The Efficacy of Platelet-Rich Fibrin (PRF) on Healing Following Surgical Removal of Third Molar Teeth

Kim Pedro-Beech

2434567

A mini-thesis submitted in partial fulfilment of the requirements for the degree of MChD in Maxillo-Facial and Oral Surgery in the Department of Maxillo-Facial and Oral Surgery, University of the Western Cape

Supervisor

Dr N Behardien

Co-Supervisor

Prof JA Morkel

TABLE OF CONTENTS

Title Page 1

Table of Contents 2

Declaration 5

Dedication 6

Acknowledgements 7

List of Abbreviations 8

Keywords 9

Abstract 10

List of Tables 12

List of Figures and Charts 13

List of Appendices 14

CHAPTER 1 15

Introduction

CHAPTER 2 19

Literature review

CHAPTER 3 36

Research design and Methodology

3.1 Introduction

3.2 Research setting

3.3 Aim

3.4 Objectives

3.5 Study design

3.6 Study participants

3.6.1 Sampling

3.7 Data collection tools

3.8 Data collection process

3.9 Clinical procedure

3.9.1 Pre-operative

3.9.2 Operative

3.9.3 Post-operative

3.10 Outcomes

3.11 Randomization

3.11.1 Allocation concealment mechanism

3.11.2 Blinding

3.12 Statistical analysis

3.13 Ethical clearance

3.14 Data management

CHAPTER 4 47

Results

CHAPTER 5 55

Discussion

CHAPTER 6 61

Conclusion

REFERENCES 62

LIST OF APPENDICES

Appendix I: Modified Archer classification for impacted maxillary third molar teeth 69

Appendix II: Pell and Gregory Classification of impacted mandibular third molar teeth 70

Appendix III: Information sheet 71

Appendix IV: Data capturing sheet – Day 2 72

Appendix V: Data capturing sheet – Day 7 73

Appendix VI: Technique to measure facial swelling 74

Appendix VII: Standard method of preparing PRF 75

Appendix VIII: Standard method of placing PRF

Appendix IX: Post-operative instruction sheet 76

Appendix X: Technique to measuring facial swelling 77

Appendix XI: Research clearance BMREC certificate 78

DECLARATION

I declare that *The Efficacy of Platelet-Rich Fibrin (PRF) on Healing Following Surgical Removal of Third Molar Teeth* is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: Kim Pedro-Beech

Date: October 2021

Signed: .....

DEDICATION

Above all, I firstly want to praise and thank God for granting me the opportunity and the ability to acquire knowledge and skill to help my fellow man.

To my husband, Clint and beautiful daughter, Eden – thank you for your support and sacrificing precious time to allow me to achieve my dreams.

To my loving and supportive parents, Franklin and Pauline, who nurtured my desire to dream big and encouraged me to be brave enough to go for it. Thank you for your sacrifices over the years, which created opportunities for me. You led by example.

ACKNOWLEDGEMENTS

I would like to thank:

My head of department and mentor, Professor JA Morkel for his unwavering support and guidance throughout the program.

Dr G Hein for the guidance and for always encouragement me to be better than my previous best.

My research supervisor, Dr N Behardien, for all the support and guidance in writing this mini-thesis.

Mr H Kruijsse for assisting with the statistical analysis and interpretation thereof.

Dr A Gaffar and Dr I Cassimjee for assisting me without hesitation. Your help in reviewing the research participants has made a huge difference in me completing this goal.

My fellow registrars (previous and current), for their support and friendship.

All the auxiliary staff in the Maxillo-Facial and Oral Surgery department at Tygerberg and Groote Schuur Hospitals. It has been a pleasure working with you all.

The University of the Western Cape for granting me the opportunity to reach my dream of becoming a maxillofacial and oral surgeon.

LIST OF ABBREVIATIONS

PRF – Platelet-rich fibrin

PRP – Platelet-rich plasma

RCT – Randomized controlled trial

AO – Alveolar osteitis

PEB – Post-extraction bleed

VEGF – vascular endothelial growth factor

TGF – transforming growth factor-beta

EGF – epidermal growth factor

PDGF – platelet-derived growth factor

MFOS – Maxillo-Facial and Oral Surgery

TOHC – Tygerberg Oral Health Centre

VAS – visual analogue scale

KEYWORDS

Third molar

Wisdom tooth

Impacted third molar

Platelet-rich fibrin

Platelet concentrates

ABSTRACT

Aim: A clinical trial to determine the post-operative outcomes of a PRF-treated socket versus a conventionally treated socket following surgical removal of third molar teeth.

Introduction: Third molar surgery is a procedure many have to endure and which often results in prolonged healing time with consequential absence from work and school. This has motivated clinicians to seek methods to enhance the healing process and in effect, reduce the healing time. Research on the use of PRF in enhancing wound healing in maxillofacial and oral surgery have shown varying results. Therefore, this study was conceived to ascertain the effect PRF has on the post-operative sequelae of third molar teeth.

Materials and Methods: This was a split mouth, prospective, single blinded, randomized control trial. The study sample was made up of 26 patients (N=26) who met the inclusion criteria. Four of the patients experienced neurosensory fallout of an associated nerve and were subsequently excluded from the sample. This resulted in the total sample size of twenty-two patients (n=22). Symmetrically impacted maxillary and mandibular third molars were removed under general anaesthesia. Patients were treated in a within subject design: when one side of a patient was treated with PRF, the other side was conventionally treated and acted as a control. The allocation of the side treated with PRF was 'random'. Patients were followed-up on Day 2 and Day 7, respectively. Pain scores were recorded on a visual analogue scale (VAS) using 0 to 10 pain score. Swelling, wound dehiscence, development of alveolar osteitis, wound infection and post-operative bleeding were compared between the intervention and control side.

Results: Twenty-two patients (females = 13 and males = nine) between the age of 16 and 31 years met the inclusion criteria. Pain experienced between the PRF and control side on Day 2 and Day 7 were not shown to be statistically significant. The reduction in pain was significant

between Day 2 and Day 7 but not between the treatment modalities. Swelling was lower on the PRF side but this was not significant compared to the control side. There was a significant reduction in swelling over time (between Day 2 and Day 7) but not between the treatment modalities. No impact was shown of PRF on wound dehiscence, infection, alveolar osteitis and bleeding.

Conclusion: Overall, this study showed no reduction in post-operative pain and swelling following placement of PRF in the sockets of third molar teeth. It also showed no effect on the incidence of wound dehiscence, infection, alveolar osteitis and bleeding, following PRF treated third molar sockets. The small sample size could be the limiting factor to reflect the true value of PRF as an intervention. However, further investigation with larger sample sizes, more meticulous measuring tools as well as longer follow-up periods are needed to assess the true effect PRF has on enhancing post-operative healing in third molar surgery.

LIST OF TABLES

Table 1: Development of platelet concentrates

Table 2: Primary outcomes measured

Table 3: Sex and age of the patients

Table 4: Descriptive statistics of swelling between treatment (PRF and control) on Day 2 and Day 7

Table 5: Pairwise comparison of swelling between treatment (PRF and control) on Day 2 and Day 7

Table 6: Modified Archer classification for impacted maxillary third molar teeth

Table 7: Pell and Gregory Classification of impacted mandibular third molar teeth

LIST OF FIGURES AND CHARTS

Figure 1: PRF Duo machine used to produce PRF

Figure 2: PRF machine settings and placement of vacutainers

Figure 3: vacutainer following centrifugation

Figure 4: PRF clot removed from vacutainer

Figure 5: Flow Chart showing sequence of steps used for randomization and blinding

Figure 6: Pain scores per patient per condition at Day 2

Figure 7: The direction and size of the difference (control – PRF) at Day 2 per patient

Figure 8: Pain scores per patient per condition at Day 7

Figure 9: The direction and size of the difference (control – PRF) at Day 7 per patient

Figure 10: Graphic description of swelling between treatment (PRF and control) and time

LIST OF APPENDICES

Appendix I: Modified Archer classification for impacted maxillary third molar teeth

Appendix II: Pell and Gregory Classification of impacted mandibular third molar teeth

Appendix III: Information sheet

Appendix IV: Data capturing sheet – Day 2

Appendix V: Data capturing sheet – Day 7

Appendix VI: Technique to measure facial swelling

Appendix VII: Standard method of preparing PRF

Appendix VIII: Standard method of placing PRF

Appendix IX: Post-operative instruction sheet

Appendix X: Technique to measuring facial swelling

Appendix XI: Research clearance BMREC certificate

CHAPTER ONE

CONTEXT

1.1 Background and Rationale

Third molar surgery is the most common surgical procedure done by oral- and maxillofacial surgeons (Al-Hammed *et al.*, 2017). The mean prevalence of impacted third molar teeth, worldwide, is 24.40% (Carter and Worthington, 2015). In South African, the prevalence of impacted third molar teeth was found to be 17% (Ishwarkuma *et al.*, 2019). The definition of an impacted tooth is a tooth that fails to erupt into the dental arch due to a physical barrier in its eruption path (Juodzbalys and Daugela, 2013).

The surgical removal of impacted third molars often results in prolonged healing time with consequential absence from work or school. This has motivated clinicians to seek methods to enhance the healing process and in effect, reduce the healing time. Despite a relatively low incidence of post-operative complications in the healing process, patients' quality of life is still significantly affected by the physiological sequelae following third molar surgery (Ogundipe *et al.*, 2011). Due to the local inflammatory response, excessive pain, oedema and trismus are the most common postoperative complications encountered (Costa *et al.*, 2015) with an incidence of 12,3%, 8,6% and 5,7% respectively (van Eeden and Bütow, 2006). Alveolar osteitis is also a common complication following third molar surgery with an incidence of 0.3 – 26%, and occur more frequently with mandibular third molar extraction sockets (Bouloux *et al.*, 2006). Other surgical complications are surgical site infection and bleeding with an incidence of 0.8 – 4.2% and 0.2 – 5.8%, respectively (Bouloux *et al.*, 2006).

This surgical procedure entails raising a full muco-periosteal flap, bone removal (depending on the degree of impaction), possible sectioning of the tooth, removal of the tooth, wound inspection and irrigation, and repositioning of the flap to its' pre-operative position (Synan and Stein, 2020).

The anatomical variation and degree of impaction can be described by various classification systems. Pell and Gregory (1933) for mandibular third molars and Archer (1966) for maxillary third molars are commonly used. These provide a prediction of the potential surgical difficulty that may be encountered (Synan and Stein, 2020).

Wound healing is the body's reparative response to tissue injury and entails four overlapping phases - hemostasis, inflammation, proliferation and remodeling (Miron *et al.*, 2017). Various cell types respond to inflammatory mediators in an attempt to repair damaged tissues, among others, platelets, which release important biomolecules needed in the initial stage of healing (Miron *et al.*, 2017). As a result, the use of platelet-concentrates in facilitating healing of tissues has come into the spotlight in various fields of medicine, including dentistry (Miron *et al.*, 2017).

Platelet-rich fibrin (PRF), a second-generation platelet concentrate, is an autologous fibrin-based biomaterial, consisting of fibrin, platelets, growth factors and a variety of cell types including stem cells and leukocytes (Hartshorne and Gluckman, 2016). It has been shown to accelerate physiological healing (Kumar *et al.*, 2016) by promoting soft and hard tissue regeneration by angiogenesis, binding circulating stem cells, guiding the immune response and providing wound protection (Suttapreyasri *et al.*, 2013). Additional to its physiological benefits, it is a safe, dependable and cost-effective way to enhance tissue repair following injury or surgical insult (Hartshorne and Gluckman, 2016).

Research on the use of PRF in enhancing wound healing in oral and maxillofacial surgery have presented both beneficial, as well as contradictory results (Varghese *et al.*, 2017). This study

was therefore conceived to determine the effect of PRF on the post-operative sequelae of extracted third molar sites.

1.2 Problem Statement

The surgical removal of impacted third molars often result in prolonged healing time with significant discomfort to patients and consequential absence from work or school. Surgical techniques or materials that could promote the healing process and reduce the symptoms associated with the surgery would benefit the patients undergoing such procedures.

1.3 Research Question

Does the use of PRF within the socket following the surgical removal of third molar teeth, contribute to reducing the inflammatory sequel (i.e. pain and swelling) and the incidence of localized complications (i.e. wound dehiscence, infection, alveolar osteitis and bleeding)?

1.4 Purpose of the Study

The findings of the study may contribute to an improved manner in treating surgical wounds of third molar teeth surgery, as well as other dento-alveolar surgical procedures, hence enhancing the healing process.

1.5 Thesis Layout

Chapter 1 describes the background and purpose of the study in addition to its aim, objectives, and its significance.

Chapter 2 reviews the literature regarding third molar surgery, the healing sequelae of third molar surgery, platelet concentrates and its' uses, as well

controversies associated with it.

Chapter 3 describes the methodological sequence of how the research was conducted.

Chapter 4 reports on the findings of the study.

Chapter 5 entails the discussion, which interprets and describes the findings of the study

Chapter 6 concludes the findings of this thesis and makes recommendations for further exploration.

CHAPTER TWO

LITERATURE REVIEW

2.1 Third Molar Surgery

2.1.1 Classification of impacted third molars

Various classifications for impacted teeth exist. It is often based on radiological and clinical findings, and is used, at times, as a predictor of the degree of surgical difficulty (Synan and Stein, 2020). Most classifications make reference to the angulation of the long axis of the third molar and the depth of impaction (Synan and Stein, 2020). To assist surgeons with treatment planning with the purpose to limit the risk of possible associated complications, Abu-El Naaj and colleagues developed a new classification. It is based on the depth of impaction and its proximity to the inferior alveolar nerve canal, as well as the type of imaging needed and surgical approach required (Naaj *et al.*, 2010). Other classification systems are created according to dental procedure codes, which are based on the degree of impaction and the type of tissue overlying the crown of the tooth, i.e. soft tissue; partial or complete bone coverage (Farish and Bouloux, 2007). The most common classifications used are Pell and Gregory (1933) (see Appendix I), and Winter (Synan and Stein, 2020). The Archer (1966) classification (see Appendix II) specifically for upper maxillary third molars is also commonly used (Siddiqi *et al.*, 2010).

