A CLINICAL & LABORATORY INVESTIGATION OF THE USE OF LEUKOCYTE AND PLATELET-RICH FIBRIN (L-PRF) IN ALVEOLAR RIDGE PRESERVATION AND MANAGEMENT OF PERI-IMPLANT DEFECTS

A thesis submitted to the University of Dublin, Trinity College Dublin, in fulfilment of the requirements for the degree of Doctor of Philosophy by

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DECLARATION

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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SUMMARY

Leukocyte-and platelet-rich fibrin (L-PRF) is an autologous platelet concentrate that has become widely used in dentistry over the past decade to enhance natural healing following a variety of periodontal treatment procedures. L-PRF may be considered an optimised blood clot: it concentrates the majority of platelets and leukocytes from whole blood and enmeshes them within a fibrin framework that allows for a gradual release of cells and growth factors over a period of up to 14 days *in vitro.* This framework also provides stabilisation to assist wound healing and regeneration at the surgical site. However, the development of new protocols for its production has resulted in multiple closely related PRF products that may have different biologic activities, and which may present differences in mechanical strength and susceptibility to chemical degradation. Furthermore, while many operators have utilised PRF variants in combination with other regenerative materials to enhance outcomes, few studies have compared the use of PRF alone with such composite materials that combine PRF with a bone substitute material.

In the project herein, a systematic review of the literature encompassing eight databases was conducted to compare the use of L-PRF alone versus L-PRF in combination with any bone substitute material for alveolar ridge preservation (ARP) at extraction sockets at any intraoral location. The literature was searched for randomised and non-randomised controlled clinical trials and prospective cohort studies of ≥3 months in duration, and pre-clinical animal studies, published in English, up to 30 June 2023. This search initially identified 7554 articles from the databases, from which 2835 articles were included for screening. Seven articles remained for retrieval for full-text review, of which four randomised controlled trials were included for analysis in the final review. A risk of bias analysis revealed that three of the four studies presented high risk (n = 1) or some concerns (n=2) of bias. The available studies demonstrated high heterogeneity in terms of the biomaterial combined with PRF, although three of the four available studies utilised alloplastic materials derived from calcium phosphate. Heterogeneity regarding tooth location, evaluation methods and outcomes was also noted between studies, rendering meta-analysis impractical. Based on the limited evidence available, the combination of PRF with another graft material does not appear to consistently offer statistically significant advantages in terms of ridge preservation or mean radiographic bone

density values compared to the use of PRF alone. One study provided data on pain and suggested that when PRF was combined with hydroxyapatite, patients had reduced discomfort and improved soft tissue healing compared with PRF used alone. The available literature does not provide information with regard to other patient-reported outcomes and only one study provided limited data on histologic findings. Additional clinical studies that more comprehensively describe the PRF preparation protocol are necessary, to elucidate whether the combination of PRF with a graft material may enhance extraction socket healing and alveolar ridge preservation.

L-PRF Block is a composite graft that combines L-PRF in equal proportions with a bovine xenograft material. Following subsequent addition of liquid fibrinogen to this homogeneous mix, rapid coagulation of the liquid fibrinogen ensues, which in turn binds the fibrin-graft mix into a block form that can be used for grafting and which has demonstrated positive results for lateral ridge augmentation. We conducted a randomised controlled clinical trial to compare the use of L-PRF and L-PRF Block in alveolar ridge preservation (ARP) of non-molar extraction sockets in 35 systemically healthy adult patients. Clinical and radiographic follow-up was conducted at 4 months with additional clinical measurements recorded at 5-6 months in 21 subjects who proceeded to dental implant placement. Although the use of L-PRF Block resulted in a trend towards improved maintenance of horizontal and vertical ridge dimensions, differences in clinical or radiographic parameters between the grafting protocols were not statistically significant. Neither protocol resulted in adverse events, while soft tissue healing after both protocols was favourable. Patient-reported outcomes did not reveal statistically significant differences between protocols, in terms of average pain, maximum pain intensity or use of analgesia. In general, pain was most severe, but generally of moderate intensity, at day 1, and tapered quickly afterwards. This study provides the first documented assessment of L-PRF Block for ARP. Our results reinforce the findings of our systematic review that question the adjunctive benefit of composite grafts compared to L-PRF used alone. However, a larger study, possibly with the adjunctive use of CBCT for volumetric analysis of dimensional ridge changes may be indicated.

An *in vitro* study was next utilised to compare enzymatic degradation of L-PRF and A-PRF membranes. Blood harvested from eight systemically healthy adult donors was used to prepare eight PRF membranes per donor. Half of the membranes were prepared using the L-PRF protocol (2700 rpm for 12 minutes; RCF-clot 408g) while

the remaining membranes represented Advanced-Platelet Rich Fibrin (A-PRF), a variant produced using a lower speed of centrifugation over a longer time period (1500 rpm for 14 minutes; RCF-clot 126g). Membranes were placed in a solution of 2µg/mL of plasmin in Dulbecco's modified Eagle's medium (DMEM), where membrane degradation was assessed at fixed time points (1d, 3d, 6d, 7d , 10 d, 14d) over a period of 14 days. DMEM-alone served as a control medium. L-PRF clots were larger at baseline but demonstrated significantly greater dimensional loss overall compared to A-PRF, most notably evidenced by changes in mean membrane weight, and visual signs of degradation. Membranes in test media demonstrated greater mean percentage weight loss than membranes in control solution. In test medium, L-PRF membranes showed a statistically significant greater proportional reduction in membrane weight at every time point and overall compared to A-PRF. Mean loss of membrane weight from baseline-7 days was significantly greater than from 7-14 days, regardless of membrane type. Changes in membrane length and width were generally minor in nature but A-PRF showed fewer instances of change in these dimensions. Visual signs of degradation were infrequently observed (17/64 membranes) with no significant difference between grafting protocols. Plasmin in DMEM appears to be a suitable medium to compare membrane degradation. Overall, while both membrane types exhibited significant loss of membrane weight, the majority of membranes maintained their physical characteristics in plasmin for at least 14 days. Further studies may change test media more frequently and/or compare degradation of alternative PRF variants.

Finally, a longitudinal analysis on the treatment of patients with peri-implantitis (26 patients, 86 implants) prospectively evaluated the medium-to-long term impact of peri-implantitis treatment upon clinical parameters and magnetic resonance frequency analysis (MRFA) in the form of implant stability quotient (ISQ) values. Four different treatment modalities were used, based on the clinical judgment of operators. These treatment modalities were: 1) Non-surgical therapy as a monotherapy; 2) Non-surgical therapy followed by open-flap debridement approach; 3) Non-surgical therapy followed by open flap debridement and the application of L-PRF only (plugs and/or membranes) and 4) Non-surgical therapy followed by open flap debridement and the application of a hard tissue regenerative material - with or without L-PRF. Measurements of clinical parameters (including probing depth, mucosal recession, bleeding, suppuration, plaque and width of keratinised tissue), in addition to implant stability measurements in the form of ISQ, were recorded at each

implant site at baseline and repeated at 6, 12 and 24-36-months following treatment. Over this follow-up period, all four treatment modalities resulted in improved clinical and radiographic peri-implant parameters. However, implant stability post-treatment, as indicated by the recorded ISQ scores, remained stable. MRFA cannot be recommended as an adjunctive diagnostic tool to periodontal and radiographic measurements to demonstrate post-operative implant stability following the treatment of peri-implant disease. The results from L-PRF treated sites indicate that this material may be considered a potential material for regeneration at peri-implantitis sites.

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LIST OF ABBREVIATIONS

AAP	American Academy of Periodontology
ABP	Alveolar bone proper
APC	Autologous platelet concentrate
a.k.a	Also known as
A-PRF	Advanced platelet-rich fibrin
ARP	Alveolar ridge preservation
BBT	Buccal bone thickness
BMP	Bone morphogenetic protein
BOP	Bleeding on probing
CAL	Clinical attachment loss
CAM	Chorioallantoic membrane
CBCT	Cone beam computed tomography
CCT	Controlled clinical trial
CEJ	Cemento-enamel junction
CGF	Concentrated growth factor
CI	Confidence interval
CP	Calcium phosphate
CPD	Cigarettes per day
CPG	Clinical practice guideline
CS	Calcium sulphate
DBBM	Deproteinised bovine bone mineral
DDUH	Dublin Dental University Hospital

DFDBA	Demineralised freeze-dried bone allograft
EAO	European Academy of Osseointegration
EFP	European Federation of Periodontology
e.g.	*Exempli gratia* (for example)
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
e-PTFE	Expanded polytetraflouroethylene
et al.	*Et alia* (and others)
etc.	*Et cetera* (and the rest)
FDBA	Freeze-dried bone allograft
FDP	Fixed dental prosthesis
FGF	Fibroblast growth factor
GBR	Guided bone regeneration
GF	Growth factor
g-force	Gravitational force
GTR	Guided tissue regeneration
HA	Hydroxyapatite
H-PRF	Horizontal platelet-rich fibrin
i.e.	*Id est* (that is)
IGF	Insulin-like growth factor
I -PRF	injectable platelet-rich fibrin
IQR	Interquartile range
ISQ	Implant stability quotient
L –PRF	Leucocyte and platelet-rich fibrin
MMP	Matrix metalloproteinase
MRFA	Magnetic resonance frequency analysis

NH	Natural healing (post-extraction)
NSS	Not statistically significant
OFD	Open flap debridement
OHIP	Oral health impact profile
OR	Odds ratio
p (p-value)	Probability value
PD	Probing depth
PET	Polyethylene tetraphthalate
PDGF	Platelet-derived growth factor
PDL	Periodontal ligament
PI	Peri-implantitis
PICO	Population, Intervention, Comparison, Outcome
PID	Peri-implant diseases
PIM	Peri-implant mucositis
PPD	Probing pocket depth
PRF	Platelet rich fibrin
PRGF	Plasma rich in growth factors
PRP	Platelet rich plasma
RBC	Red blood cell
RCF	Relative centrifugal force (a.k.a. “g-force”)
RCT	Randomised controlled trial
RR	Relative risk/Risk ratio
sd	Standard deviation
SEM	Scanning electron microscope
SLA	Sandblasted, large-grit, acid-etched
SOP	Suppuration on probing

SPC	Supportive periodontal care
SPIC	Supportive peri-implant care
SS	Statistically significant
TCP	Tricalcium phosphate
TGF-β	Transforming growth factor-Beta
TM	Treatment modality
TNF-α	Tumour necrosis factor- alpha
tPA	Tissue plasminogen activator
T-PRF	Titanium platelet-rich fibrin
uPA	Urokinase plasminogen activator
USH	Unassisted socket healing
VEGF	Vascular endothelial growth factor
WMD	Weighted mean difference

TABLE OF MATHEMATICAL SYMBOLS AND MEASUREMENTS

°C	Degrees Celsius
d	Day
h	hour
K_D	Dissociation constant
kg	Kilogram
min	Minute
mL	Millilitre
mm	Millimetre
rpm	Revolutions per minute
s	seconds
=	Equal to
>	Greater than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
%	Percent

CHAPTER 1: LITERATURE REVIEW

1.1 AUTOLOGOUS PLATELET CONCENTRATES

Autologous platelet concentrates (APCs) are biological products derived from the patient's whole blood via a process of chairside centrifugation. In recent years, APCs have gained significant attention in the medical and dental literature due to their potential to enhance and accelerate healing and to contribute to hard and soft tissue regeneration.

The first generation of APCs revolved around two main products, termed Platelet-rich plasma (PRP) and Plasma rich in growth factors (PRGF). These products had relatively complex production processes that required the use of an anticoagulant at time of blood harvest and a platelet activator prior to clinical use, meaning they could not be regarded as entirely autologous products. The additional clinical time and associated expense involved in preparation called into question the practicality of using these products in dental surgery, especially given their liquid form and short-acting nature.

The second generation of APCs saw the development of platelet-rich fibrin (PRF), an autologous platelet concentrate first described by Choukroun and colleagues (Choukroun et al. (2001). L-PRF can be created through the centrifugation of whole blood, without the need for the use of an anticoagulant at time of collection or any later use of additive agents; this results in a preparation process that is fast, cheap and easy. L-PRF has gained attention in recent years as an approach to accelerate and enhance wound healing in various medical and dental indications. In periodontology, L-PRF has been utilised and documented for indications including, but not limited to, periodontal plastic surgery, alveolar ridge preservation, sinus augmentation and the regenerative treatment of infrabony and furcation defects. However, variations in preparation protocol and inconsistency in its reporting have resulted in some discrepancies and confusion within the literature about the use and clinical outcomes of PRF products.

1.2 DEVELOPMENT OF AUTOLOGOUS PLATELET CONCENTRATES

The early stages of clinical wound healing involve successive and overlapping processes of haemostasis, inflammation and proliferation of healing cells, to stimulate formation and differentiation of tissues that provide increased strength to the wound (Bartold and Ivanovski, 2025, Aukhil, 2000). Platelets dominate in the haemostatic phase of wound healing and release cytokines and growth factors that contribute to the removal of damaged tissue and simultaneously encourage tissue regeneration. Platelet alpha-granules (α-granules) are the principal intracellular storage site of biologically active proteins, producing at least 30 growth factors that demonstrate activity in each stage of healing (Cieslik-Bielecka et al., 2012a). In addition, platelets produce a series of antimicrobial peptides that demonstrate antimicrobial activity *in vitro* (Tang et al., 2002), suggesting a possible direct antimicrobial role for APCs. Unlike pharmacologic agents, given their natural origin platelet proteins are not subject to development of antimicrobial resistance, offering promise for wound management over time (Cieslik-Bielecka et al., 2012a). The concept of producing supra-physiologic doses of platelets to enhance and accelerate wound healing both during and after surgery, in the form of platelet concentrates, was first promoted in dentistry in the late 1990s (Marx et al., 1998).

1.2.1 PLATELET-RICH PLASMA (PRP)

The first generation platelet concentrate product developed for use in dentistry was termed platelet-rich plasma (PRP) (Marx et al., 1998). As developed, creation of PRP involves a multi-step process wherein blood is firstly collected using a cell-separator device that sequentially separates blood over a 20–30 minute period. Platelets in the central PRP layer tend to mix with the upper 1 mm of the red blood cell layer, so this is included in PRP (Marx et al., 1998) and PRP also contains much of the leukocyte content of plasma (Perussolo et al., 2025). Preparation of PRP for clinical use requires a second step wherein an activator is mixed with the

PRP blood layer to stimulate the coagulation process (Marx et al., 1998). This activation step automatically activates alpha granules within platelets to release growth factors, of which platelet-derived growth factor (PDGF) is the dominant growth factor responsible for the effects of PRP (Albanese et al., 2013). The addition of anticoagulant and activator agents may interfere with natural healing and may limit the release of bioactive molecules, creating a natural limitation of action (Cavallo et al., 2016). The use of thrombin leads to a large and immediate release of growth factor that could potentially limit the beneficial effects of using PRP in combination with a graft material (Tsay et al., 2005), while the use of alternative activators may prolong the action of PRP at the surgical site compared to when thrombin is used (Calciolari et al., 2025).

Over time, many different protocols have been documented for the preparation of PRP. Commercial systems differ in terms of amount of blood collection required, the centrifugation cycle, centrifuge and blood tubes used, and the anticoagulant and activators used (Calciolari et al., 2025). The technique for PRP preparation has become simpler as manufacturers have created two-chamber systems that can accommodate both centrifugation cycles in one machine and even one-step centrifugation protocols (Gruber, 2025). Variations in activator components are one of the key features that differentiate current PRP systems and the level of growth factor release and time taken for PRP clot formation can vary significantly depending on the commercial system used for its preparation (Weibrich et al., 2002, Cavallo et al., 2016). There is no consensus regarding the optimal method to activate PRP including which anticoagulant and activator to use (Calciolari et al., 2025) and selection may thus be based on operator preference in many instances (Cavallo et al., 2016). However, multiple classification systems have been proposed to characterise the resulting array of different PRP products and the consequent lack of a consistent form of PRP challenges the ability for clinicians and researchers to determine the effectiveness of PRP (Calciolari et al., 2025).

The gelationous nature of PRP effectively mandates its combination with other biomaterials to be effective for clinical use in regeneration, while the multi-step process makes PRP production technique-sensitive, time-consuming and

expensive for use in the dental surgery, particularly in light of the clinical outcomes obtained (Albanese et al., 2013). The inclusion of bovine thrombin in the preparation process means that PRP is unsuitable for consideration as a truly autologous material, although concerns about a lack of safety data addressed in an early systematic review (Martínez-Zapata et al., 2009).

PRP produces a rapid and significant release of growth factors that can stimulate immune cells but an in vitro comparison with leukocyte-and platelet-rich fibrin (L-PRF) and advanced platelet-rich fibrin (A-PRF) membranes concluded that growth factor release from PRP Is short lasting. Furthermore, total growth factor release over the study period was lower than from L-PRF or A-PRF (Kobayashi et al., 2016). Clinical evidence supporting the use of PRP for dental indications remains low (Gruber, 2025) although one systematic review has concluded that it may have possible benefits in wound healing and regeneration (Stähli et al., 2018).

1.2.2 PLASMA RICH IN GROWTH FACTORS (PRGF)

Plasma rich in growth factors (PRGF) is an evolution of PRP, first described by Anitua and co-workers as “preparation rich in growth factors” (Anitua, 1999). The preparation of PRGF utilised calcium chloride as an activator instead of thrombin, which renders PRGF more truly autologous and also contributes to a more sustained release of growth factors than that seen with PRP (Calciolari et al., 2025). Interestingly, the production protocol for PRGF intentionally eliminated the leukocyte content to avoid the potential pro-inflammatory effects of these cells (Anitua, 1999). Subsequent evolution of the technique and armamentarium used for PRGF preparation reduced the volumes of anticoagulant and activator used, which resulted in higher release of growth factors and a reduced clotting time for PRGF (Calciolari et al., 2025). More recently, commercial centrifuge systems that accommodate the two-centrifugation steps automatically, and PRGF formulations that do not require the use of an activator, have been developed (Calciolari et al., 2025).

Discussing the potential uses of PRGF in medicine, Anitua highlighted its flexibility of form and use within medicine, depending on the extent of activation and/or coagulation of the blood samples and described liquid, clot and membrane forms. In its liquid form, PRGF may be used adjunctively to deliver growth factors during surgery (Anitua, 2006), while the clot or membrane forms are advocated for regenerative procedures (Anitua et al., 2007). In this regard, however, it is notable that a later *in vitro* study comparing the mechanical properties of L-PRF and PRGF membranes, using a universal testing machine, concluded that L-PRF membranes had increased tensile strength and better handling properties than PRGF (Khorshidi et al., 2016).

The potential limitations associated with rapid release of growth factor content from these early blood products were recognised by Anitua and colleagues (Anitua et al., 2007). A combination of PRP with an acidic gelatin (hydrogel) material was found to prolong growth factor release until the hydrogel degraded (Hokugo et al., 2007), while a collagen-PRP scaffold was also developed for orthopaedic use (Murray et al., 2007). While PRGF offered prolonged activity compared to PRP, Dohan Ehrenfest and colleagues highlighted that first-generation products may have inferior characteristics compared to L-PRF. These authors compared the release of three growth factors (TGF-β1, VEGF, and PDGF-AB) from PRGF and L-PRF membranes in culture medium over a 7-day period and observed that PRGF membranes degraded within 5 days, while L-PRF membranes remained intact up to the end of the study period at 7 days. Furthermore, L-PRF membranes released significantly larger amounts of all three growth factors, over a longer period. (Dohan Ehrenfest et al., 2012).

1.2.3 L-PRF – A "SECOND GENERATION" AUTOLOGOUS PLATELET CONCENTRATE

Leukocyte- and platelet-rich fibrin (L-PRF) is inherently biologically different from PRP and PRGF. It may be considered a "second-generation" platelet concentrate (Dohan et al., 2006b) that overcomes many of the limitations experienced with the previous products. It is a completely autologous material and is solid in nature. L-PRF requires a simple blood collection process and preparation technique, making it relatively fast, inexpensive and easy to prepare. The presence of a solid fibrin matrix contributes a supportive effect to the material, potentially reducing the need to combine it with other biomaterials for clinical use. L-PRF may be considered an "optimised blood clot", contributing the majority of platelets and leukocytes from whole blood and incorporating these within a supporting fibrin matrix. As such, L-PRF more closely approaches the concept of an ideal regenerative material for the healing wound site than PRP, as it provides the three classic features identified for regeneration, namely:

- Cells – predominantly in the form of platelets and leukocytes
- Growth factor release/stimulation – to direct angiogenesis and wound healing
- A supporting matrix to provide space maintenance/blood clot stabilisation - in the form of a fibrin clot

Fibrin plays a key role in the recruitment of multiple cell types to the wound site, including neutrophils, monocytes, endothelial cells, and fibroblasts. It also contributes to immune processes involved in granulation tissue formation and angiogenesis (Laurens et al., 2006).

1.2.4 CLASSIFICATION OF APCS

The rapid pace of development in use of autologous platelet concentrates in the first decade of this century led to some confusion as many biologically different products were grouped under umbrella terms of PRP and PRF. Dohan Ehrenfest and colleagues published a classification system to offer clarification and guide standardisation in research publications (Dohan Ehrenfest et al., 2009b). All products of the category are grouped under the general term of "platelet concentrates", whatever their form or cell content within which four families of platelet concentrates were described based on the nature of the material produced (liquid or solid) and the leukocyte and fibrin content of the material. Each product type has a "specific biological imprint" and may contain several marketed products or utilise different customized protocols (Table 1).

Table 1: Classification system for platelet concentrates (Dohan et al. 2009)

	Liquid	**Solid**
No leukocytes	Pure Platelet-rich plasma (P-PRP)	Pure Platelet-rich fibrin (P-PRF)
Leukocytes	Leukocyte and platelet-rich plasma (L-PRP)	Leukocyte and platelet-rich fibrin (L-PRF)

1.3 PREPARATION OF L-PRF CLOTS AND MEMBRANES

1.3.1 PREPARATION OF L-PRF CLOTS

To prepare L-PRF, venous blood is collected in sterile, anticoagulant-free glass tubes and centrifuged immediately following a pre-defined protocol (2700 rpm x 12 minutes; 408g). This process induces activation of platelets that are in contact with the collection tube walls and triggers the physiologic coagulation cascade. Fibrinogen is initially concentrated in the upper part of the collection tube but is subsequently acted upon by circulating thrombin within the tube, creating a fibrin clot. Following centrifugation, three distinct layers can be observed within the blood collection tube (Figure 1):

- Red blood cell (RBC) layer - occupying the lower part of the tube
- Platelet-poor plasma – a small, light coloured fluid layer occupying the top layer
- Fibrin clot – occupying the central layer of the tube contents

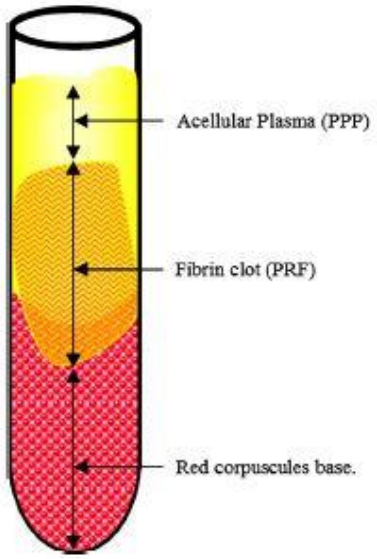

Figure 1. Layers within blood collection tube following L-PRF preparation protocol. (Reproduced from (Dohan et al., 2006c))

The fibrin clot may be removed from the tube with a surgical tweezers, and gently separated from the attached red blood cell layer. The fibrin clot – now termed L-PRF – contains a large amount of fluid. This may be expressed as an exudate by compressing the clot using gauze or, more commonly, in a specially designed box (Xpression box®; (Dohan Ehrenfest, 2010)). The Xpression box® includes a weighted metal plate which flattens the collected L-PRF clots into a standardised 1 mm-thick membrane form. A weighted metal plugger may also be used to create condensed L-PRF clots (“plugs”); the exudate expelled during the creation of plugs and membranes passes through a perforated metal tray and can be collected for later use if required (Dohan Ehrenfest, 2010).

1.3.2. COMPRESSION OF PRF CLOTS INTO MEMBRANES/PLUGS

The initial descriptions of the PRF concept, by Dohan, Choukroun and colleagues (Dohan et al., 2006b), indicated that PRF clots should be squeezed between sheets of cotton gauze to obtain fibrin membrane, and that the resulting fluid that squeezed out (PRF exudate) could be discarded. As discussed separately, it has subsequently been recognised that PRF exudate contains some cells and growth factors, and some researchers have recommended its harvest and use as an adjunct material. In a study evaluating growth factor release from PRF, Su and colleagues made suggestions for optimising PRF. This included preparing the PRF immediately before using it - to avoid wastage of growth factor release - and the potential usefulness of PRF exudate as a source of growth factors, with possible value for mixing with graft materials (Su et al., 2009). Dohan and colleagues also presented an initial discussion on different compression procedures that may be used to create more standardised PRF membranes. Methods included placement of PRF clots in a metal cup to allow exudate to drain gradually, or forcible compression of clots with the under-surface of a metal spoon. However, no significant differences in the architecture of PRF were detected based on the collection tube or the compression technique used (Dohan Ehrenfest et al., 2010a). Alternative methods for membrane preparation such as the two-spoon device (Kobayashi et al., 2012) have also been suggested in the literature. However, the PRF Box approach (commercially referred to as Xpression® box; Intra-Lock® International, Boca Raton, FL, USA), has gained widespread use across

all commercial systems in recent years and is the method recommended for standardised preparation of L-PRF membranes (Quirynen M, 2018). First introduced by Dohan and colleagues (Dohan Ehrenfest, 2010), these authors acknowledged its development by Dr Joseph Choukroun in 2007 to collect and transform multiple L-PRF clots simultaneously into membranes in sterile conditions prior to use. Clots are placed on a perforated metal plate, which allows drainage of serum exudate; this may later be collected underneath the plate. A separate weighted metal tray is applied on top of the clots; this compresses the clots under gravitational force until it meets a fixed stop, resulting in membranes of standardised thickness. The Xpression® box also has cylindrical compression wells and maces (pluggers) to compress the PRF clots into dense PRF plugs (Figure 2).

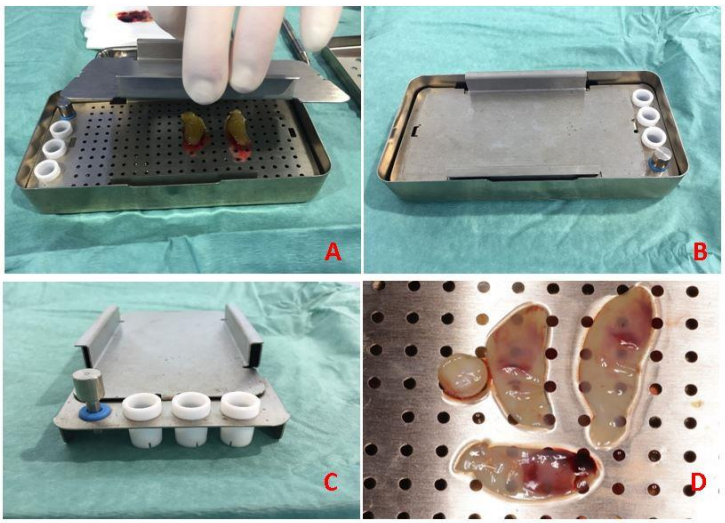

Figure 2: The Xpression® box for preparation of L-PRF membranes and plugs. A: Harvested clots are placed on the perforated metal plate. A weighted metal tray is applied on top. B: Metal plate in situ; this compresses clots under gravitational force to create membranes of standardised thickness C: Formation of L-PRF plugs: Clots are placed into cylindrical compression wells and a weighted metal plugger is applied (left) D: Prepared L-PRF plug (top left) and membranes

1.4 ARCHITECTURE AND CONSTITUENTS OF L-PRF

1.4.1 L-PRF CLOT ARCHITECTURE

Dohan and colleagues investigated the architecture of L-PRF clots using blood collected from 10 healthy volunteers (Dohan Ehrenfest et al., 2010a). Macroscopically, the LPRF clot consists of three main areas (Figure 3):

- An area of red blood cells at the lower end of the clot
- A whitish area in between the red blood cells and the main body of the clot, termed the “buffy coat”
- The main body of the fibrin clot

These authors analysed L-PRF clots using scanning electron microscope (SEM), and noted characteristic features of each layer. L-PRF clots contained approximately 97 per cent of platelets and more than 50 per cent of the leukocytes (primarily lymphocytes) present in whole blood. The “buffy coat” zone is the most cell-rich area of the L-PRF clot (Dohan Ehrenfest et al., 2010a).

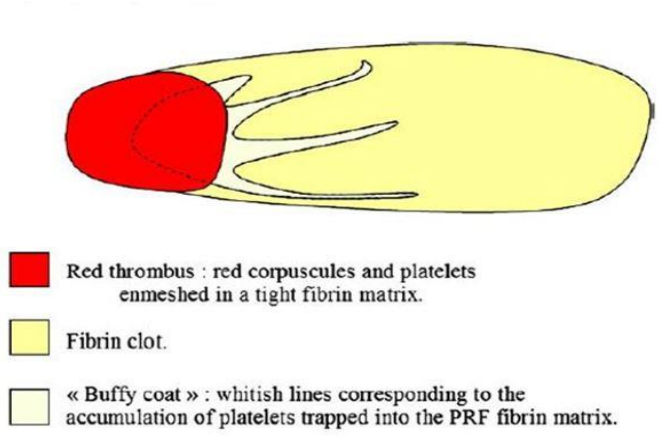

Figure 3 Macroscopic zones within the L-PRF clot. (Reproduced from Dohan et al., 2006b).

Table 2 summarises key knowledge relating to the structural make-up of each zone within the L-PRF clot.

A histomorphometric analysis of clot microstructure revealed that the L-PRF architecture consisted of structural glycoproteins (e.g. fibronectin and vitronectin) and cytokines enmeshed in a fibrin matrix. Dohan and colleagues postulated that the lower thrombin concentrations present in the collection tube during the L-PRF centrifugation process allow the formation of a fine flexible fibrin network that provides elasticity and strength and a scaffold for revascularisation. These authors suggested that the slow polymerisation process facilitates entrapment of cytokines, allowing slow and sustained release of growth factors and cytokines from L-PRF clots during the healing phase (Dohan Ehrenfest et al., 2010a).

Table 2: Overview of L-PRF Clot Structure

	Visible appearance	**SEM**
Red Blood Cells	Red area at one end of clot	▪ Immature fibrin network with enmeshed RBCs
Buffy coat	Whitish "in between" layer	▪ Small leukocytes (mainly lymphocytes) ▪ Platelet aggregation along fibrin strands
Fibrin clot	Yellowish, main body of clot	▪ Highest concentration of platelets and leukocytes in first 1 mm ▪ Essentially all cells within lower half of clot ▪ Near buffy coat, cells evenly distributed and dense ▪ Elsewhere, cells mainly concentrated along "veins" of fibrin, along outer aspects of clot

1.4.2 COMPONENTS OF L-PRF – CELLS, MATRIX, GROWTH FACTORS

L-PRF contains all three of the resources commonly cited in the literature as necessary for regeneration:

- It is a rich source of a variety of blood cells (Dohan Ehrenfest et al., 2010a)
- It is a rich source of growth factors(Dohan Ehrenfest et al., 2012, Kobayashi et al., 2016)
- It provides a supporting fibrin matrix that may provide some stabilisation and support for healing and that also enmeshes cells, allowing their release over a prolonged period at the wound site (Dohan Ehrenfest et al., 2009a).

In considering the effects of L-PRF, it is prudent to contribute the role of each of these constituent parts in turn.

1.4.2.1 The Blood Cells

Blood cells constitute about 45 per cent of blood volume, with the remainder being blood plasma. Erythrocytes (red blood cells) account for the majority of cells, with leukocytes (white blood cells) and thrombocytes (platelets) each accounting for less than one per cent of blood volume (Mescher and Junqueira, 2013).

Since PRF is prepared from whole blood, all of these cellular constituents are present in the blood collection tube, and it is prudent to consider their basic characteristics and roles in healing as a precursor to considering PRF in detail.

Erythrocytes are relatively small (7-8μm) discoid cells with a lifespan in the range of 100-120 days. They serve primarily to transport oxygen and carbon dioxide within the blood (Hughes-Jones et al., 2004).

The relative proportion of red blood cells in the blood is measured by the haematocrit test; this varies between individuals but is generally lower in women and in older individuals. This influences clot formation, as the lower the haematocrit, the larger the L-PRF clots and membranes formed (Quirynen et al, 2023).

Leukocytes are spherical, nucleated cells whose primary function is to protect the body against infection (Mehta and Hoffbrand, 2013). They also contribute to wound healing via the production of cytokines and growth factors (Bartold and Ivanovski, 2025). They may have some natural anti-microbial and anti-pain activity; the putative benefits of L-PRF use at surgical and wound sites has been attributed in part to this. Leukocytes also regulate cell proliferation and differentiation and have been described as the “turntable” around which healing revolves (Bielecki et al., 2012).

Leukocytes comprise a number of different cell types and may be first described using two main subtypes, differentiated by whether granules are present in their cytoplasm (granulocytes) or not present (agranulocytes). Granulocytes consist of three subtypes (neutrophils, eosinophils and basophils) and play a principal role in innate immunity. These subtypes derive their names from their reaction to staining with haematoxylin and eosin, wherein basophils stain blue, eosinophils stain red, and neutrophils stain a neutral pink colour (Hughes-Jones et al., 2004).Neutrophils are the most populous granular leukocyte. They have a relatively short lifespan ranging from approximately 6 hours up to a few days. Neutrophils are recruited to the wound site quickly, where they function to phagocytose bacteria, debris and necrotic tissue to clean the wound and to prevent infection. They produce a large variety of peptides and enzymes, including cytokines and growth factors (Hughes-Jones et al., 2004).

Agranulocytes consist of two cell subtypes, lymphocytes and monocytes. During natural healing, monocytes are recruited to the wound site following the initial activity of neutrophils. Monocytes can further contribute to phagocytosis. In the tissues, they develop into macrophages, which can phagocytose dead neutrophils

and help to clean the wound site. They play a role in formation of granulation tissue and help to induce angiogenesis and osteogenesis, as well as further releasing growth factors (Mescher and Junqueira, 2013, Bartold and Ivanovski, 2025). Quirynen and colleagues highlight that by applying L-PRF at a surgical site, monocytes are delivered more quickly to the "wound", possibly shortening the inflammatory phase of healing and more smoothly moving the wound into a proliferative phase. Macrophages are not present within platelet concentrates but rather are formed through development from L-PRF monocytes once applied at tissue level (Quirynen et al., 2023).

Lymphocytes engage in the immune response by directly acting on cells or through the production of antibodies. They can be short acting or persist for years as a form of immune "memory" (Hughes-Jones et al., 2004). Bielecki and co-workers have indicated that lymphocytes appear to be more concentrated in L-PRF than other leukocytes (Bielecki et al., 2012). While these authors suggested further research to determine the relative actions of leukocytes present in platelet concentrates in wound healing, they did highlight that their contribution to the activity and potential effect on healing of platelet concentrates had been largely overlooked(Bielecki et al., 2012).

Platelets are anuclear structures, whose primary function is to contribute to haemostasis. Their cytoplasm is rich in granules, which are secreted when the platelet is activated (Mehta and Hoffbrand, 2013). The alpha granules are the main intercellular storage of biologically active proteins in platelets, and at least 30 growth factors, active in each stage of healing, have been highlighted (Cieslik-Bielecka et al., 2012b). These authors have also highlighted the potential presence and activity of antimicrobial proteins in platelets (Cieslik-Bielecka et al., 2012b). Naturally, activation of platelets occurs when they contact collagen at an injured wound site. Activation releases cytokines, antimicrobial proteins and growth factors. Cytokines can stimulate migration of cells to the wound site and the proliferation of cells within the fibrin matrix of the clot, which then triggers development of the inflammatory phase of wound healing (Bartold and Ivanovski, 2025).

Table 3 lists the relative density and some other basic characteristics of blood cells.

Table 3: Characteristics of blood cells (collated from (Hughes-Jones et al., 2004, Mehta and Hoffbrand, 2013, Mescher and Junqueira, 2013)

	Red blood cell	**White blood cell**	**Platelet**
Also known as	Erythrocyte	Leukocyte	Thrombocyte
Cells/ µL	4 - 6 x 10^6	4.8 - 10.8 x 10^3	1.5 - 4.0 x 10^5
Density (kg/m^3)	1095 - 1100	1040 - 1065	1055 - 1085
Surface (µm^2)	140	28	330
Radius (µm)	4.0	11.5	5.0 - 7.5
Volume (µm^3)	92	14	200
Blood reference range	4.4 - 5.8 x 10^{12}/L (male) 4.1 - 5.2 x 10^{12}/L (female)	4.0 - 11.0 x 10^9/L	160 - 450 x 10^9/L
Lifespan in blood	100 - 120d	Varies by cell type e.g. Neutrophil $t_{1/2}$ ~ 7h Monocytes $t_{1/2}$ ~ 70h	9 - 12d

1.4.2.2 The Growth Factors

Platelets that are activated within the blood collection tube during L-PRF preparation release high (supra-physiologic) quantities of growth factors from the L-PRF clot. The PRF membrane maintains a slow release of key growth factors over a period of at least 7 days (Dohan Ehrenfest et al., 2009a).

The term growth factors (GFs) denotes a group of polypeptides of approximately 6-45kD, which have a role in controlling biological processes, such as cell growth, proliferation, differentiation and morphogenesis of tissues and organs during embryogenesis, postnatal growth and adulthood (Ghensi, 2016).

L-PRF releases a variety of growth factors active in angiogenesis and stimulation of wound healing, including:

- VEGF (vascular endothelial growth factor)
- PDGF (platelet derived growth factor)
- TGF-β1 (transforming growth factor beta)
- IGF (insulin-like growth factor)
- FGF (fibroblast growth factor)
- EGF (epidermal growth factor)

Many GFs are deposited in the extracellular matrix where they are released during matrix degradation and act as part of a complex network of signals with mutual effects during tissue remodeling and regeneration. The effect of GFs is mediated through surface receptors on the target cells by activating intracellular phosphorylating enzymes, which in turn induce an intracellular signaling pathway. Together with other transcription factors, they activate a set of genes, which then exert the changes in cellular activity or phenotype. In vivo, the effect of GFs is regulated through a complex system of feedback loops (Ghensi, 2016).

1.4.2.2.1 Description & Actions of Growth Factors in L-PRF

Platelet concentrates (PCs) contain a combination of growth factors; the concentration may vary between individual patients and according to the production method. The previously identified key growth factors released from L-PRF membranes are summarized in the following paragraphs.

Platelet-derived growth factor (PDGF)

Five PDGF isoforms have been identified to date (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD (Bartold and Ivanovski, 2025), of which the BB and AB isoforms are particularly notable; these are contained in platelet α-granules, from which they are released after adhesion of platelets to injured sites of vessel walls (Ghensi, 2016)

Multiple cell types produce PDGF, including activated platelets, macrophages and endothelial cells (Quirynen et al., 2022). PDGF functions throughout the healing process, and has key roles in enhancing fibroblast proliferation and synthesis and remodeling of the extracellular matrix (Bartold and Ivanovski, 2025), as well as functioning in wound contraction and angiogenesis (Heldin and Westermark, 1999). PDGF is chemotactic for multiple cells and attracts neutrophils and macrophages to the wound site to amplify healing response and contributing to tissue debridement and granulation tissue formation (Bartold and Ivanovski, 2025).

Transforming Growth Factor- Beta (TGF-β)

The TGF superfamily consists of more than 30 proteins, including TGF-β and some bone morphogenetic proteins (BMPs). Three TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) are primarily associated with wound healing (Bartold and Ivanovski, 2025). TGF-β is released mainly from platelets, as well as from many other cell types including macrophages, fibroblasts and lymphocytes (Bartold and Ivanovski, 2025).

The effect of TGFβ is highly variable and dependent on the type of cells and tissues. TGF–β1 contributes at all stages of wound healing and contributes to inflammation, angiogenesis, re-epithelialization, regeneration of connective tissue and extracellular matrix synthesis (Faler et al., 2006). It supports the proliferation of almost all cell types (Clark, 2001)and regulates a range of cellular activities such as cell migration, cell proliferation and cell differentiation (Ghensi, 2016)

Bone morphogenetic proteins (BMPs) play important roles in bone development and remodeling may be considered the most potent growth factors for promoting bone regeneration (Ghensi, 2016). They can promote the chemotaxis and aggregation of cells into osteogenic sites and facilitate differentiation of precursor cells into osteoblasts. They stimulate deposition of mineralized tissue by osteoblasts (Quirynen et al., 2022) and can also promote angiogenesis, regulate the activity of some growth factors and modify the production of other growth factors (Ghensi, 2016).

Vascular endothelial growth factor (VEGF)

The VEGF family consists of six related polypeptides, of which VEGF-A is most studied (Bartold and Ivanovski, 2025). VEGF is produced by multiple cell types, including platelets, neutrophils, macrophages, fibroblasts and endothelial cells (Bao et al., 2009)and has been cited as the most important regulator of blood vessel formation (Ferrara et al., 2003). VEGF has specific affinity to endothelial cells, and functions to promote proliferation of endothelial cells and their enhanced survival, stimulating the redevelopment of a blood supply (neovascularization) in an injured area. Consequently, VEGF plays a key role in angiogenesis and granulation tissue formation. (Ghensi, 2016)VEGF is also a positive regulator of bone development and stimulates the proliferation and differentiation of osteoblasts (Ghensi, 2016)

Fibroblast growth factor (FGF)

The FGF family has 23 members, of which FGF-2, FGF-7 and FGF-10 are most impactful in wound healing (Bartold and Ivanovski, 2025). They are synthesised by multiple cell types, including keratinocytes, endothelial cells and fibroblasts (Barrientos et al., 2008). FGFs contribute to cell proliferation and migration (Quirynen et al., 2022). FGFs also stimulate angiogenesis and migration of epithelial cells from wound margins to assist in wound contraction (Cao et al., 2003).

Epidermal growth factor (EGF)

EGF is primarily produced by platelets, macrophages and fibroblasts (Quirynen et al., 2022) It is mitogenic for keratinocytes and fibroblasts and contributes to the proliferation and differentiation of keratinocytes (Bartold and Ivanovski, 2025) to assist with wound re-epithelialisation (Tokumaru et al., 2000).

Insulin-like growth factor (IGF)

IGFs are simple peptides that exist in two isoforms: IGF-I and IGF-II and which are primarily secreted by the liver but also produced by macrophages and fibroblasts (Garg, 2018). They are released in high quantities from activated platelets (Miron, 2021). They stimulate glandular activities and promote metabolic and growth activity in many cell and tissue types (Giannobile et al., 1996).They stimulate fibroblast proliferation and collagen synthesis in particular (Quirynen et al., 2022).

1.4.2.2.2. Growth Factor Release from APCs

Research studies evaluating growth factor release from different platelet concentrates have produced somewhat conflicting results. The release of growth factors (PDGF – AA, PDGF – AB, PDGF – BB, TGFB1, VEGF, EGF and IGF) from different platelet concentrates (PRP, L-PRF and Advanced PRF (A-PRF)) was evaluated by Kobayashi and co-workers, who concluded that each platelet concentrate has different release kinetics for growth factors (Kobayashi et al., 2016). Although PRP releases the highest amount of growth factors at early time points (15-60 min), at all later time points, and cumulatively over a 10-day period, both A-PRF and L-PRF demonstrated greater growth factor release and they also released more as a whole over the study period than PRP. Two publications by the research team of Miron concluded that based on its low-speed centrifugation protocol, A-PRF releases significantly higher quantities of growth factors compared to L-PRF *in vitro*, particularly at later time points (Kobayashi et al., 2016, Fujioka-Kobayashi et al., 2017). In contrast, when Dohan Ehrenfest and co-workers produced L-PRF and A-PRF in the IntraSpin® centrifuge and evaluated growth factor release in Dulbecco's modified Eagle's medium, they noted greater release of growth factors from L-PRF. Furthermore, A-PRF clots dissolved in solution in as little as 3 days, while L-PRF clots remained intact in solution and continued to release growth factors up to 7 days. Notably , the conclusions of the Fujioka-Kobayashi paper ((Fujioka-Kobayashi et al., 2017) paper were challenged by another research team, on the basis that the method of L-PRF preparation did not follow its original protocol (Pinto and Quirynen, 2019). The ensuing debate led to consensus on standardised reporting of preparation protocols (Miron et al., 2019d) that should enhance interpretation of research findings in PRF studies. However, it must be noted that Kobayashi and colleagues noted large variability in growth factor concentrations between donors in their study (Kobayashi et al., 2016), and the potential role of individual variation must be considered when comparing studies that evaluate growth factor release. Similarly, it cannot be assumed that results of *in vitro* studies will be reflective of outcomes of *in vivo* studies.

More recently, Wang and colleagues described the patterns of the *in vitro* and ex vivo release of GFs, cytokines, and MMPs from L-PRF using a novel approach (Wang et al., 2022). Whereas previous studies only reported the *in vitro* concentrations of specific GFs using single-analyte, enzyme linked immunosorbent assay (ELISA), this study used multiplex immunoassay to quantify multiple biomarkers released from L-PRF in a single microplate well simultaneously. Blood from 18 systemically healthy individuals was analysed at time points up to 7 days. The participants were also taking part in a clinical trial, wherein wound fluid was sampled using sterile paper strips (Periopaper) from the wound edges of extraction sockets allowed to heal naturally or grafted with L-PRF. Analysis of growth factors and cytokines present in these samples provided the ex-vivo findings. Furthermore, based on the principle that since GFs and cytokines are also produced during the normal wound healing process, the effect of the added L-PRF was estimated by subtracting the values of the natural healing control sites from the L-PRF sites. Levels of GF, cytokine and MMP release were compared between L-PRF exudate and L-PRF membranes. Results showed that the levels of GFs in exudate were much lower than the total amounts released from L-PRF membranes, indicating that very little degranulation from platelets occurs during the clotting/preparation of L-PRF. However, some GFs (PDGF-AA, PDGF-AB/BB, TGF-β1, EGF, and VEGF) were present at higher levels in exudate than in full blood count from the same subjects' plasma, suggesting some molecular content was released from L-PRF after squeezing to flatten it into a membrane (Wang et al., 2022). The exudate might therefore have some use and might not be discarded (e.g. for mixing with graft materials since it contains some GF).

Higher rates of release were found *ex vivo*, indicating that significant amounts of GFs are produced by the local cells within a wound. The authors suggested that the initial release of GFs from L-PRF might serve as a chemoattractant for neutrophils, macrophages, and fibroblasts and then further enhance their levels in a wound. In addition, the local production of GFs also extended the half-life times for some of the GF activity (PDGF-AA, PDGF-AB/BB, and VEGF). Overall, these findings show that the release of growth factors from L-PRF *in vitro* has clinical translation- not only can L-PRF release GFs but it may also promote local host cells to produce more GFs *in vivo*. In that sense, this study provides indirect evidence that the mediators released from L-PRF are bioactive (Wang et al., 2022).

1.4.2.3 The Fibrin Matrix

For many years, it was considered that the properties of platelet concentrates mainly related to the high concentrations of growth factors in their make-up. However, the fibrin architecture itself, and the high leukocyte content of these preparations may play a key role, and growth factors alone cannot be considered the sole actors of these preparations (Cieslik-Bielecka et al., 2012a).

Fibrinogen is a soluble molecule present in large numbers in plasma and in platelet α-granules; it serves as the final substrate of all coagulation reactions and plays a key role in platelet aggregation during haemostasis. Fibrin is the activated form of fibrinogen, a conversion that is mediated by the protease thrombin. This reaction causes fibrin to polymerise; the polymerized fibrin gel represents the initial matrix that guides healing of the wounded site (Dohan et al., 2006b).

In general, fibrin is a fibrillar structure; fibrin gels have been noted to consist of fibrillae assembled in two different architectures, which may influence the nature of the matrix produced.

- Condensed tetra-molecular (bilateral) junctions, as seen in PRP and PRGF – Such junctions are strong and form a rigid fibrin network that provides a strong seal. However, this is not very favourable in limiting cellular migration or trapping cytokines within the matrix.
- Connected tri- molecular (equilateral) junctions – these junctions form in the presence of lower thrombin concentrations and allow a fine and flexible fibrin network that can support cellular migration and enmesh cytokines. This 3-D arrangement also gives elasticity and strength to the fibrin matrix. These are what are seen in PRF (Dohan et al., 2006b).

In an accompanying paper, Dohan Ehrenfest and colleagues constructed 3-dimensional computer-modelling diagrams of PRP and PRF to assist understanding of their fibrin matrix structures. The authors highlighted the presence of structural glycoproteins (e.g. fibronectin) enmeshed in the fibrin matrix of PRF; its slow

polymerization also allows glycanic chains and cytokines to be retained within the fibrin polymers. The authors concluded that PRF would therefore be very close to a natural fibrin thrombus, and that the associated biochemical components can act synergistically to potentiate healing.(Dohan et al., 2006a).

1.5 ANTIMICROBIAL PROPERTIES OF L-PRF

Antimicrobial peptides have been identified from human platelets *in vitro (Tang et al., 2002)*, suggesting a direct antimicrobial role of platelet concentrates. These peptides are also produced by some leukocytes and are a key component of human natural immunity, with a wide spectrum of activity and activities that include initiating bacterial cell lysis, inhibition of fibrinolysis, and inhibiting tissue destruction by bacterial proteases.

In the earlier medical literature, the possible antimicrobial activity of PRP products against wound bacteria was investigated, with its putative antimicrobial effects attributed to platelet activity. However, it has been suggested that some of the healing properties attributed to the PRP products were likely the result of the inclusion of leukocytes in their formulations (Bielecki et al., 2012). Leukocytes have inherent immune and antimicrobial potential but their role in antimicrobial activity may also relate to the central role they play in local regulation of wound healing (Bielecki et al., 2012). More recently, some authors have evaluated the antimicrobial activity of PRF versus oral microbes, as discussed in the following paragraph. It must be recognised that the included studies are not uniform in PRF preparation protocol and bacterial strains used, which may complicate comparison of study results.

In an early investigation, Edelblute and colleagues conducted an *in vitro* study to assess the efficacy of human platelet gel (PRP) against three opportunistic bacterial wound pathogens that included *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The authors concluded that platelet gel supernatants exhibited a significant antibacterial effect against these opportunistic pathogens (Edelblute et al., 2015). Subsequently, Badade and co-workers evaluated the antimicrobial activities of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) against periodontal disease-associated bacteria (*Aggregatibacter actinomycetemcomitans and Porphyromonas. gingivalis*) in blood samples from 10 healthy adult males; while PRP showed a strong zone of inhibition against both bacterial species, PRF did not show inhibition (Badade et al., 2016). However,

Kour compared the antibacterial capacity of PRP, PRF and i-PRF against *A. actinomycetemcomitans* and *P. gingivalis* on inoculated agar plates and found that all three platelet concentrates demonstrated some activity against both species (Kour et al., 2018). The antimicrobial capacity of L-PRF against periodontal pathogens was evaluated by Castro and colleagues (Castro et al., 2019b). Both L-PRF membranes and L-PRF exudate showed clear inhibition against *P. gingivalis*, but the effects were limited for other species tested (*Fusobacterium nucleatum, Prevotella intermedia*), and indeed *A. actinomycetemcomitans* showed an increase in growth in planktonic solution when in contact with L-PRF exudate. The latter observation may be rationalised by the knowledge that human serum is known to enhance the leucotoxic activity of *A. actinomycetemcomitans* (Castro et al., 2019b).

