possible surgical complications (Chopra *et al*., 2013). Lapid *et al*. (2013) contradicts this statement regarding BMI stating that macromastia (hypertrophy and gigantomastia) is usually correlated with a higher BMI (kg/m^2), however he does support the fact that more thin patients with large breasts present for mammary reduction surgery (Lapid *et al*., 2013).

The bra size prior to surgery ranged from a 34D-44DD. The average BMI (kg/m^2) for the participants with a 34D bra size was 20.6kg/m^2 (normal), for 38DD; 22.1kg/m^2 (normal) and

for a 44DD; 27.4kg/m^2 (overweight). Because none of the participants in the study were obese, the increase of bra sizes could not be attributed to obesity. If obesity is not related to increased breast size, the reason for enlarged breasts could be the result of hormones produced during the participants different life stages (foetal growth, pubertal expansion, pregnancy and post-menopausal). During these life stages fluctuations in hormones such as oestrogen, progesterone, prolactin, insulin, thyroxine and growth hormones, can contribute to the development of macromastia (Wolfswinkel *et al*., 2013).

Breast ptosis was determined by measuring the distance from the supra sterna notch down to the nipple in cm. If this measurement exceeds 21cm then a patient has breast ptosis (Khan and Bayat, 2008). The minimum breast ptosis value recorded for all participants were 24cm therefore, all participants in the study presented with breast ptosis (median =32cm) (Table 4.4). An increased ptosis value provides an indication for the breast tissue and amount of skin resection required during surgery and to estimate the correct nipple height and breast shape (Andrades and Prado, 2008). However, limited literature is available on the reliability of standard breast measurements, such as ptosis and suprasternal notch-nipple distance (Hansson *et al*., 2014).

Using the Regnault classification system (1976) breast ptosis can also be classified as Grade I (minor), Grade II (moderate) and Grade III (major) based on the proximal measurement from the nipple to the inframammary crease measured in cm (Castro *et al*., 2013). The degree of ptosis is determined by the plastic surgeon prior to surgery for it can provide an indication for the surgical technique (skin resection amount) and the amount of breast tissue to be removed during surgery. Ninety seven percent (97%) of the participants in the study presented with Grade III ptosis. According to Andrades and Prado (2008) more than 1000g of breast tissue can be removed when a patient present with Grade III (major) ptosis. In this study less than 1000g of breast tissue was removed in nine of the participants (30%). This could be attributed to the plastic surgeon considering patient preference and expectation with regard to desired bra size post-operatively. Between 1000-1500g of breast tissue was removed in eight (27%) participants and more than 1500g of breast tissue was removed in 13 participants (43%). The larger the amount of breast tissue and skin removed during mammary reduction surgery the higher the risk of more aggressive scar formation post-operatively due to the substantial loss of skin, tissue structure and breast function (Andrades and Prado, 2008; Murray *et al*., 2008).

5.3 Vancouver Scar Scale measurements

The researcher was not blinded to the breast receiving treatment therefore, the researcher's results were excluded from all the statistical analysis and only the plastic surgeon and independent assessor's results were included. The data measurement scale for the VSS (less is better) was maintained throughout all the statistical analysis.

The baseline [prior to treatment one (1)] to endpoint change [after treatment eight (8)] in the experimental breast was compared to the baseline to endpoint change in the control breast for the plastic surgeon and independent assessor. Both the plastic surgeon (-1.700) and independent assessor (-0.567) VSS measurements indicated an improvement (better healing or less scarring) for the experimental breast compared to the natural healing of the control breast ($p=0.0002$) (Table 4.8). This p-value ($p<0.05$) indicates a statistical significance therefore the Mesotherapy Eporex treatment was effective. If only the plastic surgeons' assessments were considered then again a significant p-value of 0.0002 was obtained, if only the assessments of the independent assessor was considered then the p-value was 0.0842.

A comparison was done between the VSS results of the plastic surgeon and independent assessor in terms of correlation, variability and average values. Although no evidence of correlation was found between the assessors ($p=0.3705$), their VSS results did not differ in terms of variability ($p=0.1950$) and average values ($p=0.0501$). Inter-observer reliability of the VSS is known to be unacceptably low and can be attributed to the fact that the VSS is a subjective assessment tool (Kaartinen *et al*., 2011, Nguyen *et al*., 2015). Subjective evaluation is known to be unreliable when done by a single observer, as is usually the case in clinical practice. By increasing the number of observers it is possible to increase the reliability of the results. However, in a study conducted by Kaartinen *et al*. (2011) they still reported that the reliability of the VSS was low even with three observers. Evidently, no matter how complicated the scar rating scales or how many observers used, we cannot overcome human limitations when fulfilling the role of an observer. Despite all the negative attributes of the VSS, the VSS remains the most well-known and mostly used scar rating scale (Idriss and Maibach, 2009; Kaartinen *et al*., 2011; Nguyen *et al*., 2015).

Because the results of the independent assessor and plastic surgeon did not correlate, the results of the plastic surgeon was excluded and a t-test and 95% CI was calculated for the total VSS score by subtracting the endpoint VSS score [after treatment eight (8)] from the baseline VSS score (prior to the 1st Mesotherapy Eporex treatment). The 95% CI for the change in baseline for the experimental and control group is -10.70 to -0.58 demonstrating that the change from baseline is at least 11% more and at least 0.6% more in the experimental group than in the control group (Table 4.11). This means that if a participant received Mesotherapy Eporex treatments she can expect a maximum of 10% improvement and a minimum of 0.6% improvement in scar appearance.

The improvement in scar appearance can be attributed to the application of the Mesotherapy Eporex treatment in combination with active ingredients [2ml *Asian Centella* (*Centella asiatica*), 5ml Vitamin C (20%) and 5ml Hyaluronic acid]. These aforementioned ingredients promote the proper formation of pro-collagen and a triple helix necessary for healing (Michels and Frei, 2013). The positive effects of the active ingredients include; reducing inflammation, assisting in collagen synthesis, restoring tissue firmness, increasing skin elasticity, decreasing collagen stiffness and acts as free radical scavengers (Guo and DiPietro, 2010; Papakonstantinou *et al*., 2012; Neuman *et al*., 2015).

The Mesotherapy Eporex machine makes use of transdermal delivery of active ingredients via isophoresis (a low frequency modulated alternating current) (Dhote *et al*., 2012; Panzade and Puranik, 2012). Transdermal delivery is when a water retaining medium encapsulates an ingredient and controls the release thereof into the skin. When the encapsulated ingredient enters the skin, it hydrates the area, allowing faster absorption of the ingredient into the blood stream via electroporation. Electroporation is a pulsating current which increases skin temperature and cell permeability which in turn temporarily disorganizes the temporary water pathways of the cellular membranes (Ita, 2016; Ma *et al*., 2014). The application of pulses stimulates the creation of new and/or creates enlargement of existing aqueous pathways in the stratum corneum. The skin's resistance decreases and water/oil soluble active ingredients are able to penetrate into the skin. Once the active ingredients have penetrated, the molecules (active ingredients) bind to natural enzymes within the body (Santra and Tseng, 2013).

5.3.1 VSS scores: vascularity, pigmentation, pliability and height

The VSS evaluate four (4) scar characteristics namely vascularity, pigmentation, pliability and scar height. A mean percentage of improvement for all four (4) categories included in the VSS was calculated using the results of the plastic surgeon and independent assessor and only the results of the independent assessor for both the control and experimental breasts. The mean percentages for each of the four (4) VSS categories were calculated by subtracting the mean results from treatment eight (8) from the mean baseline results.

All four (4) scar characteristics of the control and experimental breast improved over time but the highest percentage of improvement was seen in the experimental breast receiving Mesotherapy Eporex treatment.

5.3.1.1 Vascularity

The mean percentage of improvement (VSS results of plastic surgeon and independent assessor combined) for vascularity in the experimental breast was 75% compared to the 53% improvement seen in the control breast. The independent assessor reported a mean percentage vascularity improvement of 37% for the experimental breast and 23% for the control breast (Figure 4.4).

Blood supply is a common problem in injured skin and Goldberg *et al*. (2013) demonstrated that sufficient blood supply can increase the rate of wound healing, prevent infection and restore skin function. Improved blood supply in the area is important as it increases oxygen delivery and nutrient uptake to the injured area and assists in the removal of toxins facilitating increased cell metabolism and renewal. The improvement in vascularity can be attributed to the Mesotherapy Eporex current which creates friction (via electroporation) between the stainless steel hand piece and the skin in combination with the three (3) active ingredients selected (*Asian Centella*, Vitamin C and Hyaluronic acid). One of the characteristics of *Asian Centella* and Vitamin C is to assist in the development of blood vessels in the skin through collagen angiogenesis and epithelisation (Song *et al*., 2012).

The application of Vitamin C on incisional scars has beneficial effects on skin tissue healing, hydroxyproline levels, neovascularization, fibroblast maturation and collagen

disposition (Telang, 2013). The increase in hydroxyproline levels has the same effect as *Asian Centella*, the prevention of the over production of collagen and promotion of collagen angiogenesis epithelisation. In addition, Vitamin C connects to a hydroxylation enzyme in the skin, encouraging collagen to form a triple helix which is essential for the development and maintenance of scar tissue and blood vessels in the skin. Vitamin C stimulates dormant fibroblasts to divide and promote migration to the wound site. The fibroblasts are regenerated, promoting the healing of the skin tissue and ultimately improving the scar appearance (Sharma *et al*., 2008).

When visually assessed, the vascularity prior to the Mesotherapy Eporex treatments presented as a purple or red colour. The skin colour faded to a pink/normal skin colour (same as the surrounding skin) on completion of the treatments. This could be attributed to *Asian Centella* and Vitamin C enhancing antioxidant release in the skin by supporting and shortening the inflammatory phase (Collins, 2013). *Asian Centella* contains active components called triterpenes which include: asiaticosides, madecassoside and Asiatic acid. Triterpenes enhance normal human skin cell migration encouraging wound healing. Asiaticosides enhance antioxidant release in the skin which supports and shortens the inflammatory phase. A longer inflammatory phase allows oedema (swelling) to restrict the blood flow and oxygen levels to the wound site which results in poor wound healing (Gohil *et al*., 2010; Song *et al*., 2012).

The body's ability to absorb iron is increased by Vitamin C, contributing to higher oxygen levels in the skin (Telang, 2013). Vitamin C also acts as a potent anti-oxidant and anti-inflammatory ingredient (Guo and DiPietro, 2010). All these factors can contribute to the improvement of scar vascularity.

5.3.1.2 Pigmentation

The mean percentage of improvement (VSS results of plastic surgeon and independent assessor combined) for pigmentation in the experimental breast was 40% compared to the 13% improvement seen in the control breast. The independent assessor reported a mean percentage pigmentation improvement of 67% for the experimental breast and 40% for the control breast (Figure 4.4).

Hyper-pigmentation is a consequence of melanocyte recruitment which occurs at the wound site and can persist after the wound has successfully healed. This can be attributed to the keratinocytes situated at the wound edge that communicate a gene signature reflecting partial proliferative activation with several cell-cycle genes. The recruitment of melanocytes is generally the result of a heavy inflammatory response, particularly of neutrophills, and these cells may be phenotypically different from their equivalents in healing wound (Davis and Callender, 2010; Chadwick *et al*., 2012).

The use of Vitamin C as an active ingredient addresses pigmentation as it acts as a water soluble antioxidant protective reservoir. This action allows for the ability to lighten skin as it inhibits tyrosinase (enzyme for controlling melanin production) and assists in decreasing oxidative stress, resulting in radiant and bright skin (Lima *et al*., 2009). Copper ions at the wound site interact with Vitamin C inhibiting the action of the enzyme tyrosinase, thereby decreasing melanin formation and possible scar hyper-pigmentation (Telang, 2013). In this research study the use of Vitamin C as active ingredient in conjunction with the Mesotherapy Eporex treatment did not only contribute to wound healing but it also decreased pigmentation of scar tissue.

5.3.1.3 Pliability

The mean percentage of improvement (VSS results of plastic surgeon and independent assessor combined) for pliability in the experimental breast was 132% compared to the 81% improvement seen in the control breast. The independent assessor reported a mean percentage pliability improvement of 90% for the experimental breast and 67% for the control breast (Figure 4.4).

The increase in scar pliability could be attributed to the use of HA, because HA is known to improve tissue suppleness and contribute to the detanglement of collagen fibres formed during the healing process (Baspeyras *et al*., 2013). An investigation by Baspeyras *et al*. (2013) on the impact of HA in combination with Mesotherapy treatments showed a decrease in elastin and collagen fibre stiffness suggesting that the skin may regain suppleness by decreasing the entanglement of its collagen fibres thus restoring the mechanical behaviour of young skin. Neuman *et al*. (2015) utilized an injectable HA hydro-gel that provided an adhesion barrier in rat models following midline abdominal incision,

and this resulted in facilitated wound healing. HA absorption into the skin occurs when the Mesotherapy Eporex machine releases the conductive gel filled with active ingredients onto the skin, and via osmosis the HA is then transported by CD44 receptors to the healing site (Neuman *et al*., 2015). When HA is found in the epithelial tissue, keratinocyte proliferation is promoted, increasing the presence of retinoic acid which provides hydration in the skin. Hydrated skin cells could improve scar pliability and appearance. In addition, HA assisted in the transport of Vitamin C and *Asian Centella* across the stratum corneum skin layer with the help of naturally produced HA in the skin, including the electroporation process created by the Mesotherapy Eporex (Konda and Thappa, 2013).

Additionally, *Asian Centella* produces peptidic hydroxyproline which assists with collagen stabilization. Peptidic hydroxyproline prevents the over production of collagen which could contribute to keloid formation, and promotes collagen angiogenesis epithelisation (new blood vessel formation from pre-existing vessels). Collagen angiogenesis epithelisation assists in wound healing as the new vessels increase oxygen and nutrient delivery to the wound site resulting in improved wound healing (Song *et al*., 2012).

5.3.1.4 Scar height

The mean percentage of improvement (VSS results of plastic surgeon and independent assessor combined) for scar height in the experimental breast was 53% compared to the 33% improvement seen in the control breast. The independent assessor reported a mean percentage scar height improvement of 47% for the experimental breast and 43% for the control breast (Figure 4.4).

Scar height reflects the maturity of a scar and also the overabundance of collagen deposition (Gauglitz, 2011). During the inflammatory phase fibroblasts in the skin contribute to collagen formation in a scar which is usually circular and cyclical. Due to this process a thick, whitish collagen inside the provisional and collagen matrix results in an abundant production of packed collagen, giving rise to scars that are uneven in texture and height. Over time, during the proliferative phase the fibroblasts continue to rearrange the collagen fibres and the scar settles and becomes stiff (McDougall *et al*., 2006; Velnar *et al*., 2009). Vitamin C and *Asian Centella* prevent the overproduction of collagen, assisting in collagen

stabilization and they encourage antioxidants to be released thus improving scar appearance.

Therefore, by taking all the properties and characteristics of *Asian Centella,* Vitamin C and HA into consideration it is clear that these agents, in conjunction with Mesotherapy Eporex treatments, should have a positive effect on all four (4) scar characteristics in participants receiving mammary reduction surgery.

Due to the evidence of no correlation of the results between the independent assessor and plastic surgeon and because the independent assessor performed assessments throughout the treatment process the independent assessors results were subjected to a t-test, to determine the 95% CI. The baseline VSS scores per category for the control and experimental groups showed no statistically significant differences for vascularity (CI: 0.22; 1.24), pigmentation (CI: not calculated due to equal means and variance), pliability (CI: -2.24; 1.94) and height (CI: -1.81; 2.32) (Table 4.14). The CI was also calculated for the mean follow-up (treatment 3, 5, 7 and 8) VSS scores (per category) for the control and experimental groups. Statistically significant differences were seen for the categories pliability (CI: -3.88; -0.87) and height (CI: -2.58; -0.11) (Table 4.15).

However, it must be stated that problems with the VSS scale included separating between pigmentation and vascularity (Idriss and Maibach, 2009). Reason being that changes in both vascularity and pigmentation occur in the scar at a time overlapping each other, making colour definition difficult for the observer. Colour changes are also usually unevenly distributed across the scar, and estimating a mean value for a certain area is not evenly done by a human observer (Kaartinen *et al*., 2011).

5.4 Factors impacting the effectiveness of Mesotherapy Eporex treatment

The intercept (p=0.008), assessor (p=0.023), ptosis (p=0.024) and amount of breast tissue removed from the right breast (p=0.026) showed statistical significance therefore influencing the effectiveness of the Mesotherapy Eporex treatment (Table 4.17). Because the VSS is a subjective measurement tool and because of human inconsistency it is expected that the assessor could have an influence when evaluating the effectiveness of

the treatment. Reliability of the VSS remains a problem throughout literature (Kaartinen *et al*., 2011, Nguyen *et al*., 2015).

Ptosis, and the grade of ptosis is determine by plastic surgeons prior to surgery for it provides an estimate of the amount of skin resection and breast tissue to be removed during surgery (Andrades and Prado, 2007; Shiffman, 2009). Although ptosis alone is not the only confounding factor indicating the amount of breast tissue removed, patient preference to post-operative expected bra-size is also taken into account. All the participants in this study (100%) presented with breast ptosis. The larger the amount of breast tissue and skin removed during mammary reduction surgery the higher the risk of more aggressive scar formation post-operatively due to the substantial loss of skin, tissue structure and breast function (Andrades and Prado, 2008; Murray *et al*., 2008). In this study ptosis had a negative impact on the effectiveness of the Mesotherapy Eporex treatment.

Although the amount of right breast tissue removed had a positive effect on the Mesotherapy Eporex treatment which is contradicting the statement above, a possible reason could be that the patients receiving right treatment first may have randomly had more right (or left) breast tissue removed in general, leading to bias.

5.5 Reliability of the VSS measurement tool

Cronbach's alpha should be from 0.70 up to and including 0.90 to demonstrate consistency in a scale. An interclass correlation coefficient value of 0.70 is considered a minimum requirement for reliable results (Durani *et al*., 2009). The internal consistency of the VSS appeared to be not acceptable (Cronbach's alpha, 0.4355). The corresponding interclass correlation coefficient value for the VSS was 0.2888 with a 95% confidence interval of -0.069 to 0.583. The lack of consistency of the Vancouver scale might be explained by the fact that it includes a variable that appears to be a nominal variable (pigmentation). Consistency can be improved by increasing the number of observers. Despite the inconsistency of the VSS it remains one of the most well-known and widely used scar scales in clinical practice.

CHAPTER 6

CONCLUSION

6.1 Introduction

Enlarged breasts are a major concern for women all over the world impacting them both physically and emotionally. Mammary reduction is a well-known surgical procedure but not without complications. Various factors like age, obesity, medication, chronic diseases, nutrition, surgical procedure etc. can contribute to the development of post-operative complications. Post-operative scarring continues to be a reality and a lingering concern for many women and also one of the reasons why women sometimes opt not to go for surgery despite physical and psychological discomfort. For this reason women are continually seeking new treatment modalities to improve scar appearance post-operatively. A Mesotherapy Eporex treatment in combination with active ingredients is well established for treating cellulite, wrinkles, firming of the skin and muscle spasms. The effectiveness of the Mesotherapy Eporex treatment on post-operative scarring was assessed by evaluating scar appearance using the VSS during and after the treatment period.

No statistical significant differences were observed for demographic, anthropometric and clinical data indicating that the sample population was comparable. No difference in healing ability was observed when comparing the right experimental breast with the left experimental breast (p=0.3288). Both, the independent assessor (-0.567) and plastic surgeon (-1.700) reported improvements in scar appearance when comparing the baseline VSS results with the endpoint VSS results after applying the Mesotherapy Eporex treatment (p=0.0002). However, inter-observer reliability remains a problem due to the subjective nature of the VSS. The internal consistency of the VSS was lower than acceptable (Cronbach's alpha 0.4355). Therefore, emphasis was placed on the results of the independent assessor and because the independent assessor assessed the breasts throughout the treatment period. The independent assessor's results revealed a maximum of 11% or minimum of 0.6% improvement in scar appearance in the experimental group than in the control group after treatment (95% CI: -10.70 ; -0.58). When evaluating the 4 (four) categories if the VSS the independent assessor's results showed a statistical

significant improvement for scar pliability (95% CI: -3.88; -0.87) and height (95% CI: -2.58; -0.11) when comparing the experimental and control group after treatment. The improvement in scar appearance can be attributed to the application of the Mesotherapy Eporex treatment in combination with active ingredients for it establishes deeper product penetration into the skin to reduce inflammation, assist in collagen synthesis, restore tissue firmness, increase skin elasticity, decrease collagen stiffness and act as free radical scavengers.

The assessor (95% CI: -2.10; -0.16), ptosis (95% CI; 0.03; 0.33), and amount of right-sided breast tissue (95% CI: -0.01; -0.00) removed influenced the effectiveness of the Mesotherapy Eporex treatments ($p \leq 0.05$). The VSS is a subjective assessment tool explaining the influence of the assessor on the effectiveness of the treatment. The higher the degree of ptosis the less effective the treatment. All the participants in the study presented with ptosis. Ptosis is an indication of the amount of skin resection and breast tissue to be surgically removed and the more breast tissue removed the greater the chance of more aggressive scarring post-operatively.

In spite of the subjectivity and imprecision of the measurement system used, the effectiveness of the Mesotherapy Eporex treatment with active ingredients is not in question. There was a relative change in scar appearance over time in favour of the breast receiving Mesotherapy treatment at that time.

6.2 Limitations

The following limitations were identified in this research study:

- BMI distribution was limited as none of the 30 participants were found to be classified as underweight or obese according to the BMI rating scale.