2.1.2 Surgical technique

Surgical removal of third molar teeth should be approached in a systematic and stepwise manner (Farish and Bouloux, 2007). Prior to starting the surgical procedure, all relevant equipment must be in working order and sterilized (Farish and Bouloux, 2007). The key concern in removal of impacted teeth is accessibility (Farish and Bouloux, 2007). Firstly, a

full-thickness muco-periosteal flap should be raised to gain access and visibility to the tooth (Farish and Bouloux, 2007). This flap is most often an envelope or triangular design (Synan and Stein, 2020). Once the flap is raised, it is retracted up to the external oblique ridge, since further stripping would create dead space and worsen post-operative oedema (Farish and Bouloux, 2007). If indicated, bone is removed around the tooth, creating a buccal and distal trough up to the cemento-enamel junction of the crown, to create space for retrieval of the tooth (Farish and Bouloux, 2007). Dependent on the angulation of the long axis of the tooth, the tooth itself may have to be sectioned (Synan and Stein, 2020). This however can also be based on the tooth root anatomy, bone density, patients' age and proximity to vital structures (Synan and Stein, 2020). Purchase points can also be placed at the tooth sectioning stage, to assist in retrieval of the tooth or tooth segment (Ferish and Bouloux, 2007). Following tooth removal, the wound site requires removal of sharp bone edges, debridement of the remaining dental follicle and copious irrigation, especially under the reflected flap (Synan and Stein, 2020). The surgical procedure is completed with suturing of the flap, ideally obtaining primary closure (Ferish and Bouloux, 2007).

2.1.3 Medicaments used in third molar surgery

There has been a continuous attempt to reduce post-operative complications following third molar surgery (Gupta *et al.*, 2019). Various medicaments have been used as dressings into extraction sockets, such as antimicrobials (tetracycline, clindamycin, lincomycin, and metronidazole), hydrocortisone, chlorhexidine and Chitosan (Gupta *et al.*, 2019). These medicaments are placed into the sockets in various carriers or forms such and hydrogels, sponges, films and fibers (Gupta *et al.*, 2019). Macrolides in alveolar cones, Tetracycline powder or aqueous solutions, Lincosamines and Nitroimidazoles have been used topically into extraction sockets in an attempt to reduce the incidence of developing alveolar osteitis (Vezeau, 2000). Neomycin cones used in third molar extraction sockets were found to be useful in

preventing post-operative infections (Nordenram *et al.*, as cited in Siddiqi *et al.*, 2010). Chitosan, a derivative of chitin (natural polymer found in shells of crustaceans and walls of fungi), has been shown to improve healing by stimulating various inflammatory cells and by inhibiting organisms such as Candida, Streptococci and Staphylococci (Gupta *et al.*, 2019). The latter was confirmed in a study by Li and colleagues 2011, who assessed chitosan based microbial fiber membrane flagyl on wound healing after a tooth extraction and found that it enhances the proliferation of fibroblasts, enhances granulation tissue and epithelialization, and induces new bone formation. Another study by Gupta and colleagues 2019, found that Chitosan improved healing following its' use in sockets of erupted teeth but increased the incidence of infection when used in the sockets where impacted teeth were removed (Gupta *et al.*, 2019). Chlorhexidine gel has been used as a post-extraction socket medicament in an attempt to reduce the incidence of alveolar osteitis. In 2017, Teshome did a systematic review and meta-analysis specifically looking at patients with confounding factors (e.g. smoking, use of oral contraceptives, increased bone density, increased age) on the effect of chlorhexidine gel on preventing alveolar osteitis after mandibular third molar surgery, and found that it significantly reduced the incidence of alveolar osteitis. Anti-fibrinolytic such as epsilon-aminocaproic acid (EACA) and tranexamic acid (TEA) have been used in extraction sites in an attempt to maintain the clot formed, but has failed to do so (Vezeau, 2000). Iodine tampons have also been used as a medicament following mandibular third molar surgery, and was found to reduce post-operative pain significantly (Tuk *et al.*, 2019). The disadvantage of using an iodine tampon is that the patient needs to return to have it removed (Tuk *et al.*, 2019).

2.2 Wound Healing

Wound healing, is a dynamic restorative process in response to tissue injury, which involves a cascade of complex, orderly phases involving many cell types, which are guided by the release of mediators in an attempt to repair damaged tissue (Miron *et al.*, 2017). Wound-healing

consists of four overlapping phases, which include haemostasis, inflammation, proliferation, and remodelling (Miron *et al.*, 2017). Following the extraction, the socket fills up with blood, which coagulates and contracts. Angioblasts grow into the clot while epithelialization occurs over the clot (Vezeau, 2000). Of the various cell types involved, platelets play a key role in the haemostasis phase by stabilizing a fibrin clot, activating, and releasing a variety of essential biomolecules (Miron *et al.*, 2017). These biomolecules stimulate the proliferation and activation of cells involved in wound healing, including fibroblasts, neutrophils, macrophages, and mesenchymal stem cells (Miron *et al.*, 2017).

2.2.1 Healing of third molar extraction sites

Although the surgical removal of third molar teeth have a relatively low incidence of post-operative complications, the patients' quality of life is still significantly affected by the physiological sequelae and healing process (Ogundipe *et al.*, 2011). Pain, trismus and swelling, with an incidence of 12.3%, 8.6% and 5.7% respectively (van Eeden and Bütow, 2006), are the most common postoperative complications encountered due to the local inflammatory process, especially during the first three post-operative days (Costa *et al.*, 2015). Other postoperative side effects are bleeding, alveolar osteitis and nerve injury (He *et al.*, 2017). Alveolar osteitis, found to occur more commonly following mandibular third molar extractions, are found to have a wide range of incidence of 0.3 – 26% (Bouloux *et al.*, 2006). This wide range could be due to inconsistency in the diagnostic protocol of alveolar osteitis among studies. A recent meta-analysis reports that while PRF decreases some of these post-operative complications associated with third molar extraction, it does not prevent them from occurring (Xiang *et al.*, 2019).

2.3 Sequelae of Third Molar Surgery

2.3.1 Pain

Pain is one of the signs of the inflammatory response following tissue injury. The incidence of

excessive pain following third molar surgery is reported as 12.3% (van Eeden and Bütow, 2006). The degree of pain experienced is what surgeons want to minimize. The first 3 days following third molar surgery significantly affects the patient quality of life due to the intense pain experienced (Costa *et al.*, 2015). Pain control following third molar surgery is usually a combination of acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs), with the addition of opioids if break-through pain is experienced (van Eeden and Bütow, 2006). The study result of perceived pain should be done with caution since pain is complex and subjective; there is a difference in pain perception between different genders; there are ethnic differences in development and progression of pain; use of various analgesics; the risk of experiencing carry-across effect in pain analysis during a split-mouth design (Canellas *et al.*, 2018). A meta-analysis of pain including six randomized controlled trials (RCT's) which assessed pain by making use of a visual analog scale (VAS) and a graphic rating scale, found a significant difference between the PRF and non-PRF groups (He *et al.*, 2017).

2.3.2 Swelling

Swelling, also referred to as oedema, is another consequence of the inflammatory response following tissue injury. Maximum post-operative swelling usually presents on the second or third post-operative day and should resolve completely by day seven (Siddiqi *et al.*, 2010). In a meta-analysis including three RCT's, the postoperative swelling on day one showed no significant difference, but on day three there was a significant difference between the PRF and non-PRF group (He *et al.*, 2017). The technique by which swelling is assessed varies in literature. Various anatomical landmarks are used to draw lines, of which the sum is taken as a baseline measurement. Examples of such can be found in a study by *Costa et al.*, (2015) who used a 5-line reference technique. Lines were drawn from the angle of the mandible to 5 points: tragus; lateral canthus of the eye; alar of nose; labial commissure and soft tissue pogonion. A similar technique can be found in studies reviewed by He *et al., (2017)* which made use of a

3-line reference technique. These lines were drawn from the tragus to soft-tissue pogonion; mandibular angle to lateral canthus of the eye and tragus to the labial commissure. The total swelling is the calculated by deducting the post-operative sum from the baseline value. Ogundipe et al., (2011) who only made use of a 2-line technique drawn from tragus to the labial commissure and soft tissue pogonion, respectively described a simpler technique. A mathematical equation was used to calculate the percentage facial swelling: The sum of the post-operative values ($S_{post\text{-}op}$) is deducted from the sum of the pre-operative values ($S_{pre\text{-}op}$), which is then divided by the $S_{pre\text{-}op}$ and multiplied by 100.

2.3.3 Alveolar osteitis

Alveolar osteitis (AO) can be defined as severely increasing pain experienced between the first and third post-operative days, with an exposed socket showing a disintegrated blood clot and varying halitosis (He *et al.*, 2017). AO continues to be a relatively frequent post-extraction complication with ongoing debates regarding its' etiology, pathophysiology and ways of prevention and management (Kolokythas *et al.*, 2010). It more commonly occurs following mandibular third molar extractions (Bouloux *et al.*, 2006). The reported incidence ranges between 0.3 – 26% (Bouloux *et al.*, 2006). This wide range could be due to inconsistency in the diagnostic protocol of AO among studies. A meta-analysis of a total of 676 cases were analyzed and reported that AO was present in 9 of 338 cases that had PRF placed in the sockets and 37 of 338 cases that did not have PRF placed, concluding that applying PRF in the sockets of extracted third molar teeth will significantly reduce its incidence (He *et al.*, 2017). It is suggested that the ability of PRF to enhance angiogenesis and wound healing, are the attributing factors to the lower incidence of AO found in sockets treated with PRF (Al-Hamed *et al*, .2017). The reduction in the incidence of AO, especially following removal of mandibular third molars, is one of the most consistent findings in a systematic review by Canellas and colleagues (2018).

2.3.4 Wound dehiscence

A surgeon desires to obtain primary closure following the surgical removal of a third molar tooth. At the end of the surgical procedure, the soft tissue flap is repositioned in its' pre-operative position (Synan and Stein, 2020). Upon follow up, in the acute inflammatory healing period, wound dehiscence i.e. opening up of the wound, is assessed whether it is present or absent. PRF in the socket is inclined to accelerate soft tissue healing by stimulating angiogenesis, formation of granulation tissue and epithelialization (Moraschini *et al.*, 2015). A split-mouth comparative study was done by Varghese *et al.*, (2017) and showed a similar trend to what is noted in the literature - that soft tissue healing at the PRF site is superior. In addition to this finding, Moraschini *et al.,* (2015) found that there are also enhancements in keratinized gingiva after soft tissue surgeries using PRF when compared with control groups.

2.3.5 Surgical site infection

The surgical wound following tooth extraction is classified as a clean-contaminated wound with a surgical site infection risk of 2.4 – 7.7% (Cruse and Foord, 1980). The estimated incidence of surgical site infection following third molar surgery ranges from 1.2 – 27%, with majority reporting 5% (Susarla *et al.*, 2011). This wide range can be due to unclear diagnostic criteria (Siddiqi *et al.,* 2010). A good sterile surgical technique can reduce infection risk, and controversy continues regarding the use of prophylactic antibiotics (Siddiqi *et al.*, 2010). Antibiotics can be administered in various forms from systemic to topical. A split-mouth randomized controlled trial found overall infection rates of 2%, with no significant difference between antibiotic prophylaxes versus placebo (Siddiqi *et al.*, 2010). The PRF network consists of leukocytes and macrophages, which play an integral part in fighting infection (Miron *et al.*, 2017).

2.3.6 Bleeding

Post-extraction bleeding (PEB) is a recognized and frequently encountered complication. The rate of PEB following extraction of third molars varies in the scientific literature. The risk of PEB has been noted to be between 0.2 to 1.4% (Wells *et al.*, 2000) and as high as 2.77% (Iwabuchi *et al.*, 2014). Bleeding possibilities after dental extraction can be categorized as normal bleeding and post-extraction bleeding. Normal bleeding, with or without pressure packs, is when bleeding persists for half an hour, followed by blood tinged saliva up to eight hours (Malik, 2008). Post-extraction bleeding can be primary, reactionary/intermediate or secondary (Malik, 2008). Primary PEB occurs during and immediately after extractions (Malik, 2008). Reactionary/intermediate PEB occurs once the efficacy of the vasoconstrictor has ended, approximately two – three hours post-operative (Malik, 2008). Secondary PEB, also known as "liver clots" usually begins 7-10 days post-operative (Malik, 2008). Bleeding can be further classified as mild (oozing), moderate (persistent bleeding) and severe (hospitalization) (Abdullah and Khalil 2014). In a study by Giuffre in 2006, where PRP treatment was used in patients with hemorrhagic risk, the authors concluded that clinicians should be encouraged to use PRP regularly when carrying out surgical treatment on patient who were on warfarin therapy. PRF is also used to promote coagulation (Zhu *et al.* 2020).