A systematic review noted considerable variation in both the production methods for platelet concentrates and in the bacterial strains tested in the available literature. Overall, all of the platelet concentrates showed some reduction in the growth of microorganisms tested, without showing complete inhibition of microbes. The authors concluded that APCs therefore have a bacteriostatic rather than bactericidal activity (Varshney et al., 2019)

More recently, Mamajiwala and colleagues highlighted that the technical aspects of preparation influence PRF properties including fibrin network, platelet count and antimicrobial activity. Lower centrifugation speed and time can increase platelet concentration and concomitantly the antimicrobial activity of PRF membranes, but conversely leads to a lower density fibrin network pattern (Mamajiwala et al., 2020). In a recent development, investigators have begun to consider PRF as a possible local delivery mechanism for antimicrobial drugs. As a natural biomaterial that demonstrates sustained release of growth factors and physical characteristics that resist quick degradation, PRF offers some natural potential to serve as a conduit for application of antimicrobials locally at wound sites, while potentially avoiding the contentious and safety aspects of systemic antimicrobial use. Polak and co-workers conducted a study to explore the suitability of using PRF as a local delivery system for antimicrobials. Different volumes of metronidazole, clindamycin, or penicillin were directly added to blood

tubes before centrifugation with saline used for comparison of antibacterial activity, while a collagen sponge (Collatape) served as a control carrier for the antibiotics. Addition of antibiotic to PRF resulted in significant inhibition of both an aerobic (*S. aureus*) and anaerobic (*F. nucleatum*) species growth compared to PRF alone, suggesting PRF can bind and serve as a potentially suitable carrier for antimicrobial agents (Polak et al., 2019). More recently, Bennardo and colleagues published a recent "proof of concept" study demonstrating release of antimicrobial drugs from PRF membranes (Bennardo et al., 2023). While PRF membranes did allow release of the antimicrobial drugs at effective concentrations *in vitro*, the study also served to demonstrate the need for additional research on a drug-by-drug basis since notable variation was seen in the PRF: antimicrobial interaction, with vancomycin actually inhibiting PRF formation. Furthermore, while these authors included *S. aureus* among the species tested, indicating potential application at wound sites, no periodontal pathogens were included in their investigations.

1.6 FACTORS AFFECTING PREPARATION OF PRF PRODUCTS

Dohan and colleagues highlighted that the final composition and clinical characteristics of platelet concentrates are influenced by factors that include the speed and time of centrifugation and the g-force (relative centrifugal force, RCF) used (Dohan Ehrenfest et al., 2018). Variations in centrifuge features such as rotor diameter, rotor angulation and rotor radial vibration also influence the final PRF product. Based on a debate in the form of letters to the editor of *Journal of Periodontology* (Miron et al., 2019b), it became clear that key thought leaders in the science of L-PRF differed in opinion regarding how data relating to g-force should be measured and communicated in the dental literature. Consequently, these authors collaborated to highlight factors necessary to ensure standardised reporting of relative centrifugal forces in future studies relating to platelet-rich fibrin. Researchers are encouraged to report the centrifuge they used, the dimensions and angulation of its rotor, the revolutions per minute (rpm) and time used and the composition and size of tubes used to produce PRF. These factors collectively allow calculation of RCF values, which should also be published and therein allow direct comparison of the product used (Miron et al., 2019d).

This discussion highlights that platelet concentrates described in the literature under the same name may not always represent the same product. Indeed a study by the Dohan group, which tested PRF products created using a standardised time and rpm protocol in various centrifuges, concluded that only one centrifuge (IntraSpin® by Intra-Lock®) resulted in a product that truly represented L-PRF when characterised using light microscopy and SEM (Dohan Ehrenfest et al., 2018). It is clear that the onus is on researchers to communicate their preparation protocol clearly and comprehensively when publishing research findings.

When devised, the protocol for L-PRF aimed at rapidly separating cell layers (red blood cells from platelets and leukocytes before clotting could occur (Miron et al.,

2023). Consequently, the principles of its production involved centrifugation at relatively high speeds in blood tubes that were free of anticoagulant, a sea change from the processes used for 1st generation products. As time has passed, all of the core aspects of L-PRF preparation have been challenged, namely the centrifuge used, the preparation protocol with regard to time and revolutions per minute (rpm) used, and the blood collection tubes themselves. It is prudent to look at each of these areas in turn, accepting that this is an area of continuing development; an issue of the journal *Periodontology 2000* devotes articles to decision-making around the use of autologous platelet concentrates (Quirynen et al., 2023) and optimisation of PRF production (Miron et al., 2023).

Before considering each of these areas in turn, it is useful to consider some of the basic principles of centrifugation.

1.6.1 CENTRIFUGATION

A centrifuge spins at high speed, creating a centripetal force (i.e. directed towards the centre of the centrifuge or rotor) on the tubes. Centrifuges can hold blood tubes at a constant specified position (fixed-angle) or allow the tubes to move in line with the forces acting on them, such that at speed, the tubes move into a horizontal position (horizontal centrifuge). The forces acting on the tubes cause fluid (blood) within the tubes to come under a centrifugal force (away from the centre of the machine) that, in a fixed angle centrifuge, pushes cells towards the back of the tube and then downwards depending on their relative density. In practice, this means that heavier cells travel more rapidly to the bottom of the tube and gradually "settle" there, leading to the formation of cell layers within the tube. The separation essentially follows the fixed angle at which the tubes rotate around the rotor (e.g. 33 degrees in the case of IntraSpin®)(Quirynen et al., 2022)

Blood cells can be described in terms of their density, with water being the reference point (1000 kg/m^3 at 4°C). More than just being of academic interest, the relative figures for different blood cells immediately illustrate the expected results when whole blood is centrifuged to separate the cell layers, as in the L-PRF protocol. Red blood cells (RBCs) have the greatest relative density and form

primarily at the base of the blood tube (Quirynen et al., 2022). Leukocytes are primarily distributed above the RBC layer at its border with the L-PRF clot, while platelets are distributed at the clot too, spread throughout the clot but at diminishing amounts the further one gets from the junction of the clot and the RBC layer (i.e. clot "face") (Dohan Ehrenfest et al., 2010a)

1.6.1.1 Reporting of PRF Production Protocol and Calculation of Relative Centrifugal force (RCF)

PRF production protocols have been inconsistently reported in individual studies, complicating interpretation of the research literature (Miron et al., 2018) Furthermore, multiple different preparation protocols are used for centrifugation and while all of these products may be described using the umbrella term "PRF", it is clear that there is heterogeneity in the end-products of these protocols (Quirynen et al., 2023) . Additionally, since the dimensions of a centrifuge and the angle at which tubes are positioned during motion can influence the forces acting on the blood tube, further heterogeneity has resulted from researchers following set cycles (time and revolutions per minute) to prepare PRF clots although using different centrifuges. This can result in physical and biologic differences in PRF clots and membranes (Dohan Ehrenfest et al., 2018), which complicate interpretation of individual research publications and the cumulative literature (Miron et al., 2018, Miron et al., 2023)

Following correspondence to the Journal of Periodontology that demonstrated differences in reporting methods even among thought leaders in this field (Pinto and Quirynen, 2019, Miron et al., 2019b), these research groups reached a consensus on requirements for reporting PRF production protocol in future studies (Miron et al., 2019d). To facilitate comparison of results from individual research studies, this consensus outlined that researchers should report the following:

- Centrifuge model used
- Dimensions of the centrifuge rotor (radius to tubes) and rotor angulation for the tube holder

- Revolutions per minute (rpm) and time used
- Composition and size of tubes used
- Relative centrifugal force (RCF) value.

The relative centrifugal force (RCF) value describes the centripetal forces acting on the blood tube during centrifugation. The RCF value is calculated using a standardised formula:

$$RCF = 11.18 \times radius \times (rpm/1000)^2$$

In this formula, the radius is the distance in millimetres from the centre of the centrifuge rotor to the sample. It is clear that since a fixed-angle centrifuge inherently positions the blood tube at an angle, the radius distance from the centre of the rotor will differ along the blood tube, indicating that the RCF value will differ depending on which part of the blood tube is used to calculate the measurement. Radius distance also varies depending on centrifuge design used (Pinto and Quirynen, 2019, Quirynen et al., 2023). Even among market-leading products, the distance from the rotor to the centre of the blood tube varies widely, as follows:

- IntraSpin®: 50 mm (IntraLock, Boca Raton, FL, USA).
- DUO Quattro®: 77 mm (Process for PRF, Nice, France)
- Medifuge™: 55.5 mm (ThermoScientific™, Waltham, MA, USA)

A publication by Miron and co-workers excoriated the quality of RCF reporting in some of the landmark PRF publications (Miron et al., 2018). These authors highlighted that incorrectly reported values have subsequently been transcribed or reported by other authors cementing the inaccuracies in the literature. Miron argues that for consistency with laboratory reporting in other scientific fields, the bottom of the blood tube is the most suitable point at which to calculate the radius value; the associated RCF value is termed RCF max (Miron et al., 2023). On the other hand, Quirynen calculates the RCF value derived from the distance between the centrifuge rotor and the point within a blood tube at which a fibrin clot may be expected to form, the so-called RCF clot (Quirynen et al., 2023) and

this latter value appears to be more widely recorded in the dental literature thus far. However, Miron has opined that the use of RCF clot values has major drawbacks, since the exact level of clot formation within a blood tube cannot be standardised, and may vary due to factors such as the amount of blood collected within the tube, the time used for centrifugation and patient haematocrit values (Miron et al., 2018).

1.6.1.2 Impact of centrifugation factors on PRF architecture and cell composition

The potential impact of centrifugation protocol on the nature of PRF clots has been investigated and has been the source of considerable debate. Dohan Ehrenfest and colleagues produced L-PRF in four different commercially available centrifuges using a standardised g-force, and found differences in the size of clots produced from the different centrifuges. Furthermore, when membranes produced from the varying centrifuges were evaluated using scanning electron microscopy, variations in cellular content and architecture of the fibrin network were apparent, such that these authors questioned the suitability of all but the IntraSpin centrifuge for production of the original L-PRF clots and membranes using the original protocol (Dohan Ehrenfest et al., 2018). An earlier study by the same research team failed to find significant differences in L-PRF architecture using different blood collection tubes (Dohan Ehrenfest et al., 2010a). However, using plain glass and silica-containing plastic tubes at various centrifugation speeds, Tsujino and colleagues prepared PRF matrices for Concentrated growth factor (CGF; to represent a high-speed protocol) and Advanced PRF (A-PRF; to represent a low-speed concept), and then evaluated platelet distribution in the PRF fibrin matrix using immunohistochemistry. The low-speed protocol was associated with a homogeneous distribution of platelets regardless of tube type. However, with a high-speed protocol, platelets were located mainly on the distal surface of the glass tube but were more evenly distributed within the plastic tube. These authors concluded that both centrifuge and blood tubes used for preparation can influence platelet distribution within the PRF matrix (Tsujino et al., 2019). A later study by the same team confirmed this distribution pattern of platelets in high-speed (CGF and L-PRF) and low-speed (A-PRF) protocols. Furthermore, the distribution of activated platelets in these protocols broadly

mirrored the findings on the distribution of whole platelets, with the exception of H-PRF, where activated platelets were localised to the buffy coat region. The findings broadly suggest that platelet activation is similarly induced in the various fibrin matrices irrespective of the centrifugation cycle utilised (Sato et al., 2022).

As a rapidly developing area of regenerative dentistry, an array of commercial products have become available for the production of various PRF materials. Aizawa and co-workers have claimed that commercial interests of equipment vendors branding centrifuges and supplies limits the availability of various products in different territories and makes it challenging to replicate studies in different settings in order to compare the biomedical characteristics of the individual PRF derivatives (Aizawa et al., 2020). These authors used a novel technique (near infrared imaging) to visualise platelet distribution in PRF matrices of four types (L-PRF/A-PRF/CGF/Bio-PRF i.e. H-PRF). This method gives a fluorescent appearance to concentrated areas of cells. Low speed concepts (A-PRF/Bio-PRF) were associated with a wide and symmetrical distribution of platelets along the long axis and mainly peripherally in L-PRF/CGF primarily in the distal peripheral area. However, when fluorescence intensity was compared for three regions (upper/middle/lower) of clots, L-PRF had some platelets distributed in all three regions and was non-statistically significant from A-PRF. The principal finding is that platelet distribution is not gravity dependent. This suggests that platelets aggregate on fibrin fibres as they form and float up as a matrix. Collectively, these findings add subtlety to the generally received analysis that all cells are distributed within blood tubes by molecular weight (Aizawa et al., 2020).

1.6.2 FACTORS AFFECTING THE SIZE AND MORPHOLOGY OF PRF CLOTS

1.6.2.1 Age, gender and time taken for blood collection

Research evidence also suggests that individual characteristics of the patient or research participant may influence the size and characteristics of the clots

harvested, while increased blood collection time may also be associated with a reduction in clot size. Yajamanya and colleagues collected PRF clots from 95 patients who were subdivided by age group (20 - 39 years (y), 40 – 59y, and ≥60y); clots were subsequently fixed and processed to allow sectioning and histologic analysis. With increasing age of subjects, the fibrin network pattern became loose and increasingly irregular; fibrin became thinner, and the entrapment of platelets and leucocytes within the fibre network was progressively reduced versus younger age groups (Yajamanya et al., 2016). Miron and co-workers subdivided 60 patients into three age groups; 5 blood tubes were collected from each individual and centrifugation of one tube was commenced either immediately (0s), or after 30s, 60s, 90s, or 120s from collection. The authors concluded that age, patient gender, and time between blood draw and the start of centrifugation all influenced the size of PRF membranes obtained (Miron et al., 2019c). Specifically, blood collected from older individuals and from female subjects led to larger clots and membranes. Similarly, where centrifugation was commenced faster, larger clots and membranes were produced. At 90s following collection, a mean 13 per cent decrease in mean clot size was noted and this almost doubled by 120s (Miron et al., 2019c). Notably, guidelines from the clinical centre at Leuven produced as part of the first international conference on L-PRF suggest that users spin the first pair of tubes for a minute while collecting the remaining tubes to avoid clotting that might reduce membrane size, and this appears to be a practical solution for clinical use (Quirynen M, 2018).

1.6.2.2 Centrifuge and blood collection tubes

It appears that the size and cellular distribution within PRF clots may also be influenced by the centrifuge and blood collection tubes used and the centrifugation protocol used for PRF preparation. The research team of Miron compared PRF produced using three commercially available centrifuges using high (L-PRF) and low (A-PRF+) g-force protocols, and using blood collection tubes recommended for use by each respective manufacturer for their centrifuge (Miron et al., 2020c). All centrifuges were operated at a similar RCF and tube angulation. PRF clots produced using low centrifugation speeds (A-PRF+) produced clots of smaller size, irrespective of the centrifuge used. However,

these clots contained a higher concentration of platelets that were more evenly distributed throughout the clot, and secreted higher concentrations of growth factors over a 10-day analysis period than the L-PRF clots (Miron et al., 2020c). Interestingly, different commercial blood centrifugation tubes took differing amounts of time to fill, and given prior findings by this group that an increased time to centrifugation results in reduced clot size (Miron et al., 2019c), this difference in tube filling time may putatively have been a factor in clot sizes obtained. Furthermore, when these authors cross-compared the use of each tube design in all three of the commercial centrifuges, one of the blood tube designs (IntraSpin®) resulted in significantly smaller clots regardless of centrifuge, suggesting that the impact of blood tube may be greater than that of centrifuge in terms of clot size produced (Miron et al., 2020c).

Over several research investigations, the same research team utilised various RCF values, centrifugation times and commercial centrifuges to test alternative preparation protocols for PRF (Miron et al., 2019a, Miron et al., 2020a, Miron et al., 2020c). While the principally disseminated conclusions promote horizontal centrifugation and the associated new horizontal preparation protocols, the collected study results may inform clinical practices around PRF preparation more widely. This research team developed a novel approach that involved sequentially pipetting 1 mL samples from the blood tube post-centrifugation. Each successive sample was then analysed separately for cell counts to assess how different preparation protocols concentrate cells (Miron et al., 2019a, Miron et al., 2020b). In general, platelets could more easily accumulate in the upper layers of plasma than leucocytes; for the latter, the greatest concentration was around the buffy coat layer. These authors concluded that centrifugation time had a greater impact on the separation of the cell layers than a change of RCF (Miron et al., 2020a). Protocols of 200 g or less proved ineffective at accumulating platelets/leucocytes (Miron et al., 2020a) and this, in turn, might call into question the effectiveness of many of the established protocols for liquid PRF formulations such as i-PRF.

1.7 ALTERNATIVE FORMS OF PRF

Solid platelet concentrates - specifically the leukocyte and platelet-rich fibrin (L-PRF) family - have been the principal focus of interest among clinicians and scientists working in regenerative dentistry. Nevertheless, some challenges remain in interpreting clinical findings in the literature, as investigators have utilised different preparation protocols for L-PRF for reasons of clinical utility or in their attempts to maximise the properties of the material. This has resulted in the characterisation of a large number of solid and liquid "platelet rich fibrin" products (Quirynen et al., 2023). An early systematic review on the use of L-PRF in periodontology highlighted the need for standardised clinical preparation protocols (Castro et al., 2017a).

The following pages describe the variety of solid and liquid PRF products utilised in clinical practice; a brief understanding of the differences in these products is necessary to interpret clinical and research findings presented in the literature. Table 4 presents an accompanying summary of the different PRF products and their associated preparation protocols; some techniques describe preparation in a specific centrifuge design.

1.7.1 SOLID FORMS OF PRF

1.7.1.1 Advanced platelet-rich fibrin (A-PRF)

Developed by Ghanaati and colleagues (Ghanaati et al., 2014), A-PRF was based on the low speed centrifugation concept and uses a cycle of 14 minutes at 1500 rpm. This was developed in the "Process" centrifuge, now branded as DUO Quattro (Process for PRF, Nice, France). This cycle calculates to an RCF clot of 194g; using the IntraSpin® centrifuge, to achieve this would require 1865 rpm for 14 minutes. Sterile 10 mL glass tubes (A-PRF 10, PROCESS for PRF, Nice, France) or

9 mL glass-coated plastic tubes (Intra-Lock® International, Boca Raton, FL, USA) have been described for use depending on centrifuge used.

1.7.1.2 Advanced platelet-rich fibrin plus (A-PRF +)

First described by Fujioka-Kobayashi, A-PRF+ aimed to enhance the number of cells and growth factors released from the clot further (Fujioka-Kobayashi et al., 2017). This protocol further lowered the rpm and time used for centrifugation to 1300 rpm for 8 minutes, once again using the DUO Quattro centrifuge. This translates to an RCF Clot value of 145g; to correspond to this value, an IntraSpin would require a cycle of 1610 rpm x 8 min.

1.7.1.3 Titanium platelet-rich fibrin (T-PRF)

Developed by Tunali and co-workers the centrifugation process for T-PRF is identical to that for L-PRF. The difference is that T-PRF is prepared using titanium tubes instead of glass/glass-coated plastic. In theory, titanium tubes may be more effective in activating platelets, resulting in a stronger fibrin network (Tunalı et al., 2014). Chatterjee and colleagues conducted light microscopy analysis using cell block cytology on clots obtained from healthy patients, smokers and hypertensive patients, where samples were processed for both L-PRF and T-PRF. While the fibrin network pattern varied in healthy patients versus smokers and hypertensives, T-PRF samples generally demonstrated a more highly organised and thicker fibrin network compared to L-PRF regardless of group (Chatterjee et al., 2017a).

1.7.1.4 Concentrated growth factors (CGF)

Introduced by the team of Sacco in 2006 in the Italian language literature (Corigliano et al., 2010), CGF is produced using an alternating cycle where the speed (rpm) is changed over a period of 13 minutes in a dedicated centrifuge

(Medifuge CGF, Silfradent®, Santa Sofia, Italy), utilising borosilicate plain glass tubes (RV200R, Silfradent®). The preparation protocol involves centrifugation for 2 minutes at 2700 rpm (458 g), 4 minutes at 2400 rpm (358 g), 4 minutes at 2700 rpm (458 g) and the final 3 minutes at 3000 rpm (560 g). The rationale for alternating the centrifugation speed is that it should result in a larger and denser fibrin matrix with increased growth factor levels. Similar to L-PRF, three layers form in the blood tube, and CGF is harvested as the central layer. The presence of a fibrin network in CGF has been confirmed using SEM; this appeared as a three-dimensional network of thin and thick fibrils that trapped platelets (Bernardi et al., 2017). These authors also confirmed the presence of growth factors including VEGF and TGF-β1.

1.7.1.5 Horizontal PRF (H-PRF)

Laurenco and co-workers first showed that PRF could be produced via horizontal centrifugation, and that this produced membranes with high structural stability and cell content, that were capable of releasing growth factors and cytokines over a prolonged period of up to 21 days (Lourenço et al., 2014). Miron has championed this approach for the further development of PRF in practice. This is based on the principle that the use of a horizontal protocol for centrifugation leads to platelets and leukocytes becoming entrapped in the fibrin membrane, with a more even distribution of cell layers along the tube compared to fixed-angle centrifugation, since cells can migrate more freely within the tube, affording better separation of cell types. This can result in a higher final concentration of platelets and leucocytes in the final blood layers within the tube(Miron et al., 2019a). Furthermore, cells are less likely to be damaged even at high g-forces, as the movement within the tube during centrifugation is gentler due to the horizontal positioning.(Miron et al., 2019a).

Horizontal PRF may well signal the future direction of platelet concentrates; in a review published in a recent special issue of the journal *Periodontology 2000* on the subject of “Next generation biomaterials for bone and periodontal regeneration”, Miron argues that “PRF should not be produced using fixed angle devices” (Miron et al., 2024). However, it is clear that this opinion is not universal,

and in previous issue of the same journal, an invited review by, Quirynen and colleagues discusses centrifuge settings for preparation of different forms of L-PRF in order to standardise g-forces used across different fixed-angle centrifuges (Quirynen et al., 2023). In this context, it may be most appropriate for now to view H-PRF as a separate construct, and to view recent publications in this context, somewhat separately to the pre-existing literature, which has primarily been conducted on fixed-angle, table-top centrifuges. While it may be based on scientific principles used in biology, the development of a large number of modified protocols for horizontal centrifugation with new/proprietary terminology, and criticisms by the research team behind H-PRF of existing fixed-angle techniques and publications has added some uncertainty for clinicians and researchers alike. While L-PRF preparation was based on a defined preparation protocol of 2700 rpm for 12 minutes in the IntraSpin® centrifuge, Miron recommends that for construction of solid PRF membranes and plugs, a protocol of 700 g for 8 minutes may be used when utilising the Bio-PRF centrifuge (Bio-PRF, Jupiter, FL, USA) (Miron et al., 2020a) Miron et al, 2020).

1.7.2 LIQUID FORMS OF PRF

1.7.2.1 Liquid fibrinogen

Introduced by Cortellini and colleagues, liquid fibrinogen is prepared using the same centrifuge and settings as L-PRF but utilises a different blood tube, which is centrifuged for 3 minutes only (Cortellini et al., 2018). The tube used, often referred to as a "white cap" tube is plastic, made from polyethylene terephthalate (PET), a thermoplastic polymer that provides a hydrophobic inner surface to the tube. The hydrophobic surface delays coagulation. Liquid fibrinogen is collected as a straw-yellow fluid from the upper part of the tube and should be used relatively quickly (<30 minutes), after which some coagulation would likely occur within the tube). While the use of liquid fibrinogen has not been proposed in isolation, its use as part of a composite material has been discussed. By applying liquid fibrinogen to an existing biomaterial containing fibrin, its resulting coagulation can

be used to enhance the properties of the graft, forming L-PRF Block, as discussed below (Section 1.7.3.2).

1.7.2.2 Injectable platelet-rich fibrin (i-PRF)

This liquid material was described by Mourão and colleagues (Mourão et al., 2015) and is also prepared in a hydrophobic PET tube that slows clotting and uses a centrifugation cycle of 700 rpm x 3minutes in a DUO Quattro centrifuge. This corresponds to an RCF clot value of 60 g, implying i-PRF would require preparation at 820 rpm if an IntraSpin® centrifuge is used. The upper layer of the tube contains the liquid layer (i-PRF). This product has primarily been used outside of dentistry, in dermatology, and for injecting at painful joints including the temporomandibular joint (Albilia et al., 2020). Varela and colleagues (have demonstrated that i-PRF contains a slight increase in platelets and leukocytes compared to whole blood (Varela et al., 2019).

1.7.2.3 Concentrated platelet-rich fibrin (C-PRF)

Introduced by the research team of Miron (Miron et al., 2020b), C-PRF differs in concept from liquid fibrinogen and i-PRF. The C-PRF process seeks to harvest fluid from right above the buffy coat layer immediately after centrifugation. This is based on the idea that this zone of 0.3 to 0.5 mm is extremely cell-rich and may contain platelets and leukocytes at a multiple of 20 times their rate in whole blood; this material can then be used alone or in combination with biomaterials.

1.7.3 HYBRID PRODUCTS INVOLVING AUTOLOGOUS PLATELET CONCENTRATES

Where bone deficiencies occur or grafting is required, bone obtained from the same individual (autogenous bone) is considered the ideal regenerative grafting

material given its osteoconductive and osteoinductive capacity. However, the limited availability, high morbidity and uncertain resorption characteristics of such bone presents challenges (Benic and Hämmerle, 2014). Bone grafts and bone substitutes may alternatively be obtained from another person from the same species (allograft e.g. Freeze-dried bone allograft (FDBA); Demineralised freeze-dried bone allograft (DFDBA)), another species (xenograft e.g. Deproteinised bovine bone mineral (DBBM)) or synthetically produced (alloplast e.g. Calcium phosphate (CP), calcium sulphate (CS), hydroxyapatite (HA), tricalcium phosphate (TCP)).

The concept of constructing a hybrid or composite graft material that combines a bone substitute with autogenous material in the form of a platelet concentrate or its derivative products has received considerable attention in recent years. Brown & Barker reviewed fibrin-based biomaterials and concluded that mechanical properties of fibrin materials can be supplemented by forming hybrid materials with polymers or extracellular matrix components such as collagen. Such combinations might change the strength and durability of fibrin products *in vivo*. The addition of peptides and growth factors could also be utilised to enhance the bioactivity of fibrin constructs (Brown and Barker, 2014). Castro notes that the development of L-PRF Block derives from the concept of combining a material that provides a scaffold and osteoconductive ability with the bioactive properties of a platelet concentrate with potential osteoinductive ability (Castro et al., 2019a).

1.7.3.1 "Sticky bone"

The concept of a bone graft matrix enriched with growth factors derived from second generation autologous platelet concentrates is attributed to Sohn and colleagues (Kim, 2015). This group described "CGF-enriched bone graft matrix", the production of which involved removing a blood tube from the centrifuge after 2 minutes, from which the upper layer was withdrawn as "autologous fibrin glue". This was in turn mixed directly with particulate bone powder and with exudate derived from separately collected CGF layers, which also contained some

thrombin from the red blood cell layer. This exudate then triggered polymerisation. The authors displayed a series of cases including cases of socket preservation and ridge augmentation and indicated that this composite material had potential to minimise bone loss.

1.7.3.2 L-PRF Block

By applying liquid fibrinogen to a homogeneous mix of L-PRF membranes and a bone substitute material, coagulation of the liquid fibrinogen is triggered, converting it into a strong fibrin network that enmeshes the graft material to form a “block” of biomaterial, termed L-PRF Block (L-PRF + BioOss xenograft + liquid fibrinogen (Cortellini et al., 2018). Although this product has colloquially been referred to as “sticky bone”, the process of its generation is somewhat of an evolution from the original product developed by Sohn and colleagues (Kim, 2015), and should be considered a distinct entity.

Table 4: Platelet-rich fibrin (PRF) products and the respective protocols for their preparation. Included are details of the RCF clot (*g*-force) used for preparation and information regarding centrifugation protocol, including time, rpm used and the centrifuge on which each protocol was based. Adapted from (Quirynen et al., 2025).

Variant	Acronym	rpm	Time (min)	RCF clot (g)	Centrifuge/Additional information
Solid (Clots/plugs/membranes)					
Leukocyte and platelet-rich fibrin	L-PRF	2700	12	408	IntraSpin®
Advanced platelet-rich fibrin	A-PRF	1500	14	194	Duo Quattro®
	A-PRF+	1300	8	145	Duo Quattro®
Titanium platelet-rich fibrin	T-PRF	2700	12	408	IntraSpin® Titanium tubes used
Concentrated growth factors	CGF	2700 + 2400 + 2700 + 3000	2 +4 +4 +3	453 358 453 560	Medifuge®
Horizontal platelet-rich fibrin	H-PRF	2220	8	700	Bio-PRF
Liquid					
Injectable platelet-rich fibrin	i-PRF	700	3	60	Duo Quattro®
Liquid fibrinogen	Liquid fibrinogen	2700	3	340	IntraSpin® White cap tube used
Concentrated platelet-rich fibrin	C-PRF	Not reported	8	3000	Bio-PRF Harvest liquid buffy coat layer 0.3-0.5 mm directly above red blood cell layer

1.8 L-PRF USE IN PERIODONTICS & ORAL SURGERY

Castro et al (2017) attempted to provide some clarity on the clinical outcomes that may be attained by using L-PRF in periodontal surgery. This group published two related systematic reviews and conducted meta-analysis where possible, investigating L-PRF use in studies on periodontal plastic surgery, infrabony and furcation defects (Castro et al., 2017a) and on alveolar ridge preservation, implant therapy and sinus floor elevation (Castro et al., 2017b), respectively. Both studies employed a search of three databases and hand searching of journals for articles published in English up to July 2015. Only randomised controlled trials (RCTs) involving human subjects were included with no limitations in follow-up time applied. The authors highlighted the lack of a unified preparation protocol for L-PRF and accepted two protocols for L-PRF production (2700 rpm x 12 min/3000 rpm x 10 min) for the purposes of their investigation. Table 5 presents a summary of the key observations. Infrabony defects represented the largest single area of investigation and demonstrated some of the limitations inherent in the literature. Study follow-up was generally short-term and not uniform in period or method. Additionally, there was significant heterogeneity in defect diagnosis, method of L-PRF production and use and limitations in sample size calculation, randomisation and blinding in some instances. Many of the existing studies were conducted at a single study centre (and have yet to be replicated by other investigators). There has been little consideration of patient outcomes in studies on regeneration relating to L-PRF (Castro et al., 2017a, Castro et al., 2017b).

Table 5: Summary of findings from the Castro et al (2017) systematic reviews on the use of L-PRF in periodontics and surgery.

Indication	Number of available RCTs	Key findings
Periodontal plastic surgery	9	Various comparisons made Some positive effects of L-PRF in combination with CAF
Infrabony defects	13	(L-PRF + OFD) Vs (OFD alone): Addition of L-PRF provides SS improvements in PD reduction, AL gain and bone fill
Furcation defects	2	(L-PRF + OFD) Vs (OFD alone): Addition of L-PRF provides SS improvements in PD reduction, AL gain and bone fill
Alveolar ridge preservation	8	Most studies addressed 3rd molar teeth only; No meta-analysis possible L-PRF surgery shows trends towards decreased patient discomfort and faster soft tissue healing
Implant therapy	3	Studies had inconsistent design/aims L-PRF may accelerate osseointegration
Sinus floor elevation	3	(L-PRF + xenograft) Vs (xenograft alone): Addition of L-PRF may accelerate graft healing ; No meta-analysis possible

1.8.1 L-PRF USE IN REGENERATIVE INDICATIONS

1.8.1.1 Guided Tissue Regeneration Concept

The most studied indication for PRF use in periodontics is its use in the regeneration of infrabony defects, where it has been used as either a graft "filler" material within the bony defect itself, or as a regenerative membrane to cover a grafted defect, or both in combination. It is prudent, therefore, to evaluate briefly the use of PRF in the context of guided tissue regeneration (GTR), a concept developed by Nyman and colleagues as a method to regenerate lost periodontal tissues. In principle, by allowing cells that are capable of regenerating a particular type of tissue, to populate a defect during healing, wound healing can be "guided" to achieve a specific type of lost tissue (Nyman et al., 1982). By restricting epithelium and gingival connective tissue from contacting a surgically debrided root surface adjacent to a defect using a non-resorbable barrier material, these authors were able to demonstrate formation of new cementum with inserting periodontal ligament fibres on a previously diseased root surface (Nyman et al., 1982). In a follow-up study in monkeys, it was observed that membrane placement favoured re-population of the wound area adjacent to the roots by cells originating from the periodontal ligament (Gottlow et al., 1984). Furthermore, it was soon demonstrated that this "new attachment", gained as the result of the GTR treatment, could be maintained over periods up to 5 years (Gottlow et al., 1992).

Expanded polytetrafluoroethylene (e-PTFE) membranes were widely used to promote GTR initially; these did not elicit immunologic reactions and demonstrated good resistance to microbiologic and enzymatic degradation (Hämmerle and Jung, 2003). However, as a non-resorbable material, such membranes required a second surgical procedure to remove the membrane. For practical reasons, therefore, resorbable membranes were subsequently developed. While early studies utilised approaches such as rat collagen in canine studies (Pitaru et al., 1988), bovine collagen was soon tested in human studies, with membrane-treated sites demonstrating an increase in probing attachment level gain and bone growth at intrabony defects compared to open flap debridement alone (Chung et al., 1990). Other early choices of resorbable

material included polylactic acid (Magnusson et al., 1990), and Vicryl, a copolymer of lactide and glycolide (Fleisher et al., 1988).

In addition to negating the need for a second surgical procedure to remove the membrane, resorbable membranes may simplify the grafting technique, decrease patient morbidity and offer improved cost-effectiveness. However, they may require supporting material where defects are not self-supporting, in order to avoid a loss of mechanical strength that could disimprove the results of the regenerative procedure. Furthermore, the duration of the barrier function may be uncertain, and their resorption process may negatively affect healing (Hämmerle and Jung, 2003).

Early review articles highlighted desirable characteristics of barrier membranes used for guided tissue regeneration, including: good clinical handling properties biocompatibility and integration by host tissues, and the ability to achieve cell occlusion and maintain space to allow healing (Karring et al., 1993, Scantlebury, 1993). Reviewing the available biodegradable barriers for guided tissue regeneration in the same issue of *Periodontology 2000*, Greenstein and Caton characterised additional properties of the different available materials including sterility, good degree of stiffness, resistance to breakage, and biodegradability (Greenstein and Caton, 1993). More recently, Rakhmatia concluded that no available material meets the "ideal" criteria for all clinical applications, and every available material presents advantages and disadvantages (Rakhmatia et al., 2013). Hutmacher highlighted that biocompatibility can relate in part to the biodegradability of a material, since the by-products of a material can interact with surrounding living tissue, and leaching of degradation products or impurities appears to be the major source of inflammation as resorbable polymeric materials degrade. These authors explained that collagen materials break down enzymatically, while polyesters are thought to degrade by bulk hydrolysis of ester bonds (Hutmacher et al., 1996).

Reviewing available barrier membranes for regeneration. Retzepi & Donos (2010) explained that biodegradable polymers generally undergo 4 stages of degradation, namely those of hydration, loss of strength, loss of mass integrity and eventually, solubilisation via phagocytosis (Retzepi and Donos, 2010). The stages and rate of degradation may vary depending on the nature of the polymer

involved and characteristics of the membrane and surgical site. As such, resorption and duration of effectiveness of such membranes may be inconsistent (Hämmerle and Jung, 2003). Ideally, the rate of degradation of such membranes should match the rate of new tissue formation (Sbricoli et al., 2020). Histology of critical size defects in an animal model indicate that the wound space may be filled with reparative and regenerative tissues within 14 days (Dickinson et al., 2013), while canine studies indicate that by 4 weeks, the framework of connective tissues will have been established and started to mature (Matsuura et al., 1995). As such, the ideal resorption time of a collagen membrane for GTR has been suggested to be in the region of 28 days, albeit Sbricoli and colleagues demonstrated commercial materials may show notable ranges in duration of barrier effect ranging from 4 to as much as 38 weeks (Sbricoli et al., 2020).

1.8.1.2 Guided Bone Regeneration

Membranes are also used in guided bone regeneration (GBR) procedures. The concept of GBR arose from the GTR concept, and describes a process that excludes undesirable soft tissues from growing into an osseous defect, allowing only osteogenic cell populations from the parent bone to re-populate the site (Dahlin et al., 1988). On a practical level, GBR was first described as a method to achieve bone regeneration by surgically placing a barrier membrane material over a bony defect (Dahlin et al., 1988). This research group described the concept of "osteopromotion", whereby membranes are used to create a space in the tissue in which osteogenesis can take place unimpeded. The membrane occludes cells facing the bone surface, physically sealing the bony site. This sealed-off space then provides an environment for the recruitment and proliferation of osteoprogenitor cells, their differentiation into osteoblasts and expression of osteogenic activity (Linde et al., 1993).

Wang & Boyapati defined GBR as "a surgical technique that increases and augments alveolar bone volume in areas designated for future implant placement, or around previously placed implants". Effectively, epithelium and connective tissue are selectively excluded by the use of a barrier membrane material,

allowing bone to be “guided” into the desired position. These authors outlined four biologic principles needed to ensure the predictability of GBR, which they described using the acronym “PASS” (Wang and Boyapati, 2006).

- Primary wound closure
- Angiogenesis
- Space-creation and maintenance
- Stability (both the initial out of class and any subsequent Implant fixture)

Rakhmatia additionally summarised factors that contribute to successful outcomes of GBR procedures, including the occlusive and stability properties of the membrane barrier, the size of its perforations, the peripheral seal formed between membrane and bone (to protect the defect site), access to bone-forming cells and the ability to maintain an adequate blood supply (Rakhmatia et al., 2013).

Biomaterials used for GTR and GBR should ideally share some basic properties including biocompatibility to prevent adverse reactions in the host tissues, and properties of degradation that allow the material to survive long enough to perform its functional role but to degrade in a manner that allows its replacement with the desired host tissue. It is worthwhile to focus on general properties of existing membrane materials for regeneration, in addition to having clinical handling characteristics that make it practical for clinical use. As outlined by Scantlebury, the membrane should achieve good tissue integration, effectively creating a seal between the material and bone to protect against epithelial or connective tissue ingrowth. In terms of space creation and maintenance, the membrane should have adequate stiffness to maintain space and support the tissue, so it does not collapse into the defect. This property primarily relates to membrane thickness. The membrane should also be occlusive enough to restrict formation of undesired epithelial or connective tissue. In theory, if membrane pores are too big, faster growing cells will overpopulate, whereas pores that are too small might limit migration of all cells (Scantlebury, 1993). However, a comparison of regeneration at membranes of varying porosity in experimental rabbit defects found that although increased membrane porosity appeared to enhance osteogenesis during early healing, it did not appear to affect the final amount of new bone formed significantly. The authors concluded that the space-

maintaining properties of a membrane may be at least as important as its cell occlusive function (Lundgren et al., 1998).

More recently, Omar and colleagues conducted a review of barrier membranes used in GBR and identified that membranes may be amenable to strategies that may enhance the clinical results of GBR. Amongst the strategies identified were the incorporation of biological factors and incorporating antibacterial agents into membrane materials, and the administration of biological cues, such as growth factors and progenitor cells) into the bone defect (Omar et al., 2019). It would appear that platelet rich fibrin materials naturally provide some of these enhancements. Furthermore, since autologous platelet concentrates may be used in membrane form as adjuncts to/replacements for resorbable membrane materials, it is appropriate to investigate how their properties may influence their potential as regenerative barrier materials.

1.8.1.3 Platelet rich fibrin (PRF) - performance as a regenerative material

The use of growth factors appears to offer the potential for clinical improvements in periodontal regeneration surgery (Darby and Morris, 2013). L-PRF is an autologous material that exhibits prolonged growth factor release *in vitro* (Dohan Ehrenfest et al., 2009a), as well as possessing angiogenic (Ratajczak et al., 2018) and antimicrobial (Moraschini et al., 2023) qualities that may enhance healing at compromised sites. Systematic reviews have demonstrated that platelet rich fibrin has favourable regenerative properties in multiple dental indications (Castro et al., 2017a, Castro et al., 2017b, Miron et al., 2017b).

The use of L-PRF as an additive biomaterial following open flap debridement in infrabony defects represents its most heavily studied indication in periodontology, while its use at furcation defects has also been researched, in particular at Class II furcation defects. In these indications, when compared to open flap debridement alone, PRF has demonstrated statistically significant improvements with regard to probing pocket depth reduction, clinical attachment level gain and radiographic bone fill at both infrabony and furcation defects (Castro et al., 2017a, Quirynen et al., 2022, Miron, 2021). While these composite findings indicate a favourable role for platelet rich fibrin, clinicians may still lack clarity as to how best to utilise L-PRF to enhance healing in such indications. As such, it may be helpful to consider L-PRF used in different contexts at infrabony and furcation defects.

Recently published texts by Miron and Quirynen have systematically reviewed the available literature in a manner that facilitates such discussion (Miron, 2021, Quirynen et al., 2022),. However, it must still be recognised that the available literature contains significant heterogeneity with regard to the PRF preparation protocol, particularly in relation to centrifuge and blood tubes used and the centrifugation cycle utilised, form of platelet rich fibrin used (clot/membrane), the characteristics of the defects included and the comparators, among other features. The number of clots processed for use at each defect and the duration of study follow-up has also differed across studies (Castro et al., 2017a). Indeed, based on such heterogeneity, Quirynen notes that the large variation in how these materials are used clinically (e.g. as filler materials/as barrier materials/both, in combination/adjunctive to other grafting materials) makes it challenging to provide clear recommendations on best method of use in

regenerative surgery (Quirynen et al., 2023). Systematic reviews and meta-analyses of PRF based on clinical indications may therefore hide significant heterogeneity in terms of product used and their mode of use. While Miron included the entire range of PRF variants in his meta-analysis of study results (Miron, 2021), Quirynen and colleagues restricted their analysis to studies on L-PRF, which may afford us the opportunity to limit at least part of the associated heterogeneity (Quirynen et al., 2022). The latter analysis is consequently prioritised in the discussion of infrabony defects below; however, these authors did not separately present findings on furcation defects, so the findings of Miron and colleagues are addressed on this topic.

1.8.1.3.1 Performance of PRF as a barrier membrane alone

Three randomised controlled trials (RCTs) evaluated the adjunctive role of L-PRF used solely as a barrier material following open flap debridement (OFD) at either 1-2 wall or 2-3 wall defects, in comparison to open flap debridement alone (Arabaci and Albayrak, 2018, Kizildağ et al., 2019, Ustaoğlu et al., 2020). Studies are consistent in showing that L-PRF use was associated with a statistically significant reduction in probing depth and increase in clinical attachment approximating 1.1 mm each, with a statistically significant decrease in radiographic defect depth also noted (Quirynen et al., 2022).

This may appear to contradict the findings of a review by Agrawal who concluded that L-PRF does not possess the mechanical or degradative qualities needed to constitute a barrier membrane; however, the defects in the studies reviewed by Quirynen and colleagues represent controlled defects that may have had good inherent support (Agrawal, 2023). Quirynen notes that it is not yet possible to conclude whether the use of multiple membranes is more effective than using one membrane (Quirynen et al., 2023). However, a study by Panda and co-workers is of note as it demonstrates that using PRF along with another regenerative membrane material may enhance the regenerative benefit. These authors performed a split-mouth RCT in which 18 patients with 3-wall intrabony defects were treated with OFD and placement of a barrier membrane on the

control side; test-side defects additionally received a PRF membrane. After nine months of follow-up, the additional use of PRF was associated with statistically significant improvements in clinical attachment level gain and radiographic bone fill (Panda et al., 2016).

1.8.1.3.2 Performance as a periodontal regenerative material at infrabony defects

As evaluated by Quirynen, eight randomised controlled trials have evaluated the adjunctive role of L-PRF used as sole regenerative material to fill a 2-3 wall infrabony defect following open flap debridement, in comparison to open flap debridement alone (Rosamma Joseph et al., 2012, Pradeep et al., 2016, Martande et al., 2016, Chandradas et al., 2016, Chatterjee et al., 2017b, Patel et al., 2017, Yajamanya et al., 2017, Ahmad et al., 2019).. L-PRF used to fill the defect took the form of either a clot or a chopped membrane. Studies demonstrated that L-PRF use was associated with a statistically significant reduction in probing depth and increase in clinical attachment ranging from 1 mm to 2.5-3 mm, as well as increased radiographic defect fill noted (Quirynen et al., 2022).

The use of L-PRF for the regeneration of infrabony defects has also been compared to using various other regenerative materials in a group of head-to-head comparison studies. These other biomaterials included different bone substitutes, and enamel matrix derivative, with studies primarily focusing on 2-3 wall defects, and not utilising separate barrier membrane material (Gupta et al., 2014, Mathur et al., 2015, Shah et al., 2015, Galav et al., 2016, Chadwick et al., 2016, Yajamanya et al., 2017, Agrawal et al., 2017). In six of the 7 available randomised controlled trials, outcomes were not statistically significant between L-PRF and the comparator bone substitute, with only a small difference in the remaining study, suggesting that L-PRF may be considered a potentially suitable regenerative option at such defects (Quirynen et al., 2022).

On a related note, PRF variants have been combined with pharmacologic or therapeutic agents such as statins, and these combinations have been compared with the use of PRF alone at intrabony defect sites (Martande et al., 2016, Pradeep et al., 2016). These combination treatments appear to offer some clinically significant advantages in terms of probing depth (PD) reduction and clinical attachment level (CAL) gain, and Miron highlights that PRF may offer benefit in the future as a drug delivery system for various other therapeutic agents (Miron, 2021).

1.8.1.3.3 PRF used to obtain a combination of regenerative and barrier effects

Eight randomised controlled trials have combined L-PRF used as both a defect filler and as a barrier membrane at the same site, primarily at 2-3 wall defects (Thorat et al., 2011, Sharma and Pradeep, 2011, Pradeep et al., 2012, Ajwani et al., 2015, Pradeep et al., 2015, Kanoriya et al., 2016, Bajaj et al., 2017, Pradeep et al., 2017). It must be noted that a single research centre has provided all but one of these studies. Again, compared to OFD alone, results were more favourable with an increase in probing depth reduction (0.8 – 1.4 mm), clinical attachment gain (1.0 – 1.6 mm) and radiographic defect fill noted (Quirynen et al., 2022).

Four RCTs have additionally evaluated whether the combination of L-PRF with a bone substitute following OFD enhances outcomes versus the use of the bone substitute alone (Agrawal et al., 2017, Saravanan et al., 2019, Goyal et al., 2020, Atchuta et al., 2020) . Once more, studies utilised 2-wall and 3-wall defects and did not use a separate barrier membrane material. Each study used a different bone substitute material and these studies have provided equivocal results.

As discussed by Miron, fewer RCTs have addressed the use of PRF in furcation defects, but findings are broadly similar to those for intrabony defects. These authors presented findings collated for use in any capacity. Studies have generally focused on the treatment of Class 2 defects. Meta-analysis comparing OFD alone versus OFD with the adjunctive use of PRF found statistically significant improvements in terms of mean PD reduction for PRF equating to 1.42 mm (95%

CI 0.92, 1.92) and some clinical attachment level gain (albeit summary figures were not presented by the authors). No significant difference was found when comparing PRF alone to bone graft alone at furcation defects, whereas the addition of PRF to bone graft appeared to offer additional benefits of almost 1 mm in terms of PD reduction compared to bone graft alone in two available studies (Miron, 2021).

1.9 MECHANICAL PROPERTIES AND CHEMICAL DEGRADATION OF PRF

Platelet-rich fibrin provides many of the desirable qualities of regenerative materials outlined in early publications by Karring and Scantlebury (Karring et al., 1993, Scantlebury, 1993). PRF is a biocompatible material, with generally good handling characteristics. It is quick and cheap to produce, autologous and therefore not associated with adverse reactions (Quirynen et al., 2023). It is autologous in nature, resorbable and has antimicrobial properties (Castro et al., 2019b, Moraschini et al., 2023). Due to its accelerative effect on soft tissue healing (Miron et al., 2017a), primary closure is not mandated. PRF is an excellent source of platelets, leukocytes and their cytokines and regenerative growth factors that can direct cellular healing, promote angiogenesis and integration of the graft with the tissues, as well as maintaining blood supply and promoting bone-forming cells (Dohan et al., 2006a, Quirynen et al., 2023).

On the contrary, the impact of the PRF structure on its porosity and cell occlusion abilities remains incompletely understood. The effectiveness of PRF as a barrier material has been questioned, due to concerns about its mechanical strength and its consequent ability to maintain space (Agrawal, 2023). In particular, there is some uncertainty about the potentially fast resorption rate of PRF and its consequent ability to provide an occlusive function to survive long enough *in vivo* to replicate the inherent positive characteristics derived from *in vitro* research and alluded to above (Agrawal, 2023). In a recent narrative review article, Agrawal affirmed that PRF may better be considered a biological or supportive membrane, and that it should not be viewed as a sufficient barrier material on its own, based on current evidence (Agrawal, 2023). Other authors have also concluded that the rate of degradation of these membranes may limit its application as an effective barrier membrane (Sam et al., 2015, Yamashita Y, 2016).

Mechanical testing studies have shown PRF to have inferior stiffness and hardness relative to collagen membranes (Sam et al., 2015, Chhaya et al., 2022). Sam and colleagues concluded that lack of rigidity may result in the PRF membrane having a tendency to collapse over the bone and root surface if used for guided tissue regeneration, potentially limiting the space needed for clot maturation (Sam et al., 2015). However, Isobe and colleagues claimed that CGF and A-PRF membranes both appear to be tough enough to serve well as barrier membranes (Isobe et al., 2017). Indeed, more recent evidence suggests that the mechanical properties of PRF can be modified by preparation protocol (Ravi and Santhanakrishnan, 2020). Chemical degradation of membranes is primarily characterised by changes in weight, size and physical appearance. Evidence on PRF membrane degradation is limited, and has frequently been limited to visual observation of the degradation timeline in studies assessing growth factor release from different autologous platelet concentrates. However, a fast rate of PRF degradation *in vitro* (Sam et al., 2015, Ravi and Santhanakrishnan, 2020) and in an *in vivo* rat model (Yamashita Y, 2016) have been observed and Chhaya and colleagues (Chhaya et al., 2022) observed a higher rate of degradation of PRF membranes in comparison to collagen and chorionic membranes *in vitro*. Once again, it appears that preparation protocol (Dohan Ehrenfest et al., 2018) and cellular content and fibrin network density of the membrane material may influence degradation rate. It is possible that further research will result in modifications to preparation protocols that allow maximisation of PRF materials for regenerative purposes. Since L-PRF and its low speed protocol variant, A-PRF, represent the most commonly documented production methods for PRF, a comparison of their degradation patterns *in vitro* may be indicated as a step forward in our understanding of these materials. This is discussed in more detail in Chapter 5.

