Comparing clinical outcomes of connective tissue grafts to platelet rich fibrin in gingival recession treatment: an extended case series

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DECLARATION

I, Fatima Peer, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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Dr Fatima Peer

Student number: 00 00 587W

Signed on the _____ day of _____ 20_____ in _____

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ABSTRACT

Aim

This study set out to evaluate the clinical and aesthetic outcomes of connective tissue grafts (CTG) to platelet rich fibrin (PRF) in treating gingival recession. It was hypothesised that PRF could be as effective as CTGs in treating recession with improved aesthetic results. To the best of my knowledge this clinical study is unique in the South African setting in that an objective aesthetic scoring system was used to report on aesthetic changes and also this study was patient based to determine patient satisfaction with aesthetic outcomes.

Methods and materials

This six month study was an extended case series with a randomised split-mouth design. Six patients with a total of twenty two sites underwent treatment. However, only five patients fulfiled the study's follow-up requirements. The patient who failed to comply with the follow-up appointments was disqualified from the study. Each site was paired with a similar lesion on the opposite or contralateral side and randomly assigned to the CTG (control) or PRF (test) treatment. Six variables were recorded over the study period. These variables were probing depth, recession depth, recession width, clinical attachment level, keratinised tissue width and gingival thickness. These were measured at the following intervals: 0, 8, 12, 16 and 24 weeks. Photographs were taken at baseline and at 24 weeks to evaluate aesthetics using the Pink Esthetic Score. At the end of the study period, patients were given a questionnaire to assess their satisfaction with treatment outcomes.

Results and Conclusions

Both treatments improved the clinical outcomes but CTGs demonstrated improvements at a greater number of sites than PRF (60% to 30% respectively). The aesthetic scores improved at four sites for both CTGs and PRF with only one site in each group scoring lower at the end of the study. The aesthetic scores at the remaining sites did not change over the study period. Therefore, both CTGs and PRF demonstrated the potential to improve or maintain aesthetic results. Analyses of the patient questionnaire showed that patients were satisfied with the aesthetic outcomes of both treatments.

The results from this study indicate that both CTGs and PRF membranes can be effective in treating gingival recession and both treatments can improve clinical and aesthetic outcomes.

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List of Abbreviations

Ang: Angiotensin

A-PRF: Advanced-platelet rich fibrin

B: mid-buccal

CAF: Coronally advanced flap

CAL: Clinical attachment level

CEJ: cemento-enamel junction

CGF: Concentrated growth factors

CTG: Connective tissue graft

DB: disto-buccal

ECM: Extracellular matrix

EGF: Epidermal growth factor

FGF: Fibroblast growth factor

GT: Gingival thickness

HREC: Human Research Ethics Committee

IGF: Insulin-like growth factors

IL: Interleukin

i-PRF: injectable platelet rich fibrin

KTW: Keratinised tissue width

L-PRF: Leukocyte- and Platelet-rich fibrin

L-PRP: Leukocyte-and Platelet-rich plasma

MB: mesiobuccal

MGJ: mucogingival junction

PD: Periodontal probing depths

PDNC: Patient did not comply

PDGF: Platelet-derived growth factor

PES: Pink Esthetic Score

PIGF: Placental growth factor

PRF: Platelet-rich fibrin

P-PRF: Pure platelet-rich fibrin

PRP: Platelet-rich plasma

P-PRP: Pure Platelet-rich plasma

RCT: Randomised control trial

RD: Recession depth

RW: Recession width

RES: Root Esthetic score

TGF: Transforming growth factor

TNF: Tumour necrosis factor

VEGF: Vascular endothelial growth factor

CHAPTER 1

1 Introduction

Gingival recession is a common dental complaint and is defined as "an apical movement of the gingival margin to below the cemento-enamel junction (CEJ) thus exposing the root surface".[1-5]

Gingival recession may cause dentine hypersensitivity, root caries, decreased plaque control motivation and poor aesthetics.[6, 7] Treatment options range from the conservative and minimally invasive such as monitoring and preventative management of aetiological factors to dentine sealants and various types of restorations to the more comprehensive approach of periodontal plastic surgery.[6, 7]

Gingival recessions can lead to multiple and diverse consequences. Therefore, determining the degree of success of any chosen treatment is multifaceted. The selected treatment needs to improve the gingival architecture namely: the contours of the gingival margin and interdental papilla, gingival thickness and width in order to improve periodontal health, reduce dentine hypersensitivity and satisfy the patient's aesthetic concerns.

Traditionally, a connective tissue graft (CTG) in combination with a coronally advanced flap (CAF) is considered the surgical gold standard to treat gingival recession.[8-16] This study aims to compare this gold standard with a newer technique; a platelet-rich fibrin (PRF) membrane with a modified CAF.

PRF is a wholly autogenous biomaterial.[17] It is simple, quick and relatively inexpensive to prepare. It is made up of a 3-dimensional fibrin matrix that is rich in platelets, leukocytes and growth factors.[17, 18] Each component of the PRF biomaterial contributes factors that can enhance wound healing and tissue regeneration.[19]

Systematic reviews have highlighted a paucity of information with regards to patient-based outcomes and aesthetics in the literature.[8, 20] Together with clinical outcomes, Cortellini and Pini-Prato eloquently state that the true goal of any treatment should be 'patient satisfaction'.[20] This study aims to compare the clinical and aesthetic outcomes of CTGs and PRF in treating gingival recessions. This study will also report on patient satisfaction between the two groups.

CHAPTER 2

2 Literature review

2.1 Gingival Recession

In 1967, Gorman discussed the prevalence and aetiology of gingival recession. He defined gingival recession as exposure of a tooth's root surface as a result of apical migration of the gingiva.[5] Over a decade later, in 1979 Maynard and Wilson proposed a review of the nomenclature to update both the term gingival recession and its definition. They suggested the term "marginal tissue recession" and defined it as exposure of the root surface by apical migration of the soft tissue margin.[4] This term was widely accepted because the soft tissue margin may not always be composed of gingiva; in some instances, it may comprise of alveolar mucosa only.[4] Today, these terms are used interchangeably and "marginal tissue recession" is referred to as "gingival recession" in this paper.

Gingival recessions are a common dental problem and usually found during routine dental examinations or the symptoms caused by gingival recessions prompt patients to seek treatment.

Many epidemiological studies have shown that its prevalence varies between groups ranging from 11% to 100%.[6, 7, 21-24] Recession lesions are just as likely to present in patients with good periodontal health and good oral hygiene habits as in patients with periodontitis or gingivitis and poor oral hygiene.[3, 6] Gingival recession can present as isolated lesions on a single tooth or be widespread and include multiple teeth.[3]

2.1.1 Aetiology

Gingival recession is most likely the result of an inflammatory process that may be triggered by a variety of factors.[22] The literature is replete with ambiguity with regards to the terms 'cause/aetiology/pre-disposing factors and risk factors'. Many authors have classified and grouped these factors in various different ways. Tugnait and Clerehugh classified aetiological factors as related to either pathological or non-pathological alveolar bone loss.[6] Marini *et al*. on the other hand classified the aetiological factors according to predisposing or precipitating factors.[22] Patel *et al*. then classified aetiological factors as either a direct

result of mechanical or physical insult to the gingival tissues or as an indirect response to an inflammatory reaction in the gingival tissues.[7]

For the purpose of clarity of these terms, I will be referring to Lang and Lindhe, who categorised the aetiology of gingival recession into three main groups:[25]

1. Gingival recession associated with mechanical trauma.
2. Gingival recession associated with localised plaque-induced inflammatory lesions.
3. Gingival recessions associated with generalised types of destructive periodontal disease.

2.1.1.1 Mechanical Trauma

The mechanical trauma most commonly associated with gingival recession is aggressive oral hygiene habits.[3, 25] These types of lesions are usually found in patients with good plaque control and healthy gingiva.[21] Aggressive oral hygiene habits include aggressive brushing force and technique, increased tooth brushing frequency, increased brushing time, firmness of toothbrush bristles and using old and damaged toothbrushes.[3, 26, 27]

However, not all patients with aggressive oral hygiene habits will present with gingival recession. Anatomical factors such as a thin gingival biotype, a thinner alveolar osseous plate and a tooth in a more buccal or lingual position on the arch increase the risk of gingival recessions developing in a particular area or patient.[3, 6, 28] Thick gingival biotypes and its associated positive anatomical factors are thought to be more resistant to inflammation and trauma.[28, 29]

2.1.1.2 Localised Plaque-induced Inflammatory Lesions

These types of lesions develop in response to the presence of subgingival bacterial plaque. Plaque accumulation can induce localised areas of inflammation. In areas of anatomical vulnerability, this inflammatory response can cause localised destruction of the gingival tissues resulting in apical movement of the gingival margin.[3]

2.1.1.3 Generalised Destructive Periodontitis

Subgingival periodontal pathogens elicit a host inflammatory response that destroys alveolar bone and soft tissue attachment.[3, 6, 7] This tissue destruction can extend into the interproximal area inducing compensatory remodeling of the periodontal tissues resulting in apical migration of the gingival margin.[25]

2.1.2 Predisposing Factors

There are many predisposing factors that may lead to the development of gingival recession. Zuchelli and Mounssif divided these risk factors into three categories.[3]

1. Anatomical factors.
2. Physiological factors.
3. Pathological factors.

2.1.2.1 Anatomical Factors:

- Fenestrations or dehiscences of alveolar bone:

  A dehiscence is "a defect in which the alveolar crest of buccal bone is at least 4mm apical to the crest of interproximal bone".[30]

  A fenestration is "a localised defect of the buccal, lingual or palatal alveolar plate which exposes the root surface but does not involve the alveolar margin".[30]

  These types of bone defects can cause loss of the overlying soft tissue thus increasing the risk of gingival recession.[6, 7, 22]

- Atypical eruption pathway / abnormal tooth position in the arch:

  When a tooth follows an atypical eruption pathway, the tooth can be placed in a more buccal position.[3, 6] This placement causes the cervical portion of the root to be placed in crestal bone resulting in a thinner than usual buccal plate thereby increasing susceptibility to bone resorption.[3]

- Root morphology:

  There are two important aspects of root morphology that increase susceptibility to a tooth developing gingival recession. Teeth with long and narrow roots are more susceptible to these defects than teeth with short, broad roots. Also, teeth with roots that are equal in thickness to the thickness of the crestal bone are at an increased risk to developing gingival recessions.[3]

- Prominent frenal and muscle attachments:

  Prominent and/or additional frenal and muscle attachments are associated with localised gingival recession lesions.[6, 22, 24] The increased tension from these atypical attachments can cause direct pull on the marginal gingiva prompting apical

migration of the gingiva resulting in gingival recession. It can also hinder efficient plaque control from the discomfort whilst brushing, triggering localised areas of inflammation and subsequent gingival recession.[6]

- Shallow vestibule:

  Generally, a shallow vestibule implies an inadequate width of keratinised gingiva.[31] However, there has been much debate as to what is considered an optimum width of keratinised gingiva. Lang and Löe suggested that 2mm of keratinised gingiva is the required minimum to maintain periodontal health.[32] This theory has been challenged and it was found that periodontal health can be maintained in the absence of keratinised tissue.[33, 34]

  However, studies around peri-implant tissues have shown that insufficient keratinised tissue width is associated with higher plaque accumulation, gingival inflammation, bleeding on probing, and gingival recession.[35] Berglundh *et al.* conducted a study on beagle dogs to determine the effects of new plaque formation on gingiva around implants and teeth.[36] They found that gingiva around implants and natural teeth have the same potential to develop inflammation to plaque formation.[36] Pontoreiro *et al.* conducted a similar study in humans where gingival inflammation was experimentally induced around teeth and implants by withholding plaque control measures.[37] After three weeks, clinical examinations were done at all sites to determine the inflammatory response. There was no statistically significant difference found in tissue response between teeth and implant sites.[37]

  Therefore studies carried out on implants have a basis on natural teeth and it can be reasoned that insufficient keratinised tissue width around teeth is also associated with higher plaque accumulation, gingival inflammation, bleeding on probing, and gingival recession.[36, 37]

2.1.2.2 Physiological Factors:

- Thin gingival biotype:

  Thin, delicate gingival tissue is less resistant to microbial induced inflammation or traumatic insult whereas thick gingival biotypes are more resistant to chemical and mechanical insults such as inflammation, toothbrush abrasion, the packing of

impression cords into the gingival sulcus and poor restorative margins.[3, 6, 25, 28] Hence, thin gingival biotypes are predisposed to gingival recession.[7, 22]

- Teeth that are positioned outside of the buccal plate:

  Atypical eruption pathways or orthodontic tooth movement which retains teeth in a position outside of the buccal bone increases the risk of bone loss and subsequent soft tissue loss.[3, 6, 38]

2.1.2.3 Pathological Factors: [3]

- Physical trauma:

  Trauma to the periodontal tissues can be caused by abrasive oral hygiene techniques, traumatic occlusion, perioral and intraoral piercings or self-inflicted trauma as a result of aberrant habits such as nail-biting, toothpick use, etc.[3, 6, 22, 38]

- Chemical trauma:

  Smokers tend to exhibit increased frequency of gingival recession than non-smokers.[6] Chronic topical application of drugs or smokeless tobacco also increases the risk of gingival recessions developing in the area adjacent to where the tobacco is placed, usually the mandibular labial and buccal vestibule.[6, 39]

- Iatrogenic damage:

  Iatrogenic damage as a result of subgingival restorations or poorly designed partial dentures increases the risk of gingival recessions developing in these areas.[3 6, 7]

2.1.3 Sequelae

Gingival recession can cause a range of symptoms including dentine hypersensitivity, enamel abrasion, root caries, loss of keratinised tissue width, plaque retention with gingival bleeding and poor aesthetics in patients with high smile lines.[3, 6, 7, 13]

2.1.3.1 Dentine Hypersensitivity

Dentine hypersensitivity can be twofold; thermal and/or tactile. Thermal hypersensitivity is common on cold stimuli and causes sharp intense pain of short duration at the affected areas.[6] Tactile hypersensitivity can be determined clinically by merely running a diagnostic probe along the cervical dentine. Patients feel pain at this metallic touch. This type of hypersensitivity can make brushing uncomfortable or painful and therefore difficult for patients to effectively manage plaque control in the affected areas. This can perpetuate the

recession lesions as there will be increased plaque accumulation, eliciting an inflammatory response and possibly worsening the extent of the recession lesion.

The hydrodynamic theory of pain is the most commonly accepted theory to explain dentine hypersensitivity.[6] Stimuli on the cervical dentine causes movement of dentinal fluid within the dentine tubules. This, in turn, activates the sensory nerve fibres in the dentine and at the dentino-pulpal junction, eliciting a pain response.[6] However, pain is perceptive and not all patients experience pain at exposed root surfaces. In those who do experience pain, the intensity of pain varies from a slight sensitivity to a sharp and intense pain.

2.1.3.2 Cervical Tooth Abrasion

These dental defects are typically non-carious, wedge-shaped and appear at the cervical area of teeth.[40] These defects are usually caused by mechanical trauma typical of aggressive tooth brushing techniques. Aggressive tooth brushing can also cause trauma to the gingival soft tissues inducing gingival recessions to develop. With continued trauma after apical movement of the gingival margin, abrasion of the cervical enamel, dentine or cementum may occur.[3]

2.1.3.3 Root Caries

All tooth surfaces that are exposed to the oral environment are susceptible to decay. Gingival recession exposes root surfaces that were previously protected by the gingiva. Once the cementum on these exposed root surfaces are destroyed, pain may be felt in these areas compromising effective plaque control leading to the development of root caries.[3, 6]

2.1.3.4 Loss of Keratinised tissue Width

Keratinised tissue is firmly attached to the underlying bone and is well suited to withstand masticatory forces and resist injury from physical, thermal and chemical stimuli of everyday exposures.[41] With gingival recession, keratinised tissue is reduced or lost and the overall width of this tissue decreases. This, in turn, can cause the vestibule to narrow and can make brushing uncomfortable and painful impeding effective plaque control. Insufficient keratinised tissue width is associated with higher plaque accumulation, gingival inflammation, bleeding on probing, and gingival recession.[22, 35]

2.1.3.5 Plaque Retention and Gingival Bleeding

The combined effects of dentine hypersensitivity, reduced keratinised tissue width and a shallower vestibule can cause difficulty in maintaining effective plaque control. Plaque accumulation increases the inflammatory response causing gingival bleeding and subsequently worsening the recession lesions in a vicious cycle complex.[3]

2.1.3.6 Poor Aesthetics

The principles of smile design include aesthetic and functional components to create a harmonious integration between the two. Gingival recession affects the dental component of an ideal smile. The dental component relates specifically to teeth and their relationship to the gingival tissues.[42] The gingival perspective of aesthetics is the most quantifiable, and least prone to subjective interpretation.[43] The gingiva is the frame of the teeth and any change to the ideal level or shape of the gingival margin may disrupt the ideal proportions of the mucogingival complex compromising aesthetics.[42]

Apical movement of the gingival margin increases the clinical crown length of a tooth.[44] When this phenomena occurs in the anterior zone, this disharmony may be apparent in the patient's smile or even at a functional level affecting phonetics.[44] The exposed roots also tend to be darker and more yellow than enamel worsening overall aesthetics.[6] Gingival recession lesions can also cause loss of symmetry of the natural gingival scalloping resulting in disharmony of the gingival margin.[3, 6] A discerning patient with a high smile line often influences the "aesthetic zone". These patients usually have high expectations for treatment outcomes. Furthermore, the current age of "Extreme Makeovers" and instant fixes have made patients more aesthetic conscious and subsequently patient aesthetic expectations are higher than in the past. The aim of any treatment should be to restore the gingival architecture and holistically improve aesthetics and function.

2.1.4 Management

Gingival recession treatments include monitoring and preventative management of aetiological factors and treating symptoms with dentine sealants, restorations and periodontal plastic surgery.[6, 7]

Conservative preventative measures such as dentine sealants can reduce dentine hypersensitivity but does not restore and improve aesthetics. Class V restorations, crowns

and veneers elongate the clinical crown length and may worsen aesthetics by creating disproportionately long teeth and can result in a disharmonious smile.[6] Periodontal plastic surgery is able to restore the gingival tissue architecture and holistically improve aesthetics.

Periodontal plastic surgery is a term that encompasses a range of surgical techniques which aim to recreate and restore the gingival anatomy and morphology. Surgical treatments to restore gingival recessions fall under the umbrella of periodontal plastic surgery.

Gingival recessions can lead to multiple and diverse consequences, therefore determining the degree of success of treatment can be multifaceted. The chosen treatment has to restore gingival architecture thereby improving periodontal health in order to maintain oral health. In addition, treatment has to satisfy both the clinician's and the patient's aesthetic concerns.

2.1.5 Recession Classification System

In 1968, Sullivan and Atkins classified gingival recession into four groups: 1) Shallow-narrow. 2) Shallow-wide. 3) Deep-narrow. 4) Deep-wide.[45] This classification system is useful in categorising recession lesions but is limited in predicting prognosis of surgical treatment.[1] A more useful classification system was created by PD Miller in 1985.[46]

Miller's classification system aids in the diagnosis and prognosis of predictable outcomes of surgical treatments.[25, 46-48] Miller used the presence or absence of the interdental papilla as one of the key elements in his classification system, as the papilla level is the most significant prognostic factor for root coverage.[46] In lesions with interdental papilla loss, the aesthetic outcome is compromised as only partial root coverage is predicted. In this study, Miller's recession classification system was used to determine the severity of the recession lesions. There are four classes in this classification system.

1. **Miller's Class I**

Figure 2.1: Miller's class I.

Gingival recession that does not extend beyond the MGJ without periodontal tissue loss in the interdental area. 100% root coverage is possible.[46]

2. **Miller's Class II**

Figure 2.2: Miller's class II.

Gingival recession that extends to or beyond the MGJ without periodontal tissue loss in the interdental area. 100% root coverage is possible.[46]

3. **Miller's Class III**

Figure 2.3: Miller's class III.

Gingival recession that extends to or beyond the MGJ with periodontal tissue loss in the interdental area or malpositioning of teeth.[46] The interdental tissue loss is coronal to the apical extent of the gingival margin. These features prevent 100% root coverage and only partial root coverage can be expected.[46]

4. **Miller's Class IV**

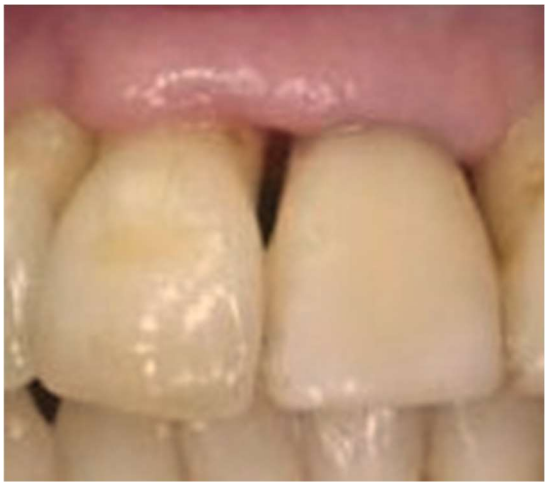

Figure 2.4: Miller's class IV.

Gingival recession that extends to or beyond the MGJ with periodontal tissue loss in the interdental area and/or malpositioning of teeth.[46] The interdental tissue loss extends to a level apical to the gingival margin. Root coverage cannot be expected in class IV lesions.[46]

2.2 The Mucogingival Complex and Gingival Biotype

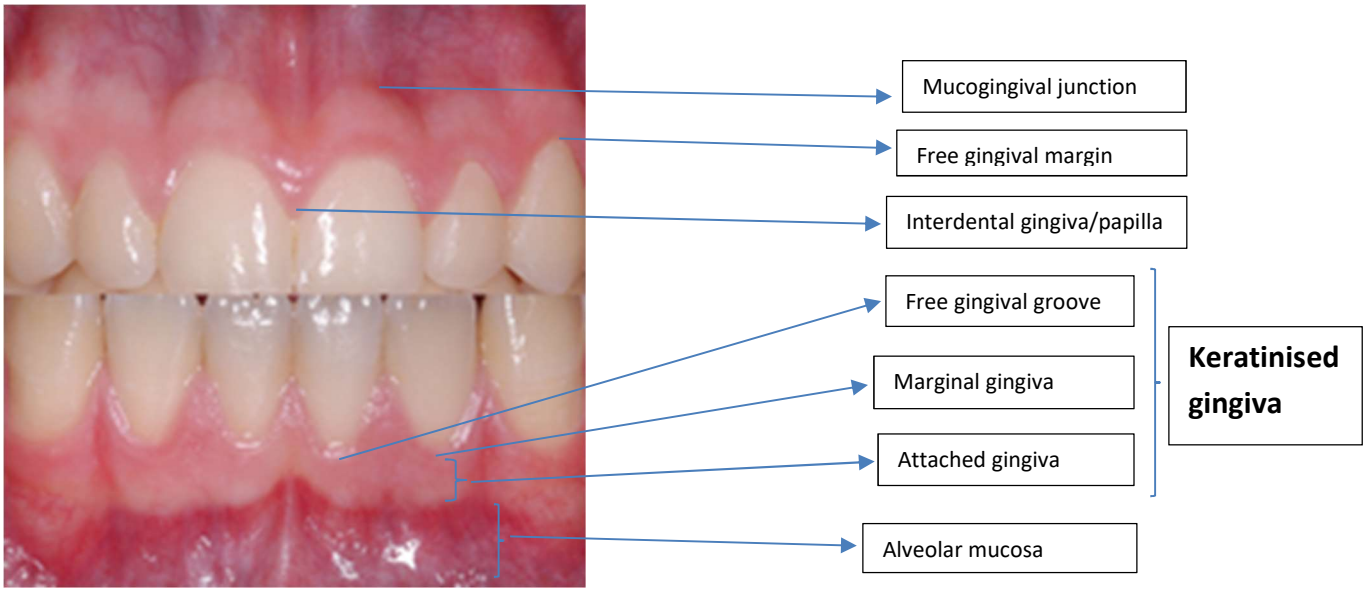

Figure 2.5: Mucogingival complex.[49]

2.2.1 Clinical Features

The gingiva is that part of the oral mucosa which covers the alveolar process and surrounds the neck of the teeth. The free gingival margin is the coronal limit of the gingiva and the apical limit is continuous with the alveolar mucosa.[50] The border between the gingiva and alveolar mucosa is usually distinct and called the mucogingival junction (MGJ). The gingiva can be divided anatomically into marginal (free), attached and interdental gingiva.[41] After tooth eruption, in health the free gingival margin is located on the enamel surface approximately 0.5 – 2mm coronal to the CEJ.[41] The free gingiva extends from the gingival margin in an apical direction to the free gingival groove creating what is known as the gingival sulcus (see figure 2.6). The soft tissue which is attached to the tooth coronal to the alveolar crest is regarded as the biological width (see figure 2.7) and is essential in maintaining periodontal health.[51] The attached gingiva extends from the free gingival groove coronally to the MGJ apically and is firm, resilient and firmly attached to the underlying alveolar bone and cementum.[50] These structural features allow the attached gingiva to meet its functional demands and withstand the shearing forces of mastication.[52] Attached gingiva is also referred to as keratinised mucosa and its width and thickness is genetically determined.[41]

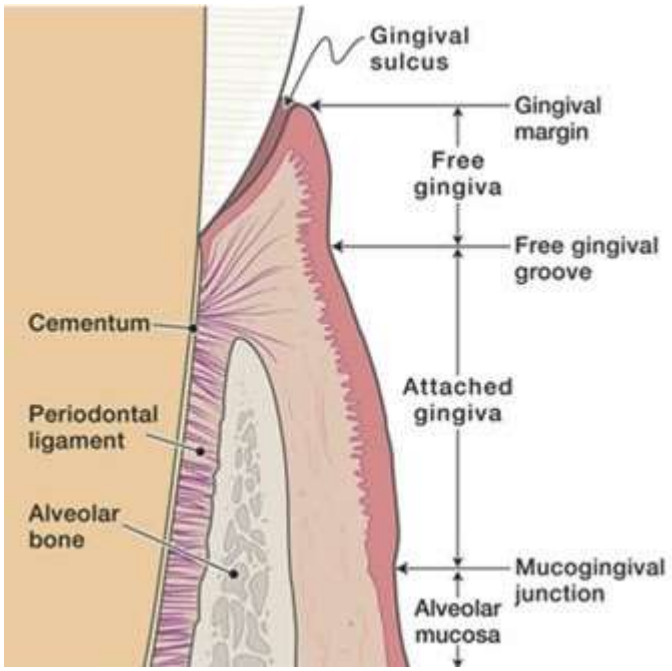

Figure 2.6: Cross-sectional view of the mucogingival complex[53]

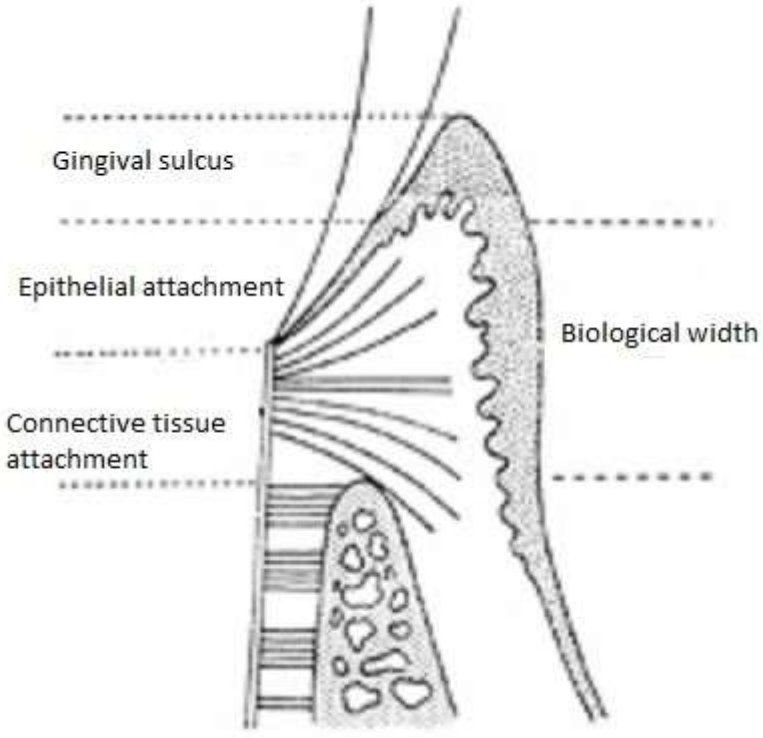

Figure 2.7: Diagrammatic representation of the biological width.[54]

2.2.2 Histological Features

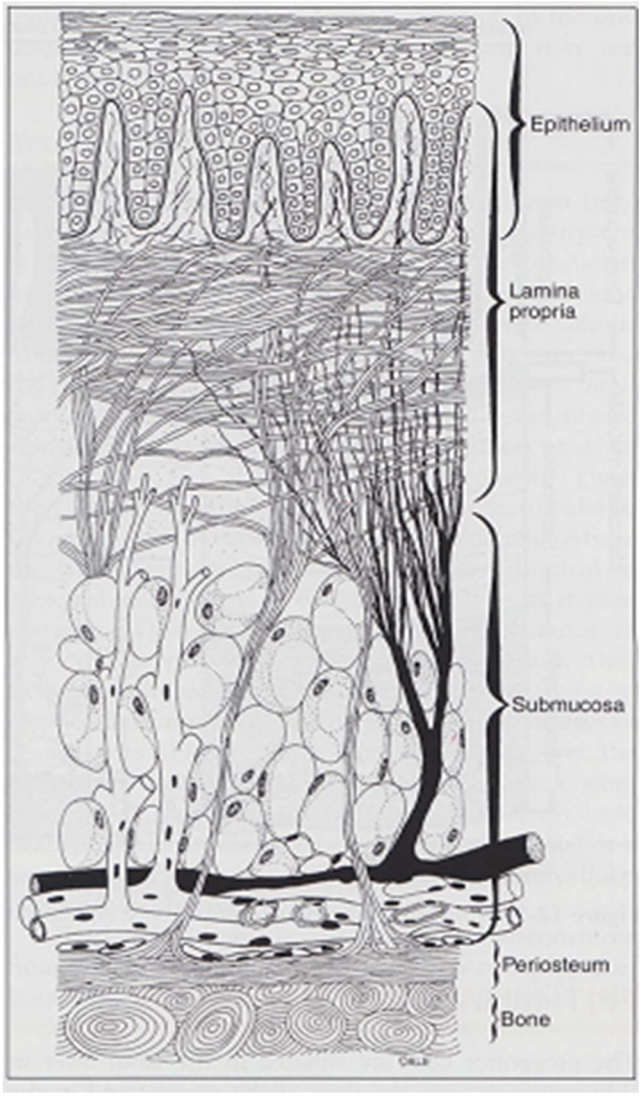

Figure 2.8: Histological appearance of the oral mucosa.[55]

Oral mucosa comprises of two distinct tissue components, the overlying epithelium and the underlying lamina propria (or connective tissue). These components are separated by a basement membrane and the oral mucosa is separated from the underlying bone or muscle by the submucosa.[50]

Oral epithelium is stratified squamous epithelium and can be nonkeratinised, keratinised, parakeratinised or a combination of keratinised and parakeratinised.[50] The masticatory mucosa of the gingiva and hard palate is keratinised and better able to withstand the shearing forces of mastication while lining mucosa may be non-keratinised or parakeratinised depending on where it is positioned in the oral cavity. The majority of cells in the oral epithelium are keratinocytes (90% of the cell population) and the remainder are

nonkeratinocytes made up of melanocytes, Langerhans cells, Merkel's cells and inflammatory cells.[25, 50]

The connective tissue lies subjacent to the oral epithelium and consists of fibres, cells, blood vessels and nerves embedded in an amorphous ground substance. The border between the oral epithelium and the connective tissue has an undulating interface. The oral epithelium lacks a blood supply and so it is the epithelial-connective tissue junction which allows for metabolic exchange between these two structures.[50]

The connective tissue has two layers:[1]

1. A superficial papillary layer below the epithelium. This layer projects connective tissue papillae that interdigitate between epithelial rete pegs. This structural arrangement of the epithelial-connective tissue junction increases the surface area of the interface than if the junction were flat. It is thought that this arrangement provides improved attachment allowing forces applied to the epithelial surface to be dispersed over a greater area of connective tissue.
2. A deeper reticular layer adjacent to the alveolar bone. Reticular refers to the net-like appearance of the collagen fibres.