2.4 Review Intervals

Clinical signs and symptoms can be assessed at various phases during the healing process. The parameter being assessed would justify the period or interval of the follow-up assessment. Significant heterogeneity regarding follow-up frequency and duration is found among studies assessing the efficacy of platelet concentrates on healing of extraction sockets. When assessing dimensional changes after extractions, the recall time is an important factor. The mean follow-up time of the studies included in the systematic review done by Moraschini *et al.*, (2015) was 2.25 months. They found that this review period might be too short to be reflective of the true

effect of platelet concentrates in the later phases of socket healing (Moraschini *et al.*, 2015). This study however, was also assessing the effect of PRF on bone healing. According to Plachokova *et al.*, as cited in Moraschini *et al.*, (2015) suggested that platelet concentrates may only have a significant effect on the initial healing phases, since studies with longer follow-up periods had poorer results when compared to studies with short follow-up periods. Studies assessing pain, swelling and soft tissue healing vary between a few days up to 14 days; whereas assessment of bone healing varied between months up to 6 months (Canellas *et al.*, 2018). The number of follow-up assessments in the total post-operative period also vary greatly among studies.

2.5 Platelet Concentrates

2.5.1 History and development

Since the 1970's the use of biological additives has been widely documented (Dohan *et al.*, 2006 and Kumar *et al.*, 2016). It was first used in the form of Platelet-rich plasma (PRP) as a "glue", which is similar to current fibrin glue, but it was made from platelet-poor plasma with different preparation protocols (Hartshorne and Gluckman, 2016). During the late 1990's great scientific advances were made by identifying PRP, first described by Whitman *et al.*, (1997), as a favourable reservoir for growth factors that could assist in wound healing of soft tissue and bone (Borie *et al.*, 2015; Singh *et al.*, 2012; Hartshorne and Gluckman, 2016). These growth factors are involved in angiogenesis (forming of new blood vessels), chemotaxis of stem cells to the site of injury, mitosis of cells, induction of cell differentiation and proliferation, and increased collagen production (Borie *et al.*, 2015; So *et al.*, 2009). The valuable growth factors that enhance the healing process are vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) (Agarwal *et al.*, 2015; So *et al.*, 2009). To optimize PRP for clinical use, which initially is in liquid form, it needs to be converted by adding bovine

thrombin (Hartsehorne and Gluckman, 2016). The addition of bovine thrombin is disadvantageous due to it delaying the diffusion of growth factors, increases risk of transmitting infections, it is technique sensitive and time consuming (Hartsehorne and Gluckman, 2016; Dohan *et al.*, 2006; Kumar *et al.*, 2016). With reduction in PRP popularity platelet-rich growth factor (PRGF) was developed by Anitua and colleagues in 1999. The latter is leukocyte-depleted to reduce the pro-inflammatory effect (Hartshorne and Gluckman, 2016). A new innovation was developed in 2006 by Choukroun and colleagues, which simplified the preparation protocol and excluded xenofactors (Hartshorne and Gluckman, 2016), thus no biochemical blood manipulation was needed (Canellas *et al.*, 2018). This second-generation platelet concentrate, Platelet-rich fibrin (PRF) is also known as Choukroun's PRF (Dohan *et al.*, 2006; Singh *et al.*, 2012). It comprises of an autologous leukocyte-platelet-rich fibrin matrix with cytokines, platelets, stem cells and growth factors (Borie *et al.*, 2015; Hartshorne and Gluckman, 2016). It acts as an ideal biodegradable scaffold, which contains all the key elements needed for tissue healing and regeneration (Borie *et al.*, 2015; Hartshorne and Gluckman, 2016).

Table 1: Development of Platelet Concentrates

Development of Platelet Concentrates			
Year	**Type**	**Description**	**Finding/use**
1970's	PRP	Platelet-poor plasma	Similar to fibrin "glue"
1990's (Whitmann 1997)	PRP	Platelet-rich plasma	Discovered it is a reservoir for growth factors. Made in liquid for by adding bovine thrombin

1999	PRGF	Leukocyte-depleted plasma	To reduce the pro-inflammatory response
2006 (Choukran)	PRF	Leukocyte-rich and platelet-rich fibrin	No biochemical blood manipulation

2.5.2 Mechanism of action

The mechanism of action of PRF is based on three elements namely, it's role as a scaffold, the secretion of growth factors and, angiogenesis. As a scaffold, the fibrin matrix acts as a mesh for aggregation of cells (epithelial, fibroblasts and endothelial cells) (Hartshorne and Gluckman, 2016). Platelets, leukocytes, neutrophils and monocytes found within the fibrin matrix secrete growth factors responsible for the recruitment of epithelial and endothelial cells as well as fibroblasts, thus enhancing healing (Hartshorne and Gluckman, 2016). This strong fibrin matrix is in control of the slow release of growth factors necessary during the proliferative stage of wound healing (Canellas *et al.*, 2018). Leukocytes contribute to the immune regulation in the wound healing process (Canellas *et al.*, 2018). Lastly, angiogenesis, which relies on a fibrin matrix and specific growth factors such as VEGF and Transforming growth factor Beta (TGF-B), occurs (Hartshorne and Gluckman, 2016). To highlight these properties an in vitro study of the effect of PRF on human dermal fibroblasts showed that PRF enhances cell proliferation, induces mitogenic activity of endothelial cells thus enhancing angiogenesis, releases growth factors into the micro-environment and has anti-inflammatory and anti-microbial properties (Miron *et al.*, 2017). More studies are needed since very little is known regarding the antibacterial properties of PRF (Miron *et al.*, 2017).

2.5.3 Uses of PRF in dentistry and medicine

PRF has been described in various clinical applications in dentistry, ranging from periodontal and alveolar bone preservation surgery following tooth extraction, periodontal reconstruction

and bone grafting, implant placement and as a potential scaffold in pulp revascularization (Borie *et al.*, 2015; Kumar *et al.*, 2016). Other applications in dentistry - in bisphosphonate related oral necrosis of the jaw (Hartshorne and Gluckman, 2016), in hyperplastic gingival tissues and, to periodontally accelerate osteogenic orthodontics (Miron *et al.*, 2017). In oral surgery, the focus of using PRF is to enhance bone healing and to minimize post-operative complications following removal of third molars.

2.5.3.1 Uses in oral surgery

Hartshorne and Gluckman (2016) highlighted the following uses specifically to oral surgery:

- In a post-extraction socket to reduce early side effect such as pain, and to enhance soft and hard tissue healing especially if implant placement is planned
- To reduce post-extraction complications in medically compromised patients
- To reduce periodontal bone-loss distal to the second molar following removal of third molars by combining PRF with grafted bone.
- Atraumatic closure of minor oro-antral communications
- In apisectomies by combining PRF with mineral trioxide aggregate (MTA)
- In alveolar cleft grafting

2.5.3.2 Uses in plastic and reconstructive surgery

Miron and colleagues (2017), discussed application of PRF in plastic and reconstructive surgery:

- As a therapeutic adjuvant in soft tissue augmentation and reconstruction of the ears' auricular by combining PRF with adipose tissue for fat pad grafting.
- Enhance deep nasolabial folds and volume-depleted midface regions
- Improve scars

- Stimulates hair growth where hair follicles are present but dormant (Karimi and Rockewell, 2019).

2.5.3.3 Uses in orthopaedic surgery

- With arthroscopic meniscal repair surgery (Kemmoch *et al.*, 2018)
- As a surgical adjuvant in rotator cuff repair (Hurley and Moran, 2018).

2.5.3.4 PRF use in other medical procedures

Miron and colleagues (2017), describes the use of PRF in other medical fields:

- Chronic leg ulcers, venous leg ulcers and diabetic foot ulcers
- Vaginal prolapse repair
- Urethra-cutaneous fistula repair
- Chronic rotator cuff repair
- Acute traumatic ear drum perforations

PRF can be used in various forms. It can be used as a clot, can be compressed to form a membrane or plug, or the supernatant can be aspirated from the vacutainer and used in an injectable form (Hartshorne and Gluckman, 2016). The protein rich exudate collected after compressing the PRF clot, can be used to flush the surgical site, hydrate biomaterials, and used to store harvested autogenous bone blocks in, (Hartshorne and Gluckman, 2016).

PRF can be used as an individual therapy, as an additive or in combination (Hartshorne and Gluckman, 2016).

- Individual treatment – e.g. placement of a fibrin-plug in an extraction socket; used as a membrane to seal of an oro-antral communication (this technique was described by Agarwal and colleagues 2016)
- Additive therapy – e.g. it can be added to bone substitutes to enhance bone regeneration
- Combination therapy – e.g. combined with biomaterial in bone augmentation and grafting sites.

2.5.4 PRF Preparation Protocol

The preparation of PRF is simple when compared to the other platelet concentrates. Immediately pre-operative or intra-operative, 10ml of blood is collected (Hartshorne and Gluckman, 2016). From collection to processing in the centrifuge should be done within 2 minutes, since the blood starts to coagulate and thus the quality of the clot will be compromised (Hartshorne and Gluckman, 2016). Dohan and colleagues (2006), also emphasizes the importance of quick handling from collecting the blood to placement of the tube into the centrifuge, to obtain a usable PRF clot. The tubes are placed into the machine in opposing slots to create balance and minimize vibrations during centrifugation (Hartshorne and Gluckman, 2016). After centrifugation, the blood samples are placed in a holder for 4-8 minutes, to allow the clot to mature, prior to taking it out of the tube (Hartshorne and Gluckman, 2016).

Hartshorne and Gluckman (2016) discuss the various centrifugation processing-protocols:

- Original Choukroun's PRF protocol (standard protocol): 3000 rpm / 10 minutes
- Dohan Ehrenfest's Group - Leukocyte- and Platelet-Rich Fibrin (L-PRF): 2700 rpm/12 minutes
- Choukroun's advanced PRF (A-PRF), enriched with leukocytes: 1300 rpm / 8 minutes
- Choukroun's i-PRF (solution/gel): 700rpm/3 minutes

Current data shows that the centrifugal force does have varying effects on the distribution of the RBC's, platelets and leukocytes, but well-controlled clinical trials are needed to assess the clinical efficacy of the various centrifugation protocols (Hartshorne and Gluckman, 2016). The quality of the clot does not seem to be affected by the type of vacutainer (i.e. glass-coated plastic tube vs dry glass) nor the force at which the clot is compressed, however research is needed to establish the effect these two factors have on the growth-factor content and the matrix

properties of the final product (Hartshorne and Gluckman, 2016). Initially the fibrinogen is concentrated in the superior part of the tube, and upon contact with thrombin that is usually in the blood, it is changed to fibrin (Giannini *et al.*, 2015). Following centrifugation, the resultant product consists of a liquid supernatant serum (also referred to as acellular plasma) on top, rigid and elastic PRF clot in the intermediate layer and the red corpuscle base below (Borie *et al.*, 2015; Dohan *et al.*, 2006; Su *et al.*, 2009). The quality of the preparation of the PRF is critical for success of its healing and regenerative properties.

Hartshorne and Gluckman (2016) describes the factors that play a critical role in the quality of PRF created:

- Limiting centrifugation vibration –fill tubes equally and create a balance by placing the tubes in opposing sites.
- Standardize the preparation
- Conserve the PRF clot – as long as the serum is in contact with the clot, the growth factor content stays stable. If prepared correctly, the PRF membrane can be conserved for many hours.
- To preserve platelets and growth factors – do not squeeze all the plasma from the clot. The highest concentration of platelets and stem cells are found adjacent to the red thrombus region, thus part of the clot nearest to the thrombus should be used if growth factors provided by platelets are desired. It is important to reserve a small RBC layer at the PRF clot end to collect the most platelets and leukocytes.
- Compression of the clot should be done gently with homogenous pressure to avoid squeezing out all the serum, to maintain a homogenously moist membrane or plug. The first 20 minutes following preparation, most of the growth factors are released. This release can be delayed when forcible extraction is not done.

2.6 Controversies

There are several advantages, as well as disadvantages reported in the use of PRF in the maxillo-facial and oral region. Advantages such as that it is prepared in a simple and cost effective manner (Borie *et al.,* 2015; Giannini *et al.,* 2015; Kumara *et al.,* 2015; Moraschini *et al.,* 2015; Singh *et al.,* 2012), that there is no risk of creating an immunological response (Borie *et al.,* 2015), and that an effective tissue regeneration environment is established as a result of sustained release of growth factors (Borie *et al.,* 2015; Kumara *et al.,* 2015; Moraschini *et al.,* 2015; Singh *et al.,* 2012). PRF is presented as a more efficient and with fewer controversies when compared to PRP (Borie *et al.,* 2015; Dohan *et al.,* 2006). The small volume of autologous blood being obtained and that the handling process plays a direct role on the success of the PRF, are disadvantages that may be encountered (Borie *et al.,* 2015). The potential benefits of the use of platelet concentrates are controversial with some studies reporting some significant improvements (Antonello *et al.,* 2013; He *et al.,* 2017; Gawai and Sobhana, 2015; Kumar *et al.,* 2015; Ogundipe *et al.,* 2011; Moraschini *et al.,* 2015; Singh *et al.,* 2012; Varghese *et al.,* 2017) and others claiming insignificant or no improvements (Suttapreyasri and Leepong, 2013). These inconsistencies are most likely linked to the variation in the preparation protocols (Gawai and Sobhana, 2015) and small sample sizes of the various studies (Al-Hamed *et al.,* 2017).

2.7 Summary

Third molar surgery is commonly done by maxillo-facial and oral surgeons, and it is an ongoing attempt to minimize post-operative complications and to lower the impact this surgery has on the quality of life of patients'.

From the thorough clinical and radiographic examination, to the meticulous stepwise surgical approach, additional attempts are made to enhance healing and reduce complications. Since the development of PRF by Choukroun in 2006 (Hartshorne and Gluckman, 2016), there has been

continuous attempts to enhance and develop this autologous biomaterial. PRF is used widely in the dental and medical fields with various indications. In oral surgery, it is mainly used to enhance healing of soft tissue and bone, and to minimize post-operative complications.