1.10 L-PRF USE TO PROMOTE OSSEOINTEGRATION OF DENTAL IMPLANTS AND IN THE TREATMENT OF PERI-IMPLANT DISEASES

The use of L-PRF in dental implant treatment has also been of interest to researchers. Davies highlighted that a critical step in osseointegration is the formation of a stable fibrin clot, which provides the initial scaffold for osteogenic cells to migrate towards the implant surface (Davies, 2003). Since L-PRF is rich in cells and growth factors, which are active in all stages of bone repair and in angiogenesis, researchers have investigated its potential to provide an osteopromotive effect during osseointegration. This potential for L-PRF to enhance osseointegration was demonstrated histologically in animals by Öncü and colleagues, using osteotomies in rabbit tibias (Öncü et al., 2016). At test implant sites, surfaces were pre-wetted with L-PRF exudate and L-PRF membranes were inserted into the osteotomy prior to implant placement. At control sites, no additional materials were used. L-PRF application significantly enhanced bone formation and bone-to-implant contact in the first 4 weeks after placement (Öncü et al., 2016). Öncü and Alaaddinoğlulul replicated the concept of coating implants with L-PRF and applying an L-PRF membrane in the osteotomy site. This was associated with increased implant stability quotient (ISQ) values, especially during early healing compared to control sites which were allowed to heal naturally (Öncü and Alaaddinoğlu, 2015)

Peri-implantitis is a frequently observed biological complication of dental implants that can affect approximately 9.6 per cent of implant sites and almost one in five patients with implants (Atieh et al., 2013). Analogous to the treatment of periodontitis, treatment of peri-implantitis aims to address the causative biofilm, and to arrest the progression of peri-implant bone loss. Researchers have evaluated clinically whether L-PRF may provide a treatment alternative for the management of peri-implantitis. In experimentally created tibial implant defects in rabbits, application of L-PRF at the defect site was associated with enhanced new bone formation and bone-to-implant contact after eight weeks of healing (Lee et al., 2012), suggesting the potential of L-PRF to enhance healing. In an

early case report, Patil and colleagues treated a 32-year-old male with a peri-implant defect using L-PRF as the sole grafting material, following surgical debridement of the affected implant. At one year follow-up, probing depth reductions were maintained and radiographs suggested an improvement in bone levels at the site (Patil et al., 2016). Hamzacebi and colleagues treated human peri-implant defects via open flap debridement, adjunctively using L-PRF in test subjects. Following six months of healing, sites with L-PRF demonstrated an increased gain of attachment and pocket depth reduction versus control sites (Hamzacebi et al., 2015).

Given these initial positive findings, researchers have worked to elucidate potential mechanisms by which L-PRF may potentiate healing. In a canine study, Neiva and colleagues found that placement of L-PRF at immediately inserted implants to fill the resulting gap defect, was associated with an enhanced bone-to-implant contact. This study suggested the possibility of a synergistic effect of L-PRF and micrometre/nanometre surfaces on healing (Neiva et al., 2016). The ability of L-PRF materials to potentiate healing via the formation of a blood clot on the implant surface was evaluated by Lollobrigida and colleagues, who compared titanium discs with machined surfaces with micro/nano-rough surfaces. Discs of each type were treated by immersion in either liquid fibrinogen, L-PRF exudate, or a combination of both. When assessed under SEM, micro/nano-rough surfaces showed an increased retention of fibrin, regardless of the treatment used. In all samples treated by liquid fibrinogen, direct contact was visible between the fibrin network and the implant surface, and erythrocytes, leucocytes and platelets were present both within the clot and on the implant surface. It appeared that adjunctive treatment of these samples with L-PRF exudate prior to liquid fibrinogen could further enhance the formation of the fibrin clot (Lollobrigida et al., 2018). These authors noted putative advantages of using liquid L-PRF materials instead of an L-PRF clot/membrane, in that fibrin polymerisation will occur in direct contact with the implant surface; this may create a strong fibrin coating that may functionalise the implant to have an osteopromotive effect. In a subsequent *in vitro* study, Andrade and co-workers utilised five commercially available dental implant designs, exhibiting different surface characteristics, and immersed them in liquid fibrinogen from healthy donors for 60 minutes. Following this, implants were fixed for scanning electron microscopy (SEM). Treatment with liquid fibrinogen promoted the formation of a fibrin mesh on all implant surfaces;

however, this initial contact differed significantly among different implant brands. Some surfaces showed zones without a fibrin coating, possibly associated with decreased hydrophilicity of the surfaces. Implants also differed with regard to the density of the fibrin mesh formed, the number of blood cells trapped inside this mesh, the thickness of the fibrin fibres and their orientation relative to the implant surface (Andrade et al., 2021).

In a case series of four patients with advanced peri-implantitis necessitating explantation (5 implants in total), Galárraga-Vinueza and colleagues tested the hypothesis that the remaining peri-implant bone surrounding peri-implantitis affected sites must adapt to the functional forces placed on it once the coronal bone surrounding the implant is lost due to disease. Histologic and histomorphometric analysis revealed signs of compaction of former cancellous bone, such that it remodels to cortical bone with few and small vascular channels (Galárraga-Vinueza et al., 2020). This so-called osteolysis may represent a functional adaptation of the bone, but could theoretically present a challenge when attempting regeneration at peri-implantitis sites. In this context, the ability of L-PRF materials to encourage neovascularisation and to functionalise the implant surface may be highly valuable in improving regenerative outcomes. The *in vivo* investigation by Francisco and co-workers is pertinent; these authors evaluated the angiogenesis activity of L-PRF using a chorioallantoic membrane (CAM) model. When L-PRF was placed in direct contact with the CAM, an increase in the number of blood vessels, and vessels of large diameter, could be observed and sprouting of vessels into the L-PRF membrane was noted (Francisco et al., 2021). The same authors used a chick-embryo femur model to assess osteogenic activity of L-PRF. Where L-PRF was placed in direct contact with the chick femur, significantly higher values were obtained for bone volume and bone mineral density compared to a group where there was indirect communication between the L-PRF and chick femur, or controls where no L-PRF was used. This finding of increased formation of bone in the direct contact group highlights that the positioning of L–PRF membranes relative to a surgical site may impact on outcomes (Francisco et al., 2021).

In light of research on the potential antimicrobial activity of L-PRF, Schuldt and colleagues investigated the potential of L-PRF to decontaminate the implant

surface. Titanium discs with a sandblasted, large-grit, acid-etched (SLA) surface were inoculated with subgingival plaque, and cultured for 21 days, after which a multi-species biofilm was attached. Discs were subsequently exposed to the cell-rich portion of L-PRF membranes for 48 hours. Under subsequent examination with SEM, platelets were identified in direct contact with bacteria and had accumulated in the lacunae of the rough SLA surface. These cells showed pseudopodia that appeared to facilitate their adaptation at these sites and exhibited a shape and size indicating these were activated cells. Adjacent bacterial cells showed widespread evidence of bacteriolysis, which was not evident in controls where L-PRF was not used. When samples were additionally rinsed with saline prior to SEM, the discs demonstrated an average 92 per cent decrease in bacterial counts, with 40 per cent of specimens, having no bacteria per field under SEM. Similar reductions were not evident in controls where no L-PRF was used and solely saline rinsing carried out (Schuldt et al., 2021). The reduction in bacterial counts on L-PRF-treated surfaces suggests that L-PRF may have anti-microbial activity and offers promise for its use at dental implants in the clinical setting.

It is clear that L-PRF has the potential to enhance healing at implants through its antimicrobial and angiogenic properties and its putative ability to biomimetically functionalise implant surfaces. However, clinical evidence for its use at peri-implantitis sites remains sparse. In their L-PRF textbook, Quirynen and co-authors acknowledge that evidence is not currently available to definitively support a specific approach to peri-implant disease treatment when using L-PRF(Quirynen et al., 2022). However, they advocate an interim approach that combines the use of L-PRF and a bone substitute material (L-PRF Block) at the peri-implant bone defect, with sealing of the graft by a double layer of L-PRF membrane (Quirynen et al., 2022). Further research is indicated to assess the clinical outcomes of patients with peri-implantitis treated with L–PRF materials.

1.11 POST-EXTRACTION HEALING

Socket healing describes the sequence of events occurring inside and outside the walls of the socket following tooth extraction. These events act to close the wound and to restore tissue homeostasis (Araújo et al., 2015).

Following tooth extraction, marked changes in alveolar bone dimensions take place. These changes involve reductions in both vertical ridge height and horizontal (bucco-lingual) ridge width. Post-extraction dimensional changes have been investigated using study casts, directly using ridge measurements at the time of surgical re-entry, using radiographs and cone beam computed tomography (CBCT) imaging. Schropp and colleagues evaluated hard and soft tissue healing at 3, 6 and 12 months following single molar/premolar tooth extraction in a prospective clinical study of 46 patients, using study casts and radiography. Mean ridge width was reduced by approximately 50 per cent over 12 months, with approximately two thirds of this change taking place during the first 3 months following extraction (Schropp et al., 2003). While it is clear that clinical resorption occurs throughout the ridge, early studies revealed that the buccal socket wall resorbed considerably more than the palatal /lingual wall and consequently the centre of the resorbed ridge was shifted lingually from its pre-extraction position (Pietrokovski and Massler, 1967). A more recent clinical follow-up study in 69 patients utilised CBCT to compare measurements of socket sites and contralateral teeth in the maxillary incisor and premolar regions following at least one year of healing (Misawa et al., 2016)[i]. Overall, the cross-sectional area of the ridge decreased by approximately one third. Compared to contralateral teeth, there was a notable reduction in both ridge height (1.6 -2.5 mm) and ridge width at socket sites. The ridge width reduction was more pronounced in the buccal and marginal portions of the ridge, and gradually decreased as one moved apically, so that the ridge took up a “triangular shape”, with its base at the basal bone of the maxilla. Although the greatest proportion of horizontal loss occurred in the marginal portion of the ridge (Approximately 60% loss), reduction in width did occur throughout the socket, such that even 7 mm apical to the CEJ, ridge width decreased by greater than 30 per cent. Extraction sites were frequently inadequate to consider implant placement (Misawa et al., 2016).

1.11.1 SYSTEMATIC REVIEWS OF POST-EXTRACTION CHANGES

Systematic reviews have attempted to quantify the hard tissue loss that occurs following tooth extraction. Van der Weijden and colleagues evaluated hard tissue changes at non-molar sites and recorded a mean horizontal bone loss of 3.87 mm (95% CI: 3.67, 4.06 mm), and clinical and radiographic decreases of 1.67 mm (95% CI: 1.43, 1.91 mm) and 1.53 mm, respectively, in the vertical dimension at 3-12 months following extraction. Mean approximal bone loss was 0.64 mm (95% CI: 0.59, 0.70 mm) (Van der Weijden et al., 2009). Tan and colleagues (2012) obtained broadly similar results following 6 months of healing but also quantified hard tissue changes as a proportion of initial ridge dimensions. Following extraction, a proportional loss of 29-63 per cent of horizontal ridge width was noted, corresponding to a clinical dimensional loss of up to 3.79 mm (±.0.23 mm). Loss of vertical ridge height was comparatively smaller, ranging from 11-22 per cent, equating to approximately 1.24 mm (±0.11 mm) at the mid-buccal aspect of the socket, with approximately 0.8mm of interproximal bone loss evident. These authors confirmed that the highest proportion of this change appears to occur in the first 3 months following extraction (Tan et al., 2012). Additional changes after the 6 month time point were more limited, although remodelling continues more slowly throughout life (Carlsson and Persson, 1967).

Couso-Queiruga and colleagues conducted a meta-analysis of post-extraction dimensional changes following natural healing, excluding sockets in the mandibular incisor and third molar regions. By not imposing a minimum number of patients for inclusion in the analysis, and by including studies with as little as 2 months of follow-up, these authors obtained an increased volume of studies (n=20) for quantitative analysis. Non-molar dimensional changes equated to a mean horizontal ridge loss of 2.73 mm and vertical change of 1.71 mm. Changes were significantly less when a thicker buccal bone plate was present at the tooth site. These authors noted that dimensional loss post-extraction varied based on tooth type, with increased horizontal dimensional changes at molar sites versus non-molar sites (mean changes of 3.61 mm in the molar region vs 2.54 mm in the anterior region, respectively). Conversely, vertical bone loss at mid-buccal and

mid-lingual sites was slightly reduced in the posterior region (Couso-Queiruga et al., 2021).

1.11.2 IMPACT OF LOCAL ANATOMY ON POST-EXTRACTION HEALING

The potential of the socket to heal may vary according to socket anatomy, and its related ability to provide stability for the healing wound site, and to provide a blood supply and bone cells for healing (Araújo et al., 2023). In this context, it is prudent to review alveolar anatomy before briefly considering factors that influence socket healing; an appreciation of these features is critical to interpreting the literature on unassisted socket healing (natural healing) and studies relating to alveolar ridge preservation.

1.11.2.1 Normal Alveolar Anatomy

The alveolar process describes the bone that surrounds the tooth root. The socket (alveolus) is a tooth-dependent structure that comprises internal and external plates of cortical bone, between which a layer of less dense, cancellous (trabecular) bone is interposed (Schroeder, 1986). Gross analysis of post-extraction sockets has revealed that the lingual cortical plate is generally 2-3 times thicker than the corresponding labial cortical plate (Atwood, 1963).

- **Alveolar bone proper (ABP)** is the cortical bone that lines the inner wall of the socket adjacent to the periodontal ligament (PDL). Histologically, this cortical bone takes the form of a cribiform plate, due to perforations that (i) allow access for blood vessels and nerves to enter and exit the PDL, and (ii) allows insertion and anchoring of PDL fibres (termed Sharpey's fibres) into the cortical bone. This engagement of the fibre bundles is responsible for the synonym "bundle bone". Bundle bone contains layers of bone deposited in an orientation parallel to the tooth socket wall, with fibre bundles emerging at right angles. It is produced and maintained primarily by periodontal ligament cells, and may be

considered part of the periodontium. As such, it is functionally dependent on the periodontal ligament and may be considered a periodontal – ligament dependent (i.e. tooth-dependent) structure that will resorb following extraction. Bundle bone varies between 0.2-0.4 mm in thickness (Schroeder, 1986).

- The external buccal and lingual **cortical plates** are continuous with the outer cortex of the body of the jaws. The alveolar wall (ABP) and cortical plate meet at the alveolar bone crest (Saffar et al., 1997).
- The **cancellous bone** surrounds the bundle bone and is a supporting bone sandwiched between the outer and inner (ABP) cortical plates, which may vary in thickness. Indeed, closer to the bony crest, as these cortical plates approach one another to intersect, the alveolar process thins and the space for cancellous bone becomes limited, such that it may even be absent at some sites, particularly at the facial aspects of teeth in the anterior regions (Schroeder, 1986, Newman et al., 2019).

Figure 4 displays some of the anatomical landmarks of the alveolus. As discussed above, approaching the crest, the alveolar bone may be restricted to fusion of the inner and outer cortical plates into a single structure. Furthermore, since the bundle bone may take a fixed dimension of up to 0.4 mm, at sites where the buccal bone is thin, a high proportion of the bone may be composed of bundle bone, a structure that is inherently dependent on the periodontal ligament, which in turn will be lost following extraction. Such resorption may result in a minimally thin remaining bone plate that is insufficient to survive post-extraction resorptive changes, and which may thereby prove unable to provide an adequate supply of cells and growth factors to encourage effective healing at the buccal aspect of the socket.

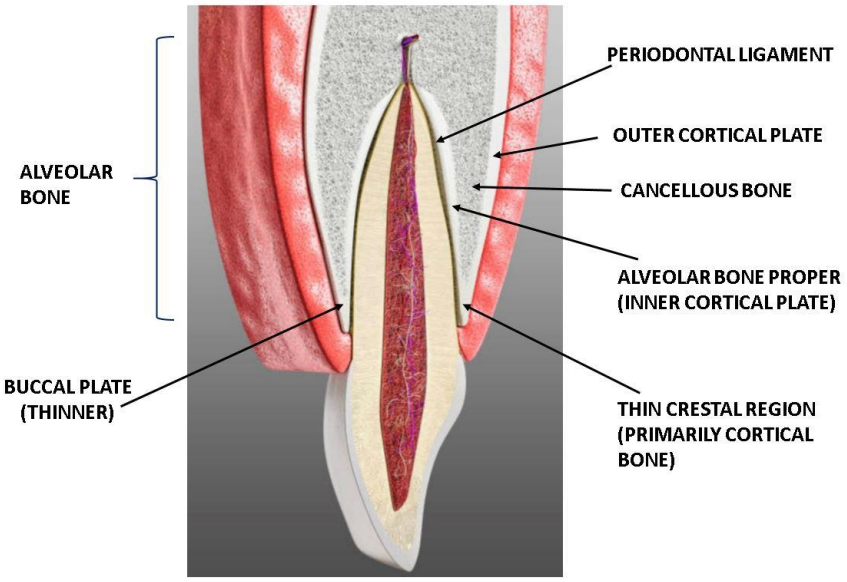

Figure 4: Basic anatomy of the alveolus

1.11.3 DYNAMICS OF UNASSISTED SOCKET HEALING POST-EXTRACTION

A group led by Mauricio Araújo & Jan Lindhe investigated the dynamics of extraction socket healing in mongrel or beagle dogs, and later in human studies. Following extraction of a premolar tooth or single molar root, varying healing protocols were followed in a sequential group of studies that evaluated natural healing, grafting and immediate implant placement using block biopsy of treated sites and histomorphometric analysis. In the first of these studies, the team investigated bone tissue formation in extraction sites up to 180 days in a canine model, using biopsies taken at varying levels of the socket (Cardaropoli et al., 2003). Initially, a blood clot was formed that was replaced sequentially by a provisional matrix, woven bone, and lamellar bone and bone marrow. Hard tissue formation had started at two weeks of healing. Woven bone filled the socket after 1 month. Over the course of healing, a hard tissue cortical) bridge formed, which closed the socket. This formed by the 3-month time point and consisted of woven and lamellar bone. After this time-point, woven bone was gradually replaced by lamellar bone and marrow. It appears that periodontal ligament cells were contributors not only to provisional matrix formation but also to hard tissue formation in the healing extraction socket, presumably as a source of mesenchymal cells. Between days 30 and 180, tissue within the socket changed from being dominated by woven bone to mature tissue that contained small amounts of mineralised bone. By day 180, this mature bone below the hard tissue bridge consisted of about 85 per cent bone marrow and 15 per cent mineralised bone. The authors suggested that the high percentage of bone marrow possibly occurs since the socket is no longer a load bearing tissue (Cardaropoli et al., 2003). In a follow-up study using a canine model at 3 months' post-extraction, healing appeared to be morphologically similar whether or not the socket was intentionally debrided of periodontal ligament (PDL) remnants. Healing at extraction sockets appeared to show greater maturation after 3 months than surgically created defects (Cardaropoli et al., 2005), suggesting that such defects may not accurately reflect healing occurring at natural teeth, and emphasising that extraction sockets should be used in research.

Araûjo & Lindhe subsequently evaluated dimensional ridge changes occurring following extraction of four mandibular premolars in 12 mongrel dogs, whose sacrifice was staggered, so that all dogs had a socket representing 1, 2, 4 and 8 weeks of healing. Over the healing period, there was a high level of osteoclastic activity, which resulted in resorption of the crestal region of both the buccal and lingual bone walls. Changes were more pronounced at the buccal aspect and vertical bone loss was consequently more noticeable at this site. In specimens taken at one week, the crestal region of the buccal wall consisted exclusively of bundle bone, while that of the lingual bone was a combination of bundle bone and lamellar borne. However, bundle bone underwent early resorption and was absent from samples by the four week time point; the authors highlighted that this explains the significant reductions of buccal bone wall height that occurred during the first four weeks (Araújo and Lindhe, 2005).

These authors described osteoclastic resorption of the buccal and lingual bone walls as occurring in two overlapping phases. In the first phase, the bundle bone of the internal walls of the socket is resorbed early in healing (within the first four weeks), to be replaced by woven bone. Since the buccal wall consisted almost solely of bundle bone, this remodelling resulted in significant vertical height change (the buccal aspect underwent an average of 2.2 mm more vertical resorption in the period from 1-week to 8 weeks of healing compared to lingual bone, which contained a greater proportion of cortical bone at baseline). In the second phase, osteoclastic activity led to resorption at the outer surfaces of the buccal and lingual walls i.e. the cortical plates (Araújo and Lindhe, 2005). In a follow-up study, these authors also revealed that immediate implant placement was unable to preserve a hard tissue dimensions of the ridge following extraction, particularly at the buccal aspect, such that at 12-weeks post-immediate implant placement, the buccal bone crest was located >2 mm apical to the implant margin (Araújo et al., 2006).

Araujo & Lindhe's findings are complemented by the findings of Scala and co-workers, who noted a broadly similar healing sequence of socket healing in primates up to 180 days (Scala et al., 2014); the primate model may more closely approximate healing events in humans. These authors highlighted that modelling

processes begin from the lateral and apical walls of the socket. A provisional matrix was evident at 10 days, with socket closure present and mineralised immature bone occupying the socket within a month. Subsequent remodelling resulted in a decrease of the amount of mineralised bone as it was replaced by trabecular bone and bone marrow. Of note, these authors highlighted that approximately half of the bundle bone was lost by 30 days and 90 per cent by 3 months post-extraction (Scala et al., 2014).

Following re-entry measurements, Kerr and colleagues noted a clinical pattern wherein buccolingual resorption decreases with increased distance from the crest. Clinically, this will impact on the shape of the residual ridge and the bone available for ideal implant placement (Kerr et al., 2008). From a research perspective, this highlights the importance of measuring near the crest to give an accurate assessment of dimensional bone loss. However, research investigators have used heterogeneous points of reference for measurements, and this is among the factors that make interpretation of measurements challenging.

1.11.4 FACTORS INFLUENCING THE EXTENT OF POST-EXTRACTION DIMENSIONAL CHANGES

The socket walls normally serve a protective function for the blood clot, granulation tissue and woven bone, in turn, at various stages of healing. Therefore, differing characteristics of socket anatomy will have a considerable impact on the ability to maintain wound stability, and supply of bone cells and blood vessels, with a consequent impact on horizontal and vertical tissue loss. Among these factors, Araujo noted the number and integrity of bone walls and the composition of the socket walls to be of prime importance, with an intact and thicker buccal bone wall desirable. The angulation of the tooth root in relation to basal bone, specifically a tooth that lies marginal to or outside the bony envelope, may predispose to resorption and compromise healing. The role of basal bone width in relation to the alveolar process is not well researched but the authors suggested that where basal bone is thinner than the alveolar process, resorption may be more likely and the supply of blood vessels and bone cells may be

impaired (Araújo et al., 2023). Some of these factors are considered in detail below.

1.11.4.1 Buccal bone thickness

The severe resorption of buccal bone that occurs post-extraction has been attributed to the observation that the buccal bone wall is frequently very thin in nature and crestally is principally comprised of bundle bone that is rapidly resorbed once the tooth is extracted (Araújo and Lindhe, 2005). This vulnerability of buccal bone to resorption is highlighted by numerous studies that have investigated buccal bone wall width (thickness), as detailed below.

Huynh-Ba and colleagues recorded the buccal bone thickness (BBT) using calliper measurements taken 1 mm apical to the alveolar crest, immediately following extraction at 93 sockets in the maxillary inter-premolar region. Mean width of buccal bone was 0.8 mm in the inter-canine region and 1.1 mm in the premolar region. The width of buccal bone was ≤1 mm in 87 per cent sites in the inter-canine region. While mean width of the palatal bone wall was significantly greater, in approximately 60 per cent of cases, this wall was also between 0.5 - 1.0 mm in thickness (Huynh-Ba et al., 2010). A subsequent study of BBT in the maxillary inter-canine region using CBCT measurements in 255 adults not only concurred with the finding that the buccal bone plate was <1 mm thick in the vast majority of cases but indeed that 40 – 60 per cent of teeth had a buccal wall thickness of <0.5 mm (Januário et al., 2011).

Two systematic reviews have evaluated studies of buccal (facial) bone thickness at the maxillary anterior teeth. In the first, Rojo-Sanchis and colleagues limited inclusion to observational studies and randomised controlled trials that utilised CBCT for measurements; this resulted in 29 studies that addressed more than 3500 patients and >17,000 teeth. These authors noted an impact of the landmark used as a reference point used for measurements - values based on the CEJ typically provided thinner measurements of BBT than studies that use the alveolar bone crest as a landmark. Based on this latter group, mean BBT was ≤1 mm in

maxillary incisors and canines (range 0.75-1.05 mm overall), while the corresponding value in the premolar region was marginally greater than one mm (range 1.09 -1.96 mm). Interestingly, these authors noted that geographical variation could explain most of the heterogeneity in studies on BBT - Asian study populations (15 of the 29 individual studies) were characterised by thinner mean BBT (Rojo-Sanchis et al., 2021). Tsigarida and co-workers utilised more flexible inclusion criteria that resulted in 50 studies, predominantly retrospective in nature, being included, presenting data on >7000 patients, but results confirmed that most maxillary anterior teeth have buccal bone thickness of ≤1 mm. These authors also noted that buccal bone tends to get thicker as the point of measurement moves more apical along the root (Tsigarida et al., 2020), in contrast to the findings of Januário and colleagues (Januário et al., 2011). Buccal bone also tended to be thicker as one moved posteriorly in the arch (Tsigarida et al., 2020). This latter observation aligned with the overall trend described in a study by Temple and co-workers, where CBCT was used to measure buccal plate thickness in posterior teeth (maxilla and mandible) at varying points apical to the alveolar crest in 265 patients and observed that the frequency of thin buccal plate (<1 mm) broadly decreased from anterior to posterior. Nevertheless, these authors noted that thin and potentially even absent buccal plate are not uncommon in the posterior regions and concluded that alveolar ridge preservation may be indicated even at posterior teeth (Temple et al., 2017). These collected observations must be noted in context of the findings of Behnia, who concluded that when residual alveolar bone plates were thicker than 1 mm clinically, CBCT demonstrated relatively good accuracy and reliability for measuring buccal bone thickness. However, given few sites exhibited buccal bone of this thickness, the authors found that overall, CBCT images tended to overestimate buccal bone thickness compared to direct clinical measurements (Behnia et al., 2015).

It is clear that a finding of thin buccal bone, may present significant challenges for subsequent treatment options. In a study that used CBCT evaluation of bounded extraction sites immediately post-extraction and at 8 weeks, in 39 patients, sites with BBT <1 mm exhibited progressive resorption leading to a median vertical bone loss of 62 per cent of facial bone height, equating to 7.5 mm. On the other hand, sites with facial bone thickness of > less than 1 mm demonstrated median

vertical bone loss of just 9 per cent or 1.1 mm (Chappuis et al., 2013). A more recent systematic review reported an additional bone loss post-extraction amounting to 2.4 mm of buccolingual width and 1.1 mm in vertical height loss at sites with a compromised buccal wall (García-González et al., 2020). A thin buccal bone plate also presents challenges for placement of dental implants, particularly if immediate implant placement is desired. At implant sites, it has been suggested that mean resorption of buccal bone was significantly greater when the remaining buccal wall was <2mm in thickness and consequently, a threshold of least 2 mm of buccal bone is desirable to predictably maintain the alveolar bone level (Qahash et al., 2008).

1.11.4.2 Other anatomical factors influencing the response to extraction

The presence of buccal fenestration or dehiscence defects at time of extraction appeared to influence dimensional changes, with greater buccolingual resorption than intact sockets at re-entry 8 weeks later (Chen and Darby, 2017). Similarly, sites with local pathology including periapical and periodontal diseases at the extraction site have been associated with compromised bone walls (Ramanauskaite et al., 2020). Indeed, a study of post-extraction healing in healthy versus periodontally diseased socket sites indicated that the rate of socket healing and bone formation at sites with severe periodontal disease destruction was much slower than at intact, healthy sites (Ahn and Shin, 2008). Despite this, ARP may still be considered for periodontally compromised sites; in a randomised controlled trial in 26 patients with stage III or stage IV periodontitis sites requiring extraction, alveolar ridge preservation (ARP) was associated with significantly less horizontal bone resorption (Ben Amara et al., 2021). It has also been highlighted that sagittal root position within the alveolus has major influence on the local bone morphology in this region, and anatomic parameters should be carefully evaluated on CBCT before confirming treatment approach (de Santana et al., 2023).

1.11.4.3 Impact of surgical flap elevation

An early study by Wood and colleagues noted that the simple act of elevating a (partial thickness or full-thickness) flap was associated with bone resorption (Wood et al., 1972). In a beagle dog model, Fickl noted that surgical trauma during extraction associated with the elevation of a surgical flap resulted in greater buccolingual ridge resorption than extractions performed without flap elevation (Fickl et al., 2008). A clinical study appears to support this finding, noting significantly less buccolingual resorption following a flapless procedure (Barone et al., 2014). However, results were based on a 3-month follow-up and it is possible that differences between groups may be negated over time. In support of this, a mongrel dog study noted similar amounts of ridge resorption 6 months following extraction, regardless of whether the procedure was flapless or flapped (Araújo and Lindhe, 2009).

1.11.4.4 Impact of buccal bone wall thickness on vertical and soft tissue changes

Chappuis and colleagues evaluated bone loss 8 weeks post-extraction in single-rooted maxillary teeth. Vertical bone loss mainly affected the mid-buccal aspect of the socket wall and was influenced by the thickness of the bone wall present at the sockets; this was described by the authors as a thin phenotype (<1 mm) or thick phenotype (>1 mm). Where a thin bone wall existed, a mean loss of vertical height of 7.5mm was found, equating to a median 62 per cent loss of the former mid-buccal height). Where a thicker bone wall was present, mean bone loss was just 1.1 mm, or 9 per cent, of buccal bone height (Chappuis et al., 2013). This change in bony dimensions may be masked in part by an increase in soft tissue thickness (Chappuis et al., 2015). These researchers noted that changes in soft tissue dimensions following single tooth extraction occur quickly, with greater than 50 per cent of total changes noted in the first 2 weeks of healing. In thick (BBT >1 mm) phenotypes, soft tissue remained essentially unchanged. However, in thin bone wall phenotypes (<1 mm), soft tissue dimensions increased dramatically (up to 7-fold) due to thickening. The authors suggested that as the thin facial bone wall resorbs, soft tissue ingrowth may be favoured due to its rapid

rate of proliferation (Chappuis et al., 2015). While this spontaneous soft tissue thickening may increase the amount of keratinised tissue and reduce the need for additional soft tissue grafting, it may mask the true extent of loss of underlying bone. This may be misleading for clinicians planning additional treatment at the site since facial soft tissue thickness may not correlate with the amount of underlying bone loss (Chappuis et al., 2017).

1.12 ALVEOLAR RIDGE PRESERVATION

Alveolar ridge preservation (ARP; also commonly referred to as socket augmentation/socket preservation), describes any procedure designed to eliminate or reduce the post-extraction alveolar ridge resorption and to promote bone formation within the socket (Horvath et al, 2013). The Glossary of Periodontal terms describes ARP as “a surgical procedure aimed at preventing ridge collapse and preserving ridge dimension after tooth extraction, typically done for the purpose of implant site development”. An Osteology group consensus identified reasons to perform ARP including a requirement to maintain the existing soft and hard tissue envelope, to maintain a stable ridge volume to optimise functional and aesthetic outcomes, and to simplify subsequent treatment procedures (Hämmerle et al., 2012).

ARP usually involves placement of a bone graft/biomaterial within the extraction socket immediately following extraction. A barrier membrane or soft tissue graft may be used in combination with the graft to seal the socket entrance (Mardas et al., 2023). The rationale for conducting this technique is that the graft material will act as a scaffold to minimise the volume loss which occurs at the socket/ alveolar ridge during natural healing, consequently reducing, or eliminating, the need for future ridge augmentation (Wang et al., 2004). Per the Osteology group consensus, primary indications for ridge preservation include sites where implant placement is planned at a later time point, sites where contouring of the ridge is required to facilitate subsequent prosthetic treatment, or to reduce the need for maxillary sinus elevation procedures (Hämmerle et al., 2012). While this characterises the ultimate aim of the procedure in terms of bone quantity, a moderate body of research has also discussed qualitative features of the bone, focusing on whether ARP techniques or biomaterials affect the percentage of vital bone at the healed socket site. In the context of increasing demand for dental implants, it is critical to understand how sockets heal and how optimally positioned implants can best be facilitated through effective planning around dental extractions and management of the ensuing socket and alveolar ridge.

1.12.1 EVALUATING THE EFFECT OF ALVEOLAR RIDGE PRESERVATION - *SYSTEMATIC REVIEWS*

1.12.1.1 Dimensional changes

Several systematic reviews have evaluated the effect of socket preservation in comparison to natural healing. All reviews have generally noted significant heterogeneity in the literature, attributable to variability in biomaterials, techniques and timeframes of evaluation used in primary studies. Nevertheless, findings are broadly consistent, and collectively confirm that ARP is beneficial in reducing the extent of alveolar dimensional changes observed following extraction.

Ten Heggeler and colleagues conducted one of the earliest systematic reviews on this topic, focussing on human studies of the non-molar regions that compared ARP to unassisted socket healing (USH)/ natural healing (NH), focusing solely on bone level changes. Although they included multiple study designs, these authors found a very limited available literature and stated that firm conclusions about dimensional changes could not be drawn. While ARP procedures produced smaller dimensional changes relative to natural healing, they did not prevent bone resorption and these authors concluded that mean dimensional loss of up to 2.64 mm in ridge height and 3.48 mm in ridge width may still be expected following ARP (Ten Heggeler et al., 2011). Vignoletti et al. subsequently evaluated both hard and soft tissue dimensional changes in studies of at least 3 months in duration. Meta-analysis demonstrated that ARP was associated with statistically significant reductions in vertical and horizontal bone loss compared to natural healing. These authors characterised benefits of ARP in terms of weighted mean differences (WMD) of 1.4 7mm (95% CI 0.95-1.98 mm) for height reduction and 1.83 mm (95% CI 0.73-2.97 mm) for reduction of alveolar width compared to natural healing. Limited data was available on soft tissue changes (Vignoletti et al., 2012).

The primary studies appraised by these two systematic reviews largely overlapped and the discussions of these two systematic reviews, conducted over a decade ago, make for illuminating reading in their presentation of limitations in the contemporaneous literature. These authors highlighted that evaluation of ridge width change is challenged by a resorption pattern, which invariably means that final measurements are performed at a position that is usually inferior and lingual to the original ridge location. Compounding this issue are factors such as the significant variation in location and nature of included extraction sockets and the methods used to evaluate dimensional changes. Furthermore, studies compared a variety of biomaterials and surgical techniques. These differences cumulatively contributed to a high level of heterogeneity that led both author teams to recommend that their results be interpreted with caution. Furthermore, adequate blinding and allocation concealment is challenging in trials where operators will likely perform both treatment and follow-up, and there may also be some risk of bias in ensuring sites in test and control groups are similar before treatment. Consequently, the majority of available individual studies were deemed at high risk of bias (Ten Heggeler et al., 2011, Vignoletti et al., 2012). Many of these issues persist in later systematic reviews on ARP and remain pertinent, contributing to some of the uncertainty that persists in making clear surgical recommendations to this day.

A systematic review by Avila-Ortiz and colleagues claimed that ARP can reduce ridge dimensional bone loss by 2.07 mm horizontally and 1.89 mm vertically (Avila-Ortiz et al., 2014). Pooled estimates from a more recent meta-analysis by the same research team concluded that ARP prevents 1.99 mm of horizontal bone loss (95% CI: 1.54, 2.44 mm) and vertically preserves an additional 1.72 mm (95% CI: 0.96, 2.48 mm) and 1.16 mm (95% CI 0.81, 1.52mm) of bone at the mid-buccal and mid-lingual aspects, respectively (Avila-Ortiz et al., 2019).

Mardas and co-workers further evaluated the impact of various ARP approaches on implant treatment outcomes. While ARP procedures significantly decreased the need for additional augmentation procedures, implant placement was generally feasible at both ARP-treated and untreated sites, while implant survival, success, and marginal bone levels were similar regardless of ARP treatment

(Mardas et al., 2015). In a complementary systematic review, this team found insufficient evidence to indicate the superiority of a specific technique in terms of changes in ridge dimension or with regard to histological findings (MacBeth et al., 2017). Although the volume of clinical studies available for analysis showed a large increase from systematic reviews conducted only a few years earlier, these authors noted many of the same challenges in their discussion, drawing attention to risk of bias issues, nature of included sockets, small sample size of many studies and the frequent absence of sample size calculations. The available literature was dominated by studies that evaluated dimensional changes using static casts; and evidence on the impact of ARP on keratinised tissue dimensions or patient reported outcomes was extremely limited. Compared to USH, ARP was found to result in a significant reduction in vertical mid-buccal bone loss following extraction with a standardised mean difference (SMD) of 0.74 mm, a benefit that is notably smaller than other systematic reviews. Interestingly, despite noting a standardised mean difference of 1.20 mm for horizontal ridge loss in favour of ARP, MacBeth failed to find a statistically significant benefit of ARP compared to unassisted socket healing for maintenance of ridge width; confidence intervals were wide and included zero. However, a statistically significant reduction in loss of proximal bone height (SMD 0.8 mm) was noted by this group (MacBeth et al., 2017). This latter finding regarding interproximal tissue is supported by a more recent review, which concluded that ARP also reduces the loss of interproximal soft tissue height (Couso-Queiruga et al., 2021).

Willenbacher and colleagues noted dimensional benefits of ARP, which amounted to a mean 1.33 mm in ridge width and 0.91 mm in mean height compared to natural healing. However, when multi-armed studies were excluded and studies were restricted to those with at least 6 months of follow-up, findings were somewhat enhanced, with a mean 1.54 mm (95% CI 0.44, 2.64 mm) benefit in ridge width and 1.12 mm (95% CI 0.62, 1.63 mm) in vertical height change (Willenbacher et al., 2016). In line with the conclusions of Mardas and co-workers (Mardas et al., 2015) these authors identified a decreased need for additional augmentation procedures at time of implant placement when ARP was conducted, concluding that the proportion of sockets requiring additional augmentation was approximately halved in ARP-treated sites versus control sites with USH (9.9% versus 20.8%, respectively) (Willenbacher et al., 2016). However,

since one study accounted for the majority of the cases requiring additional augmentation, these statistics may underestimate the benefit of ARP. Indeed, a previous DGI (German Association of Implantology) consensus conference concluded that the need for additional augmentation in ARP-treated sockets may be reduced more than 10-fold compared to natural healing (4% versus 42%, respectively) (Weng et al., 2011).

The interpretation of studies can be made more challenging by inter-individual variations in healing. Lindhe has noted that the relative volume of newly-formed bone following unassisted healing after 6 months varied by location, being higher in the mandible than maxilla, and in the anterior part of the mandible than the posterior region (Lindhe et al., 2013). Since ARP does not appear to be fully able to exclude either the possibility of ridge dimensional changes or the possibility that additional augmentation may be needed to accommodate implant placement, it may be queried whether immediate implant placement may be a feasible alternative to ARP. However, in a canine study, Araujo et al noted that loss of ridge dimensions post-extraction was not attenuated by placement of an immediate implant (Araújo et al., 2006). This finding was replicated in a later clinical study (Sanz et al., 2010)**,** although it may be noted that the amount of hard tissue alteration at immediate implant sites may also be influenced by location and buccal bone thickness (Ferrus et al., 2010, Tomasi et al., 2010).

1.12.1.2 Alveolar ridge preservation – Preservation of soft tissue

ARP can be beneficial with respect to interproximal soft tissue changes following extraction. Couso-Queiruga and colleagues used surface scans and CBCTs to digitally assess interproximal soft tissue height changes and measure facial bone thickness following extraction in 96 subjects undergoing maxillary single tooth extraction either with or without ARP. Although both the ARP and natural healing groups showed some loss of interproximal soft tissue, surface scans showed that ARP resulted in better preservation of interproximal soft tissue height, and this was most apparent where facial bone phenotype was thin. Overall, ARP reduced loss of interproximal soft tissue height, by a factor of 1.6 – 2, equating to approximately 1 mm (Couso-Queiruga et al., 2023a). In a related study, the same

research team studied the influence of various healing times up to 9 months on measurements of alveolar ridge preservation. While there was progressive horizontal dimensional ridge change over time affecting mainly the facial aspect, and greater resorption occurred where facial bone was thin at baseline, soft tissue thickness of facial and lingual aspects was found to be almost unaltered over time (Couso-Queiruga et al., 2023b).

1.12.1.3 Selection of graft material for ARP

Among their conclusions, several of the aforementioned systematic reviews noted that no clear recommendation could be made regarding a clearly superior material or technique for ARP (Mardas et al., 2015, Willenbacher et al., 2016). Stumbras and co-workers considered only studies in the time range 2007-2018 where healthy subjects had at least two extractions performed and followed for at least 2 months. This systematic review compared biomaterials in terms of radiographic dimensional changes and histology and was unable to reveal a "gold standard" material, although β-TCP was deemed the least effective approach to maintaining both vertical and horizontal ridge dimension in the comparisons performed. PRF used alone was adjudged to reduce alveolar ridge resorption vs natural healing based on two studies that met the inclusion criteria (Stumbras et al., 2019).

More recently, a systematic review by Avila-Ortiz and colleagues considered only randomised controlled trials that compared ARP to natural healing at 3-6 months, and clustered studies by intervention type. While the challenge of identifying a superior treatment approach remains, for horizontal ridge preservation, the use of a xenogeneic or allogenic material covered with an absorbable collagen membrane or sponge was associated with best outcomes. Furthermore, outcomes of ARP were enhanced where buccal bone thickness was >1 mm (Avila-Ortiz et al., 2019). In a similar vein, Canellas and colleagues conducted a network meta-analysis of various biomaterials to evaluate which graft materials may provide greatest prevention of dimensional ridge loss (Canellas et al., 2021). Grafts were studied by trade name, and each compared to natural healing and to each other. Results suggested that no grafting material can be consistently and

completely negate post-extraction dimensional resorption. However, the results did suggest variations depending on biomaterial(s) used. Specifically, eight materials showed statistically significant differences in both alveolar width and height when compared to natural healing, of which five were xenografts; these included Bio-Oss and Bio-Oss collagen, which are the most studied materials and which accounted for more than half of the available randomised controlled trials. L-PRF was also among these materials and demonstrated statistically significant benefits compared to natural healing, while the authors concluded that in head-to-head comparisons with other leading materials, L-PRF did not demonstrate significant differences in terms of preservation of ridge dimensions obtained (Canellas et al., 2021). A-PRF was found to be among the most effective materials when compared to natural healing for preserving alveolar ridge height, when used alone or in combination with allograft material, albeit the authors noted that these latter findings were based only on limited data. However, A-PRF did not show statistically significant reduction in ridge width versus natural healing in head-to-head comparisons. This study confirmed the findings of prior reviews in highlighting that no graft material can completely negate ridge resorption (Canellas et al., 2021).

A Cochrane systematic review by Atieh and colleagues evaluated randomised controlled trials of at least 6 months in duration. All 16 included trials were judged to be at unclear/high risk of bias. Overall, the authors failed to show evidence of a significant difference in the need for additional grafting prior to implant placement when a graft material was used, and they were also unable to provide evidence to support a specific biomaterial or technique (Atieh et al., 2021).

1.12.2 DYNAMICS OF POST-EXTRACTION HEALING AT GRAFTED SITES

As they did for naturally healing socket sites, the research team of Araujo & Lindhe also sequentially developed an analysis of the dynamics of socket healing events following grafting at mandibular extraction sockets in canines. These studies primarily focus on the effect of grafting using a xenograft material

containing collagen (BioOss collagen). An evaluation of the formation of bone within the extraction socket following two weeks of healing demonstrated that the apical part of the socket was healing similarly to a contralateral non-grafted site, leading the authors to speculate that the blood clot may have pushed the graft material out of this region. Graft particles were located in the central and coronal aspects of the socket, which was filled by a connective tissue that surrounded the biomass particles, forming a provisional matrix. The presence of multi-nucleated cells covering the xenograft particles suggested the graft might initially be recognised as a foreign material and thus the inflammatory phase of a foreign body reaction was part of the process of healing (Araújo et al., 2009). This may explain the observation that socket healing was slower that that seen at non-grafted sites.

These authors subsequently described the sequence of healing events in this central region of a socket grafted with BioOss collagen where primary closure was obtained, up to 4 weeks post-grafting. Following extraction, the graft was first soaked in blood, and then became trapped in the fibrin network of the blood clot. Neutrophils (PMNs) migrated to the surface of the graft particles, and were subsequently replaced with osteoclasts, commencing from about one week of healing. The osteoclasts appeared to remove mineral from the surface of the graft material and were replaced by osteoblasts after 1- 2 weeks. Osteoblasts laid down bone mineral in the collagen bundles of the provisional matrix, and in the period from 2 to 4 weeks, the amount of mineralised bone increased notably. Most of the new bone formed around the graft particles, with smaller graft particles seeming not only to serve as a scaffold for new bone to be laid down, but also to have integrated with the woven bone. Graft collagen was no longer present after 7 days. The concept of osteoclasts removing mineral from the graft surface was discussed further as a possible “surface cleaning” of any biomaterial that theoretically might be required to prepare it for deposition of new bone (Araújo et al., 2010). Healing up to 3 months can be inferred from a later study, wherein sites grafted with BioOss collagen demonstrated a delayed healing pattern with less mineralised bone evident, a decreased proportion of bone marrow and a greater amount of provisional (connective tissue) matrix still present at 3 months than at sites grafted with autologous bone chips. However, while sites grafted with autologous bone chips appeared to heal similarly to natural healing

histologically, dimensional changes were also similar to reference ranges for unassisted healing (Araújo and Lindhe, 2011).

1.12.3 HISTOLOGIC CHANGES

As noted above, studies conducted in canines indicate that placement of a biomaterial in the extraction socket may retard the rate of healing relative to unassisted natural healing (Araújo et al., 2010); this is reinforced by findings from a human trial by the same research team (Lindhe et al., 2014). In human sites grafted with Bio-Oss collagen and evaluated 6 months after extraction, it was noted that grafted sites did not show a clear cortical cap of bone, and bone maturation within the socket appeared to be delayed compared to naturally-healing sockets (Lindhe et al., 2014). In both studies, histology appeared to indicate that the biomaterial could be seen in the central zone of the socket, but following healing, a coagulum appeared between the lateral walls of the socket and the graft material. Socket healing appeared to be faster in the lateral and apical regions, where graft material was excluded. Bio-Oss particles were not resorbed and became surrounded by bone; the authors suggested that this may explain why grafted sites undergo less dimensional changes (Araújo et al., 2010, Lindhe et al., 2014).

Chan and colleagues systematically reviewed histologic outcomes in naturally healed sockets and augmented sites; available studies provided histologic data in the range 3-9 months. While no attempt was made to compare vital bone formation between the various materials, in naturally-healed sockets with ≥3 months follow-up, the mean percentage of vital bone was 38.5% +/- 13.4% and that of connective tissue 58.3% +/- 10.6%. From the 8 studies available, half provided data on xenografts which demonstrated equivocal results, with studies showing both increases and decreases in vital bone formation. Xenografts were also associated with a decreased connective tissue content and considerable residual xenograft material (36 per cent) present 5-6 months following ARP. Limited evidence was available for other materials (1-2 studies per material) but this implicated that alloplasts were associated with an increase in vital bone

fraction compared to non-grafted sites, whereas allografts and autografts did not show significant differences from sites with natural healing (Chan et al., 2013). These findings indicate that graft placement may change the proportion of vital bone compared to naturally healed sockets.

Jambhekar and co-workers also considered histologic findings based on studies conducting re-entry at or beyond 12 weeks after grafting (Jambhekar et al., 2015). Results concurred broadly with those of Chan et al., (Chan et al., 2013), with vital bone percentage in sockets with natural healing of 41 per cent. Once again, alloplasts were associated with a small increase in the proportion of vital bone, whereas xenografts showed a mean slight decrease in vital bone versus natural healing, and allografts showed the lowest percentage of vital bone. Both allografts (22%) and xenografts (19%) showed significant proportions of residual graft material at re-entry (Jambhekar et al., 2015). Willenbacher evaluated the histologic effects of ARP in comparison to unassisted socket healing as a secondary aspect of their systematic review evaluating dimensional changes. However, these authors were unable to make conclusive findings regarding the influence of ARP on the amount of vital bone in healed sockets, nor to identify the superiority of any specific ARP technique (Willenbacher et al., 2016).

As a clinician, one may question the practical impact of such histological differences. In this context, a recent review concluded that bone grafts/bone substitutes do not accelerate healing or induce greater formation of new bone compared to natural healing (Mardas et al., 2023). It has been highlighted that grafts that resorb more slowly may be more effective in providing a scaffold that helps to maintain ridge volume post-extraction. However, this must be balanced with the possible impact of these more slowly resorbing grafts on new bone formation (Avila-Ortiz et al., 2019), although others have noted that the impact of new bone formation on the feasibility of implant placement is questionable (Mardas et al., 2015).

1.12.4 ADDITIONAL CONSIDERATIONS FOR ARP

Since variability in the condition of the tooth socket may influence the process of natural healing post-extraction, as evidenced by the slower rate of socket healing and bone formation at sites with severe periodontal disease destruction (Ahn and Shin, 2008), it might be questioned if ARP can offer benefit where other local/anatomical concerns exist. However, a meta-analysis that compared natural healing and ARP at sockets of periodontally compromised teeth still demonstrated a statistically significant advantage of performing ARP. At these sites, ARP was associated with improved preservation of ridge dimensions and a reduction in the need for additional grafting at time of implant placement, albeit based on a limited dataset that allowed for only low to very low certainty evidence (Atieh et al., 2022).

1.12.5 IMPLICATIONS FOR ARP TREATMENT

A series of systematic reviews have demonstrated the beneficial effects of ARP on minimising the dimensional changes that occur post-extraction (Ten Heggeler et al., 2011, Vignoletti et al., 2012, Avila-Ortiz et al., 2014, Mardas et al., 2015, Willenbacher et al., 2016). A consensus report for the 15th European Workshop on Periodontology concluded that compared to natural healing; socket grafting may prevent 1.5-2.4 mm of horizontal bone loss and vertically may prevent 1.0 – 2.5 mm and 0.8 – 1.5 mm of mid-buccal and mid-lingual resorption, respectively (Tonetti et al., 2019). Despite this, **i**t is clear that ARP is unable to completely negate the potential for some dimensional reduction following extraction (Willenbacher et al., 2016), and its impact on the possibility for operators to place an implant without the need for additional grafting has been questioned (Atieh et al., 2015, Mardas et al., 2015). Furthermore, no “gold standard” material has been identified that provides consistently superior results to competitor materials (Mardas et al., 2015, Willenbacher et al., 2016, Avila-Ortiz et al., 2019, Canellas et al., 2021). Multiple comparisons of materials have been made, evaluating various graft materials alone, using membranes as sole agents, and their use in

combination but clinical approach and material selection remains somewhat at the preference of individual operators.

Interestingly, a recent retrospective study of 140 adult patients undergoing non-molar extraction with or without simultaneous ARP compared the efficacy of ARP with natural healing in reducing the need for further bone augmentation to facilitate dental implant placement in 140 adult patients undergoing non-molar extraction. Implant placement was deemed feasible using virtual planning on CBCT scans in all study sites. However, logistic regression revealed that the odds of not requiring additional bone augmentation were 17.8 times higher in sites that received ARP therapy. Furthermore, the need for additional bone augmentation was reduced 7.7 times for every 1 mm increase in facial bone thickness, regardless of baseline therapy. While it must be borne in mind that these results are based on digital planning rather than on clinical findings, the inference is that performing ARP may dramatically reduce the need for additional grafting at time of implant placement (Couso-Queiruga et al., 2022). A recent, commissioned narrative review for the *Periodontology 2000* journal, provocatively questioned "Is ARP an overtreatment?" highlighted that local anatomical factors and patient variability may make bone resorption and remodelling unique for each person and each tooth. Consequently, these authors suggested that the necessity for ARP should be assessed for each tooth in light of patient and site-based factors. These authors postulated that ARP may have greater benefit where a thin phenotype is present, especially where other factors are present that might impact on healing response or functional and aesthetic outcome (Mardas et al., 2023).

1.13 ALVEOLAR RIDGE PRESERVATION USING PLATELET CONCENTRATES

The sequence of events in post-extraction healing is influenced by the interplay of cytokines and growth factors that activate signalling pathways, to direct the recruitment of cells (Mardas et al., 2023). The first step of post-extraction healing is formation of a blood clot; this coagulum acts as a physical matrix to direct this migration of cells. As well as their roles in wound cleansing, neutrophils and macrophages release growth factors and cytokines that help to direct the migration and proliferation of mesenchymal cells that lead to maturation of the socket tissues (Miron, 2021).