- The VSS as a measurement tool assessing post-operative scar appearance. Internal consistency of the VSS is known to be lower than acceptable although the VSS has been validated as a post-operative assessment tool.

- The number of observers used. Reliability can be increased by increasing the number of observers however, in everyday clinical practice this is not always feasible.

- The small population size. Although it was clear that the scar appearance of the experimental breast improved after applying the Mesotherapy Eporex treatment compared to the control breast not receiving treatment. An increase in sample size could account for the internal variations seen.

6.3 Recommendations

The following recommendations can be made from this research study:

- Advanced scientific investigation into the application of Mesotherapy Eporex treatment with active ingredients to optimize the treatment effect on post-operative scar appearance.

- Re-evaluating the use of the VSS as a measurement tool for post-operative scarring. Replace subjective scar measurement tools with objective scar measurement tools.

- Incorporating more than one scar assessment tool to evaluate scar appearance (e.g. Patient Observer Scar Assessment Scale (POSAS), Wound Evaluation Scale (WES); Manchester Scar Scale (MSS).

REFERENCES

Abu-Nab Z, Grunfeld EA. 2007. Stratification with outcome and attitudes towards scarring among women undergoing breast reconstructive surgery. Patient Education and Counseling; 66: 243-249.

Ahn C, Mulligan P, Salcido RS. 2008. Smoking-the bane of wound healing: biomedical interventions and social influences. Advances in Skin and Wound Care; 21(5): 227-238.

Ahuja RB, Chatterjee P, Deraje V. 2015. A critical appraisal of nonsurgical modalities for managing hypertrophic scars and keloids. Formason Journal of Surgery; 48(2): 49-56.

American Society of Plastic Surgeons. 2011 Plastic Surgery Statistics Report [Online] Available at https://www.google.co.za/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwjXqpePutzMAhWaF8AKHR4jANMQFggaMAA&url=http%3A%2F%2Fwww.plasticsurgery.org%2Fnews%2Fplastic-surgery-statistics%2F2011-plastic-surgery-statistics.html&usg=AFQjCNEmfSIx_W24kX9eQpQAYTzFzblgCw&bvm=bv.122129774,d.d2s.[Accessed: 29/08/2014].

Anderson K, Hamm RL. 2012. Factors that impair wound healing. Journal of the American College of Clinical Wound Specialists; 4(4): 84-91.

Andrades P, Prado A. 2008. Understanding modern breast reduction techniques with a simplified approach. Journal of Plastic, Reconstructive & Aesthetic Surgery; 61(11):1284-1293.

Arnold M, Barbul A. 2006. Nutrition and wound healing. Plastic Reconstructive Surgery; 117(7 Suppl): 42S-58S.

Avsar DK, Avgit AC, Benlier E, Top H, Taskinalp O. 2010. Anthropometric breast measurement: a study of 385 Turkish female students. Aesthetic Surgical Journal; 30:44-50.

Bagwe SS, Sarawade RD. 2014. Progressive development in the field of mesotherapy – a review. International Journal for Pharmaceutical Research Scholars; 3(2): 466-474.

Baker R, Urso-Baiarda F, Linge C, Grobbelaar A. 2009. Cutaneous Scarring: A Clinical Review . Dermatology Research and Practice; 2009: http://dx.doi.org/10.1155/2009/625376.

Barbosa MVJ, Nahas FX, Neto MASN, Ferreira LM. 2010. Keyhole pattern for preoperative marking for reduction mammaplasty. Canadian journal of Plastic Surgery; 18(4): 130-134.

Baspeyras M, Rouvrais C, Liégard L, Delalleau A, Letellier S, Bacle I, Courrech L, Murat P, Mengeaud V, Schmitt AM. 2013. Clinical and biometrological efficacy of a hyaluronic acid-based mesotherapy product: a randomised controlled study. Archives of Dermatological Research; 305(8): 673–682.

Bharia N. 2012. Mesotherapy. In: Prendergast PM, Shiffman MA (Eds.). Aesthetic medicine art and techniques. Springer-Verlag Berlin Heidelberg: pp. 249–253.

Bhatia G, Banga AK. 2014. Effect of modulated alternating and direct current iontophoresis on transdermal delivery of lidocaine hydrochloride. BioMed Research International; 2014: DOI:10.1155/2014/537941.

Bian D, Zhang J, Wu X, Dou Y, Yang Y, Tan Q, Xia Q, Xia Y, Gong Z, Dai Y. 2013. Asiatic Acid Isolated From Centella Asiatica Inhibits TGF-β1-induced Collagen

Expression in Human Keloid Fibroblasts via PPAR-γ Activation. International Journal of Biological Sciences; 9(10): 1032-1042.

Brown A. 2015. Phases of the wound healing process. Nursing Times; 111(46): 12-13.

Brown JJ, Ollier W, Arscott G, Ke X, Lamb J, Day P, Bayat A. 2008. Genetic susceptibility to keloid scarring: SMAD gene SNP frequencies in Afro-Caribbeans. Experimental Dermatololgy; 17(7): 610-613.

Brown N, White J, Milligan A, Risius D, AyresB, Hedger W, Scurr J. 2012. The relationship between breast size and anthropometric characteristics. American Journal of Human Biology; 24:158-164.

Brusselaers, N. Prirayesh A, Hoeksema H, Verbelen J, Blot S, Monstrey S. 2010. Burn scar assessment: A systematic review of different scar scales. Journal of Surgical Research, 164 (1): e115-23.

Bylka W, Znajdek-Awizen, Studzińska-Sroka E, Brzezińska M. 2013. Centella asiatica in cosmetology. Advances in Dermatology and Allergology; 30(1): 46-49.

Castro M, Pesso M, Accorsi AJ, Ribeiro L, Moreira LF. 2013. Mastopexy combined with augmentation: systemic use of Ribeiro's inferiorly-based flaps. Brazilian Journal of Plastic Surgery; 28(3): 333-342.

Chadwick S, Heath R, Shah M. 2012. Abnormal pigmentation within cutaneous scars: A complication of wound healing. Indian Journal of Plastic Surgery; 45(2): 403-411.

Chopra K, Tadisina KK, Singh D. 2013. Breast Reduction Mammaplasty. Open Access Journal of Plastic Surgery; 13:ic58 (PMC3782143).

Collins N. 2013. Nutrition 411: revisiting vitamin C and wound healing. Ostomy Wound Manage; 59(9): 12-14.

Coltman CE, McGhee DE, Steel JR. 2015. Bra strap orientations and designs to minimise bra strap discomfort and pressure during sport and exercise in women with large breasts. Sports Medicine Open; 1(1): doi: 10.1186/s40798-015-0014-z.

Copcu, E. 2009. A versatile breast reduction technique: Conical plicated central U shaped (COPCUs) mammaplasty. Annals of Surgical Innovation and Research; 3(7): doi:10.1186/1750-1164-3-7.

Cope G. 2014. Effects of smoking on wound healing. Wounds UK; 10(2): 10-18.

Corbin CB, Welk GJ, Corbin WR, Welk KA. 2016. Concepts of fitness and wellness: A comprehensive lifestyle approach. 11th ed. McGraw-Hill Education. New York: pp277-295.

Crerand CE, Magee L. 2013. Cosmetic and reconstructive breast surgery in adolescents: psychological, ethical, and legal considerations. Seminars in Plastic Surgery; 27(1): 72-78.

Cutress RI, Simoes T, Gill J, Hurren JS. 2013. Modification of the Wise pattern breast reduction for oncological mammaplasty of upper outer and upper inner quadrant breast tumours: a technical note and case series. Journal of Plastic, Reconstructive and Aesthetic Surgery; 66(2): e31-36.

Czerkasij V. 2013. Skin of color: a basic outline of unique differences. The Nurse Practitioner; 38(5): 34-40.

D'Angelo JM. 2009. Spa business strategies: a plan for success. 2nd ed. Cengage Learning. United States: pp154-155.

Davalia H, Mansfield L, Minakaran N, Banerjee D. 2008. International Seminars in Surgical Oncology; 2008(5): e3 (DOI:10.1186/1477-7800-5-3.

Davis EC, Callender VD. 2010. Postinflammatory hyperpigmentation: A review of the epidemiology, clinical features, and treatment options in skin of color. The Journal of Clinical and Aesthetic Dermatology; 3(7): 20-31.

DeGeorge BR, Colen DL, Mericli AF, Drake DB. 2013. Reduction mammoplasty operative techniques for improved outcomes in the treatment of gigantomastia. Eplasty; 13(2013):e54.

Deliaert AEK, Van der Kerckhove E, Tuinder S, Noordzij SM, Dormaar TS, Van der Hulst RR. 2012. Smoking and its effect on scar healing. European Journal of Plastic Surgery; 35(6): 421–424.

Dhote V, Bhatnagar P, Mishra PK, Mahajan SC, Mishra. 2012. Iontophoresis: A Potential Emergence of a Transdermal Drug Delivery System. Scientia Pharmaceutica: 80(1): 1-28.

Donovan ML, Muller MJ, Simpson C, Rudd M, Paratz J. 2016. Interim pressure garment therapy (4–6 mmHg) and its effect on donor site healing in burn patients: study protocol for a randomised controlled trial. Trials; 17(1): 214.

Draelos Z and Pugliese PT. 2011. Physiology of the Skin. 3rd edition. Alluredbooks. United States of America: pp. 429–431.

Durani P, McGrouter DA, Ferguson WMJ. 2009. Current scales for assessing human scarring: A review. Journal of Plastic, reconstructive and Aesthetic Surgery; 62(6): 713-720.

Ellis H, Mahadevan V. 2013. Anatomy and physiology of the breast. Surgery; 31(1):11-14.

Ellsworth WA, Basu CB, Iverson RE. 2009. Perioperative considerations for patient safety during cosmetic surgery – preventing complications. Canadian Journal of Plastic Surgery; 17(1): 9-16.

Fabbrocini G, Forgione P, Capasso C. 2007. Methodologies. In: Tosti A and De Padova MP (Eds). Atlas of Mesotherapy in skin rejuvenation. 1st ed. Informa Healthcare United Kingdom: p17-19.

Fearmonti R, Bond J, Erdmann D, Levinson H. 2010. A review of scar scales and scar measuring devices. Open Access Journal of Plastic Surgery; 10:e43 (PMC2890387).

Fernandes PM, Neto MS, Veiga DF, Abla LEFA, Mundim CDA, Juliano Y, Ferreira LM. 2007. Back pain: An assessment in breast hyperthrophy patients. The Acta Ortopédica Brasileira Journal; 15(4): 227-230.

Ferriero G, Di Carlo S, Ferriero A, Salgovic L, Bravini E, Sartorio F, Vercelli S. 2015. Post-surgical scar assessment in rehabilitatioin: a systemic review. Physical Therapy and Rehabilitation; 2(2): DOI:http://dx.doi.org/10.7243/2055-2386-2-2.

Ferriero G, Vercelli S, Salgovic L, Stissi V, Sartorio F. 2010. Validation of a new device to measure postsurgical scar adherence. Physiotherapy; 90(5): 776-783.

Franz MG, Steed DL, Robson MC. 2007. Optimizing healing of the acute wound by minimizing complications. Current Problems in Surgery; 44(11): 691–763.

Gabriel V. 2011. Hypertrophic Scar. Physical Medicine and Rehabilitation Clinics of North America; 22(2): 301–310.

Gankande TU, Wood FM, Edgar DW, Duke JM, DeJong HM, Henderson AE, Wallace HJ. 2013. A modified Vancouver Scar Scale linked with TBSA (mVSS-TBSA): Inter-rater reliability of an innovative burn scar assessment method. Burns; 39(6): 1142–1149.

Gantwerker EA, Hom DB. 2012. Skin: Histology and physiology of wound healing. Facial Plastic Surgery Clinics of North America; 39(3): 441-453.

Garcia O. Management of asymmetry after breast reduction. 2016. Clinics in Plastic Surgery; 43:373-382.

Garg S, Dahiya N, Gupta S. 2014. Surgical scar revision: an overview. Journal of Cutaneous and Aesthetic Surgery; 7(1):3-13.

Garvey PB, Buchel EW, Pockaj BA, Casey WJ, Gray RJ, Hernández JL, Samson TD. 2006. DIEP and pedicled TRAM flaps: a comparison of outcomes. Plastic and Reconstructive Surgery; 117(6): 1711–1721.

Gauglitz GG. 2013. Management of keloid and hypertrophic scars: current and emerging options. Clinical, Cosmetic and Investigational Dermatology; 6:103-114.

Gauglitz GG, Korting HC, Pavicic T, Ruzicka T, Jeschke MG. 2011. Hypertrophic scarring and keloids: Pathomechanisms and current and emerging treatment strategies. Molecular Medicine; 17(1-2):113-125.

Geddes DT. 2007. Inside the lactating breast: the latest anatomy research. Journal of Midwifery and Women Health; 52(6): 556-563.

Gefen A, Dilmoney B. 2007. Mechanics of the normal women's breast. Technology Health Care; 15:259-271.

Godbout JP, Glaser R. 2006. Stress-induced immune dysregulation: implications for wound healing, infectious disease and cancer. Journal of Neuroimmune Pharmacology; 1(4): 421-427.

Gohil KJ, Patel JA, Gajjar AK. 2010. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. Indian Journal of Pharmaceutical Sciences; 72(5): 546-556.

Goldberg A, Bei M, Sheridan RL, Yarmush ML. 2013. Regeneration and control of human fibroblast cell density by intermittently delivered pulsed electric fields. Biotechnology and Bioengineering; 110(6): 1759-1768.

Goldman MP, Hexsel D. 2010. Cellulite: Pathophysiology and Treatment. 2nd ed. CRC Press. Boca Raton. Florida: pp 86-90.

Gozali MV, Zhou B. 2015. Effective treatments of atrophic acne scars. Journal of Clinical Aesthetic Dermatology; 8(5): 33-40.

Guo S. DiPietro LA. 2010. Factors affecting wound healing. Journal of Dental Research; 89(3): 219-229.

Gurtner GC, Werner S, Barrandon Y, Longaker MT. 2008. Wound repair and regeneration. Nature; 453: 314–321.

Halder S, Banerjee S, Halder A, Pal PR. 2012. Skin diseases in HIV-infected patients: Impact of immune status and histological correlation. Indian Journal of Sexually Transmitted Diseases, 33(1): 65-67.

Hallam MJ, McNaught K, Thomas AN, Nduka C. 2013. A practical approach to scar colour assessment. Journal of Plastic, Reconstructive and Aesthetic Surgery; 66(10): e271-e276.

Hammond DC, Loffredo, M. 2012. Breast Reduction. Plastic and Reconstructive Surgery; 129(5): 829e–839e.

Hammond DC. 2009. Atlas of Aesthetic Breast Surgery. 1st ed. Saunders Elsevier. Michigan USA: pp 1-9.

Handel N, Yegiyants. 2016. Managing necrosis of the nipple areaolar complex following reduction mammoplasty and mastopexy. Clinics in Plastic Surgery; 43:415-423.

Hansen JE. 2016. Avoiding the unfavorable outcome with Wise pattern breast reduction surgery. Clinics in Plastic Surgery; 43(2): 349-348.

Hansson E, Manjer J, Ringberg A. 2014. Inter-observer reliability of clinical measurements of suprasternal notch-nipple distance and breast ptosis. Indian Journal of Plastic Surgery; 47(1):61-64.

Hardy RG, Williams L, Dixon JM. 2008. Use of enoxaparin results in more haemorrhagic complications after breast surgery than unfractionated heparin. British Journal of Surgery 95(7): 834-836.

Hashim P. 2014. The effect of Centella asiatica, vitamins, glycolic acid and their mixtures preparations in stimulating collagen and fibronectin synthesis in cultured human skin fibroblast. Pakistan Journal of Pharmaceutical Science; 27(2): 233–237.

Hassiotou F, Geddes D. 2013. Anatomy of the human mammary gland: Current status of knowledge. Clinical Anatomy; 26(1): 29-48.

Herwadkar A, Banga AK. 2012. An update on the application of physical technologies to enhance intradermal and transdermal drug delivery. Therapeutic delivery; 3(3): 339-355.

Hess CT. 2011. Checklist for factors affecting wound healing. Advances in skin and wound care; 24(4):192.

Hill P, Pickart M. 2009. Milady's aesthetician series: Cosmetic Surgery and the Aethetician. 1st ed. Milady, New York USA:pp 63-66.

Hough PA, Nel N, Jooste JF, Breitenbach E, Botes R, Lamola E, Naude E, Smit M, Strydom E. 2007. The relation between breast reduction and activity participation with specific reference to leisure activities. South African Family Practice: 49(8): 17

Huang C, Murphy GF, Akaishi S, Ogawa R. 2013. Keloids and hypertrophic scars: Update and future directions. International Open Access Journal of the American Society of Plastic Surgeons; 1(4): e25 (DOI:10.1097/GOX.0b013e31829c4597).

Hunasgi S, Koneru A, Vanishree M, Shamala R. 2013. Keloid: A case report and review of pathophysiology and differences between keloid and hypertrophic scars. Journal of Oral and Maxillofacial Pathology; 17(1): 116-120.

Idriss N, Maibach HI. 2009. Scar assessment scales: a dermatologic overview. Skin Research and Technology; 15(1): 1-5.

Ikander P, Drejøe JB, Lumholt P, Sjøstrand H, Matzen S, Quirinia A, Siersen HE, Ringberg A, Lambaa S, Hölmich LR. 2014. Measurement of breast volume is a useful supplement to select candidates for surgical breast reduction. Danish Medical Journal; 61(1):A4760.

Ita K. 2016. Perspectives on Transdermal Electroporation. Pharmaceutics; 8(1): 9: DOI:10.3390/pharmaceutics8010009.

Jatoi I, Petit JY, Kaufmann M, Petit JY. 2006. Atlas of Breast Surgery. British Journal of Surgery; 93(7): 894.

Javed A, Lteif A. 2013. Development of the human breast. Seminars in Plastic Surgery; 27(1):5-12.

Jesinger RA. Breast anatomy for the interventionalist. 2014. Techniques in Vascular and Interventional Radiology; 17(1): 3-9.

Juckett G, Hartman-Adams H. 2009. Management of keloids and hypertrophic scars. American Family Physician, 80(3): 253–260.

Kaartinen IS, Välisuo PO, Alander JT, Kuokkanen HO. 2011. Objective scar assessment - a new method using standardized digital imaging and spectral modelling. Burns; 37(1):74-81.

Karaaslan O, Demirkiran HG, Silistreli O, Sonmez E, Bedir YK, Can M, Caliskan G, Aslan C, Oral MA, Kankaya. 2013. The Effect of Reduction Mammaplasty on the Vertebral Column: A Radiologic Study. Scientific World Journal; 2013:2013; DOI: 10.1155/2013/701391.

Kececi Y, Sir E. 2014. Prediction of resection weight in reduction mammaplasty based on anthropometric measurements. Breast Care; 9(1): 41-45.

Khan HA, Bayat A. 2008. A geometric method for nipple localization. Canadian Journal of Plastic Surgery; 16(1):45-47.

Khondoker S, Ahmed T. 2012. Reduction mammoplasty: desire or necessity. Bangladesh Journal of Plastic Surgery; 3(2): 29-31.

Kidd BA, Wroblewska A, Boland MR, Agudo J, Merad M, Tatonetti NP, Brown BD, Dudley JT. 2016. Mapping the effects of drugs on the immune system. Nature Biotechnology; 34(1): 47-56.

Kolarsick PAJ, Kolarsick MA, Goodwin C. 2011. Anatomy and physiology of the skin. Journal of the Dermatology Nurses Association; 3(4): 203-213.

Konda DK, Thappa DM. 2013. Mesotherapy: What is new? Indian Journal of Dermatology, Venereology, and Leprology; 79(1): 127-134.

Kopans D. Breast anatomy and basic histology, physiology, and pathology. In: Kopans D, ed. Breast imaging. 3rd ed. Philadelphia, Pa: Lippincott Williams & Wilkins, 2007; 7–43.

Kopitar AN, Kotnik V, Vidmar G, Ihan A, Novak P, Stefancic M. 2012. Therapeutic electric stimulation does not affect immune status in healthy individuals – a preliminary report. Biomedical engineering Online; 11:42: DOI: 10.1186/1475-925X-11-42.

Kose O, Waseem A. 2008. Keloids and hypertrophic scars: are they two different sides of the same coin? Dermatologic Surgery 34(3): 336-346.

Krueger E, Claudino JL, Scheeren EM, Neves EB, Mulinari E, Nohama P. 2014. Ionophoresis: principles and applications. Fisioterapia em Movimentois; 27(3): 469-481.

Lapid O, de Groof EJ, Corion LUMC, Smeuldres MJC, Van der Horst CMAM. 2013. The effect of breast hypertrophy on patient posture. Archives of Plastic Surgery; 40(5): 559-563.

Latha P, Vandana KR. 2011. Mesotherapy – a review. International journal of Advanced Pharmaceutics; 1(1): 19-29.

LeBert DC, Huttenlocher A. 2014. Inflammation and wound repair. Seminars in Immunology; 26(4): 315-320.

Levy LL, Emer JJ. 2012. Complications of minimally invasive cosmetic procedures: Prevention and management. Journal of Cutaneous and Aesthetic Surgery; 5(2): 121-132.

Levy LL, Zeichner JA. 2012. Management of acne scarring, part II: A comparative review of non-laser-based, minimally invasive approaches. American Journal of Clinical Dermatology, 13(5): 331–340.