As with oral epithelium, the connective tissue also exhibits regional variation in the proportion of its constitutional elements. The connective tissue of masticatory mucosa is firm with fewer elastic fibres. The elastic fibres of masticatory mucosa are usually associated with blood vessels. The connective tissue of lining mucosa, on the other hand, has more elastic fibres making it stretchable and able to move with muscle movements.

The oral epithelium is separated from the underlying connective tissue by a basement membrane composed of collagen, laminin, heparin sulphate proteoglycan and fibronectin.[50] The basement membrane maintains cellular organisation, acts as a filter to molecules between the epithelium and connective tissue and is a barrier to cellular migration. The basement membrane also provides the signaling essential for epithelial differentiation and the development and maintenance of epithelial cell polarity.[50]

The submucosa separates the oral mucosa from the underlying bone or muscle.[50] The submucosa is composed of loose fatty or glandular tissue, blood vessels, nerves and minor salivary glands. In the gingiva and parts of the hard palate, the oral mucosa is directly

attached to the periosteum of the underlying bone with no submucosa. This is known as mucoperiosteum and has a firm inelastic attachment.[50]

2.3 The Significance of Keratinised Mucosa

The major differences between keratinised and non-keratinised mucosa is in the structure and differentiation of the epithelium, the epithelial-connective tissue interface and the density and elasticity of the connective tissue fibres.[56] These differences allow keratinised mucosa to be tougher and better equipped to withstand the shearing forces mastication and to dissipate the pull of the alveolar musculature.[57]

Keratinised tissue width varies from between 1-9mm.[32] In 1972, Lang and Löe found that a keratinised tissue width of at least 2mm (1mm of free gingiva and 1mm of attached gingiva) was adequate to maintain gingival health.[32] As discussed previously, this theory has been challenged and it was found that periodontal health can be maintained even in the absence of keratinised tissue.[33, 34]

Berglundh *et al.* conducted a study on beagle dogs to determine the effects of new plaque formation on the gingiva around implants when compared to teeth.[36] They found that the gingiva around implants and natural teeth have the same potential to develop inflammation in response to plaque formation.[36] Pontoreiro *et al.* conducted a similar study in humans where gingival inflammation was experimentally induced around teeth and implants by withholding plaque control measures.[37] After three weeks, clinical and histological examinations were done at all sites to determine the inflammatory response. There was no statistically significant difference found in the tissue response between teeth and implant sites.[37] In 2008, Bouri *et al.* studied the relationship between keratinised tissue width and gingival health around implants. They concluded that 2mm of attached gingiva maintains peri-implant health.[58] These studies demonstrate that an insufficient width of keratinised tissue around implants is associated with higher plaque accumulation, gingival inflammation, bleeding on probing, and gingival recession.[35-37, 58] Studies carried out on implants have a basis on natural teeth and it can be reasoned that insufficient keratinised tissue width around teeth is also associated with higher plaque accumulation, gingival inflammation, bleeding on probing, and gingival recession.[36, 37]

In addition, thick keratinised gingival tissue serves as an effective barrier which is resistant to damage from the physical forces of mastication as well as thermal and chemical insults.[41] However, the point at which gingiva is determined as thick or thin is subjective.[59, 60] Some authors state that gingival tissue which is ≤1.0 mm is thin and when it is ≥1.0 mm it is thick.[60, 61] While others define thin gingiva as ≤1.5mm and thick gingiva ≥2.0mm.[29] Yet other authors state that if a periodontal probe can be seen through the gingiva when inserted into the sulcus, the gingiva is thin and if it cannot be seen it is thick.[59, 62]

Despite lack of agreement on what is thick or thin gingiva, all authors agree that thicker gingiva is important for gingival health. A positive correlation was found between gingival thickness and keratinised tissue width.[29, 63, 64] Thicker, wider gingiva implies a thick bony underlying morphology and is more resistant to traumatic and inflammatory insults.[29] On the other hand, thin and narrow gingiva implies a thin bony underlying morphology which is more susceptible to traumatic and inflammatory insults and subsequent periodontitis and greater gingival recession compared to thick gingival biotypes.[29, 59]

It is important to determine the gingival biotype in treatment planning as it is a key prognostic factor that determines the degree of success or failure of surgical treatment.[59] The importance of thick gingiva was reviewed by Hwang *et al.* The authors found a positive correlation with increased gingival thickness and root coverage.[65] They found that for good mean and complete root coverage a critical threshold for gingival thickness is greater than 1.1 mm. The thicker the gingival tissue, the easier it is to manipulate and maintain vascularity.[65]

2.4 Evolution and History of Periodontal Plastic Surgery

Periodontal plastic surgery is a collective term to describe “surgical procedures performed to prevent or correct anatomical, developmental, traumatic or plaque-induced defects of the gingiva, alveolar mucosa or bone”.[3, 13, 66-68]

Periodontal plastic surgical concepts evolved from the original principles of mucogingival surgery. Mucogingival surgery began in the 1950’s and aimed “to correct or enhance the thickness or change the position of mucogingival tissues” and was primarily concerned with improving function and did not consider gingival aesthetics.[66]

In its early days, mucogingival surgical techniques aimed to treat problems of attached gingiva, alveolar mucosa and shallow vestibules.[52] Over time, the objectives of mucogingival surgery expanded to involve procedures to correct alveolar ridge deformities, surgical exposure of unerupted teeth, to improve the contours, symmetry and colour of marginal tissues and correction of aesthetic defects around implants.[67, 69] These techniques took aesthetic problems into account. This diversity of surgical procedures propelled PD Miller to coin a new term: 'periodontal plastic surgery'.[68] Periodontal plastic surgery is a more comprehensive and descriptive term to the techniques used today.[67]

Surgical management of gingival recessions fall into this category. The aim of such surgery is to restore the gingival anatomy and achieve complete root coverage, to increase keratinised tissue width, improve aesthetics related to the adjacent gingiva, to achieve minimal probing depths after healing and prevent worsening of the lesion. [3, 70]

Surgical techniques to treat gingival recession include CAF's, pedicle flaps, lateral sliding flaps, double papilla rotational flaps, free gingival grafts, connective tissue grafts, guided tissue regeneration, allografts and xenografts.[10, 70] The gold standard of treatment is the bilaminar technique of combining a CTG with a CAF.[8-13]

2.5 Connective Tissue Grafts

Many studies have shown that CTGs combined with a CAF to be superior to other surgical techniques.[3, 13, 70] This technique was introduced by Langer and Langer in the 1980's.[71] In root coverage procedures, the avascular root surface presents a challenge for wound healing and tissue regeneration.[72] The success and predictability of a CTG with a CAF is credited to the double blood supply at the recipient site which ensures survival of the graft.[3, 71, 73] The overlying flap and the periosteal connective tissue bed below provides this dual blood supply.[71] The blood supply from the recipient bed nourishes the base of the graft while the overlying flap ensures survival of the most coronal part of the graft which lies over the avascular root surface.[3, 74] In addition, the overlying flap also masks the white scar tissue and corrugated texture of the underlying CTG.[3]

CTGs with a CAF have demonstrated significant improvements in root coverage, clinical attachment gain, keratinised tissue gain with superior colour matching to adjacent tissues

and provides the most predictable results.[3, 12, 75] It has been found that an increase in gingival tissue thickness improves long-term stability of the treated area over a ten year period.[76] This improvement in tissue thickness is a direct result of the CTG.

CTGs can be sourced from the hard palate, retromolar pad area or an edentulous space when available.[77] The retromolar pad and edentulous areas are often not large enough to produce optimal graft thickness and length. Therefore, CTGs are usually sourced from the hard palate.

2.5.1 Biological mechanisms of Connective Tissue Grafts

The success of CTGs can be explained by epithelial-mesenchymal cellular interactions. During embryogenesis, mutual inductive influences occur between epithelial and mesenchymal tissues. Induction is defined as the ability of one cell type to determine differentiation of adjacent cells of a different type[78] and it is the process that initiates differentiation[50].

Odontogenesis is an example of this type of epithelial-mesenchymal interaction. During tooth development a well- regulated, sequential and reciprocal sequence of inductive interactions between stomodeal ectoderm and ectomesenchymal tissues result in tooth development.[78, 79]

Studies have shown that it is similar in post-natal life. The first wound healing studies were carried out on dermal tissues and later on oral mucosa. These studies determined that stratified squamous epithelium of skin and oral mucosa is maintained in structure and function by epithelial-mesenchymal interactions.[56]

More than fifty years ago several researchers found that when epithelium was transplanted into a tissue type that was different in structure and function, it retained its original specificity. These studies demonstrated that the varied characteristic features of epithelium are genetically determined rather than as a result of functional adaptation.[80]

In 1971, Karring *et al.* verified this discovery.[80] The authors set out to test whether tissue specificity is pre-determined by some intrinsic factor within tissues or whether tissue specificity is determined by functional adaptation. Monkeys were used to heterotopically transplant keratinised and non-keratinised oral mucosa to different areas of the oral cavity

and they found that the transposed tissues retained their original features.[80] In addition, non-keratinised tissue that was placed in close proximity to teeth underwent a histological change and a narrow band of keratinised tissue developed in these areas. The authors deduced that the most likely origin of granulation tissue at these wound sites was from the periodontal ligament and that it was signals from this connective tissue that induced the epithelial cells of the transposed non-keratinised tissue to differentiate into keratinised epithelium. They concluded that tissue cell specificity is genetically pre-determined and that epithelial differentiation is also influenced by signals from the underlying connective tissue and basement membrane at the recipient site.[80]

Later studies which set out to determine the role of connective tissue in epithelial differentiation in humans, validated these findings. In 1974, Edel undertook a study where epithelial free CTGs from the palate were transplanted into areas that had less than 2mm of gingival keratinised tissue width.[57] The recipient sites were clinically examined over a six month period and the MGJ at one site was histologically examined at six months. The graft area appeared keratinised with an increase in keratinised tissue width. These results demonstrated that a significant increase in keratinised tissue can be achieved by palatal connective tissue alone.[57] In a similarly designed clinical study, Edel and Faccini studied the histological changes in similarly transplanted sites.[81] After six months, histological examination found that the newly formed epithelium was keratinised with a normal architecture.[81] In another study, Karring *et al.* transplanted free CTGs (void of epithelium) from keratinised and non-keratinised sources into alveolar mucosa.[82] The grafts were placed into connective tissue pouches that were created as close to the overlying epithelium as possible. The transplanted tissue was then removed and studied at varied time intervals between one and twelve months. The non-keratinised graft tissue served as the control and there was no change at these sites. At sites where connective tissue from gingival mucosa were transplanted into alveolar mucosa, the original non-keratinised epithelium that covered the transplanted gingival connective tissue developed into keratinised epithelium that was indistinguishable from the original gingival epithelium. The junction between the transplanted non-elastic gingival connective tissue and the elastic alveolar connective tissue was also distinct and supports the idea that it is the gingival connective tissue that induced histodifferentiation of the overlying epithelium.[82]

In 1980, Bernimoulin and Schroeder set out to study the different types of alterations in epithelial differentiation after transplanting palatal CTG into alveolar mucosa.[56] Six months after transplantation, these sites were biopsied and examined. The authors looked at the type of epithelial differentiation, the epithelial-connective tissue interface, the density and elasticity of the connective tissue fibres and various cellular organelles. Three of the seven test sites demonstrated characteristics almost identical to the hard palate while the remaining four test sites were different from typical alveolar mucosa but only slightly resembled the hard palate. These results also suggest that inductive stimuli originates from the connective tissue and not only influences epithelial differentiation but also the architecture of epithelial-connective tissue interface. The study also suggests that the epithelial response to these stimuli may depend on the type of epithelial differentiation pattern that exists at the recipient site.[56]

Later studies by Hill, Mackenzie and Binnie reiterates these findings.[83-86] They stated that in addition to histodifferentiation, connective tissue also directly influences mitotic activity, proliferation and migration of the overlying epithelium.[83-86] They also found that while connective tissue has directive influences on epithelial differentiation, the ability of the epithelium to respond to these influences is also critical.[84] Epithelial and mesenchymal connective tissues have a close and dependent interactive relationship that maintains its structural and functional specificity.

In clinical application, this means that it is the origin of the regenerating tissues which determines the success and predictability of mucogingival surgery. Signals from the connective tissue of keratinised mucosa will induce keratinised epithelium to develop.[82] In a study by Eren and Atilla where they treated localised gingival defects, the control group was a CTG combined with a CAF.[12] They found an increase in gingival thickness in the control group and explained this phenomenon by the type of connective tissue that was transplanted. The connective tissue was harvested from the palate and hence contained the signals that influenced the overlying epithelium to develop into keratinised tissue increasing tissue thickness.[12] This is the type of tissue that surgeons want to “re-create” when treating gingival recessions. However, there are disadvantages and complications associated with CTGs.

2.5.2 The "Dark Side" of Connective Tissue Grafts

Sourcing donor tissue from the palate increases surgical time and postoperative morbidity.[3, 77] A second surgical site prolongs pain and discomfort to the patient with an increased risk of haemorrhage and infection.[77] Other reported complications are delayed wound healing and bone necrosis with sloughing of the overlying tissues.[77] Anatomical considerations such as the position of the palatine neurovascular bundle which is in close proximity to the donor area, increases the risk of paraesthesia or permanent anaesthesia at the donor site.[12, 77] There is also a limited amount of graft tissue available from the palate limiting the number of recessions that can be treated in one sitting.[12, 77] This may necessitate multiple surgical procedures to correct multiple recession lesions in one patient.

In addition, CTGs can produce a dense and bulky tissue contour which requires a second surgery to correct.[12] A systematic review by Chambrone *et al.* reported the following adverse reactions associated with the palatal donor site: postsurgical oedema, pain and necrosis of the palatal flap during the initial healing phase.[8] Patients are usually anxious to undergo surgery in general but more so when the palate is a surgical site.[77] Eliminating the necessity of the palate as a donor site will not only reduce morbidity of this procedure but patients will be more willing to consent to treatment.

Alternatives to CTGs are commercially available barrier membranes, allografts, xenogeneic collagen membranes and enamel matrix derivative. These membranes have demonstrated success at improving gingival recessions and aesthetics.[3, 6, 10, 12, 77] However, using these membranes can be technique sensitive and complications have been documented. In cases where non-resorbable membranes are used, a second surgery is required to remove the membrane. Complications specific to these membranes are membrane exposure and contamination.[3] Newly formed periodontal tissue may be damaged when these membranes are removed or absorbed.[3] Also, these materials are regarded as foreign bodies by recipient tissues and can disrupt the natural healing process.[87] Furthermore, these membranes are expensive and most South African patients cannot afford to pursue these treatment options. Allografts and xenogeneic grafts are associated with risk of disease transmission, tissue rejection and may raise ethical concerns.[3, 77] Some patients may not be willing to use xenogeneic membranes for religious or personal reasons as they are commonly derived

from bovine or porcine sources. In sum, these alternative membranes are not an adequate substitute for CTGs. A more natural autogenous substitute is sought.

A more economical and potentially viable alternative is platelet concentrates; a natural autologous material.

2.6 Platelet Concentrates

The use of fibrin adhesives is well documented and can be said to be the precursor to the platelet concentrate evolution.[88] Fibrin adhesives were first used in surgery to amplify the natural fibrin polymerisation process during haemostasis. Fibrin adhesives act like "glue", sealing wound edges to promote healing.[88] The first fibrin adhesives were derived from allogeneic sources but with the risk of disease transmission, autologous adhesive protocols were developed. These protocols were complex, time-consuming and expensive.[19] Simplifying these protocols led to the development of platelet concentrates. When it was discovered that the growth factors within these concentrates could be harnessed, its applications expanded to tissue regeneration.[89]

Platelet concentrates have been used in surgery to augment healing for forty years.[89] In recent years, there has been a shift to utilise platelet concentrates as an adjunct to periodontal surgery to promote periodontal tissue regeneration. Platelet concentrates are autogenous biomaterials obtained from the patients' own blood. The preparation of platelet concentrates have improved and simplified over the years. It is easy, convenient and economical to produce while eliminating the risk of foreign body reactions and disease transmission.

Platelet concentrates have diverse medical applications from healing sports tendon injuries to plastic surgery to aesthetic skin treatments.[89] Oral surgical applications include but are not limited to sinus floor elevation, extraction socket preservation and augmentation, intrabony and furcation defects, peri-implant defects, regenerative endodontic treatment, root end surgery and soft tissue augmentation.[12, 18]

2.6.1 The Platelet Concentrate Evolution

- **Platelet-rich plasma (PRP)**

PRP was the first platelet concentrate and in its liquid form was originally used in transfusion medicine to treat haemorrhagic disorders such as thrombocytopenia and leukaemia and in the treatment of significant blood loss during surgery.[88, 90] Whole blood was separated to harvest platelets for transfusion. Thereafter, thrombin and calcium were added to PRP and it evolved into a fibrin concentrate. These preparations are similar to currently available fibrin adhesives and were used to control haemostasis.[19]

PRP, as we know it today, was first discussed in the literature in the 1970's for its wound healing properties. It was produced from platelet poor plasma and its production protocols were expensive, time-consuming and complex and thus its use failed to gain widespread popularity. Then, in the late 1990's, a revolution occurred when high concentrations of growth factors were discovered in PRP preparations. In 1997, Whitman introduced PRP into the field of oral surgery as an adjunct to post-surgical healing.[89] This compelled a surge in different production protocols to make it easier to produce PRP and in 1999, Anitua introduced a variation of PRP called Platelet Rich in Growth Factors.[91]

Typical PRP preparations undergo a double centrifugation process with chemical additives to create a biomaterial rich in growth factors and cytokines. It was the idea of a growth factor enriched material that prompted its initial popularity.

PRP preparation is a three-step process. It requires a double centrifugation cycle. The first cycle uses an anticoagulant and separates the platelets, the second cycle concentrates the platelets. This preparation is in a liquid form and PRP can be used as such and injected into the desired tissue but it is more commonly converted into a gel for topical application.[89] Bovine thrombin and/or calcium chloride or equivalent additives are added to the liquid PRP to initiate platelet activation and fibrin polymerisation inducing rapid gelling of the platelet concentrate.[88, 90] This gellification ensures the slow release of growth factors and cytokines trapped within the preparation.[88]

Kawase *et al.* summarised the benefits of PRP as increasing cell proliferation and upregulates extracellular matrix production thereby promoting wound healing.[92] Pradeep *et*

al. surmised that the fibrinogen in PRP reacts with thrombin thereby inducing clot formation.[93] The fibrin clot then upregulates collagen synthesis in the extracellular matrix providing a scaffold for cellular migration and adhesion.[93] PRP may also allow initial stabilisation and revascularisation of surgical flaps and grafts.[11]

However, clinical results were controversial and contradictory. Its preparation protocols were technique sensitive, time-consuming and expensive. There was also the risk of disease transmission from bovine sourced anticoagulants and immune reactions and so PRP slowly lost favour.[11, 19, 92, 94] With PRP, the role of fibrin within the preparation was not considered a necessary element and ignored. We now understand that the fibrin content and in particular its structure and arrangement is critical to the effectiveness of a platelet concentrate.

Dohan *et al.* postulated that it is the rapid polymerisation process of PRP that causes the initial rapid release of cytokines, limiting clinical effectiveness of PRP.[88] The clinical limitations have also been attributed to the high thrombin concentrations within PRP. These high thrombin concentrations create thick fibrin polymer junctions resulting in a firm and inflexible structure. This type of structure seals biological tissues very well and can control haemostasis but is not conducive to cytokine entrapment and cellular migration or interactions.[88] PRP is ultimately a low-density fibrin material enriched with growth factors and cytokines.

Anitua *et al.* also developed a type of PRP called plasma rich in growth factors (PRGF).[91] PRGF is a poor leukocyte platelet concentrate with the intent to suppress the pro-inflammatory effect.[19] Its preparation protocol involves several steps with a final step adding calcium chloride creating an unstable PRGF gel that needs to be used immediately. This protocol is not easily reproducible and is therefore not widely used and reported on.[90]

- **Platelet-rich fibrin (PRF)**

PRF was developed by Joseph Choukroun in the early 2000's. Choukroun's PRF has a simpler preparation protocol than PRP and eliminates the need for additives. PRF is a second generation platelet concentrate whose purpose is to "activate and facilitate healing and the regenerative capacity of the host tissue, by providing a strong fibrin scaffold, major growth factors and allowing space for tissue regeneration".[87] PRF is a fibrin clot enriched with

platelets, B- and T- lymphocytes, monocytes, stem cells, neutrophilic granulocytes and growth factors.[19]

When compared to PRP, PRF is a biomaterial with a high-density fibrin network. This gel-like fibrin network is created by a slow and almost natural polymerisation process. This allows the slow release of the growth factors and cytokines within the fibrin.[15] The fibrin network also acts as a scaffold for cellular migration and proliferation.

PRF is prepared from the patient's own blood and its preparation protocol is simple, efficient and relatively inexpensive. There are no anticoagulants or chemical additives added and involves only a single centrifugation step. This makes PRF wholly autogenous with an almost natural polymerisation process.[88] Choukroun *et al.* defines PRF as a healing biomaterial.[17]

- **Concentrated growth factors**

In 2006, Sacco developed another type of PRF concentrate called concentrated growth factors (CGF).[95] CGF is also an autogenous fibrin biomaterial. Its preparation protocol differs from Choukroun's PRF in that it uses variable centrifugation speeds (Choukroun uses constant speeds) and produces a larger, denser fibrin matrix with a higher growth factor concentration.[95]

2.6.2 Platelet Concentrate Classification

Different centrifugation speeds and times with or without the addition of chemical additives produces different types of platelet concentrates.[19] To help understand the different types of platelet concentrates, Ehrenfest *et al.* have proposed a simple classification system.[90] The classification system is based on: 1) The pharmacological content (platelets and leukocytes) of the concentrate and 2) The characteristics of its fibrin network.[90]

Platelet concentrates are classified into four main categories.[90]

1. Pure Platelet-rich plasma (P-PRP), also known as Leukocyte-poor plasma.
2. Leukocyte-and Platelet-rich plasma (L-PRP).
3. Pure platelet-rich fibrin (P-PRF), also known as Leukocyte-poor fibrin.
4. Leukocyte-and Platelet-rich fibrin (L-PRF).

2.6.2.1 Pure Platelet-rich plasma (P-PRP) or Leukocyte-poor plasma

P-PRP has a complex preparation protocol involving multiple steps with the addition of chemicals. Preparation is not easy, can be very expensive and lack reproducibility. Platelet collection is low and platelets are damaged in the preparation process. Also, there are limited publications on its use. Examples of P-PRP are: Vivostat PRF and Anitua's PRGF.[90, 91]

2.6.2.2 Leukocyte-and Platelet-rich plasma (L-PRP)

L-PRP is not commonly used and its preparations kits are inconvenient, inefficient, expensive and the resultant concentrate dissolves rapidly.[90] Examples of this concentrate are: Curasan, Regen PRP and Plateltex.[90]

2.6.2.3 Pure platelet-rich fibrin (P-PRF) or Leukocyte-poor fibrin

The trade name for P-PRF is Fibrinet. Its claim to fame is that it is a 'natural' platelet concentrate as it is void of bovine thrombin. However, its preparation still requires an anticoagulant and separation gel and therefore cannot be defined as 'natural'. Its preparation is difficult and expensive. There is also a lack of evidence in the literature on the efficacy of Fibrinet.[90]

2.6.2.4 Leukocyte- and Platelet-rich fibrin (L-PRF)

L-PRF is Choukroun's PRF. Its protocol is the simplest and most inexpensive of all platelet concentrates. It is a wholly autogenous and a natural biomaterial. There are no added anticoagulants or gelling agents. The resultant fibrin clot has a strong resilient matrix with a 3-dimensional structure and a high concentration of platelets and leukocytes. The PRF preparation process activates the platelets inducing platelet and leukocyte growth factors to be implanted into the fibrin matrix. PRF, when compared to PRP, has an extended working time as the resultant clot does not dissolve quickly. The PRF protocol is easily reproducible and well suited for widespread applications.[90] Its use has been well documented over recent years and includes sinus floor elevation, extraction socket preservation and augmentation, intrabony and furcation defects, peri-implant defects, regenerative endodontic treatment, root end surgery and soft tissue augmentation.[12, 18]

With its increasing popularity and a rise in commercial interests, there are several PRF kits available. The most common is Choukroun's PRF with a kit from Duo Process, Nice, France. Choukroun's protocols have evolved over time and include Choukroun's PRF; Advanced PRF

(A-PRF), and injectable i-PRF.[90] Other variants to PRF are L-PRF by Intra-spin and CGF which is a solid fibrin material enriched with leukocyte and was created by Sacco.[95]

This research report will focus on Choukroun's A-PRF.

2.7 Platelet Rich Fibrin

PRF preparation has evolved over time and different protocols are in use.[87] It has been shown that altering the centrifugation forces alters the distribution of the different cell types.[96] These different protocols attempt to optimize the ratios of blood cells, growth factors and cytokines within the fibrin matrix. This creates optimal PRF preparations specific to different clinical requirements.[19]

The current PRF protocols are:[87]

1. The original Choukroun's PRF protocol (standard protocol): 3000 rpm / 10 minutes
2. Dohan Ehrenfest's PRF (L-PRF): 2700 rpm / 12 minutes)
3. Choukroun's advanced PRF (A-PRF): 1500 rpm / 8 minutes
4. Choukroun's i-PRF (solution/gel form): 700 rpm/3 minutes

PRF is an autogenous living biomaterial.[17] It can be described as an optimised blood clot made up of a 3-dimensional fibrin matrix enriched with platelets, cytokines, growth factors, leukocytes and stem cells.[19, 96]

PRF is prepared from a patient's own blood. A small amount (9-10 ml) is extracted and undergoes centrifugation. The centrifugation process separates the blood into three distinct layers: an acellular supernatant plasma layer on top, a PRF clot in the middle and an erythrocyte layer at the bottom. The PRF clot is made up of two distinct parts: a yellow fibrin clot and a smaller red thrombus at its base. Between these two parts, there is a whitish layer called the buffy coat. The PRF clot can be used directly as is or it can be compressed into a membrane or plug depending on the clinical application. The supernatant can be aspirated and used as an injectable (i-PRF).[19]

Choukroun *et al.* summarised the clinical observations of PRF as inducing angiogenesis, enhancing natural immune support, harnessing the power of circulating stem cells and enhancing epithelial cover to protect the wound.[17] The authors state that it is not only the

platelets, growth factors and cytokines within the PRF clot that provide these benefits but that its fibrin gel matrix is the key component.

2.7.1 Structural Components of the PRF clot

The centrifugation process separates the different blood constituents into three layers: an acellular supernatant plasma layer, a middle PRF clot layer and erythrocytes below.

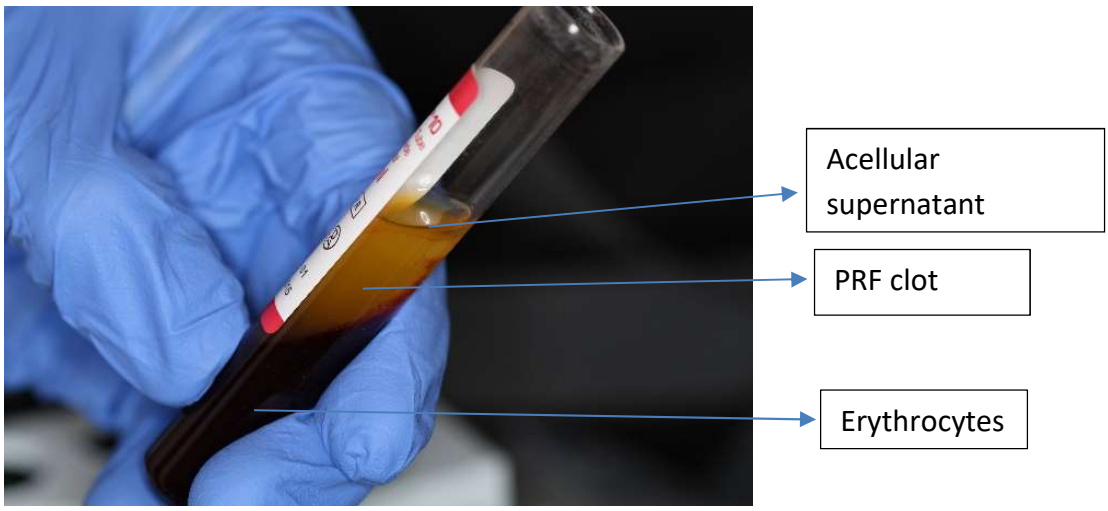

Figure 2.9: The three layers immediately after centrifugation.