L-PRF is a second-generation autologous platelet concentrate (APC) that may be considered an “optimised blood clot”; since it includes the majority of platelets and leukocytes and also results in prolonged release of growth factors that are instrumental in wound healing (Dohan et al., 2006b, Choukroun and Miron, 2017). It is prudent to briefly examine the use of PRF variants for alveolar ridge preservation, in the context of its comparison with natural healing (blood clot). Towards this aim, it is prudent to evaluate PRF used as a sole agent for ARP, and to consider its use in combination with other materials.

1.13.1 INDIVIDUAL STUDIES ON THE USE OF L-PRF AS A SOLE AGENT FOR ARP

As the early literature on L-PRF became established, research studies were initiated on a variety of potential indications, including the use of L-PRF in alveolar ridge preservation. A series of early studies evaluated healing up to 8 weeks in randomised extraction sites where L-PRF was compared to unassisted natural healing (blood clot), using measures that included clinical, radiographic and histomorphometric assessment. Hauser et al., randomised 22 premolar extraction sites to receive one of three separate protocols, including natural healing, L-PRF placement in the socket or L-PRF placement with elevation of a flap; L-PRF

membranes were also placed at all sites to seal the socket. L-PRF use was associated with a statistically significant reduction in mean horizontal bone loss; however, this benefit appeared to be negated in cases where a surgical flap was elevated (Hauser et al., 2013). The results of this often-cited investigation must be viewed in the context of the very small amount of horizontal bone loss that occurred in all study groups (with only mean 0.43 mm in natural healing controls), most probably associated with the decision to measure horizontal ridge change 4 mm apical to the gingival margin. Vertical bone loss measurements were somewhat inconclusive and neither bone biopsy nor micro-CT indicated significant differences in bone density between groups.

Suttapreyasri and Leepong also randomised paired and symmetrical premolar extractions in eight patients (20 sockets) to natural healing or L-PRF treatment, although only a single clot was used in each socket with no L-PRF socket seal. While L-PRF use was associated with faster radiographic and soft tissue healing, given the limited difference in test and control protocols, it may be unsurprising that differences observed between groups at 8 weeks in clinical dimensional changes, radiographic evidence of new bone or in soft tissue closure were not statistically significant (Suttapreyasri and Leepong, 2013). A study that compared L-PRF to natural healing at 24 randomised single extraction sites, measuring radiographic bone fill and changes in ridge width using sequential plaster casts demonstrated significantly higher radiographic bone fill and reduced mean loss of ridge width in the L-PRF group (Alzahrani et al., 2017). However, these researchers again evaluated 5 mm apical to the crest, where changes even in the natural healing control group were limited. Consequently, although statistically significant, differences between groups were small and of questionable practical relevance for clinicians. Notably, while plaster casts are widely used to assess ridge dimensional changes, they reflect a combination of hard and soft tissue dimensions and are of limited use in determining bony dimensional changes alone, since soft tissue may increase in thickness to compensate for bone resorption (Chappuis et al., 2015).

In a clinical pilot study, Anwandter et al., evaluated clinical and radiographic healing, using bone sounding and CBCT measurements respectively, in the first 4

months post-extraction in 18 single-rooted tooth sockets filled with L-PRF. Full-thickness flaps were elevated to facilitate the procedure. Several membranes were placed in the socket and an additional membrane was used to seal the socket. Although dimensional changes were limited, both clinical and radiographic measurements showed wide standard deviations, with mean clinical loss of ridge width of 1.18 mm ± 2.4 mm and vertical bone loss of 0.44 mm ± 3.5 mm respectively at 4 months (Anwandter et al., 2016). The authors concluded that the results obtained compared favourably with published outcomes following natural healing and were consistent with the dimensional changes observed with other grafting materials. The same research group also conducted a split mouth RCT, comparing natural healing with L-PRF following paired, flapless extractions in non-molar sites of 22 patients (Temmerman et al., 2016). Two to five clots were placed in test sockets with 2 to 3 membranes used to seal the socket. CBCT scans were conducted at baseline and 3-months post-operatively to investigate the benefits of L-PRF in preservation of vertical and horizontal ridge dimensions and socket fill. Horizontal changes were measured at 1, 3 and 5 mm below the crest and demonstrated reduced loss of ridge width as one moves apical from the crest, regardless of the protocol. Mean horizontal dimensional changes were greater than their previous study (Anwandter et al., 2016), with a mean loss of: 2.4 ± 2.3 mm (22.8% ± 24.3%) for L-PRF. However, this compared favourably to natural healing controls, where much greater dimensional loss was evident (5.4 ±-4.4 mm; 51.9% ± 40.3%). Vertical bone loss was only significantly different at the buccal aspect where the L-PRF group lost a mean 0.1 ± 1.6 mm of bone height versus natural healing controls who lost 1.5 ± 1.3 mm. Socket fill was almost complete (mean 94.7 per cent) at test sites and significantly greater than in controls (Temmerman et al., 2016). Zhang and colleagues compared PRF to natural healing in 28 patients undergoing molar extraction. Test sites received two PRF membranes. CBCT and soft tissue healing at 3 months was evaluated. The differences in horizontal and vertical dimensional change were not statistically significant. Use of L-PRF was observed to promote faster soft tissue healing and to be associated with reduced duration of patient discomfort, albeit no related statistics were presented. Histologically, PRF use was associated with a faster rate and a statistically significant greater area of new bone formation (Zhang et al., 2018).

While most studies have focused on results following single-tooth extraction, Castro and colleagues later compared two PRF variants (L-PRF and A-PRF+) with a natural healing control in 20 patients requiring at least 3 extractions in the premaxilla, using CBCT to assess dimensional changes at 3 months, and bone biopsy to facilitate histology and micro-CT (Castro et al., 2021). The treatment protocol was randomly assigned, leaving one empty socket or edentulous area between the test sites. Although both PRF protocols showed trends towards reduced dimensional changes and more new bone formation compared to natural healing controls, differences were not statistically significant. However, radiographic bone fill was significantly increased with both PRF protocols (Castro et al., 2021). A more recent randomised controlled trial concluded that despite an observed increase in growth factors at socket sites grafted with L-PRF, this was not translated into improved dimensional outcomes compared to sites allowed to heal naturally (Wang et al., 2022).

1.13.2 SOFT TISSUE HEALING & PAIN EXPERIENCE

According to Choukroun & Miron, direct contact of PRF with the periosteum enhances blood supply to the keratinised soft tissue, favouring its thickness, and also enhances blood supply to the underlying bone (Choukroun and Miron, 2017). The clinical impact of L-PRF on soft tissue healing and patient experience of discomfort has been investigated in many studies. Temmerman and colleagues utilised a visual analogue scale (VAS) to measure patient experience of post-operative pain following extraction and observed that subjects grafted with L-PRF reported less post-operative pain at days 3, 4 and 5 than those whose sockets were allowed to heal naturally (Temmerman et al., 2016). Marenzi et al., conducted a split-mouth randomised controlled trial in 26 patients receiving bilateral extractions (108 sockets), although limited detail was provided on how L-PRF was used. Test sites received L-PRF while contralateral sockets were allowed to heal naturally. Mean post-extraction pain, as measured by visual analogue scale (VAS) up to day 3, showed statistically significant lower values at L-PRF sites. Furthermore, grafted sockets demonstrated faster socket closure and improved healing as measured using the Wound Healing Index (Marenzi et al., 2015). However, it must be noted that many of the included patients had multiple

simultaneous paired extractions, and since different protocols were sometimes used at the midline region, pain from an adjacent site may have affected VAS responses at study sites in some instances. Consequently, the reported VAS pain measurements may not reflect the levels of pain likely to be experienced following single tooth extraction. Furthermore, since all healing index scores inferred excellent healing, the clinical significance of the differences is questionable.

A large tranche of the available evidence on the use of PRF variants at extraction sockets relates to its use at third molar sockets, where more than 20 randomised controlled trials of varying duration have been conducted comparing PRF variants to natural healing. These studies offer little value to the consideration of changes in ridge dimensions, given that these sites will not be likely to be indicated for subsequent restoration. However, although one recent meta-analysis questioned the beneficial effect of PRF in mandibular third molar sockets (Al-Hamed et al., 2017), a more recent systematic review highlighted that PRF has been associated with, enhanced soft tissue healing, decreased post-operative pain and lower rates of alveolar osteitis at third molar sites (Zhu et al., 2021).

A recent, invited narrative review addressed the role of APCs in various indications related to bone regeneration, considering only RCTs/CCTs for alveolar ridge preservation (Quirynen et al., 2023). These authors highlighted that protocols for PRF preparation and its use at tooth sockets have varied. They cited the “correct protocol” was one in which condensed membranes/plugs (rather than obtained clots) were used, in sufficient number, with sealing of the socket using L-PRF membranes. Based on this approach, these authors noted collated findings including: significantly less ridge resorption and faster soft tissue healing, higher radiographic bone density and “histologically improved bone quality”. They highlighted that results may be less impressive where the socket is inadequately filled or L-PRF is insufficiently compressed, or where the socket is not sealed. These authors attributed the observed beneficial effects to a variety of factors. Among these were the support provided by the fibrin matrix for the blood clot, and its additional haemostatic effect. The release of growth factors provides an increased opportunity for regeneration, while L-PRF also enhances neo-angiogenesis and regulates local inflammation. L-PRF has antimicrobial activity

and also acts to physically seal the socket to delay epithelial ingrowth (Quirynen et al., 2023).

1.13.3 SYSTEMATIC REVIEWS ON ALVEOLAR RIDGE PRESERVATION USING PLATELET CONCENTRATES

Given the limited number of available studies, it is perhaps unsurprising to find that systematic reviews to this point have struggled to make definitive conclusions regarding the use of L-PRF and its variants for ARP. Indeed, the literature has been complicated by heterogeneity in protocols used to prepare PRF, with many studies either not detailing their protocol or utilising PRF variants created using modified preparation protocols that intend to alter the structure, cellular content or growth factor release profile relative to L-PRF.

1.13.4 AUTOLOGOUS PLATELET CONCENTRATES - OVERALL FINDINGS

Several systematic reviews have included all autologous platelet concentrates in their analyses of alveolar ridge preservation. Moraschini & Barboza conducted one of the first such systematic reviews, including RCT/CCT/prospective cohort studies with ≥ 2 months follow up (Moraschini and Barboza, 2015). Results were based on just two studies that evaluated L-PRF (Hauser et al., 2013, Suttapreyasri and Leepong, 2013), both of which were judged to be at high risk of bias. Nevertheless, the authors concluded that there was some limited evidence for reduced discomfort with L-PRF, but no evidence of statistically significant dimensional ridge changes (Moraschini and Barboza, 2015). A later systematic review included human RCTs of ≥3 months in duration involving non-molar teeth (Niu et al., 2018). Only five studies were available for qualitative synthesis, three of which included L-PRF. Of these, only a single study (Temmerman et al., 2016) was available to compare the use of PRF to natural healing, while two additional studies evaluated L-PRF in combination with other materials, namely DFDBA

(Thakkar et al., 2016) or β-TCP (Das et al., 2016). The authors were unable to conduct meta-analysis due to the heterogeneity of available studies and made limited conclusions. However, in contrast to previous findings (Moraschini and Barboza, 2015), these authors concluded that collectively, treatment including L-PRF had a beneficial effect in preserving ridge dimension (Niu et al., 2018). Del Fabbro and colleagues observed that soft tissue healing and bone density at 1, 3 and 6 months were statistically better for sockets treated with APCs. The authors were unable to conduct meta-analysis on the benefits of APCs in reducing pain after extraction due to heterogeneity that encompassed factors such as timing of assessment, different duration of follow up, and data reporting issues, with most studies not reporting standard deviations and some reporting VAS recordings from cumulative days in one measurement. Seven studies reported a statistically significant decrease in pain while five failed to find such differences (Del Fabbro et al., 2017).

Canellas and co-workers conducted a meta-analysis on the use of APCs in various oral surgical procedures. While concluding that most available studies on ARP demonstrated reduced resorption following the use of PRF, alone or in combination with another biomaterial, limited information was available, meta-analysis was not possible and the results should be interpreted with caution (Canellas et al., 2019).

1.13.5 SYSTEMATIC REVIEWS OF PRF USE FOR ALVEOLAR RIDGE PRESERVATION

The clinical use of PRF and its variants has been specifically addressed in several more recent systematic reviews. In a systematic review addressing various surgical indications for L-PRF, Castro and colleagues found eight available randomised controlled trials that utilised L-PRF for alveolar ridge preservation. Of these, five studies used only third molar teeth, and follow-up ranged from as little as 7 days up to 4 months in duration. While no meta-analysis was possible, the authors reported that L-PRF use was associated with a decrease in healing time and patient discomfort, as well as enhanced soft tissue healing at extraction sites

(Castro et al., 2017b). As detailed below, several subsequent systematic reviews have been conducted on this subject, largely evaluating the same, limited number of primary studies, including several of those studies previously addressed in this chapter. As such, these systematic reviews have differed primarily based on their inclusion criteria, specifically in relation to the number of included subjects and follow-up duration of included individual research studies. The individual primary studies have generally been deemed to be at high risk of bias, and each review team has agreed with Castro et al., in concluding that additional high-quality trials are still required (Castro et al., 2017b).

Pan et al. evaluated PRF used alone or used in combination with other materials in ARP, and concluded that PRF may have potential value in reducing post-operative pain, and in alveolar dimensional changes. Five of the seven available trials compared PRF with natural healing while two evaluated L-PRF in combination with other biomaterials. Meta-analysis was not possible for vertical and horizontal changes and only mesial proximal bone changes were reported as statistically significant (Pan et al., 2019). Lin and colleagues included studies of ≥1 month follow up; these more lenient inclusion criteria resulted in eight eligible trials (six RCT, two CCT). While PRF appeared favourable for ARP, differences were not statistically significant compared to natural healing for change in bone height (WMD 0.28 mm benefit; 95% CI 0.0 to 0.55 mm) or bone width (WMD 1.72 mm benefit; 95% CI -0.12 to 3.58 mm). These authors concluded that PRF used alone in ARP does not provide a significant additional benefit with regard to bone volume, bone density or osteoblastic activity compared to natural healing alone (Lin et al., 2019).

Dragonas and co-workers assessed the effects of L-PRF on regeneration, soft tissue healing, and complications in patients undergoing ARP/ ridge augmentation/sinus augmentation. Third molar studies were excluded (as the authors noted these are not normally related to site preparation for future implants). The authors concluded that overall, use of L-PRF in ARP was associated with “modest” beneficial effect, by decreasing remodelling and producing some reduction in post-operative pain (Dragonas et al., 2019). Again, these authors highlighted heterogeneity related to preparation of L-PRF, the number of clots

used, and whether this was reported, and if L-PRF membranes were used to seal the socket, with all studies judged to be at unclear/high risk of bias.

More recently, Al-Badran and colleagues reported on the impact of selected PRF protocols on ARP and wound healing. These authors included all RCTs/CCTs - without restrictions on duration - that compared PRF to natural healing, and that reported on post- extraction outcomes of the soft tissues (pain/swelling/dry socket/soft tissue indices) and hard tissues (vertical change/horizontal change/socket fill/new bone). This resulted in 40 studies available for analysis. The collected outcomes were viewed as a composite group to establish the effects of PRF, and also in the context of comparing different RCF protocols (termed Low RCF/standard/high RCF/concentrated growth factors, CGF) as they relate to the relative g-forces used in their preparation. Overall, when soft and hard tissue outcomes were pooled, the data indicated positive and statistically significant outcomes from using PRF compared to natural healing, including in all four bone-related outcome variable (Horizontal and vertical resorption, socket fill and new bone formation). However, no significant differences were observed between PRF protocols on bone outcomes. PRF preparation protocols with mid-range g-forces corresponded best to clinical soft tissue healing outcomes while protocols with very low and very high g-forces presented less consistent findings. While the authors concluded that protocol type influences overall outcomes, significant findings were restricted to soft tissue healing, pain, and prevention of alveolar osteitis (Al-Badran et al., 2023). However, since the preparation protocol was often poorly described or heterogeneous -even within sub-categories - the authors advocated that clear descriptions of each aspect of preparation should be included in every publication in future (Al-Badran et al., 2023). This aligns well with a previous recommendation that all future PRF publications should report on specific key parameters to enable accurate interpretation and comparison of findings (Miron et al., 2019d).

Another recent systematic review compared PRF to natural healing and concluded that bone dimensional loss was significantly lower in PRF cases where short-term outcomes (8-15 weeks) were assessed, but not in longer-term studies (6 months). Soft tissue healing was significantly improved in the majority of studies while

approximately two thirds of studies showed improvement in post-operative pain, most notably in the first 3 days after use (Al-Maawi et al., 2021). A contemporary network meta-analysis, which compared L-PRF with natural healing in head-to-head comparisons and quantified key hard tissue-related findings, concluded that L-PRF use was associated with statistically significant reductions in ridge width (mean reduction 1.66 mm; 95% CI 0.66 – 2.67 mm), and mean ridge height (mean reduction of 0.79 mm; 95% CI 0.02 – 1.57 mm), compared with natural healing (Canellas et al., 2021).

1.13.6 PRF COMPARED WITH/ IN COMBINATION WITH OTHER MATERIALS

In principle, the addition of blood material to a bone substitute could help to bind the particles of bone substitute together and to provide regenerative growth factors to the graft; this might afford osteoinductive potential to the osteoconductive properties normally obtained in a bone substitute material. An in vivo study demonstrated that the addition of blood to a bone substitute material affected the tissue reaction to the graft, potentially enhancing its regenerative potential (Barbeck et al., 2015). Indeed, a systematic review of biologics and emerging regenerative components for implant site development concluded that autologous cell treatments and cell-enriched bone grafts enhance the effectiveness of bone regeneration (Avila-Ortiz et al., 2016). Anecdotally, this approach is widely utilised by practitioners and the idea of combining PRF with a biomaterial arises from this line of thought. In theory, PRF might enhance healing of a particulate bone substitute material due to its wealth of growth factors and cytokines that facilitate wound healing and its ability to promote angiogenesis. Platelet rich fibrin is rich in growth factors and cytokines that facilitate wound healing; these are released over a period of at least 7-14 days in vitro and could therefore further enhance the integration and clinical effect of a bone graft material (Quirynen et al., 2023). In theory, this might afford osteoinductive potential to the osteoconductive properties normally obtained in a bone substitute material. Anecdotally, this approach is widely utilised by practitioners. In turn, some authors have questioned the suitability of PRF as a standalone regenerative graft material due to its limited mechanical strength and stability,

and questions surrounding whether PRF degrades too quickly to serve a purpose as a barrier material (Agrawal, 2023). Consequently, it appears appropriate to consider the use of PRF in combination with a bone substitute material. Brown & Barker reviewed fibrin-based biomaterials and concluded that mechanical properties of these materials can be supplemented by forming hybrid materials with polymers or extra-cellular matrix components such as collagen (Brown and Barker, 2014). Such combinations might change the strength and durability of fibrin products in vivo. In theory, the combination of PRF with another graft material could offer better mechanical strength and space-making ability; this might enhance the outcomes seen with PRF alone. However, there appears to be limited information around the combination of PRF with bone substitutes for alveolar ridge preservation, as detailed below.

1.13.6.1 Comparison studies

Yelamali and Saikrishna compared the effectiveness of PRF versus PRP on bone and soft tissue healing at third molar sockets in a non-randomised clinical trial where split-mouth third molar extractions were performed. PRF provided a statistically significant improvement in both hard and soft tissue healing (Yelamali and Saikrishna, 2015). Das and colleagues randomised 26 patients (30 sockets) to receive either L-PRF or β – TCP in combination with a collagen membrane (β – TCP – Cl) in a parallel, randomised controlled trial with 6-month follow-up. Clinical results and CBCT indicated significantly greater vertical reduction at β-TCP–Cl sites, while L-PRF sites appeared to undergo significantly greater horizontal ridge reduction in the coronal third of the socket. The authors concluded that both materials appeared suitable for ARP, albeit both groups demonstrated a similar, relatively high level of buccolingual dimensional loss (Das et al., 2016). In a randomised controlled trial of ARP at single-rooted tooth sites, Girish Kumar et al., randomised 90 sockets (48 patients) to receive L-PRF used alone as a socket plug, or plaster of Paris covered by L-PRF and compared to a control group with natural healing, evaluating findings up to 6 months. Plaster of Paris is an osteoconductive, calcium sulphate material with a claimed fast resorption profile (4 weeks) that allows its replacement with new bone. However, differences between groups were not statistically significant, although a trend towards better ridge

preservation and post-operative comfort for sites treated using plaster of Paris + PRF was noted (Girish Kumar et al., 2018).

1.13.6.2 Combination studies

Using a beagle dog model, Park and colleagues evaluated histomorphometric and dimensional changes occurring at extracted molar root sockets up to 6 weeks post-extraction. Both roots of both mandibular first molars were extracted and treated using one of four approaches: natural healing, grafting with L-PRF, grafting with Bio-Oss or grafting with a combination of L-PRF and Bio-Oss. Sockets were assigned "in an interpolated fashion" and flap closure was obtained at all sites. L-PRF showed higher bone fraction area occupancy (BAFO) compared to natural healing sites but differences from Bio-Oss or Bio-Oss + L-PRF were not statistically significant. L-PRF sites demonstrated features typical of greater maturation and bone remodelling, and the authors concluded that L-PRF alone could promote socket regeneration at a level comparable to xenograft (with or without L-PRF). Xenograft material better preserved vertical dimension but differences between groups in the horizontal dimension were not significant (Park et al., 2023).

A retrospective review of 45 single posterior tooth sites treated using either PRF alone, xenograft alone or a combination of L-PRF and xenograft provided 6-month clinical and radiographic data. The L-PRF group experienced a statistically significant increase in resorption relative to the other groups, while the combination of L-PRF and xenograft was associated with less dimensional change than xenograft alone. Post-operative pain was increased and wound healing was significantly poorer in the xenograft group (De Angelis et al., 2019). Thakkar and colleagues compared DFDBA with a combination of DFDBA mixed with L-PRF, covering all sites in both groups with a collagen membrane. Both groups had a statistically significant reduction in ridge dimensions over the 6-month study period. While PRF combined with DFDBA significantly enhanced the preservation of horizontal ridge width compared to DFDBA alone, no significant differences in vertical dimensional changes were noted (Thakkar et al., 2016). The apparent beneficial effect of the combination of L-PRF and a graft material in terms of

horizontal ridge preservation may offer support for further evaluating the use of L-PRF in combination with other biomaterials.

1.13.7 COMPOSITE GRAFTS - L-PRF BLOCK

L-PRF Block is a composite material that combines deproteinised bovine bone mineral (DBBM) - a particulate xenograft material (Bio-Oss®, Geistlich Pharma, Wolhusen, Switzerland) - with chopped L-PRF membranes in equal quantities to form a homogeneous mix, to which liquid fibrinogen is then added (Cortellini et al., 2018). On contact with the chopped L-PRF, the liquid fibrinogen undergoes rapid coagulation, binding the graft-L-PRF mix together into the form of a gelatinous “block” which may offer a more supportive scaffold with space maintaining ability for application at a wound site. Cortellini and colleagues introduced L-PRF Block by conducting a proof-of-concept study that demonstrated that L-PRF Block could be effectively utilised for lateral ridge augmentation (Cortellini et al., 2018). Utilising CBCT, these authors noted that L-PRF Block provided an average linear horizontal gain of 4.6 mm immediately following grafting, with graft volume well maintained at follow-up 5-8 months later. Histologically, Castro and colleagues characterised L-PRF Block histologically as consisting of deproteinised bovine bone mineral (DBBM) particles surrounded by platelets and leukocytes, all embedded in a fibrin network. While this fibrin network was less dense than that of an L-PRF membrane, it enmeshed blood cells and maintained the biologic components of the L-PRF. The ratio of autologous tissue: DBBM particles was approximately 60:40; although this material suggests a homogeneous mix of L-PRF and DBBM, the subsequent addition of liquid fibrinogen causes additional coagulation which increases the autologous proportion (Castro et al., 2019a).

The development of L-PRF Block derives from the concept of combining a material that provides a scaffold and which provides an osteoconductive ability, with the bioactive properties of a platelet concentrate with potential osteoinductive ability (Castro et al., 2019a). L-PRF Block maintains a high concentration of leukocytes and platelets, and preserves the ability to releases growth factors up to 14 days

(Castro et al., 2019a). As such, it may represent a suitable material to enhance bone augmentation.

The selection of DBBM for this composite graft material is not arbitrary; reviewing bone grafts and bone substitutes as alternatives to the use of autogenous bone for augmentation, Benic and Hammerle noted that DBBM xenografts may be accepted as a veritable "gold standard" as the best documented and most heavily researched bone graft material (Benic and Hämmerle, 2014). Interestingly, Stephan and colleagues demonstrated in vitro that DBBM could support the attachment of osteoblastic cells and furthermore supported their proliferation (Stephan et al., 1999). Furthermore, Schwartz and colleagues decalcified DBBM bone *in vitro* and used solvents that targeted the release of bone morphogenetic proteins (BMPs). Analysis of material extracted from this process indicated that TGF-β and BMP-2 may be present within the DBBM. Subsequent implantation in nude mice indicated that as well as containing small amounts of growth factors, the extracted material appeared to have some osteoinductive potential (Schwartz et al., 2000). Jiang and colleagues incubated radiolabelled growth factors with DBBM and found that IGF-1 and PDGF-BB both adsorbed to the DBBM and their subsequent release over time could be demonstrated. Furthermore, the PDGF-BB appeared to be released more effectively and to enhance proliferation of osteoblastic cells *in vitro* compared to DBBM alone, indicating adsorbed growth factors could enhance the osteogenic qualities of a graft. Taken together, these studies indicate that DBBM may have some inherent, albeit minor, potential to offer an osteoinductive capacity and may represent an optimal graft material to combine in vivo with autogenous products (Jiang et al., 1999).

To date, there do not appear to be any studies that have investigated L-PRF Block for ARP. Indeed, there appears to be limited discussion in the literature regarding the combination of PRF with any bone substitutes for alveolar ridge preservation, where it may potentially offer practical benefits to enhance healing. In light of this, a systematic evaluation of the available research literature may be of value to compare the efficacy of platelet rich fibrin (PRF) with PRF used in combination with any other regenerative biomaterial, for the purposes of obtaining alveolar ridge preservation.

1.14 SUMMARY & OVERVIEW OF RESEARCH THESIS STRUCTURE

This PhD research aims to evaluate the efficacy of leukocyte- and platelet-rich fibrin (L-PRF) in the management of both alveolar ridge preservation (ARP) and peri-implantitis. The research thesis is divided into four individual studies. For consistency of narrative, and since some studies ran in parallel, the studies are not presented in the sequence in which they commenced/were completed

L-PRF has been researched as a regenerative material at extraction sockets, where it appears to provide benefits over unassisted natural healing with respect to soft tissue healing (Zhu et al., 2021) and post-operative pain (Marenzi et al., 2015, Temmerman et al., 2016). While some investigators have observed reduced dimensional changes post-extraction at L-PRF treated sites (Hauser et al., 2013, Temmerman et al., 2016), conflicting outcomes have been noted (Zhang et al., 2018, Castro et al., 2021). The combination of L-PRF with other biomaterials for ARP is an area of developing interest and may conceptually offer advantages for the mechanical strength and space-making ability of L-PRF grafts (Brown and Barker, 2014) . In particular, the structured combination of deproteinised bovine bone mineral with L-PRF membranes and liquid fibrinogen, termed L-PRF Block, may offer promise for enhanced results (Castro et al., 2019a, De Angelis et al., 2019).

- Chapter 3 presents a systematic review of the literature that compares the use of platelet rich fibrin (PRF) materials used alone, in comparison with PRF used in combination with any other graft biomaterial for the purpose of alveolar ridge preservation.
- Chapter 4 describes a randomised controlled trial conducted to compare the use of L-PRF used alone with L-PRF Block (a hybrid graft of L-PRF membranes and deproteinised bovine bone mineral), for alveolar ridge reservation at non-molar extraction sockets.

L-PRF membranes are frequently used to cover periodontal defects for indications including the regenerative management of infrabony and furcation defects, as well as to seal the extraction sockets following alveolar ridge preservation procedures (Quirynen et al., 2022). These indications ideally require a membrane that forms an occlusive barrier over the wound margins, and which also has sufficient strength/rigidity to maintain space for wound healing processes (Scantlebury, 1993).

- An *in vitro* study comparing degradation of L-PRF membranes with that of A-PRF (Advanced PRF) membranes - a PRF variant produced using a low-speed centrifugation process – is presented in Chapter 5. While degradation of these membranes has been compared previously, this research investigation attempted to use a more detailed assessment of physical characteristics of membranes as they degraded, and also utilised a solution of the enzyme plasmin -the enzyme principally responsible for fibrin degradation in vivo.

L-PRF materials may play a role in enhancing osseointegration at dental implants (Öncü et al., 2016) and may contribute to improved outcomes at treated peri-implant defects (Hamzacebi et al., 2015) although the current literature remains sparse.

- Chapter 6 describes a study wherein peri-implants defects treated using L-PRF and L-PRF in combination with other biomaterials (including L-PRF Block) were evaluated longitudinally as part of a prospective cohort with 2-3 year follow-up study. The principal focus of this study related to whether magnetic resonance frequency assessment (MRFA) can be utilised as an adjunctive tool to clinical and radiographic assessment to evaluate treatment response following peri-implantitis treatment. The results of regenerative approaches that included L-PRF and L-PRF in combination with other biomaterials are presented

1.15 OVERALL RESEARCH HYPOTHESIS & AIMS

1.15.1 OVERALL RESEARCH HYPOTHESIS

The principal hypotheses for this PhD research thesis are as follows:

- That L-PRF used in combination with another graft material (e.g. L-PRF Block) may provide additional dimensional benefit in alveolar ridge preservation compared to L-PRF used alone
- That PRF preparation protocol may impact on degradation rate of these membranes *in vivo*
- That L-PRF may be considered a viable adjunctive treatment material in the management of peri-implant defects

1.15.2 OVERALL RESEARCH AIMS

The overall aims of this project were as follows:

- To systematically review the literature in order to compare the efficacy of platelet-rich fibrin (PRF) with PRF used in combination with any other regenerative biomaterial for the purposes of obtaining alveolar ridge preservation
- To compare the use of L-PRF and L-PRF Block for alveolar ridge preservation at non-molar sites, in terms of clinical and radiographic outcomes, soft tissue healing and patient-reported outcomes
- To compare the degradation of PRF membranes prepared using two alternative centrifugation protocols (L-PRF versus A-PRF) in a plasmin solution of standardised concentration

- To assess whether surgical peri-implantitis treatment approaches involving L-PRF materials provide favourable treatment responses up to 2–3 years and may be considered for clinical use
- To investigate the impact of peri-implantitis treatment on clinical parameters and implant stability quotient (ISQ) values in the medium term, and to ascertain if magnetic resonance frequency analysis (MRFA) can be used as a tool to demonstrate post-operative healing following the treatment of peri-implantitis

CHAPTER 2: GENERAL METHODS

2.1 BLOOD COLLECTION METHOD

Venous blood was collected from the antecubital fossa of each participant utilising a 23G sterile butterfly needle (Vacuette®, Greiner Bio-one. Kremsmünster, Austria).

Sterile 9 mL silica-lined plastic vacuum blood collection tubes (not containing any anticoagulant or other additive agent), were used for blood collection (Red Cap 9 mL Serum Clot Activator Blood Collection Tube, IntraSpin®, Boca Raton, FL, USA). Blood was collected until it reached the level of a pre-defined mark on the external surface of each tube, to ensure tubes could remain balanced within the centrifuge during preparation. The number of tubes collected was dependent on the study in question.

2.2 CENTRIFUGATION OF COLLECTED BLOOD SAMPLES

Blood was processed in a standardised centrifuge (IntraSpin®, Intra-Lock®, Boca Raton, FL, USA) to prepare platelet-rich fibrin (PRF) products.

Following collection of the first two tubes of blood, these were immediately placed in the centrifuge, (positioned opposite one another to ensure the centrifuge was balanced), and centrifuged for up to 1 minute, while the next two blood tubes were collected. This was repeated as each subsequent pair of blood tubes were collected, depending on collection time. This approach militates against coagulation within the blood tube, as tubes do not contain any anticoagulant agent.

2.2.1 STANDARDISED RCF VALUE REPORTING

The PRF preparation protocol is defined by centrifugation rate (revolutions per minute; rpm) and time following placement of the final two tubes within the centrifuge. The centrifuge model, its rotor angulation and rotor dimensions (radius) and the composition and size of blood collection tubes all impact on the biologic PRF clot produced and by convention must be reported (Miron et al., 2019d, Miron et al., 2023).

The IntraSpin® is a calibrated, fixed-angle centrifuge with 8 blood tube buckets. It has a rotor angulation of 33° and an approximate radius from rotor to clot of 50 mm.

2.2.2 PREPARATION PROTOCOL FOR L-PRF

Venous blood was collected from the antecubital fossa as previously described. The centrifugation protocol used to prepare L-PRF was:

- Centrifugation at 2700 rpm for 12 min (RCF-clot 408g)

2.2.2.1 Preparation of L-PRF plugs/membranes

L-PRF materials were prepared according to the protocol outlined by Temmerman and co-workers (Temmerman et al., 2016). Following centrifugation, L-PRF was identifiable as a dense, yellowish clot occupying the middle layer of the blood collection tube. The L-PRF clot was retrieved from the blood tube using a surgical tissue pliers, placed on gauze and gently separated from any attached red blood cell clot using a metal spatula.

L-PRF clots were placed into a metal membrane preparation box (Xpression® box, Intra-Lock® International, Boca Raton, FL, USA) to manipulate into the desired form for surgical use. L-PRF clots are used surgically in two principal forms:

- L-PRF plug: The clot was placed within a small cylindrical tube in the Xpression® box that is perforated at its base. Each clot was condensed with a weighted plugger, expelling much of its fluid content to form a dense plug.
- L-PRF membrane: The L-PRF clot was laid flat within the Xpression® box and a light metal plate was applied to compress the clots under gravitational force. The resulting membranes are of standardised thickness (1 mm).

2.2.3 MODIFIED PROTOCOL FOR PREPARATION OF A-PRF

Venous blood was collected from the antecubital fossa as previously described. The following centrifugation protocol was used to prepare A-PRF

- Centrifugation at 1500 rpm for 14 minutes (126g)

2.2.4 MODIFIED PROTOCOL FOR PREPARATION L-PRF BLOCK

L-PRF Block was prepared according to the protocol described by Cortellini and colleagues (Cortellini et al., 2018). In addition to the collection of red cap tubes for L-PRF, one additional 9 mL plastic tube without coating (White cap plastic coated tubes (WCT), Intra-Lock® International, Boca Raton, FL, USA was collected). This tube was collected and loaded last into the centrifuge (with a balancing tube containing water opposing it on the opposite bucket of the centrifuge) and centrifuged immediately for 3 minutes at 2700 rpm (408 g). Centrifugation was interrupted after 3 minutes to remove the white cap tube; the remaining red cap tubes were centrifuged for an additional nine minutes to complete the 12-minute cycle for L-PRF.

Immediately after removal of the white cap tube, the straw-yellow coloured fluid at the top of the tube above the red blood cells was aspirated using a sterile 5 mL syringe, without collecting any of the red cell component from the tube, and was set aside for later use. This fluid is termed “liquid fibrinogen”.

Two L-PRF membranes were cut into small pieces using a surgical scissors. These were then mixed with deproteinised bovine bone mineral (DBBM; Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland), in a surgical galley pot at a ratio of 2 membranes / 0.5g biomaterial, to form a homogeneous mix. Approximately 2 mL of liquid fibrinogen was then sprayed over this mix and stirred gently for approximately 5-10 s. Due to rapid polymerisation of the liquid fibrinogen, this mix was converted into L-PRF Block material, which was ready for use after approximately 3 minutes.

Figure 5 demonstrates examples of an L-PRF clot and L-PRF Block. Figure 6 provides a composite image of the practical steps involved in L-PRF preparation.

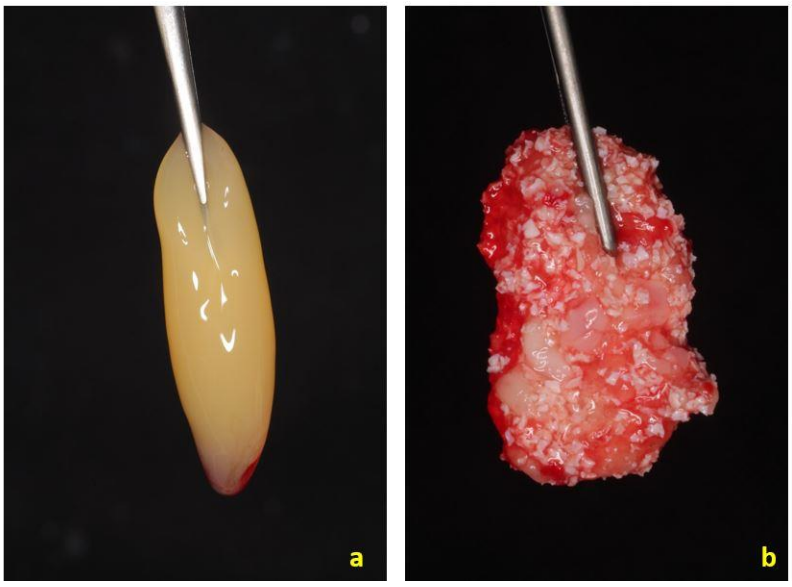

Figure 5: Examples of L-PRF clot (a) and L-PRF Block (b)

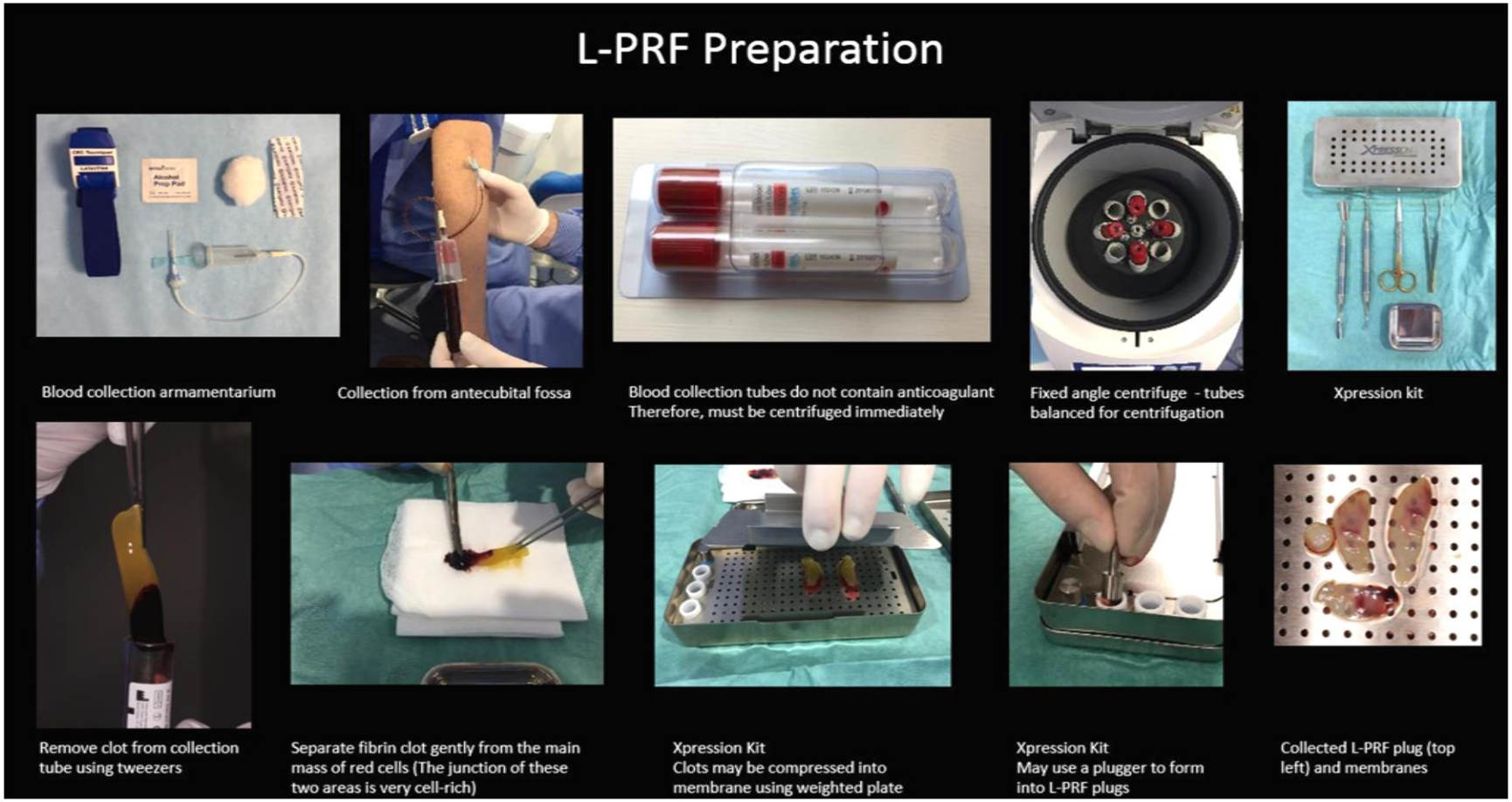

Figure 6: Composite image describing steps of blood collection and L-PRF preparation

CHAPTER 3: A COMPARISON OF THE OUTCOMES OF ALVEOLAR RIDGE PRESERVATION WITH PLATELET-RICH FIBRIN (PRF) ALONE VERSUS PRF USED IN COMBINATION WITH OTHER BIOMATERIALS: A SYSTEMATIC REVIEW

3.1 INTRODUCTION

L-PRF has been used at extraction sockets for alveolar ridge preservation (ARP), where it has been associated with reduced post-operative pain (Marenzi et al., 2015, Temmerman et al., 2016) and improved soft tissue healing (Zhu et al., 2021). However, its ability to limit post-extraction dimensional changes compared to unassisted natural healing may be questioned; with some systematic reviews concluding L-PRF may be beneficial (Dragonas et al., 2019, Canellas et al., 2021) but others providing conflicting findings (Lin et al., 2019, Al-Maawi et al., 2021).

It appears appropriate to consider the use of L-PRF in combination with a bone substitute material for the purposes of ARP. The addition of blood to a bone substitute may enhance the regenerative potential of that graft (Barbeck et al., 2015, Avila-Ortiz et al., 2016), while conversely, the mechanical properties of fibrin-based biomaterials may be enhanced by combining them with other biomaterials (Brown and Barker, 2014).

To date, there do not appear to be any studies that have investigated L-PRF Block for alveolar ridge preservation. Indeed, there appears to be limited discussion in the literature regarding the combination of any form of platelet-rich fibrin (PRF) with any bone graft or bone substitute material for alveolar ridge preservation, where it may conceivably offer practical benefit to enhance healing. In light of this, the aim of this systematic review was to compare the efficacy of PRF used alone with that of PRF used in combination with any other regenerative biomaterial, for the purposes of obtaining alveolar ridge preservation.

3.2 MATERIAL & METHODS

3.2.1 FOCUSED RESEARCH QUESTION

The present systematic review aimed to answer the following focused question, which was structured according to the PICO (Population, Intervention, Comparison, Outcome) format:

"In healthy adult patients or experimental animals undergoing tooth extraction (in the maxilla or mandible), what is the efficacy of ridge preservation with PLATELET RICH FIBRIN ALONE in comparison with PLATELET RICH FIBRIN IN COMBINATION WITH ANY OTHER ADJUNCTIVE GRAFTING MATERIAL, in terms of preservation of hard and soft tissue dimension of the residual ridge, as reported in prospective randomized controlled trials (RCT), nonrandomized controlled clinical trials (CCT) or prospective cohort (PC) studies with a minimum of 50 participants and a minimum follow-up period of 3 months; or in pre-clinical *in vivo* trials reporting on at least 5 sockets/group and a minimum of 5 animals in total, where the effect of L-PRF/L-PRF block on ridge preservation was assessed by means of clinical and/or radiographic and/ or histological/ histomorphometric analyses".

3.2.2 PICO QUESTION

The research question was structured using the PICO format as follows:

Population: Experimental animals or healthy adults patients (18 years or older) requiring tooth extraction in the maxilla or mandible.

Intervention: Ridge preservation with the use of platelet rich fibrin (PRF; any form including L-PRF, A-PRF, A-PRF+, i-PRF, T-PRF or Bio-PRF) in combination with any other bone substitute material

Comparison: Ridge preservation with the use of platelet rich fibrin (PRF; any form including L-PRF, A-PRF, A-PRF+, i-PRF, T-PRF or Bio-PRF) alone

Outcome Residual ridge width and height following a healing period of no less than 3 months

3.2.3 OUTCOME MEASURES

More specifically, outcome measures were outlined as detailed below.

Primary outcomes:

- Clinical or radiographic changes expressed in millimetres (mm) in the:
    - Buccolingual/Buccopalatal width of the alveolar ridge
    - Vertical height of the alveolar ridge

Secondary outcomes –

Potential items to assess:

- Changes in clinical attachment level at adjacent tooth sites (adjoining the extraction site)
- Soft tissue ridges dimensions at the extraction site
- Percentage of socket fill
- Density of bone at socket site
- Histologic & histomorphometric characteristics
- Soft tissue healing (speed of socket closure?) at extraction site
- Patient reported outcomes (Post-operative pain/discomfort)
- Suitability of the socket site for dental implant placement without the need for additional grafting, following healing

3.2.4 STUDY DESIGNS CONSIDERED:

Only the following research study designs were included for consideration:

- Human randomized controlled trial (RCT) or Non-randomized controlled clinical trial (CCT) studies with a minimum of 3 months follow-up and ≥10 patients in each treatment group.
- Prospective cohort (PCs) studies with a minimum of 50 participants and a minimum follow-up period of 3 months.
- Preclinical *in vivo* trials, where the L-PRF/L-PRF block on ridge preservation was assessed by means of clinical and/or radiographic and/or histological/histomorphometric analyses. Studies were included, if reporting on ≥ 5 sockets/group, ≥ 5 animals in total.

3.2.5 INCLUSION AND EXCLUSION CRITERIA

In addition to the restrictions on study designs considered, the following inclusion and exclusion criteria were applied:

Inclusion criteria:

- Studies that address the clinical and/or radiographic outcomes of alveolar ridge preservation, comparing tooth extraction followed by alveolar ridge preservation with PRF to alveolar ridge preservation with PRF in combination with any other graft material (*e.g.* xenograft, allograft, alloplast, autogenous material or any combination of the above)
- Studies that included, at a minimum, clinical and/or radiographic measurement of ridge dimensions hard and/or soft tissue dimensions at the extraction site at baseline and following a (prospectively) pre-defined healing period (≥ 3 months)

- Molar or non-molar tooth sites.

Studies were required to report one or more of the following outcome parameters:

- Radiographic or clinical changes in buccolingual width of the alveolar ridge
- Radiographic or clinical changes in vertical height of the alveolar ridge
- Changes in clinical soft tissue dimensions of the alveolar ridge
- Changes in periodontal probing pocket depth (PPD) at the proximal surfaces of adjacent teeth
- Changes in clinical or relative clinical attachment at the proximal surfaces of adjacent teeth
- Radiographic marginal bone loss (MBL) at adjacent proximal tooth surfaces
- Composite outcomes including sufficient bone to place dental implant where desired
- Patient reported outcome measures (PROMs)
- Side effects/complications

Exclusion criteria:

- Retrospective studies*, in-vitro* studies, case reports, editorials and letters
- Studies containing fewer than 10 patients per treatment group for RCTs and CCTs or fewer than 50 patients for prospective cohort studies (PCs). Preclinical *in vivo* studies including fewer than 5 animals.
- Studies with a follow-up period ≤ 3 months
- Publications not written in English
- Publications that did not include baseline measurement of ridge dimensions
- Studies that did not include data relating to changes in horizontal or vertical ridge dimensions
- Publications that did not contain statistical analysis of findings

- Studies comparing PRF to unassisted natural socket healing only
- Publications not specifying the graft material(s) used (i.e. inadequate information regarding the selected topic)
- Publications containing systemically unhealthy subjects.

All studies satisfying the exclusion and inclusion criteria described above were included in the systematic review, regardless of how the outcomes were measured.

3.2.6 INFORMATION SOURCES AND SEARCH STRATEGY

The protocol for this systematic review was registered prospectively in the PROSPERO database (# CRD 42023434015; Appendix A). The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) was used for reporting the results to ensure methodological quality (Page et al., 2021).

Three independent investigators (PH & IP & LW) performed an electronic search based on human clinical studies published in the English language until 31st August 2022. No time restriction was applied for the screening process. An additional search was later conducted extending the date of search up to 30th June 2023.

An electronic search of eight databases was conducted. Articles were identified from searching the databases using Medical Subject Headings (MeSH) and free-text search terms, in different combinations. The databases searched were:

- MEDLINE (Ovid)
- Embase
- Web of Science (WOS) Core Collection
- Web of Science Scielo
- Scopus

- PubMed
- Cochrane Reviews
- The Cochrane Library Central Register of Controlled Trials (CENTRAL)

Finally, a forward search via Science Citation Index of the selected papers was added. No manual search was conducted for this systematic review. However, the reference lists of shortlisted articles were assessed for articles of relevance. An example of a search strategy employed in the review is listed below.

MEDLINE/Embase Search Strategy

1. Exp Tooth Extraction/

2. ((tooth OR teeth OR dental OR molar OR incisor* OR premolar* OR canine*) adj6 (extract* or remov*)).map*

3. Tooth Socket/

4. ((tooth OR extract) adj6 (socket*)).mp*

5. ((socket or ridge) adj6 (graft* or preserv* or fill* or heal*)).mp.*

6. Exp Bone Regeneration/

7. ((bone adj6 regenerat*) OR Osteoconduct* OR osseointegration*).mp*

8. 1-7/OR

9. Platelet-Rich Fibrin/

10. ('Platelet rich Fibrin' OR 'Platelet-rich fibrin' OR 'L-PRF' OR LPRF OR H-PRF OR PRF OR PRGF OR PDGF OR 'Bio PRF' OR 'platelet concentrate' OR 'A-PRF*' OR APRF OR 'T-PRF' OR TPRF 'i-PRF' OR IPRF OR 'sticky bone' OR 'sticky-bone').mp*

11. 8 OR 9

12. 7 AND 10

13. 11 Limited to English

3.2.7 STUDY SELECTION

Titles and abstracts of relevant studies obtained from the electronic libraries were saved in EndNote (Clarivate Analytics, Philadelphia, USA) and duplicates were removed. The remaining studies were uploaded to the Covidence systematic review software (Himmelfarb Health Sciences Library, Washington DC, USA) for screening.

Initial calibration of reviewers was achieved by multiple in-person discussion sessions. Three reviewers (PH, IP & LW) independently screened titles and abstracts; in the event these did not provide enough information, full-texts were obtained and screened and any ambiguity was resolved by discussion (1st step).

All potentially eligible articles were reviewed and evaluated by two reviewers (PH & IP), according to the inclusion and exclusion criteria described previously. The full text of all shortlisted articles was read by the reviewers to facilitate data collection. Again, any ambiguity was resolved by discussion with a third reviewer (LW as required (2nd step).