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

3.1 Introduction

This chapter describes the setting in which the research took place, the aim and objectives, the study design, the way in which data was collected as well as the tools used to achieve it. It clarifies the PRF preparation protocol. This section also highlights the study outcomes and how randomization was achieved. The tools used for the statistical analysis is specified and it concludes with the ethical statement.

3.2 Research Setting

This research was conducted at a public health dental facility affiliated to an academic training institution. The Tygerberg Oral Health Centre is situated in Cape Town, South Africa and is one of the academic training sites of the Faculty of Dentistry of the University of the Western Cape. The maxillofacial and oral surgery department is one of only three, providing this specialized service in the Western Cape. Referrals from all public health dental facilities are channelled to these facilities.

3.3 Aim

The aim of the study was to determine the post-operative outcomes of a PRF- treated socket versus a conventionally treated socket following surgical removal of third molar teeth.

3.4 Objectives

Objectives of the study included:

1. To compare the overall pain experienced
2. To compare the pain experienced between the intervention and control sides

3. To compare soft tissue healing between the intervention and control sides
4. To determine the effect of PRF on post-operative swelling
5. To compare the incidence of alveolar osteitis of the two operated sides
6. To compare the incidence of wound infection of the two operated sides
7. To compare postoperative bleeding of the two operated sides

3.5 Study Design

This was a split-mouth, prospective, double blinded randomized control trial. A split-mouth design is an example where two treatment modalities are randomly assigned to sites of the two halves of the mouth (Lessafre *et al.*, 2009). This study design removes much of the inter-subject variability. Although patients were not randomized into treatment categories, the side was randomly selected for placement of the PRF.

3.6 Study Participants

3.6.1 Sampling

Patients recruited for the stud adhered to the inclusion criteria. Twenty-six (n=26) patients met the inclusion criteria. Population (P): patients requiring surgical removal of all 4 third molars which are mirror-image of each other; Intervention (I) and Control (C): PRF was placed in the intervention sockets following third molar surgery and the control sockets were treated conventionally; and outcomes (O): post-operative outcomes included overall pain as well as the specific side of most pain, swelling, wound dehiscence, alveolar osteitis, wound infection, postoperative bleeding and the use of the additional script for breakthrough pain. The inclusion criteria included all patients requiring the surgical removal of all four impacted wisdom teeth. The impacted teeth in the maxilla and the mandible had to be a mirror image of the teeth in the opposite side (left and right hand sides). All patients had to be 16 years and older.

The exclusion criteria include patients with known co-morbidities such as systemic infective disease, cardiovascular disease, liver disease, hematological disease, bleeding tendency, diabetes and neoplastic disease. In addition to this, patients with a history of chronic use of anti-inflammatory therapy e.g. corticosteroids, antidepressants, non-steroidal anti-inflammatory medication and homeopathic/alternative medications were also excluded. Patients who were smokers, used illicit drugs, were pregnant or who had a dento-facial pathology which could contribute to dento-facial pain (active caries, oral and jaw pathology, temporomandibular disease) were also excluded. Patients', who developed associated nerve damage to the inferior alveolar or lingual nerve respectively, were excluded from the analysis. Due to four patients experiencing neurosensory fallout of associated nerves, they were excluded from the study. The sample size was determined retrospectively due to time limitations.

3.7 Data Collection Tools

A research information sheet (see appendix III) was given to the patient as well as ethical consent (see appendix IV) was obtained on the day of consultation by the consulting practitioner. Questionnaires (see appendix V and VI) were used to capture the data. Overall pain was assessed by means of a visual analog scale (VAS), which was included on both questionnaires. A flexible tape measure was used to measure baseline facial parameters and post-operative facial parameters to calculate swelling.

3.8 Data Collection Process

On the day of surgery, the surgeon measured the baseline facial parameters, pre-operatively. This was documented on the questionnaires (see appendix V and VI). The patients drew a random card from a box to indicate which side would act as the intervention side. On Day 2 and Day 7, the examining clinician completed the questionnaire following a verbal interview,

performed clinical measurements (the same as was done by the surgeon to obtain the baseline values), and assessed all other parameters as per the questionnaires. A single staff nurse verified all questionnaires.

3.9 Clinical Procedure

The clinical aspects of the study can be viewed in three phases, namely pre-operative, intra-operative, and post-operative.

3.9.1 Pre-operative

All 26 patients underwent surgical removal of four impacted third molar teeth under general anaesthesia. At the time of initial consultation, the patients were informed of the surgical procedure and its' possible associated complications, as well as the details of the research study. Informed consent for participation in the study was obtained at this stage. Due to the COVID-19 pandemic departmental protocol dictated that a negative SARS COV2 test had to be obtained 48 hours prior to the surgical procedure.

On the day of surgery, the patients were measured with a tape measure, to determine the baseline facial diameter (oral commissure to tragus; soft tissue pogonion to tragus). These values were used in the formula to calculate post-operative swelling (see appendix VI).

3.9.2 Intra-operative

Prior to commencing the surgical intervention, all patients received an intra-operative single dose of 1.2g Amoxacillin-clavulanic acid intravenously. For patients allergic to penicillin, 600mg Clindamycin was administered intravenously.

A single operator who made use of a standard operating technique performed all surgeries. Local anaesthetic, Lidocaine – HCL 2% with adrenaline (1:80 000) was used to block the inferior alveolar nerve and to infiltrate the buccal nerve and posterior superior alveolar nerve,

respectively. For the mandibular impactions, a full-thickness muco-periosteal envelope flap design was used. For the maxillary impactions, a full-thickness muco-periosteal triangular flap design was used. This was followed by ostectomy of the surrounding bone as needed, and the tooth elevated (splitting of the crown and elevation of roots was performed as required). The sockets were debrided and the wound sites irrigated with sterile 0.9% saline. Following the extractions, the control side was treated conventionally and PRF placed into the intervention side.

During the surgical procedure, the anaesthetist collected the patients' blood to fill two vacutainer tubes. This was placed into the PRF Duo machine in opposing slots to allow balancing of the machine during the spinning process. A standard method of preparing PRF was used for all cases (see Appendix VII).

Figure 1: PRF Duo machine used to produce the PRF

The standard PRF setting was used for all patients (see figure 2).

Figure 2: PRF settings and placement of vacutainers

Following extraction of all four teeth, the undisclosed card indicating the intervention side was revealed to the surgeon. The surgeon then proceeded to place the collected PRF into the selected side. Figure 3 shows the sample following centrifugation. Figure 4 shows the sample retrieved from the vacutainer.

Figure 3: Vacutainer following centrifugation

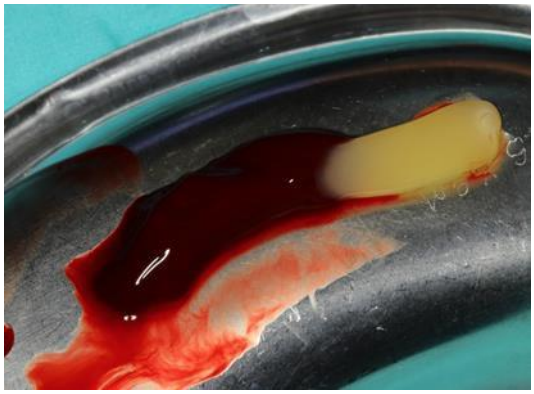


Figure 4: PRF clot removed from vacutainer

The method of placement was done as described in Appendix VIII. All sockets were sutured with 3/0 Softcat® chrome absorbable material.

3.9.3 Post-operative

Postoperative medication was given to all patients: Paracetamol 1g, 6hourly for 5 days; Ibuprofen 400mg 8 hourly for 5 days; 0.12% aqueous chlorhexidine mouth rinse 15ml 8 hourly for 1 week. A private prescription for break through pain was given: Oxynorm ®5mg per os, as needed or maximum 4 hourly, 5 tablets. The standard departmental postoperative instruction sheet (see Appendix IX) was given to all patients.

Post-operative follow-up reviews were done by fellow clinicians, on Day 2 and Day 7, respectively. A single staff nurse verified the results. The same nurse was involved for all the cases to allow for standardization and to avoid bias.

3.10 Outcomes

Table 2 presents the outcomes of the study.

Table 2: Outcome of the study

Primary outcomes measured	
Pain	A 10-point visual analog scale (VAS) with a score of 0 indicating "no pain" and 10 indicating "extreme pain" was used to assess pain (Barone *et al.*, 2010).
Facial Swelling	Facial swelling was measured according to **Appendix X** (Ogundipe *et al.*, 2011).
Soft tissue healing	assessed by stating whether it was present or absent
Alveolar osteitis	The diagnostic criteria for alveolar osteitis was the presence of halitosis, pain and a clot-less socket with exposed necrotic bone. This was also recorded as being present or absent.
Post-operative infection	the presence of suppuration was noted as present or absent
Neurosensory fallout	Inferior alveolar nerve and Lingual nerve injury were noted. If nerve injury was present, the patient was excluded from the study.
Post-extraction bleeding	Bleeding was noted as primary (during/immediately following extraction), reactionary (2-3 hours following extraction) or secondary (7-10 days following extraction). Bleeding was classified as mild (oozing), moderate (persistent bleeding), severe (needs hospitalization).
Breakthrough pain	To assess if the patient made use of the private prescription given to use additional analgesia for pain control.

3.11 Randomization

3.11.1 Allocation concealment mechanism

Thirty cards, each indicating which side (left or right hand side) the PRF was to be placed. Fifteen cards indicated the left or right side, respectively. On the morning of the procedure, the patient drew a random card and handed it to the scrub nurse.

3.11.2 Blinding

Both patient and researcher/surgeon were blinded to the side of the intervention. The surgeon was only informed of the intervention side once all four molars were removed. The patient was only informed of the intervention side after the Day 7 follow-up assessment visit.

Figure 5 shows the step by step process that was followed:

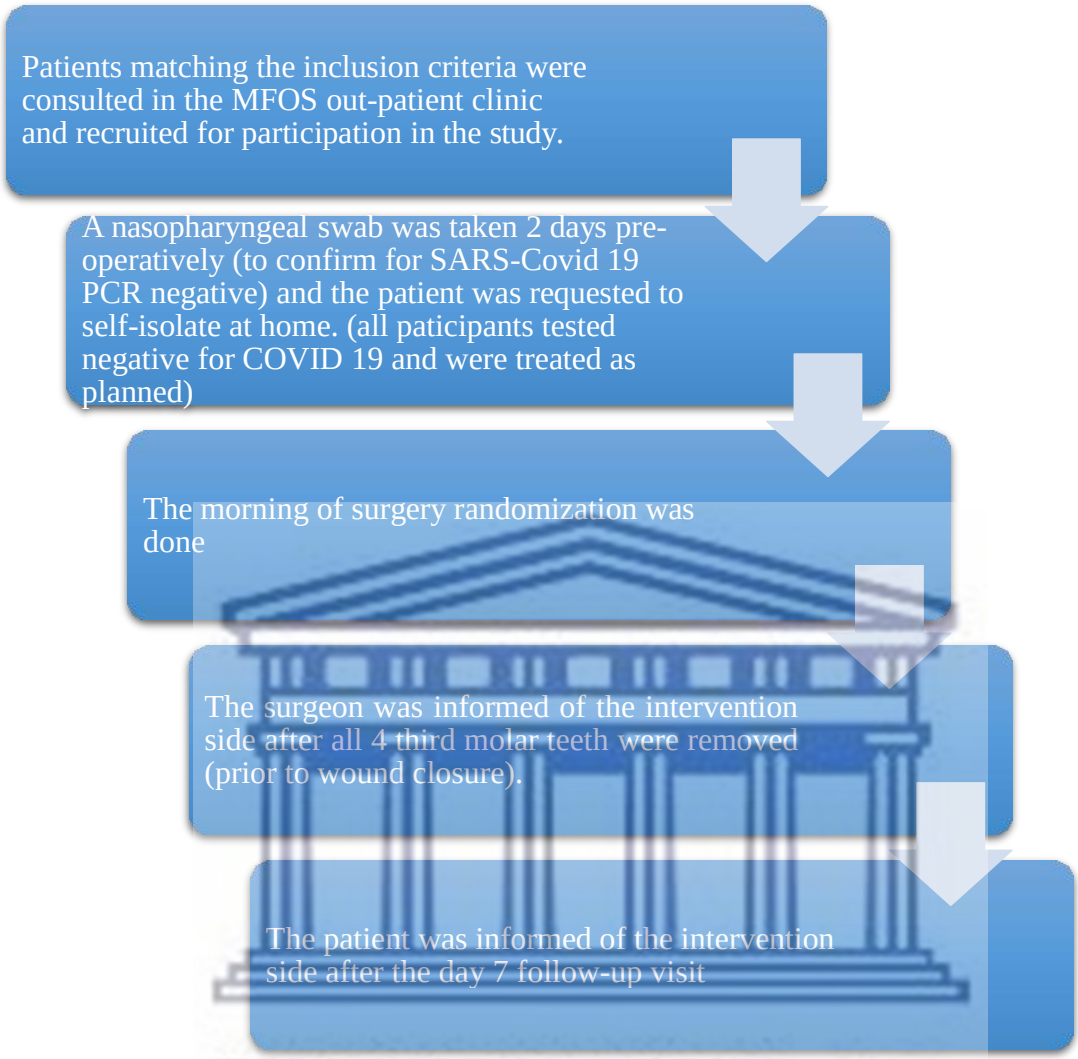


Figure 5: Flow Chart showing sequence of steps used for randomization and blinding

3.12 Statistical Analysis

Microsoft excel was used to enter the data electronically

Wilcoxon Sign Rank test was used for comparisons between treatment modalities and between time intervals.