PRF is made up of a yellow fibrin clot with a red thrombus base and a whitish buffy coat between the two.

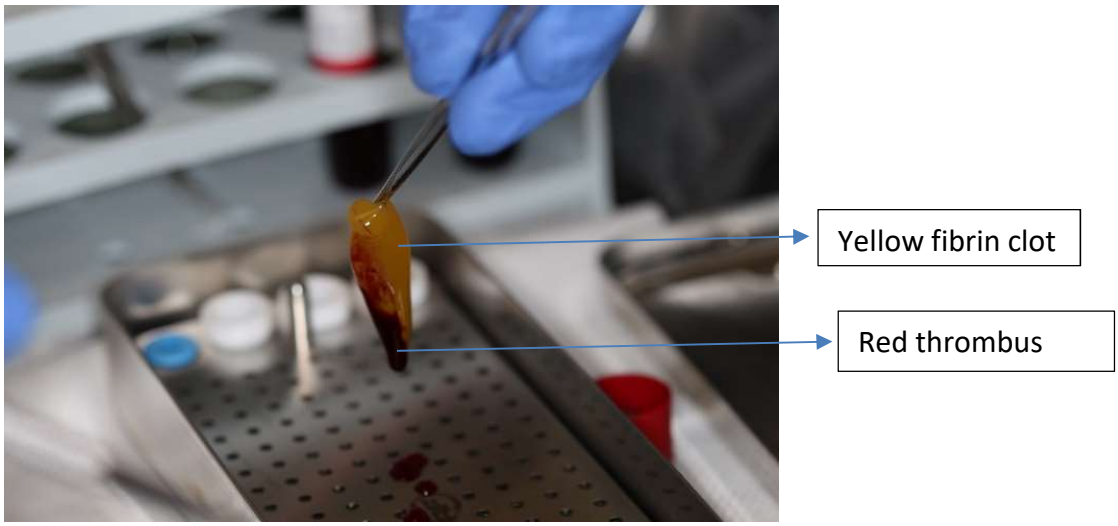

Figure 2.10: The PRF clot.

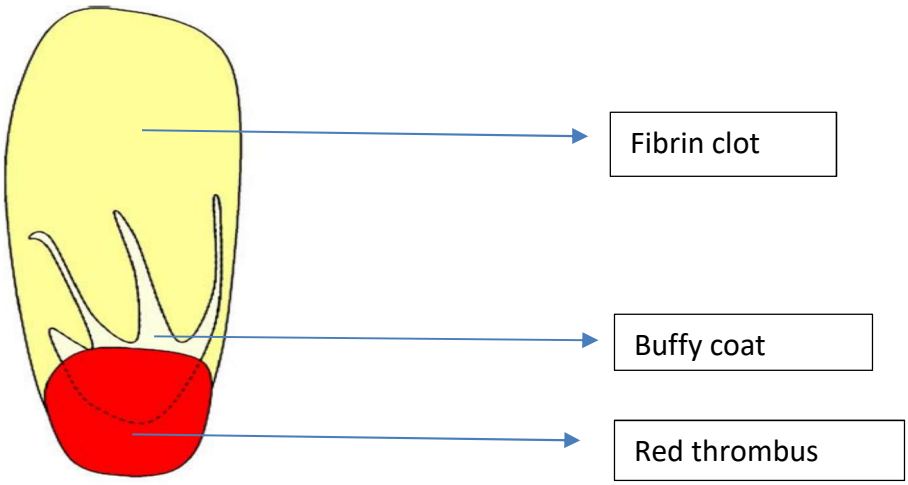

Figure 2.11: Illustration of the PRF clot.[97]

The PRF clot is a 3-dimensional fibrin matrix consisting of platelets, cytokines, growth factors, leukocytes and stem cells.[19, 96]

2.7.2 Benefits of PRF

2.7.2.1 PRF as a Tissue Regenerator

Tissue healing and regeneration requires mutual interactions between a scaffold, platelets, growth factors, leukocytes and stem cells.[18] These essential components are all found within PRF and are able to enhance tissue healing and regeneration. The key element of PRF is its 3-dimensional fibrin matrix.[17] This type of matrix gives PRF its great density, elasticity, flexibility and strength making it well suited for manipulation, handling and suturing.[18] In addition, the fibrin matrix serves as a scaffold providing a unique delivery system that gradually releases the trapped cytokines and growth factors necessary for healing.[87] It allows for the biological signals and cellular interactions necessary for healing and regeneration *i.e.* cell proliferation, differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis.[87] In addition, this fibrin matrix is able to act as an extracellular matrix creating an environment suitable for healing. Furthermore, as a scaffold for cellular interactions the fibrin matrix connects the different components within its structure to the local tissues accelerating neo-angiogenesis. This, in turn, enhances tissue healing and regeneration.[19]

Dohan *et al.* explained this 3-dimensional fibrin structure.[88] During polymerisation, fibrin fibrillae connect in one of two ways: bilateral junctions or equilateral junctions.[88, 90] The

ratio between fibrinogen and thrombin influences the rate of polymerisation. High thrombin concentrations initiate rapid fibrin polymerisation resulting in the fibrin fibrillae arranging themselves with bilateral junctions.[90] This creates a dense rigid fibrin network which is unfavourable to cytokine entrapment and cellular interactions. This type of arrangement occurs in PRP preparations.[90]

PRF, on the other hand, has low thrombin concentrations allowing for a more natural polymerisation rate, creating a higher percentage of equilateral junctions.[90] These junctions create an elastic fibrin network which supports cellular entrapment and migration. This elastic biomaterial is, therefore, a strong, flexible and malleable material that can be manipulated into a suitable delivery vehicle.[88, 90] This quality allows PRF to be manipulated and compressed into a membrane or plug to fit the clinical need and support wound healing.[93]

PRF is rich in cytokines and growth factors. Various growth factors have been identified in the PRF clot: transforming growth factor-beta 1 (TGF-β1), platelet-derived growth factor (PDGF), insulin-like growth factors (IGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF).[11, 15, 92, 97] These growth factors lie within the dense leukocyte fibrin meshwork which delivers the slow release of growth factors and cytokines.[89] These PRF properties promote homeostasis and wound healing supporting the immune response by enhancing cellular migration and proliferation, angiogenesis and regulating post-surgical inflammation.[15, 98]

Dohan *et al.* studied the distribution of platelet cytokines and found that these cytokines remain trapped within the fibrin meshwork and suggest that they may also be in the fibrin polymers.[97] Plasma analysis have shown that the supernatant and erythrocyte layers are void of platelets.[97] It was found that platelets accumulate in the lower part of the fibrin clot known as the buffy coat. It appears as whitish lines which correspond to the accumulation of platelets.[97] The authors then explain this phenomenon. PRF processing involves extracting blood into glass tubes and these residual glass particles induce immediate massive platelet activation. Platelet cytokines are small particles which imply that during centrifugation, they would accumulate in the top part of the tube *i.e*. the supernatant layer. However, plasma analysis showed that the supernatant is void of cytokines. Therefore, they

deduced that the cytokines integrate into the fibrin polymer molecular structure *i.e.* cytokines are trapped within the PRF fibrin matrix.[97]

2.7.2.2 PRF as an Extracellular Matrix

Angiogenesis is a complex biological process involving varied cellular interactions. Migration, proliferation and differentiation of endothelial cells are necessary to develop new blood vessels. In addition, a healthy extracellular matrix (ECM) is critical to support these processes. The ECM is the interstitial matrix between cells and between the basement membrane and its adjacent layers. The basic constituents of the ECM are: collagen, elastin, proteoglycans, hyaluronan, glycoproteins such as fibronectin and laminin and adhesion receptors such as integrins. Certain components of the ECM are involved in wound healing and tissue repair. These components are the proteoglycans which act as a reservoir for growth factors, glycoproteins and integrin receptors which serve to control cell interactions.

In summary, the functions of the ECM are:[99]

- To provide mechanical support for cell anchorage, migration and maintain cell polarity.
- To regulate cellular proliferation and differentiation.
- To provide a scaffold for tissue renewal.
- To create micro tissue compartments and environments.

Choukroun *et al.* claim that the PRF clot can act as an ECM.[17] Its 3-dimensional structure is enmeshed with cytokines and growth factors and provide similar functions as the ECM. Just as the ECM acts as a support structure supplying the necessary growth factors inducing angiogenesis, so too does the PRF clot act as a support structure to angiogenesis promoting cellular interactions.[17] PRF is also a reservoir for the necessary cytokines and growth factors. The authors also explain that fibrin itself is an important angiogenic conductor.[17] Fibrin stimulates expression of the integrins involved in angiogenesis. These integrins bind to the ECM components initiating signalling cascades that influence the cellular processes of angiogenesis.[99] Studies have shown that the fibrin within the PRF clot binds to growth factors such as FGF-2 and PDGF with high affinity inducing angiogenesis.[17]

A PRF clot provides angiogenic support to wounds similar to the way the ECM supports angiogenesis. The PRF clot is enmeshed with trapped cytokines and angiogenic growth

factors. Angiogenesis is the formation of new blood vessels from existing blood vessels and is essential for healing and tissue repair.[99] There are various growth factors involved in angiogenesis. Important growth factors are VEGF, FGF, PDGF and angiopoietins (Ang); Ang1 and Ang2.[17] These growth factors have been identified in PRF preparations.[97, 98, 100]

In vitro studies have shown that the structural and mechanical properties of the fibrin matrix affect angiogenesis.[17] The more rigid the matrix, as in the case of PRP or fibrin glues, the less supportive it is to new capillary formation. PRF, on the other hand, undergoes a slow polymerisation process and the resultant biomaterial is elastic. The elastic nature of PRF is favourable to cell proliferation and migration and therefore favourable to new capillary formation.[17, 97]

2.7.2.3 PRF as an Immune Enhancer

PRF acts as an adjunct to wound healing aiding the immune response. Dohan *et al.* found that there was an increase secretion of these cytokines within the PRF clot.[98] As these cytokines are leukocytic in origin, it can be inferred that the PRF process causes leukocyte activation and degranulation.[98] These cytokines are then entrapped within the fibrin network during PRF processing and are slowly released during healing. The authors conclude that PRF can be considered as an inflammatory regulator and immune response stimulator.[98]

Choukroun *et al.* also explain that fibrin is a natural immune enhancer.[17] Fibrin has positive effects on inflammation. It has been shown that fibrin and fibrinogen degradation products stimulate neutrophil migration, adhesion and transmigration. Fibrin degradation products also promote phagocytosis of neutrophils. Fibronectin and chemotactic substances that are trapped within the fibrin matrix promote macrophage wound colonisation. These actions have been documented by the accelerated wound healing properties of PRF.[17]

In vitro studies have shown that fibroblasts are able to migrate efficiently within a fibrin gel culture model system.[101] The fibrin culture was able to stabilise, reorganise and produce a matrix similar to that of naturally repairing tissue.[101] The PRF fibrin gel matrix behaves similarly by organising into a strong fibrin clot providing a scaffold for epithelial cell proliferation and ultimately wound coverage.[17] The fibrin matrix directs epithelial coverage by altering the metabolic processes of epithelial cells and fibroblasts. In summary, PRF

stimulates angiogenesis, epithelial cell migration and collagen synthesis while providing enhanced immune support. These properties are best demonstrated in a tooth extraction socket where it has been reported that PRF treated sockets heal quicker with reduced pain, dryness or purulent complications than sockets without PRF.[17]

2.7.2.4 PRF as a Source of Circulation Stem Cells

In healthy individuals, haemorrhage leads to haemostasis. The resulting fibrin blood clot traps circulating stem cells. Stem cells are created in bone marrow and are mesenchymal in origin. They have the ability to differentiate into various cell types depending on the signals they receive. Stem cells are an integral part of the healing and repair process.[99] Similar to a natural blood clot, PRF is a naturally derived fibrin matrix which also traps circulating stem cells. It has been demonstrated that the PRF fibrin matrix provides optimal support for stem cells to differentiate.[17] Clinically, PRF has the potential to surpass the natural healing phenomenon. A PRF clot is better organised than a natural blood clot and so able to direct stem cell activities more efficiently, accelerating the healing process.[17]

2.7.2.5 PRF as a Healing Biomaterial

Choukroun *et al.* summarise the benefits of PRF as conducive to all types of superficial cutaneous and mucous membrane healing.[17] With its molecular structure, PRF provides an optimal fibrin matrix for endothelial cell and fibroblast migration, it supports rapid angiogenesis and easily remodels fibrin into a stronger connective tissue.[17]

Dohan *et al.* state that a PRF clot is more supportive to healing than natural fibrin clots.[97] The authors explain that the PRF centrifugation process allows for slow polymerisation of the fibrin and hence greater integration of cytokines into the fibrin network. This integration enables these cytokines to be kept *in situ* until healing is initiated. Once healing is initiated and the fibrin matrix undergoes remodelling, these cytokines are progressively released from the PRF clot. In this way, the lifespan of the entrapped cytokines are increased.[97] These cytokines play a key role in healing as they are anti-infectious and can regulate the immune response.[90] The PRF matrix with its molecular and cellular components provides synergistic effects on healing.[97]

A key factor in healing is inflammation. Inflammation is a protective immune response to any injury. There are three key inflammatory phases: vascular phase, cellular phase and healing phase.[98]

The vascular phase of inflammation is characterised by vasodilation and increased vascular permeability.[99] These vascular changes result in an increase of leukocytic cells at the wound site which secrete inflammatory cytokines and growth promoters. Similarly, a PRF clot contains these cell mediators.[98]

Dohan *et al.* studied the distribution of the five key cell mediators within PRF.[98] The following cytokines were identified: Interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor α (TNF-α), interleukin-4 (IL-4) and VEGF.[98]

IL-1β, IL-6 and TNF-α are pro-inflammatory mediators. These cytokines are produced by leukocytic cells in response to tissue injury and promote expression of selectins and integrins on endothelium, increase avidity of integrins for their ligands and promote chemotaxis.[99] The combination of these effects promotes inflammation, cellular destruction and remodelling.[98] IL-4 is an anti-inflammatory mediator and inhibits the inflammatory signal pathways and counteracts their amplification.[98] VEGF is an angiogenic growth promotor important in the healing phase of inflammation and promotes endothelial cell proliferation, migration and differentiation.[98] VEGF is a potent angiogenic initiator and is involved in the development of initial healing structures.[98] Hence, PRF has the necessary cytokines to support inflammation and healing. These inflammatory regulators may also decrease post-operative pain making the post-operative phase of healing more tolerable to patients.[98, 102]

It can be said that PRF is a "healing" biomaterial.[17, 89, 97] PRF membranes are cost-effective to produce with a short chairside time and can make periodontal surgery affordable. PRF is being used increasingly by clinicians as an adjunctive autologous biomaterial to enhance bone and soft tissue healing and regeneration.[18] The most widely reported PRF technique is Choukroun's PRF with his latest updated protocol being Advanced PRF (A-PRF).[89]

2.8 Platelets and Cytokines

Platelets are normally found in circulating blood and are discoidal, anuclear cell fragments produced by megakaryocytes in bone marrow.[99] Platelets are primarily involved in haemostasis. Activated platelets aggregate at an injured site forming a haemostatic plug. This seals vascular defects and creates a surface to recruit and concentrate coagulation factors. Platelets consist of a contractile cytoskeleton, cytoplasmic granules and have a double phospholipid membrane layer which serves as receptor sites for many molecules.[97, 99]

A platelet's primary function ultimately leads to the coagulation cascade. However, its activation releases cytokines contained within its cytoplasmic granules. These cytokines stimulate cellular migration and proliferation initiating wound healing and tissue repair.[97] Platelets have two types of cytoplasmic granules:

1. α granules which contain platelet specific and non-platelet specific proteins. Platelet specific proteins are β-thromboglobulin and non-platelet specific proteins are fibrinogen, fibronectin, coagulation factors, PDGF, TGF-β, thrombospondin, and various other growth promotors
2. δ granules which contain adenine nucleotides, calcium, serotonin, histamine and epinephrine.[97, 99]

On activation, platelets aggregate at the injury site initiating and supporting haemostasis and undergoes degranulation releasing its cytokines to stimulate cellular interactions within the fibrin matrix and is critical for the initial phase of healing.[97]

In PRF preparation, the centrifugation forces expose platelets to the glass tube wall thereby initiating their activation. In the absence of anticoagulant, there is massive platelet activation and these activated platelets up-regulate tissue factor expression of leukocytes. As the platelets activate, their α-granules burst releasing cytokines and the various growth factors contained within these granules.[89]

2.8.1 Transforming Growth Factor β (TGF-β)

The TGF-β superfamily is a large group of cell regulatory proteins with more than thirty isoforms. The most commonly produced isoform is TGF-β1. It is produced in platelet α-

granules and has pleiotropic functions.[103] TGF-β1 is a potent fibrosis mediator and induces increased synthesis of collagen type 1 and fibronectin in osteoblasts and fibroblasts.[97] It also induces fibrin matrix remodelling in wound repair. TGF-β1 supports angiogenesis by suppressing endothelial cell proliferation and migration and increases extracellular matrix (ECM) protein production.[99] TGF-β1 is intricately involved in the fibrous healing process and considered to be a key regulator of inflammation and wound healing.[97, 103]

2.8.2 Platelet Derived Growth Factor (PDGF)

PDGF regulates cellular migration, proliferation and survival of mesenchymal cell lineages. Their specific receptor systems enable them to be either cell stimulators or inhibitors depending on which receptor is engaged. Hence, PDGF plays a critical role in regulating development during embryogenesis and in physiological tissue remodelling during healing.[97] PDGF also plays a role in angiogenesis by recruiting smooth muscle cells and thereby enabling new blood vessel maturation.[99]

2.8.3 Insulin-like Growth Factors (IGF)

IGF I and II functions as growth factors and hormones of energy metabolism and in this way positively regulates cellular proliferation and differentiation of most cell types.[89, 97] IGF also induces surviving signals which protect cells from matricial apoptotic stimuli and in this way is an important apoptotic regulator.[97]

2.8.4 Vascular Endothelial Growth Factor (VEGF)

VEGF is a family of growth factors that include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PIGF).[99] VEGF-A is an important initiator of angiogenesis following injury and is most commonly referred to as VEGF. VEGF-C and –D initiate angiogenesis and lymphangiogenesis while VEGF-B and PIGF is involved in embryonic angiogenesis.[99]

VEGF has a three-fold mechanism of action. It initiates angiogenesis by directly stimulating proliferation, migration and differentiation of endothelial cells, stimulates vasodilation by producing nitric oxide and aids in development of the vascular lumen.[99] VEGF is also important for endothelial cell survival and its various isoforms allow it to guide the development of the capillary network and its branches.[98]

2.8.5 Fibroblast Growth Factor (FGF)

FGF is a family of 20+ subtypes and its most common isoforms are acidic FGF (FGF-1) and basic FGF (FGF-2).[99] FGF-2 is involved in angiogenesis by stimulating proliferation of endothelial cells. FGF-2 also induces migration of macrophages and fibroblasts to the injury site and stimulates epithelial cell proliferation.[99]

2.8.6 Angiotensin 1 and 2 (Ang1 and Ang2)

Ang1 and Ang2 are involved in angiogenesis. Angiogenesis is a key biological process for normal development, wound healing and organ regeneration.[100] Angiogenesis is involved in the development and structural maturation of new blood vessels.[99]

2.9 The Importance of Cytokines in PRF

Dohan *et al.* undertook a study to determine the distribution of these cytokines within the different parts of the PRF layers *i.e.* the supernatant layer, the PRF exudate (by allowing the PRF solution time to exude out of the clot) and the PRF clot itself.[97] After PRF processing each layer was examined. The authors concluded that the cytokines remained trapped within the fibrin matrix and deduced that they must be intricately enmeshed within the fibrin polymers.[97] The fibrin clot was then stained with alcian blue and this revealed the distribution pattern of the glycoproteins and glycanic chains within the PRF clot. These molecules were trapped within the fibrin mesh following the fibrillary architecture of the mesh. Such an intricate distribution of the cytokines imply an increased lifespan of these cytokines as they would only be released at the time of matrix remodelling aiding cellular proliferation and migration during healing.[17, 97]

In sum, various platelet cytokines are trapped within the PRF fibrin matrix and polymers. These cytokines play vital roles in homeostasis, inflammation, wound healing and tissue repair. By using PRF, there is the potential to use the slow release of these cytokines to amplify and improve post-surgical healing. PRF is thought to be a healing biomaterial.[17] Can these properties enhance the surgical outcomes of periodontal plastic surgery? Hence, I was prompted to test this hypotheses clinically to determine if PRF can improve surgical outcomes in treating gingival recessions.

2.10 Aesthetic evaluation of surgical results

Soft tissue aesthetics is not routinely evaluated after gingival recession surgery.[13, 104] However, with increasing aesthetic demands of patients and clinicians, it is advantageous to evaluate and compare aesthetic results between different surgical techniques.

The proverb, "beauty is in the eye of the beholder", aptly describes the subjective nature of acceptable aesthetics. The idea of what personifies beauty is intrinsically subjective and can be intensely affected by cultural factors.[105] There are limited studies that have systematically evaluated aesthetic outcomes using any type of visual scoring system. Studies simply comment on aesthetic outcomes using general terms such as ''aesthetically pleasing'' and ''good aesthetic results''.[72] In a study by Rosetti *et al.* aesthetic results were evaluated on a good, regular and poor scale.[106] Most sites (80%) were rated as good and 20% of sites were rated regular, no sites were rated poor. The authors state that this scoring system is dependent on a subjective clinical impression.[106] Also, the degree of aesthetic expectations can vary not only between patients and clinicians but between different clinicians as well. Fürhauser *et al.* set out to determine the objectivity and reproducibility of an aesthetic scoring system: the Pink Esthetic Score.[107] Four different types of dental observers (prosthodontists, orthodontists, oral surgeons and dental students) were asked to evaluate aesthetics using photographs. It was found that orthodontists are the most critical assessors of soft tissue aesthetics.[107] Therefore, a more objective means of assessing aesthetics is needed. Kerner *et al*. stated that a before and after scoring system is a reliable and adequate way of evaluating aesthetic outcomes.[108]

There are two scoring systems that may be used to assess gingival aesthetics. 1) The root coverage esthetic score (RES) and 2) The pink esthetic score (PES).

2.10.1 The RES System

The RES system was proposed to evaluate aesthetic outcomes of root coverage procedures. This scoring system uses five variables to evaluate gingival aesthetics: gingival margin, marginal tissue contour, soft tissue texture, MGJ alignment and gingival colour.[104] Points are allocated as follows: with regards to gingival margin, 6 points for complete root coverage, 3 points for partial root coverage and 0 points for no root coverage. The remaining variables

are scored in the following way: 1 point for a normal appearance of the tissue and 0 points when the tissue deviates from this. The highest possible score is 10 points. See table below.

Table 2.1: Root Esthetic Score by Cairo *et al.*[104]

Variables	Description
Gingival margin	**0 points**: root coverage failed *i.e.* the gingival margin is apical or equal to the baseline level and/or if there was complete or partial loss of the interproximal papilla. **3 points:** partial root coverage. **6 points:** complete root coverage with the gingival margin at or slightly coronal to the CEJ and the site has a physiological sulcus depth.
Marginal Tissue Contour	**0 points:** irregular gingival margin that does not follow the CEJ. **1 point:** the gingival margin follows the CEJ contours.
Soft Tissue Texture	**0 points:** scars or keloids impair the gingival appearance. **1 point:** normal gingival texture.
Mucogingival junction alignment	**0 points:** MGJ of the tooth in question does not line up with the MGJ of adjacent teeth. The new MGJ is either coronal or apical to the adjacent MGJ. **1 point:** the MGJ lines up with the adjacent MGJ.
Gingival Colour	**0 points:** the grafted tissue is a different colour from the adjacent tissue. **1 point:** the grafted tissue matches the adjacent tissue in colour and is well integrated.

2.10.2 The PES System

The PES system is an alternative scoring system to evaluate gingival aesthetics. This scoring system was created to evaluate the soft tissue around single-tooth implant crowns.[107] Since successful aesthetic outcomes (whether on an implant or after root coverage procedures) is determined by how close to natural a tooth looks, its application may be extended to evaluate aesthetic outcomes of root coverage procedures. This system uses a seven variable system to evaluate aesthetics: mesial papilla, distal papilla, level of soft tissue margin, soft tissue contour, alveolar process, soft tissue colour, soft tissue texture.[107] The scoring system is a 2-1-0 score with 2 being the best and 0 being the worst. Variables are assessed in comparison to a reference tooth, which can be the corresponding tooth in another quadrant or the adjacent tooth. The highest possible score is 14 points. See Table below.

Table 2.2: Pink Esthetic Score by Fürhauser *et al.*[107]

Variables	Details	0	1	2
Mesial papilla	Shape vs reference tooth	Absent	Incomplete	Complete
Distal papilla	Shape vs reference tooth	Absent	Incomplete	Complete
Marginal Tissue Level	Level vs reference tooth	Major discrepancy >2mm	Minor discrepancy 1-2mm	No discrepancy <1mm
Soft Tissue Contour	Natural matching reference tooth	Unnatural	Fairly natural	Natural
Alveolar Process	Alveolar process deficiency	Obvious	Slight	None
Soft Tissue Colour	Colour vs reference tooth	Obvious difference	Moderate difference	No difference
Soft Tissue Texture	Texture vs reference tooth	Obvious difference	Moderate difference	No difference

On photographs, it can be challenging to determine the MGJ because the border between the keratinised tissue and the alveolar mucosa is often unclear. Cairo *et al.* tested the predictability of the RES with photographs and found that the assessment of the MGJ position varied amongst examiners.[109] The authors attributed this discrepancy to the difficulty in determining the MGJ on photographs.[109] Hence, to avoid this bias, this study used the PES to evaluate aesthetic changes.

2.11 Modified Tunnel Flap

CAF's provide the most predictable and improved aesthetic outcomes in correcting gingival recessions.[3] This technique was first described by Langer and Langer.[71] Their technique produced predictable and acceptable outcomes. This technique uses vertical relieving incisions to mobilise the flap and these incisions can result in scarring compromising aesthetics. In a study by Zucchelli *et al.* where CAFs with and without vertical incisions were compared, the authors found that keloid formation due to the vertical incisions resulted in the poorest aesthetic outcomes.[110] In 1985, Raetzke introduced the envelope flap eliminating the vertical incision.[74] This technique was simple and improved aesthetic results.

However, the envelope flap is used to treat single recession lesions. In 1994, Allen modified this flap design by connecting multiple adjacent envelope flaps to create a tunnel flap.[111] This flap design allows for multiple adjacent recession lesions to be treated at once. Then, in

1999, Zabalegui described connecting multiple envelope flaps while keeping the interdental papilla intact creating the modified tunnel flap.[112] This type of flap is minimally invasive and prevents potential scarring improving the aesthetic result. In addition, Zuhr *et al.* state that the blood supply to the graft tissue is the key element to the success of this procedure.[113] A modified tunnel flap avoids vertical and horizontal incisions preserving continuity of the collateral blood supply to the graft allowing for improved healing.[114] Zucchelli *et al.* in their study to compare outcomes between CAF's with and without vertical relieving incisions, found that although both techniques were effective in improving recessions, the envelope type CAF increases the probability of complete root coverage with an improved result.[110]

In recent years there has been a shift towards microsurgical dentistry. This concept uses microsurgical instruments, materials and visual aids to allow more delicate and precise access to the surgical site potentially improving clinical and aesthetic outcomes.[113] Tunnel flaps which are created with microsurgical blades and specially developed tunnelling instruments allow for precise intrasulcular incisions which can help minimize soft tissue ruptures that would compromise aesthetic results.[113] Burkhardt and Lang compared microsurgical and macrosurgical techniques in treating recessions and found that the microsurgical approach improved vascularisation of the grafts minimizing the risk of graft necrosis thereby improving wound healing.[115]

In this study a modified tunnel flap was created with microsurgical instruments in order to minimise postoperative scarring to allow for the best possible clinical and aesthetic outcomes.

CHAPTER 3

3 AIMS AND OBJECTIVES

3.1 Aim

To evaluate the clinical efficacy of PRF membranes and CTGs in treating gingival recession.

3.2 Hypothesis

PRF membranes can be as effective as CTGs in gingival recession treatment with improved aesthetic results.

3.3 Objectives

1 To determine the clinical efficacy of PRF membranes in treating gingival recession.

2 To evaluate clinical and aesthetic outcomes between the control and test within each patient.

3 To determine patient satisfaction with regards to aesthetic outcomes between the control and test groups.

CHAPTER 4

4 METHODS AND MATERIALS

4.1 Study design

This project is an extended case series designed as a randomised split-mouth study to evaluate the clinical efficacy of PRF membranes and CTGs in treating gingival recessions.

4.2 Study population

Patients over the age of 18 years who presented at the Wits Oral Health Centre and fulfilled the selection criteria.

4.3 Sampling

All patients that fulfilled the selection criteria were invited to participate in this study.

4.3.1 Inclusion criteria

- Patients who presented with at least two Miller's class I and/or class II gingival recession lesions that were bilateral or contralateral to each other but not excluding recession lesions that were in the same quadrant and a reasonable distance apart. {*The interdental papilla level is the most significant prognostic factor for root coverage.*[46] *In lesions with interdental papilla loss, the aesthetic outcome is compromised as only partial root coverage is predicted. Therefore, in order to effectively test clinical outcomes between the groups, only Miller's class I and II lesions (see page 10) were included in the study.}*
- Patients who were at least 18 years old.
- Patients who were willing to give written informed consent.
- Patients who were in good systemic health.
- Patients with good periodontal health.
- Non-smokers.
- Patients who were motivated and able to maintain good oral hygiene.