3.2.8 DATA COLLECTION

Data collection was completed using a specifically designed Excel spreadsheet (Microsoft Corporation. Redmond, WA, USA). This included aspects related to methods and results from the selected studies. Two independent reviewers (PH & IP) extracted data addressing the primary and secondary outcomes in duplicate for analysis. Additional information like country, setting and source of funding was also recorded. Where the reviewers noted articles contained missing data, an attempt was made to contact the primary authors

3.2.9 RISK OF BIAS IN INDIVIDUAL STUDIES

Risk of bias assessment was conducted independently by the two review authors (PH & IP). The Cochrane risk-of-bias tool for randomised trials (RoB2) Sterne et al, 2019)) was utilised to assess the risk of bias in the included randomised trials (Sterne et al., 2019). Each study was assessed based on 5 domains, as follows:

- Bias arising from the randomisation process (selection bias)
- Bias due to deviations from intended intervention (effect of assignment and adhering to the intervention)
- Bias due to missing outcome data
- Bias in measurement of the outcome
- Bias in selection of the reported result

For each domain, each study was categorised as being either at “low risk” of bias, presenting “some concerns” or “high risk” of bias. Any disagreement was resolved by discussion between the reviewers.

An online risk of bias visualisation tool (Robvis; accessed at https://mcguinlu.shinyapps.io/robvis/) (McGuinness and Higgins, 2021) was used for graphic display of the results of the risk-of-bias assessment conducted in this systematic review (Traffic Light Diagrams; Summary Plots)

3.2.10 DATA SYNTHESIS

Data extraction was performed by the two study reviewers (PH& IP) independently, using a pre-designed data collection form. The information items recorded on the data collection sheet included:

- Year of publication
- Characteristics of study participants

- Biomaterial used for alveolar ridge preservation (and control arm, if relevant)
- Duration of follow-up
- Preparation methods used for PRF (if outlined)
- Dimensional changes at the alveolar ridge (changes in width or height of ridge)
- List of other assessment parameters/outcomes

The mean treatment effect (i.e. the mean of the group receiving any ADJUNCTIVE GRAFT MATERIAL IN COMBINATION WITH PRF following extraction), the standard error of the mean difference, and 95 per cent confidence interval were calculated. Analysis was performed using the patient as the statistical unit.

3.3 RESULTS

The initial search identified 7037 articles from the databases. Following removal of duplicates, 2622 articles were included for screening. Following exclusion of irrelevant articles, six articles remained for retrieval for full text review. Three of these articles were not relevant and were excluded (reasons given in Table 6). Consequently, three articles remained for analysis. The repeat search to June 30th 2023 identified an additional 517 articles, of which 213 articles were included for screening following removal of duplicates. One further article was deemed relevant for full-text review and was deemed relevant for inclusion in the final review. Figure 7 is a composite PRISMA diagram summarising the search process.

Table 6: Reasons for exclusion of articles following full-text review (n=3)

Study	**Reason for exclusion**
Clark et al (2016)	Research abstract (Relates to partial results of study used for full-text review)
Ahmed et al (2019)	Does not meet inclusion criteria – no bone substitute used (simply collagen plug)
Park et al (2022)	Does not meet inclusion criteria – preclinical trial with insufficient follow-up period

3.3.1 DATA EXTRACTION

Table 2 summarises data extraction & results from the 4 studies included in the final analysis. All four included studies were randomised controlled clinical trials. The selected papers were heterogeneous and each evaluated a different graft material. The preparation protocol used to prepare PRF was not consistent across studies and was incompletely documented in two of the four trials. Of the four studies, one (Clark et al., 2018) presented a clinical investigation of non-molar extraction sockets with mean 15-week follow-up, in which dimensional ridge measurements were recorded at baseline and follow-up with the aid of a customised stent. In the remaining 3 studies post-operative evaluation of primary outcome was based on CBCT measurements at 6-months post-extraction; however all 3 studies utilised different graft materials and different tooth socket locations (Ibrahim et al., 2022, Alam et al., 2022, Jouliq et al., 2023).

A meta-analysis was not deemed appropriate as there was insufficient data on individual graft materials and significant diversity in the way outcomes were assessed. Since combining outcome data was not possible, a narrative summary of key findings from each study is presented below, with accompanying tables to describe key outcomes from the individual studies. Table 2 describes outcomes from the 3 studies that provided data on horizontal ridge width changes. Table 3 provides corresponding information on vertical dimensional changes, while Table 4 describes findings relating to radiographic bone density.

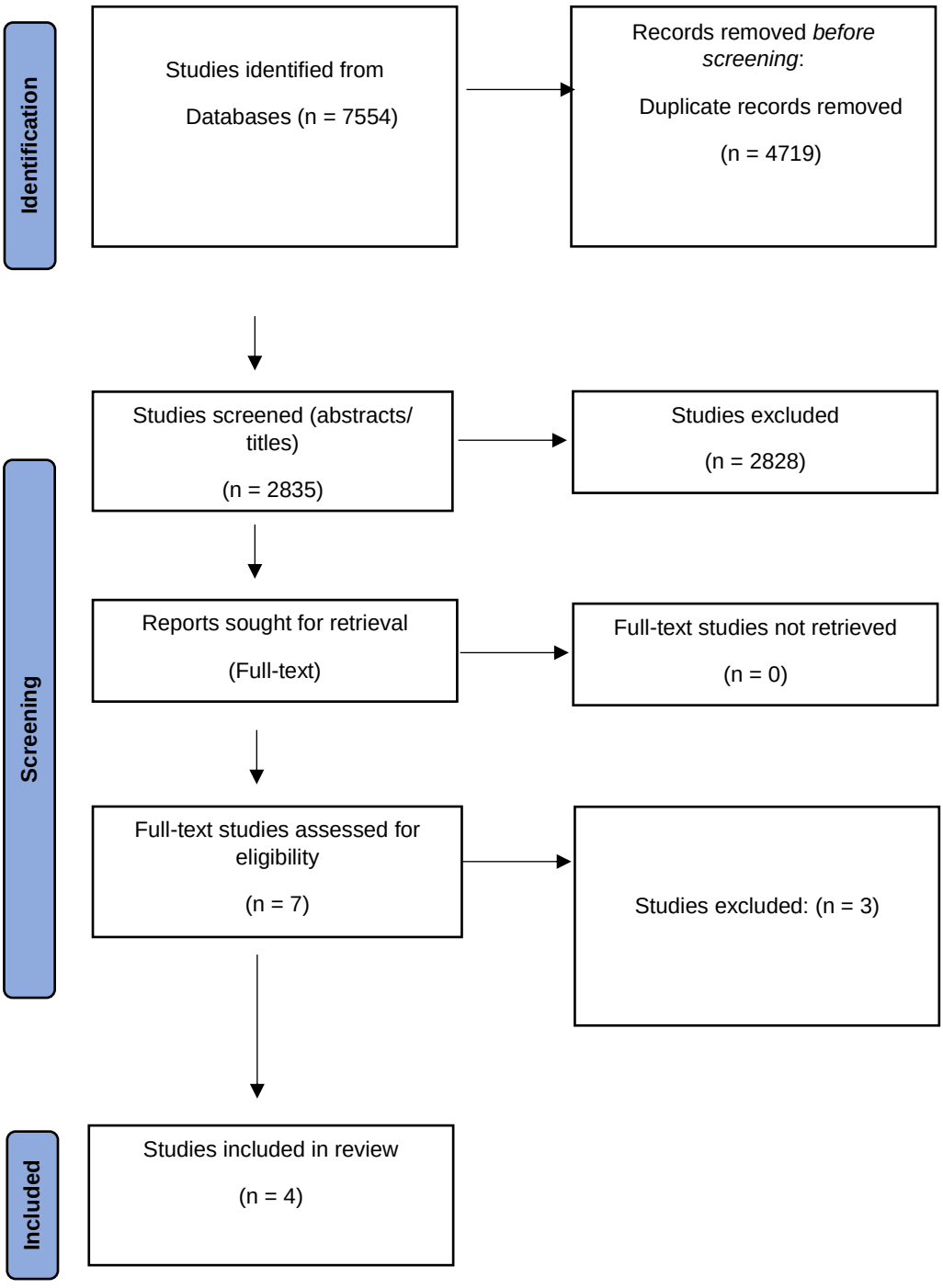

Figure 7: PRISMA Flow diagram (Adapted from: (Page et al., 2021))

3.3.2 RISK OF BIAS ASSESSMENTS

A risk of bias analysis was conducted for each study. First, each randomised controlled trials were assessed using the RoB2 tool and the results were visually documented with the Robvis tool (McGuinness and Higgins, 2021) in the form of a Traffic Light diagram (Figure 8) and Summary Plot (Figure 9). The traffic light diagram presents a judgement of each study under each of five domains, with a final judgement of overall risk of bias. The summary plot is a complimentary figure that presents data by domain, categorising the proportion of studies judged in each risk of bias category for that domain.

One study (Clark et al., 2018) was deemed at overall low risk of bias. The three remaining randomised controlled trials each presented more than one domain where some concerns were present, and were consequently assessed as presenting some concerns (Ibrahim et al., 2022, Alam et al., 2022) or high risk of bias overall (Jouliq et al., 2023).

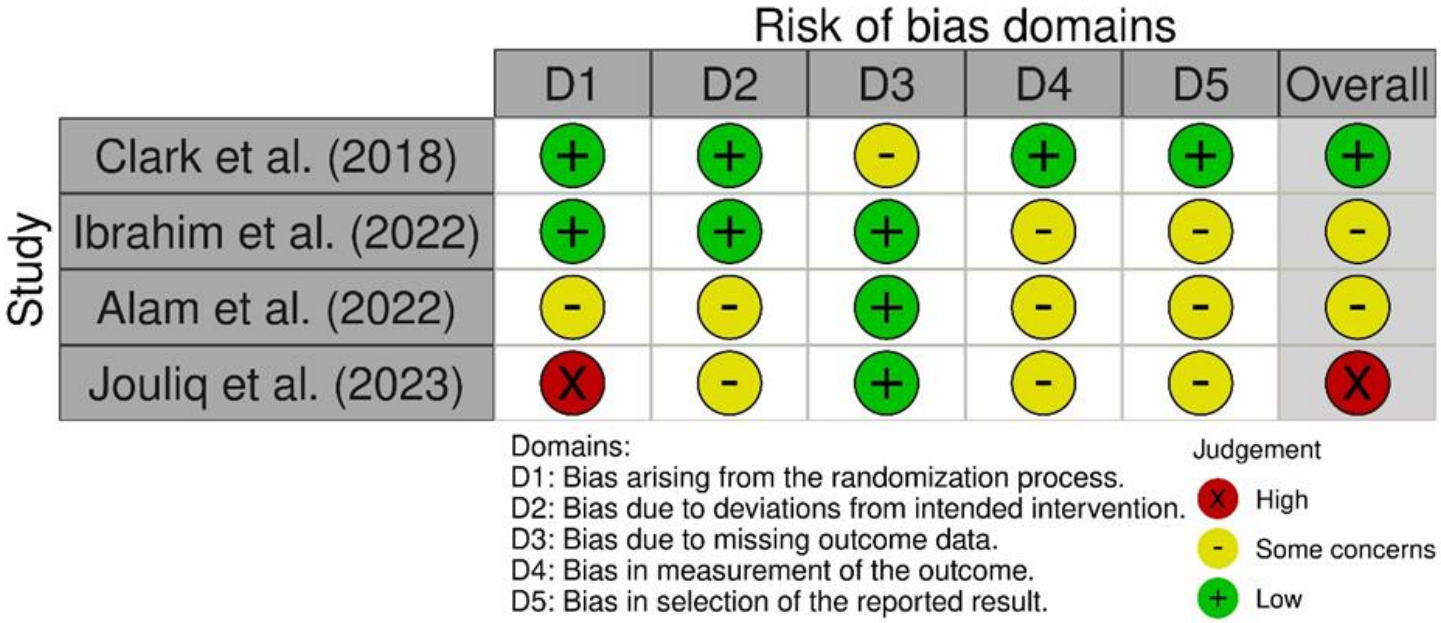

Figure 8: Traffic light diagram describing risk of bias assessment of randomised controlled trials (n=4)

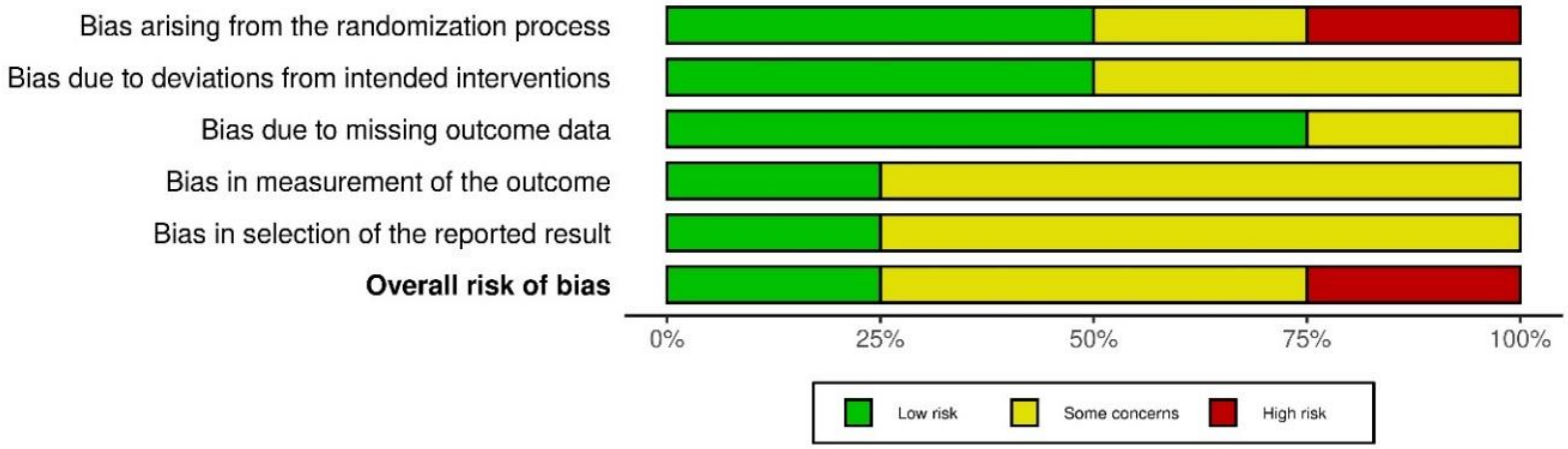

Figure 9: Summary plot (bottom) describing risk of bias assessment of randomised controlled trials (n=4). Horizontal bars describe domains of bias, collating findings of all randomised controlled trials considered in this review. Colour coding displays demonstrate proportion of trials corresponding to each risk of bias judgement for that domain (Green = Low risk; Yellow = some concerns; Red = High risk).

3.3.3 NARRATIVE REVIEW OF INCLUDED STUDIES

Clark and colleagues compared A-PRF and freeze-dried bone allograft (FDBA) used alone, and in combination for alveolar ridge preservation in a parallel, randomised controlled trial (RCT), where natural healing (blood clot) served as a control (Clark et al., 2018). Forty patients completed this study, although the authors noted that 45 were enrolled, with a loss of two patients due to systemic health issues, and a further 3 due to non-compliance; these were lost across three groups. Clinical measurements were made at baseline and immediately prior to implant placement at a mean of 15 weeks later, using callipers to measure buccolingual ridge width with three sites per tooth (coronal, mid, and apical), and probe used to measure vertical distance from a measurement stent constructed for each patient. Only mean changes at follow-up were reported and baseline data was not presented in the publication.

Mean horizontal changes were greater at the coronal aspect of the socket for each graft material, but no statistical significance was seen versus the control group (blood clot i.e. natural healing) or between study groups with regard to ridge width changes. Mean changes in vertical ridge height showed greater variation, albeit standard deviations were wide. The mean decrease in ridge height for the natural healing control group was 3.8 mm ± 2.0 mm. This exceeds the mean findings reported in a systematic review of natural healing by van der Weijden and colleagues (Van der Weijden et al., 2009), and is at the upper limit of the range cited in a later review (Ten Heggeler et al., 2011), although a stent was used to ensure measurement accuracy. Loss of vertical ridge height was statistically significantly greater in the control group than in both the A-PRF group and (A-PRF + FDBA) groups; the latter showed the lowest mean vertical dimensional loss overall. This research team also harvested bone cores at time of implant placement. These were analysed using micro-CT and histologically. Histology of a “representative area” showed that where FDBA was part of the graft, residual particles were frequently evident at 15 weeks. A-PRF and (A-PRF + FDBA) resulted in a denser trabecular structure, while A-PRF also demonstrated a higher percentage of vital bone. Overall, (A-PRF + FDBA) demonstrated the least reduction of ridge height (1.0 +-2.3 mm) and of ridge width (1.9 +- 1.1) but A-PRF alone appeared equivalent to FDBA alone from the perspective of dimensional changes, but was better at forming a vital bone. The combination of

(A-PRF + FDBA) did not significantly decrease vital bone versus A-PRF alone, although residual particles of graft material were a frequent finding.
Micro-CT analysis revealed no statistically significant difference in bone mineral density for treatment groups. FDBA values were statistically significantly higher than the natural healing control. All groups demonstrated values below 600 mg/cm^3 at 15 weeks; these values are all below the value identified for mature, non-grafted bone identified in a cadaver study by Blok et al., where a value greater than 800 mg/cm^3 was suggested to represent mature bone (Blok et al., 2013). In conclusion the authors stated A-PRF is a suitable biomaterial for ridge preservation; used alone, it preserved ridge dimensions similarly to FDBA and better than natural healing. The combination of (A-PRF + FDBA) had the best results overall but mean findings were not statistically significantly different than A-PRF alone (Clark et al., 2018).

Ibrahim and co-workers compared bone dimensional changes following extraction of non-molar teeth in 30 systemically healthy adults, comparing calcium phosphosilicate morsels (CPS), alone and in combination with PRF, against a control group of PRF alone (Ibrahim et al., 2022). It must be noted that PRF was additionally placed as a socket seal in all 3 groups. Dimensional changes were computed by comparing baseline CBCT (taken one week following extraction) with those at the 6-month follow-up time point. PRF sites lost a mean of 2.03 mm ± 0.95 mm (approximately 24 per cent) ridge width and 1.84 mm ± 1.16 mm height (approximately 10 per cent). CPS used alone preserved horizontal ridge dimensions better than PRF or CPS + PRF, although differences in vertical dimensional change were not statistically significant. Mean bone density, as measured by Hounsfield units (HU), was also statistically significantly greater for CPS alone, albeit the authors acknowledged that all three groups had findings equating to a similar level of bone density. Overall, the authors concluded that CPS alone provided more predictable socket preservation than the other groups. The authors were unable to demonstrate a statistically significant benefit from using a combination of CPS + PRF compared to the use of PRF alone (Ibrahim et al., 2022).

Alam and colleagues compared PRF alone with PRF in combination with hydroxyapatite (HA) following mandibular third molar extraction (Alam et al., 2022). The authors described this hydroxyapatite material (G-Bone; SHAG-31, G.Surgiwear

Limited) as a granular biomaterial containing a combination of HA, TCP, calcium carbonate and bi-calcium phosphate. Extracted teeth were mesioangular, distoangular or horizontally impacted teeth of similar difficulty index. Forty patients were treated by triangular or envelope flap, with buccal guttering to facilitate extraction, following which patients were randomised to receive one of the respective graft materials. The study did not measure dimensional ridge changes, but instead focused on post-operative pain, swelling and soft tissue healing. Pain was measured at 1d, 3d, 7d by visual analogue scale (VAS), while swelling was measured at the same time points using assessment of facial landmarks. Both groups demonstrated a reduction in pain intensity scored by VAS at successive time points, with consistently lower values in the PRF + HA group. No baseline data was presented and although the authors noted statistically significant findings for pain and swelling, these were not clarified in the article text. However, comparison of group means indicates that subjects grafted with PRF + HA exhibited significantly less pain at days 3 and 7, and significantly less swelling on the first and third days post-extraction. Soft tissue healing was assessed at 3d, 7d and 14d using the Landry index (Landry, 1985). The authors noted impaired healing in the PRF + HA group at days 7 and 14. Socket healing was assessed radiographically for "osseous regeneration" at 1mo, 3mo and 6mo by allocating scores for lamina dura, overall density and trabecular pattern. While the authors reported that the PRF + HA group demonstrated significantly greater osseous regeneration at 6-month follow-up, the statistics presented in the research paper do not clearly demonstrate this.

Finally, Jouliq and colleagues evaluated the efficacy of a combination of Beta tricalcium phosphate (β-TCP) and PRF on alveolar ridge preservation, comparing with a natural healing control, and with each material used alone (Jouliq et al., 2023). The study was conducted at an Egyptian men's hospital, where 40 systemically healthy adult patients indicated for extraction of a mandibular molar tooth were included, and randomised into one of the four study groups. Pain and oedema on the day of procedure and at 2d, 5d, 7d, and 10d were recorded at follow-up evaluations. Results were only partially presented, but indicated an absence of infections, and that β-TCP used alone, was associated with highest mean pain at baseline and 3 days. Both PRF and (PRF + β-TCP) patients scored statistically significantly less pain on the day of procedure and at 2d but were not statistically significantly different from each other. No differences between groups were seen after day 2, while no differences between

groups were seen with regard to oedema at any time point. Dimensional changes were evaluated radiographically using CBCTs taken at time of extraction and six months later. Buccolingual bone width, vertical height from base of socket to "highest point" of bone, and bone density were presented at baseline and 6M, with data compared in the form of mean percentage reduction for each parameter.

Reductions in buccolingual bone were limited in nature, and even the natural healing group demonstrated a mean reduction of only 27.8% ± 7.6%; it must be borne in mind that all sites involved mandibular molars, which may have had thicker buccal bone present at baseline; no data for this was presented. At 6 months, the lowest percentage of bone loss was seen for the combination of (PRF +β-TCP), where mean buccolingual bone loss was negligible (2.6% ± 5.2%). Loss of ridge width in the PRF-only group was approximately 15% ± 6%; this increased loss of ridge width relative to the combination of PRF and β-TCP was statistically significant ($p< 0.015$), with β –TCP used as a sole agent demonstrating an intermediate amount of dimensional change. All experimental groups demonstrated a statistically significant reduction in loss of ridge width relative to control (natural healing) sockets. However, it must be noted that at baseline, the mean alveolar ridge width of the (PRF + β–TCP) group was greater than that of control group, with differences statistically significant even at baseline. Data for bone height indicated reductions less than 10 per cent for all groups at 6 months, and no consequent statistically significant differences for inter-group comparisons. Bone density data was presented that indicated the β–TCP and (PRF + β–TCP) groups had statistically significantly greater density values relative to control sockets at baseline, and this relationship remained at 6 months. At this point, (PRF + β–TCP) had a greater mean density than PRF or β-TCP alone; the differences were statistically significant. The density values for β-TCP were also greater than PRF values (Jouliq et al., 2023).

Table 7 provides an overview of the individual study designs and conclusions. Tables 8-10 summarise selected outcome measurements from the selected studies, relating specifically to horizontal ridge width (Table 8), vertical ridge height (Table 9) and bone density (Table 10).

Table 7: Overview of included studies and key conclusions

(RCT= Randomised controlled trial; SS = statistically significant; HA = Hydroxyapatite; β-TCP = Beta tricalcium phosphate)

Author (year) Title	Design & Evaluation period Country	No. of Subjects Gender Age range	PRF protocol	Comparison (No. sites)	Conclusions
Clark et al. (2018) Advanced platelet-rich fibrin and freeze-dried bone allograft (FDBA) for ridge preservation: A randomized controlled clinical trial	RCT Non-molar teeth 15 weeks USA	40 (45 enrolled) 18M, 22F Mean age: 58y	A-PRF 1300 rpm x 8 min (200 g) Process for PRF centrifuge	Blood clot (control) (n=10) FDBA alone (N=10) A-PRF alone (N=10) FDBA + A-PRF (n=10) All sockets covered by collagen plug (Collaplug)	▪ A-PRF alone preserved ridge dimensions similarly to FDBA alone and better than blood clot ▪ A-PRF produced more vital bone than FDBA ▪ (FDBA + A-PRF) provided best results overall; ○ Showed SS reduced loss of height versus control. ○ No other groups was SS vs control in terms of height or width reduction
Ibrahim et al. (2022) Comparative evaluation of bone dimensional changes in extraction sockets preserved with calcium phosphosilicate (CPS) bone substitute with and without platelet rich fibrin: A randomised controlled clinical trial	RCT Non-molar teeth 6 months India	30 15M, 15F Age range: 19-55y	Not explained	PRF (Control) (n=10) CPS morsels (n=10) CPS + PRF (n=10)	▪ Sockets grafted with CPS alone showed smaller horizontal and vertical bone changes than PRF alone and (CPS+ PRF) ▪ CPS showed SS less horizontal change than other groups
Alam et al. (2022) A comparative study of platelet-rich fibrin and platelet-rich fibrin with hydroxyapatite (HA) to promote healing of impacted mandibular third molar socket	RCT Mandibular third molar 6 months India	40 19M, 21F Age range: 17-35y	3000 rpm x 10min Process for PRF centrifuge (original L-PRF production cycle) 5-10 mL only (1 plug)	PRF (n=20) PRF + HA (n=20)	▪ (PRF + HA) appeared to reduce pain and swelling relatively more (SS at 1d, 3d, 7d) and in soft tissue healing (SS 3d, 7d) ▪ Also showed greater radiographic evidence of osseous regeneration at 6 months
Jouliq et al. (2023) Evaluation of effect of Beta-tricalcium phosphate (β-TCP) with platelets rich fibrin on alveolar ridge post extraction	RCT Mandibular molar 6 months Egypt	40 40M (not clarified) Age range: 18-40y	Not explained	Blood clot (Control) (n=10) B-TCP alone (n=10) PRF alone (n=10) B-TCP + PRF (n=10)	▪ (B-TCP + PRF) demonstrated improved maintenance of ridge width than other groups and increased relative bone density ▪ No SS differences in pain or swelling noted between groups

Table 8: Clinical outcomes with respect to horizontal ridge width (in millimetres) (SS = (Statistically significant; NSS = Not statistically significant; FDBA = Freeze dried bone allograft; CPS = Calcium phosphosilicate; β-TCP = Beta tricalcium phosphate; PRF = Platelet rich fibrin; CBCT = Cone beam computed tomography)

Author (year)	Measurement tool/index Time	Groups/intervention		Baseline (mm)	End (mm)	Change (mm)	Time	Comment
Clark et al (2018)	Callipers at apical, middle, coronal thirds; sounded to bone Stent used to guide positioning	Blood clot	coronal middle apical			2.9 ± 1.7 1.8 ± 1.3 1.5 ± 1.6	15 weeks	Greater horizontal change coronally for all groups – corresponds to the crest-1 mm position
		FDBA	coronal middle apical			2.5 ± 1.1 1.5 ± 1.2 1.2 ± 1.3		All test groups NSS vs control in any width parameter
		A-PRF	coronal middle apical			2.8 ± 1.2 1.8 ± 1.8 1.8 ± 1.5		
		FDBA + A-PRF	coronal middle apical			1.9 ± 1.1 1.7 ± 1.2 1.6 ± 1.2		
Ibrahim et al (2022)	CBCT measurements	PRF (control) CPS CPS + PRF		8.56 ± 1.55 8.76 ± 1.61 8.65 ± 1.62	6.53 ± 1.61 7.80 ± 1.45 6.92 ± 1.36	2.03 ± 0.95 0.96 ± 0.53 1.73 ± 0.75	6 months (Note baseline CBCT taken 1 week after extraction)	Inter-group mean difference p<0.013 in favour of CPS (CPS +PRF) NSS from PRF
Jouliq et al (2023)	CBCT measurements	Blood clot (control) B-TCP PRF B-TCP +PRF		8.78 ± 1.26 8.80 ± 1.10 9.48 ± 1.65 10.08 ± 1.65	6.40 ± 1.52 7.86 ± 0.78 8.12 ± 1.30 9.78 ± 1.39	Percentages 27.8 ± 7.6 10.5 ± 2.8 15.0 ± 6.0 2.6 ± 5.2	6 months	(β-TCP + PRF) width SS different than control at baseline All test groups SS less percentage reduction vs control at 6 M (β-TCP + PRF) Vs PRF (p=0.015)

Table 9: Clinical outcomes with respect to vertical ridge height (in millimetres). (SS = statistically significant; NSS = Not statistically significant; FDBA = Freeze dried bone allograft; CPS = Calcium phosphosilicate; β-TCP = Beta tricalcium phosphate; PRF = Platelet rich fibrin; CBCT = Cone beam computed tomography)

Author (year)	Measurement tool/index Time	Groups/intervention		Baseline	End	Change (mm)	Time	Comment
Clark et al (2018)	Measurement stent used; probe from stent to mid-buccal wall intra-op	Blood clot FDBA A-PRF FDBA +A-PRF				3.8 ± 2.0 2.2 ± 1.8 1.8 ± 2.1* 1.0 ± 2.3**	15 weeks	Vs blood clot *p <0.05 **p<0.01
Ibrahim et al (2022)	CBCT measurements	PRF (control) CPS CPS + PRF		19.33 ± 1.52 18.33 ± 1.27 19.43 ± 1.94	17.49 ± 1.57 17.31 ± 1.26 18.00 ± 1.90	1.84 ± 1.16 1.02 ± 0.32 1.43 ± 0.69	6 months Note baseline CBCT taken 1 week after extraction	While CPS showed less vertical change, NSS between groups
Jouliq et al (2023)	CBCT measurements	Blood clot (control) B-TCP PRF B-TCP + PRF		11.64 ± 1.70 12.38 ± 0.88 11.88 ± 1.69 12.92 ± 2.11	10.44 ± 0.93 11.52 ± 0.82 10.84 ± 1.68 12.48 ± 2.15	Percentages 9.6 ± 7.4 6.9 ± 3.6 8.9 ± 1.9 3.5 ± 2.7	6 months	(β-TCP + PRF) showed least percentage height reduction but all groups NSS from control and NSSS from each other.

Table 10: Outcomes with respect to bone density. SS = statistically significant NSS = Not statistically significant; FDBA = Freeze dried bone allograft; CPS = Calcium phosphosilicate; β-TCP = Beta tricalcium phosphate; PRF = Platelet rich fibrin; CBCT = Cone beam computed tomography; 6M = 6-month; HU = Hounsfield units)

Author (year)	Measurement tool/index Time	Groups/intervention	HU Baseline	HU End	Mg/cm^3 End	Comment
Clark et al (2018)	Micro-CT analysis of harvested bone cores at 15 weeks	Blood clot FDBA A-PRF FDBA + A-PRF			487 ± 64 551 ± 58 493 ± 70 521 ± 58	FDBA had greatest density and was only group SS Vs control (p<0.05) NSS between graft/test groups
Ibrahim et al (2022)	CBCT Hounsfield units (HU) - compare baseline (1 week post-extraction) and 6 months	PRF (control) CPS CPS + PRF	87 ± 14.4 837 ± 60.8 630.5 ± 63.9	403.9 ± 35.4 525 ± 48.1 408.5 ± 47.4		SS differences between groups at 6M with CPS SS Higher density than other groups However, authors note all groups in range indicating similar clinical density (D3 bone)
Jouliq et al (2023)	From CBCT via "bone density window in software) - assumed Hounsfield units (HU) - compare baseline and 6 months	Blood clot (control) B-TCP PRF B-TCP + PRF	139.4 ± 38.8 255 ± 90.8 209 ± 57.5 287.8 ± 49.5	318.7 ± 97.3 466 ± 38.2 336.6 ± 66.7 620 ± 31		Both β-TCP and (β-TCP + PRF) show SS higher density than control at baseline. (Still SS at 6M) At 6M: β-TCP SS higher vs PRF (p= 0.026) (β-TCP + PRF) SS higher vs PRF (p< 0.001) (β-TCP + PRF) SS higher vs β-TCP (p< 0.008)

3.4 DISCUSSION

Despite an extensive literature on platelet rich fibrin (PRF), there is limited evidence on its use in combination with other materials in alveolar ridge preservation following tooth extraction. The present systematic review searched for randomised controlled clinical trials, non-randomised controlled clinical trials or prospective cohort studies of at least 3 months' duration, in which PRF in combination with any bone substitute material was compared to PRF alone for alveolar ridge preservation. In addition, pre-clinical *in vivo* trials reporting clinical or radiographic findings were considered. Following exclusion of research studies that did not meet the inclusion criteria, a search of 8 databases uncovered only four randomised controlled trials that addressed the research question.

All four studies utilised a different bone substitute material and none of the combination materials corresponded to the concept of L-PRF Block, which has been documented clinically (Cortellini et al., 2018), histologically and biochemically (Castro et al., 2019a) in the literature as a structured biomaterial for regenerative grafting. The study of Clark and colleagues (Clark et al., 2018) was conducted prior to publication of the L-PRF Block concept, while the remaining studies combined their respective biomaterial with PRF but did not describe this process in detail and did not utilise liquid fibrinogen to bind the mixed material into block form. Nevertheless, PRF offers the potential to enhance the regenerative capacity of a bone substitute material, since it contains an array of platelets and leukocytes that may enhance the early healing processes following extraction, and an array of growth factors that may promote angiogenesis and osteogenesis. With the exception of autogenous graft materials and some allograft materials, bone graft materials are osteoconductive in nature (Newman et al., 2019); the addition of PRF may afford osteoinductive potential to the composite material that in theory might result in improved healing outcomes. In the present review, three of the four trials presented data on dimensional changes of the alveolar ridge post-extraction, and also assessed bone density in the extraction socket, with

time points ranging from 15 weeks to 6 months post-extraction (Clark et al., 2018, Ibrahim et al., 2022, Jouliq et al., 2023).

Freeze dried bone allograft (FDBA) is an allograft material that may be considered osteoconductive in nature. A randomised controlled clinical trial that compared FDBA and PRF for alveolar ridge preservation after non-molar extraction failed to reveal statistically significant differences between groups in ridge width or height changes (Azangookhiavi et al., 2020). The potential to combine these materials for additional benefit may be inferred from an early case series which concluded that clinical benefits could be obtained by combining a biologic mediator (enamel matrix derivative) with FDBA in the regeneration of infrabony defects (Rosen and Reynolds, 2002).

Clark and colleagues (Clark et al., 2018) compared Advanced platelet-rich fibrin (A-PRF) and freeze-dried bone allograft (FDBA) used alone, and in combination, evaluating dimensional changes clinically at a mean of 15 weeks post extraction; natural healing (blood clot) served as a control. Based on mean clinical findings, A-PRF alone preserved horizontal ridge dimensions similarly to FDBA alone and to blood clot (natural healing), and preserved vertical ridge height similarly to FDBA and better than natural healing, although the only finding that was statistically significant was the comparison to natural healing for vertical dimensional change. While the combination of A-PRF with FDBA produced the best mean preservation of ridge dimensions, again only the comparison with natural healing for vertical bone loss was statistically significant. No statistically significant differences in dimensional changes or final bone density were observed when comparing A-PRF with A-PRF+ FDBA. Study group size was limited to 10 subjects and confidence intervals were relatively wide, and in light of trends favouring A-PRF + FDBA, notably for vertical ridge preservation, it is possible that significant differences might be found should further, larger trials be conducted in future. At this point, based on comparison with an established bone substitute material in FDBA, it is apparent that both A-PRF used alone and A-PRF in combination with FDBA may be considered for alveolar ridge preservation. Clark and colleagues reported their methods in detail, and a

power calculation was reported. An established preparation protocol was used for A-PRF and the FDBA material was identified (AlloOss, Ace Surgical Supply, Brockton, MA, USA). FDBA and A-PRF were mixed at a 1:1 ratio, although only a single tube of blood appears to have been collected from each individual, possibly limiting the availability of graft material. It must be noted that all groups received a collagen dressing (Collaplug, Zimmer Dental, Warsaw, IN, USA) to close the socket, and cyanoacrylate (PeriAcryl 90, GluStitch, Delta, Canada) to seal the margins, which may have had a notable bearing on clinical outcomes.

Ibrahim and colleagues (Ibrahim et al., 2022) compared bone dimensional changes following extraction of non-molar teeth in 30 systemically healthy adults, comparing calcium phosphosilicate morsels (CPS), alone and in combination with PRF, against a control group of PRF alone. The authors did not clarify the production protocol for PRF, and all three groups had PRF placed as a socket seal, which complicates interpretation and comparison of findings with other studies. Furthermore, while CBCT was used to compare baseline measurements with those at the 6-month follow-up time point, these authors used a baseline CBCT taken one week after extraction and placement of the graft material, and results must be interpreted with this in mind. Since intra-surgical bone measurements were taken at baseline using callipers, one might assume that these were used to confirm the accuracy of baseline CBCT measurements, although this was not reported in the publication. PRF was associated with a mean 2.03 mm, or approximately 24 per cent, reduction in ridge width and 1.84 mm (10 per cent) reduction in ridge height, which compares favourably with meta-analyses on outcomes of natural healing (Ten Heggeler et al., 2011, Tan et al., 2012). The authors highlighted that CPS used alone appeared to provide more predictable socket preservation than the other groups and produced statistically significant benefits in terms of horizontal ridge dimensions and bone density relative to PRF or CPS + PRF, although differences in vertical dimensional change were not statistically significant. The combination of CPS + PRF did not demonstrate statistically significant advantages compared to use of PRF alone.

This study presents several methodological issues of concern, including the absence of a power calculation and a lack of any reporting on how PRF was produced. Furthermore, the methods section noted recording of periodontal data, yet this is not presented in the findings, and while buccal soft tissue thickness was measured at baseline, and CBCT measurement of buccal bone thickness was recorded, these too were omitted in the final report and do not appear to have been correlated with dimensional changes. This may present some concerns regarding the possibility of selective outcome reporting and concerns were consequently recorded in the relevant domains of the risk of bias assessment. It must also be noted that all teeth were extracted following mucoperiosteal flap elevation; conflicting findings have been presented regarding the potential of flap elevation to modify the dimensional changes following extraction and ridge preservation procedures (Fickl et al., 2008, Vignoletti et al., 2012). To seek further clarification on research methods used, I contacted the corresponding author for clarification but did not receive a response.

Alam and colleagues compared PRF alone with PRF in combination with hydroxyapatite following mandibular third molar extraction (Alam et al., 2022) This study focused on post-operative pain and swelling over the first 7 days post-operatively, and soft tissue healing over a 14-day period. The PRF preparation broadly aligns with the original approach used by Choukroun and colleagues (Choukroun et al., 2001) for L-PRF. However, it must be noted that although the centrifuge used was not outlined, other studies from the same research group utilised a REMI R23 fixed-angle centrifuge (Remi Laboratories, Mumbai, India), which may provide further variation in end-product. The combination of PRF + HA produced statistically significant reductions in pain and swelling at 1d, 3d, 7d and in soft tissue healing at 3d and 7d versus PRF alone. The authors also reported that (PRF+ HA) demonstrated greater radiographic evidence of osseous regeneration at 6 months, albeit limited detail was presented to describe the method of assessment. Patients included in this study had third molars that were distoangular, mesioangular or horizontally impacted; however, the authors indicated extractions were of similar difficulty, and that the same process of flap elevation was used for all patients. No presentation of dimensional changes was conducted; this is

practical given the tooth location. This study can nevertheless affirm that the combination of PRF and bone substitute is not harmful with regards to pain and swelling; conversely, the combination of (PRF + HA) showed statistically significant improved results compared to healing with PRF alone.

Jouliq and colleagues evaluated the efficacy of a combination of Beta-tricalcium phosphate (β-TCP) and PRF on alveolar ridge preservation at mandibular molar sites, comparing with a natural healing control, and with each material used alone (Jouliq et al., 2023). Patients in the PRF and combination (PRF + B– TCP) groups scored statistically significantly reduced pain during early healing (day of procedure and at 2d) but were not statistically significantly different from each other in this regard. Dimensional changes from baseline to 6 months were evaluated using CBCT and indicated only limited dimensional change in all groups. The lowest percentage of bone loss was seen for the combination of (PRF + B– TCP) which was statistically significantly less than that observed in the PRF-only group. Data for vertical bone height changes failed to show statistically significant inter-group differences, with all groups demonstrating vertical reduction of less than 10 per cent. Bone density data was not well explained, but rather was simply defined as being calculated "via bone density window in the software", with no details of the software used, the socket location measured or the measurement units. Based on the figures presented, data appears to align with HU values. Bone density of both the β–TCP and (PRF + β–TCP) groups was greater than at control sockets at 6 months; however, differences between controls and these groups were also statistically significant at baseline. At 6 months, (PRF + β–TCP) demonstrated greatest mean density and differences from the PRF-alone or β-TCP-alone groups were statistically significant. Bone density values of PRF are broadly in the same range of those presented by Ibrahim and colleagues (Ibrahim et al., 2022), who also used a six-month assessment via CBCT. However, a notable difference for this study is that the combination of PRF with a biomaterial (β-TCP in this instance) was associated with greater bone density than the use of the bone substitute alone. In contrast, both Clark et al (Clark et al., 2018) and Ibrahim et al (Ibrahim et al., 2022) found highest 6-month values for bone substitute used alone.

This publication presents notable discrepancies or omissions in the description of methods and lacks detail regarding PRF preparation and use, including the centrifuge and preparation protocol utilised, as well as post-operative analgesia and antimicrobial use, which may have influenced post-operative healing, including pain scores. Furthermore, randomisation, power calculation, and study measurements were only cursorily addressed in the publication, rendering it difficult to assess study quality. Indeed, the risk of bias assessment deemed this study to present "some concerns" in almost every domain, in most instances due to lack of information, and the study was adjudged at "high risk" of bias overall. Once again, in an effort to obtain clarification on the research methods used by these authors, I contacted the journal that published this paper, as no corresponding author details were listed in the article. I requested correspondence details to afford me contact with the research team in question. However, I did not receive a response to my request.

Three of the evaluated studies utilised an alloplastic material. Alloplasts are a chemically diverse group of synthetic biomaterials with different properties as grafts. Alloplasts are biocompatible and exhibit a chemical similarity with bone that rationalises their use in grafting procedures (Haugen et al., 2019). However, they are brittle materials and this limits their use in load-bearing applications; while this does not typically exclude their use in treatment of extraction sockets, their somewhat unpredictable resorption may be a limitation (Nasr et al., 1999, Haugen et al., 2019). Hydroxyapatite – used by Alam and co-workers (Alam et al., 2022) - and beta-tricalcium phosphate (β-TCP) – used by Jouliq and colleagues (Jouliq et al., 2023) - are bioceramic alloplasts derived from calcium phosphate, while calcium phosphosilicate particulate material - used by Ibrahim (Ibrahim et al., 2022) - is characterised as a bioactive glass material. Since these synthetic materials function primarily as defect fillers, the consensus of the 1996 World Workshop in Periodontics was that alternative materials would be recommended "if regeneration is the desired outcome" (Garrett, 1996). In an early study, Nevins and colleagues histologically evaluated 5 clinical cases where bioactive glass was used at infrabony defects; although clinical improvements were evident and radiopacity was evident at the defects, histology revealed that

this material appeared to have limited regenerative capacity (Nevins et al., 2000). However, subsequent research has documented some potential for alloplast use in alveolar ridge preservation. Among these studies, Horowitz and colleagues reported positive findings in a cohort of patients treated using β-TCP covered with either a resorbable collagen or dense polytetrafluoroethylene (d-PTFE) membrane, reporting grafts were well-tolerated, maintained 91 per cent of ridge width at 6 months and facilitated implant placement (Horowitz et al., 2009). Kotsakis and colleagues failed to note statistically significant differences in clinical or radiographic outcomes between sockets grafted with anorganic bovine bone mineral or calcium phosphosilicate (Kotsakis et al., 2014). A randomised controlled trial with re-entry at 8 months compared DBBM and a bioceramic consisting of hydroxyapatite/tricalcium phosphate (HA/TCP) for ARP at non-molar sockets. In both groups, a collagen membrane was applied. Trephine biopsies at re-entry showed new bone formation in the apical part of both socket types and both materials preserved ridge dimensions. HA/TCP demonstrated significantly better maintenance of horizontal ridge dimension (Mardas et al., 2010).

As outlined above, the available studies demonstrate significant heterogeneity in biomaterials used in combination with platelet rich fibrin. Furthermore, with the exception of the study of Clark and colleagues, each trial publication provides limited detail on the preparation and clinical use of PRF in their respective studies. The potential for bias arises primarily from the domains involving measurement of outcomes and selection of the reported results. While the study of Clark et al. was deemed at overall low risk of bias, the three remaining randomised controlled trials each presented more than one domain where some concerns were present, and the study of Jouliq and colleagues (Jouliq et al., 2023) was deemed to be at high risk of bias overall.

Although this systematic review did not aim to compare the use of PRF alone in combination with natural healing or the use of a bone graft material alone, based on the limited evidence available from the included studies, some

inferences may be made regarding the use of PRF as a sole grafting material for alveolar ridge preservation. Based on clinical assessment at mean 15 weeks post-extraction and alveolar ridge preservation, Clark et al demonstrated that A-PRF alone preserved ridge dimensions similarly to FDBA alone, and better than blood clot alone (natural healing), although the latter difference was not statistically significant (Clark et al., 2018). Jouliq and colleagues demonstrated a statistically significant reduction in horizontal bone loss at 6 months following extraction, as evaluated with CBCT, when PRF was used alone in comparison to natural healing; changes were not statistically different in comparison with β-TCP alone (Jouliq et al., 2023). Conversely, Ibrahim et al (2022) demonstrated horizontal dimensional changes were more favourable with CPS morsels used alone in comparison with PRF alone at 6 months post alveolar ridge preservation (Ibrahim et al., 2022). Available studies have mixed PRF with graft materials using individualised approaches; in this context, studies utilising more standardised approaches such as the L-PRF Block concept may be of additional value. Several systematic reviews have been unable to demonstrate a “gold standard” approach to ARP (Mardas et al., 2015, Willenbacher et al., 2016, Canellas et al., 2021, Atieh et al., 2021). In this context, further studies that compare L-PRF or its analogues to other biomaterials for ARP will continue to have merit.

3.5 CONCLUSIONS

This systematic review found only 4 studies in which the sole use of a form of platelet rich fibrin (PRF) was compared with Platelet-rich fibrin (PRF) used in combination with a bone substitute material for the purposes of alveolar ridge preservation.The available studies demonstrated high heterogeneity in terms of the biomaterial combined with PRF, although three of the four available studies did utilise alloplastic materials derived from calcium phosphate. Heterogeneity in tooth location, evaluation methods and outcomes was also noted between studies, rendering meta-analysis impractical. The methods used for PRF preparation were unclear or incompletely described in some studies, making it difficult to reach clear conclusions on the effectiveness of PRF for this indication.

Based on the limited evidence available, the combination of PRF with another graft material does not appear to consistently offer statistically significant advantages in terms of ridge preservation or mean radiographic bone density values compared to the use of PRF alone. Only one of the available studies addressed pain and soft tissue healing, with data suggestive of reduced patient discomfort and improved soft tissue healing noted when PRF was combined with hydroxyapatite compared with PRF used alone. The available literature does not provide information with regard to other patient-reported outcomes. One study additionally described histologic findings following core biopsy of grafted sites at mean 15 weeks post-alveolar ridge preservation. While biopsies obtained from sites preserved with a combination of A-PRF and freeze-dried bone allograft (FDBA) exhibited a denser trabecular structure than sites grafted with A-PRF alone, the latter sites demonstrated a higher percentage of vital bone.

Additional clinical studies that more comprehensively describe the PRF preparation protocol are necessary, to elucidate whether the combination of PRF with a graft material may enhance extraction socket healing and alveolar ridge preservation.

CHAPTER 4: A COMPARATIVE STUDY INVESTIGATING THE EFFECTS OF L-PRF AND L-PRF BLOCK ON THE REGENERATIVE TREATMENT OF NON-MOLAR EXTRACTION SOCKETS

4.1 INTRODUCTION

Following tooth extraction, the alveolar ridge, which is a tooth-dependent structure (Schroeder, 1986), undergoes significant remodelling. This natural healing process is associated with changes in both the horizontal and vertical dimensions (Schropp et al., 2003). These changes can be sizeable: systematic reviews have noted mean horizontal resorption ranging from 3.79 to 3.87 mm, equating to 29 to 63 per cent of ridge dimensions after 6 months. The comparative vertical change was 1.24 to 1.67 mm, equating to 11 to 22 per cent of ridge height (Van der Weijden et al., 2009, Tan et al., 2012). The major proportion of these changes occurs in the first 3 months post-extraction (Schropp et al., 2003) and most changes appear to have occurred by 6 months, with limited, gradual and slow progression thereafter (Carlsson and Persson, 1967). Tooth type and location can also affect healing and increased loss of horizontal ridge dimension has been noted at molar sites versus non-molar sites (Couso-Queiruga et al., 2021). Bone loss appears to be most severe in the coronal and facial aspects of the socket, with the result that the crest of the alveolar ridge moves apically and lingually from its original position over the post-extraction healing period (Pietrokovski and Massler, 1967, Misawa et al., 2016). This can present challenges when treatment planning future restoration of these sites, and may influence, for example, the suitability of such sites for dental implant placement, from both a practical and aesthetic standpoint; consequently, much of the research has focused on changes occurring in the anterior regions.

Local characteristics can influence the dimensional changes observed at the socket site. A large volume of research has focused in particular on the thickness of the buccal bone wall of the socket, which is frequently less than 1 mm in dimension in the inter-premolar region (Huynh-Ba et al., 2010, Januário et al., 2011). At this dimension, the buccal bone wall is composed primarily of so-called bundle bone, which resorbs rapidly in the weeks following extraction (Araújo and Lindhe, 2005, Araújo et al., 2023). Indeed, it has been highlighted that where the buccal bone wall is thinner, it will be lost more rapidly during healing, with a concomitant impact on stability of the

socket wound, since the blood clot, granulation tissue and woven bone all represent fragile, non-load-bearing tissues that require protection during healing (Araújo et al., 2023). In cases with thin buccal bone, the consequent bone loss can result in an additional loss of horizontal ridge width and vertical height post-extraction (Chappuis et al., 2013, García-González et al., 2020). This is frequently most pronounced at the mid-buccal aspect of the socket, often resulting in a residual ridge that takes on an inverted "V" shape at the buccal aspect of the socket (Farmer and Darby, 2014).

Alveolar ridge preservation (ARP) aims to reduce the loss of ridge dimension changes that occur post-extraction (Horvath et al., 2013), in order to facilitate subsequent restorative treatment (Hämmerle et al., 2012). ARP procedures usually involve placing a biomaterial within the extraction socket immediately following extraction; a barrier membrane material or soft tissue graft may also be used to seal the socket entrance (Mardas et al., 2023). A series of systematic reviews have compared socket preservation with natural healing. The included studies have investigated various biomaterials, and heterogeneity is evident in the design of both the primary clinical trials and, indeed, in the systematic reviews themselves. Nevertheless, some general trends emerge with respect to socket preservation:

- Alveolar ridge preservation (ARP) does appear to decrease both horizontal and vertical hard tissue loss, albeit not always to a statistically significant extent (Vignoletti et al., 2012, Willenbacher et al., 2016). Furthermore, the reduction is horizontal dimensional loss may be more variable (MacBeth et al., 2017).
- However, ARP does not eliminate hard tissue loss following extraction – bone loss may still be detected in both the vertical and horizontal dimensions (Willenbacher et al., 2016).
- ARP may provide a reduction in the number of sites requiring further augmentation prior to implant placement (Willenbacher et al., 2016), although evidence to support a clinical benefit of these techniques over natural healing in improving the clinical outcomes of treatment is limited and indeed may be questioned (Atieh et al., 2015, Mardas et al., 2015).

- No specific technique/biomaterial has been unequivocally identified as the “gold standard” for ARP (Vignoletti et al., 2012, Atieh et al., 2015, Mardas et al., 2015, MacBeth et al., 2017).
- The relative hard tissue benefits of grafting appear to be maintained even when ARP is compared to natural healing at sites where flapless (atraumatic) tooth extraction is performed (Jambhekar et al., 2015).