3.13 Ethical Clearance

Ethics approval was obtained from the University of the Western Cape's Biomedical Research Ethics Committee (project registration number: 130416-050). Patient participation in this study

was voluntary. All patients received an information letter outlining the risks of the study. Informed consent was obtained from all participants.

3.14 Data Management

Patient confidentiality was protected at all times. Patients had the right to withdraw from the study at any stage. The data collected was stored in a password-protected computer. No identifiable patient information such as name and identity number was used. Instead, a record number was allocated to each participant.

CHAPTER

FOUR RESULTS

4.1 Introduction

The findings of the study are presented in this chapter.

4.2 Recruitment

Twenty-six patients met the inclusion criteria and entered the study. Four of the total were excluded due to experienced transient paraesthesia. This resulted in a total sample size of twenty-two patients (n=22) (females = 13 and males = nine). Recruitment commenced November 2020, with the first surgical procedure done in December 2020. The study was conducted between November 2020 and September 2021. Due to the COVID-19 pandemic departmental protocol dictated that a negative SARS COV2 test had to be obtained 48 hours prior to the surgical procedure. All recruited patients tested negative and received surgical intervention as scheduled.

4.3 Demographic information

The ages and gender distribution are presented in table 3. The age of the sample subjects ranged from 16-31 years.

Table 3. Gender and age of the patients

Patient	Gender	Age		Patient	Gender	Age
1	F	20		12	F	27
2	M	21		13	M	22
3	F	21		14	M	17
4	M	23		15	F	20
5	F	16		16	M	23

6	M	19		17	F	22
7	F	22		18	F	20
8	M	20		19	F	18
9	M	18		20	F	31
10	F	19		21	F	23
11	F	21		22	F	23

4.4 Parameters measured

4.4.1 Pain

Each patient was instructed to rate the overall pain and the pain of each treatment side. Subjects were unaware of which side was treated with PRF. The Visual Analogue Scale (VAS) was used to collect pain measurements: a score on a zero to ten scale.

In this analysis the distance and direction between two pain-scores (within subjects) was used to determine the effect of the PRF side and control side treatment.

Figure 6 presents the pain scores per patient per condition at day 2. The differences between patients are erratic as well as the differences between the treatment within each patient.

Figure 7 shows the direction and size of the difference (control - PRF) at day 2 per patient.

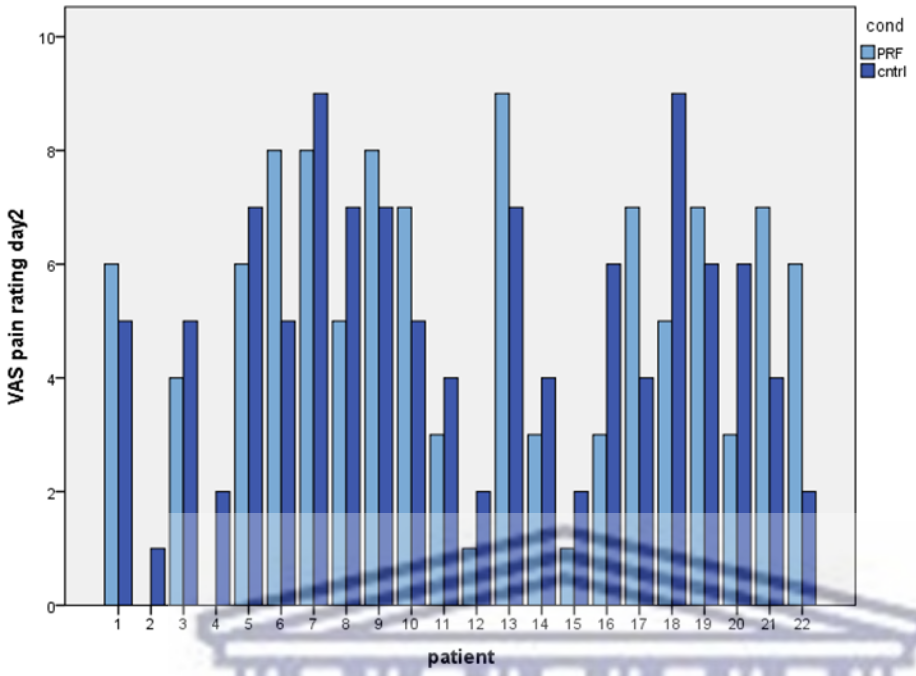


Figure 6. Pain scores per patient per condition at Day 2

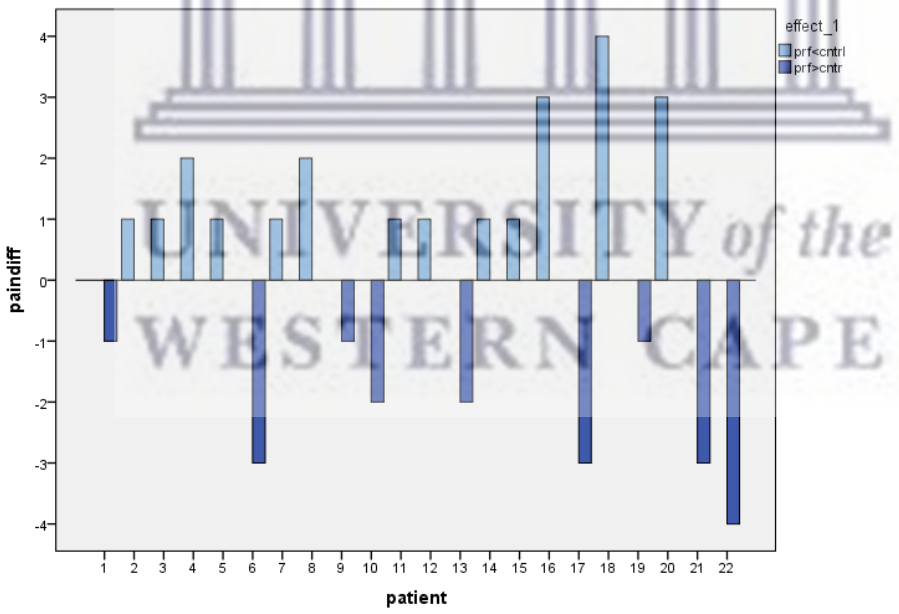


Figure7. The direction and size of the difference (control - PRF) at Day 2 per patient

Thirteen patients had less pain at the side treated with PRF. The remaining nine patients had more pain on the side treated with PRF. The probability that nine patients or more, when N=22, have a prf>cntrl pain rating, is p[y >= n-k] = 0.52.

The null-hypotheses that pain at the PRF treatment side is equal to the pain at the control side at day 2 cannot be rejected.

Figure 8 presents the pain scores per patient per condition at Day 7. The pain ratings differ from the ratings at Day 2, but the ratings remain erratic. The size difference between the PRF treated side and the control side appear more extreme: some differences are small other large. Figure 9 shows the direction and size of the difference (cntrl-PRF) at Day 7 per patient.

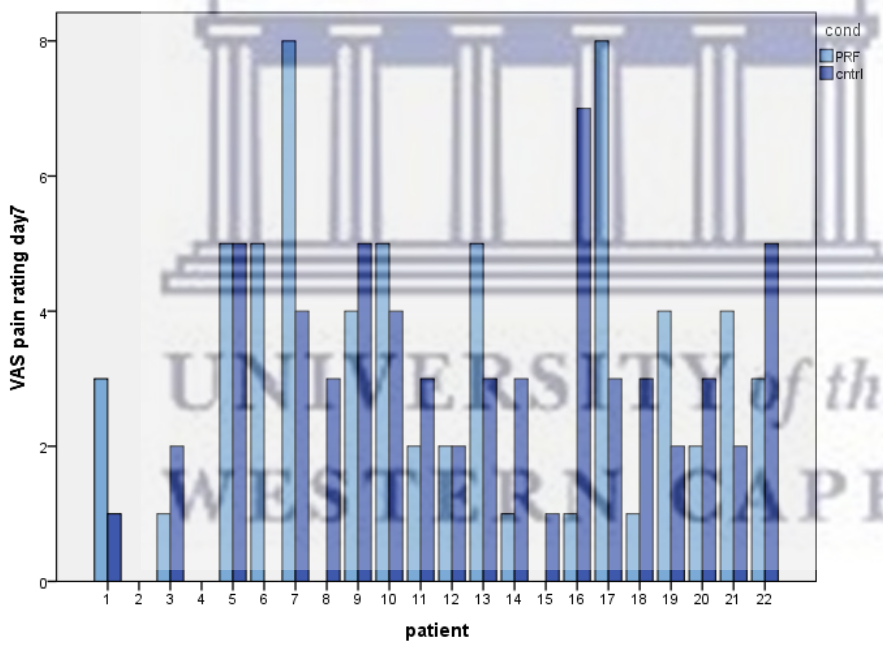


Figure 8: Pain scores per patient per condition at Day 7

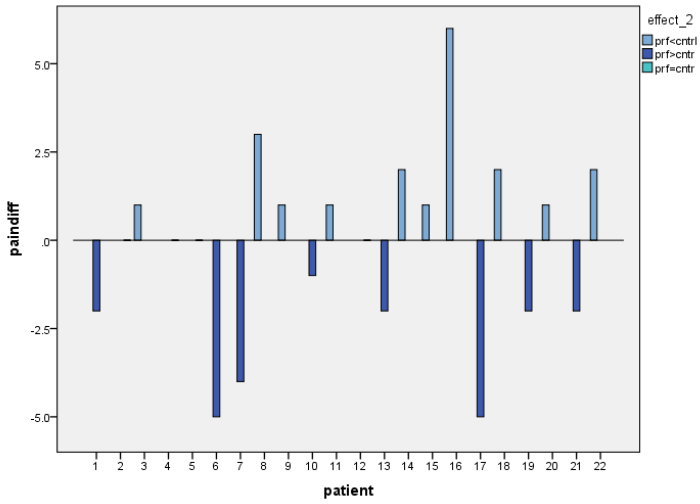


Figure 9: The direction and size of the difference (control - PRF) at Day 7 per patient

Four patients judged the pain between the PRF treatment and control as equal (ties). Ignoring the size of the difference and ties, and testing the direction only (sign) shows that eight patients or more, when n=18, have a prf>cntrl pain rating, is p[y >= n-k] = 0.81.

The null-hypotheses that pain at the PRF treatment side is equal to the pain at the control side at Day 7 cannot be rejected.

The difference between the PRF treatment at Day 2 and the PRF treatment at Day 7 was tested using Wilcoxon Sign Rank test. The pain experienced at the PRF treated side on Day 2 decreased significantly on Day 7: pain day7 > pain day2, Z =-3.57, p<0.01.

However, the pain experienced on the control side on Day 2 also decreased significantly on day 7: pain day7 > pain day2, Z = -3.45, p<0.01.

Thus both PRF and control side experienced a significant reduction in pain between Day 2 and Day 7.

4.4.1.1 Breakthrough pain

All patients received a script to use for breakthrough pain. On Day 2, four patients (three female; one male) reported making use of the script for breakthrough pain. On Day 7, two patients (both female) reported using the script for breakthrough pain.

4.4.2 Swelling

Facial swelling was determined by the measurements of the Tragus Labial & Tragus-soft tissue pogonion on a time scale: t_1 (pre-op), t_2 (day 2 post-op) and t_3 (day 7 post-op).
The sum of both measurements of the Tragus Labial & Targus-soft tissue pogonion is used as the dependent variable of facial swelling in the following analysis.

Both factors, treatment (PRF versus control) and time (day 2 and day 7), were treated as within subjects in a GLM repeated measurement design without between variables.

The descriptive statistics and pairwise comparisons are presented in Table 4 and Table 5. Figure 10 presents the results graphically.

Table 4. Descriptive statistics of swelling between treatment (PRF and control) and Day 2 and Day 7

	Mean	Std. Deviation	N
PRFd2	273.77	16.303	22
PRFd7	267.95	17.270	22
cntrld2	274.00	17.227	22
cntrld7	269.00	18.817	22

Table 5. Pairwise comparison of swelling between treatment (PRF and control) and Day 2 and Day 7

Exp	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
PRF	1	273.773	3.476	266.544	281.001

	2	267.955	3.682	260.298	275.611
Control	1	274.000	3.673	266.362	281.638
	2	269.000	4.012	260.657	277.343

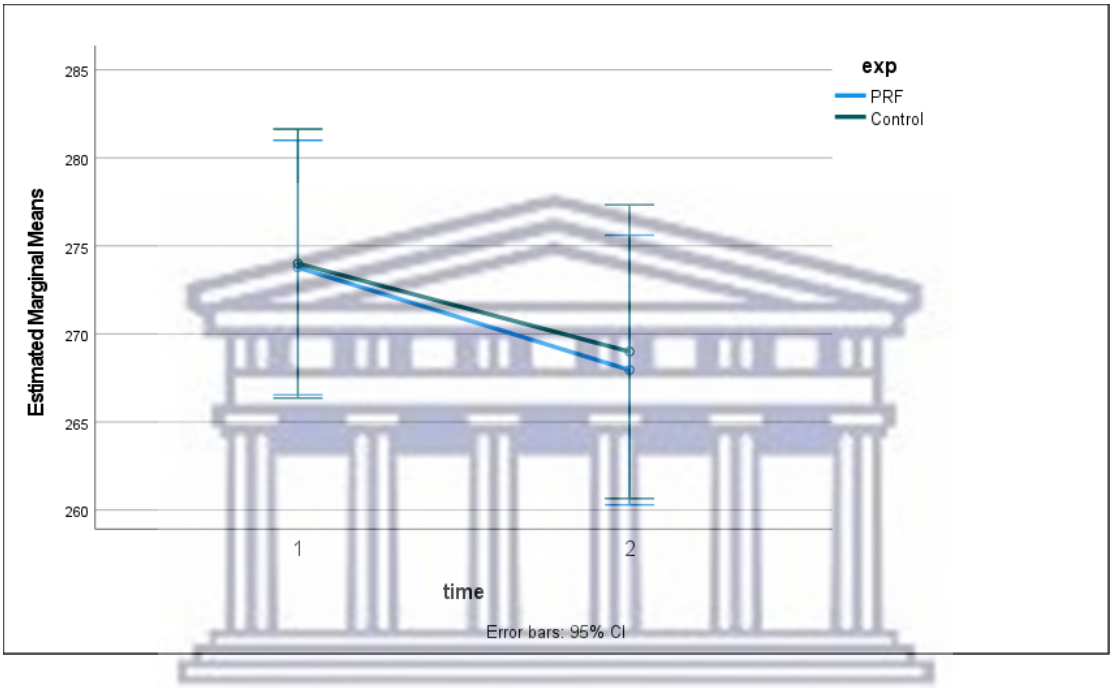


Figure 10: Graphic description of swelling between treatment (PRF and control) and time

The difference between PRF treatment and the control was not significant $F_{1,21}$=0.165 ; P>0.05. The difference between time (day 2 and day 7) was significant $F_{1,21}$=16.48 ; P<0.01. The interaction between treatment and time was not significant $F_{1,21}$=0.413 ; P>0.05.