4.3.2 Exclusion criteria

- Those patients who presented with Miller's class III and class IV (see page 11) gingival recessions.
- Pregnancy.
- Patients with bleeding disorders.
- Recession sites on posterior teeth with furcation involvement.
- Patients who have had previous periodontal surgery in an attempt to correct gingival recessions in the areas of interest.

4.4 Patient recruitment and enrolment

Patients were sourced from the Wits Oral Health Centre. Patients with gingival recession lesions were identified and screened to determine if they qualified for the study as per the inclusion and exclusion criteria. Once identified, the study was explained to patients with possible alternative treatment options and if interested in this surgical treatment, they were enrolled into the study. Informed consent was obtained with signed consent forms (appendix 1). A case report file was created for each patient where all relevant information was kept and recorded with anonymity. Patient names were not recorded on the case report file and a reference code was assigned to each file to ensure anonymity. This case report form had a detailed breakdown of each appointment (appendix 2). A clinical checklist was used to ensure all necessary procedures were carried out at the relevant appointments (appendix 3).

A comprehensive clinical examination was carried out on each patient. Aetiological factors were identified and addressed accordingly. A scale and polish was done and patients were given oral hygiene instructions and motivation.

The plaque control in these patients were ≤20% [116] and well controlled. These patients were instructed to use a soft bristle toothbrush with the modified Bass brushing technique.[48] The toothbrush is placed at 45° angle to the long axis of the tooth in an apical direction partly covering the gingiva and the cervical area of the tooth. The toothbrush is moved with short back and forth strokes rolling over the gingiva in an occlusal direction with light pressure.[48]

Photographs and alginate impressions were taken. The laboratory work was done at the Wits Dental Laboratory. Acrylic palatal stents and measuring guides (see figures 4.1 and 4.2) were made. The palatal stent was used to protect the palatal donor site post-surgery and the measuring guides were used to ensure reproducible clinical measurements. Each recession lesion was identified clinically. Some patients had lesions in all four quadrants and these were paired and each recession recorded as a separate site.

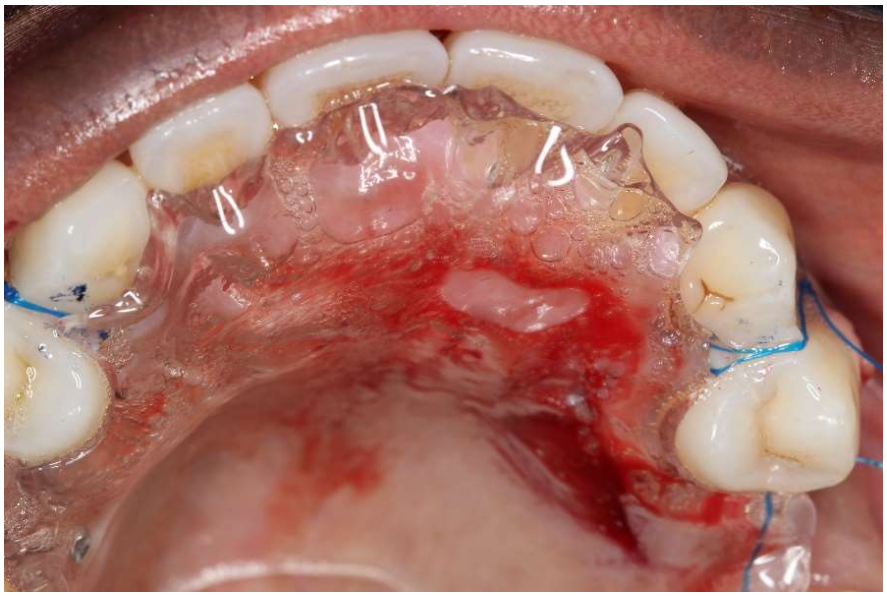

Figure 4.1: Acrylic palatal stent in place to protect palatal surgical wound.

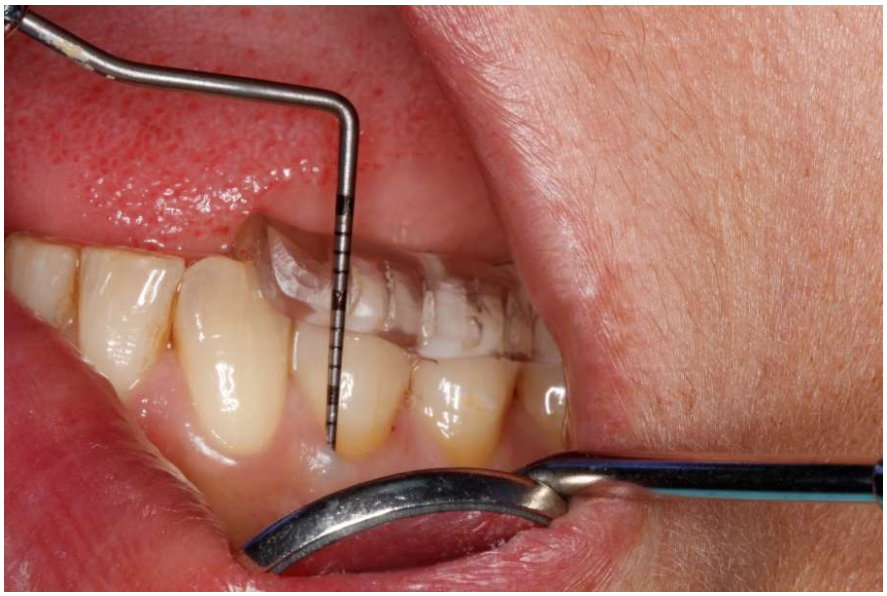

Figure 4.2: Acrylic measuring guide with probe.

4.5 Randomisation

Once patients agreed to take part in this study, their anonymity was ensured by giving each patient an alphabetical reference *i.e.* A, B, C, D, E or F. Each paired site was given a numerical value from 1 to 11 (e.g. A1, B2, C3 etc. see appendix 5) and randomly allocated to the control or test site using the RAND function in Microsoft Excel 2016, see figure 4.3.

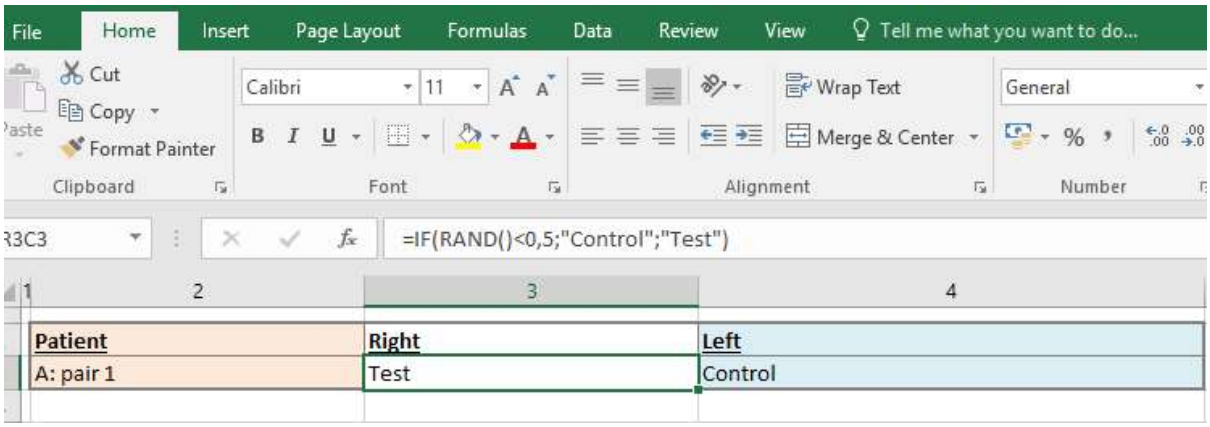

Figure 4.3: Example of randomisation function using Microsoft Excel 2016.

4.6 Clinical measurements

The clinical measurements were recorded using a University of North Carolina probe (UNC 15). This probe is 15mm long with shaded markings at every mm and wider shaded bands at 5, 10 and 15mm.

Figure 4.4: University of North Carolina probe (UNC 15).

The probe was placed within narrow grooves created on the buccal aspect of the measuring guide (see figure 4.2).

The following parameters were measured and recorded to nearest millimetre (mm):

4.6.1 Periodontal probing depths (PD)

PD were measured at three points: mesiobuccal (MB), mid-buccal (B) and distobuccal (DB). Periodontal probing depth is the distance measured from the gingival margin to the base of the gingival sulcus.[117]

4.6.2 Recession depth (RD)

Recession depths were measured at three points: MB, B, and DB. Recession depth is the distance from the CEJ to the gingival margin.[117] Identifying the CEJ can be challenging. The CEJ can be lost due to tooth brushing habits that erode enamel or a restoration may mask the junction. In scenarios where the CEJ was lost, the crown lengths of adjacent or contralateral teeth were used as a guide to determine the position of the CEJ.

4.6.3 The clinical attachment level (CAL)

CALs are indicators of periodontal tissue destruction and can also be used to monitor disease progression and tissue response to periodontal therapy.[118] CAL is measured from the base of the sulcus to the CEJ (which is a fixed point and does not change throughout life). The aim of periodontal therapy is to increase periodontal attachment and this can be determined by a 'gain' or 'loss' in CALs.

In this study, the CAL was recorded as clinical attachment loss. CALs are calculated by adding the probing depth to the recession depth values.[47] The CAL values were determined at three points: MB, B, and DB.

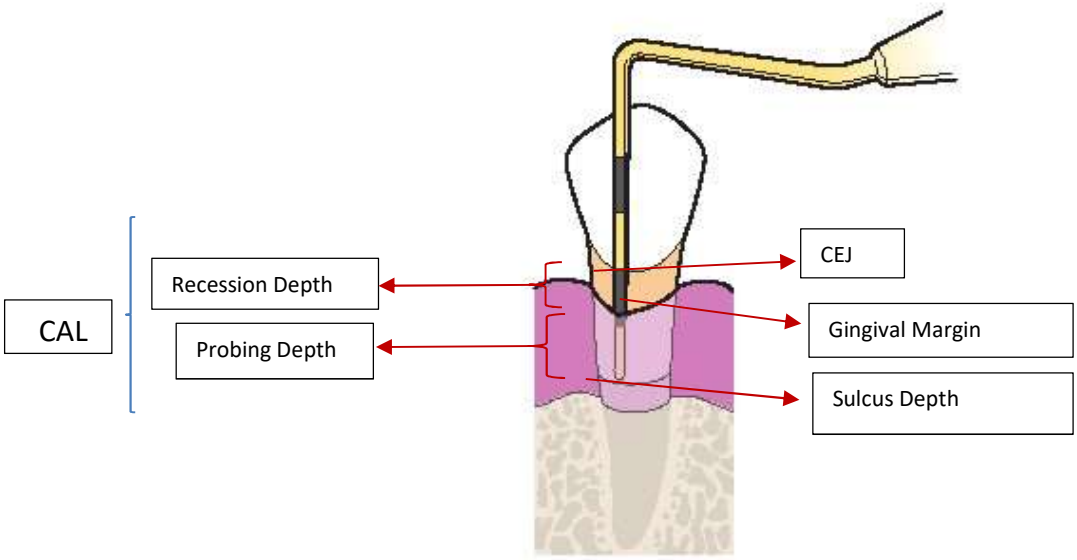

Figure 4.5: Diagrammatic representation of CAL[117]

4.6.4 Recession width (RW)

RW was measured at the widest horizontal distance from the mesial to distal margins of the recession defect.[6]

4.6.5 Keratinised tissue width (KTW).

The MGJ was determined with a combination of the visual and rolling method.[35, 119] The visual method involves identifying the colour change between the alveolar mucosa and the keratinised gingiva. The keratinised gingiva is a pale pink coral colour. In contrast, alveolar mucosa is darker, shiny and smoother than keratinised gingiva. In the rolling method, the side aspect of the UNC-15 probe was used to gently "roll" the gingiva horizontally from the vestibule towards the gingival margin. This action causes the loose alveolar mucosa to roll and the mucogingival line is then identified at the point where this movement stops creating a crease and the attached gingiva is immoveable.[35] The KTW was measured along the vertical mid-buccal groove of the measuring guide.

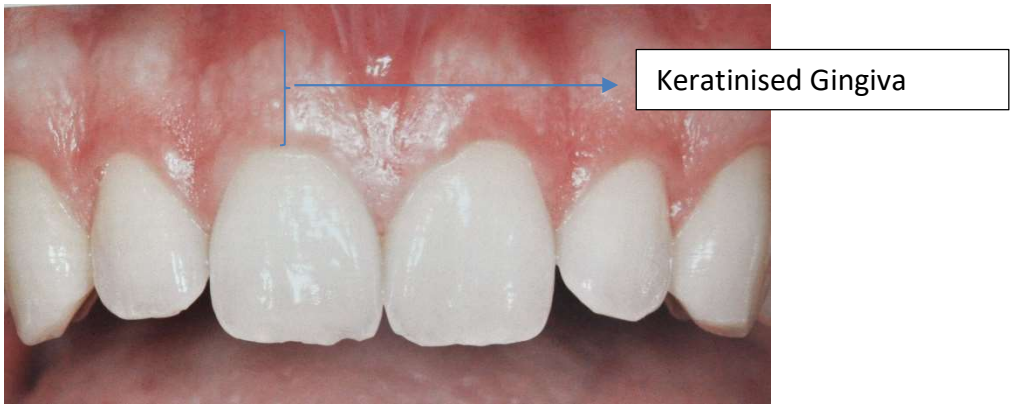

Figure 4.6: Keratinised Tissue Width

4.6.6 Gingival thickness (GT)

GT was recorded using rubber stoppers at the end of endodontic reamers.[11, 15]

Figure 4.7: Endodontic reamer with rubber stopper

Under local anaesthesia, the reamer was pushed into the attached gingiva at the point corresponding to the vertical line of the mid-buccal groove of the measuring guide and the mid-point of the apico-coronal width of keratinised gingiva. Tactile sensation was used to determine the point at which the reamer made contact with bone. The rubber stopper demarcated the distance from the bone to the gingival surface and this distance was measured.

4.6.7 Summary of variables

Table 4.1: Clinical parameters

Clinical Parameters	Mesio-buccal (MB)	Buccal (B)	Disto-buccal (DB)
PD (periodontal probing depth)*	MB	B	DB
RD (recession depth)*	MB	B	DB
CAL (clinical attachment level)*	MB	B	DB
RW (recession width)**	RW was measured using a UNC 15 probe at the widest horizontal distance from the mesial to the distal margins of the recession defect		
KTW (keratinised tissue width)**	KTW was measured using a UNC 15 probe along the vertical mid-buccal groove of the measuring guide.		
GT (gingival thickness)**	GT was recorded using rubber stoppers at the end of endodontic reamers at the point corresponding to the vertical line of the mid-buccal groove of the measuring guide and the mid-point of the width of keratinised gingiva.		

** Variables measured at three points*
*** Variables measured at one point*

All clinical measurements were independent variables and were measured in millimetres (mm). All measurements except GT were measured to the nearest millimetre and recorded at baseline pre-operatively, 8 weeks, 12 weeks, 16 weeks and 24 weeks. GT measurements were recorded to the nearest 0,5mm at baseline and at 24 weeks.

Prior to surgery, a statistician randomly assigned a recession site to the control or test treatment. The site allocation envelope was opened at the surgical appointment. This randomisation removed investigator bias.

4.7 Evaluating and comparing aesthetics

Photographs were taken prior to surgery and six months later at the final recall appointment. These were printed for evaluation. As discussed in chapter 2 (pages 39-41), there are two scoring systems to evaluate gingival aesthetics: the RES and PES. The RES system was created specifically to evaluate overall aesthetic outcomes after root coverage procedures. However, this scoring system uses the MGJ as one of its variables and it can be challenging to determine the position of the MGJ on photographs. Poor image contrast, lack of MGJ visibility or colour contrast increases the difficulty in clearly identifying the MGJ.[109] Cairo *et al.* tested the predictability of the RES with photographs and found that although the RES system is useful for gingival aesthetic evaluation, it posed a limitation when using

photographs as opposed to direct clinical assessment.[109] The authors found that assessment of the MGJ position varied amongst assessors and attributed this to the difficulty in determining the MGJ on photographs.[109] On the other hand, the PES system does not use the MGJ as a variable and was therefore used to evaluate aesthetic outcomes in this study.

Table 4.2: Variables of the Pink Esthetic Score[107]

Variables	Details	0	1	2
Mesial papilla	Shape vs reference tooth	Absent	Incomplete	Complete
Distal papilla	Shape vs reference tooth	Absent	Incomplete	Complete
Marginal Tissue Level	Level vs reference tooth	Major discrepancy >2mm	Minor discrepancy 1-2mm	No discrepancy <1mm
Soft Tissue Contour	Natural matching reference tooth	Unnatural	Fairly natural	Natural
Alveolar Process	Alveolar process deficiency	Obvious	Slight	None
Soft Tissue Colour	Colour vs reference tooth	Obvious difference	Moderate difference	No difference
Soft Tissue Texture	Texture vs reference tooth	Obvious difference	Moderate difference	No difference

4.8 Patient questionnaire

A patient questionnaire was used to determine patient satisfaction or dissatisfaction with the results (see appendix 4). A Likert type scale was used for analysis. Likert Scales are routinely used to measure attitudes with a range of responses to a particular question, usually a five or seven point pre-coded scale.[120, 121]

This study's questionnaire used a five point scale to evaluate the following variables: level of gingival recession, dentine sensitivity, gingival colour and gingival contour. See table 4.3 below for the five point scale.

Table 4.3: Likert 5 point scale for patient questionnaire

Much worse than before	Slightly worse than before	No change	Slight improvement	Noticeable improvement
1	2	3	4	5

4.9 Data analysis

Descriptive statistics was presented for each variable in each of the six patients at baseline and at 24 weeks. Each patient was a unit of analysis. The outcomes for each patient was summarised and analysed independently. Outcomes between the baseline and 24 week measurements were compared in each patient. In patients with multiple paired sites (patient E and F), the mean of the each variable was used for statistical analysis and t-tests were used to compare baseline and 24 week values. In addition, the percentage root coverage, as well as root coverage (yes/no) was derived.

The success of recession treatment is considered to be any decrease in the amount of exposed root surface.[70] The primary variables that were analysed to determine this change was RD and RW.

Data analysis was carried out with Microsoft Excel 2016 statistical analysis software. A 5% significance level was used. The skewness and kurtosis of the distribution was considered while determining if the null hypothesis can be accepted. Skewness explains whether the distribution conforms to a typical bell curve, or is asymmetrical. Most of the data typically appears to be on one side of the hypothesised mean. Kurtosis describes how often samples may appear at either ends (the tails) of the curve.

The patient questionnaire was based on a Likert scale.[120] A Likert scale assumes that the power of experience is linear *i.e.* a patients satisfaction or dissatisfaction can be measured.[121] It cannot be presumed that the intervals are equal.[120] The data was summarised and analysed for each patient. As the data was ordinal, it was summarised as a mode in patients with multiple sites.

4.10 Surgical procedure

Both control and test treatments were done at the same time by the same operator. This was part of the research protocol so as to remove bias that may arise should different patients or different sites in the same patient be treated by different surgeons. Both recipient sites were prepared in the same way. As per discussion in chapter 2 (pages 41-42), a modified tunnel flap as described by Zuhr *et al.* was used.[113]

Once adequate local anaesthesia was achieved, intrasulcular incisions were made around the necks of the teeth using 15c Swann-Morton® surgical blades and Keydent® microsurgical discoid blades. The incision was extended to the adjacent teeth on both sides. The flap was mobilised by extending it into the mucosal tissues. Hurzeler tunnelling instruments were used, minimizing perforations into the marginal tissues.[113] The interdental papillae remained intact and connected. The papillae were detached from the periosteum and the buccal gingiva was completely mobilised allowing for tension free coronal displacement of the flap.

Figure 4.8: 15c Swann-Morton® blade

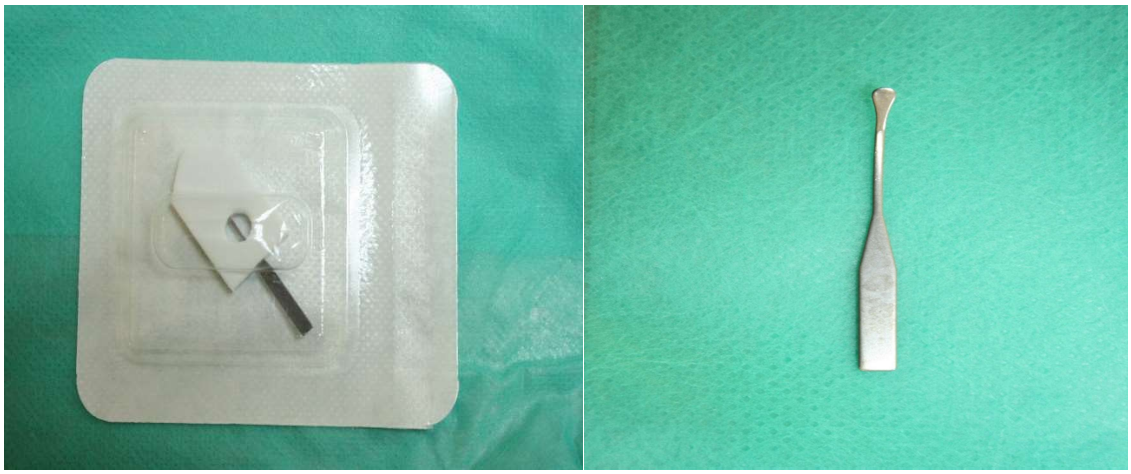

Figure 4.9: Keydent® microsurgical blade

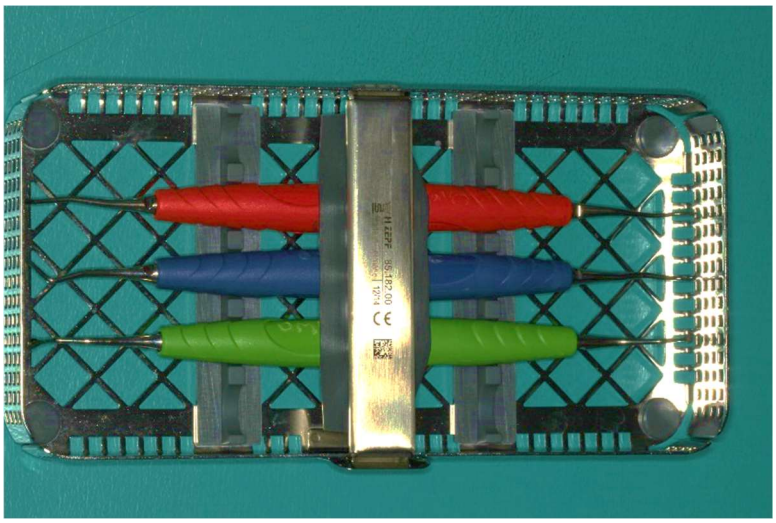

Figure 4.10a: Hurzeler tunnelling instruments

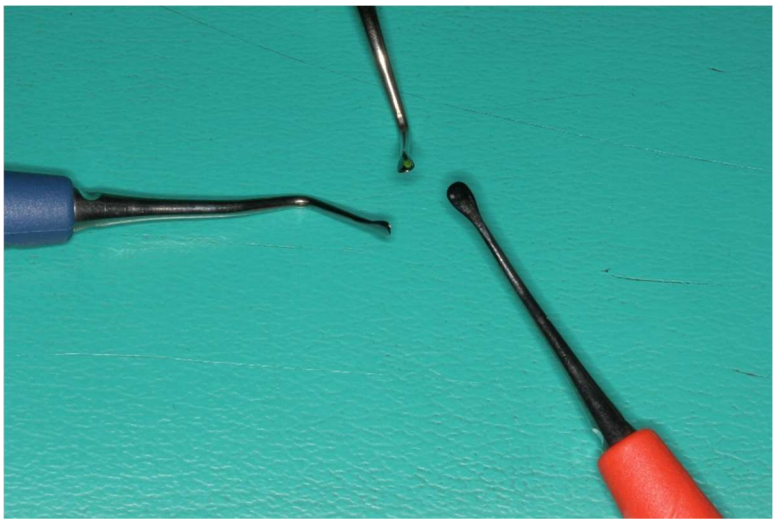

Figure 4.10b: Hurzeler tunnelling instruments

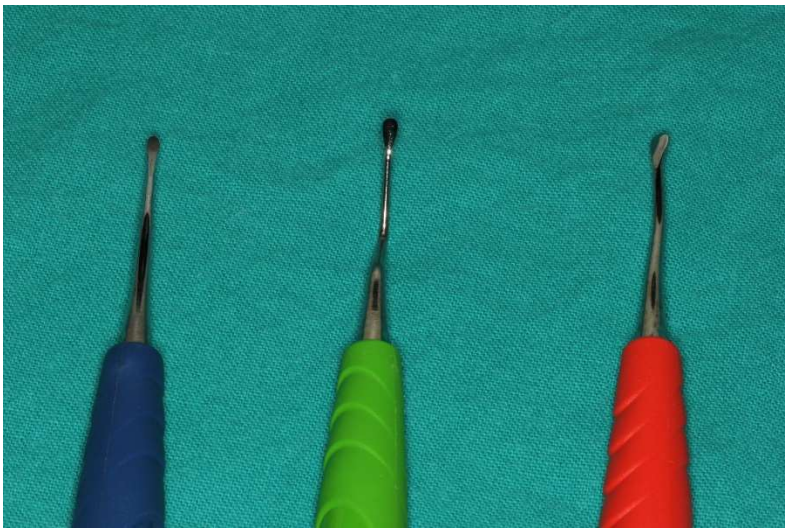

Figure 4.10c: Hurzeler tunnelling instruments

For the control, the CTG was obtained from the palate. The trap-door technique was used. This involved making an 'L' shaped incision adjacent from the distal aspect of the canine up to the mesial root of the first molar depending on the length of tissue needed. The length of tissue was determined by the mesio-distal extent of the recession defect at the recipient site. A split thickness flap was raised and the subepithelial connective tissue was carefully dissected. This type of access flap allowed the donor site to be completely covered after the graft tissue was removed. An acrylic stent was placed *in lieu* of sutures (see figure 4.1). The stent exerted pressure onto the donor site aiding haemostasis and pain management. Patients were instructed to keep the acrylic plate in place continuously for five days. They were given a 0.2% chlorhexidine mouthwash to aid plaque control instead of conventional oral hygiene measures which would be difficult to carry out while wearing the acrylic plate.

The CTG was trimmed as necessary and transferred to the recipient site and carefully placed and threaded into the gingival tunnel created. The flap was secured with Seralon® monofilament 4/0 DS 18 sutures using the double-crossed suture technique described by Zuhr and Hürzeler and Zuhr *et al*.[122, 123] Prior to surgery, composites rests were created on the interproximal contact points. The enamel surface was etched for 10 seconds and bonded. 3M flowable composite was used and a probe was placed in a bucco-palatal direction on the composite surface while light curing. This created a groove which provided a fulcrum to help advance the flap coronally and support the graft.

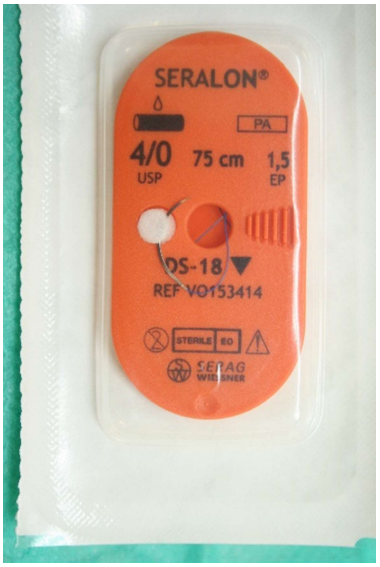

Figure 4.11: Seralon® suture

The needle was guided through the buccal soft tissue about 5mm apical to the tip of the papilla. The needle was pushed through to emerge at the base of the palatal papilla (figure 4.12a). The needle was then looped around the composite rest and slid underneath the contact point to reappear at the palatal side (figure 4.12b). The procedure was repeated starting from the palatal side. The needle was guided from the palatal side to emerge at the base of the buccal papilla and then looped around the composite rest and slid underneath the contact point to re-emerge on the buccal side (figure 4.12c). The suture was closed and knotted on the buccal side (figures 4.12d and 4.12e).[122]

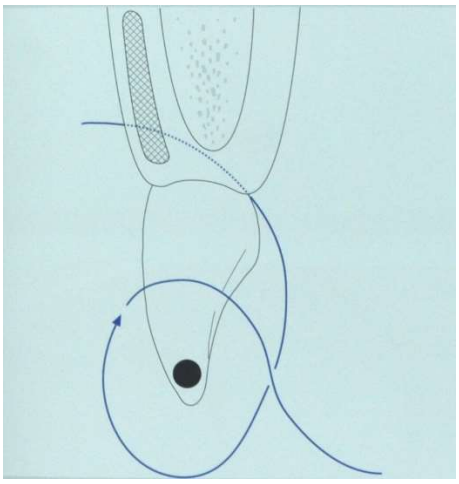

Figure 4.12a[122]

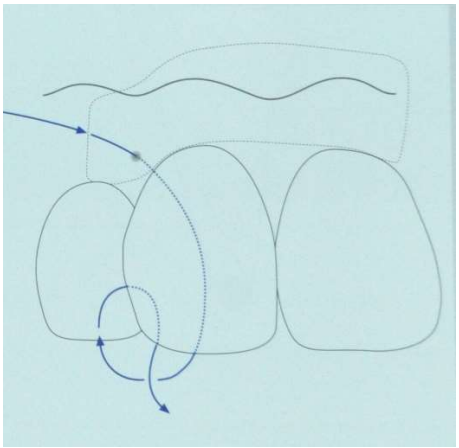

Figure 4.12b[122]

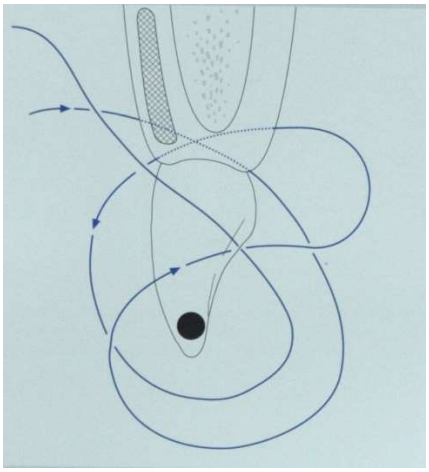

Figure 4.12c[122]

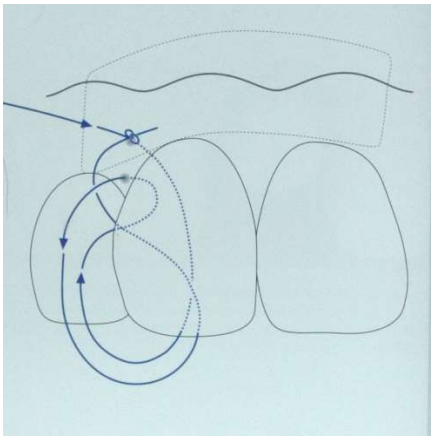

Figure 4.12d[122]

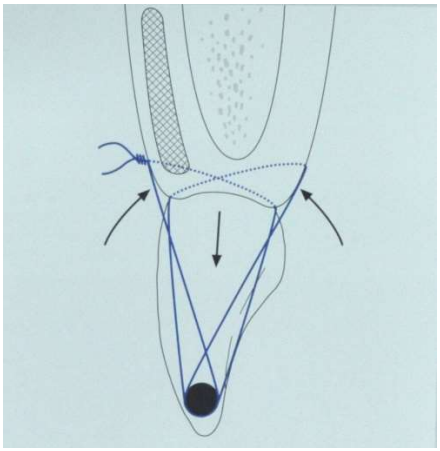

Figure 4.12e[122]

This suture technique offers the following advantages:

- It creates a coronal anchor point allowing for maximum coronal flap mobilisation and stabilisation while exerting gentle pressure.[123]
- Crossing the suture over ensures wound stabilisation and improved flap and graft adaptation. This close tissue and flap adaptation increases revascularisation of the

graft tissue improving healing. It also encourages a thin blood clot to form which improves tensile strength and stability of the wound.[123]

- Since the suture passes through the papilla twice, the knot tension is distributed more equally sparing the fragile buccal soft tissue.[123]

The test site received the PRF membrane. Choukroun's A-PRF preparation protocol was used. Just prior to surgery, intravenous blood was collected from the antecubital fossa in 10 ml A-PRF+ blood vials and immediately centrifuged at 1500 rpm for eight minutes. At least two vials (always an even number) were filled equally for each patient. The vials were placed directly across from each other to ensure balance in the centrifuge.