Several of these available systematic reviews addressing alveolar ridge preservation have attempted to quantify the relative dimensional benefits to the patient in terms of post-extraction healing. Considering their collected findings, the following benefits may be identified: in terms of additional preservation of tissue following extraction and healing at 3 to 6 months:

- Benefit in horizontal width ranging from 1.20 mm (Mardas et al., 2015) to 1.83 mm (Vignoletti et al., 2012)
- Benefit in vertical height ranging from 0.74 mm (Mardas et al., 2015) to 1.47 mm (Vignoletti et al., 2012)
- Benefit in proximal height of 0.80 mm (Mardas et al., 2015)
- Reduction in need for additional grafting (Mardas et al., 2015, Willenbacher et al., 2016, Weng et al., 2011)

It must be noted that histologically, the placement of a bone substitute into a socket may delay the rate of healing within the socket (Lindhe et al., 2014), and consequently change the proportion of vital bone compared to naturally healing sockets (Chan et al., 2013), most notably at early time points. Grafts that resorb more slowly may beneficially provide a structural scaffold during healing that helps to maintain alveolar ridge dimensions post-extraction (Avila-Ortiz et al., 2019). However, such materials may consequently be associated with a higher proportion of residual graft material in surgical re-entry studies, as is typically evident for allograft and xenograft materials (Jambhekar et al., 2015). While the presence of residual graft material may in theory impact on the potential for new bone formation, researchers have noted the difficulty of making conclusive findings in this regard (Willenbacher

et al., 2016), and its impact on the feasibility of subsequent implant placement has been questioned (Mardas et al., 2015).

The use of leukocyte and platelet-rich fibrin (L-PRF) and its variants for alveolar ridge preservation has been investigated in a series of studies. An early study by Hauser et al., which compared L-PRF to natural healing at premolar extraction sockets found that L=PRF use was associated with a statistically significant reduction in mean horizontal bone loss, although the associated benefit was negated where surgical flap elevation was required (Hauser et al., 2013). Other research teams have also demonstrated statistically significant dimensional benefits in the horizontal dimension compared to natural healing, although standard deviations in these studies were wide, and vertical benefits were more limited (Anwandter et al., 2016, Temmerman et al., 2016). While several other research studies have noted trends towards improved healing at L-PRF sites, they have been unable to demonstrate statistically significant benefits in dimensional changes at non-molar (Wang et al., 2022) or molar sites (Zhang et al., 2018), or following multiple tooth extractions (Castro et al., 2021).

Systematic reviews have consequently struggled to make clear conclusions regarding the benefits of using PRF in comparison to natural healing in terms of the hard tissue dimensional changes observed. This is unsurprising since the number of well-conducted primary research studies remains somewhat limited and there is significant heterogeneity in their conduct, not least in inconsistency in PRF preparation protocols and reporting, an issue that blights the entire PRF literature. This has led to recommendations by thought leaders in this field that all future PRF-related publications should report on specific preparation parameters, to enable interpretation of study results and their comparison to future studies (Miron et al., 2019d). The systematic review by Al-Maawi and colleagues concluded that evidence of hard tissue dimensional benefits was only present in studies where short-term outcomes (8-15 weeks) were assessed, and not in trials of longer duration (Al-Maawi et al., 2021). However, a recent systematic review that included all clinical trials, without restriction on duration, concluded that PRF provided statistically significant

benefits over natural healing in terms of horizontal and vertical resorption, socket fill and new bone formation, although differences between different PRF protocols were not significant (Al-Badran et al., 2023). Canellas and colleagues quantified the benefits of PRF compared to natural healing as mean reductions of 1.66 mm in horizontal ridge loss and 0.79 mm in vertical dimensional change, both of which were statistically significant (Canellas et al., 2021). The available systematic reviews also indicate that PRF use at extraction sockets is associated with additional benefits, such as enhanced soft tissue healing and reductions in post-operative discomfort (Castro et al., 2017b, Dragonas et al., 2019, Al-Maawi et al., 2021, Al-Badran et al., 2023). However, it must be noted that much of the available research on these outcomes is derived from studies of third molar extraction sites, where reductions in alveolar osteitis have also been described (Zhu et al., 2021).

The ability of PRF to provide cells and growth factors that may stimulate and accelerate post-extraction healing is clear (Dohan et al., 2006a, Quirynen et al., 2023), and has been described in detail elsewhere in this thesis. However, the limited mechanical strength of PRF and its consequent ability to form and maintain a scaffold for healing has been presented as a limitation (Agrawal, 2023). In this context, the combination of PRF with other biomaterials represents a logical step for its use in ARP, where maintenance of hard and soft tissue dimensions is the core aspiration (Horvath et al., 2013). Such hybrid or composite materials might change the strength and durability of fibrin products *in vivo* (Brown and Barker, 2014). In turn, a bone graft/bone substitute that provides a scaffold and osteoconductive ability may be enhanced by its combination with a platelet concentrate that possesses such bioactive properties and potential osteoinductive ability (Castro et al., 2019a).

Chapter 3 presented a systematic review of the English language literature up to 30 June 2023 addressing the comparison of PRF used alone versus the combination of a bone substitute material and PRF for the purposes of obtaining alveolar ridge preservation. This review uncovered only 4 randomised controlled trials that evaluated such a comparison in a controlled clinical trial with at least 3 months of follow-up. Furthermore, the included

studies each utilised a different graft material, and demonstrated notable heterogeneity in preparation protocol for PRF (or failed to clearly describe their protocol). Despite this, the information presented in the publications does suggest that all of the identified studies utilised a protocol where leukocytes were present in the autologous platelet concentrate, and as such represent L-PRF or one of its modifications, allowing analysis to proceed (Quirynen et al., 2023). However, these studies also lacked uniformity in the follow-up period or methods of evaluation used to assess dimensional changes. Three of the four studies were deemed at some/high risk of bias, and meta-analysis of results was not deemed possible.

The present study aimed to compare L-PRF and L-PRF Block for the purposes of alveolar ridge preservation at non-molar sites, in terms of clinical and radiographic outcomes (including dimensional changes), soft tissue healing and patient reported outcomes.

4.2 MATERIAL & METHODS

4.2.1 OVERVIEW OF STUDY

This study was a parallel-arm, single-blinded, randomised controlled trial with clinical and radiographic follow-up to compare the effectiveness of L-PRF (control) and L-PRF Block (test) as materials for socket preservation following single tooth extraction at bounded non-molar extraction sites. All study procedures were conducted at a single centre in a university clinic setting (Dublin Dental University Hospital).This study received research ethics approval from the St James's Hospital/Adelaide & Meath National Children's Hospital (SJH/AMNCH) Research Ethics Committee (Ref 2018/04/04; Appendix B).

This study aimed to compare the clinical and radiographic outcomes of L-PRF and L-PRF Block as socket preservation materials following extraction of *bounded* non-molar teeth. Study objectives also included the following:

- Investigation as to whether the choice of regenerative graft material (L-PRF Vs L-PRF Block) influences patient reported outcomes and/or healing in the early post-operative period
- Clinical assessment of horizontal and vertical ridge measurements (hard and soft tissue combined) of patients at 4 months post-extraction, and radiographic measurements of vertical ridge changes at this time point
- In patients proceeding to dental implant treatment, record the vertical and horizontal changes in ridge dimensions occurring at the socket at time of implant placement (5-6 months following extraction).
- Visual assessment of wound healing at 7 days and 14 days following the surgical procedure

- Evaluation of patient-reported outcomes in the first 7 days following the surgical procedure utilizing a patient diary of symptoms and consumption of pain medication

4.2.2 INCLUSION AND EXCLUSION CRITERIA

The following inclusion criteria were outlined:

Patient Level	Site Level
▪ Age ≥ 18 years ▪ Planned for extraction with socket preservation ▪ Capacity to provide informed consent ▪ Willing to comply with study appointment schedule ▪ Willing to maintain a diary of symptoms	▪ Bounded extraction site (adjacent tooth present on both sides) ▪ Maxillary or mandibular location ▪ Non-molar teeth only (excluding mandibular incisors) i.e. 5 4 3 2 1 | 1 2 3 4 5 5 4 3 | 3 4 5

The following exclusion criteria were outned*:*

Patient Level	Site Level
▪ Tobacco smoking ≥10 cigarettes per day ▪ Systemic antibiotics within previous 3 months ▪ Pregnant/breastfeeding females ▪ Uncontrolled systemic disease ▪ Use of systemic medications with an expected impact on bone healing/platelet count	▪ Acute infection at surgical site ▪ Sinus tract present ▪ Dehiscence ≥3 mm present at buccal bone wall following extraction

4.2.3 OUTCOME MEASURES

Primary outcome measures:

- Mean reduction in horizontal ridge width 1 mm below bone crest (millimetres)
- Mean reduction in combined hard + soft tissue thickness (mm)
- Mean reduction in radiographic height of bone crest (mm)

Secondary outcome measures:

- Mean vertical bone loss at proximal surface of teeth adjacent to extraction socket
- Mean change in clinical measurements (probing depth/attachment level) at adjacent tooth surfaces (mm)
- Post-operative adverse events, swelling or visual signs
- Patient reported outcomes
- Resonance frequency analysis of implants at time of placement
- Requirement for additional grafting at time of implant placement

4.2.4 NULL AND ALTERNATIVE HYPOTHESES

The null hypothesis was that there would be no difference in the dimensional ridge change evident following alveolar ridge preservation with L-PRF Block compared to L-PRF alone. The alternative hypothesis was that alveolar ridge preservation with L-PRF Block would result in reduced changes in ridge dimensions compared to L-PRF alone.

4.2.5 POWER CALCULATION

Power calculation was based on the detection of a clinically significant difference of 3.0 mm in mean crest dimension difference between the two treatment groups, assuming a standard deviation of 2.5 mm, as reported by the study of Schropp and colleagues (Schropp et al., 2003), with an alpha-value of 0.05 and a Beta-error of 0.20.

To achieve 80 per cent power, a sample of 12 subjects were required in each group. To compensate for potential patient dropout, a minimum recruitment of 14 participants were planned for enrolment in each study group.

4.2.6 ENROLMENT & PREPARATORY STEPS

Participants were recruited sequentially from patients referred to the periodontal department at Dublin Dental University Hospital (DDUH) for extraction of a non-molar tooth and alveolar ridge preservation of the site to facilitate an option for future dental implant placement. All patients were attending for comprehensive restorative care at DDUH via undergraduate, postgraduate or staff restorative clinics when the need for referral was identified. The dominant reasons for referral were un-restorable caries and fractured and un-restorable teeth.

Following discussion of alternative treatment options with their DDUH clinician, participants attended a brief screening appointment to confirm their suitability for surgery. As part of the assessment for extraction procedure, patients received clinical assessment and radiographic assessment of the socket site.

A customised radiographic stent/jig was fabricated by adapting addition silicone material (VPS Hydro Putty Vinyl polysiloxane impression material, Henry Schein®, Melville, NY, USA) to the bite plane of a standardised intra-oral periapical holder (Hawe Super-Bite,

Hawe Neos Dental, Bioggio, Switzerland). A digital radiographic film sensor was placed in the periapical holder to simulate the desired radiographic position in the mouth. The impression material was allowed to set, the stability against the teeth was verified, and the periapical holder was removed and repositioned in place to verify the desired seating intra-orally could be reliably obtained. Accurate seating was confirmed on a study cast (Figure 10).

Patients were given opportunity to discuss the study in detail and were provided with a written copy of the Patient Information Leaflet (PIL) to take home. Patients were given ample time to consider their participation and contacted the study gatekeeper if they chose to participate. Patients signed a freely given informed consent form prior to their enrolment in the study (Appendix C).

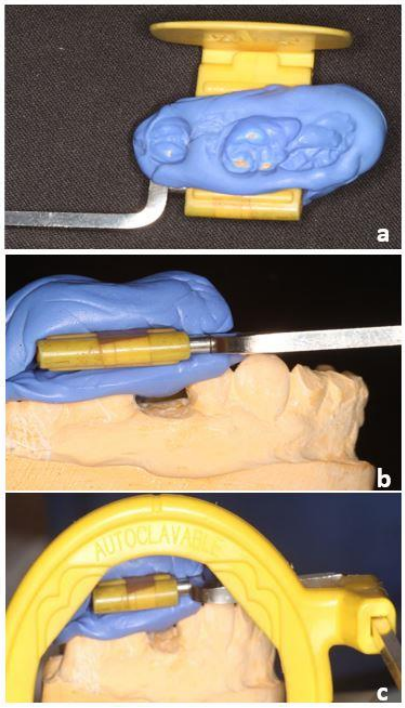

Figure 10: Example of radiographic stent a. Impression made on radiographic holder intra-orally b. Confirmation of seating on study cast c. Assessment of radiographic positioning on study cast

4.2.7 SURGICAL PROCEDURE

All patients rinsed preoperatively with 0.2% chlorhexidine digluconate mouthwash (Perio-Kin, Laboratorios Kin, Barcelona, Spain). Blood was collected from the antecubital fossa and processed to create L-PRF/L-PRF Block, as previously described (Chapter 3). Each patient was randomly allocated to receive either L-PRF or L-PRF Block graft material, as directed by a pre-determined randomisation schedule (www.randomization.com) Patients were sequentially assigned a number in order of participation in the study; operators were unaware of individual patient randomisation until the procedure commenced. Patients were not made aware of their treatment group at any time; all patients donated blood for the investigation to create L-PRF membranes and the final graft material was not prepared within direct sight of the patient

Each tooth identified for extraction was extracted under local anaesthesia, using a minimally traumatic technique, utilising a periotome. The marginal gingival tissue at the socket site was reflected only sufficiently enough to allow adequate access for instrument placement and surgical measurements, and no conventional flap therapy was provided.

Following tooth removal, the extraction socket was debrided and irrigated with saline to remove any debris/inflammatory tissue. Socket walls were assessed for the presence of fenestration/dehiscence to confirm suitability for the research study.

4.2.8 INTRA – SURGICAL MEASUREMENTS

Intra-surgical clinical measurements were recorded at the socket and adjacent tooth sites using the surgical data collection sheet (Appendix D). Measurements recorded at baseline included:

- Probing pocket depth and gingival margin position - recorded at mid-col, proximal surface of tooth immediately mesial and distal to the tooth adjoining the extraction site
- Ridge width – soft and hard tissue combined (buccal-lingual)
- Ridge width – hard tissue only (measured 1 mm below ridge crest)
- Socket depth (Buccal mid-crest to socket apex)
- Socket internal width (buccal-lingual aspect)
- Socket internal width (mesial-distal aspect)
- Buccal bone wall thickness
- Lingual bone wall thickness

A standard periodontal probe (UNC-15, Hu-Friedy, Chicago, USA) and callipers (Straight Castroviejo Calliper, Hu-Friedy, Chicago, USA), were used for all clinical and intra-surgical measurements in this study. A previously calibrated independent examiner confirmed all measurements intra-surgically. All clinical and intra-surgical measurements were recorded in duplicate and were verified by two previously calibrated examiners. Figure 11 illustrates the measurements described above.

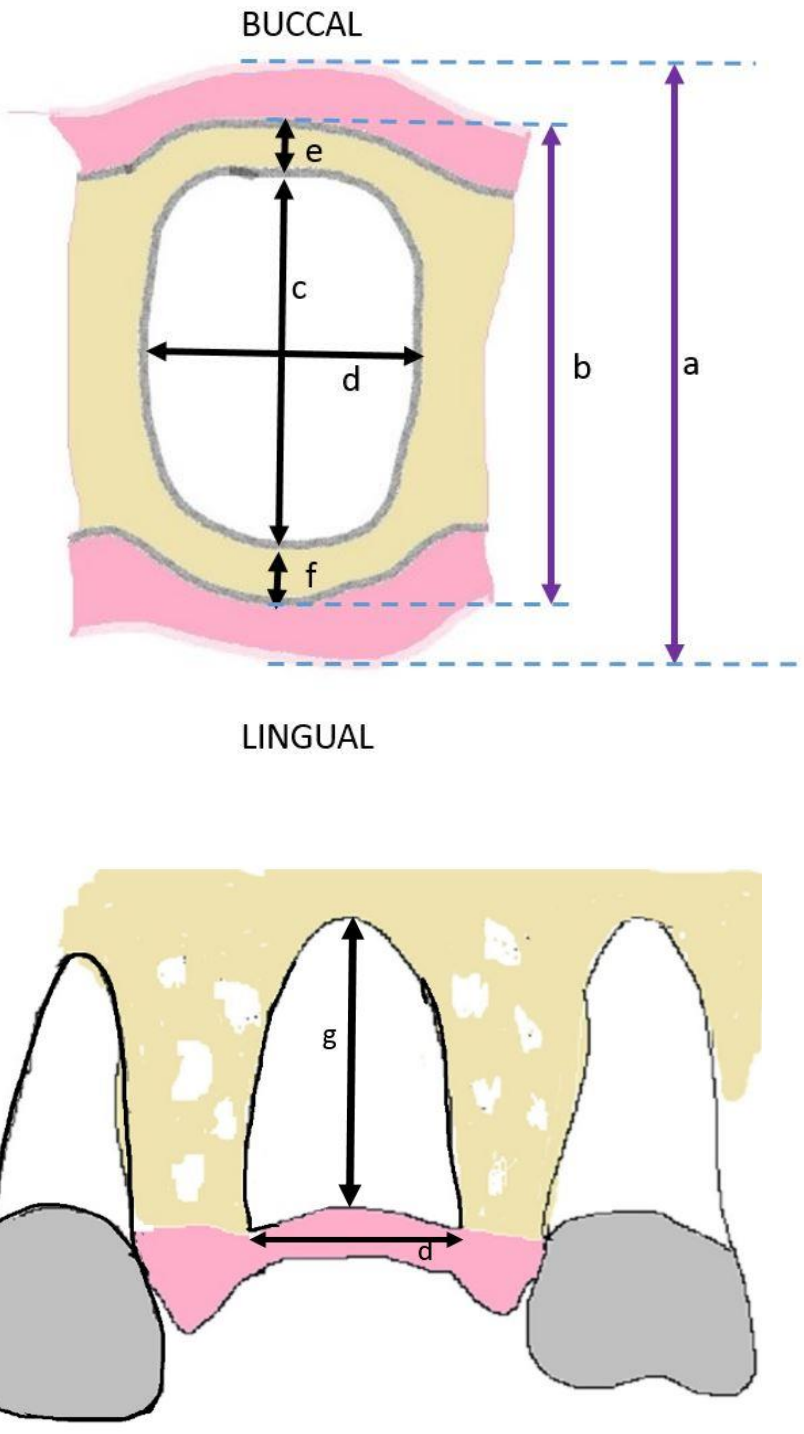

Figure 11: Baseline intra-surgical measurements. a Ridge width (hard & soft tissue) b Ridge with (hard tissue only) c Internal socket width (buccal-lingual) d Internal socket width (mesial-distal) e Buccal bone wall thickness f Lingual bone wall thickness g Socket depth (mid-crest to apex)

4.2.9 GRAFTING PROCEDURE

Following socket measurements, the socket was then grafted following the appropriate study protocol, as follows:

Group 1: L-PRF

- L-PRF membranes were (prepared as previously described in section 3.2.2.1, Membranes were condensed within the socket (with the clot face directed towards bone) to the level of the bone crest. No fewer than two membranes were used, with the number used depending on the size of the socket and of the harvested membranes. The socket was sealed with at least two layers of L-PRF membranes; these were tucked under the periosteum at the socket margins.

Group 2: L-PRF Block

- L-PRF Block was prepared as previously described in section 3.2.4. The socket was filled with the required amount of L-PRF Block material to the level of the bone crest. The socket was then sealed with at least two layers of L-PRF membranes; these were tucked under the periosteum at the socket margins.

4.2.10 BASELINE RADIOGRAPH

A baseline intra-oral periapical radiograph of the socket site was taken prior to patient discharge from surgery using the previously described positioning stent

4.2.11 SUTURING & POST-OPERATIVE MANAGEMENT

Suturing was completed without any attempt to obtain primary closure at the socket. A criss-cross suture was placed to stabilise the marginal tissues and secure the L-PRF membrane material; this was supported at the mesial and distal aspects by simple interrupted sutures (4/0 Coated VICRYL™ (polyglactin 910), Ethicon, J&J MedTech, New Brunswick, NJ, USA).

Haemostasis was confirmed before the patient was discharged, and verbal and written post-operative instructions were given. Participants were prescribed 0.2% chlorhexidine digluconate mouthrinse; they were advised to commence this on the third post-opeartiv day and to rinse twice daily for 14 days post-operatively. Participants were advised not to use this mouthwash on the first two post-operative days due to guidelines that this may not be appropriate where PRF materials are used (Quirynen M, 2018)Participants were also prescribed post-operative antibiotics (Amoxicillin 500mg t.i.d. 5 days) and were advised to use analgesic medication (Paracetamol 500mg/Ibuprofen 200mg) as required. Temporary restoration of the extraction site was offered to each patient at initial screening. Restoration was by means of removable orthodontic plastic (Essix) retainer or by a resin-retained fixed dental prosthesis only, in order to avoid direct application of pressure over the healing wound site.

4.2.12 PATIENT-REPORTED POST-OPERATIVE OUTCOMES

Each participant was provided paperwork for completion of a daily record of symptoms for the first seven post-operative days (Appendix F). The sheet - and how to complete it - was discussed with each patient. A separate sheet/symptom diary was recorded each day.

This included:

- Rating of (i) worst pain and (ii) average pain in the previous 24-hour perio. This was a visual analogue scale (VAS), recorded by the patient placing a mark (X) corresponding to their response, on a 100 mm line with the end-points “No pain” and “Worst pain imaginable”.
- Rating of how pain interfered with routine activities in the previous 24 hours (recorded by VAS)
- Rating of how the graft procedure interfered with the patient’s ability to do simple tasks in the previous 24 hours; eight items were included (e.g. “sleep”, “open your mouth wide”, “chew the foods you want”. Answers were recorded on a 5-point Likert scale with end-points “None” and “Lots”. Patients were requested to circle the outcome that best corresponded to the level of interference they had experienced in the previous 24 hours.
- Diary of pain medication. This was a free-text section, where patients were asked to record daily the name of pain medication, its strength and the number of pills taken in the previous 24 hours.

4.2.13 POST-OPERATIVE REVIEW AND ASSESSMENT OF HEALING

Review visits were conducted as follows:

- 7 days post-extraction:
    - Collection of patient record of symptoms;
    - Review and irrigation of surgical site;
- 14 days post-extraction:
    - Suture removal
    - Evaluation of post-operative healing by the study team

Two clinicians scored visual features of post-operative healing at the wounds site and recorded an agreed finding on the post-operative healing sheet (Appendix G)

- Post-operative swelling:
    - This was assessed visually and recorded on a Likert scale running from 0 (“No swelling”) to 3 (“Severe swelling”).

- Wound healing:
    - The surgical site was visually assessed for presence or absence of several features, including bleeding, suppuration, bruising, wound dehiscence, and difference in visual tissue colour compared to the contralateral side tissues. This was recorded on a dichotomous scale

4.2.14 ASSESSMENT OF HEALED SITES

Clinical and radiographic review of the participant/ site was conducted at 4 months following alveolar ridge preservation. At this point, the patient also confirmed their interest/readiness to proceed to implant placement at the healed site. Basic clinical information was recorded at this appointment on the post-operative assessment (healed site) data sheet (Appendix E):

- Probing pocket depth and gingival margin position - recorded at mid-col, proximal surface of tooth immediately mesial and distal to the tooth adjoining the extraction site
- Ridge width – soft and hard tissue combined (buccal-lingual)

A radiograph of the site (intra-oral periapical) was taken using the customised positioning stent used at baseline.

Where participants committed to proceed to implant placement, the patient was reappointed for surgical implant placement, which took place at 5-6

months post extraction. In these cases, additional data was collected intra-surgically using the post-operative assessment (healed site) data sheet (Appendix 5), as detailed below.

4.2.15 DENTAL IMPLANT SURGERY

Dental implant positioning was restoratively-driven. Following elevation of a mucoperiosteal flap, the site was prepared for placement of a tapered, non-platform switched implant fixture with a standard 4 mm platform and an internal connection (Certain® T3® BNST, ZimVie, Palm Beach Gardens FL, USA).

During surgery, additional measurements of the healed site were made as follows:

- External width of ridge – bone only (outermost buccal wall to outermost lingual wall)
- Height to mid crest from adjacent tooth CEJ
- Operator perception of bone density (Poor/Medium/Good)
- Assessment of adequacy of available bone to place a dental implant in the restoratively-driven implant position (Yes/No)
- Need for additional grafting to accommodate implant placement (Yes/No)
- Implant stability quotient (ISQ) value
    - Following implant placement, an implant stability quotient score (ISQ; Osstell IDx, Osstell, Göteborg, Sweden) was recorded to assess primary implant stability at the grafted site. Measurements were made in the bucco-oral and mesio-distal directions; the average of these measurements was recorded as a single ISQ score.

A standard periodontal probe and callipers (UNC-15, Hu-Friedy, Chicago, USA), were used for intra-surgical measurements as previously described. All data at review and intra-surgically was recorded using the post-healing data sheet.

4.2.16 ANALYSIS OF RADIOGRAPHS

Radiographs (digital intra-oral periapical) were automatically imported into a digital imaging software programme (Romexis®, Planmeca, Helsinki, Finland) equipped with magnification capability and measurement tools. Radiographic landmarks, including socket apex, crestal bone level and cemento-enamel junction (CEJ) of adjacent teeth, were identified by the study clinician (PH) and were outlined on the digital image files in the software programme. A second, independent periodontist examiner, who was blinded to study group allocation, reviewed all digital measurements for accuracy and detailed the following measurements, which were recorded/calculated automatically within the software:

- Distance from CEJ to bone crest at proximal surface of adjacent tooth (mesial and distal tooth)
- Distance CEJ to mid-crest - This was calculated by tracing a line between at a fixed landmark (CEJ) at the proximal surface of each adjacent tooth, and dropping a line perpendicular from this to reach the mid-crestal aspect of the socket (Figure 12)

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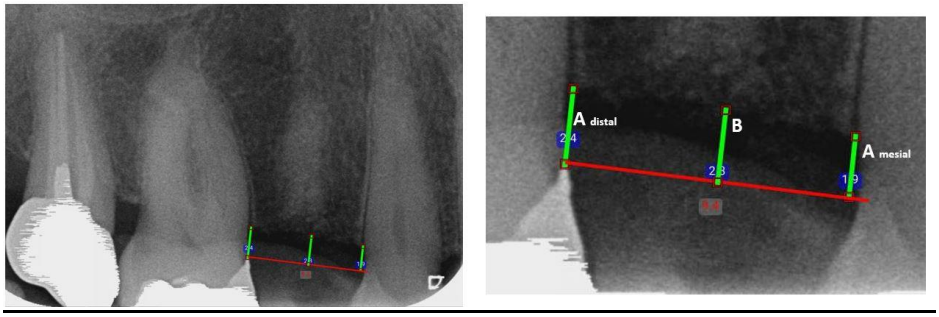

Figure 12: Measurement of distance from CEJ to bone crest. Left image: normal view. Right: cropped/enlarged view A: CEJ-proximal bone crest B: CEJ-mid-crest

4.2.17 STATISTICAL ANALYSIS

Appropriate measures of central tendencies (mean, median) and dispersion (standard deviation, interquartile range) were used to descriptively summarise the data.

Changes from baseline to follow-up were established either using actual measurements or percentage of baseline values.

The comparisons between protocols at each time point (baseline, 4 months) for changes observed were performed using either independent samples t-test or Mann-Whitney U test, as appropriate. The 5 per cent level of significance was used for all tests. Where data was not normally distributed, median and interquartile range values were provided. Where data was normally distributed, mean and standard deviation was used.

4.3 RESULTS

4.3.1 PARTICIPANTS

Thirty-three patients were recruited and enrolled to participate in the study. Three participants were referred on two separate occasions for separate extractions on contralateral sides and were accepted for participation in each instance as timelines ensured treatment at one site would not impact assessment of healing or patient reported outcomes in the other site. One patient presented a buccal bone dehiscence of >3 mm following extraction and was excluded at time of surgery before the grafting protocol was exercised.

Consequently, 32 systemically healthy adult patients (20 female, 10 male; age range 18-82 years) requiring single-tooth extraction of a non-molar tooth and simultaneous grafting of the socket site (alveolar ridge preservation; 35 sites total) participated in the trial.

Only 21 patients (21 sites; 10 L-PRF, 11 L-PRF Block) proceeded to dental implant placement within the study timeline as highlighted below (Figure 13). The principal reasons why patients did not proceed to implant included:

- COVID-19 Clinical restrictions/patient-mediated deferral - Delay in treatment/alternative restoration type chosen
- Restorative stability/compliance was deemed insufficient to proceed to implant – treatment deferred/alternative restoration selected
- Patient selected alternative treatment option

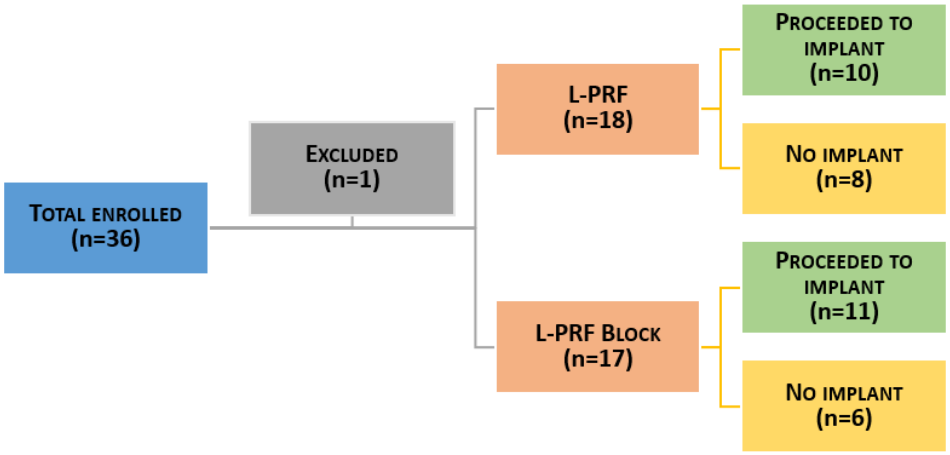

Figure 13: Flowchart indicating sockets treated by protocol.

4.3.2 DEMOGRAPHIC CHARACTERISTICS BY STUDY PROTOCOL

Demographic characteristics (gender, age) of participants at baseline were analysed by study protocol and are summarised in Table 11.

Gender and age were normally distributed. The independent samples t-test confirmed that gender was not statistically significantly different between groups. Similarly, mean age compared between protocols was not statistically significant between groups (p=0.128). Within study protocols, any differences in the mean age of male and female participants were not statistically significant

Table 11: Demographic characteristics of participants at baseline and the result of independent samples t-test for comparison between protocols. No significant difference observed at 5% level of significance.

	L-PRF	L-PRF Block	t (df)	p-value
Participants	18	17	1.56 (32)	0.128
Gender	6M, 12 F	7M, 10F		
Age(years)	47.8 ± 17.4	56.1±13.4		

4.3.3 ADVERSE EVENTS

No adverse events were observed following either treatment protocol.

4.3.4 POST-OPERATIVE HEALING - FINDINGS AT DAY 7 AND DAY 14

Visual features of post-operative healing at extraction sites were assessed at 7d and 14d. The association between study material and visual findings in relation to post-operative swelling or signs of wound healing (bleeding, suppuration, bruising, wound dehiscence or difference in visual tissue colour) was assessed with the Chi-squared test of association. Patients did not demonstrate swelling, and visual signs were limited to localised redness in a small number of study participants at day 7. There was no statistically significant association observed between study material and visual findings on day 7 ($p = 0.959$) or on day 14 ($p = 0.922$).

4.3.5 CHANGES IN CLINICAL AND RADIOGRAPHIC PARAMETERS

4.3.5.1 Dimensional changes (in millimetres) by protocol

Dimensional changes in clinical parameters from baseline to 4 months (in millimetres) were compared by graft protocol (L-PRF Vs L-PRF Block). The measurement of horizontal external ridge width (bone only) was recorded at time of implant placement (5-6 months).

Median clinical changes

Data was not normally distributed. Consequently, the most appropriate statistical measures were median and interquartile range, along with using Mann-Whitney test. When analysed using this approach, the difference between graft protocols was not statistically significant for any of the observed parameters (all values p >0.05). However, the difference between protocols for change in horizontal ridge dimensions (bone only and bone + soft tissue) showed marginally non-significant (p= 0.051 and p=0.057 respectively), with the median value favouring L-PRF Block. Table 12 includes median changes for clinical parameters measured from baseline (immediately post-extraction) to follow-up (4 months, with exception of ridge width (bone only) measurement).

Table 12: Median (interquartile range) values for clinical and radiographic changes from baseline to follow-up. Due to non-normality of measures, median and IQR used, as well as non-parametric tests for comparison of protocols. No significant difference observed at 5% level of significance. Some significant differences revealed at 10% level of significance.

	Change in Parameter	L-PRF Median (IQR) (mm)	L-PRF Block Median (IQR) (mm)	p-value (Mann-Whitney)
Clinical	Distal tooth PD	0.50 (1.00)	0.00 (1.00)	.684
	Mesial tooth PD	1.00 (1.00)	1.00 (1.00)	.422
	Distal tooth CAL	0.00 (1.00)	0.00 (2.00)	.103
	Mesial tooth CAL	-0.50 (2.00)	0.00 (1.00)	.347
	External ridge width (bone only)	2.00 (1.00)	1.50 (1.00)	.051
	Ridge width (bone + soft tissue)	2.00 (2.00)	1.50 (0.50)	.057
Radiographic	Mesial tooth CEJ – Bone Crest	0.00 (0.20)	0.00 (0.10)	.732
	Distal tooth CEJ – Bone Crest	0.00 (1.00)	0.00 (0.10)	.503
	Mid-socket height relative to adjacent CEJs	-0.40 (0.60)	-0.40 (0.50)	1.00
	Socket depth crest-apex*	0.70 (0.70)	0. 55 (0.45)	.224

Mean clinical findings

It was recognised that presentation of median and interquartile range values only, while statistically appropriate, presents challenges for comparison of the study results with the existing literature. Consequently, mean values for change in each clinical and radiographic parameter were also calculated using the independent samples t-test, with accompanying standard deviation values. Mean findings for clinical changes are included in Table 13.

All parameters remained non-statistically significant between graft protocols. Mean pocket depth at the adjacent tooth surfaces mesial and distal to treated sockets showed a slight improvement at follow-up in the range of 0.4 to-0.78 mm for L-PRF and 0.35 to 0.44 mm for L-PRF Block. Mean clinical attachment levels demonstrated some inconsistency in healing response, with a mean attachment level gain at distal tooth sites, but slight attachment loss at mesial sites. Clinically, mean loss of horizontal ridge dimension (bone + soft tissue) for sockets grafted with L-PRF was 3.80 mm (± 1.96 mm), while the comparative value for L-PRF Block was 2.85 mm (±1.73 mm). Comparative values for horizontal bone–only measurements among the 21 subjects proceeding to dental implant placement were 2.15 mm (± 2.02 mm) for L-PRF, while the comparative value for L-PRF Block was 1.20 mm (±1.79 mm).

Radiographic findings

Radiographically, both protocols were associated with vertical mid-crestal bone loss, as measured relative to a line connecting the cementoenamel junctions (CEJ) of adjacent teeth.

L-PRF treated sockets exhibited mean 0.48 mm (± 2.03 mm) vertical bone loss, while sockets treated with L-PRF Block had a mean 0.40 mm (± 1.65 mm) vertical bone loss change at mid-crest Neither protocol was associated with notable radiographic bone loss at the interproximal surfaces. Mean values for

additional proximal bone loss at both mesial and distal sites for both protocols were in the range of 0.06 to 0.14 mm, as measured from the CEJ to the most superior aspect of the proximal crestal bone.

Radiographic findings are also summarised in Table 12 (median findings) and Table 13 (mean findings).

Table 13: Mean (sd) values for clinical and radiographic changes from baseline to follow-up compared by graft protocol. Paired samples t-test used. No significant difference observed at 5% level of significance. "+" = Increase in dimension "-"= decrease in dimension measured

	Change in Clinical Parameter	L-PRF Mean (sd) (mm)	L-PRF Block Mean (sd) (mm)	p-value (t-test)
Clinical	Distal tooth PD	-0.44 (.93)	-0.35 (1.07)	0.79
	Mesial tooth PD	-0.78 (.80)	-0.44 (1.01)	0.28
	Distal tooth CAL	-0.39 (2.22)	+0.41 (2.28)	0.98
	Mesial tooth CAL	+0.33 (1.89)	+0.03 (2.06)	0.66
	External ridge width (bone only)	-2.15 (2.02)	-1.20 (1.79)	0.27
	Ridge width (bone + soft tissue)	-3.80 (1.96)	-2.85 (1.73)	0.14
Radiographic	Mesial tooth CEJ – Bone Crest	+0.11 (1.90)	+0.06 (1.77)	0.94
	Distal tooth CEJ – Bone Crest	+0.09 (1.51)	+0.14 (1.62)	0.93
	Mid-socket height relative to adjacent CEJ	+0.48 (2.03)	+0.40 (1.65)	0.90
	Socket depth crest-apex*	-0.76 (2.88)	-0.62 (2.87)	0.89

4.3.5.2 Relative changes (percentage) by protocol

Relative (percentage) change in vertical bone height - measured radiographically by socket depth (mid-crest to apex) and horizontal ridge dimension (bone + soft tissue combined) were calculated based on values recorded at baseline and 4-month follow-up, and compared by graft material (L-PRF Vs L-PRF Block).

In patients who proceeded to dental implant placement, relative change in horizontal ridge width (bone only) was also calculated. The mean percentage change in vertical and horizontal ridge dimensions between protocols was not statistically significant for any of these parameters (Table 14).

Table 14: Mean (sd) dimensional changes calculated by percentage. Values represent change from baseline value. Paired samples t-test. No significant difference observed at 5% level of significance.

Parameter	Mean % change		t (df)	p-value
	L-PRF	L-PRF Block		
Vertical ridge height (CEJ-CREST)	23.9 ± 26.9	18.2 ± 17.4	0.74 (33)	0.46
Socket depth	6.6 ± 4.0	5.0 ± 4.5	1.07 (32)	0.29
Horizontal ridge width (Bone + soft tissue)	17.3 ± 10.0	12.1 ± 8.4	1.65 (33)	0.11
Horizontal ridge width (Bone only)	22.7 ± 11.2	15.1 ± 10.7	1.64 (20)	0.12

4.3.5.3 Changes relative to baseline Buccal bone thickness

Relationship of buccal bone thickness (BBT) relative to actual change (baseline to 4 months) in selected clinical or radiographic parameters was assessed based on all participants. Based on limited sample size and non-significance of clinical and radiographic findings between protocols, all subjects were collected for this analysis. Changes in horizontal and vertical dimensions were compared for situations where buccal bone thickness was <1 mm at time of extraction, versus situations where BBT was ≥1 mm at time of extraction (Table 15). Buccal bone thickness was not associated with statistically significant differences in horizontal or vertical ridge changes, nor with differences in implant stability at time of placement.

Change in ridge dimensions trended in favour of situations where BBT was less than 1 mm but values were based on a small number of subjects in each group overall.

Eighteen subjects had L-PRF grafting – of these 10 had BBT< 1 mm and 8 had BBT ≥1 mm. Ten L-PRF participants proceeded to implant placement; 5 of these had BBT < 1 mm and 5 had BBT ≥ 1 mm. Seventeen subjects had L-PRF Block grafting – of these 14 had BBT < 1 mm and 3 had BBT ≥ 1 mm. Of these subjects, 11/17 proceeded to implant placement; 10 of whom had BBT< 1 mm and one had BBT ≥ 1 mm. Based on these low numbers, no inter-protocol comparisons between buccal bone thicknesses were statistically significant or approached it.

4.3.5.4 Adequacy of bone for implant placement/Need for additional grafting

In all cases (n = 21) proceeding to dental implant, operators deemed it feasible to place a dental implant fixture. Adequate bone was present for implant placement regardless of the initial ARP protocol used, and limited

additional ridge augmentation was required only in a single instance to facilitate more optimal implant positioning. Consequently, there was no statistically significant difference in adequacy of bone for implant placement nor in need for additional grafting between protocols.

Table 15: Mean (sd) clinical and radiographic findings as they relate to buccal bone thickness (BBT) at baseline. Independent samples t-test. No significant difference observed at 5% level of significance.

	Overall - Mean(SD)		
	BBT < 1	**BBT≥ 1**	**p-value**
Change in External ridge width (bone only)	1.44 (1.05)	2.20 (0.91)	0.161
Change in Ridge width (bone + soft tissue)	1.50 (0.91)	1.68 (1.29)	0.634
Change in Mid-socket height relative to adjacent tooth CEJs	-0.49 (0.53)	-0.33 (0.25)	0.333
Change in Socket depth crest-apex	0.74 (0.51)	0.58 (0.53)	0.401
Mean Osstell reading	68.1 (6.6)	67.2 (6.5)	0.779

4.3.5.5 Implant Stability Quotient (ISQ) reading (Osstell®)

In participants proceeding to dental implant, an implant stability quotient (ISQ) reading was calculated at time of surgery using an Osstell device. Mean Osstell readings were compared by protocol (L-PRF Vs L-PRF Block) to evaluate if graft material/protocol was associated with differences in implant stability at time of placement. Differences in mean Osstell values were not statistically significant by protocol (Table 16).

Table 16: Mean ISQ by grafting protocol. Independent samples t-test. No significant difference observed at 5% level of significance.

Protocol	Mean (sd)	p-value
L-PRF	67.8 (6.2)	1.000
L-PRF Block	67.8 (6.9)	

4.3.6 PATIENT REPORTED OUTCOMES

Patients completed a self-answered questionnaire on each of days 1 to 7 inclusive. Patients reported few negative findings overall. Key findings are outlined below.

4.3.6.1 Post-operative pain

The association between study material and the patient-reported outcomes was assessed for days 1 -7. On each of these days, each participant recorded their worst pain in the previous 24 hours and their average pain in previous 24 hours using VAS (Scott and Huskisson, 1976). From the collected data, mean values were calculated for each protocol for each day. The normality assumption for distribution of the data was not met and therefore non-parametric testing (Mann-Whitney U test) was employed. Consequently, median data for worst pain and average pain by graft protocol is summarised in Table 17.

Worst pain in previous 24 hours

The difference between protocols was not statistically significant on any day ($p>0.05$ on all days)

Average pain in previous 24 hours

The difference between protocols was not statistically significant on any day ($p>0.05$ on all days).

Use of pain medication

Participants also recorded their use of any pain medication on each of the post-operative days 1-7. The percentages of patients who self-reported the use of any pain medication at any time on each of days 1 to 7 is compared between protocols in Table 18 If patients did not complete their data sheet on a particular day, they were excluded from the analysis of data on that day. The majority of patients grafted with either protocol used pain medication on day 1 and day 2. At day 5, approximately one in three subjects still reported that they required pain medication. After this point, the number of patients requiring pain medication showed little change in the L-PRF Block group. In the L-PRF group, patients had generally stopped pain medication by day 7.

Table 17: Worst (left side of table) and average pain (right side of table) experienced by patients at each time point, analysed by graft protocol. Mean values were calculated for each protocol for each day, but the normality assumption for distribution of the data was not met and therefore non-parametric testing (Mann-Whitney ~U test) was employed. Data presented as median VAS with interquartile ranges. No significant differences observed at 5% level of significance.

	Worst pain previous 24 hours (VAS ex 100) Median (IQR)			**Average pain previous 24 hours (VAS ex 100) Median (IQR)**		
	L-PRF	**L-PRF Block**	**p - value**	**L-PRF**	**L-PRF Block**	**p - value**
Day 1	24 (61)	30 (44)	0.528	17 (34)	28 (41)	0.224
Day 2	5 (35)	33 (30)	0.175	5 (35)	27 (30)	0.198
Day 3	3 (36)	25 (51)	0.101	2 (20)	14 (39)	0.078
Day 4	4 (24)	10 (31)	0.551	2 (22)	8 (29)	0.331
Day 5	5 (14)	4 (14)	0.823	2 (14)	2 (11)	0.882
Day 6	3 (12)	0 (11)	0.433	0 (5)	2 (10)	0.655
Day 7	0 (11)	1 (4)	0.843	0 (4)	2 (4)	0.270

Table 18: Proportion (percentage) of patients in each group using any dose of any pain medication on each of the first 7 days post-extraction and alveolar ridge preservation, analysed by graft protocol.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
L-PRF (% Yes)	72	61	37.5	41.2	35.2	23.5	18.8
L-PRF Block (% Yes)	81.2	62.5	57.1	46.7	33.3	33.3	35.7

4.4 DISCUSSION

The present study was a parallel-arm, single-blinded, randomised controlled trial with clinical and radiographic follow-up that aimed to compare the effectiveness of L-PRF (control) and L-PRF Block (test) as materials for socket preservation following single tooth extraction at bounded non-molar extraction sites. Evaluation included clinical and radiographic dimensional changes, operator assessment of adverse events and of patient soft tissue healing recorded at 7 and 14 days post-operatively. Patient-reported outcomes in the first 7 days following their procedure were also collected in the form of a questionnaire self-recorded daily by patients. Thirty-two systemically healthy adults participated, providing 35 sockets for analysis. Only 21 participants proceeded to implant placement at the socket site. To the author's knowledge, this is first study that compares L-PRF and L-PRF Block as materials for alveolar ridge preservation at extraction sockets.

4.4.1 DIMENSIONAL CHANGES

The principal outcomes of interest to clinicians in evaluating a study of this nature is whether the "new" grafting approach (L-PRF Block in this instance) may offer any dimensional benefits over and above the existing "control" approach (L-PRF). First, it must be noted that data on dimensional changes in the present investigation was not normally distributed. This is most likely a consequence of the limited sample size and individual variation in response among participants. As such, reporting median findings with interquartile ranges represents the most appropriate approach from a statistical perspective (Table 12). However, this limits comparison with the existing literature, which has used mean findings when reporting on dimensional changes (Ten Heggeler et al., 2011, Vignoletti et al., 2012, Mardas et al., 2015). Three of the four studies in our systematic review reported on changes in ridge dimension (Clark et al., 2018, Ibrahim et al., 2022, Jouliq et al., 2023). These studies demonstrated a trend in which the best mean clinical results

were obtained when PRF was used in combination with a bone substitute material. However, when compared to PRF used alone, differences in mean horizontal or vertical bone loss were not statistically significant, with the exception of the study by Jouliq and colleagues, who reported that maintenance of vertical socket height was statistically superior following the use of PRF + β-TCP compared to PRF alone (Jouliq et al., 2023).

Our results indicate that the combination of a bovine xenograft and L-PRF with liquid fibrinogen (L-PRF Block) provided greater mean reduction in vertical and horizontal changes post-extraction when compared to L-PRF used alone. However, the difference between grafting protocols was not statistically significant for vertical or horizontal changes. The mean findings for each of the study protocols is discussed in further detail below. Median findings for horizontal and vertical mid-crestal dimensional change are also reported for completeness. While it is not appropriate to make direct comparison with individual studies conducted using different participant samples, PRF preparation protocols and grafting materials, the discussion of dimensional changes in the current study (below) is framed in the context of the relevant literature to enable a more comprehensive assessment of our findings.

4.4.1.1 Horizontal changes

Mean horizontal (buccolingual) dimensional change in the L-PRF subgroup at 4 months, when hard and soft tissue was combined was 3.8 mm ± 1.96 mm (median value 2.00 mm (IQR 2.00 mm). In subjects proceeding to dental implant (n = 10), bone level (hard tissue only) changes were recorded intra-surgically at 5 to 6 months (time of implant placement); a mean value of 2.15 mm ± 2.20 mm was determined (median value 2.00 mm (IQR 1.00 mm)). The mean hard tissue value is within the range of 2.03 to 2.8 mm observed for bone-only change in the randomised controlled trials documented in Chapter 2 (Ibrahim et al., 2022, Clark et al., 2018), albeit only the latter study, by Clark and colleagues, utilised clinical measurements, while Ibrahim and colleagues

measured bone dimensions using CBCT. The study by Jouliq and colleagues (Jouliq et al., 2023) will not be considered in this discussion of dimensional comparisons, since these authors only included mandibular molar sites in their research investigation.

Considering only the L-PRF Block subgroup, mean horizontal change was 2.85 mm ± 1.73 mm (hard and soft tissue combined) at 4 months post-extraction (median value 1.50 mm (IQR 0.50 mm)). In the participants who proceeded to implant surgery (n = 11), the mean change in bone-level assessed intra-surgically at 5 to 6 months was 1.20 ± 1.79 mm (median value 1.50 mm (IQR 1.00 mm). The individual trials uncovered in our systematic review (Chapter 2) that investigated the combination of PRF with a bone substitute demonstrated horizontal bone-level change ranging from 1.73 mm to 1.9 mm (Ibrahim et al., 2022, Clark et al., 2018).

4.4.1.2 Vertical changes

Vertical bone loss was evaluated radiographically using the distance from a line drawn between adjacent CEJs to the superior border of the bone crest at the mid-crestal region. Both treatment protocols were associated with vertical bone loss from baseline (immediately post-extraction) to follow-up radiograph at 4 months. The median loss of vertical bone height (interquartile range in brackets) was 0.40 mm (0.60 mm) and 0.40 mm (0.50 mm) for L-PRF and L-PRF Block, respectively. Corresponding mean values amounted to 0.48 mm ± 2.03 mm for L-PRF and 0.40 mm ± 1.65 mm for L-PRF Block, respectively. Neither median nor mean values demonstrated statistically significant differences between protocols. Mean mid-crestal vertical dimensional changes for the L-PRF subgroup compare favourably with mean changes noted in the randomised controlled trials that described non-molar extraction sockets and which were documented in Chapter 2 (Clark et al., 2018, Ibrahim et al., 2022); these ranged from 1.80 mm to 1.84 mm at the mid-crestal region. Corresponding mean mid-crestal vertical changes for the L-PRF Block subgroup also compare favourably to mean vertical changes

noted in the same randomised controlled trials documented in Chapter 2 wherein PRF was combined with a regenerative graft material, which ranged from 1.00 mm – 1.43 mm (Clark et al., 2018, Ibrahim et al., 2022)

Mean vertical bony changes measured radiographically at the proximal aspect of the tooth site adjacent to the socket were in the range of 0.09-0.11 mm for L-PRF and 0.06 – 0.14 mm for L-PRF Block. Differences between protocols were not statistically significant and mean changes support the concept that ARP limits hard tissue loss at the proximal aspect (Mardas et al., 2015). An early systematic review of natural healing concluded mean interproximal bone loss was 0.64 mm when assessed clinically and 0.70 mm radiographically (Van der Weijden et al., 2009). A systematic review on alveolar ridge preservation considered proximal bone loss and characterised a standardised mean difference (SMD) between ARP and natural healing (control) of 0.8 mm in favour of ARP, although confidence intervals were wide and included dimensional changes that implied additional bone loss with ARP (MacBeth et al., 2017).

Comparison with mean findings reported in the general literature on L-PRF variants remains challenging due to the limited number of primary studies and heterogeneity of included socket types, PRF preparation protocols, measurement tools and follow-up duration, among other factors. Aravena and colleagues attested that in terms of dimensional and volumetric ridge changes, L-PRF behaved similarly to natural healing (blood clot) when used for ARP (Aravena et al., 2021). While systematic reviews by Pan and colleagues, and Lin and co-workers (Pan et al., 2019, Lin et al., 2019) noted that PRF appeared to be favourable for ARP, meta-analysis was not possible in the former study, while the latter failed to demonstrate statistically significant benefits relative to natural healing.