The Null-hypothesis that facial swelling remains the same under both treatment conditions and does not interact with the treatments over time cannot be rejected.

4.4.3 Wound dehiscence

On day 2, only one patient presented with wound dehiscence at the right mandibular extraction site. This occurred on the control side. Due to no surgical intervention done to close this wound,

it remained open at Day 7, as expected. On Day 7, another patient presented with wound dehiscence at the left mandibular extraction sockets. This occurred on the PRF side.

The hypothesis that wound dehiscence at the PRF side is less than wound dehiscence at the control side cannot be rejected.

4.4.4 Wound infection

None of the patients developed wound infection during the follow-up period. One patient returned with wound infection, two weeks post-operatively. This was however not included in the results, as it falls out of the assessment period.

4.4.5 Alveolar osteitis

None of the patients developed alveolar osteitis, hence no comparison could be made.

4.4.6 Paraesthesia

Four patients experienced transient paraesthesia. Full nerve recovery only occurred after the assessment period thus these patients are excluded from the study.

4.4.7 Bleeding (PEB)

Bleeding was noted as primary (during/immediately following extraction), reactionary (2-3 hours following extraction) or secondary (7-10 days following extraction). On day 2, only one patient reported reactionary bleeding, bilaterally. All other patients reported primary bleeding. No secondary bleeding was encountered.

CHAPTER FIVE

DISCUSSION

Third molar surgery, one of the most frequent procedures conducted by maxillofacial and oral surgeons, is associated with several post-operative complications, thus many pre- and post- operative strategies have been established to reduce it (Zhu *et al.*, 2020).

It was hypothesized that the local application of PRF into third molar extraction sockets would reduce the post-operative complications. Pain, swelling, wound dehiscence, alveolar osteitis, surgical site infection and bleeding were the parameters assessed in this study.

The current study showed that the use of PRF made no difference in the acute healing phase following third molar surgery. This is contradictory to the findings of a similar study by Kapse and colleagues (2018), who found that PRF significantly reduced over-all pain and swelling following third molar surgery.

Pain

Pain was rated overall as well as the pain of each treatment side. Assessment of the overall pain proved to be irrelevant as pain was compared within a subject and not between subjects. Hence, overall pain was not included in the results.

When assessing and comparing the pain experienced between the intervention and control sides, the patients were unaware of which side was treated with PRF. This blinding prevented the effect that knowledge of the intervention side would have on the subjective reporting of pain. In this study, the pain reported between and within individuals were erratic both on Day 2 and Day 7. Pain experienced in this study, both on Day 2 and Day 7, proved to be influenced significantly by the placement of PRF. A study done by Singh *et al.* (2012) showed pain reported less on the PRF side, but it was not statistically significant (P = 0.005) when compared

to the control side. On the contrary, a similar study done by Kapse *et al.* (2018) showed that PRF had a significant effect on post-operative pain. This contradiction, despite using the same visual analogue scale measuring tool could be due differences in the sample size. Another cause could be that the preceding question of overall pain, in this study, could have been misleading the true reflection of comparing pain experienced on the individual sides.

As expected, pain reduced over time on both PRF and control sides. This study however failed to prove a greater reduction on the PRF side, as hypothesized. Ogundipe *et al.* (2011) showed a significant difference of PRF on pain reduction over a two-week period. The longer duration of assessment in Ogundipe *et al.'s* study could be the factor contributing to the contradicting outcome. The varying effect of PRF on pain in literature could be due to a variety of methods used to assess pain and the variation in different time intervals. The varying methods seen in literature included a visual analogue scale (VAS) (0-10) by Singh *et al.*, 2012; VAS with a graphic rating scale by Uyanik *et al.*, 2015; a modification of a VAS with additional descriptions by Kumar *et al.*, 2015.

Swelling

Swelling is a consequence of the inflammatory response and whether it truly is a complication of third molar surgery is debatable. The degree of swelling and rate of its reduction are parameters assessed by many studies assessing the effect of PRF use in third molar surgery (He *et al.*, 2017; Al-Hamed *et al.*, 2017; Kapse *et al.*, 2018; Zhu *et al.*, 2020).

Literature shows heterogeneity in the techniques used for measuring swelling. Some studies make use of the arithmetic sum based on 2 measurements (Ogundipe *et al.*, 2011); based on 3 measurements (Kapse *et al.*, 2017); based on 5 measurements (Bilginaylar *et al.*, 2016; Ozgul *et al.*, 2015; and Uyanik *et al.*, 2015 – as cited in He *et al.*, 2017). Another method used is a VAS scale with a description linked to a value (zero – 5) (Pasqualini *et al.*, 2005). This study

made use of the measuring technique described by Ogundipe and colleagues (2011). No difference in swelling when comparing treatment modalities were shown in this study. The interaction between treatment and time on swelling was also not significant (P>0.05). On the contrary the similar study by Kapse *et al.* (2017), showed that the mean percentage of swelling was lower on the PRF side at all times (p<0.05). Kumar and colleagues (2015) showed a significant reduction in swelling on the PRF side on day one. Whereas Uyanik and colleagues (2015), proved the effect of PRF on swelling to be significant on the second post-operative day but not significant on day one, three and seven, respectively. It's not only the measuring tool, but also the time frame of assessing swelling that influences the heterogeneity seen in literature.

Wound dehiscence, wound infection, alveolar osteitis

In this study, wound dehiscence, wound infection and alveolar osteitis were noted as present or absent. The true effect of PRF on these parameters were not shown. This can be attributed to the small sample size. Al-Ahmed and colleagues (2016) used a Landry index to assess soft tissue healing which placed tissue colour, bleeding on palpation, presence of granulation tissue, wound margin epithelialization and suppuration, under one banner.

Alveolar osteitis is considered a common complication following third molar surgery, with an estimated reported incidence of 0.3 – 26% (Bouloux *et al.*, 2006). None of the research participants developed alveolar osteitis. Although the exact etiology of alveolar osteitis is controversial, links have been made with smoking as a causative factor (Kolkythas *et al.*, 2010). All patients in this study were non-smokers and were given thorough verbal and written post-operative instructions, which could have contributed to this success. Surgical site infection and post-operative bleeding are important complications that could occur (Bouloux *et al.*, 2006). The risk of surgical site infection following third molar surgery has been reported as 5%

(Susarla *et al.*, 2011). All research candidates were young, healthy, non-smoking individuals. These factors combined with a meticulous sterile surgical technique could contribute to no surgical site infection developing. The small sample size of this study also contributes to the lack of statistical significant outcomes of the above-mentioned parameters.

Paraesthesia

The incidence of inferior alveolar nerve involvement during the first week following third molar surgery is approximately 1 – 5%, and involvement persisting longer than six months, up to 0.9% (Schultze-Mosgau *et al.*, 1995). The reported incidence of lingual nerve involvement day one post-operative is 0.4 – 1.5% (Queral-Godoy *et al.*, 2006).

In this study three (11.5%) and one (3.8%) patient(s) experienced transient involvement of the inferior alveolar nerve and lingual nerve, respectively. This incidence is relatively high when comparing to incidence reported in literature. The small sample size could be skewing the results.

Associated nerve injury, despite the degree, would influence the pain experienced, thus these four patients were excluded. If the follow-up time was of longer duration one could have included these patients by assessing the duration it took for full nerve recovery.

Bleeding

Filho and colleagues (2021) did a systematic review and meta-analysis on the effect that PRF has on post-operative bleeding in patients on anti-coagulants, and found that PRF does not prevent hemorrhagic complications following tooth extraction.

This study did not include patients with any underlying coagulopathies, but it did assess if PRF played a role in the control of bleeding following third molar surgery. Only one patient

reported reactionary bleeding (2-3 hours following extraction) bilaterally. Thus, no conclusion can be made on the effect of PRF on bleeding in this study.

A number of limitations plagued this study.

- The unexpected COVID 19 pandemic resulted in limitations in access to theatre, due to governmental restrictions on elective surgery in theatre under certain lockdown levels. This resulted in a much smaller sample size than anticipated.
- Limitations of a split-mouth design is that it limits the patients included to having mirror-image pathology, on the other hand, however, it eliminates much of the inter-subject variability (Lessafre *et al*., 2009).
- The short duration of follow-up, (seven days) resulted in certain parameters being missed i.e. late presentation of wound infection, complete resolution of associated nerve injury.
- Parameters such as the effect of PRF on bone preservation or bone healing could not be included due to the short duration of the study.
- The measurement of pain remains complex due to its subjective nature. Whether the assessment of pain experienced on individual sides was obscured or misled by the preceding question of the overall pain score, is debatable.
- The measuring tool used for swelling, which was done by various clinicians, posed room for inter-examiner errors.

Overall, this study did not show any value in placing PRF in the sockets of third molar teeth.

It is recommended that future research on this subject should involve a larger sample size and longer duration of assessment to truly reflect the effect on possible complications. A more reliable, reproducible measuring tool needs to be employed when assessing swelling in order to minimize inter-examiner errors. Alveolar bone regeneration and periodontal ligament

attachment posterior to the second molar teeth are important aspects that should be assessed following third molar surgery. These parameters however, need a much longer follow-up period, which this study was unable to achieve.

CHAPTER SIX

CONCLUSION

The aim of this study was to determine the post-operative outcomes of a PRF-treated socket versus a conventionally treated socket following surgical removal of third molar teeth. In recent years, the use of PRF has been popularized over a broad scope in the dental and medical fields.

This study rejected our hypothesis that PRF would enhance the sequelae of third molar surgery in the acute phase of healing and reduce the incidence of associated complications. This failure can be attributed to the limitation of the sample size, the short duration of follow-up and the restriction of access to theatre due to the SARS COVID-19 pandemic.

Before popularizing this simple and affordable material into routine clinical practice, more quality research is needed. Further investigations with larger sample sizes, more meticulous measuring tools as well as longer follow-up periods are needed to assess the true effect PRF has on enhancing post-operative healing in third molar surgery.

REFERENCES

Abdullah W.A., Khalil H. Dental extraction in patients on warfarin treatment. *Journal of Clinical, Cosmetic and Investigational Dentistry* 2014; 6:65-9.

Agarwal B., Gagnani S., Roychoudhury A., Bhutia O. Technical note Optimum use of platelet-rich fibrin: technical note. *British Journal of Oral and Maxillofacial Surgery* 2015; 53: 664–665.

Al-Hamed F.S., Tawfik M.A., Abdelfadil E., Al-Saleh M.A.Q. Efficacy of Platelet-Rich Fibrin after Mandibular Third Molar Extraction: A Systematic Review and Meta-Analysis. *Journal of Oral and Maxillofacial Surgery* 2017; 75: 1124-1135.

Antonello G., Torres Do Couto R., Giongo C.C., Corrêa M.B., Chagas Júnior O.L., Lemes C.H.J. Evaluation of the effects of the use of platelet-rich plasma (PRP) on alveolar bone repair following extraction of impacted third molars: Prospective study. *Journal of Cranio-Maxillofacial Surgery* 2013; 41(4): 70 –75.

Barone, A., Marconcini, S., Giacomelli, L., Rispoli, L., Calvo, J. and Covani, U. A Randomized Clinical Evaluation of Ultrasound Bone Surgery versus Traditional Rotary Instruments in Lower Third Molar Extraction. *Journal of Oral and Maxillofacial Surgery* 2010; 68(2): 330-336.

Bilginaylar, K. and Uyanik, L. Evaluation of the Effects of Platelet-Rich Fibrin and Piezosurgery on Surgical Outcomes after Removal of Impacted Mandibular Third Molars. *British Journal of Oral and Maxillofacial Surgery* 2016; 54(10): e99.

Borie E., Oliví D.G., Orsi I.A., Garlet K., Weber B., Beltrán V., Fuentes R. Platelet-rich fibrin application in dentistry: a literature review. *International Journal of Clinical and Experimental Medicine* 2015; 8(5): 7922-7929.

Bouloux, G., Steed, M. and Perciaccante, V. Complications of Third Molar Surgery. *Oral and Maxillofacial Surgery Clinics of North America* 2007; 19(1): 117-128.

Canellas, J., Ritto, F. and Medeiros, P. Evaluation of postoperative complications after mandibular third molar surgery with the use of platelet-rich fibrin: a systematic review and meta-analysis. *International Journal of Oral and Maxillofacial Surgery* 2017; 46(9): 1138-1146.