Lima CC, Pereira AP, Silva JR, Oliveira LS, Resck MC, Grechi CO, Bernardes MT, Olimpio FM, Santos AM, Incerpi EK, Garcia JA. 2009. Ascorbic acid for the healing of skin wounds in rats. Brazilian Journal of Biology; 69(4); 1195–1201.

Liu YJ. 2009. Aesthetics of the female breast correlation of pluralistic evaluations with volume and surface area. Yale Medicine Thesis Digital Library. Paper 172.

Ma S, Schroeder B, Sun C, Loufakis DN, Cao Z, Sriranganathan N, and Lu C. 2014. Electroporation-based delivery of cell-penetrating peptide conjugates of peptide nucleic acids for antisense inhibition of intracellular bacteria. Integrative Biology 6(10): 973-978.Vel

MacGregor JL, Tanzi EL. 2013. Microfocused ultrasound for skin tightening. Seminars in Cutaneous Medicine and Surgery; 32(1): 18-25.

Mammucari M, Gatti A, Maggiori S, Bartoletti CA, Sabato AF. 2011. Mesotherapy, definition, rationale and clinical role: A consensus report from the Italian Society of Mesotherapy. European Review for Medical and Pharmacological Sciences; 15(6): 682–694.

Mansel RE, Webster DJT, Sweetland HM. 2009. Breast anatomy and physiology. In: Mansel RE, Webster DJT, Sweetland HM (Eds.). Hughes, Mansel & Webster's Benign Disorders and Diseases of the Breast. 3rd ed. Saunders: Elsevier: p25-40.

McDougall S, Dallon J, Sherratt J, Maini P. 2006. Fibroblast migration and collagen deposition during dermal wound healing: mathematical modelling and clinical implications. Philosophical transactions. Series A, Mathematical, Physical, and Engineering Sciences; 364(1843): 1385-13405.

McLafferty E, Hendry C, Alistair F. 2012. The integumentary system: anatomy, physiology and function of skin. Nursing Standard; 27(3): 35-42.

Mehmet K, Sevin ÖG, Okan Y, Gözde U, Hüsnü FA, Özgen IK, Ahmet I, Ersin E. 2015. Patient perception, satisfaction and cosmetic results of surgical atrial septal defect closure: Minithoracotomy versus sternotomy. Turk Gogus Kalp Dama; 23(1): 1-8.

Michels AJ, Frei B. 2013. Myths, artifacts, and fatal flaws: Identifying limitations and opportunities in Vitamin C research. Nutrients; 5(12): 5161–5192.

Mistiaen P, Van Halm-Walters M. 2010. Prevention and treatment of intertrigo in large skin folds of adults: a systematic review. BMC Nursing; 9(12): DOI:10.1186/1472-6955-9-12.

Moio M, Schonauer F. 2015. Correlation between nipple elevation and breast resection weight: How to preoperatively plan breast reduction. Journal of Plastic, Reconstructive and Aesthetic Surgery; 68(8): 1127-1131.

Monstrey S, Middelkoop E, Vranckx JJ, Bassetto F, Ziegler UE, Meaume S, Téot L. 2014. Updated scar management practical guidelines: Non-invasive and invasive measures. Journal of Plastic, Reconstructive & Aesthetic Surgery; 67: 1017-1025.

Morien A, Garrison D, Smith NK. 2008. Range of motion improves after massage in children with burns: A pilot study. Journal of Bodywork and Movement Therapies; 12(1): 67-71.

Murray JD, Elwood ET, Barrick R, Feng J. 2008. Predicting breast reduction weight using mass of breast ptosis. Canadian Journal of Plastic Surgery; 16(1):18-22.

Nafae R, Mohamed NA, Amany FM, Gad DM, Aly AM, Mahmoud AA, Abdin H. 2013. The effect of weight reduction versus reduction mammoplasty on the pulmonary function parameters and Leptin level in obese-macromazia subjects. Egyptian Journal of Chest Diseases and Tuberculosis; 62(4): 723-729.

Necas J, Bartosikova L, Brauner P, Kolar J. 2008. Hyaluronic acid (hyaluronan): a review. Veterinarni Medicina, 53(8): 397–411.

Nelson RA, Colohan SM, Sigurdson LJ, Lalonde DH. 2008. Practice profiles in breast reduction: A survey among Canadian plastic surgeons. Canadian Journal of Plastic Surgery; 16(3): 157-167.

Neuman MG, Nanau RM, Oruña-Sanchez L, Coto G. 2015. Hyaluronic acid and wound healing. Journal of Pharmacy & Pharmaceutical Sciences; 18(1): 53–60.

Nezhadhoseini SE, Fotohi K, Vejdani M. 2015. Risk factors associated with surgical site infection after breast surgery. Reviews in Clinical medicine; 2(1): 45-48.

Nguyen TA, Feldstein SI, Shumaker PR, Krakowski, AC. 2015. A review of scar assessment scales. Seminars in Cutaneous Medicine and Surgery; 34(1): 28-36).

Ogawa R, Chin M. 2008. Animal models of keloids and hypertrophic scars. Journal of Burn Care and Research; 29(6): 1016-1017.

Oultram S. 2009. All hail the new flesh: some thoughts on scarification, children and adults. Journal of Medical Ethics; 35(10): 607-610.

Paiva GS, Taft CA, Carvalho MC, de Souza IA, da Silva ECB, Cavalcanti KP, Ronaldo FL, De la Cruz NM. 2013. A Comparative Study of the effects of Vitamins C and E in the Development of Sarcoma 180 in Mice. Journal of Cancer; 4(9): 724-726.

Panzade P, Puranik P. 2012. Iontophoresis: A Functional Approach for Enhancement of Transdermal Drug Delivery. Asian Journal of Biomedical and Pharmaceutical Sciences; 2(11): 1-8.

Papakonstantinou E, Roth M, Karakiulakis G. 2012. Hyaluronic acid: a key molecule in skin aging. Dermato Endocrinology: 4(3):253-258.

Penn JW, Grobbelaar AO, Rolfe KJ. 2012. The role of the TGF-β family in wound healing, burns and scarring: a review. International Journal of Burns and Trauma; 2(1): 18-28.

Perry DM, McGrouther DA, Bayat A. 2010. Current tools for noninvasive objective assessment of skin scars. Plastic Reconstructive Surgery; 126(3): 912–913.

Pierpont YN, Dinh TP, Salas E, Johnson EL, Wright TG, Robson MC, Payne WG. 2014. Obesity and Surgical Wound Healing: A Current Review. International Scholarly Research Notices; 2014: http://dx.doi.org/10.1155/2014/638936.

Purohit S. 2008. Reduction mammoplasty. Indian Journal of Plastic Surgery; 41: S64-S79.

Rabello FB, Souza CD, Júnior JAF. 2014. Update on hypertrophic scar treatment. Clinics; 69(8): 565-573.

Radosa JC, Radosa MP, Baum S, Mavrova R Camara O. 2013. Reduction mammoplasty for symptomatic macromastia: which factors influence the post-operative outcome? Archives Gynecology Obstetrics; 278(4): 715-722.

Rawat S, Vengurlekar S, Rakesh B, Jain S, Srikarti G. 2008. Transdermal delivery by iontophoresis. Indian Journal of Pharmaceutical Sciences; 70(1): 5–10.

Reinke JM, Sorg H. 2012. Wound repair and regeneration. European Surgical Research; 49(1): 35-43.

Rinker B, Veneracion M, Walsh CP. 2008. The effect of breastfeeding on breast aesthetics. Aesthetic Surgical journal; 28(5): 534-537.

Rukari TG, Alhat BR. 2014. Iontophoresis: An electrically assisted drug delivery system. Journal of Advanced Drug Delivery, 1(1): 58-70.

Saariniemi KM., Joukamaa M, Raitasalo R, Kuokkanen HO. 2009. Breast reduction alleviates depression and anxiety and restores self-esteem: a prospective randomized clinical trial. Scandinavian Journal of plastic and reconstructive surgery and hand surgery, 43:320-324.

Sahin I, Iskender S, Ozturk S, Balaban B, Isik S. 2013. Evaluation of breast reduction surgery effect on body posture and gait pattern using three-dimensional gait analysis. Aesthetic Plastic Surgery; 37(3): 549-553.

Saleem L, John JR. 2013. Unfavourable results following reduction mammoplasty. Indian Journal of Plastic Surgery; 46(2): 401–407.

Santra TS, Tseng FG. 2013. Recent trends on micro/nanofluidic single cell electroporation. Micromachines; 4(3): 333-356.

Savolainen-Peltonen H, Vihma V, Leidenius M, Wang F, Turpeinen U, Hämäläinen E, Tikkanen MJ, Mikkola TS. 2014. Breast adipose tissue estrogen metabolism in postmenopausal women with or without breast cancer. Journal of Clinical Endocrinology and Metabolism; 99(12): E2661-2667.

Scurr J, Brown N, Smith J, Brasher A, Risius D, Marczyk. 2016. The Influence of the Breast on Sport and Exercise Participation in School Girls in the United Kingdom. Journal of Adolescent Health; 58(2): 167-173.

Seevaratnam V, Banumathi P, Premalatha MR, Sundaram SP, Arumugam T. 2012. Functional properties of centella asiatica (L.): a review. International Journal of Pharmacy and Pharmaceutical Sciences; 4(5): 8–14.

Setälä L, Papp A, Joukainene S, Martikainen R, Berg L, Mustonen P, Härmä M. 2009. Obesity and complications in breast reduction surgery: are restrictions justified? Journal of Plastic, Reconstructive and Aesthetic Surgery; 62: 195-199.

Seth AK, Kim JYS. 2010. Acute symptomatic hematoma with defined etiology seven years after breast reconstruction: A case report and literature review. Canadian Journal of Plastic Surgery; 18(2): e27-e29.

Sharma M, Wakure A. 2013. Scar revision. Indian Journal of Plastic Surgery; 46(2):408-418.

Sharma S, Parvez N, Sharma PK. 2015. Iontophoresis-Models and Applications: A Review. African Journal of Basic and Applied Sciences; 7(1): 1-7.

Sharma SR, Poddar R, Sen P, Andrews JT. 2008. Effect of vitamin C on collagen biosynthesis and degree of birefringence in polarization sensitive optical coherence tomography (PS-OCT). African Journal of Biotechnology; 7(12): 2049-2054.

Shen J, Lian X, Sun Y, Wang X, Hu K, Hou X, Sun S, Yan J, Yu L, Sun X, Li W, Wang X, Guan Q, Pang T, Zhang F. 2015. Hypofractionated electron-beam radiation

therapy for keloids: retrospective study of 568 cases with 834 lesions. Journal of Radiation Research; 56(5): 811-817.

Shermak MA, Chang D, Buretta K, Mithani S, Mallalieu J, Manahan M. 2011. Increasing age impairs outcomes in breast reduction surgery. Plastic Reconstructive Surgery; 128(6): 1182-1187.

Shiffman MA. 2010. History of Breast Augmentation with Autologous Fat. In: Breast Augmentation. 1st ed. Springer-Verlag, Berlin Heidelberg: pp.437-441.

Shih B, Bayat A. 2010. Genetics of keloid scarring. Archives of Dermatological Research; 302(5): 319–339.

Sivagnanam G. 2010. Mesotherapy – the French connection. Journal of Pharmacology and Pharmacotherapeutics; 1(1): 4-8.

Sofianos C, Zinn RJ, Geoffreys DA, Kruger D. 2015. Pathological findings in reduction mammoplasty specimens: A South African perspective. South African Medical Journal; 105 (4): 308-311.

Solorzano CMB, McCartney CR. 2010. Obesity and the portal transition in girls and boys. Reproduction; 140(3): 399-410.

Somboonwong J, Kankaisre M, Tantisira B, Tantisira M. 2012. Wound healing activities of different extracts of Centella asiatica in incision and burn wound models: an experimental study. BMC Complimentary and Alternative Medicine; 12(103): DOI: 10.1186/1472-6882-12-103.

Son D, Harijan A. 2014. Overview of surgical scar prevention and management. Journal of Korean Medical Science; 29(6): 751-757.

Song J, Xu H, Lu Q, Xu Z, Bian D, Xia Y, Wei Z, Gong Z, Dai Y. 2012. Madecassoside suppresses migration of fibroblasts from keloids: Involvement of p38 kinase and PI3K signaling pathways. Burns; 38(5): 677–684.

Sørensen LT, Jørgensen S, Petersen LJ, Hemmingsen U, Bűlow J, Loft S, Gottrup F. 2009. Acute effects of nicotine and smoking on blood flow, tissue oxygenation, and aerobe metabolism of the skin and subcutis. Journal of Surgical Research; 152(2): 224-230.

South African Good Clinical Practice Guidelines. 2006. Guidelines for good practice in the conduct of clinical trials with human participants in South Africa. 2nd ed. Department of Health.

Srinivasaiah N, Iwuchukwu OC, Stanley PRW, Hart NB, Platt AJ, Drew PJ. 2014. Risk factors for complications following breast reduction: results from a randomized control trial. The Breast Journal; 20(3): 274-278.

Stein C, Küchler S. 2013. Targeting inflammation and wound healing by opoids. Trends in Pharmacological Sciences; 34(6); 303-312.

Strong B, Hall-Findlay EJ. 2015. How does volume of resection relate to symptom relief for reduction mammoplasty patients? Annals of Plastic Surgery; 75(4): 376-382.

Sutradhar, A. & Miller, M.J., 2013. In vivo measurement of breast skin elasticity and breast skin thickness. Skin Research and Technology, 19(1): e191-9. doi: 10.1111/j.1600-0846.2012.00627.x.

Tan PH, Lai LM, Carrington EV, Opaluwa AS, Ravikumar KH, Chetty N, Kaplan NV, Kelley CJ, Babu ED. 2006. The Breast; 15: 313-318.

Tekchandani S. 2016. Study of Clinical Cosmotology – 1: A Hands-on Guide. 1st ed. Jaypee Bothers Medical Publishers (Ltd). Philadelphia. United States of America: pp 29-32.

Telang PS. 2013. Vitamin C in dermatology. Indian Dermatology Online Journal; 4(2): 143-146.

Thompson CM, Hocking AM, Honari S, Muffley LA, Ga M, Gibran NS. 2013. Genetic risk factors for hypertrophic scar development. Journal of Burn Care and Research; 34(5): 477-482.

Tripathi G, Mishra S, Upadhyay P, Purohit S, Dubey GP, Agrawal A. 2015. Ethnopharmacological Importance of Centella asiatica with Special Reference to Neuroprotective activity. Asian Journal of Pharmacology and Toxicology; 3(10): 49-53.

Truong PT, Abnousi F, Yong CM, Hayashi A, Runkel JA, Phillips T, Olivotto I. 2005. Standardized assessment of breast cancer surgical scars integrating the Vancouver Scar Scale, Short-Form McGill Pain questionnaire, and patients' perspectives. Plastic Reconstructive Surgery; 116(5): 1291-1299.

Tziotzios C, Profyris C, Sterling J. 2012. Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics Part II. Strategies to reduce scar formation after dermatologic procedures. Journal of the American Academy of Dermatology; 66(1); 13-26.

Ud-Din S, Bayat A. 2014. New insights on keloids, hypertrophic scars, and striae. Dermatologic Clinics, 32(2): 193–209.

Ud-Din S, Giddings Dip P, Colthurst J, Whiteside S, Morris J, Bayat A. 2013. Significant reduction of symptoms of scarring with electrical stimulation: evaluated with subjective and objective assessment tools in a prospective noncontrolled case series. Wounds; 25(8): 212-224.

Vachiramon V, Thadanipon K. 2011. Postinflammatory hypopigmentation. Clinical and Experimental Dermatology; 36(7): 708-714.

Van den Broek LJ, Niessen FB, Scheper RJ, Gibbs S. 2012. Development, validation and testing of a human tissue engineered hypertrophic scar model. Altex; 29(4): 389–402.

Van der Veer WM, Bloemen MC, Ulrich MM, Molema G, van Zuijlen PP, Middelkoop E, Niessen FB. 2009. Potential cellular and molecular causes of hypertrophic scar formation. Burns; 35(1): 15–29.

Vedamurthy M. 2007. Mesotherapy. Indian Journal of Dermatology, Venereology and Leprology; 73(1): 60-62.

Velnar T, Bailey T, Smrkolj V. 2009. The wound healing process: an overview of the cellular and molecular mechanisms. Journal of International Medical Research; 37(5): 1528-1542.

Verhaegen PD, Schouten HJ, Tigchelaar-Gutter W, Van Marle J, Van Noorden CJ, Middelkoop E, Van Zuijlen PP. 2012. Adaptation of the dermal collagen structure of human skin and scar tissue in response to stretch: An experimental study Wound Repair and Regeneration; 20(5):658-666.

Vileikyte L. 2007. Stress and wound healing. Clinical dermatology; 25(1): 49-55.

von Sperling ML, Høimyr H, Finnerup K, Jensen TS, Finnerup NB. 2011. Chronic postoperative pain and sensory changes following reduction mammoplasty. Scandinavian Journal of Pain; 2(2); 57-61.

Wade TD, Zhu G, Martin NG. 2010. Body mass index and breast size in women: same or different genes? Twin Research and Human Genetics; 13(4): 450-454.

Wei Y, Li-Tsang, Luk DCK, Tan T, Zhang W, Chiu TW. 2015. A validation study of scar vascularity and pigmentation assessment using dermoscopy. Burns; 41(8): 1717-1723.

Weissman O, Winkler E, Teot L, Remer E, Farber N, Bank J, Hundeshagen G, Zilinsky I, Haik J. 2014. Treatment of wounds following breast reduction and Mastopexy with subsequent wound dehiscence with charged polystyrene microspheres. Wounds; 26(2): 37-42.

Wigston C, Hassan S, Turvey S, Bosanquet D, Richards A, Holloway S, Harding K. 2013. Impact of medications and lifestyle factors on wound healing: A pilot study. Wounds UK; 9(1): 22-28.

Wolfswinkel EM, Lemaine V, Weathers WM, Chike-Obi CJ, Xue AS, Heller L. 2013. Hyperplastic breast anomalies in the female adolescent breast. Seminars in Plastic Surgery; 27(1): 49-55.

Wong C, Vucovich M, Rohich R. 2014. Mastopexy and reduction mammoplasty pedicles and skin resection patterns. International open access Journal of the American society of Plastic Surgeons; 2(8): e202.

World Medical Association Declaration of Helsinki. 2013. Ethical principles of Medical Research Involving Human Subjects. [Online] Available at http://jama.jamanetwork.com/.[Accessed:15/06/2016].

Xu X, Jha AK, Harrington DA, Farach-Carson MC, Jia X. 2012. Hyaluronic Acid-Based Hydrogels: from a natural polysaccharide to complex networks: 8(12); 3280-3294.

Yang JA, Kim ES, Kwon JH, Kim H, Shin JH, Yun SH, Choi KY, Hahn SK. 2012. Transdermal delivery of hyaluronic acid – human growth hormone conjugate. Biomaterials; 33(25): 5947–5954.

Young A, McNaught CE. 2011. The physiology of wound healing. Surgery; 29(10): 475–479.

APPENDICES

Appendix A – Mesotherapy Eporex manual A1

Mesotherapy Eporex manual A2

Appendix B – Participant information card

Appendix C – Mammary reduction surgical technique

Appendix D – Vancouver Scar Scale (VSS)

Appendix E – Vancouver Scar Scale (VSS) assessment card with colour index

Appendix F – Breast photographic observations

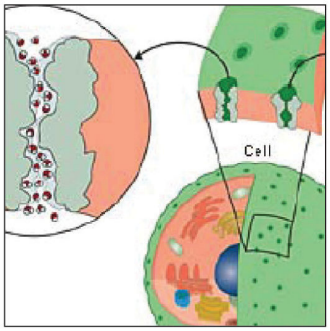
Appendix G – Ethical Clearance

Mesotherapy Eporex Manual A1

Central University of Technology, Free State

eporex

TECHNICAL & SCIENTIFIC



Eporex

Delivering Wellbeing through Technology

This is not just a slogan, it represents the aim we set ourselves and the goal finally reached after the incorporation of a variety of various synergies and types of co-operation. In fact, modern research in the electro-medical field at the moment offers various tools for medical professionals using machines with varying degrees of innovative technology.

Nowadays, much is being said regarding how skin and intracellular absorption takes place and there are various theories, even if they are not all sustainable. Herein, we will only discuss scientifically tested theories.

Eporex is an optimized system of cutaneous transport based upon a large number of accredited studies and research, in particular the research in the field of physical mechanisms that increase the penetration and spread of active principles into the epidermis:

With **EPOREX** we are creating a new generation of transdermal delivery system, bringing about the synergy of already known techniques such as Ionophoresis, Iontophoresis, and Electroporation, through an innovative method for "active" molecular transcutaneous transport that we call:

"ISOPHORESIS"

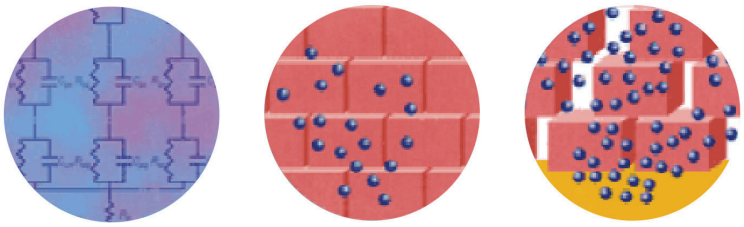
The electronic transdermal method has been used for decades, beginning in the medical field for the administration of painkilling drugs and for rehabilitative therapy, but recently it was found that it could be applied in the dermatology and professional aesthetics fields.