Figure 4.13: PC-O2 centrifuge used to create A-PRF (Process, Nice, France)

The resultant fibrin clot was removed and compressed into a membrane using a PRF metal fabrication box.[124] The compressed membrane was placed at the recipient site and sutured in the same way as the control site.

Figure 4.14: Separated blood components immediately after centrifugation

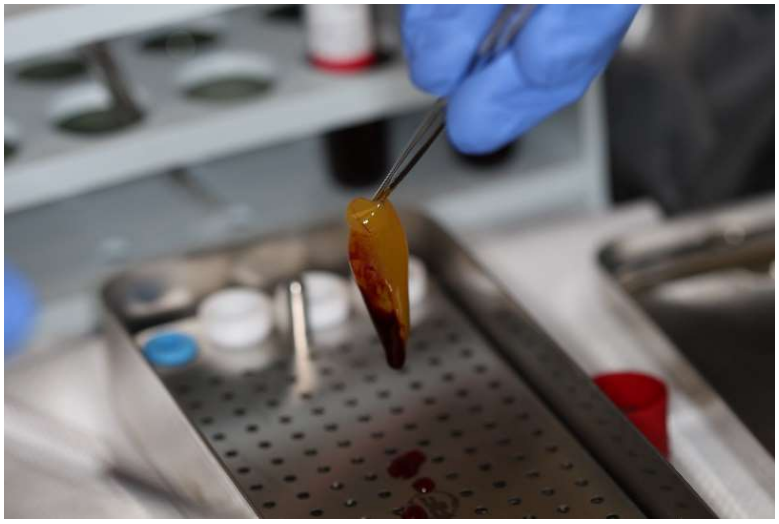

Figure 4.15: PRF clot removed from glass vial

Figure 4.16: PRF clot placed in PRF membrane fabrication box

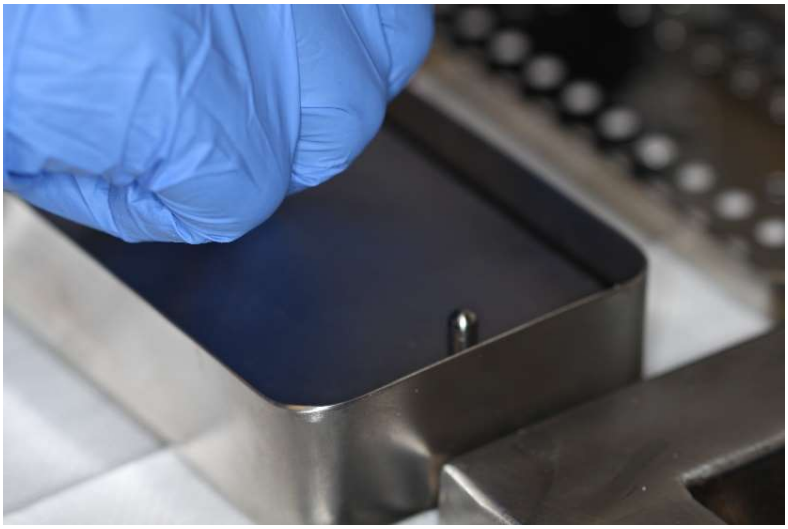

Figure 4.17: Compression plate placed on PRF clot

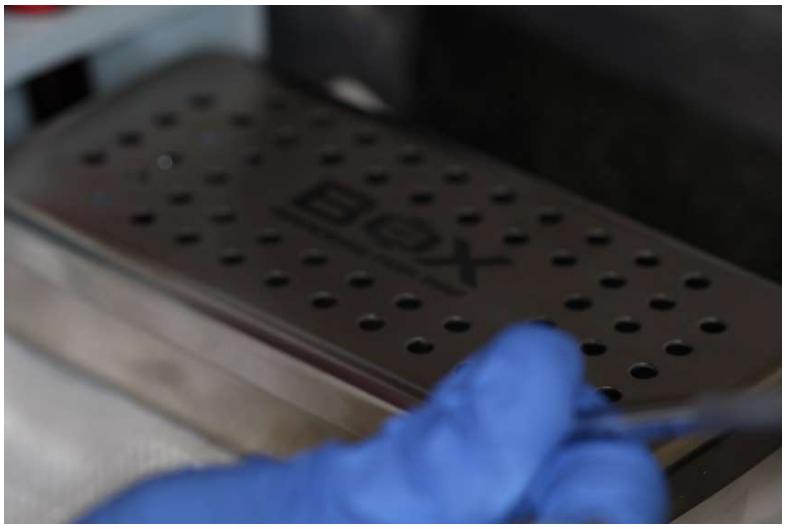

Figure 4.18: PRF box was closed and left until the membrane was needed

Figure 4.19: The PRF clot was compressed into a membrane

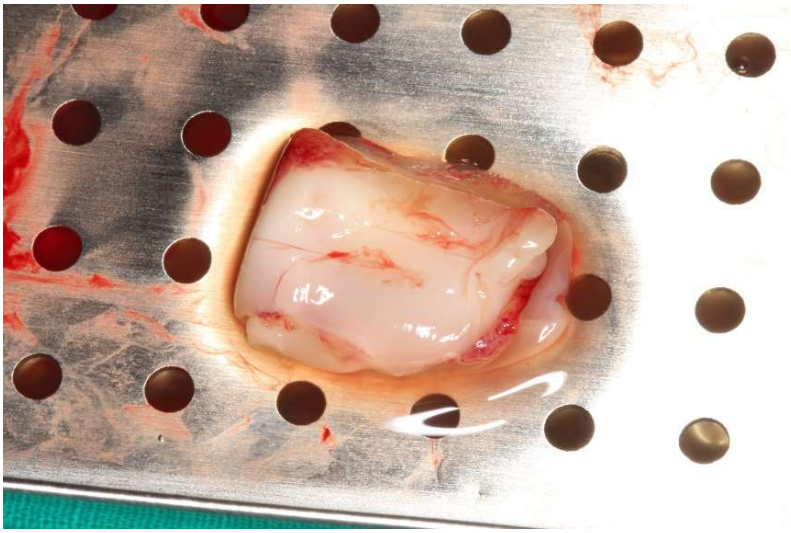

Figure 4.20: PRF membrane

4.11 Post-surgical protocol

Patients were prescribed analgesics: paracetamol 500mg and ibuprofen 200mg, and a 0.2% chlorhexidine gluconate mouthwash. The ibuprofen was prescribed for three days postoperatively and the paracetamol prescribed for five days.

Patients were instructed to avoid brushing the surgical site for two weeks, the chlorhexidine provided the necessary plaque control measures.

At the two week recall appointment, sutures were removed, healing was assessed and plaque control measures were carried out. Oral hygiene instructions were reinforced and a modified brushing technique was explained to patients.

Recall appointments were set up for 8, 12, 16 and 24 weeks postoperatively. At the 8, 12 and 16 week appointments, all clinical parameters except for GT were recorded. GT was not measured at these appointments as I did not want to interrupt new gingival attachment by probing into the gingiva with a sharp endodontic reamer. Plaque control measures were carried out. An added advantage of a strict postoperative follow-up schedule such as this encourages long-term patient compliance and maintenance.[3]

At the 24 week recall appointment, all clinical variables were measured, photographs were taken and patients completed a written questionnaire.

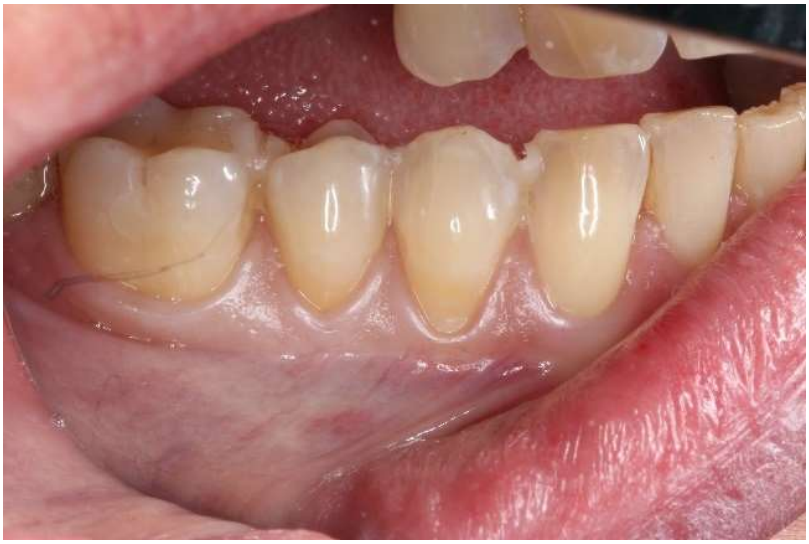

Figure 4.21: Pre-operative photograph of the E6 and E7 control site (tooth 45 and 44 respectively)

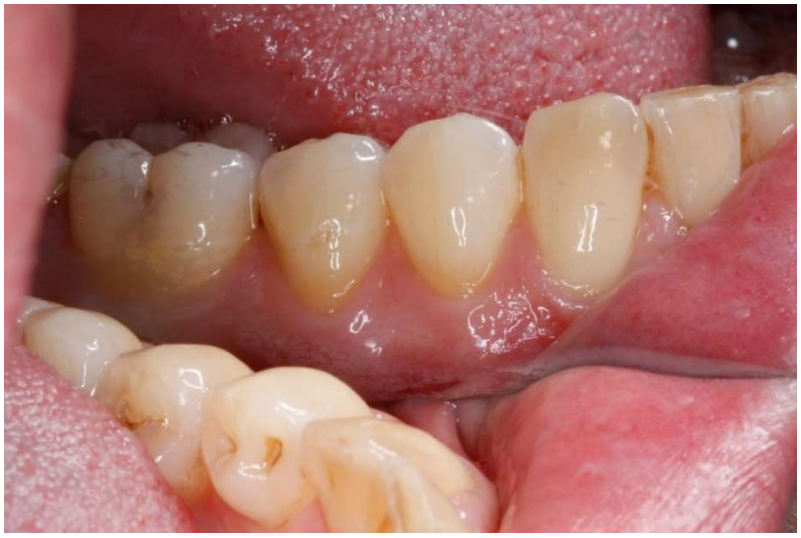

Figure 4.22: Postoperative photograph of the E6 and E7 control site (tooth 45 and 44 respectively)

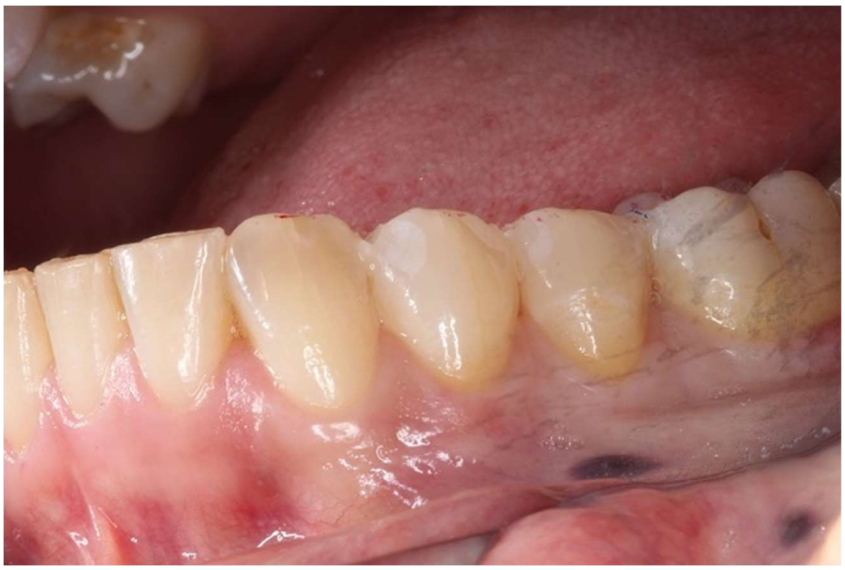

Figure 4.23: Pre-operative photograph of the E6 and E7 test site (tooth 35 and 34 respectively)

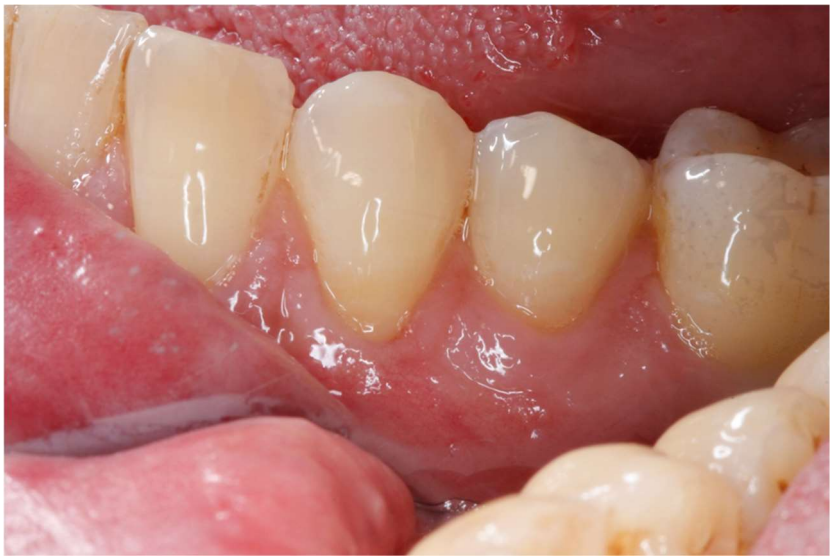

Figure 4.24: Postoperative photograph of the E6 and E7 test site (tooth 35 and 34 respectively)

4.12 Ethical considerations

Ethical clearance was applied to and approved by the Human Research Ethics Committee (HREC). Certificate no M150506 (appendix 6). As per the requirements of the HREC, investigators completed Investigator Training Programs for Good Clinical Practise (appendix 7).

CHAPTER 5

5 RESULTS

Table 5.1: Patient demographic information.

Patient Demographics					
Patient	Pair	Age	Gender	Tooth	Control (C) or Test (T)
A	A1	69	M	13 23	C T
B	B2	66	M	23 13	C T
C	C3	29	F	14 24	C T
D	D4	40	F	35 45	C T
E	E5	41	F	14 24	C T
E	E6	41	F	45 35	C T
E	E7	41	F	44 34	C T
F	F8	47	M	15 45	C T
F	F9	47	M	14 24	C T
F	F10	47	M	13 23	C T
F	F11	47	M	34 44	C T

The sample population consisted of six patients, three males and three females aged between 29 and 69 years (mean age was 48.67). Patients were enrolled and surgical treatments completed between January and June 2016. Patients were seen for a total of five recall appointments (at 2, 8, 12, 16 and 24 weeks) over six months and the observational period concluded in December 2016. One patient (patient D) failed to comply with the recall appointments and was therefore disqualified from the study but was included in this report for statistical purposes.

Five patients with a collective total of twenty sites (*i.e.* ten paired sites) completed the study. Three patients (patients A, B and C) presented with two recession sites each (*i.e.* one paired site), one patient (patient E) presented with six recession sites (*i.e.* three paired sites) and one patient (patient F) presented with eight recession sites (*i.e.* four paired sites).

In patients E and F, sites on different quadrants were paired. Each set of paired sites were treated as a single pair and labelled according to patient and site number *i.e.* E5, E6, E7, F8, F9, F10 and F11.

Six of the twenty sites were located at upper canines, while recessions at the remaining fourteen sites were spread between the upper and lower premolars. The most commonly treated premolar was the upper first premolars with six sites, seven sites were distributed between the lower first and second premolars and one upper second premolar was treated.

For each site, the following six clinical parameters were recorded in millimetres (mm), see appendices 8-13:

Table 5.2: Clinical parameters

Clinical Parameters	Mesio-buccal (MB)	Buccal (B)	Disto-buccal (DB)
PD (periodontal probing depth)*	MB	B	DB
RD (recession depth)*	MB	B	DB
CAL (clinical attachment level)*	MB	B	DB
RW (recession width)**	RW was measured using a UNC 15 probe at the widest horizontal distance from the mesial to the distal margins of the recession defect		
KTW (keratinised tissue width)**	KTW was measured using a UNC 15 probe along the vertical mid-buccal groove of the measuring guide.		
GT (gingival thickness)**	GT was recorded using rubber stoppers at the end of endodontic reamers at the point corresponding to the vertical line of the mid-buccal groove of the measuring guide and the mid-point of the width of keratinised gingiva.		

* *Variables measured at three points*

** *Variables measured at one point*

The Pink Esthetic Score (PES) was calculated for each site before treatment and again at 24 weeks (appendix 14). Patient perception responses were tabulated in Appendix 15. For each patient, the clinical measurements, PES and patient questionnaire responses were summarised and are presented and discussed in this chapter.

Each patient was a unit of analysis. In patients with multiple paired sites (patients E and F), the mean of the each variable will be used for statistical analysis. Success of recession treatment is considered to be any decrease in the amount of exposed root surface.[70] Hence, while all variables are discussed, the primary variables that were analysed to determine root coverage was RD and RW.

The sample size was not large enough to prove whether there was a variance in the rate of improvement between the individual points of measure; namely (MB, B and DB). Therefore, an assumption was made that the rate of improvement or lack thereof was the same between the three points and the mean was used for analysis.

Statistical analysis was done to compare the baseline values to the 24 week values for each single paired patient (A to C); and for those patients with multiple paired sites (E and F), the patient's mean values were analysed as a group. This model is similar to Hirsch *et al.* wherein each patient was a unit of analysis and when more than one site was treated in a patient, the patient mean values were analysed.[125]

With regards to the patient questionnaire, the data was summarised and analysed for each patient. The patient questionnaire was based on a Likert scale.[120] A Likert scale assumes that the power of experience is linear *i.e.* a patients satisfaction or dissatisfaction can be measured.[121] As the data was ordinal, it was summarised as a mode in patients with multiple sites.

5.1 Patient A

5.1.1 Observation and Results

At the 2 week appointment, patient A reported intense pain at the palatal donor site during the first ten days. The pain gradually subsided between days eleven and fourteen. The patient also reported that the sutures at the test site loosened and some tissue was lost.

Table 5.3: Tabulation of clinical results for pair A1

		Control	Test
PD	0 weeks	2	2
	24 weeks	2	2
RD	0 weeks	3,67	2,67
	24 weeks	2,33	2,33
RW	0 weeks	7	7
	24 weeks	6	6
CAL	0 weeks	5,67	4,67
	24 weeks	4,33	4,33
KTW	0 weeks	2	2
	24 weeks	2	2
GT	0 weeks	1	0,5
	24 weeks	1,5	1
PES	0 weeks	11	10
	24 weeks	12	12

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

5.1.2 Data Summary and analysis

There was root coverage (yes) at both sites. At the control site, there was a 36.5% reduction in mean RD from 3,67mm to 2,33mm. At the test site, mean RD decreased from 2,67mm to 2,33mm; a 12.8% improvement. While the final result at both sites is the same, the control site showed greater reduction in RD. Both sites displayed identical improvements in RW from 7mm to 6mm. CAL also improved at both sites but was greater at the control with 23.6% improvement (from 5,67mm to 4,33mm) and only a 7.3% improvement at the test site. The mean PD measurements remained unchanged at baseline and 24 weeks at both the control and test site. KTW remained unchanged (2mm) at both sites.

Overall, the control site showed greater improvement in recession measurements than the test site. GT improved at both sites. At the control site, GT changed from 1mm to 1.5mm (50% improvement) and 0.5mm to 1mm (100% improvement) at the test site. The PES

improved at both sites. At the control site there was a 1 point improvement and a 2 point improvement at the test site.

5.1.3 Summary of Patient Perceptions

The patient scored both sites the same. He scored dentine sensitivity with 'slight improvement' while there was 'no change' in all other soft tissue variables.

Table 5.4: Tabulation of scores from patient questionnaire

	Level of Gingival Recession	Dentine Sensitivity	Gingival Colour	Gingival Shape
Control	3	4	3	3
Test	3	4	3	3

The scores are based on the Likert key tabulated on a 5 point scale: 1= much worse than before, 2= slightly worse than before, 3= no change, 4= slight improvement, 5= noticeable improvement.

5.2 Patient B

5.2.1 Observations and Results

As a point of interest, patient B was diabetic and his disease was well-controlled. Diabetes was neither included nor excluded from the study cohort by design and as such patients B's diabetic status was not a limiting factor.

At the 2 week recall appointment, patient B said that the palatal wound reportedly bled for one and a half days. His face was swollen on both sides for three days postoperatively and pain was felt at all three surgical sites. The most intense pain was felt at the donor palatal site. In this patient, the acrylic stent did not fit as well as on the other patients and it was slightly loose. This reduction in pressure against the palate could be the reason for the extended bleeding time and pain. Postoperative swelling is expected after surgery as the gingival tissues and blood vessels are sharply dissected when creating the tunnel flap.[113] This was managed with anti-inflammatory drugs. Pain at the test site resolved fairly quickly but pain at the control site was still felt at 2 weeks postoperatively. He recounted that the distal suture at the control site loosened after a few days and subsequently some tissue was lost. He also reported that there was loss of sensation at the palatal donor site. At subsequent

appointments, this was evaluated and some sensation began to return at the 8 week recall appointment and sensation was back to normal at 24 weeks.

Table 5.5: Tabulation of results for pair B2

		Control	Test
PD	0 weeks	2	3,33
	24 weeks	2	3
RD	0 weeks	2,67	3
	24 weeks	1,33	1,33
RW	0 weeks	5	5
	24 weeks	4	4
CAL	0 weeks	4,67	6,33
	24 weeks	3,33	4,33
KTW	0 weeks	3	4
	24 weeks	2	5
GT	0 weeks	0,5	1,5
	24 weeks	1	1
PES	0 weeks	8	11
	24 weeks	8	12

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

5.2.2 Data Summary and analysis

There was root coverage (yes) at both sites. Mean RD improved at both sites, 50.19% at the control site and 55.67% at the test site. Although there were mean RD improvements at both sites, the test site demonstrated a 5.48% greater improvement over the control site. Both sites displayed identical improvements in RW from 5mm to 4mm. CAL also improved at both sites but was greater at the test site with 31.9% improvement (from 6,33mm to 4,33mm) and 28.7% improvement at the control site.

The mean PD at the control site remained unchanged over the study period, while at the test site it reduced by 10% (from 3,33mm to 3mm). KTW decreased at the control site, from 3mm at baseline to 2mm at 24 weeks while at the test site, KTW increased from 4mm to 5mm over this period. GT improved by 0.5mm (from 0,5mm to 1mm) at the control site but decreased by 0.5mm (from 1,5mm to 1mm) at the test site.

At the control site, the PES score remained unchanged at 8 points. At the test site, the score improved from 11 to 12 points over the study period.

5.2.3 Summary of Patient Perceptions

The patient scored both sites the same. He scored the level of gingival recession as 'slight improvement'. There was a 'noticeable improvement' in gingival colour. Dentine sensitivity and gingival shape were both scored as 'no change'.

Table 5.6: Tabulation of scores from patient questionnaire

	Level of Gingival Recession	Dentine Sensitivity	Gingival Colour	Gingival Shape
Control	4	3	5	3
Test	4	3	5	3

The scores are based on the Likert key tabulated on a 5 point scale: 1= much worse than before, 2= slightly worse than before, 3= no change, 4= slight improvement, 5= noticeable improvement.

5.3 Patient C

5.3.1 Observations and Results

During the surgical appointment, there was some difficulty in isolating the cubital veins of patient C. Eventually, we managed to collect two vials of blood and this was sufficient in this patient because there was only one site to treat with PRF.

At the 2 week recall appointment, patient C reported that she experienced severe pain at the palatal donor site. She needed to take analgesics for longer than the prescribed period. The pain subsided 1 week postoperatively.

Table 5.7: Tabulation of results for pair C3

		Control	Test
PD	0 weeks	2	2
	24 weeks	2	2,33
RD	0 weeks	0,67	0,67
	24 weeks	0,33	0,67
RW	0 weeks	3	4
	24 weeks	0	3
CAL	0 weeks	2,67	2,67
	24 weeks	2,33	3
KTW	0 weeks	4	4
	24 weeks	6	6
GT	0 weeks	1	1
	24 weeks	1,5	1
PES	0 weeks	14	14
	24 weeks	13	14

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

5.3.2 Data Summary and Analysis

There was root coverage (yes) at the control site but no root coverage at the test site.

At the control site, there was a 50.75% reduction in mean RD from 0,67mm to 0,33mm over the study period. At the test site, mean RD remained unchanged at 0,67mm. RW reduced a 100% at the control site while there was only a 25% improvement (from 4mm to 3mm) at the test site. CAL improved by 12.3% (from 2,67mm to 2,33mm) at the control site and worsened by 12.36% (from 2,67mm to 3mm). Both sites displayed identical improvements in KTW from 4mm to 6mm. Mean PD at the control site remained unchanged over the study period (at 2mm) while at the test site, mean PD worsened from 2mm to 2,33mm over the same period. GT increased by 50% (from 1mm to 1,5mm) at the control site with no change at the test site. Overall the control site demonstrated superior results than the test site.

The PES score decreased by 1 point at the control site. There was slight change to the natural soft tissue contour with loss of some gingival scalloping, hence the decrease. It was so minor that the patient did not notice it and scored the gingival shape as ‘no change’ on the questionnaire (see table 5.8). The test site remained a perfect score of 14. In patient C,

the baseline aesthetic scores was already high and neither treatment worsened the aesthetic result significantly.

On the questionnaire, the only change the patient noted was that dentine sensitivity at the control site had 'noticeably improved'. She commented that the sensitivity at this site resolved completely while the test site was still sensitive. As her motivation for the treatment was to improve dentine sensitivity, she was interested in undergoing further treatment at the test site to improve sensitivity at this site and she wanted the same treatment that was done at the controls site *i.e.* CTG. She was happy with the aesthetic result.

5.3.3 Summary of Patient Perceptions

The patient scored all sites as 'no change' except for dentine sensitivity at the control site. This was scored with a 'noticeable improvement'.

Table 5.8: Tabulation of scores from patient questionnaire

	Level of Gingival Recession	Dentine Sensitivity	Gingival Colour	Gingival Shape
Control	3	5	3	3
Test	3	3	3	3

The scores are based on the Likert key tabulated on a 5 point scale: 1= much worse than before, 2= slightly worse than before, 3= no change, 4= slight improvement, 5= noticeable improvement.

5.4 Patient D

5.4.1 Observations and results

At the 2 week appointment, the patient reported that the test site was very swollen for a few days after the surgery. The control site healed uneventfully.

Patient D was seen until the 8 week recall appointment. She then failed to comply with any of the subsequent appointments and was disqualified from the study. The measurements recorded at baseline and 8 weeks are tabulated below to complete data summary.

Table 5.9: Tabulation of results for patient D at the control site

		Control	Test
PD	0 weeks	1	1,33
	8 weeks	1,33	1,67
RD	0 weeks	1,67	1,33
	8 weeks	0,33	1,33
RW	0 weeks	4	5
	8 weeks	3	4
CAL	0 weeks	2,67	2,67
	8 weeks	1,67	3
KTW	0 weeks	0,5	0,5
	8 weeks	1	1
GT	0 weeks	0,5	0,5
	8 weeks	Gt measured at 24 weeks	
PES	0 weeks	14	14
	8 weeks	Photographs not taken	

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

5.5 Patient E

5.5.1 Observation and Results

Patient E was treated at six sites. She was able to remove the palatal stent after three days, as the pain at the palatal site had subsided. At the 8 week appointment, she reported numbness at the palatal donor site. There was some sensation when the site was stroked with a probe validating the perception, and full sensation returned by week 24.