Canellas J., Medeiros P., Figueredo C., Fischer R., Ritto F. Platelet-rich fibrin in oral surgical procedures: a systematic review and meta-analysis. *International Journal of Oral and Maxillofacial Surgery* 2018; 48(3): 395-414.

Costa, F.W.G., Soares E.C.S., Esses D.F.S., Silva P. G.D.B., Bezerra T. P., Scarparo H. C., Ribeiro T. R., Fonteles C. S.R. A split-mouth, randomized, triple-blind, placebo-controlled study to analyze the pre-emptive effect of etoricoxib 120 mg on inflammatory events following removal of unerupted mandibular third molars. *International Journal of Oral and Maxillofacial Surgery* 2015; 44(9): 1166-1174.

Cruse P. J. and Foord R. The epidemiology of wound infection. A 10-year prospective study of 62,939 wounds. *Surgical Clinics of North America* 1980; 60(1):27.

Eshghpour, M., Dastmalchi, P., Nekooei, A. and Nejat, A. Effect of Platelet-Rich Fibrin on Frequency of Alveolar Osteitis Following Mandibular Third Molar Surgery: A Double-Blinded Randomized Clinical Trial. *Journal of Oral and Maxillofacial Surgery* 2014; 72(8): 1463-1467.

Faizel, S., Thomas, S., Yuvaraj, V., Prabhu, S. and Tripathi, G. Comparision Between Neocone, Alvogyl and Zinc Oxide Eugenol Packing for the Treatment of Dry Socket: A Double Blind Randomised Control Trial. *Journal of Maxillofacial and Oral Surgery* 2014; 14(2): 312-320.

Farish, S. and Bouloux, G. General Technique of Third Molar Removal. *Oral and Maxillofacial Surgery Clinics of North America* 2007; 19(1): 23-43.

Filho, E., Franco, J., Ribeiro, T., Silva, P. and Costa, F. Does Platelet-Rich Fibrin Prevent Hemorrhagic Complications After Dental Extractions in Patients Using Oral Anticoagulant Therapy? *Journal of Oral and Maxillofacial Surgery* 2021; 1 – 12.

Gawai K.T., Sobhana C.R. Clinical Evaluation of Use of Platelet Rich Plasma in Bone Healing. *Journal of Maxillofacial and Oral Surgery* 2015; 14(1): 67-80.

Giannini S., Cielo A., Bonanome L., Rastelli C., Derla C., Corpaci F., Falisi G. Comparison between PRP, PRGF and PRF: Lights and shadows in three similar but different protocols. *European Review for Medical and Pharmacological Sciences* 2015; 19(6): 927-930.

Giuffrè G., Caputo G., Misso S., Peluso F. Platelet-rich plasma treatment and hemostasis in patients with hemorrhagic risk. *Minerva Stomatol* 2006; 55(11-12): 599-609.

Gupta, A., Rattan, V. and Rai, S., 2019. Efficacy of Chitosan in promoting wound healing in extraction socket: A prospective study. *Journal of Oral Biology and Craniofacial Research* 2019; 9(1): 91-95.

Hartshorne J and Gluckman H. A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part 1: Definition, development, biological characteristics and function. *International dentistry-African edition.* 2016; 6(5)14-24.

Hartshorne J and Gluckman H. A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part II: Preparation, optimization, handling and application, benefits and limitations of PRF. *International Dentistry- African Edition* 2016; 6(5):34-48.

Hartshorne J and Gluckman H. A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part III: Clinical indications of PRF in implant dentistry, periodontology, oral surgery and regenerative endodontics. *International Dentistry – African edition* 2016; 6(5):64-79.

He Y., Chen J., Huang Y., Pan Q., Nie M. Local Application of Platelet-Rich Fibrin During Lower Third Molar Extraction Improves Treatment Outcomes. *Journal of Oral Maxillofacial Surgery* 2017; 75:2497-2506.

Hurley, E., Lim Fat, D., Moran, C. and Mullett, H. The Efficacy of Platelet-Rich Plasma and Platelet-Rich Fibrin in Arthroscopic Rotator Cuff Repair: A Meta-analysis of Randomized Controlled Trials. *The American Journal of Sports Medicine* 2018; 47(3): 753-761.

Ishwarkumar, S., Pillay, P., Haffajee, M. and Satyapal, K., 2019. Prevalence of impacted third molars in the South African Indian population of the eThekwini Metropolitan Region. *South African Dental Journal*, 74(6).

Iwabuchi H., Imai Y., Asanami S., Shirakawa M., Yamane G.Y., Ogiuchi H, *et al.* Evaluation of postextraction bleeding incidence to compare patient receiving and not receiving warfarin therapy: a cross-sectional, multicenter, observational study. *BMJ Open* 2014; 4(12):e005777.

Kapse, S., Surana, S., Satish, M., Hussain, S., Vyas, S. and Thakur, D. Autologous platelet-rich fibrin: can it secure a better healing? *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* 2009; 127(1):8-18.

Karimi, K. and Rockwell, H. The Benefits of Platelet-Rich Fibrin. *Facial Plastic Surgery Clinics of North America* 2019; 27(3): 331-340.

Kaul R.P., Godhi S., Singh A. Autologous Platelet Rich Plasma after Third Molar Surgery: A Comparative Study. *Journal of Maxillofacial and Oral Surgery* 2012; 11(2): 200-205.

Kolokythas, A., Olech, E. and Miloro, M. Alveolar Osteitis: A Comprehensive Review of Concepts and Controversies. *International Journal of Dentistry*, 2010:1-10.

Kumar Y.R., Mohanty S., Verma M., Kaur R.R., Bhatia P., Kumar V.R., Chaudhary Z. Platelet-rich fribrin: the benefits. *British Journal of Oral and Maxillofacial Surgery* 2016; 54: 57–61.

Lim A. A.T., Wong C. W., Allen J. C. Maxillary Third Molar: Patterns of Impaction and Their Relation to Oroantral Perforation. *Journal of Oral and Maxillofacial Surgery* 2012; 70: 1035-1039.

Lesaffre, E., Philstrom, B., Needleman, I. and Worthington, H. The design and analysis of split-mouth studies: What statisticians and clinicians should know. *Statistics in Medicine* 2009; 28(28): 3470-3482.

Malik N.A. Textbook of Oral and Maxillofacial Surgery. Second edition. New Delhi: Jaypee Brothers Medical Publishers, 2008.

Melville, J., Mañón, V., Blackburn, C. and Young, S. Current Methods of Maxillofacial Tissue Engineering. *Oral and Maxillofacial Surgery Clinics of North America* 2019; 31(4): 579-591.

Miron, R., Fujioka-Kobayashi, M., Bishara, M., Zhang, Y., Hernandez, M. and Choukroun, J. Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. *Tissue Engineering Part B: Reviews* 2017; 23(1): 83-99.

Miron, R., Zucchelli, G., Pikos, M., Salama, M., Lee, S., Guillemette, V., Fujioka-Kobayashi, M., Bishara, M., Zhang, Y., Wang, H., Chandad, F., Nacopoulos, C., Simonpieri, A., Aalam, A., Felice, P., Sammartino, G., Ghanaati, S., Hernandez, M. and Choukroun, J. Use of platelet-rich fibrin in regenerative dentistry: a systematic review. *Clinical Oral Investigations* 2017; 21(6): 1913-1927.

Miron, R., Fujioka-Kobayashi, M., Bishara, M., Zhang, Y., Hernandez, M. and Choukroun, J. Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. *Tissue Engineering Part B: Reviews* 2017; 23(1): 83-99.

Moraschini V., Barboza E. Effect of autologous platelet concentrates for alveolar socket preservation: A systematic review. *International Journal of Oral and Maxillofacial Surgery* 2015; 44(5): 632-641.

Ogundipe O., Ugboko V.I., Owotade F.J. Can Autologous Platelet-Rich Plasma Gel Enhance Healing After Surgical Extraction of Mandibular Third Molars? *Journal of Oral and Maxillofacial Surgery* 2011; 69: 2305-2310.

Pasqualini, D., Cocero, N., Castella, A., Mela, L. and Bracco, P. Primary and secondary closure of the surgical wound after removal of impacted mandibular third molars: a comparative study. *International Journal of Oral and Maxillofacial Surgery* 2005; 34(1): 52-57.

Susarla, S., Sharaf, B. and Dodson, T. Do Antibiotics Reduce the Frequency of Surgical Site Infections After Impacted Mandibular Third Molar Surgery? *Oral and Maxillofacial Surgery Clinics of North America* 2011; 23(4): 541-546.

Schultze-Mosgau S., Schmelseizen R., Frolich J.C. Use of ibuprofen and methylprednisolone for the prevention of pain and swelling after removal of impacted third molars. *Journal of Oral and Maxillofacial Surgery* 1995; 53(1): 2-7.

Siddiqi A., Morkel J.A., Zafar S. Antibiotic prophylaxis in third molar surgery: A randomized double placebo-controlled trial using split mouth technique. *International Journal of Oral Maxillofacial Surgery* 2010; 39(2): 101-114.

Singh A., Kohli M., Gupta N. Platelet Rich Fibrin: A Novel Approach for Osseous Regeneration. *Journal of Maxillofacial and Oral Surgery* 2012; 11(4): 430-434.

Su C.Y., Kuo Y.P., Tseng Y.H., Su C., Burnouf T. In vitro release of growth factors from platelet-rich fibrin (PRF): a proposal to optimize the clinical applications of PRF. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* 2009; 108: 56-61.

Suttapreyasri S., Leepong N. Influence of Platelet-Rich Fibrin on Alveolar Ridge Preservation. *The Journal of Craniofacial Surgery* 2013; 24: 1088-1094.

Synan, W. and Stein, K. Management of Impacted Third Molars. *Oral and Maxillofacial Surgery Clinics of North America* 2020; 32(4): 519-559.

Teshome, A. The efficacy of chlorhexidine gel in the prevention of alveolar osteitis after mandibular third molar extraction: a systematic review and meta-analysis. *BMC Oral Health* 2017; 17(1).

Tuk, J., Lindeboom, J., Sana, F., van Wijk, A. and Milstein, D. Alveolar Iodine Tampon Packing Reduces Postoperative Morbidity after Third Molar Surgery. *Journal of Oral and Maxillofacial Surgery* 2019; 77(12): 2401-2411.

Queral-Godoy, E., Figueiredo, R., Valmaseda-Castellón, E., Berini-Aytés, L. and Gay-Escoda, C. Frequency and Evolution of Lingual Nerve Lesions Following Lower Third Molar Extraction. *Journal of Oral and Maxillofacial Surgery* 2006; 64(3): 402-407.

Üstün, Y., Erdoğan, Ö., Esen, E., & Karsli, E. D. Comparison of the effects of 2 doses of methylprednisolone on pain, swelling, and trismus after third molar surgery. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 2003; 96(5): 535–539.

Uyanık, L., Bilginaylar, K. and Etikan, İ. Effects of platelet-rich fibrin and piezosurgery on impacted mandibular third molar surgery outcomes. *Head & Face Medicine* 2015; 11(1).

Varghese M.P., Manuel S., Kumar S. Potential for Osseous Regeneration of Platelet-Rich Fibrin—A Comparative Study in Mandibular Third Molar Impaction Sockets. *Journal of Oral and Maxillofacial Surgery* 2017; 75:1322-1329.

Vezeau, P. Dental extraction wound management: Medicating postextraction sockets. *Journal of Oral and Maxillofacial Surgery* 2000; 58(5): 531-537.

Wells D., Cape J., Powers M. Complications of dentoalveolar surgery. In: Fonseca R editor(s). Oral and Maxillofacial Surgery. Vol. 1, Philadelphia: WB Saunders, 200: 421-38.

White R.P., Fisher E.L., Magraw C.B.L., Phero J., Abdelbaky O., Sherwood C, Nelson B. Special contribution: Third molar clinical trials annotated bibliography. *Journal of Oral and Maxillofacial Surgery* 2016; 74(1): 4-12.

Xiang X., Shi P., Zhang P., Shen J. and Kang J. Impact of platelet-rich fibrin on mandibular third molar surgery recovery: a systematic review and meta-analysis. *BMC oral health* 2019; 19(163):1-10.

Zhu, J., Zhang, S., Yuan, X., He, T., Liu, H., Wang, J. and Xu, B. Effect of platelet-rich fibrin on the control of alveolar osteitis, pain, trismus, soft tissue healing, and swelling following mandibular third molar surgery: an updated systematic review and meta-analysis. *International Journal of Oral and Maxillofacial Surgery* 2021; 50(3): 398-406.