The main difficulty for the penetration of substances through the human skin is the external layer, i.e. the corneal layer that is a tough barrier to the transport of substances; its lipid-corneocyte matrix has been the subject of studies regarding:

- Variations of impedance of the dermis subject to impulse charges, and
- The consequent variation of its permeability;
- Determination of the wave shapes useful for transport purposes; creation of a wave shape generator

SKIN BARRIER FUNCTION

HYDROPHYLIC KERATINOCYTES



With ISOPHORESIS an attempt has been made to optimize the transport method and avoid the limits posed by techniques used in the past, i.e. the poor concentration of active principle delivered, the limited depth reached in the tissue, & the damage of the tissue due to the intense currents induced. For these reasons, the Isophoretic method represents the avant-garde in skin treatments: new research has already been carried out in various countries with outstanding results representing the future of this new needle- free technique.

IONOPHORESIS, IONTOPHORESIS, ELECTROPORATION

Ionophoresis was first described by A. Volta and A. Galvani in 1707 and laws were formulated about 1900 by Leduc. This technique exploits the electrical principle by which electrically charged "ions" move at variable speeds in an electrical field generated by a continuous galvanic current, but it is limitations are:

- Poor penetration for fixed frequency use (max 5 mm.)
- The need for polarized products
- High concentration of active principle required
- "Saponification" of the skin and burns due to the use of energy over 0.1 mA/cm
- Formulation of substance: pH, viscosity, presence of other ions

Iontophoresis uses the same electrical principles as ionophoresis, in as much as the substance is ionized by means of a continuous galvanic current using, however, a developed wave no longer continuous but modulated in impulse trains. This reduces the incidence of surface burns but has the same limited capacity for penetration due to the wave frequency.

The limits are the same as for ionophoresis, especially in the need to use products that are already polarized but, above all, the limit is that only a low quantity of active principle can be carried and the depth reached in the corneal layer is quite limited.

With iontophoresis, the importance of using the electronic transdermal method for the administration of drugs in medical therapy, in various specialties, was recognised first of all in genetic-molecular therapy, as demonstrated by more than 1400 U.S. patents and more than 5,600 articles in scientific journals and magazines.

ELECTROPORATION

This methodology is employed in medical therapy to increase in the permeability of the skin tissue: Electroporation of the skin occurs when, by the art of an electric pulse between 0,5 and 1,5 Volts, a transmembrane potential is generated within an epithelial cell structure.

As a result, the lipid layer of the cell membrane is subjected to an alteration: the formation of the well known aqueous vessels, hence called "electropores".

The creation of electropores occurs at a subsequent time, after the induction of the electric pulse, and takes only a few minutes, thus leading to a diffusive permeability of the molecules, even of high weight, that enables them to penetrate through the entire tissue ''targeted'' by the electric pulse.

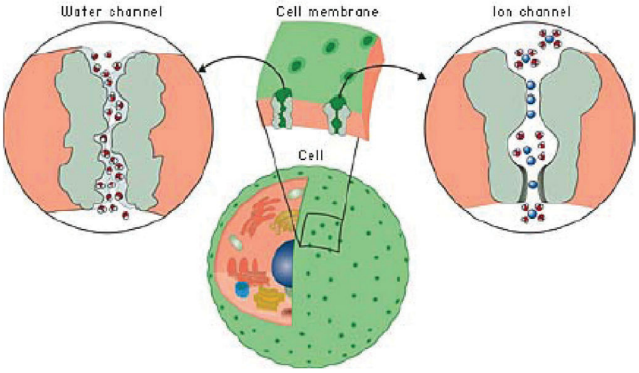
The aqueous vessels will preserve themselves for a period directly proportional to the wave length of the pulse itself; the time-span ranges from a few seconds to about ten minutes. This has been recently established by chemical-biological field researches in the well known study on "vessels dedicated to transport of water into cells''.

This study was awarded the Nobel Prize for Chemistry in 2003, which went to two eminent American researchers, Roderick MacKinnon and Peter Agre. In fact, thanks to this study, it was possible to discover the existence of molecular vessels which enable the cellular membrane to let in or out vital substances as water and salts (membrane proteins).

The control of the crossing-over of ions and molecules through these structures is regulated by chemical impulses that allow cell-cell contacts. These impulses appear to be ions or small molecules that induce a series of "cascade reactions" inside the cell, this event leading to the emergence of a macroscopic effect, such as, muscular tension or biochemical or metabolic reactions in our body or in the brain.

SKIN BARRIER FUNCTION

HYDROPHYLIC KERATINOCYTES



In consideration of the above, thanks to the methodology developed with EPOREX, i.e. ISOPHORESIS, it is possible to transport water-soluble molecules through the epidermal barrier, which it is known, shows an extremely low permeability to water-soluble substances, due to its extracellular lipid matrix (cholesterol, fatty acids), thereby allowing the introduction of active pharmacological principles at varied depths.

EPOREX AND ISOPHORESIS

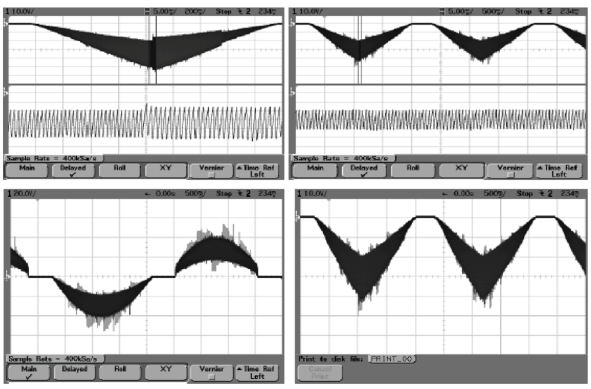
With the method we have tested called ISOPHORESIS, a pulsed and modulated current is used: i.e. a low frequency wave with elastomodulated wave shape and with a modulated intensity which is variable by the operator.

Its shape and particular trend is able in this way to electronically activate molecules with both low and high molecular weight and allows them to pass into the extra-cellular compartment at depths never reached before: up to 9 to 10 cm, while introducing 90% of the active substance necessary for the treatment. In this way a double objective is achieved: the increase in the permeability of skin tissue even at the deepest levels of the dermis and the capacity of introducing active hydrosoluble substances with a high molecular weight (e.g. hyaluronic acid, collagen, etc.)

EPOREX differs considerably from other systems in two essential features:

1) THE WAVE MODULARITY: non continuous negative/positive or alternate, constituted by a pulse train with a sinusoidal mode separated by intervals that can be modified in a frequency that accords to the wave equation Y=X – 180 + 2000.

Where Y is the frequency in Hertz and X the depth in cm, the product is carried without thermal damage for several centimeters: in fact, the wave modulation avoids the absorption of too much energy into tissue (maximum for human tissue is 0,1mA/cm2) in the time of phase “off”. The Eporex system makes it possible to automatically vary the wave length frequency in relation to the set penetration depth.



2) THE PRESENCE OF A IONIZATION CHAMBER in the hand piece that polarizes and prepares molecules electrically, with the possibility of thereby carrying any hydrosoluble substance through the opening of the so called "electropores" and easier intracellular penetration: the "in dissociate" molecules actively brought to and into the cells will modulate the metabolism in relation to their characteristics and concentration and they will move towards the dermal matrix by electro-osmosis.

In brief, in ISOPHORESIS, the transmission process occurs according to a synergy of steady processes:

1. IONIZATION OF THE SUBSTANCE
The elasto-pulsated current meets the molecule then it ionises it and makes it "active" and ready for transport.

2. TRANSMISSION
The ionised molecule is transferred to the optimal depth.

3. ELECTROPORATION
After a few minutes, electropores are created: the molecules transfer the substances from one cell to the other by osmosis.

EPOREX HANDPIECE & THE IONIZATION CHAMBER

The ionization chamber is a true innovation; here the osmotic and conductive processes of the substances gradually take place.

Attached to the ionization chamber, a bottle of conductive gel is mixed with dehydrated powder or mesotherapy ampoules, immediately before use. This ensures the freshness of the product. The conductive gel is free of preservatives, which might create allergies.

EPOREX is supplied with specifically prepared gel, powders and ampoules and utilizes traditional mesotherapy protocols.

The ionization chamber features structured metal armatures and the mix of actives is subjected to fixed and constant induction by the elasto-modulated current.

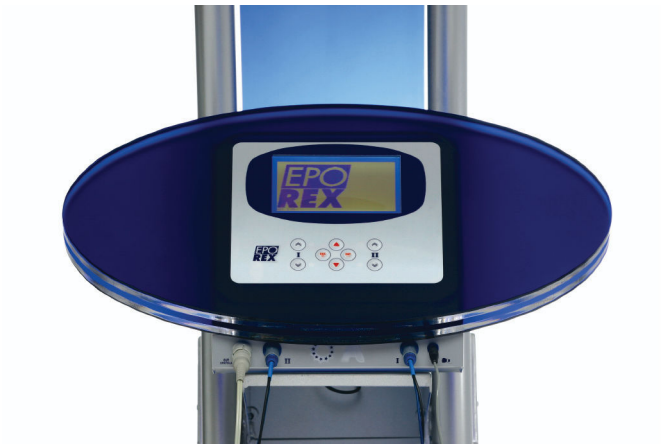
The hand piece is in fact structured in such a way that the armature of the ionization chamber is fixed and constantly in contact with the mix of actives and ensures a constancy of ionisation of the active principle for transmission.

The Eporex hand piece can carry out:

- Constant and graduated transmission of active substances, as its steel roller carries out the electroporation on the skin, with constant release of the mix, with perfect adhesion to the skin and at fixed distance

- No dispersion or concentration of the ingredients is created. It ensures a constant quantity of electric power and thereby, of ionised substance

- Furthermore, the hand piece ensures all sanitary standards, in compliance with health regulations. In fact, its special stainless steel parts are removable and can be cleaned and sterilized.



Methodology's objective:

- Transmission of the active principle directly and in depth, into the target tissue
- Extended action of active principles
- Reduced quantity of active principle used
- Higher concentration in treated site
- Less systemic absorption and toxicity
- Total absence of any pain or discomfort during treatment
- Hygiene

BRIGHT SKIN ULTRASOUND

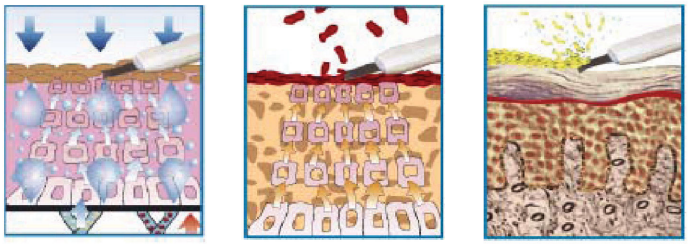
Eporex is successful in combining different methodologies and is able to enhance the efficacy of Isophoresis treatments through ultrasound waves combined with galvanic current by means of BRIGHT SKIN. This technological device is patented and supported by registered protocols.

The BRIGHT SKIN ultrasound device promotes the rejuvenation of the skin and the prevention of cutaneous ageing through the use of Ultrasound waves: It consists of a metal strip in the shape of a spatula, oscillating at a frequency of 25,000 K hertz. The spatula is bent 45° at its end, and allows the spreading of the ultrasonic waves along the tissue surface, exploiting the principle of CAVITATION.

This phenomenon occurs when a liquid is subject to a significant depression. When the absolute pressure becomes less than the ''tension'' of the liquid, a force field is created which transforms, copiously, the liquid into vapour, in the form of micro-bubbles of gases. This enables the removal of dead skin cells and residues of make-up and impurities deposited in the sebaceous follicles.

Furthermore, with the Bright SKIN handset it is possible to combine, in relation to the selected programme, the emission of galvanic micro-currents for transdermal delivery or electric peeling, exploiting the synergy between the action of ultrasound (sonic thrust) and galvanic micro-current (ionic thrust).

CAVITATION PHASES



BRIGHT SKIN TREATMENT PROGRAMME

PROGRAMME	WAVE	TREATMENT
Dermopurification	Continuous	Exfoliation & cleanse
Deep dermo-purification	Continuous combined with galvanic current	Exfoliation & deep cleanse
Deep purification & Tone-up	Pulsating wave with galvanic current	Exfoliation, cleanse & tone-up with mechanical massage
Tone-up action	Pulsating wave	Toning with deep moisturization (active principles penetrate into the skin)
Skin reactivation	Continuous wave with galvanic current	Cellular stimulation with deep moisturization (active principles penetrate into the skin)
Reactivation & Tone-up	Pulsating wave with galvanic current	Cellular stimulation and toning with deep moisturization

THE SINERGIC TECHNIQUE: ULTRASOUND + ISOPHORESIS

In conclusion, applications with EPOREX in combination with ultra-sound are particularly effective:

ULTRASOUND= Bright Skin is utilised initially for cleansing with sonic waves, removing dirt and oil from the skin's surface as well as a portion of corneal layer in a method similar to that of a slight microdermabrasion of the skin.

WAVE FORM - EPOREX In the second phase of the treatment, the sequenced wave shapes are able to penetrate 90% of the substance to a chosen depth.

The wave shapes are used in combination with a conducting current in gel, thus permitting the treatment of the active agents to the depth required. In the applied clinical studies it is shown that a range of depth from 0.5 cm to 10 cm might be carried in the tissue.

The key for optimum transmission in aesthetic treatments is the EPOREX patented hand piece incorporating a dispenser with 60 ml of conductive gel.

The hand piece, made with a "roller" mechanism, distributes the mix of conductive gel and the important activated ingredients to be transferred, over the surface of the tissue.

The treatment time is approximately 20 minutes and the operator can select the treatment parameters according to the special needs of the patient. Furthermore, no visible sign of the treatment remain on the surface of the skin and the patient's experience is totally painless and not unpleasant.

APPLICATION FIELDS AND ACTIVITIES

MEDICINE

Analgesic

Anaesthetic

Anti-Inflammatory

Antiphlogistic

Muscle Relaxant

Antieoedematous

AESTHETIC MEDICINE

Cellulite Reduction

Targeted Fat Reduction

Body Toning

Stretch Marks

Acne

Anti-Ageing

Facial Rejuvenation

Mesolift

Tired Eye Treatment

Hair Restoration

Breast Firming

FINAL CONSIDERATIONS

Eporex is the first computer-driven, transdermal delivery system that can perform a variety of different types of treatments.

Its microprocessor allows the setting of delivery treatments with different frequencies, penetrating the actives to the required depth without tissue damage.

Each operation is visible on the contemporary designed control panel, where the entire electronic functions are set.

Easy to use and handy: thanks to its portability and its streamlined software, enables clear and simple employment.

Its innovative, patented hand-piece, featured by a constant capacity of carrying performance, ensures optimal sanitary standards, in compliance with health regulations, since its parts are removable, and therefore can be cleaned and sterilized.

Central University of Technology, Free State

MEDICAL & TECHNOLOGY srl

Strumenti tecnologici per la medicina e l'estetica

EPOREX K9E

User Manual

CE

Kind Client,

We thank you for the preference reserved to our equipment and we wish that it can respond to Your expectations.

What customer of Medical & Technology you now have the privilege to have in every moment a staff of specialized technicians to Your complete disposition.

For every question don't hesitate to contact us with the means that you will think fitter and opportune; to the purpose of our answers to make more exhaustive we beg you to always quote the model and the serial number of Your instrument.

We invite you to meticulously follow the indications brought in the present user manual , this will be for you a valid help for the optimal use and maintain unchanged in the time the characteristics of Your equipment.

Cordially we wish you a good job.

User Manual

CONTENT

MEDICAL & TECHNOLOGY srl

2. DICHIARAZIONE DI CONFORMITA'

Declaration of conformity

Medical & Technology
Via della fiera 87
47900 Rimini (RN)

Dichiara sotto la propria responsabilità che il prodotto
EPOREX K 69-E
Declares on it's own sole responsibility that the product
EPOREX K 69-E

dichiara sotto la propria responsabilità che il dispositivo di cui all'oggetto soddisfa tutte le disposizioni applicabili nelle direttive

2006/95/CEE concernente la bassa tensione

89/336/CEE concernente la compatibilità elettromagnetica

Standard CEI 62-24: Norma particolare per la sicurezza degli stimolatori neuromuscolari

Standard CEI 62-23 Norme particolari per la sicurezza delle apparecchiature per terapie a ultrasuoni

Declares on it's own responsibility that the mentioned device satisfies all the dispositions directives:

2006/95/CEE concerning the low voltage

89/336/CEE concerning the electromagnetic compatibility

Standard CEI 62-24: Particular safety for the nerve and muscle stimulators

Standard CEI 62-23: Particular requirement for the safety of ultrasonic physiotherapy equipment

A tale scopo la scrivente garantisce e dichiara sotto la propria responsabilità quanto segue:
For this purpose the undersigned guarantees and declares on it's own responsibility the following:

- Tipologia del dispositivo: dispositivo per veicolazione transdermica
- Type of device: device for transdermical electric veiculation
- il dispositivo in oggetto soddisfa i requisiti essenziali richiesti dall'allegato I della direttiva 2006/95/CEE e dell'allegato III della Direttiva 89/336/CEE
The present device complies with the essential requirements in the attachment 1 of the 2006/95/CEE Directive and the attachment III of the 89/336/CEE Directive;

Rimini il 01/09/2006

CE

ROSSI FIORENZO
CEO
Medical & Technology S.r.l.

3. INSTRUCTIONS AND SAFETY PRECAUTIONS

BEFORE PROCEEDING TO THE USE OF THE DEVICE READ CAREFULLY THIS USER MANUAL AND THE FOLLOW THE INSTRUCTIONS AND PRECAUTIONS

- Avoid the exposure to excessive heat sources. The temperature of use must be between 10 and 40 °C;
- Use only the present device in conformity to its destination of use, Medical & Technology does not assume any responsibility for damages to things or people caused by a non correct use of the device;
- Use only the device with the original accessories and suitable substances listed in the manual present of use;
- Regarding the choice of the substances to use, follow to the dispositions for the intramuscular therapies;
- Eporex K69-E can delivery any substance water soluble;
- The device must be used only from qualified and educated personel on the techniques of application;
- Verify that the characteristics of the electrical system are suitable to the requisites of the device, written on the label and in the present user manual;
- Any manipulation, substitution, intervention on the device not performed from authorized personnel by Medical & Technology involves the decadence of the warranty and thus the manufacturer will be not responsible for damages to people or things that can derive of it;
- Don't use the device in presence of inflammable mixtures;
- Don't use on the patient other devices besides Eporex K69-E;
- Don't use the device in the case in which it presents breaking on the enclosure, on the handle dispenser, on the spatula: call immediatly the Medical & Technology technical service;
- Don't use the device in environments with elevated electromagnetic fields that could cause malfunctions of the Eporex K69-E and of the other instruments in the surrounding environment;
- A simultaneous connection of a Patient to an Instrument of high frequency electrosurgery device, can result in burns in correspondence of the electrodes of the device and can result on damages of Eporex K69-E;
- Operating in proximity of devices for short or micro waves therapy (eg. 1 m) can produce instability in the outputs of the Eporex K69-E;

- Absolutely do not to use the device on people carriers of peace-maker or other devices on whose use of device can interferes with the electromagnetic fields and the electrostatic current. In doubtful case you have to consult the physician.

- Treatments to the face could interfere with acoustic instruments that must be removed before the application.

Adviced against use:

- Contour of the eyes, heart disease people, pregnancy, areas to be treated interesting infections, neoplasticis, ulcers and bedsores, abrasions, wounds, obliterating arteriopathie, phlebitis, trombophlebitis, suspicious snows, subjects with redoubt cutaneous sensibility, subjects unable to communicate. (different medical prescription excepted);
- Prosthesis or metallic osteosintesys (different medical prescription excepted);
- Spastic paralysis (different medical prescription excepted);
- Avoid the use on patient connected with equipments of monitoring of the vital parameters;

Don't apply the electrodes on:

- Traumatized parts, cuts, lips and mucous generally, carotid and its proximities, area of the breast, central anterior surface of the neck, cardiac area;

- Don't use the spatula Brightskin in presence of liquids or in immersion in liquid. Brightskin is not protected against the liquid penetration.The degree of protection IP is 0. If Brightskin shold be in contact with a liquid, immediately suspend the treatment and to contact the technical support of Medical & Technology.

- Don't use Brighskin on the wet skin

4. INTENDED USE

Eporex K69-E is a device for transdermical electroveiculation and ultrasounds treatments. The principles on which it founds its employment are those diffused in the scientific community of the ionoforesi, elettrorepulsion and elettroporation.

The shape of wave created by the generator allow to transport any subatances (applied on an electrode with equal polarity to that of the ions of the substance) solution from an electrode to another of opposite polarity through the technique of the transdermical electroveiculation.

The device can be used by aesthetical qualified personnel, Eporex K69-E allows to use the transdermical electroveiculation for the subcutaneous transfer of cosmetic substances, for the contrast of the beauty flaw of the cellulitis, to improve the cutaneous and muscular tone.

The application of the ultrasounds spatula allows

- A deep purification of the skin
- An invigorating action for the derma
- A cutaneous reactivation

5. USED SYMBOLS

In the present user manual symbols are used for recalling the attention of the user on information and observations of particular importance, the symbols are the followings:


Warning – High Voltage


Warning – see the documentation


Follow the instruction for use


CE Marking of European Conformity


Fuse


Equipotential


Aplies part BF type


Fig. 6 Waste Electric and Electronic Directive Symbol Dir. 2002/96/CE

6. DESCRIPTION OF THE PRODUCT

6.1 General Features

Eporex K69-E is a device that allows to make to penetrate, through the generation of pulsated currents, substances to high and low molecular weight through the dermo-epidermal barrier without occur any damage to it.