Since there were multiple sites for patient E (and F), the data collected and statistics derived are more comprehensive than in the previous patients. Specifically, data was collected for all sites and there were two types of mean calculations determined as presented in the tables below. The mean in the top row (headings) is the mean of the MB, B and DB measurements per site. The mean in column 1 (second last row) is the mean of the pairs (E5, E6 and E7). Further, the control and test data was separated into their own tables as presented below.

Table 5.10: Tabulation of results for patient E at the control site

	Mean PD		Mean RD		RW		Mean CAL		KTW		GT		PES	
Weeks	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**
Pair E5	2,00	2,00	1,33	1,33	4,00	4,00	3,33	3,33	4,00	1,00	1,00	1,00	11,00	12,00
Pair E6	2,00	2,33	1,33	2,33	3,00	3,00	3,33	4,67	2,00	3,00	1,5	1,5	14,00	14,00
Pair E7	2,33	1,67	1,33	1,00	3,00	3,00	3,67	2,67	1,00	1,00	0,5	0,5	14,00	14,00
Mean	2,11	2,00	1,33	1,55	3,33	3,33	3,44	3,56	2,33	1,67	1,00	1,00	13,00	13,33
STD Dev	0,19	0,33	0,00	0,69	0,58	0,58	0,20	1,02	1,53	1,15	0,50	0,50	1,73	1,15

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

Table 5.11: Tabulation of results for patient E at the test site

	Mean PD		Mean RD		RW		Mean CAL		KTW		GT		PES	
Weeks	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**
Pair E5	1,67	2,00	1,00	0,67	3,00	3,00	2,67	2,67	3,00	5,00	1,00	1,00	14,00	14,00
Pair E6	2,33	2,00	1,67	1,33	4,00	4,00	3,67	3,33	2,00	2,00	1,00	1,00	14,00	14,00
Pair E7	2,00	2,00	1,33	1,33	4,00	3,00	3,33	3,33	2,00	2,00	1,00	1,00	14,00	14,00
Mean	2,00	2,00	1,33	1,11	3,67	3,33	3,22	3,11	2,33	3,00	1,00	1,00	14,00	14,00
STD Dev	0,33	0,00	0,34	0,38	0,58	0,58	0,51	0,38	0,58	1,73	0,00	0,00	0,00	0,00

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

5.5.2 Data Summary and analysis

There was root coverage (yes) at one of the three control sites (E7) and two of the three test sites (E5 and E6).

The summary statistics show that in the control group, the mean baseline RD measurement was 1.33mm ±0.00mm and at 24 weeks the mean RD measurement was 1.55 ±0.69mm. In the test group, the mean baseline RD measurements were 1.33 ±0.34mm and 1.11 ±0.38mm at 24 weeks. The control group was on average 16% worse while the test group was 16.54% better. By virtue of the resultant standard deviations for the two groups, it appears that the test result is more consistent. There was no change in mean RW of the control group while there was a 9% improvement in mean RW in the test group. CAL worsened slightly (3.4%) in the control group and improved slightly (3.4%) in the test group. KTW worsened in the control group by 28.3% while it improved in the test group by 28.76%. Overall, in patient E, PRF demonstrated better results than CTG.

Table 5.12: Tabulation of descriptive statistics and t-test results for patient E

		RD		CAL		RW		KTW		GT	
Patient E	P(T<=t) two-tail	0,35		0,71		0,42		0,55		N/A	
	Kurtosis	-0,01	0,73	-1,25	1,13	U	U	U	U	U	U
	Skewness	-0,50	1,62	-0,68	0,02	U	1,73	1,29	-1,73	U	U

Descriptive statistics and t-tests revealed that while the improvement for both control and test was the same, *i.e.* the high p-value indicates that the null hypothesis is supported (table 5.12), skewness and kurtosis could only be calculated for the RD and CAL distributions. However, these distributions differed in that the control favoured negatively scored distributions while the test positively scored. This supports that the test sample variance results were slightly lower if not similar and the mean is lower than the hypothesised mean. t-Tests cannot be reliably used for the RW, KTW and GT since Microsoft Excel returned undefined (divide by zero) scores for the distributions. These are noted as “U” in the table.

The PES increased in the CTG group from a baseline mean of 13.00 ±1.73 to 13.33 ±1.15. In the PRF group, the PES remained unchanged at perfect score of 14.

According to the patient questionnaire, patient E scored the test sites has having improved over the control site. She stated that dentine sensitivity and the gingival margin improved at the test sites while these same variables worsened slightly at the control sites.

5.5.3 Summary of Patient Perceptions

Patient E scored the level of gingival recession and dentine sensitivity at the test sites as improved while the control sites worsened. The variables gingival colour and shape remained unchanged at all sites. This perception correlates to the clinical results as the test group demonstrated improvements in RD while the control group worsened. RW decreased in the test group and remained unchanged in the control group. Further, the PES remained unchanged or improved (control site E5) correlating to the patients perception of "no change" in terms of gingival colour and shape.

Table 5.13: Tabulation of scores from patient questionnaire

Pair	Level of Gingival Recession		Dentine Sensitivity		Gingival Colour		Gingival Shape	
	Control	Test	Control	Test	Control	Test	Control	Test
E5	2	4	2	5	3	3	3	3
E6	2	4	2	5	3	3	3	3
E7	2	4	2	5	3	3	3	3
Mode	**2**	**4**	**2**	**5**	**3**	**3**	**3**	**3**

The scores are based on the Likert key tabulated on a 5 point scale: 1= much worse than before, 2= slightly worse than before, 3= no change, 4= slight improvement, 5= noticeable improvement.

5.6 Patient F

5.6.1 Observation and results

At the 2 week recall appointment, patient F reported that he was experiencing severe pain in the upper right quadrant. On examination, the 23 and 24 area (test sites) was inflamed and there was gingival sloughing in the area. The other surgical sites had satisfactory healing. At subsequent appointments, healing at the sites 23 and 24 improved (without additional treatment).

Table 5.14: Tabulation of results for patient F at the control site

	Mean PD		Mean RD		RW		Mean CAL		KTW		GT		PES	
Weeks	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**
Pair F8	2,00	2,00	1,67	1,33	5,00	5,00	3,67	3,33	4,00	4,00	1,00	2,00	8,00	11,00
Pair F9	1,67	2,00	2,33	2,33	5,00	5,00	4,00	4,33	3,00	4,00	1,5	2,00	10,00	10,00
Pair F10	2,00	2,67	0,67	1,33	4,00	5,00	2,67	4,00	4,00	4,00	1,00	1,5	10,00	10,00
Pair F11	2,00	2,67	2,67	2,00	5,00	5,00	4,67	4,67	3,00	1,00	1,00	1,00	11,00	13,00
Mean	1,92	2,34	1,84	1,75	4,75	5,00	3,75	4,08	3,50	3,25	1,13	1,63	9,75	11,00
STD dev	0,17	0,39	0,88	0,50	0,50	0,00	0,83	0,57	0,58	1,50	0,25	0,48	1,26	1,41

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

Table 5.15: Tabulation of results for patient F at the test site

	Mean PD		Mean RD		RW		Mean CAL		KTW		GT		PES	
Weeks	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**
Pair F8	1,67	2,33	1,67	1,00	4,00	5,00	3,33	3,33	2,00	2,00	0,5	1,00	11,00	11,00
Pair F9	1,67	2,00	1,33	1,33	5,00	5,00	3,00	3,33	6,00	5,00	1,5	2,00	9,00	10,00
Pair F10	2,00	4,67	1,67	2,00	6,00	6,00	3,67	6,67	4,00	3,00	1,00	1,00	11,00	10,00
Pair F11	2,00	2,33	1,00	1,33	4,00	4,00	3,00	3,67	3,00	3,00	1,00	1,00	12,00	13,00
Mean	1,84	2,83	1,42	1,42	4,75	5,00	3,25	4,25	3,75	3,25	1,00	1,25	10,75	11,00
STD dev	0,19	1,23	0,32	0,42	0,96	0,82	0,32	1,62	1,71	1,26	0,41	0,50	1,26	1,41

Abbreviation key: PD= probing depth, RD= recession depth, RW- recession width, CAL= clinical attachment level, KTW= keratinised tissue width, GT= gingival thickness, PES= pink esthetic score.

5.6.2 Data Summary and analysis

Two of the four control sites showed root coverage (yes) and one of the four test sites had root coverage.

The summary statistics show that in the control group, mean baseline RD measurement was 1.84mm ±0.88mm and at 24 weeks the mean RD measurement was 1.75 ±0.50mm. In the

test group, mean baseline RD measurement was 1.42mm ±0.32mm and at 24 weeks the mean RD measurement was 1.42 ±0.42mm.

Table 5.16 Tabulation of descriptive statistics and t-test results for patient F

		RD		CAL		RW		KTW		GT	
Patient F	P(T<=t) two-tail	0,79		0,12		1,00		0,79		0,18	
	Kurtosis	-0,19	-0,86	-0,25	-1,28	4,00	4,00	2,23	-6,00	1,50	-6,00
	Skewness	-0,09	0,00	-0,14	-0,46	-2,00	-2,00	1,13	0,00	0,00	0,00

Descriptive statistics and t-tests revealed that while the improvement for both control and test was the same, *i.e.* the high p-value indicates that the null hypothesis is supported (table 5.16), skewness and kurtosis could only be reliably used for the RD and CAL distributions. The RD and CAL distributions exhibited similar scores. This supports the use of the findings. However, t-Tests cannot be reliably used for the RW, KTW and GT the score for the other three measures were fairly extreme.

In the control group, mean baseline RW measurement was 4.75mm ±0.50mm and at 24 weeks the mean RW measurement was 5.00 ±0.00mm. In the test group, mean baseline RW measurement was 4.75mm ±0.96mm and at 24 weeks the mean RW measurement was 5.00 ±0.82mm. RW worsened in both groups.

In the control group, mean baseline CAL measurement was 3.75mm ±0.83mm and at 24 weeks the mean CAL measurement was 4.08 ±0.57mm. In the test group, mean baseline CAL measurement was 3.25mm ±0.32mm and at 24 weeks the mean RW measurement was 4.25 ±1.62mm. CAL worsened in both groups.

Mean GT and PES improved in both sites.

5.6.3 Summary of Patient Perceptions

The patient scored all control and test sites as “noticeable improvement”.

Table 5.17: Tabulation of scores from patient questionnaire

Pair	Level of Gingival Recession		Dentine Sensitivity		Gingival Colour		Gingival Shape	
	Control	Test	Control	Test	Control	Test	Control	Test
F8	5	5	5	5	5	5	5	5
F9	5	5	5	5	5	5	5	5
F10	5	5	5	5	5	5	5	5
F11	5	5	5	5	5	5	5	5
Mode	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**

The scores are based on the Likert key tabulated on a 5 point scale: 1= much worse than before, 2= slightly worse than before, 3= no change, 4= slight improvement, 5= noticeable improvement.

CHAPTER 6

6 DISCUSSION

The purpose of this study was to evaluate and compare the clinical outcomes of CTGs and PRF in treating gingival recession. The study set out to: 1) Determine the clinical efficacy of PRF membranes, 2) Compare the clinical and aesthetic outcomes between the two groups and 3) Determine patient aesthetic satisfaction between the two groups.

6.1 Clinical Outcomes

As discussed in chapter *4.9 Data Analysis,* this study is an extended case series and therefore, each patient was a unit of analysis and was analysed independently of each other.[125] There were three patients with single paired sites (A1, B2 and C3) while two patients had multiple paired sites. Patient E had three paired sites (E5, E6 and E7) and patient F had four paired sites (F8, F9, F10 and F11). Patient D4 was excluded from data analysis as she failed to comply with the study's requirements.

The primary variables analysed to determine clinical improvements in root coverage were RD and RW. Keratinised tissue changes (KTW and GT) were secondary outcomes.

With regards to the recession measurements, there were improvements at both CTG and PRF sites but there were a greater number of CTG sites which showed improvements. Of the total sites treated, six of the ten control sites and only five of the ten test sites demonstrated root coverage. In the single paired sites A1 and C3, the control sites showed greater improvements in RD measurements than the test sites while test site B2 showed a greater improvement than its corresponding control site. In patients A1, B2 and C3, RW improved at all control and test sites. RW changes between the control and test sites for A1 and B2 were the same. However, control site C3 showed a 100% improvement while test site C3 showed a less favourable 25% improvement. Overall in the single paired sites, CTG performed better with only one PRF site (B2) performing better than its control. At the 2 week recall appointment, patient B reported that some tissue was lost from the CTG site. The poorer result at this CTG site (B2) could be due to loss of some graft tissue and therefore a compromised outcome at this particular CTG site.

CAL (recorded as a loss) decreased at all single paired sites. In patient A1, the control site performed better with a 24% improvement whereas the test site had only a 7% improvement. In patients B2 and C3, the control and test sites showed similar decreases in CAL (B2: 29% at the control and 32% at the test site, C3: 12% at the control and 13% at the test site). This demonstrates that both CTGs and PRF have the potential to improve CALs.

In patients E and F, descriptive statistics and t-tests revealed that the improvement for both control and test was the same, *i.e.* the high p-value indicates that the null hypothesis is supported. In patient E, the individual test sites displayed slightly better results in RD and CAL than the control sites (the test sample variance results were slightly lower if not similar and the mean was lower than the hypothesised mean). These results in patients E and F are mirrored in a similarly designed study by Jankovic *et al.* where CTGs was compared to PRF.[102] It was found that RD improved in both groups with no statistical difference between groups.[102] In patients E and F, RW measurements remained unchanged at most sites (11 of the 14 sites). Of the three sites that demonstrated a change in RW, only one site improved (test site E7 improved by 1mm). The other two sites worsened by 1mm each (test site F8 and control site F10). It was not possible to test statistical significance of RW in patients E and F as Microsoft Excel 2016 returned undefined (divide by zero) scores for the distributions. These results in clinical measurements demonstrates that both CTGs and PRF have the potential to reduce recession measurements.

There were improvements in GT at many sites, 60% of CTG sites demonstrated an increase in GT while only 30% of the PRF sites increased in GT. CTGs showed a greater inclination to increase GT than PRF. Few studies report on changes in GT. While reviewing the literature, I found only two studies that reported on changes in GT. Both studies also used a 6 month observation period and GT was recorded at baseline and 6 months. One of the studies was conducted by Eren and Atilla, wherein CTGs and PRF in combination with a CAF was also evaluated and compared.[12] The authors found similar statistically significant improvements in GT measurements in both groups.[12] The present study, on the other hand, showed a greater increase at the CTG sites. The second study; by Aroca *et al.* tested a CAF alone to a CAF combined with PRF and found statistically significant improvements in GT in the PRF

group.[11] These results demonstrate that PRF in combination with a CAF can be more beneficial than using a CAF alone in treating gingival recession.

The present study's results demonstrates that CTGs provide greater improvements in GT over PRF. The increase in GT at the CTG sites can be explained by the influence of the type of connective tissue used. Connective tissue from the palate contains the biological signals that induce the overlying epithelium to differentiate into keratinised epithelium.[12, 56, 80, 82] In a study by Zucchelli *et al*. where de-epithelised CTGs were compared to epithelised CTGs, it was found that GT increased in patients with de-epithelised CTGs.[126] The present study also used de-epithelised palatal CTGs. The increase in the PRF group may be explained by the influences of the growth factors trapped within the PRF membrane.[12] These growth factors positively influence proliferation and differentiation of the gingival and periodontal ligament fibroblasts and epithelial cells encouraging angiogenesis.[102] The dense 3-dimensional fibrin structure of PRF may also function in a similar fashion to an extracellular matrix by providing stability to the wound and acts as a scaffold for cellular interactions thereby increasing the thickness of the overlying epithelium.[17] Previous studies have emphasised the benefits of increasing the amount of keratinised tissue. An increase in GT contributes to complete root coverage, long-term periodontal stability and helps prevent further gingival recession over time.[102, 127] Therefore, increases in GT is a desired outcome of any surgical treatment. Although both CTGs and PRF demonstrated the potential to do so, CTGs performed better than PRF.

The KTW results of this study showed improvements in only six of the twenty sites (three control and three test sites). There were decreases in KTW in three control sites and two test sites while the remaining sites stayed the same (four control sites and five test sites). In their CTG and PRF study, Jankovic *et al.* observed a different outcome over 6 months with statistical gain in KTW in both groups.[102]

Overall, both CTGs and PRF can improve clinical outcomes in treating gingival recessions but CTGs demonstrated improved clinical outcomes at a greater number of sites. These findings supports the view by many authors that CTGs with a CAF produces the best results in treating recessions.[8, 11, 13, 112, 128, 129]

A clinical limitation of PRF is the phlebotomy component of its preparation. In some patients, (as in patient C in this study) it can be challenging to not only isolate the required veins but also in obtaining the required volume of blood. This can pose a limitation chair side and can make completing treatment difficult. Enhancing the localisation of subsurface veins may make obtaining blood easier. A Doppler ultrasound uses high-frequency sound waves to measure blood flow and can be used to identify the position of blood vessels.[130] However, this technique requires additional training and/or assistants with added expense. A simpler alternative may be devices that use non-invasive near-infrared light.[130] In the wavelength of 700-900nm, haemoglobin and oxy-haemoglobin are the main absorbers of light.[131] When this infra-red light is placed over skin, blood flowing in the area absorbs light in this wavelength spectrum causing blood vessels to appear much darker than the surrounding tissues.[130] This type of device is economical and much easier to use than a Doppler ultrasound. There are home devices that use this technology to aid in the early detection of breast cancer. These devices are small, relatively inexpensive and are meant to be used easily and correctly by anyone.[131] Hence it is a viable aid for isolating veins for easier blood withdrawal.

6.2 Aesthetic Outcomes

According to Chambrone *et al.* improved gingival aesthetics is considered the primary goal of root coverage procedures.[132] Furthermore, Zucchelli *et al*. states that root coverage success should be determined not only by reductions in recession measurements but also by soft tissue coverage, the thickness and colour of which should be indistinguishable from those of adjacent soft tissue.[44]

This study used the PES as an objective and reproducible aesthetic scoring system and demonstrated that both CTGs and PRF can improve aesthetic outcomes. In a comparative type study by Kerner *et al.*, it was found that a before and after scoring system is an acceptable and reliable method for aesthetic assessment of root coverage treatment.[108]

The aesthetic results in both groups were similar and no patient was dissatisfied with the aesthetic outcomes. Across the control and test groups, there were eight sites with an increased PES, ten sites with no change and two sites that decreased in the PES. The

increases in the PES were distributed equally between the control and test sites *i.e.* four control sites A1, E5, F8 and F11 while the four test sites were A1, B2, F9 and F11.

The decreases in the PES were at control site C3 and test site F10. At both sites, there was a change from a perfect score of two ("natural") on soft tissue contour to a "fairly natural" score of one. This change in tissue contour may be the result of the surgical incisions into the sulcus and not necessarily as a result of the graft or PRF membrane. The remaining sites displayed an improvement in the PES or an unchanged aesthetic score *i.e.* the aesthetic score did not worsen. Therefore, both CTGs and PRF have the potential to improve gingival aesthetics or at the very least maintain aesthetics at any given site.

There are limited studies that have systematically evaluated aesthetic outcomes using any type of visual scoring system. Studies simply comment on aesthetic outcomes using either general terms such as ''aesthetically pleasing'' or ''good aesthetic results'', or a by using a scale similar to "good", "regular" or "poor".[72, 106] In a study by Rosetti *et al.* aesthetic results were evaluated on a good, regular and poor scale. The authors recognised that this type of scoring system is dependent on a subjective clinical impression.[106] Another study by Bouchard *et al*. used a scoring system of good, moderate and poor. They also acknowledged that this type of aesthetic evaluation was subjective and tried to eliminate this bias by using independent examiners to evaluate the aesthetic outcomes.[133] These methods of scoring aesthetics is not ideal as it is not only subjective but also not reproducible. In contrast, the present study is unique in that I used a more objective means to evaluate aesthetics. The PES is a reproducible, reliable and predictable scoring system which evaluates seven specific characteristics of gingival tissue to calculate a maximum score of fourteen.[107] Studies which evaluated aesthetic results pre-date aesthetic scoring systems and therefore the results of this study cannot be compared to any other studies.

6.3 Patient-Based Outcomes

According to Bouchard *et al.* the success of root coverage procedures should be determined by the patient and not the clinician.[72] The ultimate goal of any treatment is patient satisfaction. To this aim, patients were given a questionnaire to score treatment outcomes on a Likert type scale (appendix 4). The questionnaire was given to patients at the end of the

study period. Patients were asked to score four features of each site: marginal gingival level, dentine sensitivity, gingival colour and gingival shape. The scoring system was a five point scale: 1 – much worse than before, 2 – slightly worse than before, 3 – no change, 4 – slight improvement and 5 – noticeable improvement.

All patients appeared satisfied with the aesthetic results. According to the patient questionnaire, most sites (both control and test sites) either improved or remained unchanged. Patient E was the exception. She scored the gingival level at all three control sites as "slightly worse than before" and the corresponding test sites as "slightly improved". Her perception is mirrored in the clinical results where statistical analysis showed that the control group worsened by 16% and the test group improved by 16.54%. With regards to aesthetic outcomes, she scored gingival colour and shape as unchanged at all sites. Again, this is reflected in the PES. The PES for these sites showed either an improvement (control site E5) or it remained unchanged at a perfect score of 14. Analysis of patient E's questionnaire supports the concept that patients can constructively contribute to determining the success or failure of clinical outcomes.

On the other hand, patient F scored all control and test sites as 5 -"noticeable improvement". The clinical results differed in that some of these sites worsened (*i.e.* control site F10 and test sites F10 and F11), three sites improved (control sites F8 and F11 and test site F8) and two sites remained unchanged (both control and test sites F9) over the study period. These incongruities between clinical results and the patient's perceptions show a tendency of bias on the part of the patient. He may be bias in wanting to favour a good result for me as an investigator. This shows that a patient's perceptions can be skewed to the reality of outcomes. Similarly, patient B scored the gingival margin as "slightly improved" and gingival colour as "noticeable improvement" at the CTG site even though there was no change in the PES. Therefore, as a clinician, I may not deem this site an aesthetic success (due to the weak PES of 8 at the control site) but the patient seemed satisfied with the overall result. Again, the patient may be bias, wanting to favour good study results for me as an investigator. This concept is known as participant bias and is well documented in psychological studies. Participant bias occurs when participants want to make a useful contribution to the study and so will provide what they think is the "correct"

answers to provide the researcher with what he or she wants.[134] In this study, patients B and F might have thought that the researchers wanted treatment outcomes to be excellent and therefore provided answers to this end.

Despite this limitation, patients appeared satisfied with the aesthetic outcomes. There were no scores in the PRF group that worsened. Chambrone *et al.* consider aesthetics as the primary goal of root coverage procedures.[132] Yet, very few studies consider aesthetic outcomes from a patient-based approach. In their systematic review of root coverage procedures, Chambrone *et al.* found only three RCTs that evaluated aesthetic outcomes according to patient opinions. Similar to the present study, Chambrone *et al.* also found that when patients were asked to evaluate aesthetic results, most patients were satisfied with the aesthetic outcome.[132] Rotundo *et al.* set out to determine aesthetic perceptions after root coverage and interestingly found that clinicians can only expect patients to be fully satisfied with aesthetic results when complete root coverage is achieved.[105] However, the present study found that patients can be satisfied with the aesthetic result even in the absence of complete root coverage.

Another frequent goal of root coverage treatment is to reduce dentine sensitivity.[132] As part of determining overall patient satisfaction, dentine sensitivity was one of the variables evaluated in the patient questionnaire. Dentine sensitivity is rarely reported on and yet it is one of the main factors that prompt patients to seek treatment.[20] Of the twenty sites treated, fourteen sites were scored as improved (slight and noticeable improvement) with regards to dentine sensitivity. Six control sites and eight test sites were marked as improved. These results indicate that both CTGs and PRF can improve dentine sensitivity.

Of all the patients treated, only patient C's main concern was dentine sensitivity and she was very pleased with the result at the control site. In fact, she expressed interest to re-treat the test site to improve sensitivity at this site. In patient C, recession measurements at the control site also showed greater reduction than the test site. There was almost complete resolution of the recession at the control site with improvements in KTW and GT. In patient C, these results suggest that CTG may provide better results with regards to dentine sensitivity than PRF. However, at this site, the PES decreased lowering the aesthetic

outcome but the patient was still happy with the result because her main complaint was addressed *i.e.* dentine hypersensitivity. This highlights that in addition to aesthetic outcomes, addressing a patient's main complaint is also important in determining overall success or failure of treatment outcomes.

CHAPTER 7

7 CONCLUSIONS

To the best of my knowledge, this study is unique in the South African setting. Both CTGs and PRF demonstrated clinical and aesthetic improvements in gingival recession coverage. The results obtained for RD, CAL and GT were better in the CTG group whereas PRF demonstrated better results for RW and KTW. The aesthetic outcomes were the same for both groups. With regards to patient perceptions, PRF performed better than CTGs.

The results from this study indicate that both CTGs and PRF membranes can be effective in treating gingival recession and both treatments can improve clinical and aesthetic outcomes.

7.1 Limitations and recommendations

7.1.1 The small sample size

Small sample sizes are a recurring limitation of most dental studies.[135, 136] The ability to enrol a sufficient number of patients into a clinical study who meet the selection criteria can be a key limiting factor for obtaining a powerful study. Under-powered studies have a low probability of detecting true differences between treatments.[135] This means that a study demonstrating results of 'no difference' between groups may, in fact, have too small a sample size to detect true differences between the groups and the overall estimate effect of the study is less precise.

For future studies, a similarly designed RCT with a larger sample size and higher statistical power may provide a better understating of PRF efficacy. Hujoel *et al.* provide guidelines to increase a study's power.[135] They suggest that in addition to a rigid selection criteria for patient enrolment, the size of the lesions between groups and patients should be similar and in patients with multiple sites, the numbers of sites in each patient should be also be similar.[135]

7.1.2 Method of measuring gingival recession depth

In this study, small changes were noted in recession measurements over the study period. This study used a UNC-15 probe that is marked in 1mm increments. This type of measuring method was not able to detect smaller than 1mm changes in measurements. Perhaps a different method whereby smaller increments can be measured may provide a more accurate analysis of clinical outcomes. 3D digital measurements could help in this regard.

7.1.3 A relatively short study time

Changes in soft tissue at treated sites were observed and recorded over a six month period. This is a relatively short observational time and studies have shown that there could be progressive coronal movement of the attached gingiva over a longer period of time.[137] This phenomena has been termed creeping attachment and was first described in 1973 by Goldman and Cohen.[138] They defined creeping attachment as "the post-operative migration of gingival marginal tissue in a coronal direction over portions of a previously denuded root."[138] The authors stated that this migration may be observed for extended periods after surgery until a constant marginal level is reached.[138] This gingiva is usually firm, well attached with a healthy sulcus depth.[139] Any improvement in recession measurements that occur after 4 weeks postoperatively is considered to be creeping attachment.[137] In a study to determine the rate of creeping attachment, Bell *et al*. found that creeping attachment occurs at an average rate of 1mm over 12 months.[140] In a retrospective study by Agudio *et al.* creeping attachment was observed at treated sites during the entire follow-up period (10 to 25 years).[141] However, creeping attachment is not predictable.[137] Factors associated with increased creeping attachment are narrower recessions, younger patients, single tooth recession correction, tooth position and level of oral hygiene.[137, 142] The studies by Lee *et al.* and Harris suggest that creeping attachment may occur commonly in areas where CTGs are placed.[137, 143] Therefore, longer observational periods may produce different results.

7.1.4 Using photographs to evaluate aesthetics

Another limitation of the present study may be associated with the use of photographs to evaluate aesthetics as opposed to using direct clinical assessment of the patient. Clinical assessment would allow identification of the MGJ and this would allow using the RES (by

Cairo *et al.*[104]) as opposed to the PES which was used in this study. The RES was specifically designed to evaluate aesthetic outcomes of root coverage procedures.[104] The RES uses MGJ alignment as one of its variables to evaluate aesthetic success. If aesthetics is evaluated clinically, the aesthetic score may be more representative of the outcomes of root coverage treatments.

7.1.5 Investigator bias when evaluating aesthetic outcomes

The aesthetic evaluator was not blinded to the control and test sites. This could have created bias favouring one treatment over the other. Ensuring evaluator blindness can eliminate this bias.

7.1.6 Patient bias when answering the patient questionnaire

Another limitation of this study was the method used to determine patient satisfaction. Patients were asked to complete a questionnaire based on a Likert-type scale. When analysing the results, it appeared that some patients may have been bias in their answers. This limitation has been recognised in psychological studies and is known as "participant bias".

Participant bias is when patients may consciously or unconsciously want to provide the researcher with what they perceive to be the desired outcome.[134] A way to overcome this bias is to ensure complete anonymity with regards to every aspect of the questionnaire. All patients were familiar with the researchers as a relationship usually develops between doctor and patient. Therefore, a person not known to the patients should hand out and collect the questionnaire without any familiar persons around. Patients should also see that there would be no means for the researcher to identify their answers. Study participants might be influenced to answer questions more objectively if they are unfamiliar with the person in charge of the questionnaire.

7.1.7 Alternate way to determine patient-based outcomes

In this study, patients were questioned on their aesthetic perception after treatment. In future studies, an additional patient questionnaire before treatment to assess their initial

perceptions of the aesthetic appearance of the oral tissues may be helpful to compare their perceptions on treatment outcomes.

7.1.8 Lack of histological evaluation

This study has no histological component and is unable to determine the regenerative capacity of CTGs and PRF. Miller stated that although new connective tissue attachment is the ultimate goal of root coverage treatment, healing by long junctional epithelium is also acceptable.[144] However, without histological data, the nature of any new gingival attachment is unknown. However, this is no different to other similar studies as ethical human research prevents this type of histological evaluation.

8 REFERENCES

1. Newman M, Takei H, Carranza F. Carranza's Clinical Periodontology. 9th ed. Philadelphia: W.B. Saunders; 2002.

2. Kassab MM, Cohen RE. The etiology and prevalence of gingival recession. J Am Dent Assoc. 2003;134(2):220-5.

3. Zucchelli G, Mounssif I. Periodontal plastic surgery. Periodontol 2000. 2015;68(1):333-68.

4. Maynard Jr JG, Wilson RDK. Physiologic dimensions of the periodontium significant to the restorative dentist. J Periodontol. 1979;50(4):170-4.