With regard to dimensional changes in the current study, perhaps the most meaningful comparisons are with systematic reviews of natural healing and alveolar ridge preservation, in turn, to contextualise the findings of this study.

Systematic reviews of natural healing demonstrate mean vertical changes of 1.24-1.53 mm and of 0.64 – 0.80 mm at the mid-crestal and interproximal regions respectively, and mean horizontal changes of 3.79 - 3.87 mm (Van der Weijden et al., 2009, Tan et al., 2012). Considering this, the changes seen in the present study using both protocols (L-PRF or L-PRF Block) clearly appear to offer dimensional benefits relative to expected results for natural healing.

Systematic reviews of ARP note that these procedures may decrease both horizontal and vertical hard tissue loss (Vignoletti et al., 2012, Avila-Ortiz et al., 2014, Willenbacher et al., 2016, MacBeth et al., 2017), but few have clearly quantified the dimensional loss that may occur despite conducting ARP. An early systematic review concluded that dimensional changes of up to 2.64 mm in vertical ridge height and 3.48 mm in mean ridge width may still be expected (Ten Heggeler et al., 2011). In this context, the results of both study subgroups in the present trial appear favourable. An AAP consensus report quantified the mean benefit of ARP as preventing 1.5 - 2.4 mm of horizontal bone loss and 1.0 - 2.5 mm of vertical mid-buccal bone loss (Tonetti et al., 2019). The results obtained for L-PRF and L-PRF Block in the present study meet these criteria when compared to the aforementioned systematic review findings noted for natural healing (Van der Weijden et al., 2009, Tan et al., 2012). Taken in full, the dimensional changes noted in the present study appear to advocate that both L-PRF and L-PRF Block may be considered as effective biomaterials for ARP. This complements the findings of a recent network meta-analysis (Canellas et al., 2021), which demonstrated that L-PRF alone demonstrated statistically significant benefits in comparison to natural healing in head-to-head comparisons, and furthermore, could not be deemed inferior to other graft materials for ARP from the perspective of statistical outcomes.

4.4.1 3 Clinical parameters at adjacent tooth sites

Both grafting protocols were associated with small mean decreases in probing pocket depth at adjacent proximal tooth sites. At distal sites, these changes amounted to mean 0.44 mm (± 0.93 mm) for L-PRF sites compared to 0.35 mm (± 1.07 mm) for L-PRF Block. At mesial sites, mean changes were 0.78 mm (± 0.80 mm) for L-PRF and 0.44 mm (± 1.01 mm) for L-PRF Block.

Clinical attachment level changes showed an even lower mean change, and indeed the median measurement was 0 mm at both sites for L-PRF Block and distal proximal surfaces using L-PRF. Changes were not statistically significant between protocols for any of these parameters, whether measuring at median level (appropriate, since data was not normally distributed) or mean level.

Direct comparison of such measurements with other studies may not be appropriate, since probing pocket depth and clinical attachment level changes may be considered a function of the initial clinical status of the participants and specific to this cohort. However, it is notable that a recent investigation that involved surface tissue scans of maxillary single tooth extraction sites that were allowed either to heal naturally or assigned to ARP, demonstrated that ARP reduced the loss of interproximal soft tissue height by a factor of up to 2-fold, equating to a benefit of approximately 1 mm (Couso-Queiruga et al., 2023a). On this basis, the soft tissue loss in the present study with both protocols is within the level inferred for ARP cases by Couso-Queiruga and colleagues.

4.4.1.4 Relative change in dimensions

The relative percentage change from baseline to 4 months in radiographic bone height (change in socket depth) and in horizontal ridge dimension (change in bone + soft tissue measurement) was also calculated. Similarly, in patients proceeding to dental implant, change in buccolingual bone dimension (horizontal ridge width) was calculated. As was the case for the dimensional changes in millimetres, none of these parameters was statistically significant by protocol.

Mean horizontal ridge width (bone + soft tissue; all subjects) decreased by 17.3% (± 10%) for L-PRF and 12.15% (± 8.4%) for L-PRF Block, respectively. When only participants providing direct measurements for hard tissue (bone only) at time of implant placement were analysed, the proportional loss of ridge width was slightly greater: 22.7% (± 11.2%) for L-PRF and 15.1% (± 10.7%) for L-PRF Block. This latter finding concurs with the previous observation that soft tissue thickness may in part mask the changes in bone dimension at extraction sites (Chappuis et al., 2015). However, the percentage bone loss observed for both treatment protocols is notably less than the proportional loss of ridge width at naturally healing sockets of 29 to 63% observed in the systematic review by Tan and colleagues (Tan et al., 2012).

The mean proportional loss of vertical dimensions was notably dependent on the method used to characterise such change. The literature has classically used percentage loss in socket depth (measured from bone crest to apex of socket), with changes for natural healing described in the range 11-22 per cent (Tan et al., 2012). In the current investigation, vertical change using this method averaged 6.65% (±4.0%) for L-PRF and 5.05% (± 4.5%) for L-PRF Block, respectively; these values compare favourably to the results of Tan et al. However, socket depth measurements are inherently challenging in ARP studies, since the landmark of the socket apex may become less well defined radiographically as socket remodelling occurs. Hence, using the proportional

change in vertical ridge height (measured as the distance from CEJ to bone crest) may be more likely to give an accurate perspective on bone loss. However, since this is of smaller dimension at baseline, the percentage changes observed may be greater and show more variability, and this method is not widely referenced in the literature. Interestingly, in the current study, the mean percentage change in this dimension was 23.9% (± 26.9%) for L-PRF and 18.2% (± 17.4%) for L-PRF Block.

Comment on participant inclusion criteria and dimensional changes

In recruiting for this study, inclusion criteria permitted the participation of smokers of less than 10 cigarettes per day, to reflect a "real world" scenario where almost 20 per cent of the Irish population are smokers (Ipsos, 2023). However, few of those enrolled were smokers. It must be acknowledged that smoking may influence ARP outcomes and as such, if smokers are included, a low threshold for daily consumption is appropriate. Smoking has been characterised as a factor that may negatively affect wound healing (Ahn and Shin, 2008, Guo and Dipietro, 2010). In a prospective clinical study, Saldanha and co-workers investigated the dimensional changes occurring following non-molar extraction and concluded that smokers exhibited an increased reduction in alveolar ridge width equating to approximately 0.5 mm (Saldanha et al., 2006).

4.4.2 SOFT TISSUE HEALING (VISUAL SIGNS) AND POST-OPERATIVE COMPLICATIONS (ADVERSE EVENTS)

In the present study, no adverse events were observed with either grafting protocol. Furthermore, when visual signs of swelling and local tissue signs (including bleeding, bruising, suppuration, wound dehiscence and tissue colour) were evaluated at 7 and 14 days post-extraction, swelling was not observed. Indeed, aside from a small number of cases where tissue margins appeared redder than the gingival tissue on the contralateral side of the mouth, no other local tissue signs were noted. We can infer that neither grafting protocol is associated with complications, and consequently, no statistical significance was observed between grafting protocols. Since both study protocols involved sealing of the socket with L-PRF membranes, these positive healing responses align with prevailing outcomes noted in the scientific literature. A systematic review on the effect of PRF on soft tissue healing in regenerative treatment highlighted that the majority of available clinical studies demonstrated better soft tissue healing at PRF sites versus natural healing controls and supported PRF use for wound healing and soft tissue regeneration (Miron et al., 2017a).

Although complications following vertical and horizontal ridge augmentation procedures may be relatively common, evidence for complication rates following alveolar ridge preservation is sparse (Jepsen et al., 2019). A Cochrane systematic review of ARP did not report any serious adverse events following ARP. These authors commented that individual trials noted complications including post-operative pain swelling, redness and oedema (Atieh et al., 2021). Barootchi and colleagues deemed that such complications are infrequent regardless of materials used, are largely preventable with planning, and mainly limited to infection, exposure or loss of membranes, and discharge of bone graft particles (Barootchi et al., 2023). The absence of adverse events with these materials is perhaps unsurprising, given the autologous nature and antimicrobial properties of L-PRF. The consequent ability to leave L-PRF exposed when used as a membrane intra-orally

(Quirynen et al., 2023), and the binding of graft particles by liquid fibrinogen in the preparation phase of L-PRF Block (possibly limiting the potential for loose graft particles) are also helpful in this regard. Nevertheless, our results reinforce that both L-PRF and L-PRF Block appear to be “safe” options for alveolar ridge preservation when sockets are additionally sealed with L-PRF membranes.

Given the lack of clear superiority of any technique or materials evidenced in systematic reviews, decision trees have been suggested for use in ARP (Jung et al., 2018, Juodzbalys et al., 2019) but have yet to be validated or widely accepted. However, Jung’s recommendations that clinical decision-making for ARP begins prior to tooth extraction in the aesthetic zone appears prudent, particularly since alveolar ridge preservation procedures may not be without some complications, particularly in relation to soft tissue healing and material-related complications (Leblebicioglu and Tatakis, 2023). Given the lack of a “gold standard” treatment for ARP overall, cost-effectiveness may be a tool used by practitioners to rationalise the material used case-by-case. Barootchi and colleagues evaluated the cost effectiveness of different modalities of ARP, correlating assumed material costs with mean changes in ridge dimensions following four months of healing. Unsurprisingly, the use of allograft and xenograft materials was associated with increased cost, but this was not found to translate into statistically significant increased effectiveness. The authors did observe some statistically significant, albeit limited, association between expenditure on using a barrier membrane and ensuing reductions in both vertical and horizontal ridge resorption. Nevertheless, as the cost of such barrier materials increased, the relative cost-effectiveness of their use exhibited diminishing returns (Barootchi et al., 2023). As a low-cost, autologous material, it is easy to see that L-PRF offers advantages in this context. Given the similarity in outcomes between L-PRF and L-PRF Block in the current trial, were this to be reflected in larger scale trials or in clinical practice, the additional preparation time and cost of xenograft material required for L-PRF Block preparation could be questioned.

4.4.3 PATIENT-REPORTED OUTCOMES

In recent years, it has been recognised that evidence on patient-reported outcomes in regenerative studies is limited. A 2015 AAP Regeneration Workshop called for greater inclusion of such outcomes in the design of regenerative periodontal studies (Inglehart, 2015). More recently, a commissioned review for the journal *Periodontology 2000* highlighted that such information remains sparse in the area of guided bone regeneration and other bone regenerative procedures (Gotfredsen, 2023). The inclusion of patient-reported outcomes in studies on tooth extraction and alveolar regeneration is somewhat challenged by the small number of established evaluative tools; furthermore, the applicability of available tools to outcomes of extraction and ridge preservation might be questioned (Gotfredsen, 2023). A positive aspect of the current study design was the collection of PROMs at an early point in the post-operative treatment and over a continuous period of several days. The importance of contemporaneous recording of these measures has been highlighted (McGuire et al., 2014).

Among the patient reported outcomes in the current investigation, patients reported worst and average pain using a 100 mm visual analogue (VAS) scale on each of the first 7 post-operative days, and also provided information on their consumption of analgesic medication. Since data was not normally distributed, median responses are presented. No statistically significant differences were noted between protocols when comparing median VAS scores for “worst pain” or “average pain” at each time point. However, levels of post-operative pain appeared relatively high for both groups at day 1. In respect of average pain experience, the median VAS reported at day 1 was 17 (ex 100) for L-PRF and 28 for L-PRF Block, respectively. By day 2, median values for average pain were 5 and 27 respectively, but while the values for days 1-3 were all lower for L-PRF, these were not statistically significant. Similarly, median values for worst pain in the previous 24-hour period were notably higher at days 2 and 3 for L-PRF Block group but values were again not statistically significant. As this may be reflective of the limited size of the

study subgroups, further evaluation may be indicated to confirm these findings in future studies.

It has been suggested that implant placement with guided bone regeneration (GBR) may be perceived by patients as being more painful than implant placement without GBR (De Bruyckere et al., 2020). Since ARP aims to reduce the need for additional, separate grafting procedures, it is interesting to note that patient reports for worst pain and average pain in the current study suggest that median pain levels were relatively low at day 1, decreased quickly over subsequent days and were negligible following day 3. While there was a trend for higher pain scores in both worst and average pain for the L-PRF Block subgroup in the first 3 days post-operatively, these findings were not statistically significant. Furthermore, since the rate of post-operative complications after regeneration procedures may be relatively low, it may be difficult to demonstrate statistically significant findings in such studies (Payer et al., 2020).

The presence of pain measured by VAS appeared relatively well reflected in pain assessed by analgesic use by patients. When considering findings relating to the proportion of patients using analgesics, it must be noted that this was categorised dichotomously, such that consumption of any dose of any pain medication represented a positive response for this measure. As such, this data does not quantify the severity of pain nor the strength of pain medication required. Patients were advised to use paracetamol and ibuprofen on the evening of surgery and as needed thereafter. However, over the ensuing days, some patients utilised other pain medications, so analysis by any pain medication at any time was deemed more appropriate. Similarly, the relative percentage of patients using any analgesic (any pain medication, any dosage) at each time point did not show statistically significant differences between protocols, although there was a trend towards increased prevalence of analgesic use among the L-PRF Block subgroup.

Data on mean pain and analgesic use with these biomaterials may assist clinicians to communicate the risks of post-operative discomfort following alveolar ridge preservation with L-PRF/L-PRF Block. Based on the present information, patients may be advised that the majority of patients treated with these materials for ARP appear to require pain medication on days 1 and 2, following which most patients discontinue the use of pain medication. Nevertheless, a sizeable minority may use pain medication on subsequent days such that approximately 1 in 3 patients may still require some level of pain relief at day 5. Pain can be characterised as being of low-to-moderate intensity based on mean VAS scores recorded in the present investigation.

Kofina and colleagues evaluated post-operative symptoms following single tooth extraction and guided bone regeneration at bounded, single extraction sites exhibiting buccal dehiscence >4m. These authors described a relatively long mean procedure time and required flap advancement.to obtain closure, which may have resulted in an increased likelihood of discomfort. Although the majority of patients experienced no or only mild (VAS scores 1-3) symptoms over the 14-day healing period, at day 2, almost half described moderate or severe pain (VAS score ≥4), with approximately one-third still describing moderate/severe pain by day 7 (Kofina et al., 2023). These findings serve as useful context for assessing pain levels in the current study following flapless grafting, which appears to be associated with a relatively lower frequency and intensity of pain. The inclusion of L-PRF in the grafting approaches described in the current study might naturally be expected to result in reduced pain levels; in a recent systematic review, PRF use was associated with enhanced soft tissue healing and lower levels of post-operative pain at third molar extraction sites (Zhu et al., 2021). Of the four studies reported in Chapter 2 that have directly compared PRF in combination with a bone substitute with PRF alone for ARP, two of the studies reported on pain, with conflicting findings. Jouliq and colleagues presented limited description of their assessment method but appeared to score pain on a 10-point visual analogue scale (VAS) on the day of the extraction/graft procedure (day 0), and at 2, 5 and 7 days later. Mean VAS for the PRF group was significantly lower than for natural healing at day 0 and day 2, but not thereafter. Differences between PRF and PRF + β-TCP were not significant at

any time point. Overall, pain appeared to be of limited severity with mean scores in the range of 2.6 (PRF) to 3.4 (PRF + β-TCP) at days 0 and 2. Values declined to a VAS score <1 by day 5 (Jouliq et al., 2023). Conversely, Alam and co-workers showed much higher mean VAS values using the same scale, although this study encompassed mandibular third molar extraction sites. Both PRF and PRF + hydroxyapatite (HA) groups scored mean VAS >5 on the first post-operative day, with pain persisting at a more moderate level up to day 7 (Mean 3.1 in PRF group, with mean 1.4 in PRF + HA group). Pain scores were significantly lower in the PRF + HA group than with PRF alone (Alam et al., 2022).

Overall, quantification of patient pain is difficult since it has physical and psychological components and is subjective in nature; this also makes it challenging to generalise findings. Large variation in pain tolerance among patients has been noted even with simpler procedures such as periodontal probing (Heins et al., 1998). An early study of pain experience among 200 patients following periodontal surgery noted that pain was experienced by 73.5 per cent of patients and was severe in 21.0 per cent. Pain was more likely to be experienced in patients undergoing complex surgery compared to those having simpler procedures. Clinicians were frequently incorrect in the level of pain they forecasted for patients; this may be impactful, since it was revealed that many patients were prepared to accept pain rather than to take pain medication if this had not been specifically advised (Strahan and Glenwright, 1967). Assessing pain associated with implant placement or mucogingival procedures, Beaudette noted that patient anticipation of pain was correlated with pain experienced post-surgery, which in turn was significantly associated with use of analgesia. However, the actual pain reported by patients was frequently lower than anticipated (Beaudette et al., 2018).

Our finding that average pain and worst pain are most notable on the first post-operative day and taper off following 3 days align well with a past study on pain levels following implant treatment conducted at this research centre (Hashem et al., 2006). Interestingly, the VAS scores for average pain levels at

days 1 (Mean VAS 24) and day 3 (Mean VAS 12) align relatively closely with levels reported following L-PRF Block placement in the current study. Indeed, the research literature also demonstrates that using arbitrary thresholds of VAS ≥40 mm to indicate a notable pain experience for patients, a sizeable proportion - in the range of 15-33 per cent of patients - may record even routine periodontal procedures such as supportive periodontal care appointments (Karadottir et al., 2002), initial periodontal probing (Chung et al., 2003, Harrison et al., 2020) and recall probing (Hassan et al., 2005) to be painful experiences.

4.4.4 POSSIBILITY OF IMPLANT PLACEMENT AT GRAFTED SITES/ NEED FOR ADDITIONAL GRAFTING

Of the 21 cases where implant placement was planned, all sites ultimately accommodated implant placement. Only one of these sites, grafted with L-PRF Block, required additional grafting to facilitate implant placement. It must be noted that at baseline, the ridge in question had a limited buccolingual bone dimension of 7 mm, with a thin buccal bone width of 0.5 mm, so it is perhaps unsurprising that this site may have been vulnerable to requiring additional grafting.

Bone density at the sites proceeding to dental implant was judged clinically to be medium or good in almost all instances by the operator. For more objective measurement, an implant stability quotient (ISQ) reading was calculated at time of surgery using an Osstell ™ device; this is a third-generation resonance frequency analysis (RFA) system, developed by Huang and colleagues (Huang et al., 2003). RFA measures implant stability as a function of the interface between an implant and its surrounding bone (Sennerby and Meredith, 2008). While values may differ between implant systems, a recent investigation at this university determined a range of normative values of 61-85 for the Osseotite Zimmer Biomet implant system used in the current investigation (Reynolds et al., 2023).

In the present investigation, differences in mean Osstell readings were not statistically significant by protocol. Furthermore, mean values were 67.8 in both groups, indicating bone was within the satisfactory range and sufficient to allow implant placement and loading if required.

4.4.5 BUCCAL BONE THICKNESS

Of the 35 sockets assessed at time of extraction, 24 sites exhibited buccal bone thickness of less than 1 mm, with the remaining 11 sites exhibiting buccal bone thickness (BBT) ≥1 mm. This translates to 68.6 per cent of study sites having BBT<1 mm, which aligns broadly with findings indicating the majority of teeth in the inter-premolar regions have BBT<1 mm (Huynh-Ba et al., 2010, Januário et al., 2011). Despite this finding, of the 21 cases proceeding to implant, only the single case described above required additional grafting.

Sites with a compromised buccal bone wall have been associated with additional horizontal and vertical bone loss in the current period (Chappuis et al., 2013, García-González et al., 2020). The findings of the current study indicate that median and mean mid-buccal vertical bone loss was limited to less than 0.5 mm in both treatment subgroups, despite the narrow BBT at baseline. Although vertical changes were limited, it cannot be excluded that the changes in horizontal ridge dimension seen in the study were impacted by the lack of buccal bone thickness (García-González et al., 2020).

4.4.6 CLINICAL APPROACH USED

Given the relative lack of available literature making the comparison between L-PRF use and L-PRF Block, it may be prudent to rationalise some of the other procedural aspects of this study for clarity or to facilitate reproducibility by other researchers. The approach used for grafting in the present study

utilised recommendations previously outlined by Quirynen and colleagues at the inaugural “Enhanced Natural Healing in Dentistry” (ENHD) workshop (Pinto, 2018) and in a subsequent commissioned review publication by the same research group (Quirynen et al., 2023). These recommendations advise the use of L-PRF plugs or membranes rather than non-condensed L-PRF clots, the use of more than one membrane or plug to ensure adequate socket filling and the sealing of the extraction socket with an L-PRF membrane. No attempt was made to obtain primary closure at the extraction socket. Quirynen and colleagues have highlighted that when L-PRF is used in alveolar ridge preservation, primary closure is not required. Combined with the sealing of the alveolus with L-PRF membranes, this provides additional advantages in maintaining gingival width and vestibular depth (Quirynen et al., 2023).

All subjects in this study rinsed post-operatively for the first 2 weeks post-extraction with chlorhexidine. This may itself putatively assist in obtaining positive healing outcomes. In a randomised controlled trial in subjects following dental extraction, while the control group lost almost 1 mm of bone height at proximal sites of adjacent teeth over 6 months, the test group who rinsed twice daily with 0.12% CHX over the first month maintained the crestal alveolar bone level (Brägger et al., 1994).

In the current trial, there was no attempt to perform flap advancement to obtain primary closure. Sockets were sealed with the use of L-PRF as a barrier membrane. Martins and colleagues conducted a network meta-analysis of ARP techniques to compare the effectiveness of flap advancement, open healing following graft placement with a barrier material and open healing following graft placement without a barrier material in terms of the horizontal ridge width changes obtained. Randomised controlled trials of ≥ 2 months duration that compared two or more of the techniques against each other or against spontaneous (natural) healing were evaluated (Martins et al., 2022). Open healing following graft placement without the use of a barrier did not show statistically significant differences compared to natural healing, whereas the remaining techniques showed significantly less horizontal ridge width resorption than natural healing (Martins et al., 2022). The increased

cost, surgical time, patient morbidity and risk of adverse events, and the impact on soft tissue landmarks such as decreasing the zone of keratinised tissue render it questionable to recommend the use of flap advancement over open healing with a barrier material following graft placement in the socket. The latter provides almost equivalent clinical benefits of >1.1 mm of horizontal ridge width preservation versus natural healing and may represent the best choice for clinicians (Martins et al., 2022).

The current trial used an L-PRF barrier and was not associated with any adverse events. As such, the techniques used in the current study may represent appropriate and practical clinical approaches to ARP. The results of both treatment approaches compare favourably with the literature describing mean dimensional changes following extraction and natural healing (Van der Weijden et al., 2009, Tan et al., 2012), suggesting both techniques were effective for ARP. It must also be noted that included sockets had an intact buccal wall – fenestration or dehiscence >3 mm was an exclusion factor for our study, since socket sites with a buccal fenestration or dehiscence defect prior to extraction may demonstrate greater buccolingual resorption following extraction (Chen and Darby, 2017).

Systemic antibiotics were prescribed as part of the treatment protocol for all participants in the current investigation, based on the use of a xenograft material in the L-PRF Block group. However, a recent systematic review and meta-analysis gives some clarity on decision-making in this area, noting that the use of adjunct systemic antibiotics in regenerative periodontal surgery does not appear to enhance clinical outcomes; consequently, these authors suggested that the use of adjunct systemic antibiotics as part of regenerative periodontal treatment may be reconsidered (Chiou et al., 2023).

4.4.7 LIMITATIONS

Based on the primary outcome measure of assessment of change in horizontal bone dimensions, the current study may be considered underpowered. Power calculation indicated 12 patients per study group would be required for this purpose; however of the 35 sockets (32 patients) treated, only 21 participants (21 sockets) proceeded to implant placement, where intra-surgical measurements could be recorded. The reasons for patients not opting to proceed to implant were many but primarily driven by finances and/or poor compliance with other aspects of their restorative treatment plan. In some cases, this related to the timing of treatment in these patients, which overlapped with national Covid-19 restrictions, and led to delays/changes in treatment undertaken by patients. This provides the principal limitation of the study. Our initial plan was to recruit 28 participants for this study; while a small number of additional patients were recruited in an attempt to overcome this, a decision was made not to limit recruitment when some of these additional patients also opted to change to a removable treatment option.

Despite this, in comparison to the randomised controlled trials considered in our systematic review (Chapter 2), the size of study groups in the current study is not unfavourable. In these other investigations, study groups generally consisted of 10 subjects per group, with 3 of the trials (Clark et al., 2018, Ibrahim et al., 2022, Jouliq et al., 2023) using a multi-arm approach, where the combination of PRF and graft substitute were compared to each material used alone, and in some cases also compared to natural healing. The exception was the study of Alam and colleagues which used a parallel-arm approach and a larger group size (n=15) to compare PRF with a composite graft in third molar extraction sockets (Alam et al., 2022). In the current study, a positive feature was that despite the limited number (n=21) of patients proceeding to implant therapy, the size of the subgroups proceeding to implant remained similar (10 L-PRF subjects, 11 L-PRF Block subjects) and inter-group comparison was feasible. However, it must still be acknowledged

that the limited number of cases treated challenges the ability to make definitive conclusions from the current study.

In the present investigation, we used simple assessment measures based on recording of patient pain/discomfort, and on the impact of treatment on daily activities and life. These measures have previously been used in a study on dental implant treatment wherein patient reported outcomes were evaluated (Nolan et al., 2014). Patients were requested to complete an evaluation form each day for the first seven days post- extraction. Although the question format and contents of the daily questionnaire were clearly explained to patients at time of surgery, responses were not reviewed in person with the patient by the researchers when returned, to avoid any potential for coercion, response bias or impact on how discussion/instructions would be provided to subsequent patients. However, it is apparent that some patients either struggled with the questions that utilised multiple statements or Likert scales, or else completed these without due care. This may be noted in the inconsistency of some responses, where participants expressed an absence of symptoms or effects on daily activities in free text on the form but still cited a significant impact on their daily activities when they recorded Likert scale measurements. Consequently, for future research, the researchers would recommend that an independent clinician/blinded examiner could engage in a meeting/telephone conversation with each patient to discuss any questions relating to the evaluation form and to ensure it can be completed as accurately and comprehensively as possible.

4.5 CONCLUSIONS

Results failed to demonstrate statistically significant differences between treatment approaches with respect to horizontal ridge dimensional changes measured clinically at soft tissue level (bone + soft tissue; measured at 4 months) or intra-surgically at hard tissue level (bone-only; measured at time of 5-6 months). Despite this, mean loss of buccolingual ridge width at bone level trended lower for L-PRF Block subjects compared to L-PRF subjects. Only one of 21 sites required additional grafting to place an optimally located implant. Primary stability of implants was similarly high at sites with both grafting protocols.

Radiographic measurements of vertical bone loss was lower for L-PRF Block grafted sites, compared to L-PRF sites but not statistically significant.

Differences in probing depth changes and clinical attachment level measurements were minimal, and not statistically significant between protocols. No adverse events were noted and visual assessment indicated favourable soft tissue healing at grafted sites at both 7 and 14 days.

Average and worst pain levels experienced by patients did not differ significantly between protocols. As expected, pain was most severe - but typically of moderate intensity - at day 1, and tapered quickly such that mean intensity was low from day 3 onwards. Despite this, approximately one in three patients self-reported use of some pain medication at day 7, suggesting VAS levels and analgesic use did not correlate well in this cohort.

Based on the overall findings, both L-PRF and L-PRF Block appear to offer promise as grafting materials for alveolar ridge preservation. Further studies of larger size will be indicated to establish if trends towards reduced horizontal dimensional changes with L-PRF Block use may offer a statistically

significant benefit that justifies the additional preparation time and cost associated with its use for ARP.

CHAPTER 5: AN *IN VITRO* COMPARISON OF THE DEGRADATION OF L-PRF AND A-PRF MEMBRANES IN PLASMIN

5.1 INTRODUCTION

As noted in Chapter 1, the most widely researched indication for the use of PRF in periodontics is in the regeneration of osseous defects. Osseous defects describe a situation wherein there is a reduction or deficiency in the bony architecture around teeth/implants caused by disease or trauma (**AAP**, **2024**).These defects can be infrabony or inter-radicular in nature and appear to be common among patients with periodontitis (Vrotsos et al., 1999). Untreated infrabony defects may represent a risk factor for attachment loss and progression of periodontal disease (Papapanou and Wennström, 1991). Treatment of infrabony defects incorporates techniques to remove infected/inflammatory material from the defect site and in more recent years attempts to obtain regeneration of lost tissues at the site. Periodontal regeneration refers to the re-establishment of a periodontal ligament, root cementum and bone at a tooth root site previously denuded due to periodontitis (Claffey, 1992). The migration of epithelial cells into the wound site at a rate faster than the mesenchymal cells that form new connective tissues is believed to be the primary factor that limits the chances of effective regeneration (Listgarten and Rosenberg, 1979). Consequently, membrane materials are frequently placed in conjunction with grafting procedures to cover the treated defect site and provide a barrier to epithelial downgrowth; a procedure termed guided tissue regeneration. Consensus indicates that such regenerative therapy can improve tooth prognosis and represents a successful approach to the management of infrabony defects (Reynolds et al., 2015, Wu et al., 2017).

Guided bone regeneration (GBR) is “a surgical technique that aims to enhance alveolar bone volume at sites planned for future implant placement, or around previously placed implants” (Wang and Boyapati, 2006). Membranes are also used in GBR procedures where their use aims to exclude the undesirable growth of soft tissues into an osseous defect, allowing only osteogenic cell populations from the parent bone to re-populate the site. Effectively, membranes are used to create a space in the tissue in which osteogenesis can take place unimpeded. The membrane occludes cells facing

the bone surface, physically sealing the bony site (Dahlin et al., 1988) and selectively excluding epithelium and connective tissue, providing an environment for the recruitment and proliferation of osteoprogenitor cells, their differentiation into osteoblasts and expression of osteogenic activity (Linde et al., 1993). The additional use of bone substitute materials may provide a scaffold to facilitate the ingrowth and deposition of bone (osteoconduction); with a very limited number of materials potentially offering osteoinductive ability, wherein progenitor cells at the healing wound site may be stimulated to transform to cells capable of forming osseous tissue (Nasr et al., 1999). GBR finds use beyond infrabony defects in various indications including augmentation of deficient alveolar ridges and in alveolar ridge preservation, where the use of an occlusive membrane material remains a core component of treatment protocol.

The desirable characteristics of barrier membranes used for guided tissue regeneration were highlighted in early reviews (Karring et al., 1993, Scantlebury, 1993), and include good clinical handling properties, biocompatibility and integration by host tissues, and the ability to achieve cell occlusion and maintain space to allow healing. Additional characteristics such as sterility, stiffness and resistance to breakage, and biodegradability are also important (Greenstein and Caton, 1993). In the absence of an “ideal” membrane barrier material (Rakhmatia et al., 2013, Mizraji et al., 2023), it is merited to characterise strengths and weaknesses of platelet-rich fibrin as a regenerative material.

The potential to enhance regenerative outcomes through measures such as incorporating biological factors and antibacterial agents into existing membrane materials and the use of growth factors and progenitor cells that may direct healing events has been highlighted (Omar et al., 2019). Platelet rich fibrin membranes possess many of these characteristics; they are autologous, biocompatible, resorbable materials with generally good handling characteristics, which are not associated with adverse reactions (Quirynen et al., 2023). Since PRF membranes also possess some antimicrobial properties (Castro et al., 2019b, Moraschini et al., 2023) and appear to accelerate soft tissue healing (Miron et al., 2017a), primary closure is not mandated. PRF is

quick and cheap to produce, and contains the majority of platelets and leukocytes from whole blood. These cells are enmeshed in a fibrin clot that facilitates their sustained release, in addition to cytokines and a range of regenerative growth factors (Dohan Ehrenfest et al., 2018). These agents can collectively direct cellular healing and promote angiogenesis (Ratajczak et al., 2018) and integration of PRF with the tissues, maintaining blood supply and promoting bone-forming cells (Dohan et al., 2006a, Dohan Ehrenfest et al., 2010b, Quirynen et al., 2023). Systematic reviews have demonstrated that platelet rich fibrin (PRF) has favourable regenerative properties in multiple dental indications (Castro et al., 2017a, Castro et al., 2017b, Miron et al., 2017b, Miron, 2021).

However, the impact of the PRF structure on its porosity and cell occlusion abilities remains incompletely understood. Its effectiveness as a barrier material has been questioned, due to concerns about its mechanical strength and its consequent ability to maintain space to facilitate bone growth within a wound defect. In particular, the degradation profile of PRF membranes has been highlighted, since its putative fast resorption rate may lead to questions about its capacity to serve an occlusive function and to survive long enough *in vivo* to replicate the outcomes seen *in vitro*. A recent review affirmed that PRF may be better considered a biological or supportive membrane and not viewed as a sufficient barrier material on its own based on current evidence (Agrawal, 2023). Indeed, reviewing the use of PRF in guided bone regeneration for his current textbook, Miron affirmed that no data yet demonstrates any advantage in the use of PRF in GBR procedures in terms of new bone formation, faster bone healing, or final amount of augmented bone (Miron, 2021).

Mechanical testing has revealed that PRF has somewhat inferior mechanical properties relative to collagen membranes in terms of stiffness (modulus of elasticity measured using a universal testing machine at < 1 μN load), and hardness (measured by indentation testing using a nano-indenter at < 1 μN load) (Sam et al., 2015, Chhaya et al., 2022). Indeed, these authors suggested that lack of rigidity may result in the PRF membrane having a tendency to

collapse over the bone and root surface if used for guided tissue regeneration, potentially limiting the space needed for clot maturation. In a later study though, Isobe and colleagues concluded that CGF and A-PRF membranes both appear to be tough enough to serve well as barrier membranes (Isobe et al., 2017). It has been demonstrated that the tensile strength and stiffness of PRF properties can be modified by preparation protocol (Ravi and Santhanakrishnan, 2020). However, these authors found that PRF membranes created using titanium tubes (T-PRF) had a significantly higher tensile strength and modulus of elasticity than those created using standard glass-coated plastic tubes that used the same centrifugation cycle (L-PRF). Similarly, A-PRF membranes produced using the low-speed centrifugation concept had higher tensile strength and modulus of elasticity than L-PRF membranes. The values for modulus of elasticity found by Sam (0.35 GPa) and Ravi & Santanakrishnan (290 MPa) are broadly similar and suggest that alternative PRF variants may offer some mechanical advantages over L-PRF (Sam et al., 2015, Ravi and Santhanakrishnan, 2020). This may occur despite the observation that growth factor release from A-PRF membranes may be increased relative to L-PRF clots (Fujioka-Kobayashi et al., 2017), a feature previously speculated to correlate with an increased degradation rate (Pluemsakunthai et al., 2013). Further attempts have been made to enhance the mechanical properties of PRF through modifications in preparation protocol, such as the use of heat treatment, albeit this may occur with some challenge to the biologic signature of PRF (Kawase et al., 2015, Gheno et al., 2021).

Information regarding the chemical degradation of PRF variants has been derived from simple visual observations of membrane degradation reported in publications that focused on measurement of growth factor release from PRF membranes (Dohan Ehrenfest et al., 2018, Pluemsakunthai et al., 2013). These studies utilised Dulbecco's modified Eagle's medium (DMEM) but since media were changed at multiple time-points to accommodate growth factor collection, the rate of membrane degradation may be difficult to infer. A second group of studies that focused on chemical degradation have primarily utilised the percentage reduction in membrane weight as an outcome measure. These studies have typically been of short duration, employing

immersion in phosphate buffered saline (pH 7.4) and vibration on an orbital shaker. The findings of these studies have differed notably, with reductions in in L-PRF membrane weight after one week ranging from 363 4e to 85 per cent (Sam et al., 2015, Chhaya et al., 2022, Ravi and Santhanakrishnan, 2020). Interestingly, the latter study used a comparatively higher value of rotations at the orbital shaker. Although none of these studies reported total degradation of the PRF membranes, it must be suspected that this would have occurred if the study timelines were extended. The selection of study medium is important; Csönge and co-workers used 0.1% v/v trypsin with EDTA in DMEM and reported that only four of 5 A-PRF membranes degraded within 5 days (Csönge et al., 2021). Conversely, Isobe and colleagues immersed A-PRF an d CGF membranes in 0.05% trypsin plus EDTA and utilised a CO_2 incubator, and reported complete degradation of both membrane types within 120-180 minutes (Isobe et al., 2017). The comparison of membrane degradation in more clinically relevant solutions appears of value.

Fibrinolysis describes proteolytic degradation of the blood clots, of which fibrin is the dominant structural component (Risman et al., 2023). Plasmin is the primary enzyme responsible for fibrinolysis (Chapin and Hajjar, 2015), although its action is not specific for fibrin and it can also degrade fibrinogen, factors V and VII, and extracellular matrix proteins such as laminin and fibronectin (Virtanen et al., 2006). Plasmin is generated from activation of its inactive pro-enyzme precursor, plasminogen. Physiologically, plasminogen is activated by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA), and these activators in turn are regulated in plasma by corresponding (plasminogen activator) inhibitors (Virtanen et al., 2006)

An *in vitro* study wherein fibrin plates containing plasminogen were exposed to human parotid saliva demonstrated fibrinolytic activity within saliva and this appeared to be regulated by tPA. (Kjaeldgaard and Kjaeldgaard, 1987) . The same research team demonstrated that tPA appeared to be physiologically active in sampled human saliva, while uPA was generally not present (Kjaeldgaard and Kjaeldgaard, 1986). It has been argued that the presence of plasminogen and tPA in saliva implies that when fibrin is present

following oral surgical procedures, fibrinolysis may be triggered (Sindet-Pedersen, 1991). Plasmin may potentially represent a clinically relevant enzymatic model for investigating degradation of PRF clots. Human plasmin in solution has been used successfully to degrade gauze-compressed and heat-compressed PRF membranes in a previous study, although only visual observation of complete degradation was recorded (Kawase et al., 2015).

For clinicians, L-PRF and A-PRF may represent the most commonly used PRF production methods and in the context of the above evidence, it is pertinent to evaluate the limited literature that exists describing mechanical properties and chemical degradation of these PRF membranes. Consequently, the current in vitro study aimed to compare the degradation of A-PRF and L-PRF membranes in plasmin solution over a 14-day period to simulate more closely the enzymatic environment present in early wound healing.

5.2 METHODS – STUDY DESIGN & PREPARATION

5.2.1 STUDY DESIGN

This investigation was an in vitro comparison of the degradation of platelet-rich fibrin membranes in an enzyme (plasmin) solution. The study aimed to compare the extent of degradation of PRF membranes produced using two alternative preparation (centrifugation) cycles (respectively termed A-PRF and L-PRF protocols) over a 14-day period. Membranes degradation was assessed using relative loss of membrane weight as a primary outcome. Additionally, change in membrane length and width, and the presence of visual signs of degradation were assessed at fixed time points over the study period (1d, 3d, 6d, 7d, 10d, 14d) as secondary measures of membrane degradation.

The null hypothesis was that there would be no difference in degradation seen between L-PRF and A-PRF as measured by mean loss of membrane weight.

5.2.2. ETHICAL APPROVAL

This study received preliminary approval from the School of Dental Science Ethics Committee. Based on the collection of a blood sample from each participant, this was deemed beyond the scope of the internal school committee and referral to the Faculty of Health Sciences Research Ethics Committee (REC) was mandated. The study received ethical approval from the Faculty of Health Sciences REC, Trinity College Dublin (#210208; Appendix H).

5.2.3 LABORATORY AGREEMENT & TRAINING

A Visiting Scientist Agreement was completed with the tissue laboratory (Tissue Engineering Research Group Laboratory (TERG Lab), Royal College of

Surgeons in Ireland), to allow site access and use of laboratory facilities for the purposes of this investigation. Online and on-site preparatory training in laboratory procedures was undertaken prior to commencement of the study activity.

5.3 METHODS – PILOT STUDY & PRELIMINARY STEPS

5.3.1 PILOT STUDY TO DETERMINE MEDIA CONCENTRATION

Fifty millilitres (50 mL) of blood was collected from a single volunteer for a pilot investigation following the techniques described below. The purpose of the pilot study was:

(i) To verify that 2 μg/mL plasmin represents an optimal concentration in the test medium

(ii) To facilitate standardisation of study protocol and procedures

In summary, eight blood tubes were collected and processed using the L-PRF protocol (2700 rpm for 12 minutes, RCFclot 408g). Clots were compressed to form L-PRF membranes.

The orientation of membranes within the collection box and timing of transport of membranes to the laboratory was confirmed.

Following transport to the laboratory, membranes were placed in 6-well cell culture plates. Two membranes served as controls and were placed in a well containing 5 mL Dulbecco's modified Eagle's medium (DMEM) alone; the remaining membranes each were placed in duplicate in a medium of DMEM with plasmin at 3 different concentrations, as detailed below.

- Dulbecco's modified Eagle's medium (DMEM) alone (Control)
- 2 μg/mL plasmin in DMEM (Test 1)
- 5 μg/mL plasmin in DMEM (Test 2)
- 10 μg/mL plasmin in DMEM (Test 3)

Membrane weight was recorded at time-points over 14-day period (at 2d, 5d, 7d and 14d) to evaluate the characteristics of membrane degradation at each concentration of plasmin.

The pilot study additionally allowed verification that PRF did not float and could be suspended within both the control and test media for the purposes of the investigation. Similarly, the pilot study allowed verification of preferred methods of conducting membrane measurements.

5.3.2 PLASMIN PREPARATION AND ESTABLISHMENT OF DESIRED PLASMIN CONCENTRATION

A 750 µg package of plasmin (P1867 Plasmin from human plasma; CAS number: 9001-90-5, Sigma-Aldrich [Merck KGaA], Darmstadt, Germany), taking the form of a lyophilised powder of ≥2.0 units/mg protein, was delivered as a frozen package and was stored at -20°C, sealed in its commercial packaging sealed (Eppendorf tube) until ready for use. Upon removal from the -20°C freezer, the plasmin was thawed gradually at room temperature and then mixed in solution as follows. The plasmin pellet was mixed with distilled water to 1.5 mL (to give a concentration of 500µg/mL). Even distribution was ensured with gentle movement of the Eppendorf tube. This tube was not vibrated during the process to avoid the potential for protein to denature (takes form of foaming at top of the tube solution).

An optimal test solution containing 2 µg/mL plasmin in DMEM was selected for the final in vitro investigation of participant membranes, as determined following the pilot study. This aligned with a previous study that evaluated PRF degradation in plasmin (Kawase et al., 2015).

Aliquots of 150 µL of 2 µg/mL plasmin were prepared and stored in individual (1.5 mL capacity) Eppendorf tubes (Eppendorf® microtube, Sigma-Aldrich, St Louis, MO, USA) for storage at -20°C until use.

5.3.3 PREPARATION OF CULTURE MEDIA IN 6-WELL CELL CULTURE PLATES

Individual wells on the 6-well cell culture plates (Cellstar®, Greiner Bio-One, Kremsmünster, Austria) were prepared to contain 5 mL of (either control or test) solution. Dulbecco's modified Eagle's Medium – Low Glucose (DMEM; Sigma-Aldrich, St Louis, USA) was used as the control medium in this investigation.

Control wells

For control wells, 5 mL of DMEM solution was pipetted to the well.

Test wells

For test wells, 5 mL of medium was established at a concentration of 2µg/mL plasmin in DMEM. The following equation was used to calculate how much plasmin should be added to create a medium of desired concentration:

(Initial concentration x Initial volume) = (Final concentration x Final volume)

To establish a concentration 2 µg/mL based on a 5 mL well, the volume of plasmin calculated to be added to each well was 20 µL plasmin per 5 mL (i.e. 4980 µL of DMEM, 20 µL plasmin per test well)

At time of use, the Eppendorf tubes containing 150 µL aliquots of 2 µg/mL plasmin were allowed to thaw at room temperature before gentle movement was again applied to distribute plasmin evenly and 20 µL was removed using a laboratory pipette and added to a well in the cell culture plates as indicated. Separate 6-well sterile cell culture plates (Cellstar®, Greiner Bio-One, Kremsmünster, Austria) were prepared for A-PRF and L-PRF to avoid any potential for error during measurement episodes. Control medium was added to one well on each plate. Test medium was added to 3 separate wells on

each plate. Each well was numbered so that the corresponding membrane could be identified and measured at each successive time point without error.

All preparation of study media and measurement of membranes was conducted in a vertical laminar airflow system cabinet (SafeFAST Classic Bio Safety Cabinet, Faster S.r.l, Cornaredo, Italy) to ensure sterility of the processes.

5.4 METHODS - PRINCIPAL INVESTIGATION

5.4.1 STUDY POPULATION

Eight donor individuals (6 females, 2 male) provided blood samples for this investigation.

All participant donors were systemically healthy members of staff at Dublin Dental University Hospital who volunteered to donate blood for the purposes of this research investigation. Per the terms of ethical approval, no personal or demographic data was collected from donor individuals but all were adult patients in age range 21-60 years). All patients give written informed consent prior to blood collection. Sample size (number of participants) was advised by the collaborating laboratory.

5.4.2 BLOOD COLLECTION

Eight tubes of blood were collected from the ante-cubital fossa of each participant. Sterile (9 mL silica-coated, plastic) tubes without anticoagulant (BVBCTP-2, (red cap), IntraSpin, Intra-Lock, Florida, USA - confirm) were used for blood collection and platelet-rich fibrin preparation using the L-PRF and A-PRF protocols, as previously described. Each tube was marked externally prior to collection of blood at a point that corresponded to 6 mL, in line with the maximum 50 mL volume blood sample collection threshold, specified by the local ethical review committee. Tubes were also labelled with numbers 1–8, specifying the order of collection from the patient, (although no identifiers were used to link to a specific patient).

5.4.3 PREPARATION OF L-PRF AND A- PRF

Two centrifuges were used for this study. Both were calibrated, fixed angle centrifuges of the same design (IntraSpin®, Intra-Lock®, Boca Raton, Florida, USA). These were designated centrifuge A and centrifuge B as follows:

- Centrifuge A was used for the L-PRF protocol: 2700 rpm for 12 minutes (RCF-clot 408 g)
- Centrifuge B was used for the A-PRF protocol: 1500 rpm for 14 minutes (RCF-clot 126 g).

Upon collection, blood tubes were immediately inserted into the centrifuge in alternate pairs (to ensure balancing within the centrifuge bucket) and were centrifuged according to the appropriate protocol.
Tubes 1, 2, 5 and 6 (first and third pairs) were inserted in one centrifuge while tubes 3, 4, 7 and 8 (second and fourth pairs) were placed in the remaining centrifuge. Coin-toss randomisation was used to determine which centrifuge was used for the first pair of tubes in each case.
Four tubes were inserted in Centrifuge A, and processed to create L-PRF, while the remaining four tubes were inserted in Centrifuge B and processed to create A-PRF.

Following centrifugation, clots were identified as the middle layer of biologic material within each tube and were gently separated from the red blood cell layer using a metal spatula. Clots were placed into a metal membrane preparation box (XPression® Box, Intra-Lock®, Boca Raton, FL, USA) for processing into membranes. A light metal plate was applied to compress the clots under gravitational force, resulting in PRF membranes of standardised thickness (1 mm). Membranes were separated by centrifugation (preparation) protocol, such that all membranes prepared for A-PRF were collected in a separate XPression® Box from those prepared for L-PRF. Membranes were placed in a consistent orientation and were placed according to sequence of collection to assist record keeping. A standardised compression time (15

minutes) was used to allow elimination of clot fluid/exudate from the membrane).

5.4.4 TRANSFER TO LABORATORY AND PREPARATION FOR MEASUREMENT AND CULTURE

Membranes were immediately transferred to the tissue laboratory (TERG Lab, RCSI) for baseline measurements and placement in solution (test medium/control medium, as appropriate), where their degradation was studied over a 14-day period. A key was used that allowed identification of the protocol and sequence of collection of each membrane following transfer.

5.4.5 LABORATORY PROCESS – BASELINE PREPARATION & MEASUREMENT

Following transport to the laboratory, the biosafety cabinet was prepared. The external surface of the Xpression Box® was sterilised with 70% alcohol before placement in the biosafety cabinet. Each membrane was placed on gauze tissue and was gently compressed for 60 s using another piece of gauze to remove any excess fluid.

Measurement of maximum membrane width and length was conducted (as detailed in the measurements section below). The baseline weight of each membranes was recorded as outlined below. Following measurement of physical membranes characteristics, each membrane was placed in its individual well of the 6-well cell culture plate containing a static medium. Culture plates were then transferred to a CO_2 incubator at 37°C (Panasonic, Oizumi-Machi, Japan).

5.4.6 LABORATORY PROCESS - PHYSICAL MEASUREMENT & VISUAL ASSESSMENT OF MEMBRANES

Physical measurements were recorded for each membrane at each designated time point, as detailed below. At each time point, the 6-well plates were retrieved from the incubator. The external surface of the 6-well plate was disinfected with 70% alcohol and was transferred to the biosafety cabinet. Each membrane was removed from its well using a sterile spatula Membranes were then placed on gauze tissue and gently compressed with a second piece of gauze to remove excess fluid, as previously described. As well as baseline measurements, membranes were assessed at the following fixed time points:

- Day 1
- Day 3
- Day 6
- Day 7
- Day 10
- Day 14

A sterile metal spatula (Hu-Friedy, Chicago, USA) was opened within the biosafety cabinet and were used to remove the membrane from the Xpression Box® at baseline and for transfer of the membrane to and from its respective well in the 6-well tray at subsequent measurement time-points. This was utilised to avoid potential compression/traumatic damage to the membrane surface associated with the use of tweezers to pick up each membrane, particularly since some membranes folded on themselves in solution (Figure 14). (A sterile non-toothed tweezers (Hu-Friedy, Chicago, USA) was maintained in the event that a membrane folded in a fashion that limited the possibility of recording physical measurements or was not accessible for lifting from its well using the metal spatula).

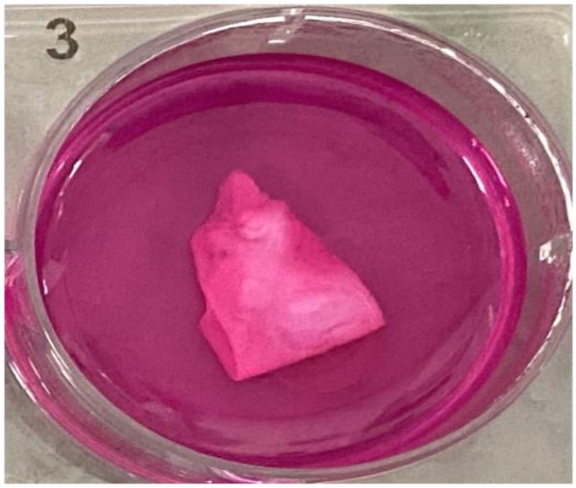

Figure 14: Membranes often folded on themselves, making handling difficult

The following measurements/observations were recorded for each membrane at each time point:

- Assessment of visual signs of degradation
- Maximum membrane length (mm)
- Maximum membrane width (mm)
- Measurement of membrane weight (mg)

5.4.7 ASSESSMENT OF VISUAL SIGNS OF DEGRADATION

Membranes were visually assessed against a blue surgical drape sheet for signs of physical degradation, namely:

- Perforation of the membrane
- Major change in membrane shape
- Complete dissolution/degradation in the medium

Visually translucent membranes were additionally documented at day 14. The presence of any visual sign of degradation at any time point was recorded to allow comparison between membrane types. If visual physical signs were noted, the first time-point at which changes were noted was recorded and the membrane was reviewed for further signs at subsequent time points, for descriptive purposes. Photographs were taken where perforation, major

changes in membrane shape or complete degradation were noted. Examples of such changes are presented in Section 5.5.6 (Figures 15 & 16).