The penetration of the substances through the derma is obtained by using the transdermical electroveiculation, such penetration happens thanks to the increased ability of transcutaneous absorption that follows the deliver of periodic current impulses.

Such impulses allow the opening of opportune "hydroelecttropores", through which penetrate the select substances.

The active substance, dissolved in specific gel are electrically loaded by the application of pulsated currents and made ready to the ionic transport.

Eporex K69-E can deliver any product dissolved in watery base, to subdermals depth and therefore to allow the veiculation of a cosmetic substabces without any invasivity for the patient.

The possibility to deliver substances in subdermals depths allows applications in all that fields in which it is necessary to transfer a substance through the derma.

The use of Eporex K69-E in aesthetical applications, is obtained by the mean of the cavitation principles, through the use of an ultrasounds spatula. The cavitation is the formation and activity of bubbles (or hollow) inside a liquid submitted to an intense ultrasonic field.

In a liquid, the ultrasonic waves produced by a transducer, create high pressure and low depression waves at high speed. These waves of pressure and depression in the liquid originate the phenomenon of cavitation. During the phase of depression, are created inside the liquid a multitude of little bubbles. During the second phase of ultrasonic compression, the enormous pressure stored into the little bubble, compresses the same up to make it implode with consequent release of energy directed to the object to clean.

The output signals, generator of currents are available on two separate channels and galvanically isolated to the device, the negative electrode is constituted by a plate of plastic material, the positive electrode is constituted by the re-usable handle dispenser.

6.2 Content of the package

The package of Eporex K69-E contains the following elements:

The package of Eporex K69 contains the following elements:

- n° 1 Base Unit
- n° 1 Power supply cable
- n° 1 Transdermic handle dispenser
- n° 2 Rolls Electrodes for body application of the transdermic handle dispenser
- n° 2 Bull Electrodes for face application of the transdermic handle dispenser
- n° 1 Ultrasonic Spatula
- n° 1 Conductor wrist strap
- n° 2 Complete Connection cables for transdermic handle dispenser
- n° 2 Ground plates
- n. 2 Sponges for ground plate
- n .2 Power key for the base unit
- n. 1 500 ml Ultrapeel
- n. 1 Ultragel skin for face
- n. 1 Ulragel white
- n. 1 Ultragelo striae
- n° 25 packages of 60 ml conductive gel
- n° 5 packages of face treatments powder
- n° 5 packages of breast treatments powder
- n° 5 packages of stretch marks treatments powder
- n° 5 packages of toning treatments powder
- n° 5 packages of cellulites treatments powder

Powder packages and gel bottles are intended only for the first use of the system focused to:

Test the functionality and setting the system components

Execute the training of the user from authorized and skilled M&T technicians

Fig 1. Base Unit

Fig.2 Connection cables for transdermic handle dispenser

Fig.3 Wrist strap with earth cable

Fig.4 Rolls Electrode for body application of transdermic handle dispenser

Fig.5 Bull Electrode for face application of transdermic handle dispenser

Fig.6 Mass Plate

Fig.7 Ultrasonic Spatula

7. TECHNICAL SPECIFICATIONS

MODEL	Eporex K69-E
CENTRAL UNIT	
POWER SUPPLY VOLTAGE	90V,220V c.a.
WORKING FREQUENCY	50 Hz, 60 Hz
INTERNAL WORKING VOLTAGE	24 Vcc
MAX POWER ABSORBED	40 VA
FUSES	2x R 2A 250V
ELECTRICAL SECURITY CLASS	I BF
MAX OUTPUT VOLTAGE	45 V
MAX OUTPUT CURRENT (R=1K)	45mA
MAX WORKING FREQUENCY	1760 Hz
BRIGHTSKIN SPATULA	
POWER SUPPLY VOLTAGE	24 V c.c.
WORKING VOLTAGE	5 V c.c.
WORKING FREQUENCY	25KHz
MAXIMUM POWER BRIGHTSKIN SPATULA	0,1 mW
CENTRAL UNIT DIMENSIONS (LxAxP)	550x260x230 mm
CENTRAL UNIT WEIGHT	6 Kg
BRIGHTSKIN SPATULA DIMENSIONS(LxAxP)	50x20x150 mm
BRIGHTSKIN SPATULA WEIGHT	0,2 Kg

8. PANEL AND CONTROLS

8.1 Frontal panel

Fig.8 Front panel

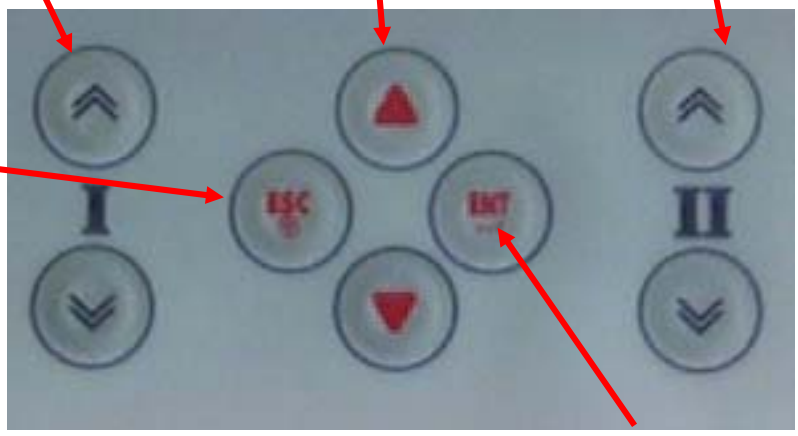


Fig.9 Keys of the command

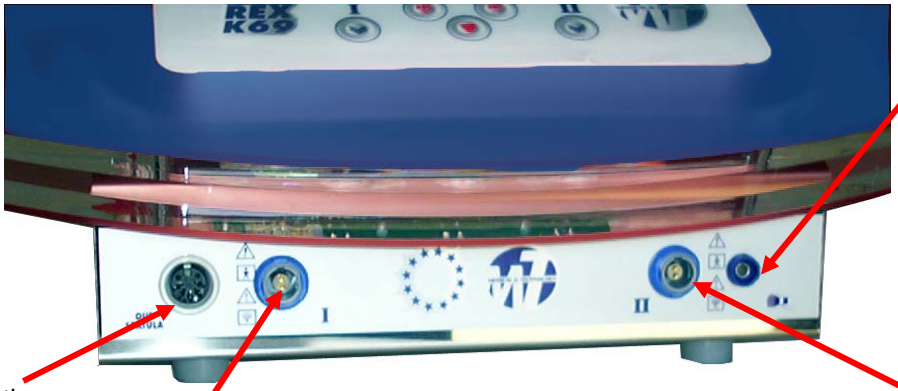


Fig.9 Sockets

8.2 Rear panel

Fig.10 Rear Panel

9. LABELS

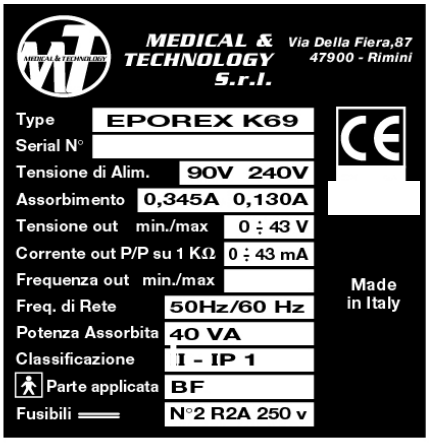


Fig.11 Device Label

Fig. 12 Label, Warning high voltage. Waste Electric and Electronic Directive Simbol (Rif. Directive 2002/96/CE)

10. INTRODUCING THE SYSTEM

10.1 Generalities on the transdermic veiculation

The technique of the transdermic veicultation allows to make to penetrate "actively" substances to high and low molecular weight through the dermo-epidermal barrier, without that such barrier suffers any type of damage.

The transport of substances happens thanks to the peculiar association of electropulsed wave – chamber of ionization, that promote the increase of the ability of transcutaneous absorption, following the delivery of electric periodic impulses. Such impulses allow the opening of "hydroelecttropores", electric intracellular doors, through which penetrate the active principles mixed preventively to a specific loaded gel and made ready to the ionic transport.

The advantages of the transdermic veiculation are :

- Delivery of the active principle directly in the extracellular compartment. The high concentrations obtained with this technique allow to quickly activate the metabolism of the cells target which begin to immediately use the communicated substances
- Possibility to deliver any type of molecules avoiding painful somministration approach
- Fast results, in clinical and aesthetical circle
- Possibility to be able to choose the depth of penetration of the substance from 0.1 cm up to 10 cm
- High concentration of the quantity of substance that reaches the zone to treat (90% against 5% of the traditional techniques)

10.2 Generalities on the cavitation

The phenomenon of the cavitation consists in the formation of bubbles (or hollow) inside a liquid submitted to an intense ultrasonic field. This phenomenon manifests when a liquid is submitted to a notable depression; in the moment in which the absolute pressure becomes inferior to the intramolecular tension of the liquid, an intense energetic field is created which creates a pressure and depression waves to high speed. These waves of pressure and depression in the liquid originate the phenomenon of cavitation. During the phase of depression, are created inside the liquid little bubbles. During the second phase of ultrasonic compression, the enormous pressure stored into little bubbles, compresses the same one up to make them collapse with consequent release of energy that strikes the object to clean. The created energy is used then for promote the separation of died cells on the epidermal surface.

11. TURNING ON THE SYSTEM

11.1 Base Unit installation

After having fixed the basic unit to the support, extract from the suitcase all the components of the system.

- Connect the power supply cable to the relative socket on the back of the basic unit and to the electrical system;
- Insert the key-switch of the system;
- Rotate the switch in hourly sense to turn on the system, the light indicator will turn on;
- Press the enter key to enter the main menù

Fig.14 Initail screen

Fig.15 Main Menù

11.2 Connceting the transdermic handle dispenser

For the conncecton of the transdermic veicultation handle dispenser, proceed as follows:

Connect the connection cable for transdermic handpice to the special connector as shown in figure:

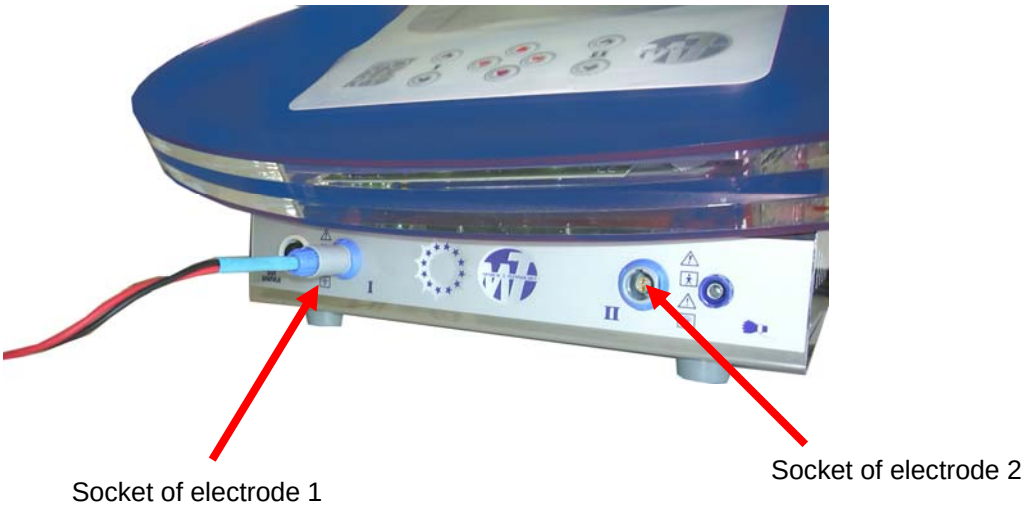


Fig.16 Connection of cables to base unit

- Connect tje red plug of the cables fo the transdermic handle dispenser acting on the knob on such plug

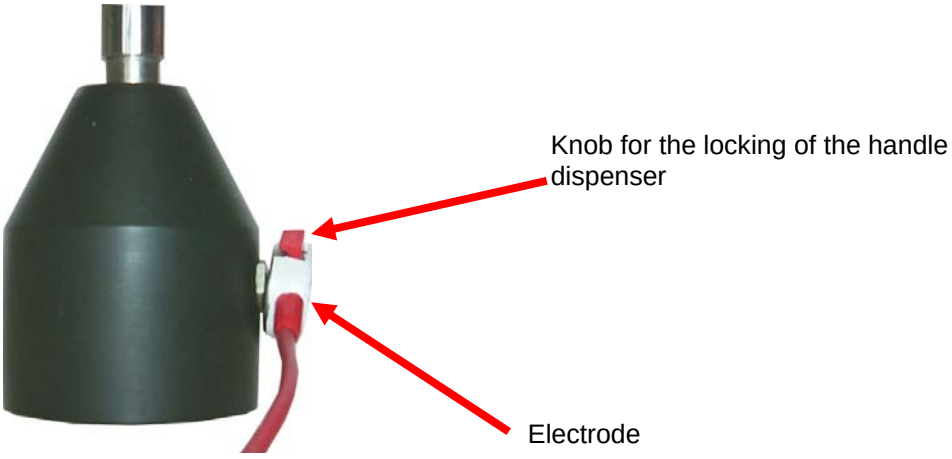


Fig.17 Connection of the cables to the transdermic handle dispenser

- Choose the type of handle dispenser according to the part of the body to treat, single roll handle dispenser for treatments of the face, double roll handle dispenser for treatments to the body
- Connect the black cable in the special lodging of the plate of mass.
- Put the plate of mass in a position diametrically opposite on the body of the patient to the area of treatment, having care to have a contact uniform between the whole surface of the plate and the body of the patient. Interpose between plate and the body a conductive gel.

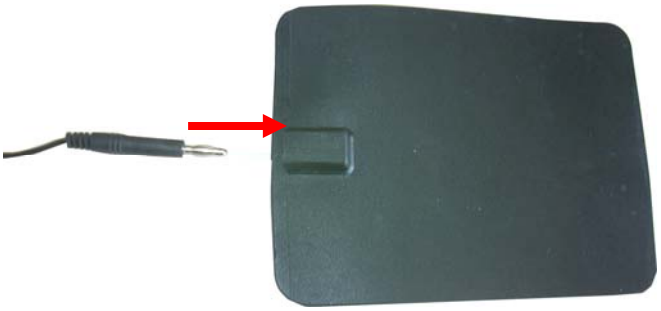


Fig.18 Connection of the cables to the earth plate

- Insert the solution in the transdermic handle dispenser

For the installation of the second electrode perform the same operations described in precedence, connecting the electrode to the socket with the Roman number 2

11.3 Installation of the ultrasound spatula

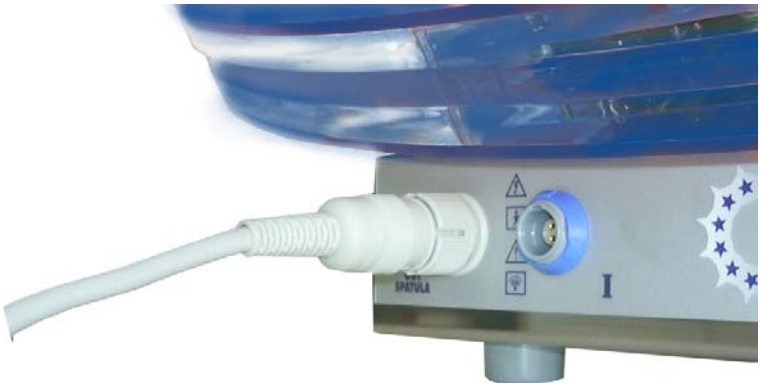


Fig.19 Connection of the ultrasonic spatula to the base unit

For the connection of the ultrasounds spatula connect the connector of the spatula to the socket denominated "spatula"

IN SOME THERAPIES IT IS NECESSARY TO CONNECT THE WRIST STRAP OF MASS TO THE DEVICE IT IS TO MAKE IT WEAR TO THE PATIENT. THE NECESSITY OF THE CONNECTION IS VISUALIZED BY THE DEVICE THROUGH THE FOLLOWING WARNING ON THE DISPLAY

Fig.20 Screen that indicates to connect the earth wrist strap

⚠ THE WRIST STRAP MUST BE WET WITH WATER BEFORE BEING WORN TO THE PATIENT, THIS TO AVOID IRRITATIONS ON THE POINT OF CONTACT WITH THE SKIN. FOR A CORRECT HYGIENE PUT AMONG THE WRIST STRAP AND THE SKIN A ROLL OF WET TNT WITH WATER THAT MUST HAVE REPLACED TO EVERY TREATMENT

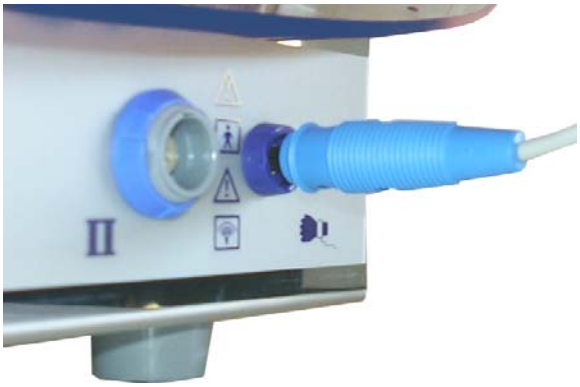


Fig.21 Connection between the earth wrist strap and the base unit

Connect the wrist strap of mass to the special connection as illustrated in figure

12. TREATMENT PROCEDURES

Before starting:

The areas destined to the treatment must carefully be cleaned with suitable solutions to remove impurity and possible residues of makeup (in the case of treatment revitalising for the face).

To facilitate the cavitation process it is to recommends to use rinsing detergents, such process will precede the treatment of veiculation.

Make to strip the patient of every metal object in contact with the skin

Before beginning the treatment with the ultrasounds, we recommend to prepare the choosen gels for the treatment of the veiculation, by the mean of a good mixing, vigorously shake them.

12.1 TRANSDERMIC VEICULATION MODE

12.1.1 P.E.F.S. / Cellulite

The methodic is suitable in the treatment of patients with located adiposity and P.E.F.S. After having developed the preliminary procedures of preparation, make to stretch prone the patient for the treatment of the trocanter zone, or supine for the treatment of the intern part of the thigh and of the knee. Carefully mix the select substances (in powder) with the gel, and screw the bottle dispenser to the transdermic veiculation handle dispenser.
Position the plate of mass and select the electroveiculation program.

- From the main menu, select, the desired treatment;
- Press the key ENT to confirm the choice;

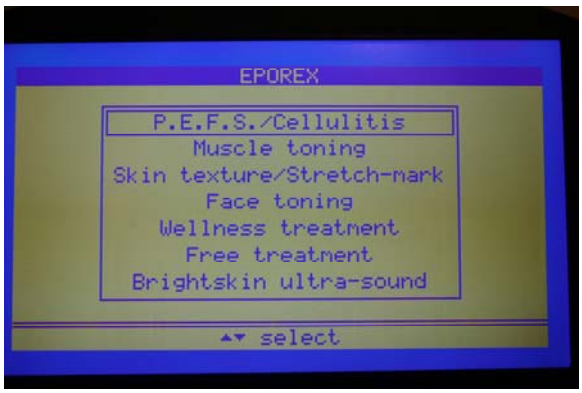
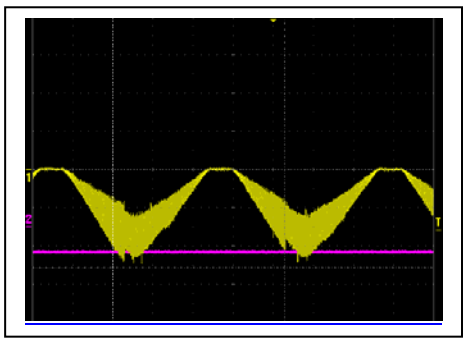


Fig.22 Choosing of the treatment mode from the main menu



Waveforms

- Set the duration of the treatment through the keys of selection;
- Set the intensity of treatment acting on the keys of regulation intensity;
- Set the correct intensity of treatment to begin from the low level of intensity up to make to warn a light feeling of prickle to the patient watching out for not to make it become annoying.

Fig.23 Setting of the treatment duration

- Press the key ENT to begin the treatment, an intermittent symbol will flash at the beginning of the treatment;
- The time of treatment on the display start to increase;

Fig.24 Setting the treatment intensity

- To interrupt the treatment press the key ESC.
- Press the key ESC to return to the principal menu

Begin the treatment putting the steel part of the handle dispenser in contact with the zone to treat of the patient and apply a massage contemporarily practicing a light pressure on the dispenser to facilitate the spillage of the gel.
At the end of the time of treatment, massage the zone treated for favoring the absorption of the residual product.

12.1.2.Muscle Toning

After having developed the preliminary procedures of preparation, make to stretch prone the patient for the treatment of the trocanter zone, or supine for the treatment of the part intern thigh and of the knee. Carefully mix the select substances (in powder) with the gel, and to screw the bottle dispenser to the transdermic veiculation handle dispenser.
Position the plate of mass and select the electroveiculation program.