5. Gorman WJ. Prevalence and etiology of gingival recession. J Periodontol. 1967;38(4):316-22.

6. Tugnait A, Clerehugh V. Gingival recession—its significance and management. J Dent. 2001;29(6):381-94.

7. Patel M, Nixon P, Chan M-Y. Gingival recession: Part 1. Aetiology and non-surgical management. Br Dent J. 2011;211(6):251-4.

8. Chambrone L, Chambrone D, Pustiglioni FE, Chambrone LA, Lima LA. Can subepithelial connective tissue grafts be considered the gold standard procedure in the treatment of Miller Class I and II recession-type defects? J Dent. 2008;36(9):659-71.

9. Jankovic S, Aleksic Z, Milinkovic I, Dimitrijevic B. The coronally advanced flap in combination with platelet-rich fibrin (PRF) and enamel matrix derivative in the treatment of gingival recession: a comparative study. Eur J Esthet Dent. 2010;5(3):260-73.

10. Patel M, Nixon P, Chan M-Y. Gingival recession: part 3. Surgical management using free grafts and guided tissue regeneration. Br Dent J. 2011;211(8):353-8.

11. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. J Periodontol. 2009;80(2):244-52.

12. Eren G, Atilla G. Platelet-rich fibrin in the treatment of localized gingival recessions: a split-mouth randomized clinical trial. Clin Oral Investig. 2014;18(8):1941-8.

13. Roccuzzo M, Bunino M, Needleman I, Sanz M. Periodontal plastic surgery for treatment of localized gingival recessions: a systematic review. J Clin Periodontol. 2002;29(suppl 3):178-94.

14. Pini-Prato G, Nieri M, Pagliaro U, Giorgi TS, La Marca M, Franceschi D, et al. Surgical treatment of single gingival recessions: Clinical guidelines. Eur J Oral Implantol. 2014;7(1):9-43.

15. Keceli HG, Kamak G, Erdemir EO, Evginer MS, Dolgun A. The adjunctive effect of platelet-rich fibrin to connective tissue graft in the treatment of buccal recession defects: Results of a randomized, parallel-group controlled trial. J Periodontol. 2015;86(11):1221-30.

16. Chambrone L, Faggion Jr CM, Pannuti CM, Chambrone LA. Evidence-based periodontal plastic surgery: an assessment of quality of systematic reviews in the treatment of recession-type defects. J Clin Periodontol. 2010;37(12):1110-8.

17. Choukroun J, Diss A, Simonpieri A, Girard M-O, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e56-60.

18. Hartshorne J, 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. Int Dent Afr. 2016;6(5):66-82.

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

20. Cortellini P, Pini Prato G. Coronally advanced flap and combination therapy for root coverage. Clinical strategies based on scientific evidence and clinical experience. Periodontol 2000. 2012;59(1):158-84.

21. Serino G, Wennström JL, Lindhe J, Eneroth L. The prevalence and distribution of gingival recession in subjects with a high standard of oral hygiene. J Clin Periodontol. 1994;21(1):57-63.

22. Marini MG, Greghi SLA, Passanezi E, Sant'Ana ACP. Gingival recession: prevalence, extension and severity in adults. J Appl Oral Sci. 2004;12(3):250-5.

23. Susin C, Haas AN, Oppermann RV, Haugejorden O, Albandar JM. Gingival recession: epidemiology and risk indicators in a representative urban Brazilian population. J Periodontol. 2004;75(10):1377-86.

24. Toker H, Ozdemir H. Gingival recession: epidemiology and risk indicators in a university dental hospital in Turkey. Int J Dent Hyg. 2009;7(2):115-20.

25. Lindhe J, Karring T, Lang NP. Clinical Periodontology and Implant Dentistry. 5th ed. Chicester: Wiley-Blackwell; 2003.

26. Rajapakse PS, McCracken GI, Gwynnett E, Steen ND, Guentsch A, Heasman PA. Does tooth brushing influence the development and progression of non-inflammatory gingival recession? A systematic review. J Clin Periodontol. 2007;34(12):1046-61.

27. Khocht A, Simon G, Person P, Denepitiya JL. Gingival recession in relation to history of hard toothbrush use. J Periodontol. 1993;64(9):900-5.

28. Kao RT, Pasquinelli K. Thick versus thin gingival tissue: A key determinant in tissue response to disease and restorative treatment. J Calif Dent Assoc. 2002;30(7):521-6.

29. Abraham S, Deepak K, Ambili R, Preeja C, Archana V. Gingival biotype and its clinical significance–A review. Saudi J Dent Res. 2014;5(1):3-7.

30. Davies R, Downer M, Hull P, Lennon M. Alveolar defects in human skulls. J Clin Periodontol. 1974;1(2):107-11.

31. Khan MA, Tripathi AK, Jaishwal RK, Agrawal P. Single-stage surgical procedure for increasing depth of vestibule and the width of attached gingiva. J Oral Res Rev. 2015;7(2):58-61.

32. Lang NP, Löe H. The relationship between the width of keratinized gingiva and gingival health. J Periodontol. 1972;43(10):623-7.

33. Miyasato M, Crigger M, Egelberg J. Gingival condition in areas of minimal and appreciable width of keratinized gingival. Journal of Clinical Periodontology. 1977;4(3):200-9.

34. Kennedy JE, Bird WC, Palcanis KG, Dorfman HS. A longitudinal evaluation of varying widths of attached gingiva. Journal of Clinical Periodontology. 1985;12(8):667-75.

35. Adibrad M, Shahabuei M, Sahabi M. Significance of the width of keratinized mucosa on the health status of the supporting tissue around implants supporting overdentures. J Oral Implantol. 2009;35(5):232-7.

36. Berglundh T, Lindhe J, Marinell C, Ericsson I, Liljenberg B. Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. Clin Oral Implants Res. 1992;3(1):1-8.

37. Pontoriero R, Tonelli M, Carnevale G, Mombelli A, Nyman S, Lang N. Experimentally induced peri-implant mucositis. A clinical study in humans. Clin Oral Implants Res. 1994;5(4):254-9.

38. Slutzkey S, Levin L. Gingival recession in young adults: occurrence, severity, and relationship to past orthodontic treatment and oral piercing. Am J Orthod Dentofacial Orthop. 2008;134(5):652-6.

39. Robertson P, Walsh M, Greene J, Ernster V, Grady D, Hauck W. Periodontal effects associated with the use of smokeless tobacco. J Periodontol. 1990;61(7):438-43.

40. Lussi A, Schaffner M. Progression of and risk factors for dental erosion and wedge–shaped defects over a 6–year period. Caries Res. 2000;34(2):182-7.

41. Camargo PM, Melnick PR, Kenney EB. The use of free gingival grafts for aesthetic purposes. Periodontol 2000. 2001;27(1):72-96.

42. Bhuvaneswaran M. Principles of smile design. J Conserv Dent. 2010;13(4):225-32.

43. Ahmad I. Anterior dental aesthetics: gingival perspective. Br Dent J. 2005;199(4):195-202.

44. Zucchelli G, Amore C, Sforza N, Montebugnoli L, De Sanctis M. Bilaminar techniques for the treatment of recession-type defects. A comparative clinical study. J Clin Periodontol. 2003;30(10):862-70.

45. Sullivan HC, Atkins JH. Free autogenous gingival grafts. 3. Utilization of grafts in the treatment of gingival recession. Periodontics. 1968;6(4):152-60.

46. Miller Jr PD. A classification of marginal tissue recession. Int J Periodontics Restorative Dent. 1985;5(2):8-13.

47. Latha TA, Sudarsan S, Arun K, Talwar A. Root coverage in class I gingival recession defects, combining rotated papillary pedicle graft and coronally repositioned flap, using a micro surgical approach: A clinical evaluation. J Indian Soc Periodontol. 2009;13(1):21-6.

48. Claydon NC. Current concepts in toothbrushing and interdental cleaning. Periodontol 2000. 2008;48(1):10-22.

49. Kleinheinz J, Büchter A, Fillies T, Joos U. Vascular basis of mucosal color. Head Face Med. 2005;1(1):4.

50. Nanci A. Ten Cate's Oral Histology: Development, Structure, and Function. 6th ed. St. Louis: Mosby; 2003.

51. Nugala B, Kumar BS, Sahitya S, Krishna PM. Biologic width and its importance in periodontal and restorative dentistry. J Conserv Dent. 2012;15(1):12-7.

52. Ochsenbein C. Newer concepts of mucogingival surgery. J Periodontol. 1960;31(3):175-85.

53. Van Dyke TE. Pro-resolving mediators in the regulation of periodontal disease. Mol Aspects Med. 2017;58:21-36.

54. Abel MG. Metal-Free Dentistry: Restoration of Congenitally Missing Lateral Incisors [image on the internet] 2005 [cited 2018 Feb 20]. Available from: http://www.dentistrytoday.com/aesthetics/168--sp-35889872.

55. TenCate A. Oral Histology Development, Structure and Function. 4th ed. St. Louis: Mosby; 1994.

56. Bernimoulin J-P, Schroeder H. Changes in the differentiation pattern of oral mucosal epithelium following heterotopic connective tissue transplantation in man. Pathol Res Pract. 1980;166(2-3):290-312.

57. Edel A. Clinical evaluation of free connective tissue grafts used to increase the width of keratinised gingiva. J Clin Periodontol. 1974;1(4):185-96.

58. Bouri Jr A, Bissada N, Al-Zahrani MS, Faddoul F, Nouneh I. Width of keratinized gingiva and the health status of the supporting tissues around dental implants. Int J Oral Maxillofac Implants. 2008;23(2):323-6.

59. Frost NA, Mealey BL, Jones AA, Huynh-Ba G. Periodontal biotype: Gingival thickness as it relates to probe visibility and buccal plate thickness. J Periodontol. 2015;86(10):1141-9.

60. Kan JY, Morimoto T, Rungcharassaeng K, Roe P, Smith DH. Gingival biotype assessment in the esthetic zone: visual versus direct measurement. Int J Periodontics Restorative Dent. 2010;30(3):237-43.

61. Vandana KL, Gupta I. The relation of gingival thickness to dynamics of gingival margin position pre-and post-surgically. J Indian Soc Periodontol. 2016;20(2):167-73.

62. Singh J, Rathod VJ, Rao PR, Patil AA, Langade DG, Singh RK. Correlation of gingival thickness with gingival width, probing depth, and papillary fill in maxillary anterior teeth in students of a dental college in Navi Mumbai. Contemp Clin Dent. 2016;7(4):535-8.

63. Penmetsa GS, Supriya MS, Doraiswamy DC. Correlation of width of zone of keratinized tissue and gingival tissue thickness with periodontal status in anterior teeth. J Evolution Med Dent Sci [Internet]. 2016 [cited 2017 15 Aug]; 5(47). Available from: https://jemds.com/data_pdf/1_1_Sai%20Supriya.pdf.

64. Egreja AMC, Kahn S, Barceleiro M, Bittencourt S. Relationship between the width of the zone of keratinized tissue and thickness of gingival tissue in the anterior maxilla. Int J Periodontics Restorative Dent. 2012;32(5):573-9.

65. Hwang D, Wang H-L. Flap thickness as a predictor of root coverage: a systematic review. J Periodontol. 2006;77(10):1625-34.

66. American Academy of Periodontology. Chicago IL: AAP Connect; 2017 [cited 2017 1 Oct]. Available from: https://members.perio.org/libraries/glossary/search?executeSearch=true&ProductList=Announcement%2cBlog%2cCommunity%2cEgroup%2cCalendarEvent%2cGlossary%2cNavigation%2cLibrary%2cLibraryEntry&SearchTerm=periodontal+plastic+surgery&SearchMatch=all.

67. Miller Jr PD, Allen EP. The development of periodontal plastic surgery. Periodontol 2000. 1996;11(1):7-17.

68. Miller Jr PD. Regenerative and reconstructive periodontal plastic surgery. Mucogingival surgery. Dent Clin North Am. 1988;32(2):287-306.

69. Prato GP, Clauser C, Cortellini P. Periodontal plastic and mucogingival surgery. Periodontol 2000. 1995;9(1):90-105.

70. Patel M, Nixon P, Chan M-Y. Gingival recession: part 2. Surgical management using pedicle grafts. Br Dent J. 2011;211(7):315-9.

71. Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. J Periodontol. 1985;56(12):715-20.

72. Bouchard P, Malet J, Borghetti A. Decision-making in aesthetics: root coverage revisited. Periodontol 2000. 2001;27(1):97-120.

73. Nelson SW. The subpedicle connective tissue graft: A bilaminar reconstructive procedure for the coverage of denuded root surfaces. J Periodontol. 1987;58(2):95-102.

74. Raetzke PB. Covering localized areas of root exposure employing the “envelope” technique. J Periodontol. 1985;56(7):397-402.

75. Gholami GA, Saberi A, Kadkhodazadeh M, Amid R, Karami D. Comparison of the clinical outcomes of connective tissue and acellular dermal matrix in combination with double papillary flap for root coverage: A 6-month trial. Dent Res J (Isfahan). 2013;10(4):506-13.

76. Schlee M, Ghanaati S, Willershausen I, Stimmlmayr M, Sculean A, Sader RA. Bovine pericardium based non-cross linked collagen matrix for successful root coverage, a clinical study in human. Head Face Med [Internet]. 2012 [cited 2014 Oct 5]; 8(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3311137/.

77. Ramachandra SS, Rana R, Reetika S, Jithendra K. Options to avoid the second surgical site: a review of literature. Cell Tissue Bank. 2014;15(3):297-305.

78. Moss ML. Phylogeny and comparative anatomy of oral ectodermal-ectomesenchymal inductive interactions. J Dent Res. 1969;48(5):732-7.

79. Santosh ABR, Jones TJ. The epithelial-mesenchymal interactions: insights into physiological and pathological aspects of oral tissues. Oncol Rev [Internet]. 2014 [cited 2015 10 Nov]; 8(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4419607/.

80. Karring T, Östergaard E, Löe H. Conservation of tissue specifically after heterotopic transplantation of gingiva and alveolar mucosa. J Periodontal Res. 1971;6(4):282-93.

81. Edel A, Faccini JM. Histologic changes following the grafting of connective tissue into human gingiva. Oral Surg Oral Med Oral Pathol. 1977;43(2):190-5.

82. Karring T, Lang N, Löe H. The role of gingival connective tissue in determining epithelial differentiation. J Periodontal Res. 1975;10(1):1-11.

83. Hill MW, Mackenzie IC. The influence of differing connective tissue substrates on the maintenance of adult stratified squamous epithelia. Cell Tissue Res. 1984;237(3):473-8.

84. Mackenzie IC, Hill MW. Connective tissue influences on patterns of epithelial architecture and keratinization in skin and oral mucosa of the adult mouse. Cell Tissue Res. 1984;235(3):551-9.

85. Hill MW, Mackenzie IC. The influence of subepithelial connective tissues on epithelial proliferation in the adult mouse. Cell Tissue Res. 1989;255(1):179-82.

86. Mackenzie I, Binnie W. Recent advances in oral mucosal research. J Oral Pathol. 1983;12(6):389-415.

87. Hartshorne J, 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. Int Dent Afr. 2016;6(5):34-50.

88. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):37-44.

89. Du Toit J, Gluckman H, Salama M. Platelet-rich fibrin (PRF): a growth factor-rich biomaterial. Part 1–the platelet concentrates milieu & review of the literature. Int Dent Afr.5(5):62-74.

90. Ehrenfest DMD, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte-and platelet-rich fibrin (L-PRF). Trends Biotechnol. 2009;27(3):158-67.

91. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants. 1999;14(4):529-35.

92. Kawase T, Okuda K, Wolff LF, Yoshie H. Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol. 2003;74(6):858-64.

93. Pradeep A, Rao NS, Agarwal E, Bajaj P, Kumari M, Naik SB. Comparative evaluation of autologous platelet-rich fibrin and platelet-rich plasma in the treatment of 3-wall intrabony defects in chronic periodontitis: a randomized controlled clinical trial. J Periodontol. 2012;83(12):1499-507.

94. Huang L-H, Neiva RE, Soehren SE, Giannobile WV, Wang H-L. The effect of platelet-rich plasma on the coronally advanced flap root coverage procedure: a pilot human trial. J Periodontol. 2005;76(10):1768-77.

95. Rodella LF, Favero G, Boninsegna R, Buffoli B, Labanca M, Scari G, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. Microsc Res Tech. 2011;74(8):772-7.

96. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, et al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol. 2014;40(6):679-89.

97. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):45-50.

98. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):51-5.

99. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology. 9th ed. Philadelphia: Elsevier Saunders; 2012.

100. Mammoto T, Jiang A, Jiang E, Mammoto A. Platelet rich plasma extract promotes angiogenesis through the angiopoietin1-Tie2 pathway. Microvasc Res. 2013;89:15-24.

101. Tuan T-L, Song A, Chang S, Younai S, Nimni ME. In VitroFibroplasia: Matrix Contraction, Cell Growth, and Collagen Production of Fibroblasts Cultured in Fibrin Gels. Exp Cell Res. 1996;223(1):127-34.

102. Jankovic S, Aleksic Z, Klokkevold P, Lekovic V, Dimitrijevic B, Barrie Kenney E, et al. Use of platelet-rich fibrin membrane following treatment of gingival recession: a randomized clinical trial. Int J Periodontics Restorative Dent. 2012;32(2):e41-50.

103. Sculean A, Gruber R, Bosshardt DD. Soft tissue wound healing around teeth and dental implants. J Clin Periodontol. 2014;41(Suppl 15):S6-22.

104. Cairo F, Rotundo R, Miller Jr PD, Pini Prato GP. Root coverage esthetic score: a system to evaluate the esthetic outcome of the treatment of gingival recession through evaluation of clinical cases. J Periodontol. 2009;80(4):705-10.

105. Rotundo R, Nieri M, Mori M, Clauser C, Pini Prato G. Aesthetic perception after root coverage procedure. J Clin Periodontol. 2008;35(8):705-12.

106. Rosetti EP, Marcantonio RAC, Rossa Jr C, Chaves ES, Goissis G, Marcantonio Jr E. Treatment of gingival recession: comparative study between subepithelial connective tissue graft and guided tissue regeneration. J Periodontol. 2000;71(9):1441-7.

107. Fürhauser R, Florescu D, Benesch T, Haas R, Mailath G, Watzek G. Evaluation of soft tissue around single-tooth implant crowns: the pink esthetic score. Clin Oral Implants Res. 2005;16(6):639-44.

108. Kerner S, Katsahian S, Sarfati A, Korngold S, Jakmakjian S, Tavernier B, et al. A comparison of methods of aesthetic assessment in root coverage procedures. J Clin Periodontol. 2009;36(1):80-7.

109. Cairo F, Nieri M, Cattabriga M, Cortellini P, De Paoli S, De Sanctis M, et al. Root coverage esthetic score after treatment of gingival recession: an interrater agreement multicenter study. J Periodontol. 2010;81(12):1752-8.

110. Zucchelli G, Mele M, Mazzotti C, Marzadori M, Montebugnoli L, De Sanctis M. Coronally advanced flap with and without vertical releasing incisions for the treatment of multiple gingival recessions: a comparative controlled randomized clinical trial. J Periodontol. 2009;80(7):1083-94.

111. Allen AL. Use of the supraperiosteal envelope in soft tissue grafting for root coverage. II. Clinical results. Int J Periodontics Restorative Dent. 1994;14(4):303-16.

112. Zabalegui I, Sicilia A, Cambra J, Gil J, Sanz M. Treatment of multiple adjacent gingival recessions with the tunnel subepithelial connective tissue graft: a clinical report. Int J Periodontics Restorative Dent. 1999;19(2):199-206.

113. Zuhr O, Fickl S, Wachtel H, Bolz W, Hurzeler M. Covering of gingival recessions with a modified microsurgical tunnel technique: case report. Int J Periodontics Restorative Dent. 2007;27(5):457-63.

114. Zuhr O, Rebele SF, Schneider D, Jung RE, Hürzeler MB. Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivative for root coverage: a RCT using 3D digital measuring methods. Part I. Clinical and patient-centred outcomes. J Clin Periodontol. 2014;41(6):582-92.

115. Burkhardt R, Lang NP. Coverage of localized gingival recessions: comparison of micro-and macrosurgical techniques. J Clin Periodontol. 2005;32(3):287-93.

116. Montevecchi M, Moreschi A, Gatto MR, Checchi L, Checchi V. Evaluation of clinical effectiveness and subjective satisfaction of a new toothbrush for postsurgical hygiene care: a randomized split-mouth double-blind clinical trial. ScientificWorldJournal [Internet]. 2015 [cited 2018 Feb 21]; 2015. Available from: https://www.hindawi.com/journals/tswj/2015/828794/.

117. Nield-Gehrig JS. Fundamentals of periodontal instrumentation & advanced root instrumentation. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2008.

118. Ryan M. Clinical attachment level change as an outcome measure for therapies that slow the progression of periodontal disease. J Int Acad Periodontol. 2005;7(4 Suppl):162-71.

119. Bhatia G, Kumar A, Khatri M, Bansal M, Saxena S. Assessment of the width of attached gingiva using different methods in various age groups: A clinical study. J Indian Soc Periodontol. 2015;19(2):199-202.

120. Jamieson S. Likert scales: how to (ab) use them. Med Educ. 2004;38(12):1217-8.

121. Allen IE, Seaman CA. Likert scales and data analyses. Qual Prog. 2007;40(7):64-5.

122. Zuhr O, Hürzeler M. Plastic-Esthetic Periodontal and Implant Surgery: A Microsurgical Approach. Surrey: Quintessence Publishing; 2012.

123. Zuhr O, Rebele S, Thalmair T, Fickl S, Hürzeler M. A modified suture technique for plastic periodontal and implant surgery - the double-crossed suture. Eur J Esthet Dent. 2009;4(4):338-47.

124. Ehrenfest DMD. How to optimize the preparation of leukocyte-and platelet-rich fibrin (L-PRF, Choukroun's technique) clots and membranes: introducing the PRF Box. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110(3):275-8.

125. Hirsch A, Brayer L, Shapira L, Goldstein M. Prevention of gingival recession following flap debridement surgery by subepithelial connective tissue graft: Consecutive case series. J Periodontol. 2004;75(5):757-61.

126. Zucchelli G, Mele M, Stefanini M, Mazzotti C, Marzadori M, Montebugnoli L, et al. Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: a comparative randomized-controlled clinical trial. J Clin Periodontol. 2010;37(8):728-38.

127. Agudio G, Nieri M, Rotundo R, Franceschi D, Cortellini P, Pini Prato G. Periodontal conditions of sites treated with gingival-augmentation surgery compared to untreated contralateral homologous sites: a 10-to 27-year long-term study. J Periodontol. 2009;80(9):1399-405.

128. Chambrone L, Pannuti CM, Tu Y-K, Chambrone LA. Evidence-based periodontal plastic surgery. II. An individual data meta-analysis for evaluating factors in achieving complete root coverage. J Periodontol. 2012;83(4):477-90.

129. Cheung WS, Griffin TJ. A comparative study of root coverage with connective tissue and platelet concentrate grafts: 8-month results. J Periodontol. 2004;75(12):1678-87.

130. Ai D, Yang J, Fan J, Zhao Y, Song X, Shen J, et al. Augmented reality based real-time subcutaneous vein imaging system. Biomed Opt Express. 2016;7(7):2565-85.

131. Manohar S, Vaartjes SE, van Hespen JC, Klaase JM, van den Engh FM, Steenbergen W, et al. Initial results of in vivo non-invasive cancer imaging in the human breast using near-infrared photoacoustics. Opt Express. 2007;15(19):12277-85.

132. Chambrone L, Sukekava F, Araújo MG, Pustiglioni FE, Chambrone LA, Lima LA. Root-coverage procedures for the treatment of localized recession-type defects: a Cochrane systematic review. J Periodontol. 2010;81(4):452-78.

133. Bouchard P, Etienne D, Ouhayoun J-P, Nilvéus R. Subepithelial connective tissue grafts in the treatment of gingival recessions. A comparative study of 2 procedures. J Periodontol. 1994;65(10):929-36.

134. Dell N, Vaidyanathan V, Medhi I, Cutrell E, Thies W. "Yours is better!" participant response bias in HCI. In: Proceedings of the SIGCHI Conference on Human Factors in Computing Systems [Internet]. 2012 [cited 2018 Feb 20]:[1321-30 pp.]. Available from: https://www.microsoft.com/en-us/research/wp-content/uploads/2016/02/CHI2012-Dell-ResponseBias-proc.pdf.

135. Hujoel P, Baab D, DeRouen T. The power of tests to detect differences between periodontal treatments in published studies. J Clin Periodontol. 1992;19(10):779-84.

136. Koletsi D, Fleming PS, Seehra J, Bagos PG, Pandis N. Are sample sizes clear and justified in RCTs published in dental journals? PLoS One. 2014;9(1):e85949.

137. Harris RJ. Creeping attachment associated with the connective tissue with partial-thickness double pedicle graft. J Periodontol. 1997;68(9):890-9.

138. Goldman HM, Cohen DW. Periodontal Therapy. 5th ed. St. Louis: C. V. Mosby Co; 1973.

139. Goldman HM, Isenberg G, Shuman A. The gingival autograft and gingivectomy. J Periodontol. 1976;47(10):586-9.

140. Bell LA, Valluzzo TA, Garnick JJ, Pennel BM. The presence of “creeping attachment” in human gingiva. J Periodontol. 1978;49(10):513-7.

141. Agudio G, Nieri M, Rotundo R, Cortellini P, Pini Prato G. Free gingival grafts to increase keratinized tissue: a retrospective long-term evaluation (10 to 25 years) of outcomes. J Periodontol. 2008;79(4):587-94.

142. Matter J, Cimasoni G. Creeping attachment after free gingival grafts. J Periodontol. 1976;47(10):574-9.

143. Lee Y-M, Kim JY, Seol Y-J, Lee Y-K, Ku Y, Rhyu I-C, et al. A 3-year longitudinal evaluation of subpedicle free connective tissue graft for gingival recession coverage. J Periodontol. 2002;73(12):1412-8.

144. Miller PD. Root coverage grafting for regeneration and aesthetics. Periodontol 2000. 1993;1(1):118-27.

CHAPTER 9

9 APPENDICES

9.1 Appendix 1: Patient Information Sheet and Consent Form

Good day!

This is an information document for patients invited to participate in a research project.

The title of this research project is **"Comparing clinical outcomes of connective tissue grafts to platelet rich fibrin in gingival recession treatment".**

This form has two parts:

1. Information Sheet (to share information about the research with you)
2. Participant informed consent form (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form.

PART 1: Information Sheet

Introduction

I, Dr. Fatima Peer, am conducting research on a new technique to treat gum recession. The results obtained in this study will contribute towards a Masters research report and is a requirement to my completing an MSc (Dent) degree. In this study, I want to compare a new type of tissue regrowth material; platelet rich fibrin to the common technique. You were selected as a possible participant in this study because you have the type of gum recession that is most likely to show good results after the operation.

You are invited to participate in this study. Participation in this study is voluntary and no person will be advantaged or disadvantaged for choosing to participate or not participate in this study. The information sheet will provide you with the details of this study as well as what is required of the participant. You do not have to decide today whether or not you will participate in this study. Let me know within two (2) weeks of your decision. My contact number is 0116467560. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me.

Purpose of the research

Gum recession is a common problem that exposes a tooth's root surface. The common technique uses tissue from the palate (roof of the mouth). Using this type of tissue has a risk of complications and can produce unfavourable aesthetic results. The new type of material that I want to test is platelet rich fibrin. Platelet rich fibrin is a substance found in blood that can stimulate bone and soft tissue (like gums) to regrow. This membrane is easily prepared from a small amount of your own blood and can eliminate the potential risks of grafting tissue from the palate. Its preparation is also simple and inexpensive and can reduce the costs of this type of oral surgery. It also has the potential to produce a more superior aesthetic result than the common technique. The purpose of this research is to find out if this new material is better than or at least equal to the common technique used to treat gum recession.

What is involved in this research?

If you volunteer to participate in this study, the study will first be explained to you. You will need to sign a consent form. You will then have to be available to have the surgery to treat your gum recession. You will need to have follow-up appointments to monitor healing. These appointments will be scheduled for 2, 8, 12, 16 and 24 weeks after your surgery.

Before surgery, you will have a full dental examination and photographs of your mouth will be taken. I will ensure that your face will not be in any of the photographs and no-one will be able to identify you from the photographs. A cleaning will be done in preparation for the surgery. At the 24 week appointment, a full dental examination and photographs will be taken again to compare the before and after results.

Procedures

What is involved in the surgical procedure?

Your gum recessions will be treated with 2 types of treatments. One side of your mouth will receive the common treatment (control) and the other side will receive the new material (test).

I, Dr Fatima Peer, am the Researcher and will not be performing the operation. My supervisor, Dr Mohangi, will perform the operation. He is a specialist Periodontist and specialises in gum surgery. I will analyse and report on the data collected.

The common treatment:

Under local anaesthesia (your mouth will be numb), the surgical area will be prepared. A thin gum flap will be created and the root of the tooth being treated will be cleaned. A thin strip of gum will be taken from the roof of your mouth. You will be given a plastic to wear on your top jaw to protect the open wound during healing. The strip of gum will be placed at the base of the exposed tooth and covered with the gum flap and stitched together.

The test procedure:

Just before surgery, a small amount of your blood (2 teaspoons) will be collected from your arm using a syringe and needle. This blood will be used to make a thin membrane that will be used as the graft. Any leftover blood will be destroyed.

As described above, under local anaesthesia, the surgical area will be prepared. A thin gum flap will be created and the root of the tooth being treated will be cleaned. The membrane that was made from your blood will be placed at the base of the exposed tooth and covered with the gum flap and stitched together.

After surgery, there will be some pain in the recession area and in the roof of your mouth as it heals. The pain will be managed with pain killers and the plastic plate you will wear to protect the roof of your mouth will also help manage the pain in this area.

Bleeding is a risk of surgery and can occur in the roof of your mouth. If this happens, continue wearing the plastic plate as this plate will apply pressure to stop the bleeding and contact me or Dr Mohangi immediately. Bleeding at the surgical area is less likely as stiches will protect the gum, but if bleeding does occur call me or Dr Mohangi and come to the Wits Dental School (during office hours) or to the outpatient (OPD) clinic at the Charlotte Maxeke Hospital (after hours).