APPENDIX I

Table 6: Modified Archer classification for impacted maxillary third molar teeth	
Relative depth of the maxillary third molar in bone	Class A: The lowest portion of the crown of the impacted maxillary third molar is on a line with the occlusal plane of the second molar Class B: The lowest portion of the crown of the impacted maxillary third molar is between the occlusal plane of the second molar and the cervical line. Class C: The lowest portion of the crown of the impacted maxillary third molar is between the cervical line of the second molar and the mid third of the root of the second molar Class D: lowest portion of the crown of the impacted third molar is at or above the apical third of the second molar
The position of the long axis of the impacted maxillary third molar in relation to the long axis of the second molar tooth	I – vertical II – horizontal III – mesioangular IV – distoangular V – transverse or buccolingual angular
Relationship of the impacted maxillary third molar to the maxillary sinus	I - Sinus approximation - No bone or a thin partition of bone between the impacted maxillary third molar and the maxillary sinus II - No sinus approximation - 2 mm or more of bone between the impacted maxillary third molar and the maxillary sinus

Modified classification of impacted maxillary third molar teeth by Archer (Lim *et al.* 2012)

APPENDIX II

Table 7: Pell and Gregory Classification of impacted mandibular third molar teeth	
Based on the space between the second molar and the anterior aspect of the ramus	Class I: the mandibular third molar has sufficient space anterior to the anterior aspect of the ramus to erupt Class II: the space between the anterior aspect of the ramus and the second molar is insufficient for the third molar to erupt Class III: the impacted third molar is completely embedded in the ramus of the mandible
Based on the relationship of the occlusal plane of second and third molars	Class A: occlusal plane of the third molar is in line with that of the second molar Class B: occlusal plane of third molar is between the occlusal plane of the second molar and its cervical line Class C: occlusal plane of the third molar is below the cervical line of the second molar
The position of the tooth in relation to the long axis of the second molar tooth	I – vertical II – horizontal III – inverted IV – mesioangular V – distoangular May also occur in: a – buccal deflection b – lingual deflection c - Torsion

APPENDIX III

Department of Maxillo-Facial and Oral Surgery Faculty of Dentistry and WHO Oral Health Collaborating Centre, University of the Western Cape, Cape Town

Patient Information Letter

I, Dr. K Pedro-Beech (currently a qualified dentist enrolled in a specialist training program), plan to conduct a clinical study to assess if using autologous protein rich fibrin would enhance the healing after surgical removal of third molar teeth.

During the initial healing period a patient experiences pain and swelling. There is also a risk of developing a dry socket (alveolar osteitis) after the extraction of teeth.

Protein rich fibrin (PRF) is created from drawing your blood and placing it into a test tube which is spun in a centrifuge. Theoretically, protein rich fibrin (PRF) has multiple properties that should enhance healing.

We would like to apply this PRF within the sockets on one side of your mouth to assess if it does reduce your pain, swelling, enhances soft tissue healing and reduce the risk of developing a dry socket. You will be given an envelope which will indicate which side the PRF will be placed (left or right).

We will assess this impact by reviewing you 2 days and 7 days after surgery.

Your participation will help us assess the impact PRF will have on post extraction healing. If it is significant, patients undergoing surgical removal of third molars could receive PRF therapy to enhance their healing and reduce their pain allowing them to return to their day-to-day function sooner.

Participating in the study is on a voluntary basis. You may withdraw from the study at any time.

Participating in the study or refusing to participate will not harm or prejudice you in any way.

Participating in the study will definitely benefit future patients. All information will be kept strictly confidential.

Thanking you in anticipation.

Dr. K Pedro-Beech (Researcher)
Registrar (Maxillo-Facial and Oral Surgery)
Department of Maxillo-Facial and Oral Surgery
Oral Health Centre Tygerberg
Contact details: Tel: (021) 937 3119 Mobile: 0727085878
If you have any other queries, you are welcome to contact my supervisor, Dr. N. Behardien at 021 938 3119

I, (Patient name) ....., fully understand the information supplied to me by Dr. K Pedro-Beech in the above information letter.

Signature:..... Date:.....

APPENDIX IV

UNIVERSITY *of the*
WESTERN CAPE

Department of Maxillo-Facial and Oral Surgery Faculty of Dentistry and WHO Oral Health Collaborating Centre

University of the Western Cape, Cape Town

Consent form

I, Mr/Mrs/Miss.....

Date of Birth: .....

File no./Hosp. Sticker.....

am willing to participate in the study as described to me in the patient information letter by Dr K Pedro-Beech. I understand that participation in the study is voluntary.

The study is approved by the Ethical and Research Committee of the University of the Western Cape and participation in this study is on a voluntary basis. I have been adequately informed about the objectives of the study. I also know that I have the right to withdraw from the study at any stage which will not prejudice me in any way regarding future treatments. My rights will be protected and all my details will be kept confidential. No personal information will be published.

I hereby consent to be part of the research/study.

Patient name: .....

Patient signature: .....

Witness name: .....

Witness signature: .....

Researcher signature: .....

Dr. K Pedro-Beech

Date:.....

APPENDIX V

UNIVERSITY of the WESTERN CAPE

Department of Maxillo-Facial and Oral Surgery Faculty of Dentistry and WHO Oral Health Collaborating Centre, University of the Western Cape, Cape Town

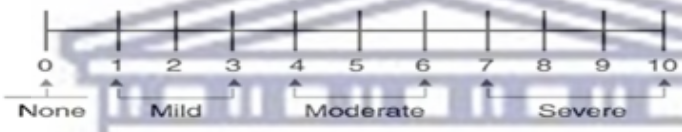
QUESTIONNAIRE DAY 2

1. **RECORD NUMBER:** _____

2. **GENDER:** F / M

3. **AGE:** _____

4. **PAIN EXPERIENCED:**



5. **WHICH SIDE ARE YOU EXPERIENCING MORE PAIN?** L / R

6. **WOUND DEHISCENCE:**

	MAXILLA		MANDIBLE	
LEFT SIDE	PRESENT	ABSENT	PRESENT	ABSENT
RIGHT SIDE	PRESENT	ABSENT	PRESENT	ABSENT

7. **ALVEOLAR OSTEITIS:**

	MAXILLA		MANDIBLE	
LEFT SIDE	PRESENT	ABSENT	PRESENT	ABSENT
RIGHT SIDE	PRESENT	ABSENT	PRESENT	ABSENT

8. **PUS DISCHARGE:**

	MAXILLA		MANDIBLE	
LEFT SIDE	PRESENT	ABSENT	PRESENT	ABSENT
RIGHT SIDE	PRESENT	ABSENT	PRESENT	ABSENT

9. **FACIAL SWELLING:**

PRE-OPERATIVE	LEFT	RIGHT
TRAGUS - LABIAL COMMISSURE	mm	mm
TRAGUS - SOFT TISSUE POGONION	mm	mm
DAY 2		
TRAGUS - LABIAL COMMISSURE	mm	mm
TRAGUS - SOFT TISSUE POGONION	mm	mm
TOTAL PERCENTAGE SWELLING	%	%

10. **PARAESTHESIA OF INFERIOR ALVEOLAR AND/OR LINGUAL NERVE** PRESENT / ABSENT

11. **POST OPERATIVE BLEEDING**

LEFT	PRIMARY	REACTIONARY	SECONDARY
	MILD	MODERATE	SEVERE
RIGHT	PRIMARY	REACTIONARY	SECONDARY
	MILD	MODERATE	SEVERE

12. **SCRIPT USED FOR BREKTHROUGH PAIN** YES / NO

APPENDIX VI

UNIVERSITY of the WESTERN CAPE

Department of Maxillo-Facial and Oral Surgery Faculty of Dentistry and WHO Oral Health Collaborating Centre, University of the Western Cape, Cape Town

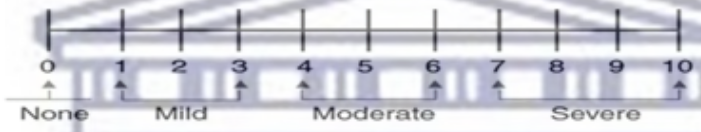
QUESTIONNAIRE DAY 7

1. **RECORD NUMBER:** _____

2. **GENDER:** N/A

3. **AGE:** N/A

4. **PAIN EXPERIENCED:**



5. **WHICH SIDE ARE YOU EXPERIENCING MORE PAIN?**

L	R

6. **WOUND DEHISCENCE:**

	MAXILLA		MANDIBLE	
LEFT SIDE	PRESENT	ABSENT	PRESENT	ABSENT
RIGHT SIDE	PRESENT	ABSENT	PRESENT	ABSENT

7. **ALVEOLAR OSTEITIS:**

	MAXILLA		MANDIBLE	
LEFT SIDE	PRESENT	ABSENT	PRESENT	ABSENT
RIGHT SIDE	PRESENT	ABSENT	PRESENT	ABSENT

8. **PUS DISCHARGE:**

	MAXILLA		MANDIBLE	
LEFT SIDE	PRESENT	ABSENT	PRESENT	ABSENT
RIGHT SIDE	PRESENT	ABSENT	PRESENT	ABSENT

9. **FACIAL SWELLING:**

PRE-OPERATIVE	LEFT	RIGHT
TRAGUS - LABIAL COMMISSURE	mm	mm
TRAGUS - SOFT TISSUE POGONION	mm	mm
DAY 7		
TRAGUS - LABIAL COMMISSURE	mm	mm
TRAGUS - SOFT TISSUE POGONION	mm	mm
TOTAL PERCENTAGE SWELLING	%	%

10. **PARAESTHESIA OF INFERIOR ALVELOAR AND/OR LINGUAL NERVE** N/A

11. **POST-OPERATIVE BLEEDING**

LEFT	PRIMARY	REACTIONARY	SECONDARY
	MILD	MODERATE	SEVERE
RIGHT	PRIMARY	REACTIONARY	SECONDARY
	MILD	MODERATE	SEVERE

12. **SCRIPT USE FOR BREAKTHROUGH PAIN**

YES	NO

APPENDIX VII

Standard method of preparing PRF

The anaesthetist drew venous blood intra-operatively, to fill 2 glass coated red top vacutainer tubes. The Duo PRF machine was used. The tubes were placed into opposing slots in the machine to allow for balancing during centrifugation.

Standard settings for PRF as per the Duo PRF Machine: 1300rpm for 8min.

Following centrifugation, the tubes were maintained in the holder until ready for placement.

APPENDIX VIII

Standard method of placing PRF

The PRF was picked up with a non-toothed forceps and placed onto the stainless tray. The PRF section was cut from the sample and placed into the selected intervention side sockets.

APPENDIX IX

Post-operative information sheet

1. It is normal for your face to swell up. The swelling will most probably increase in size for the first two days after the operation. This is normal. Please do not worry.
2. You may have slight bloody ooze with blood stained saliva for the first 24h00. This is within normal limits. If there is constant red bloody ooze or blood clots, apply pressure over the area by placing a dampon pad/swab overthe wound and closing the teeth firmly over the pad for 15minutes: it can help. Contact us if you are concerned.
3. You may place ice pacs on the side of your face for the first 24 hours following the operation. After that use them only when necessary.
4. Use the pain tablets which has been prescribed and given to you for the first 48 hours. After that use them only when necessary.
5. Sleep on as many pillows as possible. When you slep with your head raisedyou will find that this will help to minimise the post-operative swwelling and it will also ease the discomfort which you may experience at night-time.
6. Please use mouthwash and rinse gently only after each meal, as indicated on the label (even use half teaspoon of salt in ons gless of warm water).
7. Brush your teeth regularly and you may find a little tender at first but within a few days the tenderness will disappear. The brushing will not tear out the stitches. If one or two of the stitches dislodges after 3-4 days, do not worry about this.
8. The chewing gum can help to prevent jaw stiffness. Buy large, flat sticks of gum and use 2-3 packets per day.
9. Eat normal diet from day of surgery. The only foods to avoid eating are those with seeds, pips, or grain. If you are a diabetic, maintain your normal diet and take your medication as usual.
10. After a few days, if you develop a bad taste, swelling, a discharge around the stitches, or a high temperature, please contact us immediately.
11. If you had your operation under general anaesthetic or with intravenous sedation you must not drive a motor vehicle, or use any tools or machinery, do not make any important decisions or sign any iportant documents for 24 hours following the operation.
12. Do ot take any alcohol while you are on the post-operative medication.
13. Reduce strenuous activity for at least 3 days after surgery. This will reduce bleeding and help your blood clot and wound to stabalise.
14. If you have any problems please contact the department at 021 937 3119/ 3120.
15. Please do not phone your dentist or house doctor (there is always an on-call dentsit available should you for any reason not get hold of us). The on call dentist should be able to conatct a dentist in the department.

APPENDIX X

Technique to measuring facial swelling

As described by Ogundipe *et al.* (2011): Two lines were drawn – (1) from the tragus to the labial commissure and (2) from tragus to the soft tissue pogonion.

A flexible tape measure was used to measure the length of the lines drawn.

Baseline measurements were taken pre-operatively on the day of surgery. The follow-up measurements were done on Day 2 and Day 7, respectively.

The following mathematical equation was used to calculate the percentage facial swelling:

$$\frac{S_{post\text{-}op} - S_{pre\text{-}op}}{S_{pre\text{-}op}} X\ 100 =$$

This percentage was calculated using day 2 and day 7 values, respectively.

APPENDIX XI

UNIVERSITY of the WESTERN CAPE

50 YEARS of hope, action & knowledge

27 November 2020

Dr K Pedro-Beech
Faculty of Dentistry

Ethics Reference Number: BM19/3/8

Project Title: The efficacy of platelet-rich fibrin (PRF) on healing following surgical removal of third molar teeth.

Approval Period: 18 September 2020 – 18 September 2023

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report annually by 30 November for the duration of the project.

Permission to conduct the study must be submitted to BMREC for record-keeping.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

Director: Research Development
University of the Western Cape
Private Bag X 17
Bellville 7535
Republic of South Africa
Tel: +27 21 959 4111
Email: research-ethics@uwc.ac.za

NHREC Registration Number: BMREC-130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE.

The Efficacy of Platelet-Rich Fibrin (PRF) on Healing Following Surgical Removal of Third Molar Teeth

ORIGINALITY REPORT

22%	18%	13%	9%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	hdl.handle.net Internet Source	4%
2	Submitted to University of the Western Cape Student Paper	1%
3	www.moderndentistrymedia.com Internet Source	1%
4	Omer Demirtas, Abubekir Harorli. "Evaluation of the maxillary third molar position and its relationship with the maxillary sinus: a CBCT study", Oral Radiology, 2015 Publication	1%
5	www.science.gov Internet Source	1%
6	etd.uwc.ac.za Internet Source	1%
7	repository-tnmgrmu.ac.in Internet Source	1%