- From the main menu, select, the desired treatment;
- Press the key ENT to confirm the choice;

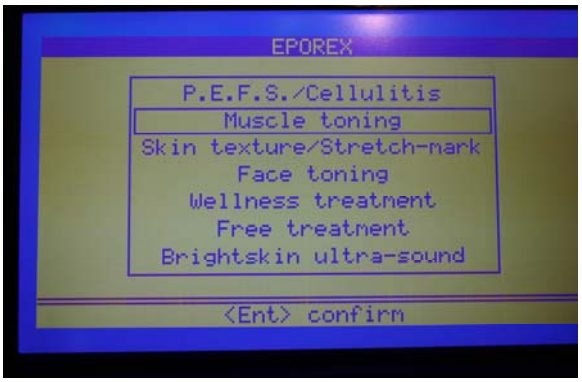
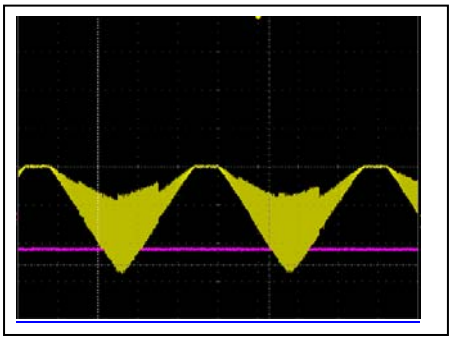


Fig.25 Choosing of the treatment mode from the main menu



Waveforms

- Set the duration of the treatment through the keys of selection;
- Set the intensity of treatment acting on the keys of regulation intensity;
- Set the correct intensity of treatment to begin from the low level of intensity up to make to warn a light feeling of prickle to the patient watching out for not to make it become annoying.

Fig.26 Setting of the treatment duration

- Press the key ENT to begin the treatment, an intermittent symbol will flash at the beginning of the treatment;
- The time of treatment on the display start to increase;

Fig.23 Setting the treatment intensity

- To interrupt the treatment press the key ESC.
- Press the key ESC to return to the principal menu

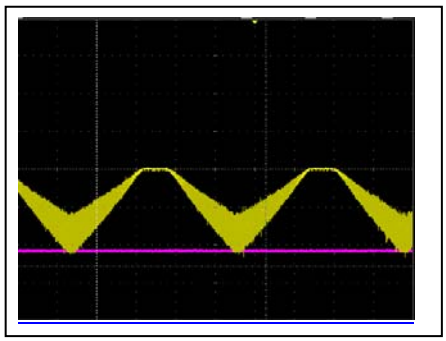
Begin the treatment putting the stell part of the handle dispenser in contact with the zone to treat of the patient and make the massage contemporarily practicing a light pressure on the dispenser to facilitate the spillage of the gel.
At the end of the time of treatment, massage the zone treated for favoring the absorption of the residual product.

12.1.3 Skin Texture/Stretch-mark

After having developed the preliminary procedures of preparation, make to stretch prone the patient for the treatment of the trocanter zone, or supine for the treatment of the part intern thigh and of the knee. Carefully mix the select substances (in powder) with the gel, and to screw the bottle dispenser to the transdermic veiculation handle dispenser.
Position the plate of mass and select the electroveiculation program.

- From the main menu, select, the desired treatment;
- Press the key ENT to confirm the choice;

Fig.27 Choosing of the treatment mode from the main menu



Waveforms

- Set the duration of the treatment through the keys of selection;
- Set the intensity of treatment acting on the keys of regulation intensity;
- Set the correct intensity of treatment to begin from the low level of intensity up to make to warn a light feeling of prickle to the patient watching out for not to make it become annoying.

Fig.28 Setting of the treatment duration

- Press the key ENT to begin the treatment, an intermittent symbol will flash at the beginning of the treatment;
- The time of treatment on the display start to increase;

Fig.29 Setting the treatment intensity

- To interrupt the treatment press the key ESC.
- Press the key ESC to return to the principal menu

Begin the treatment putting the stell part of the handle dispenser in contact with the zone to treat of the patient and make the massage contemporarily practicing a light pressure on the dispenser to facilitate the spillage of the gel.
At the end of the time of treatment, massage the zone treated for favoring the absorption of the residual product.

12.1.4 Face Toning

For this treatment we recommend the use of the handle dispenser with single (to see page 9) roll. Carefully mix the select substances (in powder) with the gel, and screw the bottle dispenser to the transdermic handle dispenser.
Position the electrode of mass and predispose the use of the program for the ettroveiculation.

- From the main menu, select, the desired treatment;
- Press the key ENT to confirm the choice;

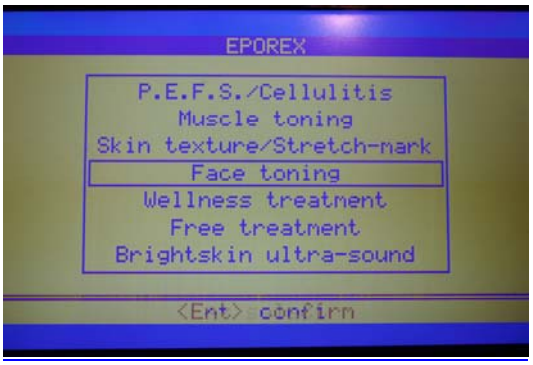
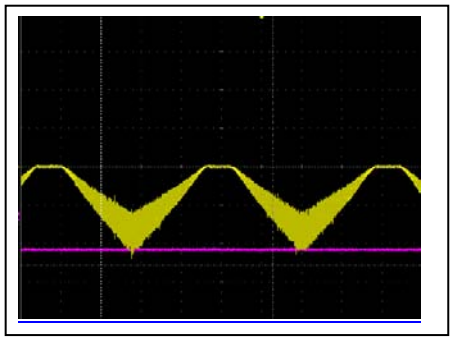


Fig.30 Choosing of the treatment mode from the main menu



Waveforms of selection;

- Set the intensity of treatment acting on the keys of regulation intensity;
- Set the correct intensity of treatment to begin from the low level of intensity up to make to warn a light feeling of prickle to the patient watching out for not to make it become annoying.

Fig.31 Setting of the treatment duration

- Press the key ENT to begin the treatment, an intermittent symbol will flash at the beginning of the treatment;
- The time of treatment on the display start to increase;

Fig.32 Setting the treatment intensity

- To interrupt the treatment press the key ESC.
- Press the key ESC to return to the principal menu

Begin the treatment putting the stell part of the handle dispenser in contact with the zone to treat of the patient and make the massage contemporarily practicing a light pressure on the dispenser to facilitate the spillage of the gel.
Move the handle dispenser slowly following the tension face lines, treat singles areas by dividing the total time previously selected in the treatment mode selected.
At the end of the time of treatment, massage the zone treated for favoring the absorption of the residual product.

12.1.5 Wellness Treatments

After having developed the preliminary procedures of preparation, make to stretch the prone patient for the treatment of the zone trocantera, or supine for the treatment of the part intern thigh and of the knee. Carefully mix the select substances (in powder) with the gel, and screw the bottle dispenser to the transdermic veicultation handle dispenser.
Position the electrode of mass and predispose the use of the electroveicultation program.
Il trattamento è indicato, ad esempio, nel trattamento di stati dolorosi infiammatori.

- From the main menu, select, the desired treatment;
- Press the key ENT to confirm the choice;

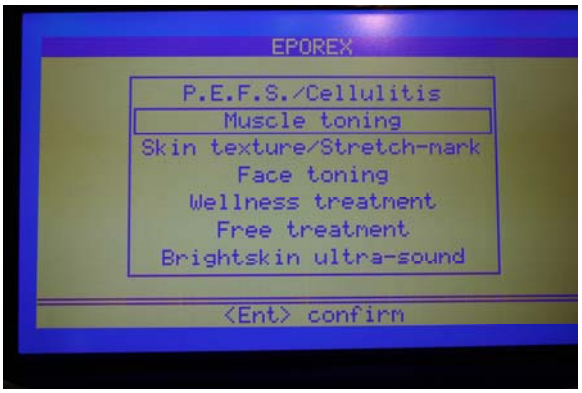
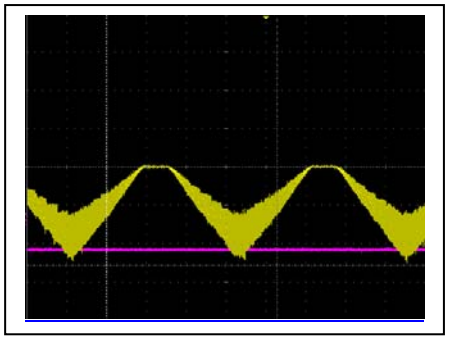


Fig.33 Choosing of the treatment mode from the main menu

- Once pressed the key ENT will appear another menu for the choice of the depth of penetration of the substance; are available three options
  - Low Level (For light pain)
  - Medium Level
  - High Level (For deep pain)
- Choose the preferred formality through the keys of selection
- Confirm pressing the key ENT.

Fig.34 Setting of the treatment level



Waveforms

- Set the duration of the treatment through the keys of selection;
- Set the intensity of treatment acting on the keys of regulation intensity;
- Set the correct intensity of treatment to begin from the low level of intensity up to make to warn a light feeling of prickle to the patient watching out for not to make her/it become annoying.
- Press the key ENT to begin the treatment, an intermittent symbol will start flashing during the execution; the time of suitable treatment on the display will start to increase;

Fig.35 Setting the treatment intensity

- To interrupt the treatment press the key ESC.
- Press the key ESC to return to the principal menu

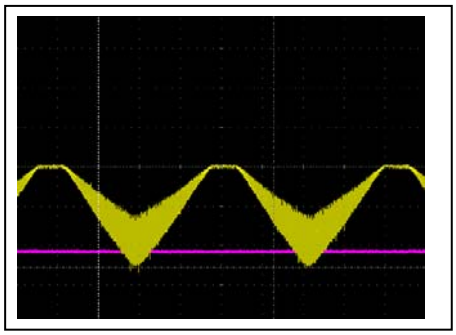
Begin the treatment putting the stell part of the handle dispenser in contact with the zone to treat of the patient and make the massage contemporarily practicing a light pressure on the dispenser to facilitate the spillage of the gel.
Move the handle dispenser slowly following the tension face lines, treat singles areas by dividing the total time previously selected in the treatment mode selected.
At the end of the time of treatment, massage the zone treated for favoring the absorption of the residual product.

12.1.6 Free Treatment

After having performed the preliminary procedures of preparation, make to stretch the prone patient for the treatment of the zone trocantera, or supine for the treatment of the part intern thigh and of the knee. Carefully mix the select substances (in powder) with the gel, and screw the bottle dispenser to the transdermic veicultation handle dispenser.
Position the electrode of mass and predispose the use of the electroveicultation program.

- From the main menu, select, the desired treatment;
- Press the key ENT to confirm the choice;

Fig.36 Choosing of the treatment mode from the main menu



Waveforms

- Once pressed the ENT key, will appear another menu for the choice of the depth of penetration of the substance; are available three options

Fig.37 Setting of the treatment deep

- Set the duration of the treatment through the selection keys;
- Set the intensity of treatment acting on the keys of regulation intensity;
- Set the correct intensity of treatment to begin from the low level of intensity up to
- make to warn a light feeling of prickle to the patient watching out for not to make it become annoying.

Fig.38 Setting of the treatment duration

- Press the key ENT to begin the treatment, an intermittent symbol will start flashing during the execution; the time of suitable treatment on the display will start to increase;

- 

Fig.39 Setting the treatment intensity

- To interrupt the treatment press the key ESC.
- Press the key ESC to return to the principal menu

Begin the treatment putting the stell part of the handle dispenser in contact with the zone to treat of the patient and make the massage contemporarily practicing a light pressure on the dispenser to facilitate the spillage of the gel.
Move the handle dispenser slowly following the tension face lines, treat singles areas by dividing the total time previously selected in the treatment mode selected.
At the end of the time of treatment, massage the zone treated for favoring the absorption of the residual product.

12.2 BRIGHTSKIN ULTRASOUND TREATMENT

The system Eporex K69-E, thanks to the use of the Ultrasounds handle dispenser and principles of the cavitation is able to remove died cells by the horny layer.
The ultrasonic handle dispenser, equipped with a piezoelectric oscillating device at 25 KHz, has in the distal part a metallic spatula, that allows an accurate job of cleaning and preparation to the transdermical electroveiculation.

Don't use the Brightskin spatula in presence of liquids or in immersion in liquid. Brightskin is not protected against the liquids, the immersion or penetration of liquids. If Brightskin should be put in contact with a liquid, immediately suspend the treatment and contact the technical support Medical & Technology.

WARNING: read carefully the instructions

- **Do not use the ultrasounds probe on the skin without water, gel or appropriate product;**
- **Do not dip the probe in water, the degree of protection is IP 0. Only the tip can be brought in contact with liquid;**
- **Absolutely keep away from children;**
- **Do not use the spatula in perpendicular position referred to the surface to treat;**

Fig.40 Wrong use of the ultrasonic spatula

- **Do not use the probe on subjects carriers of pacemaker or other devices whose operation could be influenced by electrostatic electromagnetic or current interferences;**

- **The treatment to the face could interfere with acoustic instruments, it is necessary to remove them before the beginning of the treatment;**

- **Don not to use the spatula in the immediate contour eyes, on patient with heart disease, on pregnancy patients, patient with affections on the treatment area like neoplasticis, ulcers and bedsores, abrasions, wounds, obliterating arteriopatie, phlebitis, trombophlebitis, varicouse,suspicious snows;**
- **Do not use the spatula on subjects with reduced cutaneous sensibility, unable to communicate or affected by paralysis (different medical prescription excepted);**

- **Avoid the use on subjects connected to equipments of monitoring of the vital parameters;**
- **Do not apply the electrodes on traumatized parts, cuts, lips and mucous generally, carotid and its proximities, area of the breast, cardiac area;**

12.2.1 Bightskin Menu

From the principal menu, acting on the selection buttons , set the "Brightskink ultra-sound" mode, push the ENT key to confirm

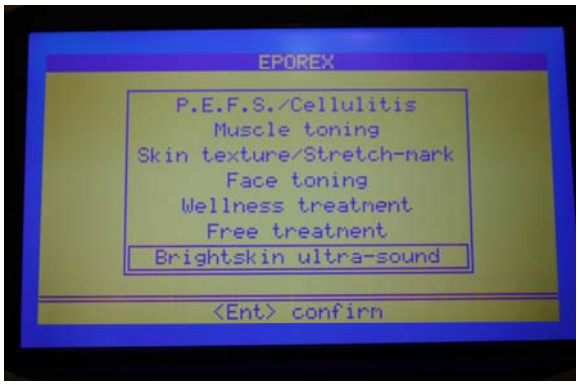


Fig.41 Choose of the treatment mode from the main menu

Once confirmed the choice, set the type of skin to treat, using the keys of selection and confirm by pressing the ENT key;

Fig.42 Setting of the type of skin

The following menu shows the available types of treatment in the brightskin ultrasound treatment mode.
Choose the desired treatment by pressing the selection keys and confirm the choice pressing the key ENT:

Fig.43 Treatment modalities of the brightskin menu

12.2.2 Dermopurification

The Dermopurification mode is suitable for the execution of peeling and cleaning of the treated areas.

- From the menu Brightskin select dermpurification and press ENT
- Set the time of treatment acting on the keys of selection
- Begin the treatment pressing the ENT key
- To end the treatment press the ESC key
- Press the ESC key to return to the Brightskin menu

Fig.44 Dermopurification treatment screen

12.2.3 Deep Purification

The Deep Dermopurification treatment is suitable for the execution of peeling and deep cleaning of the treated areas.

- From the menu Brightskin select deep dermpurification and press ENT
- Set the time of treatment acting on the keys of selection
- Begin the treatment pressing the ENT key
- To end the treatment press the ESC key
- Press the ESC key to return to the Brightskin menu

Connect the **wrist strap with the mass cable** to the wrist or on the arm of the patient when required by the selected treatment; **the application is recommended from the presence on the display of the sign + or -**

Fig.45 View of the deep dermopurification treatment

The wrist strap must be wet with water before being worn to the client, this to avoid irritations on the point of contact with the skin. In order to keep a correct hygienic behavior always interpose between the wrist strap and the skin a roll of well wet TNT (Fabric not Fabric) with water, this has to be replaced after every treatment.

12.2.4 Deep Purification Tone-up

The Deep Ton up Dermopurification is suitable for the execution of peeling and deep cleaning of the treated areas and for a firm of the same.

- From the menu Brightskin select deep tone up dermpurification and press ENT
- Set the time of treatment acting on the keys of selection
- Begin the treatment pressing the ENT key
- To end the treatment press the ESC key
- Press the ESC key to return to the Brightskin menu

Connect the **wrist strap with the mass cable** to the wrist or on the arm of the patient when required by the selected treatment; **the application is recommended from the presence on the display of the sign + or -**

Fig.46 View of the deep tone up dermopurification treatment

The wrist strap must be wet with water before being worn to the client, this to avoid irritations on the point of contact with the skin. In order to keep a correct hygienic behavior always interpose between the wrist strap and the skin a roll of well wet TNT (Fabric not Fabric) with water, this has to be replaced after every treatment.

12.2.5 Tone Up Action

The tone up action treatment is suitable for the firm of the treated areas.

- From the menu Brightskin select tone up treatment action and press ENT
- Set the time of treatment acting on the keys of selection
- Begin the treatment pressing the ENT key
- To end the treatment press the ESC key
- Press the ESC key to return to the Brightskin menu

o

Fig.47 View of the tone up action treatment

12.2.6 Skin Reactivation

- From the menu Brightskin select cutaneous treatment reactivation and press ENT
- Set the time of treatment acting on the keys of selection
- Begin the treatment pressing the ENT key
- To end the treatment press the ESC key

Press the ESC key to return to the Brightskin menu

Connect the **wrist strap with the mass cable** to the wrist or on the arm of the patient when required by the selected treatment; **the application is recommended from the presence on the display of the sign + or -**

Fig.48 View of the cutaneous treatment reactivation

⚠ The wrist strap must be wet with water before being worn to the client, this to avoid irritations on the point of contact with the skin. In order to keep a correct hygienic behavior always interpose between the wrist strap and the skin a roll of well wet TNT (Fabric not Fabric) with water, this has to be replaced after every treatment.

12.2.7 Reactivation tone-up

- From the menu Brightskin select tone up reactivation treatment and press ENT
- Set the time of treatment acting on the keys of selection
- Begin the treatment pressing the ENT key
- To end the treatment press the ESC key

Press the ESC key to return to the Brightskin menu

Connect the **wrist strap with the mass cable** to the wrist or on the arm of the patient when required by the selected treatment; **the application is recommended from the presence on the display of the sign + or -**

Fig.49 View of the tone up reactivation screen

The wrist strap must be wet with water before being worn to the client, this to avoid irritations on the point of contact with the skin. In order to keep a correct hygienic behavior always interpose between the wrist strap and the skin a roll of well wet TNT (Fabric not Fabric) with water, this has to be replaced after every treatment.

For a correct use in the folloqing treatments:

- Dermopurification
- Deep Dermopurification
- Toning Action

Use the ultrasonic handle dispenser as shown in the figure:

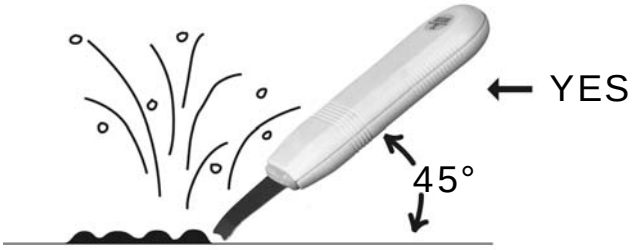


Fig.50 Correct use handling of the ultrasonic handle dispenser

For a correct use in the folloqing treatments:

- Toning Action
- Cuteneous Reactivation
- Toning reactivation

Use the ultrasonic handle dispenser as shown in the figure:

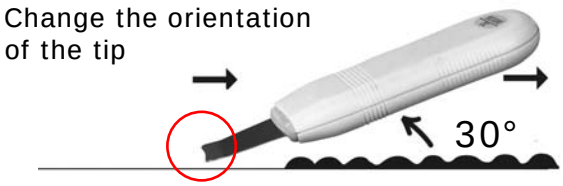


Fig.51 Correct use handling of the ultrasonic handle dispenser

13. MAINTENANCE

13.1 Ordinary Maintenance

The Eporex K69-E system has been developed for working in reliable way with a minimum maintenance. We advise to perform periodic interventions of maintenance from specialized personnel authorized by the Medical & Technology. The Medical & Technology can instruct personnel for technical interventions and ordinary maintenance.

Note: The Eporex K69-E system doesn't contain sharp parts that must have submitted to maintenance. Every attempt of reparation, restoration or change of the system, that is not contemplated in this user guide, from not authorized Medical & Technology personnel will make void the guarantee.

Don't remove the covering to prevent the risk of electric shocks. Every technical intervention must be entirely effected by skilled personnel. For every intervention contact a representative of the company.

13.2 Cleaning and Disinfection of the device

Warning: before effecting the cleaning or the disinfection of the unit, disconnect the equipment from the power supply. Keep the equipment away from water.

The wrap of the console can be clean with a damp cloth. Is possible to use an antiseptic detergent or a non aggressive detergent. Avoid use chemical detergents, aggressive chemical detergents or rough cloths that could damage the surface of the wrap.

The device is not protected against the penetration of liquids (IP 20), avoid spray or infiltrations of liquids or immersions of any type.

Cleaning and hygienization of the handle dispensers

Make an accurate cleaning of the metallic rolls, inside the chamber of ionization with the purpose to avoid possible stagnations of gel e/o substances treating.

With the purpose to avoid the solidification of gel residues inside the chamber of ionization, it is recommended to dip the handle dispensers (Fig.4. Fig. 5) completely in water at the end of the treatment

Don't effect the disinfection with corrosive products

Cleaning and hygienization of the ultrasound probe

Clean the ultrasounds probe with a soft cloth dampened with water and neutral detergent. Clean the metallic spatula with non-corrosive products not nuked, applied with cloths and napkins humidified.
In the case in which infiltration of liquid had to be verified in the probe, leave it suspended with the spatula head orientated toward earth to make t the liquid flow out, do not it until the probe is completely dry.