Our numbers are Dr Fatima Peer: 011 646 7560 / 079 527 4957
Dr Mohangi 011 488 4886 / 083 777 1771

After 2 weeks, I will ask you to return for your first follow-up appointment. At this appointment, I will assess healing and the stitches will be removed. I will give you oral hygiene instructions and show you how to keep the surgical area clean. You will be expected to maintain a high standard of oral hygiene through the length of the study. You will be expected to brush, floss and use a mouthwash. This is important to ensure the best possible results from the surgery. I will ask you to schedule appointments for 8, 12, 16 and 24 weeks. I will assess healing and remove any plaque in the area. . At the 24 week (6 month) appointment, I will carry out a full dental examination and take photographs again.

After this appointment, I will ask you to complete a short questionnaire to determine your satisfaction/dissatisfaction with the aesthetic results. It will take approximately 10 minutes to complete the questionnaire.

Participant selection

You are invited to participate in this study because the gum recession that you have is most likely to show good results after the operation.
There will be 16 people taking part in this study.
The procedures will be done at the Wits Dental School. A fee of R305 will be paid by the participant.
There will be no financial compensation to anyone who chooses to take part in this study.

Time needed for each appointment:

- First appointment – 1 hour
- Surgical appointment – 2 hours
- 2 week follow-up appointment – 30 minutes
- 8, 12, 16, week follow-up appointment -30 minutes
- 24 week appointment – 1 hour

Voluntary Participation
Your participation in this research is entirely voluntary and refusal to participate will involve no penalty or loss of benefits. It is your choice whether to participate or not. You may change your mind later and withdraw from the study at any time without penalty or loss of benefits. You will not need to answer any questions should you not wish to. It is your choice and all of your rights will always be respected.

Risks and complications
Some patients do not respond successfully to gum surgery. Sometimes there is no change to the gum recession.

There are some complications that may result from the surgery and/or any drugs used. These complications may include, but are not limited to, infection; bleeding; swelling; pain; temporary discoloration of the face; increase tooth looseness; tooth sensitivity to hot, cold, sweet or acidic foods; shrinkage of the new gum upon healing, resulting in elongation of some teeth and greater spaced between some teeth. Allergic reactions and accidental swallowing or inhaling of foreign matter are also possible. The duration of complications cannot be determined, and complications may be irreversible. Should any complications arise, they will be managed accordingly at the Wits Dental School.

The success of gum surgery can be affected by medical conditions, dietary and nutritional problems, smoking, alcohol consumption, clenching and grinding of teeth, inadequate oral hygiene, and medications that you may be taking.

Benefits
The purpose of gum surgery is to create an amount of attached gum tissue that is wide enough to cover the exposed root surfaces, to improve aesthetics, to allow easier plaque removal, to reduce tooth sensitivity and pain, to prevent root decay and to prevent the possibility of further gum recession.

Although there is no guarantee that treatment will be successful, in most cases the treatment should provide benefit in reducing the cause of your gum condition and should produce healing.

Confidentiality
Any information that is obtained in connection with this study and that can be identified with you will remain confidential. Absolute confidentiality cannot be guaranteed.

I will not be sharing the identity of those participating in the research. The information that I collect from this research project will be kept confidential. Any information about you will have a number on it instead of your name keeping you anonymous. Information about you that will be collected during the research will be locked away in cabinet and no-one but the researchers will be able to see it. Organisations or persons that may inspect and/or copy my research records for quality assurance and data analysis include groups such as the Research Ethics Committee and my supervisors.

Sharing the Results
All participants will be given important information on the study while involved in the project and after the results are available. The results will be available in a short (1page) summary to all participants approximately 6 months after the data has been collected. My research findings may be shared through publications.

Who to Contact

Researcher contact details:
If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact me Dr. Fatima Peer on 011 646 7560 / 079 527 4957.

You may also contact me or Dr Mohangi should you experience any complications after surgery and during the healing period.
Dr Mohangi's contact numbers are: 011 488 4886 / 083 777 1771

Research ethics committee:
If you have any complaints or problems that you wish to report, contact the Research Ethics Committee administrator, Professor Peter Cleaton-Jones on 011 717 2301.

PART II: Patient informed consent form

I have read the information above, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I understand that there are risks associated with the procedure. I understand that I can withdraw from participating in this study at any point without penalty or loss of benefits. I consent voluntarily to participate as a participant in this study.

Print Name of Participant _____

Signature of Participant _____

Date _____

Time _____

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Time _____

9.2 Appendix 2: Case Report Form

CASE REPORT FORM

Protocol number: M150506

Comparing clinical outcomes of connective tissue grafts to platelet rich fibrin in gingival recession treatment

Participant number: _ _ _

Participant number: _ _ _

Date of visit: _____

Demography:

Sex: M / F

Date of birth: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

Initial of Principal Investigator (PI) _____

Initial of Sub Investigator (SI) _____

Participant number: _ _ _

Medical History:

	Yes	**No**
Does the participant have any significant medical history?		

If yes, what medical conditions has the participant been diagnosed with?

	Past	**Present**	
Diagnosis		**Controlled**	**Active**

	Yes	**No**
Does the participant have any allergies?		

If yes, what allergies does the participant have?

_____

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

Inclusion Criteria:

	YES	**NO**
· 2 contralateral/bilateral Miller's class I or II lesions		
· 18 years old		
· Good systemic health		
· Good periodontal health **see periodontal chart*		
· Non-smoker		
· PI ≤ 20 %		

Any 'no' response in the above section, disqualifies this participant from the study.

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

Exclusion criteria:

	YES	**NO**
· Miller's class III or IV recession lesions		
· Pregnancy		
· Bleeding disorders - uncontrolled		
· Furcation involvement - related to maxillary 1st premolars		
· Previous periodontal surgery - related to treatment of the recession lesions in the areas of interest		

Any 'yes' response in the above section, disqualifies this participant from the study.

_____

We certify that this participant meets the study selection criteria to the best of our knowledge.

Signature of PI: _____ Date of signature: _____

Signature of SI: _____ Date of signature: _____

Participant number: _ _ _

	YES	**NO**
Informed consent		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

Pre-surgical appointment

Date: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

	YES	**NO**
Review medical history		
Scale and polish		
Plaque control instructions		
Impressions		
Photographs		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

Surgical appointment

Date: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

	YES	**No**
Review medical history		

Measure clinical parameters:

	Control Site	**Test site**	**YES**	**NO**
· Probing depth (mm)				
· Recession depth (mm)				
· Recession width (mm)				
· Clinical attachment level (mm)				
· Keratinised tissue width (mm)				
· Gingival thickness (mm)				

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

	YES	**NO**
Randomisation		
Open envelope just before surgery		

Which quadrant is assigned to the control or test procedure?

	Control	**Test**
1st quadrant		
2nd quadrant		
3rd quadrant		
4th quadrant		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

Procedure

	YES	**NO**
Control:		
· Local anaesthetic : type		
· Tunnel flap		
· Root conditioning		
· Palatal connective tissue graft		
· Sutures		
Test:		
· PRF preparation just before surgery		
· Immediately centrifuged at 1500 rpm for 8 min		
· PRF membrane created with metal box		
· Local anaesthetic		
· Tunnel flap		
· Root conditioning		
· PRF membrane placement		
· Sutures		
Photographs during procedure		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

Post-surgery protocol

	YES	**NO**
· Paracetamol 500mg -1g every 4-6 hours for 7 days		
· Ibuprofen 200mg 3 X dly for 3 days		
· 0.2% chlorhexidine gluconate mouthwash; 15ml BD		
· Instruct participant on modified brushing technique for surgical area		
· Set up 2 week recall appointment		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

2 week recall appointment

Date: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

	YES	**NO**
Review medical history		
· Remove sutures		
· Supragingival plaque removal		
· Assess healing _____		
· Oral hygiene motivation		
· Photographs		
· Set up 8 week (from date of surgery) recall appointment		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

8 week recall appointment

Date: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

	YES	**No**
Review medical history		

Measure clinical parameters:

	Control Site	**Test site**	**YES**	**NO**
· Probing depth (mm)				
· Recession depth (mm)				
· Recession width (mm)				
· Clinical attachment level (mm)				
· Keratinised tissue width (mm)				
· Gingival thickness (mm)				

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

	YES	**NO**
Healing _____		
Supragingival plaque removal		
Oral hygiene motivation		
Photographs		
Set up 12 week (from date of surgery) recall appointment		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

12 week recall appointment

Date: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

	YES	**No**
Review medical history		

Measure clinical parameters:

	Control Site	**Test site**	**YES**	**NO**
· Probing depth (mm)				
· Recession depth (mm)				
· Recession width (mm)				
· Clinical attachment level (mm)				
· Keratinised tissue width (mm)				
· Gingival thickness (mm)				

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

	YES	**NO**
Healing _____		
Supragingival plaque removal		
Oral hygiene motivation		
Photographs		
Set up 16 week (from date of surgery) recall appointment		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

16 week recall appointment

Date: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

	YES	**No**
Review medical history		

Measure clinical parameters:

	Control Site	**Test site**	**YES**	**NO**
· Probing depth (mm)				
· Recession depth (mm)				
· Recession width (mm)				
· Clinical attachment level (mm)				
· Keratinised tissue width (mm)				
· Gingival thickness (mm)				

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

	YES	**NO**
Healing _____		
Supragingival plaque removal		
Oral hygiene motivation		
Photographs		
Set up 24 week (from date of surgery) recall appointment		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

24 week recall appointment

Date: _____

Vital signs:

Sitting blood pressure: _____ mmHg

Sitting pulse: _____/ min

	YES	**No**
Review medical history		

Measure clinical parameters:

	Control Site	**Test site**	**YES**	**NO**
· Probing depth (mm)				
· Recession depth (mm)				
· Recession width (mm)				
· Clinical attachment level (mm)				
· Keratinised tissue width (mm)				
· Gingival thickness (mm)				

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

	YES	**NO**
Healing _____		
Supragingival plaque removal		
Photographs		
Participant questionnaire		

Initial of PI _____

Initial of SI _____

Participant number: _ _ _

Adverse event

	YES	**NO**
Did the participant experience any adverse event during the study period?		

If 'yes' complete the table below

Event	**Was event serious?**	**Start date**	**End date**	**Related to the surgery**	**Action taken**	**Outcome**
1	**Yes* No**			**Yes* No**		
2	**Yes* No**			**Yes* No**		
3	**Yes* No**			**Yes* No**		

***explain further**

Initial of PI _____ Initial of SI _____

Participant number: _ _ _

Notes on adverse events

Initial of PI _____ Initial of SI _____

9.3 Appendix 3: Clinical Checklist

Protocol activities to be completed	Procedure appointment (Day)			Recall appointments - (weeks after procedure)				
	-14	-7	0	2	8	12	16	24
Medical history	X							
Vital signs: BP and Pulse	X							
Periodontal examination	X							
Inclusion and exclusion criteria		X						
Informed consent		X						
Randomisation		X	X					
Scale and Polish		X						
Plaque control Instructions		X						
Impressions		X						
Photographs		X	X	X	X	X	X	X
Probing depth measurements			X		X	X	X	X
Recession depth measurements			X		X	X	X	X
Recession width measurements			X		X	X	X	X
Clinical attachment level measurements			X		X	X	X	X
Keratinised tissue width measurements			X		X	X	X	X
Gingival thickness measurements			X		X	X	X	X
Surgical procedures : control and test			X					
Prescribing medication			X					
Setting up recall appointments			X	X	X	X	X	
Suture removal				X				
Supragingival plaque removal				X	X	X	X	X
Healing assessment				X	X	X	X	X
Oral hygiene motivation				X	X	X	X	X
Participant questionnaire								X
Adverse event				X	X	X	X	X

9.4 Appendix 4: Patient Questionnaire

Participant Questionnaire

Dear Participant

This questionnaire will assess your satisfaction or dissatisfaction of the results of the surgery done to treat your gum recession. Kindly take a few minutes of your time to complete this form.

Complete the table by rating your experience using the scale below:

1 – Much worse than before

2 – Slightly worse than before

3 – No change

4 – Slight improvement

5 – Noticeable improvement

	Common treatment	New treatment
level of gum recession		
dentine sensitivity		
gum colour		
gum shape		

Are you satisfied with the overall aesthetic result?

_____

_____

Do you have any further information you would like to share?

_____

_____

Thank you again for your time and co-operation.

9.5 Appendix 5: Patient demographics and allocation of patient and pair numbers

Patient Demographics					
Patient number	Pair	Age	Gender	Tooth	Control (C) or Test (T)
A	A1	69	M	13	C
				23	T
B	B2	66	M	23	C
				13	T
C	C3	29	F	14	C
				24	T
D	D4	40	F	35	C
				45	T
E	E5	41	F	14	C
				24	T
E	E6	41	F	45	C
				35	T
E	E7	41	F	44	C
				34	T
F	F8	47	M	15	C
				45	T
F	F9	47	M	14	C
				24	T
F	F10	47	M	13	C
				23	T
F	F11	47	M	34	C
				44	T

9.6 Appendix 6: Ethical Clearance Certificate.

R14/49 Fatima Peer

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150506

NAME: (Principal Investigator)	Fatima Peer
DEPARTMENT:	Oral Health
PROJECT TITLE:	Comparing Clinical Outcomes of Connective Tissue Grafts to Pletelet Rich Fribin in Gingival Recession Treatment
DATE CONSIDERED:	29 May 2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr G U Mohangi
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	17/07/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

Date 16-07-2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

9.7 Appendix 7: Good Clinical Practise certificates.

Pfizer Investigator Training Program

Certificate of Training Completion

This is to certify that

FATIMA PEER

DP0087254

has successfully completed the

INVESTIGATOR TRAINING PROGRAM WORKSHOP

16 hours of instruction on 18-19 April 2015, Sandton

SAMA CPD Accreditation Ref. MDB015/249/02/2015; 11 Clinical; 6 Ethics

The workshop is based on principles of ICH-GCP and includes the following topics:

- The Drug Development Process
- Planning and preparation
- Recruitment and enrollment
- In-trial procedures
- Safety in clinical trials
- Monitoring, audits, inspections, and publication
- SA GCP 2006, SA ETHICS IN HEALTH RESAERCH, HPCSA guidelines and other guidelines

Course Leader: _____

Thanyani N Ramuthaga, *Med.Sci. & Pr.Sci.Nat.*

Sr. Director Compliance Oversight Africa & Middle East

Pfizer Investigator Training Program

GOOD CLINICAL PRACTICE

Basic Course

Dr GU Mohangi

DP 0039055

attended the course and achieved 98% for the assessment

2 & 3 September 2015

Venue: Wits Health Consortium, 8 Blackwood Ave, PARKTOWN

Facilitator: Lesley Burgess

SACRA GCP Registration No: SACRA/GCP/74/2015

The Health Professions Council of South Africa approved CPD reference is as follows:

MD808/027/01/2015 Activity No: 33528 Category: 1B Points: 11
MD808/028/01/2015 Activity No: 33531 Category: 2L Points: 4 Ethics

This ICH E6 GCP Investigator Site Training meets the Minimum Criteria for ICH GCP Investigator Site Personnel Training identified by TransCelerate BioPharma as necessary to enable mutual recognition of GCP training among trial sponsors

COURSE CONTENT:

Drug Development Process
Historical Review of GCP
South African GCP
Regulatory process in South Africa
Study Documents
Patient Recruitment and Retention
Informed Consent
Investigational Product
Safety Reporting
Responsibility of the Study Team
Standard Operating Procedures
Laboratory Issues
Monitoring
Audits

academic advance
A division of Wits Health Consortium (Pty) Ltd

GENERAL MANAGER –TRAINING

Date of Issue: 7 September 2015

TRAINING COORDINATOR

Contact: 011 274 9200 or training@witshealth.co.za

9.8 Appendix 8: Tabulation of PD measurements.

Pair		0 weeks				8 weeks				12 weeks				16 weeks				24 weeks			
		MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave
A1	C	2	2	2	2,00	3	3	3	3,00	2	2	2	2,00	3	2	3	2,67	2	2	2	2,00
	T	2	2	2	2,00	3	3	2	2,67	2	2	2	2,00	2	1	1	1,33	2	2	2	2,00
B2	C	3	2	1	2,00	4	4	3	3,67	3	1	2	2,00	3	1	3	2,33	2	2	2	2,00
	T	3	3	4	3,33	2	2	1	1,67	2	2	2	2,00	2	3	2	2,33	3	3	3	3,00
C3	C	2	2	2	2,00	1	1	1	1,00	2	2	2	2,00	2	2	2	2,00	2	2	2	2,00
	T	2	2	2	2,00	2	2	1	1,67	3	2	1	2,00	3	3	2	2,67	3	2	2	2,33
D4	C	1	1	1	1,00	1	1	2	1,33	Patient did not comply with these recall appointments											
	T	1	1	2	1,33	2	1	2	1,67												
E5	C	2	2	2	2,00	2	2	2	2,00	2	2	2	2,00	2	2	1	1,67	2	2	2	2,00
	T	2	1	2	1,67	2	2	2	2,00	3	2	3	2,67	3	1	2	2,00	2	2	2	2,00
E6	C	2	2	2	2,00	2	2	3	2,33	1	1	2	1,33	2	2	3	2,33	2	2	3	2,33
	T	2	2	3	2,33	3	2	3	2,67	2	3	2	2,33	2	1	2	1,67	2	2	2	2,00
E7	C	3	1	3	2,33	3	1	2	2,00	1	1	2	1,33	2	1	2	1,67	2	1	2	1,67
	T	2	2	2	2,00	1	2	1	1,33	1	1	2	1,33	1	1	1	1,00	2	2	2	2,00
F8	C	2	2	2	2,00	2	1	1	1,33	2	1	1	1,33	2	1	2	1,67	2	2	2	2,00
	T	1	2	2	1,67	3	1	2	2,00	3	2	2	2,33	3	2	2	2,33	2	2	3	2,33
F9	C	2	1	2	1,67	2	2	2	2,00	2	2	2	2,00	2	2	2	2,00	2	2	2	2,00
	T	2	2	1	1,67	2	1	2	1,67	2	1	1	1,33	2	1	2	1,67	3	1	2	2,00
F10	C	2	2	2	2,00	3	3	2	2,67	2	3	3	2,67	3	2	3	2,67	2	3	3	2,67
	T	2	2	2	2,00	8	8	2	6,00	7	5	1	4,33	6	5	1	4,00	5	5	4	4,67
F11	C	2	2	2	2,00	1	2	2	1,67	2	2	2	2,00	2	2	1	1,67	4	2	2	2,67
	T	2	2	2	2,00	2	2	2	2,00	3	2	2	2,33	2	2	2	2,00	2	2	3	2,33
Ave		2,00	1,82	2,05		2,45	2,18	1,95		2,35	1,95	1,90		2,45	1,85	1,95		2,40	2,15	2,35	
St Dv		0,53	0,50	0,65		1,47	1,53	0,65		1,27	0,94	0,55		1,00	0,99	0,69		0,82	0,81	0,59	

9.9 Appendix 9: Tabulation of RD measurements

Patient		0 weeks				8 weeks				12 weeks				16 weeks				24 weeks			
		MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave
A1	C	4	4	3	3,67	3	2	2	2,33	3	3	1	2,33	2	2	2	2,00	2	3	2	2,33
	T	3	3	2	2,67	4	4	2	3,33	3	2	1	2,00	3	3	1	2,33	3	3	1	2,33
B2	C	3	3	2	2,67	1	2	2	1,67	0	1	2	1,00	0	1	2	1,00	1	2	1	1,33
	T	3	4	2	3,00	0	1	1	0,67	1	1	0	0,66	0	1	0	0,33	0	2	2	1,33
C3	C	0	1	1	0,67	1	1	1	1,00	0	0	0	0,00	0	1	1	0,67	0	1	0	0,33
	T	0	1	1	0,67	0	1	1	0,67	0	0	1	0,33	0	0	1	0,33	0	1	1	0,67
D4	C	1	3	1	1,67	0	1	0	0,33	Patient did not comply with these recall appointments											
	T	1	2	1	1,33	1	2	1	1,33												
E5	C	1	2	1	1,33	1	2	2	1,67	1	2	2	1,67	0	1	1	0,67	2	1	1	1,33
	T	1	1	1	1,00	1	1	1	1,00	0	1	1	0,67	0	1	1	0,67	0	1	1	0,67
E6	C	1	2	1	1,33	0	1	1	0,67	1	2	1	1,33	3	2	2	2,33	3	2	2	2,33
	T	2	2	1	1,67	1	2	0	1,00	0	2	0	0,66	2	2	0	1,33	1	2	1	1,33
E7	C	0	3	1	1,33	0	2	2	1,33	1	2	1	1,33	1	2	2	1,67	0	2	1	1,00
	T	1	2	1	1,33	0	2	1	1,00	1	2	1	1,33	0	2	1	1,00	1	2	1	1,33
F8	C	2	2	1	1,67	2	2	1	1,67	2	2	2	2,00	2	3	1	2,00	1	2	1	1,33
	T	1	3	1	1,67	0	2	2	1,33	0	2	2	1,33	0	2	2	1,33	1	2	0	1,00
F9	C	2	4	1	2,33	1	2	1	1,33	2	4	2	2,67	2	4	1	2,33	2	4	1	2,33
	T	1	1	2	1,33	1	2	2	1,67	0	2	2	1,33	0	2	2	1,33	0	2	2	1,33
F10	C	0	2	0	0,67	2	4	2	2,67	3	4	2	3,00	1	4	0		1	3	0	1,33
	T	1	3	1	1,67	1	2	1	1,33	1	2	1	1,33	1	2	1	1,33	1	3	2	2,00
F11	C	3	3	2	2,67	3	3	1	2,33	3	3	1	2,33	2	3	0	1,67	2	3	1	2,00
	T	1	2	0	1,00	2	1	2	1,67	1	3	2	2,00	1	2	1	1,33	1	2	1	1,33
Ave		1,45	2,41	1,23		1,14	1,91	1,32		1,05	2,00	1,25		1,00	2,00	1,10		1,10	2,15	1,10	
St Dv		1,14	0,96	0,69		1,13	0,87	0,65		1,08	1,08	0,72		1,08	1,03	0,72		0,97	0,81	0,64	

9.10 Appendix 10: Tabulation of RW measurements.

Patient		0 weeks	8 weeks	12 weeks	16 weeks	24 weeks
A1	C	7	6	6	6	6
	T	7	7	6	7	6
B2	C	5	5	4	4	4
	T	5	3	4	4	4
C3	C	3	4	0	3	0
	T	4	4	4	2	3
D4	C	4	3	Patient did not comply with these recall appointments		
	T	5	4			
E5	C	4	4	4	3	4
	T	3	3	3	3	3
E6	C	3	3	4	3	3
	T	4	4	3	3	4
E7	C	3	4	4	3	3
	T	4	4	4	4	3
F8	C	5	5	5	4	5
	T	4	4	5	3	5
F9	C	5	4	5	5	5
	T	5	4	5	4	5
F10	C	4	5	5	6	5
	T	6	6	6	6	6
F11	C	5	5	5	5	5
	T	4	5	4	4	4
Ave		4,50	4,36	4,30	4,10	4,15
St Dv		1,14	1,05	1,34	1,33	1,42

9.11 Appendix 11: Tabulation of KTW measurements

Pair		0 weeks	8 weeks	12 weeks	16 weeks	24 weeks
A1	C	2	5	3	2	2
	T	2	8	2	2	2
B2	C	3	2	5	1	2
	T	4	4	6	5	5
C3	C	4	5	6	5	6
	T	4	5	5	5	6
D4	C	0,5	1	Patient did not comply with these recall appointments		
	T	0,5	1			
E5	C	4	1	1	2	1
	T	3	3	3	3	5
E6	C	2	2	1	3	3
	T	2	2	2	2	2
E7	C	1	1	1	1	1
	T	2	2	2	2	2
F8	C	4	4	4	5	4
	T	2	1	4	2	2
F9	C	3	4	5	5	4
	T	6	7	4	5	5
F10	C	4	3	3	4	4
	T	4	0	2	2	3
F11	C	3	1	4	2	1
	T	3	1	2	2	3
Ave		2,86	2,86	3,25	3,00	3,15
St Dv		1,35	2,14	1,62	1,49	1,63

9.12 Appendix 12: Tabulation of GT measurements

Pair		0 weeks	24 weeks
A1	C	1,00	1,50
	T	0,50	1,00
B2	C	0,50	1,00
	T	1,50	1,00
C3	C	1,00	1,50
	T	1,00	1,00
D4	C	0,50	PDNC
	T	0,50	
E5	C	1,00	1,00
	T	1,00	1,00
E6	C	1,50	1,50
	T	1,00	1,00
E7	C	0,50	0,50
	T	1,00	1,00
F8	C	1,00	2,00
	T	0,50	1,00
F9	C	1,50	2,00
	T	1,50	2,00
F10	C	1,00	1,50
	T	1,00	1,00
F11	C	1,00	1,00
	T	1,00	1,00
Ave		0,95	1,23
St Dv		0,34	0,41

9.13 Appendix 13: Tabulation of CAL measurements.

Patient		0 weeks				8 weeks				12 weeks				16 weeks				24 weeks			
		MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave	MB	B	DB	Ave
A1	C	6	6	5	5,67	6	5	5	5,33	5	5	3	4,33	5	4	5	4,67	4	5	4	4,33
	T	5	5	4	4,67	7	7	5	6,33	5	4	3	4,00	5	4	2	3,67	5	5	3	4,33
B2	C	6	5	3	4,67	5	6	5	5,33	3	2	4	3,00	3	2	5	3,33	3	4	3	3,33
	T	6	7	6	6,33	2	3	2	2,33	3	3	2	2,67	2	4	2	2,67	3	5	5	4,33
C3	C	2	3	3	2,67	2	2	2	2,00	2	2	2	2,00	2	3	3	2,67	2	3	2	2,33
	T	2	3	3	2,67	2	3	2	2,33	3	2	2	2,33	3	3	3	3,00	3	3	3	3,00
D4	C	2	4	2	2,67	1	2	2	1,67	Patient did not comply with these recall appointments											
	T	2	3	3	2,67	3	3	3	3,00												
E5	C	3	4	3	3,33	3	4	4	3,67	3	4	4	3,67	2	3	2	2,33	4	3	3	3,33
	T	3	2	3	2,67	3	3	3	3,00	3	3	4	3,33	3	2	3	2,67	2	3	3	2,67
E6	C	3	4	3	3,33	2	3	4	3,00	2	3	3	2,67	5	4	5	4,67	5	4	5	4,67
	T	4	4	3	3,67	4	4	3	3,67	2	5	2	3,00	4	3	2	3,00	3	4	3	3,33
E7	C	3	4	4	3,67	3	3	4	3,33	2	3	3	2,67	3	3	4	3,33	2	3	3	2,67
	T	3	4	3	3,33	1	4	2	2,33	2	3	3	2,67	2	3	2	2,33	3	4	3	3,33
F8	C	4	4	3	3,67	4	3	2	3,00	4	3	3	3,33	4	4	3	3,67	3	4	3	3,33
	T	2	5	3	3,33	3	3	4	3,33	3	4	4	3,67	3	4	4	3,67	3	4	3	3,33
F9	C	4	5	3	4,00	3	4	3	3,33	4	6	4	4,67	4	6	3	4,33	4	6	3	4,33
	T	3	3	3	3,00	3	3	4	3,33	2	3	3	2,67	2	3	4	3,00	3	3	4	3,33
F10	C	2	4	2	2,67	5	7	4	5,33	5	7	5	5,67	4	6	3	4,33	3	6	3	4,00
	T	3	5	3	3,67	9	10	3	7,33	8	7	2	5,67	7	7	2	5,33	6	8	6	6,67
F11	C	5	5	4	4,67	4	5	3	4,00	5	5	3	4,33	4	5	1	3,33	6	5	3	4,67
	T	3	4	2	3,00	4	3	4	3,67	4	5	4	4,33	3	4	3	3,33	3	4	4	3,67
Ave		3,45	4,23	3,23		3,59	4,09	3,32		3,50	3,95	3,15		3,50	3,85	3,05		3,50	4,30	3,45	
St Dv		1,37	1,11	0,92		1,92	1,93	1,04		1,54	1,54	0,88		1,32	1,31	1,15		1,19	1,30	0,94	

9.14 Appendix 14: Tabulation of PES scores

Pair		0 weeks	24 weeks
A1	C	11	12
	T	10	12
B2	C	8	8
	T	11	12
C3	C	14	13
	T	14	14
D4	C	14	PDNC
	T	14	
E5	C	11	12
	T	14	14
E6	C	14	14
	T	14	14
E7	C	14	14
	T	14	14
F8	C	8	11
	T	11	11
F9	C	10	10
	T	9	10
F10	C	10	10
	T	11	10
F11	C	11	13
	T	12	13

9.15 Appendix 15: Tabulation of questionnaire answers

Pair	Q1 Level of gum recession- Control	Q1 Level of gum recession- Test	Q2 dentine sensitivity- Control	Q2 dentine sensitivity- Test	Q3 gum colour-Control	Q3 gum colour-Test	Q4 gum shape-Control	Q4 gum shape-Test
A1	No change	No change	Slight improvement	Slight improvement	No change	No change	No change	No change
B2	Slight improvement	Slight improvement	No change	No change	Noticeable improvement	Noticeable improvement	No change	No change
C3	No change	No change	Noticeable improvement	No change	No change	No change	No change	No change
D4	Did not complete the study							
E5	Slightly worse than before	Slight improvement	Slightly worse than before	Noticeable improvement	No change	No change	No change	No change
E6	Slightly worse than before	Slight improvement	Slightly worse than before	Noticeable improvement	No change	No change	No change	No change
E7	Slightly worse than before	Slight improvement	Slightly worse than before	Noticeable improvement	No change	No change	No change	No change
.8	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement
F9	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement
F10	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement
F11	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement	Noticeable improvement
Count (N)	10	10	10	10	10	10	10	10
Not answered	1	1	1	1	1	1	1	1
Total	**11**	**11**	**11**	**11**	**11**	**11**	**11**	**11**

9.16 Appendix 16: Turnitin Report

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