For the substitution of the ultrasounds probes Brightskin contact the distributor of zone or to the laboratories Medical & Technology.

13.3 Fuses replacement

For the fuses replacement

Pull and change the fuses

Fig.52 Replacement of fused

13.4 Planned Maintenance

Perform, with illustrated cadency in the following chart the following operations:

Maintenance	Personal	Lilt
Cleaning and hygienization of the console	Operator	Daily
Cleaning and patient hygienization of the cables	Operator	Daily
Cleaning, hygienization or substitution of the accessories to direct contact with the patient	Operator	At the end of every session
Visual control of the integrity of the power supply cables	Operator	Weekly
Visual control of integrity of the cables in contact with the patient	Operator	Weekly
Inside cleaning of the console, verification of the functionality, revision and setting	Laboratories Medical & Technology	Annual
Verification of the current of dispersion and the electric safety	Laboratory qualitficato	Annual
Calibration ultrasound probe, Examination of treatments head -examination of the cables	Laboratories Medical & Technology	Annual

Fig.53 Programmed maintenance table

13.5 Technical assistance

Medical & Technology on request, will make available schematics diagrams of connection, lists of the parts of exchange, descriptions and other information that can allow the qualified technical personnel to effect the ordinary maintenance of the device Eporex K69-E. If the Medical & Technology had to believe that the parts cannot be repaired or if a training were necessary or a special equipment to effect the reparation or the regulation, the Medical & Technology can refuse the information for safety motives. If is necessary the restitution of the device Eporex K69-E to the Medical & Technology, the unity must be accompanies from a declaration on the state of contamination.

13.6 Disposal

As required by the Directive 2002/96 pertaining the Waste of Electronic and Electric (RAEE) Equipments, are necessary:

not do dispose the RAEEs as common refuse and effect a separate disposal of such RAEE;

Contact your own City of Residence for information around the centers of separate disposal for the RAEEs;

This symbol stand for indicate that the waste ha sto be trated separatly from other discards (Ref.Directive 2002/96/CE)

The right differed disposal for the following start of the negleted instrument to the recycling, to the treatment and the disposal environmentally compatibilie contribute to avoid negative effects on the environment and on the health and it favors the recycle of some materials which the product is composed.

14. RESOLUTION OF THE PROBLEMS

1. The device does not turn on:
· check to have connected the power supply cable to the socket of the electric power net
· verify the presence of tension on the electric net
· verify the integrity of the fuses

2. The substance doesn't flow out from the handle dispenser
· Verify the cleaning of the nozzles of the rolls
· Verify the connections of the cables of connection between the handle dispenser and base unit

3. The ultrasounds spatula doesn't work
· Verify the correct connection of the spatula to the base unit
· Verify to have connected the wrist strap of mass for the applications that ask for it

4. The handle dispensers (Fig.4. Fig.5) doesn't transmit energy and/or the red cable deattach from the electrode
· Replace the terminal part of the red cable with a new one

15. WARRANTY

Medical & Technology guarantees the system Eporex K69-E against defects of material and production for a period of 24 months. During this period you can be agreed with the Medical & Technology contract of extension of guarantee and programmed assistance.

The guarantee starts from the date of the installation.

Note: every attempt of reparation, restoration or change of the system, that is not contemplated in this user guide, from not authorized personnel by Medical & Technology, will make decay the warranty.

To Obtain the warranty intervention, the buyer, immediately after the accertain of the event related to the claim, must contact the Medical & Technology via mail or telephone.

Under no circumstances Medical & Technology, will be responsible of direct damages, indirect, special, accidental, consequential from convention, from civil crime or from other legal concept.

MEDICAL & TECHNOLOGY srl

Strumenti tecnologici per la medicina e l'estetica

Via della Fiera 87- 47900 Rimini(Rn)
WWW.medical-technology.it
info@medical-technology.it

APPENDIX B

Participant information card

CLIENT CARD CONSULTATION SHEET	
Identification number:	Date of Birth:
Race:	Date:
Consultant:	

CONTRA-INDICATIONS

Epilepsy	Jaundice/ hepatitis/TB/HIV	Back/neck pain
Pacemaker	High blood pressure	Menopause
Cancer	Ulcer	Cuts/sores/abrasions
Pregnant/ breastfeeding	Rheumatic	Asthma
Serious medical conditions	Hormonal problems/ imbalance	Drink alcohol
Infectious conditions	Diabetic	Smoker
Menstruation	Thyroid problems	Metal implants
Stroke	Bra size/ptosis	Reduction amount
Height	Weight	BMI

Do you take any medication including vitamins? If yes, please list.

Are you currently under a physician's care? (Y/N)

Are you allergic to anything (e.g. metal, penicillin) (Y/N)

Do you have a tendency to develop scars / healing ability? (e.g. Good, poor)

Notes

Participants Identification number: _____

Treatment number	Date	Intensity
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		

Comments / observations prior to treatment:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

Comments / observations after completion of treatment:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

A summary of the Wise pattern with inferior pedicle operative technique

- Once in theatre the pedicle was measured (anatomical position) 2cm above the nipple areola complex with 6-8cm measured in width on either side of the nipple in order to centre the nipple areola complex on the breast meridian (Figure C1).
- Participants were then placed in the supine position, anaesthetized and intubated before surgery commenced.

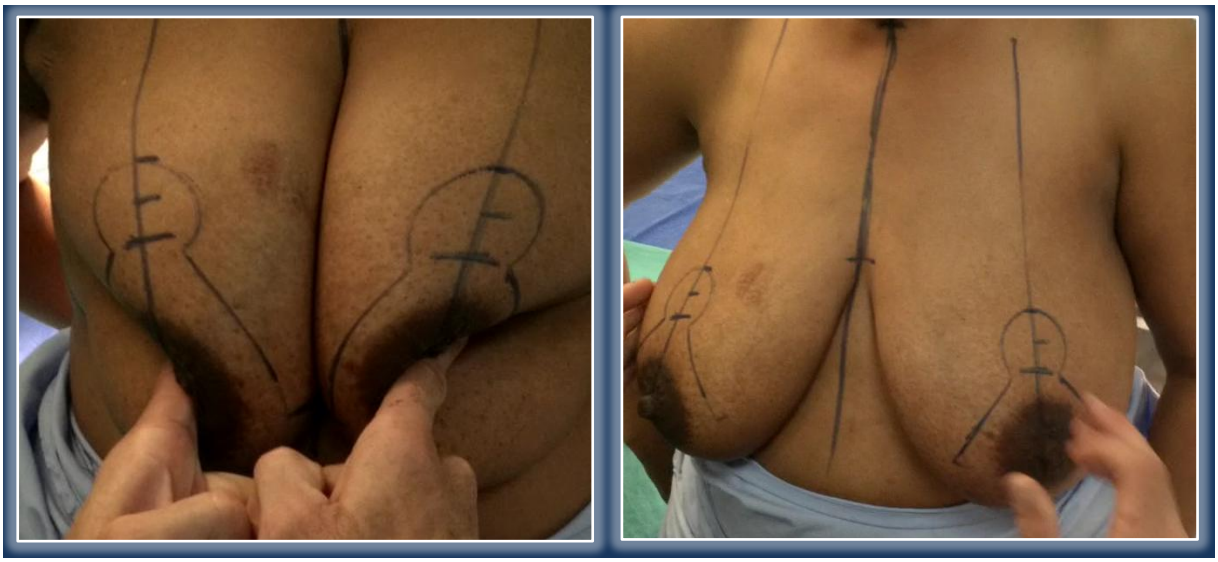


Figure C1 Markings of the inferior skin resection pattern

- The surgeon started with the U-shape procedure (inferior pedicle incision), starting at the inframammary fold; from the fold, the incision moved up and around the nipple area.

- The areola was stretched, and depending on the participant"s breast size, a circular marking of 3.5-4.5cm in diameter was made around the areola (Figure C2).

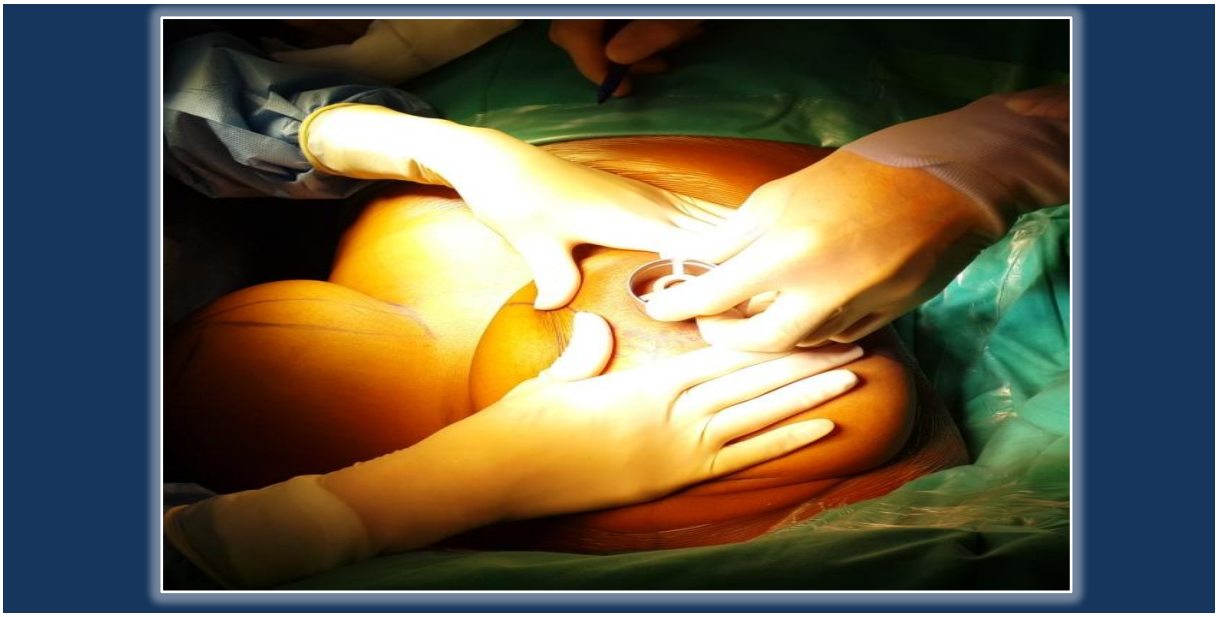


Figure C2 Marking of the areola complex using a cookie cutter

- Following the markings, the skin was incised up to the dermal layer.
- De-epithelialisation of the inferior pedicle skin followed and incisions were made on both the lateral and medial sides of the breast (Figure C3).

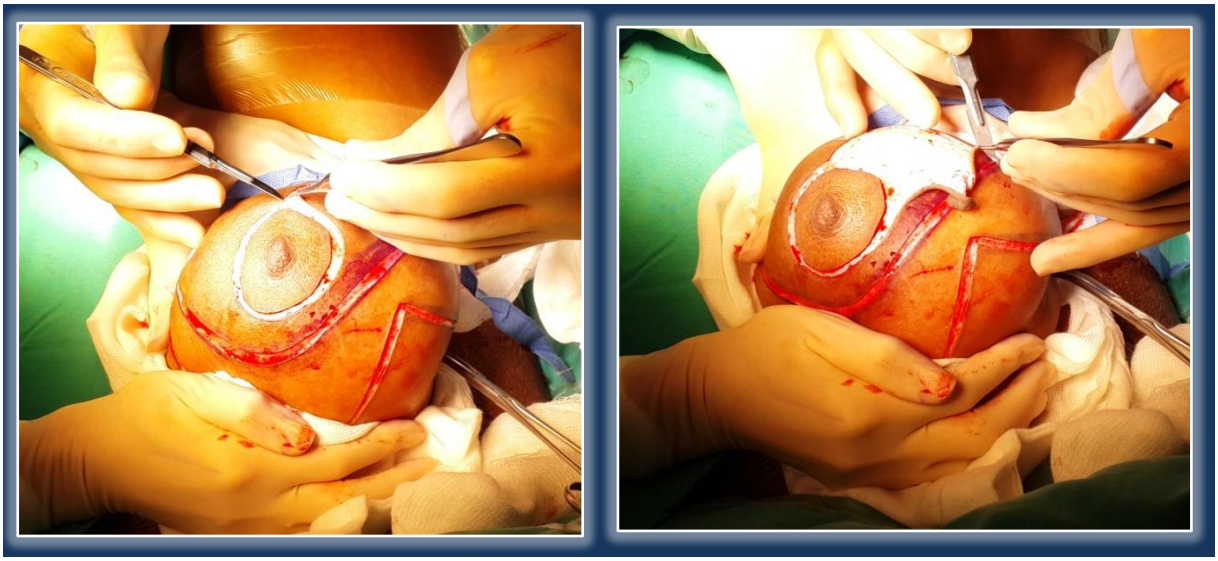


Figure C3 De-epithelialisation of the inferior pedicle skin

- The breast tissues of the superior flaps were then thinned out to achieve the natural breast shape and size, as determined prior to surgery (Figure C4).

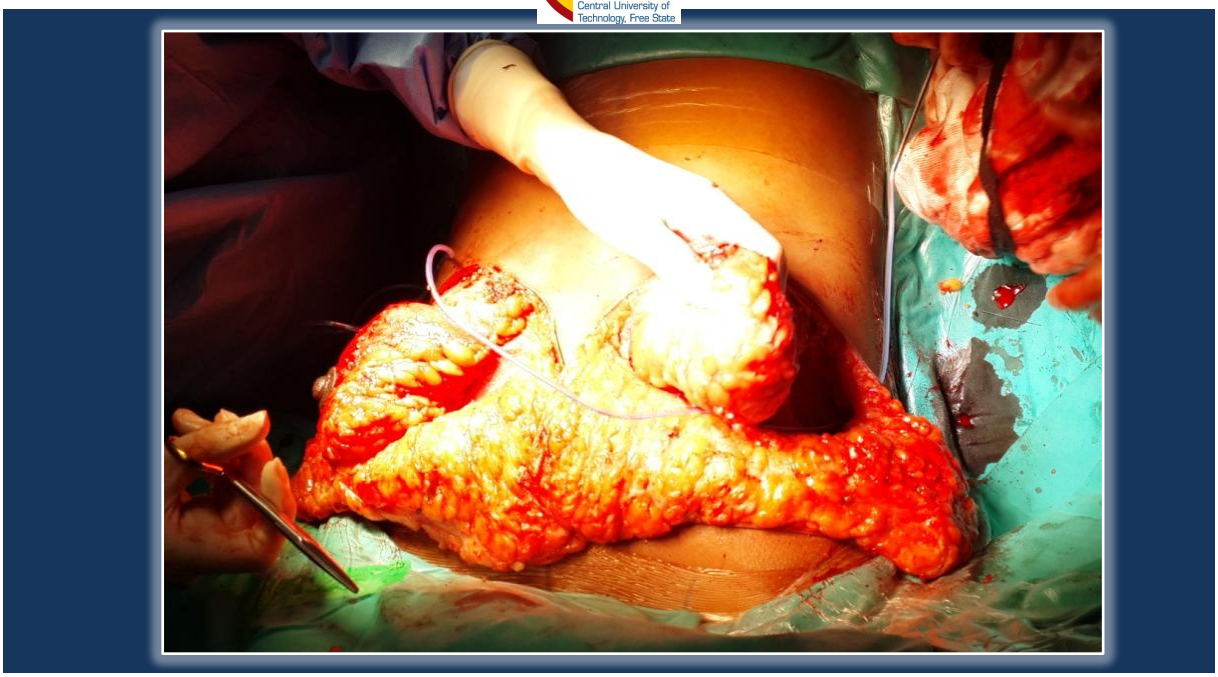


Figure C4 Thinning out breast tissue on the superior flaps

- On completion of the breast tissue removal, a latex free surgivac drainage system (DJH, Defty Chemist, Bloemfontein, Free State) was inserted below the superior flaps of both breasts to ensure proper drainage of the serous fluid (Figure C5).

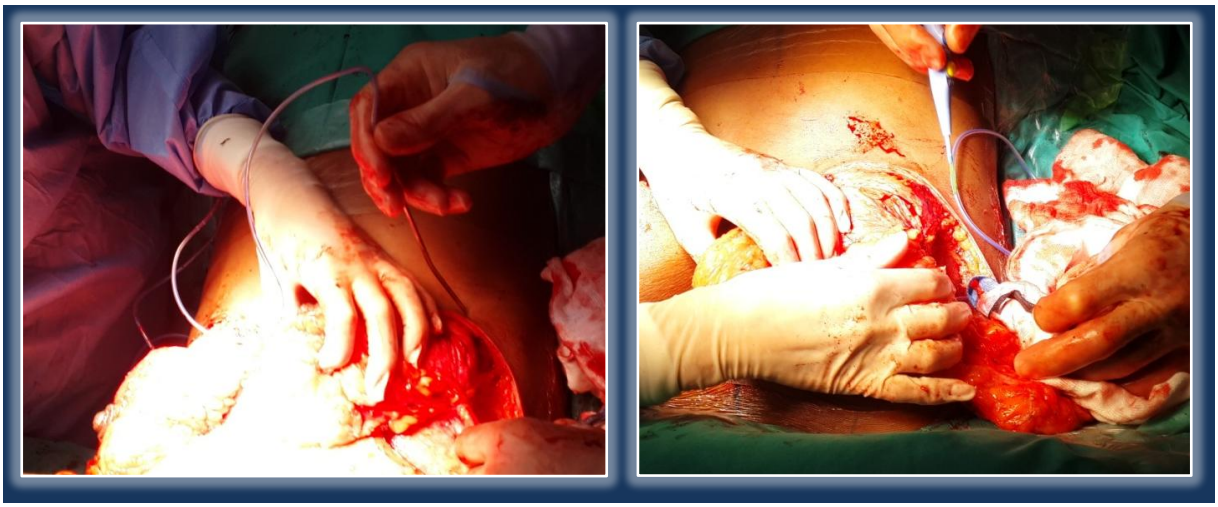


Figure C5 Insertion of the surgivac drainage system

- To ensure an adequate opening for the „new" nipple areola complex, the thinned out superior flaps were pulled towards each other utilizing a 2/0 mocryl monofilament absorbable surgical suture (Ethicon, Johnson & Johnson, Bloemfontein, Free State). One suture was inserted (Figure C6).

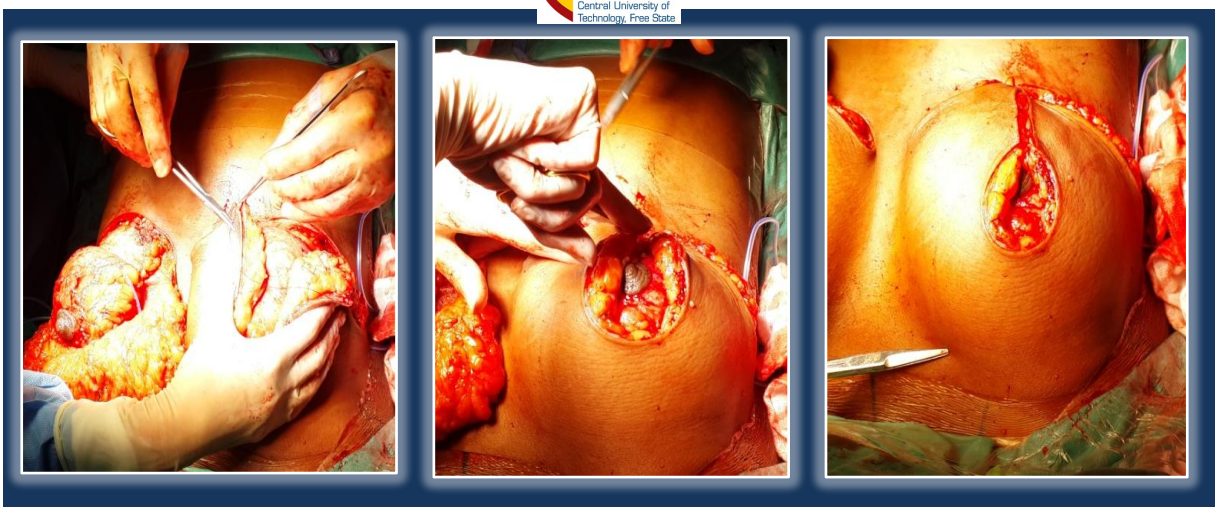


Figure C6 **Insertion of 2/0 mocryl surgical suture pulling superior flaps towards each other**

- Once the nipple areola complex was in position, the vertical line down to the inframammary fold was sutured. This was followed by suturing the inframammary fold creating an inverted T (Figure C7). Continuous intra-dermal sutures (4/0 mocryl monofilament absorbable surgical sutures; Ethicon, Johnson & Johnson, Bloemfontein, Free State) were used.

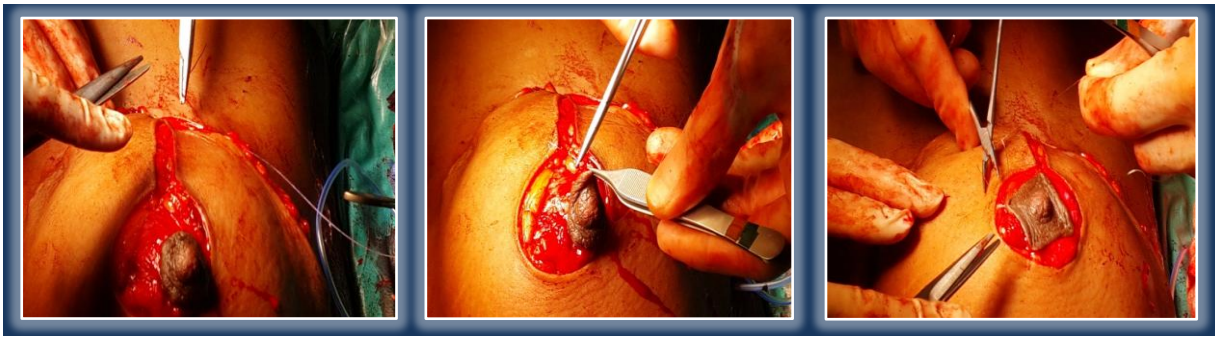


Figure C7 **Positioning of the new nipple areola complex**

- The same procedure was followed on the second breast.
- The amount of breast tissue removed (g) was weighed and recorded.
- To support healing of the breast tissue a silver acticoat plaster (Figure C8 A) was placed on the sutured lines. Further, to encourage natural breast shape development, a white micro foam plaster (DJH, Defty Chemist, Bloemfontein, Free State) was applied in a figure 8 around the breasts (Figure C8 B).

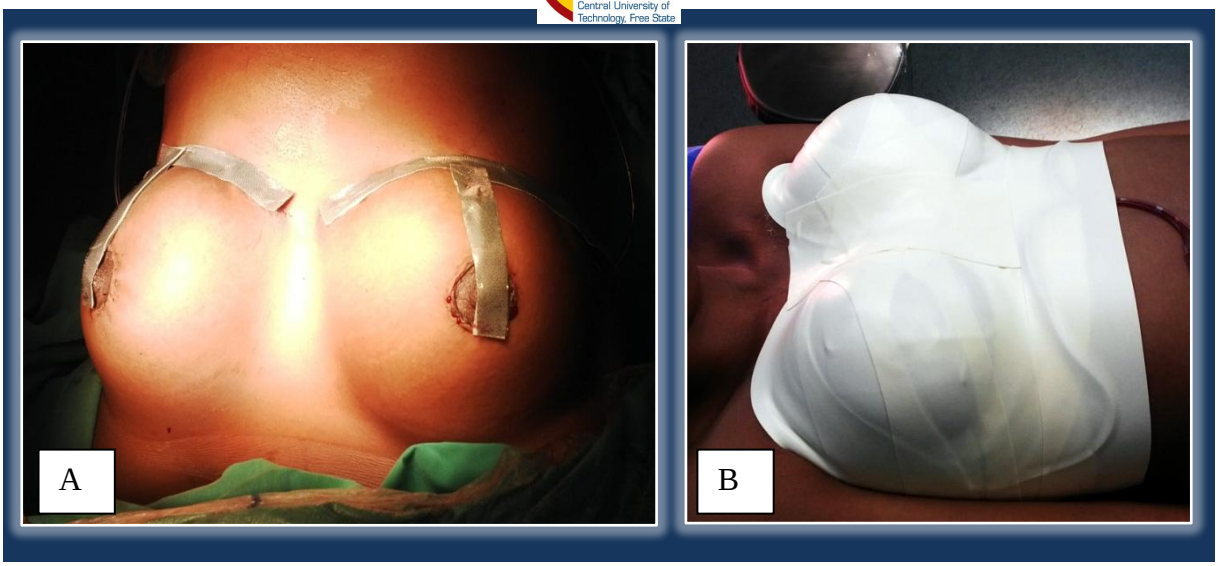


Figure C8 **Application of plaster on surgical incisions after mammary reduction surgery** (A – silver acticoat plaster, B – white micro foam plaster

- One week post-operatively, the latex free surgivac drainage system (DJH, Defty Chemist, Bloemfontein, Free State) was removed and to maintain breast support tan micro pore surgical tape (GMS Medical, Bloemfontein, Free State) was placed on the inverted T incision to assist in wound healing and limit scarring.
- A “Yummy mummy” bra (Conquest surgical, Johannesburg, Gauteng) was provided to each participant to support the breasts and provide comfort once the micro foam plaster was removed.

Vancouver Scar Scale

PARAMETER	DESCRIPTOR	POINTS
Vascularity	Normal	0
	Pink	1
	Red	2
	Purple	3
Pigmentation	Normal	0
	Hypo-pigmentation	1
	Hyper-pigmentation	2
Pliability	Normal	0
	Supple	1
	Yielding	2
	Firm	3
	Ropes	4
	Contracture	5
Height	Flat	0
	<2mm	1
	2-5 mm	2
	>5 mm	3
Total score		**/13**

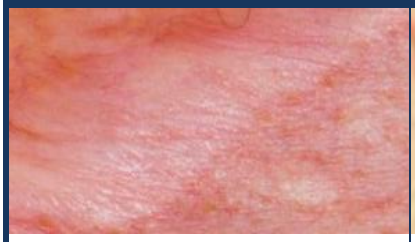
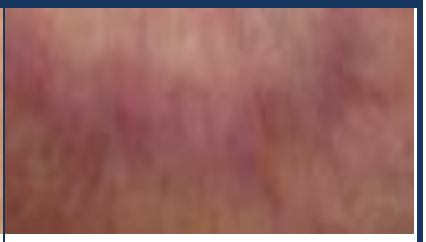
Pre set VSS Vancouver Scar Scale (VSS) assessment card with colour index

Participant Number: _____ Observer: _____

Treatment number: _____ Date of assessment: _____

Scale characteristics		Score	R	L
Vascularity	Normal	0		
	Pink	1		
	Red	2		
	Purple	3		
Pigmentation	Normal	0		
	Hypo pigmentation	1		
	Hyper pigmentation	2		
Pliability	Normal	0		
	Supple	1		
	Yielding	2		
	Firm	3		
	Ropes	4		
	Contracture	5		
Height	Flat	0		
	<2mm	1		
	2-5 mm	2		
	>5 mm	3		
Total score		**13**		

Colour index assessing vascularity using the Vancouver Scar Scale

Pink	Red	Purple
		
Pink refers to a light shade of red	Red refers to a colour or pigment; the chromatic colour resembling the hue of blood.	An intermediate colour between red and blue, however more red than blue.

**The amount of redness assessed in scar tissue is dependent on the presence of blood vessels in the area, which is tested by how quickly the skin turns red after blanching (pressure applied to the scar using the pointer finger and then releasing allowing blood flow to return to the area)*

Breast Photography

Photographic assessments were performed to visually compare the control breasts to the experimental breasts prior to the first, and after the last, treatment. Note that these photographs are not regarded as a scientific result but only added for illustration purposes.

Photographs of both breasts were taken prior to the 1st Mesotherapy Eporex treatment and after the 8th treatment for all 30 participants. This was done to visually compare the experimental breast with the control breast to evaluate whether an improvement on scar appearance was visible with the naked eye after the Mesotherapy Eporex treatments. The visual images of the three participants displayed in Figures F1, F2 and F3 were randomly selected and the left breast (B1: before treatment and B2: after 8th treatment) served as the experimental breast and the right breast (C1: control breast before treatment of experimental breast and C2: control breast after 8th treatment of experimental breast) served as the control breast (natural healing). Each participant"s breasts were photographed from a frontal view (Figures F1 A, F2 A and F3 A). Close-up photographs were taken of the left experimental breasts (Figures F1, F2 and F3 - B1 before and B2 after Mesotherapy Eporex treatments).

Visual improvement can be seen when comparing the experimental breasts before and after images (red arrows). Improvement is seen in the vascularity, pigmentation and height of the scar appearance. Natural healing (black arrows) is visually evident on the control breasts. Although the experimental breast healed quicker, the control breast continued healing naturally thus supporting the results presented in Figures F1, F2 and F3 - C1 before and C2 after Mesotherapy Eporex treatments.

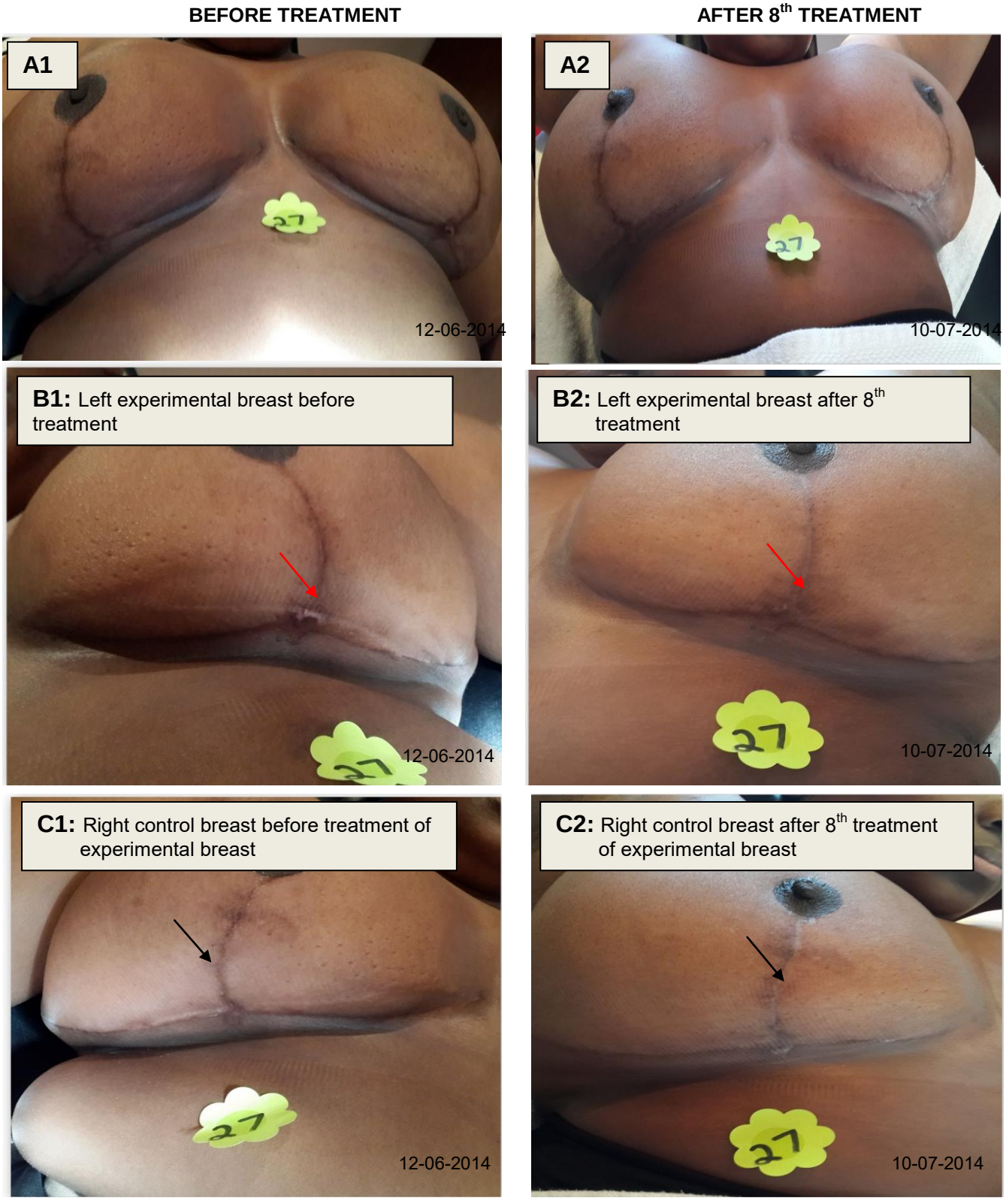


Figure F1 **Participant 27- A 36 year old patient with a Grade 3, major (16cm) ptosis. Patient underwent mammary reduction (Left breast - 530g, Right breast -502g) using the Wise pattern with inferior pedicle technique.** *[A1 - frontal view before treatment; A2 - frontal view after treatment; B1 - left breast before treatment; B2 - left breast after treatment, C1 and C2- right breasts natural healing]*

BEFORE 1st TREATMENT AFTER 8th TREATMENT

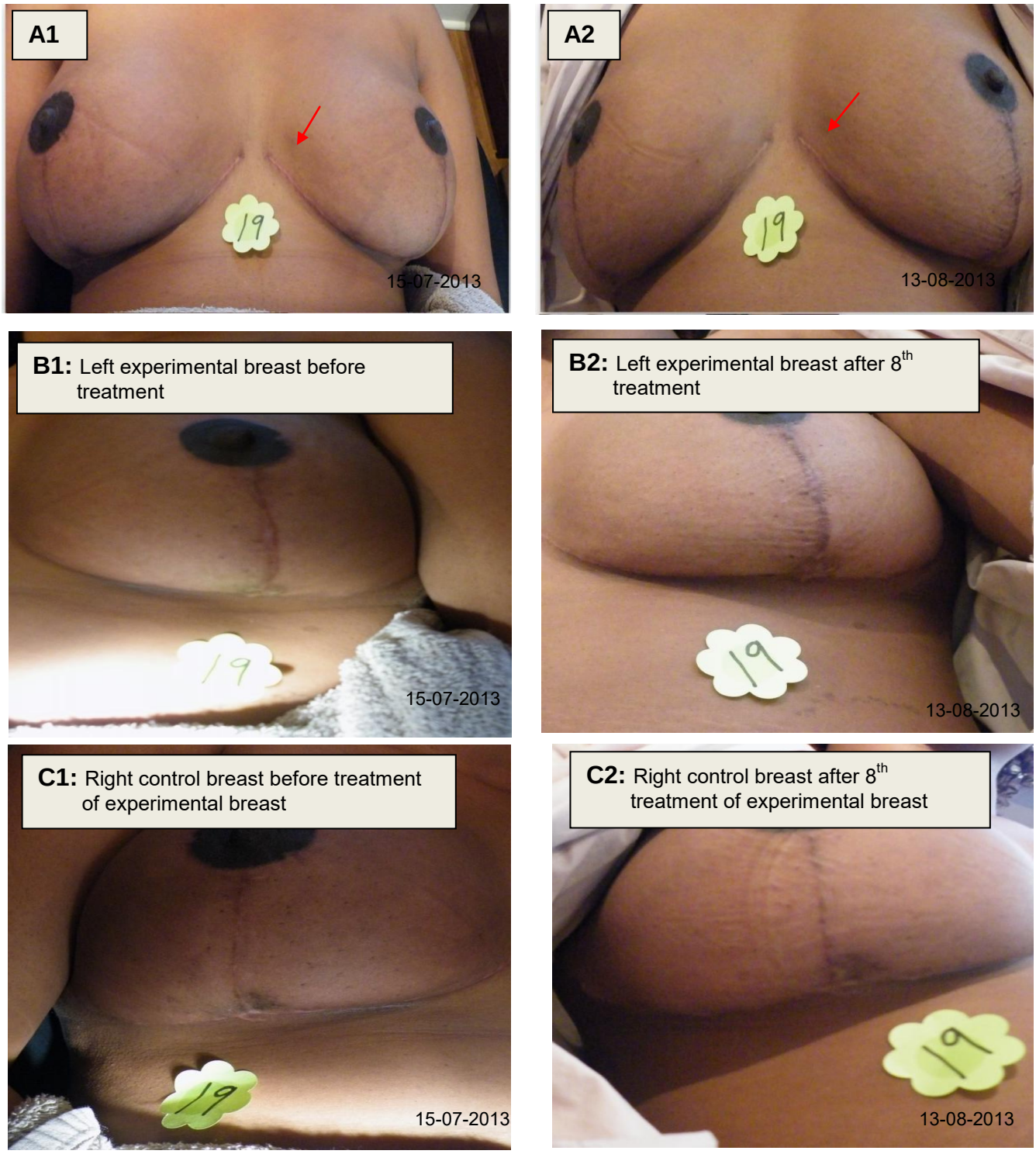


Figure F2 **Participant 19 - A 35 year old patient with a Grade 3, major (44cm) ptosis. Patient underwent mammary reduction (Left breast - 2398g, Right breast -3446g) using the Wise pattern with inferior pedicle technique.** *[A1 - frontal view before treatment; A2 - frontal view after treatment; B1 - left breast before treatment; B2 - left breast after treatment, C1 and C2- right breasts natural healing]*

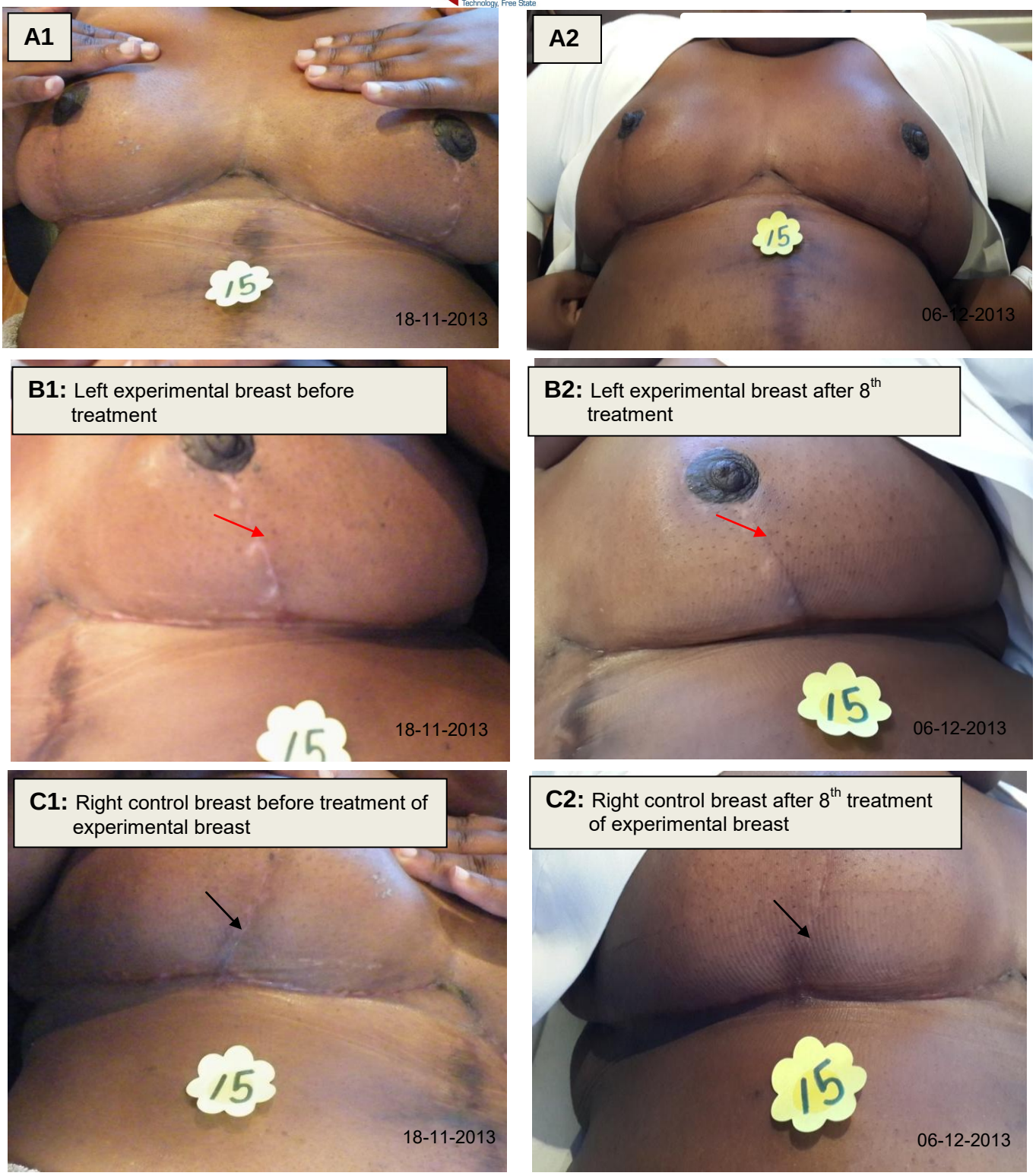


Figure F3 **Participant 15- A 37 year old patient with a Grade 3, major (21cm) ptosis. Patient underwent mammary reduction (Left breast - 2173g, Right breast -2207g) using the Wise pattern with inferior pedicle technique.** *[A1 - frontal view before treatment; A2 - frontal view after treatment; B1 - left breast before treatment; B2 - left breast after treatment, C1 and C2- right breasts natural healing]*

UNIVERSITY OF THE FREE STATE
IIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS UV

Research Division
Internal Post Box G40
☎(051) 4052812
Fax (051) 4444359

E-mail address: StraussHS@ufs.ac.za

Ms H Strauss/hv

2012-11-08

REC Reference nr 230408-011
IRB nr 00006240

MS TM BOSHOFF
c/o DR L BOTES
DEPT OF HEALTH AND ENVIRONMENTAL SCIENCE
CENTRAL UNIVERSITY OF TECHNOLOGY
BLOEMFONTEIN
9301

Dear Ms Boshoff

ECUFS NR 185/2012
PROJECT TITLE:THE EVALUATION OF MESOTHERAPY EPOREX TREATMENTS ON POST-OPERATIVE MAMMARY REDUCTION SCARRING

- You are hereby kindly informed that the Ethics Committee approved the above project at the meeting held on 6 November 2012.
- Committee guidance documents: Declaration of Helsinki, ICH, GCP and MRC Guidelines on Bio Medical Research. Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition (2006); the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.
- Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.
- The Committee must be informed of any serious adverse event and/or termination of the study.
- A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.
- Kindly refer to the ECUFS reference number in correspondence to the Ethics Committee secretariat.

Yours faithfully

.....
PROF WH KRUGER
CHAIR: ETHICS COMMITTEE

cc. Dr L Botes

)5 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
O. Box/Posbus 339, Bloemfontein 9300, South Africa/Suid-Afrika, T: +27(0)51 401 9111, www.ufs.ac.za