5.4.8 MEASUREMENT OF MAXIMUM MEMBRANE WIDTH AND MAXIMUM MEMBRANE LENGTH

Measurement of maximum membrane length and maximum membrane width was conducted in the biosafety cabinet. Each membrane was placed flat on a sterile sheet and measured using callipers. Each membrane was also positioned beside a ruler with millimetre markings, and maximum length and width were again recorded to confirm accuracy.

5.4.9 MEASUREMENT OF MEMBRANE WEIGHT

Weight measurements were conducted using a pre-calibrated electronic analytical (Measuring) balance with readability of 0.01mg/0.1mg (XS205 DualRange, Mettler Toledo, Greifensee, Switzerland). This balance has repeatability of ±0.02mg and a settling time of 1.5s.

A spatula was used to lift the membrane onto a sterile petri dish lid to transfer each membrane for weighing on the electronic analytic balance. The balance reading was zeroed each time with the petri dish in place before the membrane was added to the petri dish and the weight calculated. Membrane weight measurements were conducted in triplicate to ensure accuracy. Membrane weight was recorded in milligrams (mg) before the membrane was returned to its respective medium in the 6-well cell culture plate. Culture plates were then returned to a 37°C incubator until the next measurement time point

All physical measurements and visual signs were recorded on a template form at each time point (Figure 15). Data was transferred to an Excel spreadsheet (Microsoft, Redmond, WA, USA) for subsequent analysis.

Subject number						
Time-point						
Membrane ID	Protocol	Weight (mg)	Max length (mm)	Max width (mm)	Visual change evident?	If YES: Describe visual change

Figure 15: Image of template form used for data collection at each time point

5.4.10 STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS® Statistics software version 29 (IBM®, Armonk, NY, USA).
For measurements of membrane weight, length and width, mean and standard deviations were calculated for both membrane types (A-PRF and L-PRF), and for subgroups of study media (test medium vs control medium) within each membrane type.

The independent samples t-test was used to compare characteristics by membrane type (L-PRF versus A-PRF) at baseline and at each time point.
The paired samples t-test was used to compare measurements within each protocol at each time point. The Chi-squared test was used to study the association between membrane type and whether they exhibited dimensional changes above pre-determined thresholds.

5.5 RESULTS

5.5.1 PILOT STUDY TO DETERMINE MEDIA CONCENTRATION

At the 2-day measurement, it was noted that weight of some membranes actually increased. This was postulated to relate to fluid uptake/coating at the L-PRF membranes, despite placement on gauze before measurement. A protocol modification was made whereby membranes were placed on gauze for 60 s before measurement. During this time, another sheet of gauze was used to gently compress the membranes on their superior aspect to express any excess fluid prior to measurement.

In comparison to the control medium (DMEM alone), all test media (containing plasmin) were associated with an increased percentage of weight loss over the duration of the pilot study (i.e. at 14 days).

Membranes (test and control media) continued to degrade (as measured by loss of membrane weight) up to 14 days. The rate of membrane degradation from baseline to 7 d and from 7 d to 14 d was broadly similar (Table 19). An increase in the plasmin concentration from 2 µg/mL to 5 µg/mL or 10 µg/mL was not associated with additional percentage weight decrease over the duration of the study.

It was determined that the addition of plasmin solution was associated with an increase in mean degradation and appeared to be a valid model to test. A concentration of 2 µg/mL was chosen for the test medium in the subsequent investigation

Table 19: Mean percentage weight loss from baseline to 7 days and 14 days - pilot data

Time-Point	DMEM (Control)	2 µg/mL Plasmin	5 µg/mL Plasmin	10 µg/mL Plasmin
7 days	29.5	30.5	40	29
14 days	52.5	62.5	61.5	63

5.5.2 BASELINE WEIGHT

Baseline weight was normally distributed for both membrane types. As demonstrated in Table 20 the mean weight of L-PRF membranes at baseline was greater than that of A-PRF membranes, this difference was statistically significant ($t(62) = 3.63$; $p<0.001$).

Table 20: Mean baseline weight of L-PRF and A-PRF membranes. Independent samples t-test. Mean membrane weight of L-PRF membranes at baseline was greater than A-PRF membranes; statistically significant at 5% significance level.

	Mean weight ± sd (mg)	t	df	p
L-PRF	101.5 ± 18.0	3.63	62	<0.001
A-PRF	85.0 ± 18.3			

5.5.3 CHANGES IN MEAN MEMBRANE WEIGHT

5.5.3.1 Mean membrane weight by time-point

Comparison by membrane type

Mean membrane weight was calculated at each time point for each PRF preparation protocol (A-PRF and L-PRF). Mean membrane weight overall showed a consistent decrease at each successive time point for both A-PRF and L-PRF membranes, indicating degradation continued to 14 days. L-PRF membranes remained heavier, on average, that A-PRF membranes up to day 3, after which mean membrane weight was similar up to day 14, when mean weight of A-PRF membranes was greater than that of L-PRF membranes (Table 21).

Table 21: Mean weight (± standard deviation, sd) of L-PRF and A-PRF membranes overall, compared by time-point. This data combines mean changes noted for membranes regardless of study medium.

	A-PRF Weight (mg)	L-PRF Weight (mg)
Baseline	85.0 ± 18.3	101.5± 18.0
Day 1	74.8 ± 15.6	80.8 ± 13.2
Day 3	65.8± 15.0	69.3 ± 13.1
Day 6	58.8 ±15.5	57.9 ± 12.8
Day 7	54.2 ± 14.4	54.0 ± 11.1
Day 10	49.9 ± 13.8	51.2 ± 10.8
Day 14	43.3 ± 14.2	39.4 ± 16.4

Comparison by membrane type and study medium

When analysed by the medium used (test vs control medium) and within protocols, a pattern of progressive decrease in mean membrane weight at successive time points was evident for both A-PRF and L-PRF membranes, regardless of the medium in which they had been incubated (Table 22).

Table 22: Mean weight (± sd) of L-PRF and A-PRF membranes compared by time-point, and sub-analysed by test and control medium.

	A-PRF Test Weight (mg)	A-PRF Control Weight (mg)	L-PRF Test Weight (mg)	L-PRF Control Weight (mg)
Baseline	84.0 ± 18.1	88.1 ± 19.8	100 ± 17.8	105.8 ± 19.1
Day 1	73.3 ± 15.3	79.2 ± 16.9	79.2 ± 13.8	85.7 ± 10.5
Day 3	65.2 ± 15.4	67.8 ± 14.5	68.2 ± 13.5	72.8 ± 11.9
Day 6	57.3 ± 15.7	63.2 ± 15.0	54.9 ± 12.4	66.9 ± 9.9
Day 7	52.6 ± 13.6	59.1 ± 16.5	51.5 ± 11.1	61.7 ± 6.7
Day 10	47.8 ± 13.2	56.1 ± 14.7	49.2 ± 10.7	57.4 ± 9.1
Day 14	41.1 ± 13.3	49.8 ± 16.0	35.9 ± 16.8	50.2 ± 9.5

5.5.3.2 Percentage change in mean membrane weight over time

Table 23 demonstrates the mean change in relative weight (by percentage) at each time point compared to baseline membrane weight, analysed by membrane type (L-PRF vs A-PRF). This is expressed as the percentage weight decrease at each time point relative to baseline weight.

Comparison by membrane type

Both A-PRF and L-PRF membranes showed a consistent decline in mean weight from baseline to 14 days with mean reductions noted between every successive time point. Overall, membranes produced using the L-PRF preparation protocol demonstrated greater mean weight change from baseline to 14 days and at each time point.

Table 23: Mean percentage weight change (decrease vs baseline) of L-PRF and A-PRF membranes overall compared by time-point. This data combines mean changes noted for membranes regardless of medium. Independent samples t-test. Statistical significance at 5% significance level observed at all time-points, with greater mean percentage loss observed for L-PRF membranes.

	A-PRF % decrease	**L-PRF % decrease**	**p-value**
Day 1	11.3 ± 10.3	19.7 ± 9.5	0.001
Day 3	22.4 ± 8.1	31.1 ± 10.6	< 0.001
Day 6	31.0 ± 9.4	42.4 ± 11.4	< 0.001
Day 7	36.5 ± 7.8	46.0 ± 10.4	< 0.001
Day 10	41.5 ± 8.7	48.9 ± 10.2	0.003
Day 14	49.4 ± 10.4	61.6 ± 16.0	< 0.001

Comparison by membrane type and medium

The mean percentage weight decrease of A-PRF and L-PRF membranes was analysed further, to separate membranes degraded in test media from those degraded in control media at each time-point. Within each protocol, membranes degraded in test media showed greater proportional weight decrease than those in control media. Comparing protocols, L-PRF membranes showed greater proportional weight loss than A-PRF; this was consistent for each time point and was noted regardless of medium used (Table 24).

Table 24: Mean percentage weight change (decrease vs baseline) of L-PRF and A-PRF membranes detailed by time-point, and sub-analysed by test and control medium

	A-PRF Test (% decrease)	A-PRF Control (% decrease)	L-PRF Test (% decrease)	L-PRF Control (% decrease)
Day 1	11.9 ± 10.8	9.5 ± 9.0	20.2 ± 10.1	18.2 ± 8.0
Day 3	22.2 ± 9.2	22.9 ± 3.9	31.4 ± 10.1	30.1 ± 12.7
Day 6	31.9 ± 10.3	28.4 ± 6.1	44.7 ± 10.7	35.6 ± 11.3
Day 7	37.5 ± 7.7	33.5 ± 8.1	47.9 ± 10.1	40.5 ± 10.0
Day 10	43.1 ± 8.6	36.6 ± 7.4	50.3 ± 9.8	44.7 ± 11.0
Day 14	51.1 ± 10.5	44.4 ± 8.9	65.0 ± 15.6	51.3 ± 12.8

In test medium, the difference in mean weight decrease between L-PRF and A-PRF was statistically significant at all time-points for test groups as determined by the independent samples t-test. L-PRF membranes showed a greater reduction in mean weight versus baseline than A-PRF at every time point.

Comparison by membrane type in test media only

The mean percentage decrease in weight of membranes degraded in test media was analysed further at each time point, to evaluate if there was a significant difference in the mean percentage weight decrease when comparing A-PRF and L-PRF membranes. At each time point from day 1 to day 14, L-PRF membranes demonstrated greater mean percentage of weight loss than A-PRF membranes. These differences were statistically significant at every time point, as demonstrated in Table 25.

Table 25: Comparison of mean percentage weight change (decrease vs baseline) of L-PRF and A-PRF membranes in test medium at each time point. Independent samples t-test. Statistically significant at 5% significance level at all time-points with greater mean percentage change for L-PRF.

Time	A-PRF Test	L-PRF Test	t (df)	p	Effect size (Cohen's d)
Day 1	11.9 ± 10.8	20.2 ± 10.1	2.75 (46)	0.009	10.45
Day 3	22.2 ± 9.2	31.4 ± 10.1	3.28 (46)	0.002	9.66
Day 6	31.9 ± 10.3	44.7 ± 10.7	4.21 (46)	< 0.001	10.47
Day 7	37.5 ± 7.7	47.9 ± 10.1	4.02 (46)	< 0.001	8.94
Day 10	43.1 ± 8.6	50.3 ± 9.8	2. 69 (46)	0.010	9.25
Day 14	51.1 ± 10.5	65.0 ± 15.6	3.63 (46)	< 0.001	13.31

Figures 14, 15 and 16 document graphically the proportional change (decline) in membrane weight at each time point. Using baseline weight as 100 per cent, mean membrane weight at subsequent time-points are demonstrated as a percentage of initial weight remaining at this time point. Standard deviations are not presented; these overlapped at each time point, and it may be interpreted that differences in mean percentage weight change were not statistically significant between protocols.

Figure 16 utilises a trend graph to compare the mean percentage weights of A-PRF and L-PRF membranes overall. Both membrane types showed a consistent decline from baseline to 14 days with mean reductions between every successive time point. L-PRF membranes showed a greater mean change from baseline to 14 days than A-PRF membranes (61.6 per cent versus 49.2 per cent decline respectively – resulting in residual proportional weight at 14d of 38.4 per cent and 50.8 per cent respectively). Figures 17 and 18 graphically represent changes in relative membrane weight between membranes placed in control versus test media for each study protocol.

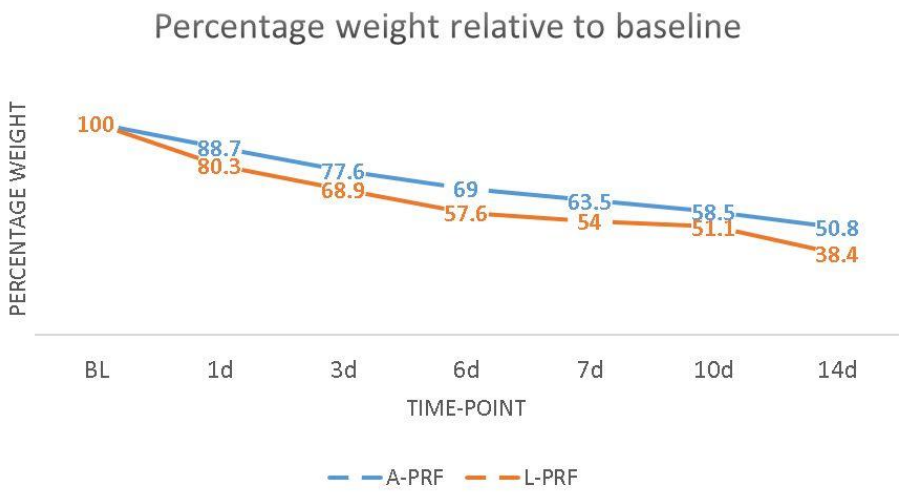

Figure 16: Trend graph demonstrating the mean percentage weight relative to baseline, sub-analysed by test protocol (L-PRF vs A-PRF)

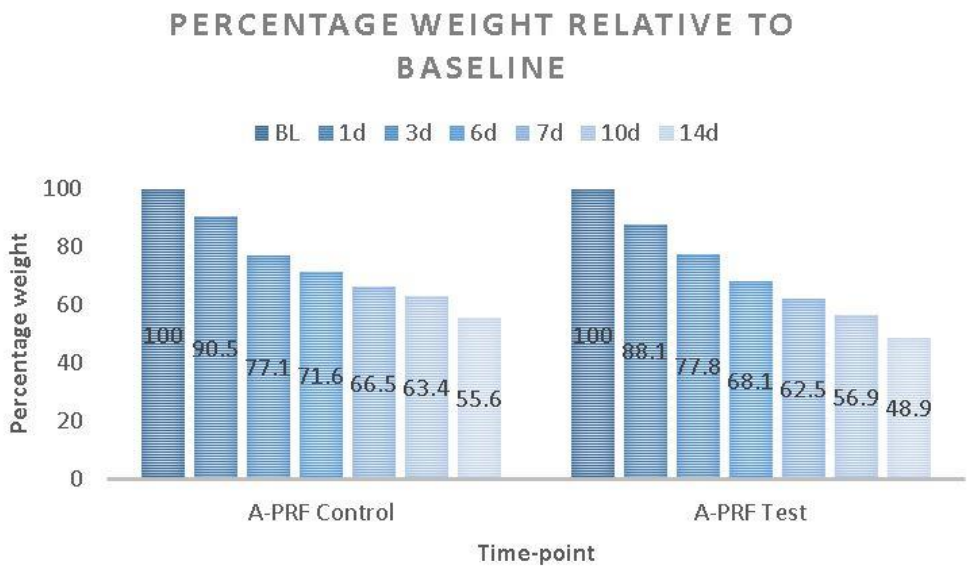

Figure 17: Comparative analysis of percentage decline in mean membrane weight at each time point for A-PRF Control medium and A-PRF Test medium

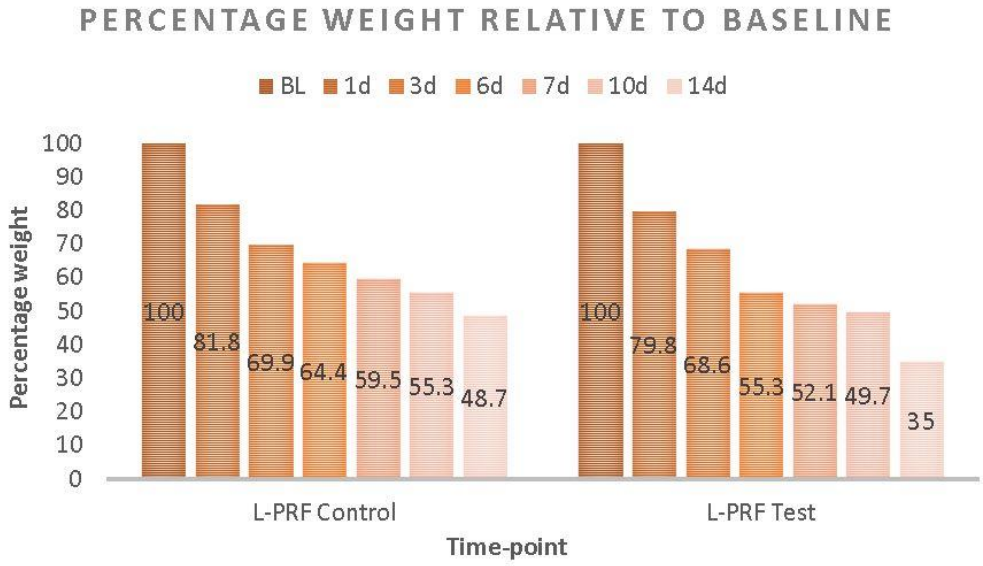

Figure 18: Comparative analysis of percentage decline in mean membrane weight at each time point for L-PRF Control medium and L-PRF Test medium

5.5.3.3 Mean percentage weight reduction (Baseline – 7 days) and (Baseline – 14 days)

Mean reduction in membrane weight from baseline-7 days was compared with mean reduction in weight from 7 days to 14 days: This aimed to test if the rate of degradation declined in the second week of the investigation, in order to assess for future studies if medium should be changed after 7 days if the study was to be repeated. The paired samples t-test was used per protocol. Results show that relative reduction in mean weight was significantly greater from baseline-7 days than from 7-days to 14 days, for both test protocols (Table 26).

Table 26: Comparison of relative mean reduction in membrane weight for membranes in test media in first and second 7-day periods. Comparison within each protocol. Paired sample t-test. Statistically significant at 5% significance level with greater percentage change during period from baseline to day 7 for both protocols

		Mean	**sd**	**t (df)**	**p-value**
L-PRF	Day 7 - Baseline	47.9	10.1	2.46 (23)	.022
	Day 14 – Day 7	30.1	29.1		
A-PRF	Day 7 - Baseline	37.5	7.7	6.03 (23)	<.001
	Day 14 – Day 7	22.2	12.1		

5.5.4 MEMBRANE LENGTH AND WIDTH

5.5.4.1 Baseline Characteristics

The independent samples t-test was used to compare membrane length and membrane width by preparation protocol at baseline only (Table 27). L-PRF Membranes were significantly longer at baseline ($p < 0.001$). L-PRF membranes were also significantly wider at baseline ($p < 0.001$). Mean membrane length and width for membranes in test media was reflective of the overall findings.

Table 27: Mean membrane length and width at baseline by preparation protocol. Independent samples t-test. L-PRF membranes longer and wider at baseline. Statistical significance at 5% significance level for both overall and test membranes.

	A-PRF Overall	L-PRF Overall	p-value	A-PRF Test Medium only	L-PRF Test Medium only	p-value
Mean Length (mm)	19.84 ± 4.46	23. 81 ± 2.80	< 0.001	19.58 ± 4.50	23.83 ± 2.65	< 0.001
Mean Width (mm)	9.06 ± 0.88	9.96 ± 0.78	< 0.001	9.08 ± 0.88	9.88 ± 0.80	< 0.001

5.5.4.2 Changes in Membrane Length

Thresholds for decrease in membrane length were investigated for each protocol overall and for membranes in test media, to assess if either protocol was associated with greater likelihood of clinically relevant changes in membrane length over time.

As a first step, the percentage of membranes losing ≥ 20 per cent or ≥ 50 per cent of their initial length was calculated (Table 28). This showed a greater proportion of L-PRF membranes showed a reduction in membrane length that exceeded both thresholds. When change in mean length among membranes in test media only was assessed, data was not normally distributed.

Table 28: Percentage of membranes losing ≥20% or ≥50% of their initial length analysed by preparation protocol.

	L-PRF Overall	L-PRF Test	A-PRF Overall	A-PRF Test
Loss ≥ 20% weight	15%	13%	3%	4%
Loss ≥ 50% weight	9%	25%	3%	4%

As a second step, the threshold for change in membrane length from baseline to 14 days was re-set at zero percent (0%) to determine the proportion of membranes that showed any decrease in length over the study period, by protocol. A-PRF membranes were statistically significantly more likely not to demonstrate loss of membrane length (i.e. more likely to maintain their length) than L-PRF membranes ($p < 0.001$; Table 29)

Table 29: Percentage of membranes losing 0% of their initial length, analysed by preparation protocol, for membranes in test media only. "No" indicates no loss of length, "Yes" implies membrane decreased in length from baseline-14 days. Chi-squared test of independence. L-PRF membranes were more likely to show a decrease in length; statistically significant at 5% significance level.

Protocol		No	Yes	Chi-sq (df)	p-value
L-PRF	Count	6 (25%)	18 (75%)	19.05 (1)	< 0.001
A-PRF	Count	21 (87.5%)	3 (12.5%)		

5.5.4.3 Changes in Membrane Width

The threshold for change in membrane width from baseline to 14 days was set at zero per cent (0%) to determine the proportion of membranes that showed any decrease in width over the study period, by protocol. A-PRF membranes were more likely not to demonstrate loss of membrane width (i.e. more likely to maintain their width) than L-PRF membranes; this was marginally statistically significant ($p = 0.046$).

With a threshold of ≥ 10 per cent change in width, the differences between protocols was no longer statistically significant ($p = 0.149$).

5.5.5 VISUAL PHYSICAL SIGNS OF DEGRADATION – ANY SIGN AT ANY TIME

Visual signs of degradation (membrane perforation, major change in membrane shape or complete membrane dissolution) were assessed at each time point. Examples of different visual signs of membrane change are demonstrated in Figures 19 and 20.

In total, only 17 of the 64 membranes (11 L-PRF, 6 A-PRF) showed one or more of these visual signs at any time point. This included four membranes (in two subjects) that showed complete dissolution in their medium. The first time point at which any membrane displayed visual signs of degradation was on day 10. The effect of PRF preparation protocol on observing visual physical signs (any physical signs at any time point) was not found to be statistically significant overall (p = 0.157; Table 30)

Table 30: Percentage of membranes where visual signs of physical membrane change were observed overall, comparing L-PRF and A-PRF membranes. Chi-squared test of independence. No statistical significance at 5% significance level.

Signs of physical change	**Yes** n (%)	**No** n (%)	**Chi-sq (df)**	**p-value**
L-PRF Overall	11 (34.4)	21 (65.6)	2.00 (1)	0.157
A-PRF Overall	6 (18.8)	26 (81.2)		

The effect of preparation protocol was further assessed considering only membranes placed in test medium. The effect of protocol on observing visual physical signs (any physical signs at any time point) was not statistically significant by PRF preparation protocol in these circumstances (p = 0.204; Table 31)

Table 31: Percentage of membranes where visual signs of physical membrane change were observed overall, comparing L-PRF and A-PRF membranes placed in test medium only. Chi-squared test of independence. No statistical significance at 5% significance level.

Signs of physical change	Yes n (%)	No n (%)	Chi-sq (df)	p-value
L-PRF Test	9 (37.5)	15 (62.5)	1.61 (1)	0.204
A-PRF Test	5 (20.8)	19 (79.2)		

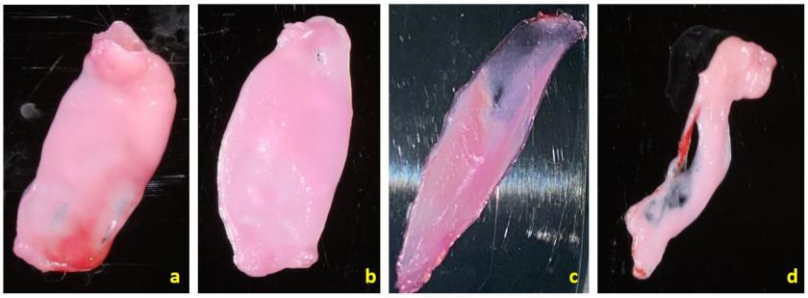

Figure 19: Membranes demonstrating visual signs of change (all images collected at 10-day time-point). a. Minor areas of translucency b. Minor perforation c. Minor perforation, translucency d. Multiple perforations, translucency, change in membrane shape

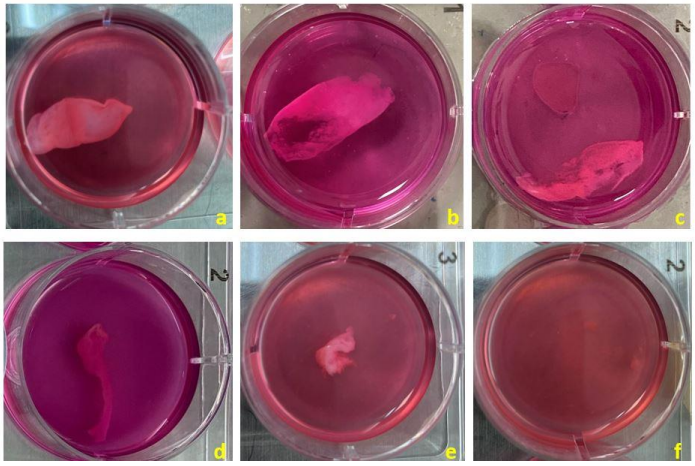

Figure 20: Various PRF membranes imaged at day 14 time-point. a. Control L-PRF membrane b. Perforated membrane c. Perforation, translucency and early loss of integrity d. Major change in membrane shape e. Partial degradation f. Completely degraded membrane

5.6 DISCUSSION

This in vitro study aimed to compare the degradation of A-PRF and L-PRF membranes in vitro over a 14-day period. Membranes were placed in an enzymatic solution (2 µg/mL plasmin) and were assessed for visual signs of degradation at fixed time points over a 14-day period (1d, 3d, 6d, 7d, 10d and 14d). Degradation was also characterised using physical measurements (maximum membrane length, maximum membrane width and membrane weight) at each time point. Dulbecco's modified Eagle's medium (DMEM) was used as a control medium; this is a basal medium widely used for cell culture (Eagle, 1959, Dulbecco and Freeman, 1959). Membrane degradation was measured using reduction in mean membrane weight (primary outcome), while measurements of maximum length and weight were recorded to establish if these might account for any changes observed in mean weight. Given that membranes were flattened at baseline to a uniform thickness of just 1 mm, it was not expected that membrane thickness could be reliably measured without potential damage to the membranes themselves. Hence, visual signs of physical degradation were used to characterise any changes observed in membrane appearance that could not be characterised in dimensional measurements.

5.6.1 MEMBRANE SIZE

L-PRF membranes were significantly larger than A-PRF membranes at baseline, as measured by mean maximum length ($p < 0.001$), mean maximum width ($p < 0.001$) and mean membrane weight ($p < 0.001$). This is consistent with the findings of Dohan Ehrenfest and colleagues who studied growth factor release from L-PRF and A-PRF membranes produced in the IntraSpin® centrifuge and observed that L-PRF membranes were consistently larger in size that A-PRF (Dohan Ehrenfest et al., 2018). Demographic characteristics of donors can influence membrane dimensions, with larger membranes reported to be produced from females and older individuals, which has been attributed to a lower haematocrit among these groups (Miron et al., 2019c).

In the present investigation, no comparison was made with the dimensions of membranes in other studies. This is not a productive exercise since membrane size is also influenced by other factors, including time taken from blood collection to commencement of centrifugation and the type of centrifuge and blood tubes used for PRF production (Dohan Ehrenfest et al., 2018, Miron et al., 2019c, Miron et al., 2023). Additionally, since both L-PRF and A-PRF membranes were prepared from each participant, donors acted as their own control for membrane size.

Membrane length and width was also recorded at each subsequent time point. Membranes generally maintained these characteristics, with the majority of membranes demonstrating only minor changes, if any, over the study period. Nevertheless, statistical analysis demonstrated that A-PRF membranes were statistically more likely not to demonstrate any loss of membrane length ($p < 0.001$) or membrane width ($p = 0.046$) over the study period. Since changes were in the order of only 1 mm in many instances, the possibility that findings of length and width may be statistically significant but not clinically meaningful was raised. As a result, thresholds were set to establish scenarios where dimensional change via shrinkage of membrane length or width might potentially compromise coverage of a defect etc. At a threshold of just 10 per cent loss of membrane width, the difference between A-PRF and L-PRF membranes was no longer statistically significant. However, membrane length was more clearly affected by study preparation protocol – a greater proportion of L-PRF membranes demonstrated loss of membrane length ≥ 20% and at ≥ 50% of initial length. Were these scenarios to apply in practice, this might manifest in compromised healing. However, the limited changes observed in membrane length and width suggest that when L-PRF and A-PRF membranes are used for the purpose of defect coverage, they are capable of maintaining their dimension over time and do not appear to be particularly vulnerable to shrinkage.

It must be acknowledged, however, that measurement of membrane length and width was not without some challenge at later time points. As membranes started to become lighter, membrane handling became

increasingly difficult as some membranes tended to fold and avoidance of iatrogenic perforations/tears of these fragile membranes became increasingly critical. Membranes were lifted from their wells using a metal spatula to minimise risk, a non-toothed tweezers or the rolled edge of a periodontal probe tine were gently used to “open” membranes to full length if required. Furthermore, an additional limitation was that only maximum width and length was recorded, since avoidance of manipulation of membranes was desired. The high percentage of loss of membrane weight, in conjunction with limited changes in width and length may suggest that membrane degradation may primarily occur through loss of membrane thickness rather than loss of length or width. Membranes were of standard 1 mm dimension at baseline, following compression in the Xpression ®box, and assessment of changes in this dimension were impractical in the current design.

5.6.2 MEMBRANE WEIGHT

When all collected membranes were considered, the mean membrane weight showed a consistent decrease at each successive time point for both A-PRF and L-PRF membranes, indicating degradation continued to 14 days. This pattern was also evident when only membranes exposed to test media were assessed. L-PRF membranes showed a greater reduction in mean membrane weight, as measured in milligrams using an electronic analytical balance. This relative decline in weight was such that the mean weight of A-PRF membranes was greater overall at day 14; this was also the case when the subgroup of membranes that were placed in test medium was analysed separately.

Membrane degradation was also measured by evaluating the relative reduction (percentage decrease) in mean membrane weight over the study period. There was a statistically significant difference between L-PRF membranes and A-PRF membranes in this regard; with L-PRF membranes showing a greater percentage decrease in weight from baseline-14 days overall, and for both test and control media. L-PRF membranes in test

medium demonstrated a mean weight decrease of 65% ± 16.6% at day 14, whereas A-PRF membranes showed a mean decrease of 51.1% ± 10.5%; this difference between protocols was highly statistically significant ($p < 0.001$). The increased degradation of L-PRF is in contrast to Dohan Ehrenfest and colleagues who reported that L-PRF maintained its shape better and degraded more slowly than A-PRF when immersed in a test tube of DMEM (Dohan Ehrenfest et al., 2018). It must be noted that the medium was changed frequently in Dohan Ehrenfest's study, since collection of released growth factors was the primary focus of their research; perhaps this may have impacted on degradation patterns. Ravi & Santakrishnan demonstrated similar degradation rates between L-PRF and A-PRF in phosphate buffered saline (PBS) placed on an orbital shaker; however, degradation rates for both membranes approximated 85 per cent membrane weight after one week, which is notably greater than the rate found in the present investigation (Ravi and Santhanakrishnan, 2020). In contrast, two other studies that compared PRF membrane degradation to that of collagen materials using a PBS medium reported mean weight reductions of 36% and 55% respectively at one week (Sam et al., 2015, Chhaya et al., 2022). This is broadly similar to findings in the present investigation (33.5% for A - PRF controls, 40.5% for L - PRF controls at 7 days). To the authors' knowledge, this is the first study that has directly compared degradation in the context of loss of membrane weight at various time points, while comparing protocols in test and control media.

5.6.3 SIGNS OF PHYSICAL CHANGES IN MEMBRANE APPEARANCE

Signs of physical changes in membrane appearance ("visual changes") were also recorded. These included membrane perforation, major changes in membrane shape and total dissolution of membranes. The primary determination for each membrane was the presence of any visual signs of physical changes at any time. Only 17 of the 64 membranes demonstrated one or more visual signs of change over the study period. None of the visual changes recorded in this investigation were noted before day 10 indicating that all membranes maintained their properties for at least 7 days in plasmin.

Indeed, only four membranes in two subjects underwent complete dissolution. This is in contrast to other investigators (Dohan Ehrenfest et al., 2018, Pluemsakunthai et al., 2013), who have noted major degradation as early as 3 days and complete dissolution of A-PRF membranes with a 7-day period.

An additional eight membranes showed major changes in shape (e.g. significant loss of width or length, gross change in morphology etc.), while a further 5 membranes demonstrated perforations in the membrane at days 10 and/or 14. However, it must be recognised that some of the membranes that showed major shape change or dissolution might have evinced perforation as a precursor stage. These membranes might also represent situations where a practical complication of regenerative surgery might be expected as a result i.e. where shape change might result in a membrane ineffectively occluding a defect. Major translucency of membranes was also seen in an additional 10 membranes at day 14. It is conceivable these membranes may have demonstrated perforation or major shape change at subsequent time points had the study continued. This arguably reinforces the concept that degradation/weight loss may be primarily accounted for through loss of membrane thickness.

5.6.4 LIMITATIONS

This investigation is not without limitations, which primarily relate to treatment of the study media, and challenges relating to measurements. Isobe and colleagues noted that PRF clots consist primarily of fluid; after excess exudate was absorbed by dry gauze, these authors recorded the wet weight of clots using an electric balance. Later, the clots were compressed to membranes and these were dried by heating and weighed again. This showed that the water content of A-PRF (and CGF) clots was approximately 98 per cent (Isobe et al., 2017). It is not known if limited fluid uptake remains a possibility for already fabricated PRF membranes; this could obviously impact membrane weight measurements in any study where membranes are placed in media. Furthermore, given the mean membrane weight at baseline was just 101.5 mg for L-PRF and 85 mg for A-PRF, and since these values showed a gradual decline to a mean of approximately 54 mg by day 7 and close to 40 mg by day 14, it is apparent that measurements could be sensitive to the presence of fluid droplets on the surface of the membranes following their removal from their respective media at each measurement point. During the pilot study, it was noted that some membrane weight measurements were higher at day 2 than at baseline (day 0). In the current investigation, all membranes were gently compressed with gauze prior to weighing to minimise the potential for surface fluid from media to distort measurements. Based on the pilot study observations, the final protocol was modified from placing the membrane on gauze to its placement on gauze followed by gentle compression with a second piece of gauze on the superior surface in order to remove any excess fluid that might introduce inaccuracy into weight measurements. Given the delicate nature of membranes, care was taken not to over-compress the membranes such that they might become stuck to the gauze or suffer any traumatic impact.

It must be noted that an IntraSpin® centrifuge was used to produce both L-PRF and A-PRF in the current investigation. A-PRF was produced following the classic protocol of 1500 rpm for 14 minutes. Recent publications by the teams of Miron and Quirynen have highlighted that since various protocols were

developed using different proprietary centrifuges, which differ with regard to features such as dimension of the rotor and rotor angulation, the end-product created following the same time and rpm protocol will differ between centrifuges (Miron et al., 2023, Quirynen et al., 2023). It must be noted that the A-PRF production cycle used in the present study translated to an RCF-clot of 126 g. Quirynen and colleagues have calculated that as developed, A-PRF was created at an RCF-clot of 194 g, which would correspond to a preparation cycle of 1865 rpm for 14 minutes in the IntraSpin® centrifuge (Quirynen et al., 2023). However, it must be noted that the degradation studies referred to in this chapter were not based on RCF-clot calculations and that the study of Dohan Ehrenfest and colleagues in which A-PRF membranes showed degradation at 3 days in DMEM (Dohan Ehrenfest et al., 2018) was produced using the same centrifuge and preparation cycle as used in the study herein.

Mean reduction in membrane weight from baseline-7 days was compared with mean reduction in weight from 7 days to 14 days. This analysis was performed for membranes in test media only (n =24 for each protocol), comparing within each protocol. Results show that relative reduction in mean weight was significantly greater from baseline-7 days than from 7- 14 days, for both A-PRF and L-PRF. Relative decrease in membrane weight slowed in the second 7-day period. While membranes in both protocols still decreased in weight over this period, this decline in rate of degradation may advocate for changing plasmin medium at 7 days in future studies. Similar conclusions may be made when considering historic studies that have shown faster rates of degradation in Dulbecco's modified Eagle's medium (Dohan Ehrenfest et al., 2018, Pluemsakunthai et al., 2013), and may consequently be directly compared to membranes in control medium in the present study. Such studies were designed to measure expressed growth factors from PRF membranes; perhaps the expression and removal of growth factors from test medium facilitates faster degradation. In theory, then the non-replacement of media in the present investigation may have restricted growth factor expression from PRF, maintaining cells within the fibrin meshwork and consequently, maintaining some weight.

The current investigation used the same plasmin concentration (2 μg/mL) as Kawase and colleagues, albeit that study used plasmin in combination with Hank's balanced salt solution (Kawase et al., 2015). DMEM was selected as medium for the current study based on its use as a supportive medium for cell culture and its use in the majority of studies evaluating PRF growth factor release. A pilot study was used to evaluate the use various plasmin concentrations in DMEM solution. The results of this pilot study indicated that an increased plasmin concentration was not associated with increased percentage of membrane weight reduction at 14 days. Furthermore, since mean degradation during week 2 of the pilot study was equivalent to that in week 1, a decision was made not to change test media. With a greater number of membranes in the final investigation, it appears that future studies should modify this aspect as previously discussed.

5.6.5 FURTHER CONSIDERATIONS

Degradation of PRF in vitro has generally been conducted in standard media, albeit Isobe and team utilised trypsin to accelerate enzymatic degradation and observed complete degradation within 3 hours (Isobe et al., 2017). Plasmin was selected for the current study as an enzyme representative of a wound site to which PRF membranes would be exposed in vivo. Plasmin is provided for research purposes as a lyophilised powder that can be reconstituted from frozen for use; however, manufacturers highlight that there is not clear documentation to its duration of activity in vitro (Personal communication, Merck Life Science Technical Service Scientist, 3 December 2021). However, that membranes in plasmin-containing test media continued to demonstrate greater mean degradation than those in control solutions of DMEM alone may suggest that plasmin retained some activity in solution over the study period.

As fibrin is degraded, it appears that fibrin degradation products are produced; using a chick embryo chorioallantoic membrane assay, Thompson and colleagues demonstrated these FDPs are themselves capable of promoting angiogenic activity (Thompson et al., 1992). However, their impact in a degradation study in vitro has not been addressed in the literature. The impact of good laboratory practices is an important step in cell culture care was, and taken to maintain sterility and follow best practice at all times (Baust et al., 2017). While plates and membranes were not evaluated with light microscopy, no evidence of change to study media such as turbidity of media or suggestion of bacterial or fungal colonisation, as might be evidenced via a change in colour of the phenol red within the DMEM solution.

A recent review by Mizraji and colleagues overviewed available biomaterials for guided bone regeneration. The authors outlined that a membrane is more than just an occlusive barrier and that a membrane also plays an active role in modulating bone regeneration during GBR (Mizraji et al., 2023). Methods to enhance the function of membranes in this realm can include the

incorporation of antimicrobial agents or of growth factors, including bone morphogenetic protein (BMP), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF). It may be argued that PRF contains these components already. However, the mechanical properties of PRF membranes appear to be relatively poor and in a recent narrative review, Agrawal concluded that they lack the mechanical properties to constitute an effective barrier membrane used in isolation, and may best be used adjunctive to other materials (Agrawal, 2023). While some authors have advocated techniques such as heat compression to aid mechanical strength and to provide greater resistance to degradation (Kawase et al., 2015, Gheno et al., 2021), this may occur at the cost of growth factor release from the membranes, increased preparation time to develop usable membranes and increased equipment costs. As such, it remains of interest to compare the degradation properties of easily obtainable forms of PRF.

One of the challenges of membrane use is the ability to control membrane degradation. Resorbable membranes have relatively unpredictable degradation characteristics and given the cost advantages and simplicity of PRF preparation, it is merited to investigate its membrane degradation properties. The current study confirmed that preparation protocol can affect membrane degradation, as measured by initial size and mean membrane weight. While both L-PRF and A-PRF membranes demonstrated progressive degradation over time, and lost a mean 51 per cent (A-PRF) to 65 per cent (L-PRF) of their mean weight over a 14-day period in plasmin solution, the majority of membranes retained their length and width over the study period. Only 17 of 64 membranes demonstrated visual signs of perforation, major shape change or complete dissolution. In this regard, A-PRF membranes appear clearly superior in maintaining their physical dimensions and undergo statistically reduced degradation as determine by relative loss of weight. As such, they may represent a superior option to L-PRF when used for regenerative indications. However, further studies are indicated to refine the use of the plasmin degradation model employed herein, and to determine how resistance to degradation can be further enhanced in these materials.

5.7 Conclusions

- L-PRF membranes were significantly larger than A-PRF membranes at baseline, as measured by mean maximum length, mean maximum width and mean membrane weight.

- Membranes exhibited only minor changes in length or width over the study period regardless of protocol, but A-PRF membranes were statistically more likely not to demonstrate any change, or change above a set threshold, over the study period

- Mean membrane weight decreased at each successive time point for both A-PRF and L-PRF membranes up to 14 days. L-PRF membranes showed a greater reduction in mean membrane weight, such that the mean weight of A-PRF membranes was greater at day 14

- The membrane degradation process resulted in visual signs of degradation at day 14 in about one quarter of the tested membranes. Only four membranes in two subjects underwent complete dissolution. Other visual signs included major changes in shape and perforations in the membrane at days 10 and/or day 14.

- Membranes showed limited change in length or width over the study period. It may be assumed that degradation occurs primarily through a loss of membrane thickness and/or release of cellular contents over time. Nevertheless, the majority of both L-PRF and A-PRF membranes appeared to retain their basic membrane form at day 14, indicating they are capable of reliably maintaining defect coverage when used clinically.

CHAPTER 6: A CLINICAL AND RADIOGRAPHIC EVALUATION OF THE USE OF L-PRF AND L-PRF BLOCK IN THE REGENERATIVE TREATMENT OF PERI-IMPLANT DEFECTS

6.1 INTRODUCTION

A dental implant is an alloplastic material or device that is surgically placed into the oral tissues within the bone for functional, therapeutic, or aesthetic purposes (**AAP**, **2024**).
A dental implant achieves osseointegration with its surrounding bone - the direct structural and functional connection between ordered, living bone and the surface of an immobile, load-bearing implant (Brånemark, 1983).

6.1.1 IMPLANT SURVIVAL

Implant survival may be considered simply as any implant that remains in situ at the time of assessment, regardless of signs, symptoms or a history of past complications or treatment need. Dental implants have high survival rates, with early studies demonstrating a survival of 90–94 per cent at 10 years (Lekholm et al., 1999, Lang et al., 2004), with a trend for prospective studies to propose an even higher survival rate of up to 98–99 per cent (Blanes et al., 2007, van Velzen et al., 2015). Systematic reviews in recent years have concurred, indicating a survival rate of 94–99 per cent at 10 years (Busenlechner et al., 2014, Moraschini et al., 2015), while the survival rates for implant restorations also appears to exceed 90 per cent at 10 years (Jung et al., 2012, Pjetursson et al., 2012, Romeo and Storelli, 2012). It has been suggested that implant survival may be lowered among patients with periodontitis (Ong et al., 2008), as well as among smokers (Strietzel et al., 2007, Moraschini and Barboza, 2016) and bruxism patients (Chrcanovic et al., 2016).

6.1.2 IMPLANT SUCCESS

Dental implant success criteria were presented in the 1980s by Albrektsson and colleagues (Albrektsson et al., 1986). Despite subsequent attempts to

modernise these, most notably at a 2007 consensus conference of the International Congress of Oral Implantologists (ICOI) (Misch et al., 2008), the Albrektsson criteria remain the most widely used. These define that for success an implant should be clinically immobile, with no radiographic evidence of peri-implant radiolucency and exhibiting vertical bone loss of less than 0.2 mm annually, following the first year of service. Additionally, there should be an absence of persistent and/or irreversible signs and symptoms, such as pain, infection, neuropathy and paraesthesia. Based on these features, the authors stated that a success rate of 85 per cent at five years, and 80 per cent at 10 years should be the minimum criteria for success (Albrektsson et al., 1986). These success criteria do not specifically consider biological and technical complications, and consequently, such aspects were reported infrequently in the early years of implant research (Berglundh et al., 2002). In a frequently cited study, Goodacre described the occurrence of a relatively high frequency and wide range of technical complications at implant restorations, especially at implant overdentures (Goodacre et al., 2003). However, in the periodontal field, biological complications have been the focus of the implant literature in recent years, as concerns regarding the potentially high prevalence of such complications came to light by virtue of the increasing number of implants in longer-term service. Indeed, a bibliometric analysis of the literature on peri-implant diseases up to 2019 concluded that publications had increased “exponentially” in the previous 5 years (Tarazona-Álvarez et al., 2021).

6.1.2.1 Case Definitions for peri-implant health and diseases

Despite the large number of peri-implant disease publications, quantifying the prevalence of peri-implantitis has been a major challenge, largely due to heterogeneity in the definitions of peri-implant health and disease. Despite peri-implant diseases first being defined in 1994 (Albrektsson, 1994), the 2017 Classification of periodontal and peri-implant diseases and conditions was the first classification system to provide clear case definitions for peri-implant health and peri-implant diseases for researchers and dental health

professionals in clinical practice (Berglundh et al., 2018) These case definitions are summarised in Table 32.

Table 32: Case definitions for peri-implant health and diseases - adapted from 2017 Classification (Berglundh et al., 2018)

	Characteristic features
PERI-IMPLANT HEALTH	▪ No clinical signs of inflammation ▪ No bleeding/suppuration on probing ▪ No increase in probing depth Vs previous examination ▪ No bone loss beyond crestal bone level changes resulting from initial bone remodelling
PERI-IMPLANT MUCOSITIS	▪ Bleeding and/or suppuration on probing ▪ With/without increase in probing depth Vs previous examination ▪ No bone loss beyond crestal bone level changes resulting from initial bone remodelling
PERI-IMPLANTITIS	▪ Bleeding and/or suppuration on probing ▪ Increased probing depth Vs previous examination ▪ Bone loss extends beyond crestal bone level changes resulting from initial bone remodelling Where previous clinical/radiographic data is not available ▪ Bleeding and/or suppuration on probing ▪ Probing depths ≥6 mm ▪ Bone levels ≥3 mm apical to the most coronal portion of the intraosseous part of the implant.

6.1.2.2 Prevalence of peri-implant diseases

Cosgarea et al., used the case definitions for peri-implant diseases outlined at the 2017 World Workshop (Berglundh et al., 2018) as the basis for conducting a review of peri-implant disease prevalence in studies with a minimum follow-up of one year or more, to account for the recency of the publication of new diagnostic terminology (Cosgarea et al., 2019). No published study had yet fully applied these criteria and the authors again noted that a wide range of other case definitions and prevalence was in use in the dental literature. They suggested that multiple factors can influence reported prevalence data in individual studies, notably the definitions used for disease, the time point of assessment (time in function), reporting at implant versus patient subject level and the use of convenience versus randomly selected populations in research (Cosgarea et al., 2019). Indeed, the authors of one systematic review highlighted the range of definitions used for peri-implantitis across the literature, by virtue of which prevalence figures of peri-implantitis ranged from 1 to 47% (Derks and Tomasi, 2015).

We must recognise, therefore, that existing prevalence statistics are primarily based on inconsistent criteria and/or older case definitions, and our appreciation of the incidence and prevalence of disease, as well as contributory factors, is likely to evolve over time. Nevertheless, a number of recent systematic reviews have provided broadly consistent estimates of the prevalence of peri-implantitis, approximating 18–20% at patient level and 9–13% at implant level (Atieh et al., 2013, Derks and Tomasi, 2015, Lee et al., 2017, Rakic et al., 2018, Diaz et al., 2022). It has also been suggested that implant location may influence peri-implantitis prevalence, with an increased number of cases being associated with implants in anterior regions (Song et al., 2020). Table 33 presents a summary of selected systematic reviews that have detailed the prevalence of peri-implantitis.

The impact of peri-implant disease processes on patient quality of life has been questioned; a recent prospective trial noted that mean scores utilising

the fourteen question-form of the Oral Health Impact Profile questionnaire (OHIP-14) were low both before and after peri-implant treatment, indicating the impact of both peri-implant disease and its treatment on patients is low (Rustand et al., 2022). Furthermore, the pain levels measured by VAS appear to be low-to-moderate for most patients following non-surgical or surgical treatment of peri-implantitis (Norum et al., 2019). Although the current estimates of peri-implantitis represent a notable decrease compared to early studies (Zitzmann and Berglundh, 2008), the figures still imply that approximately 1 in 5 patients, and up to 1 in 8 implants may develop peri-implantitis. This will inherently create a heavy clinical workload for practitioners and place a financial burden on patients to treat and maintain these cases; therefore, research on the prevention, treatment and maintenance of peri-implant diseases is merited.

Table 33: Summary of systematic reviews on prevalence of peri-implantitis. PI = Peri-implantitis

SYSTEMATIC REVIEW	LANGUAGE OR DATE RESTRICTIONS	INCLUDED STUDY DESIGNS	MINIMUM FOLLOW-UP	PERI-IMPLANTITIS CRITERIA	NO. OF STUDIES/NO. OF PARTICIPANTS	PREVALENCE OF PERI-IMPLANTITIS	
						PATIENT	IMPLANT
Atieh et al (2013)	English language	Cross sectional, cohort	≥ 5 years	Inflamed mucosa with BOP; PD≥ 5 mm Bone loss ≥ 2 mm ± ≥3 threads	9/ ~ 1500	18.8%	9.6%
Derks & Tomasi (2015)	Not specified (none apparent)	Cross sectional ≥ 100 participants	Not specified	Various	11/ > 2000	Patient level data only - Range 1-47% Weighted mean prevalence 22% (CI 14%–30%)	
Lee et al (2017)	English language	Cross sectional, cohort ≥ 30 participants	≥3 years	Various	47/ > 6300	19.8%	9.25%
Rakic et al (2018)	No restrictions	Cross-sectional, cohort, case-control, RCT ≥ 20 participants	Not specified	Sanz & Chapple (2012): BOP, PD≥ 5 mm Bone loss ≥ 2 mm	29/ > 5800	18.5%	12.8%
Cosgarea et al (2019)	English language	Not specified (but selected studies included cross-sectional, cohort, RCT)	≥ 1 year	Various - clear definition for PI with a clear cut-off for bone level changes	25/> 6800	No overall summary statistic presented Study designed to assess prevalence using 2017 World Workshop criteria (Berglundh et al, 2018) - No published study had yet applied these criteria	
Diaz et al (2022)	English language 2005-2021	Cross-sectional, longitudinal, cohort, case-control, RCT ≥ 10 participants	≥ 5 years	Various (once including any measure of BOP, PD, bone loss)	57 /> 10,600	19.5%	12.5%
Song et al (2020)	No restrictions	Cross-sectional, cohort, case-control Implant location specified ≥ 30 participants	≥ 1 year	Various	10/ > 2000	Aimed to compare prevalence in anterior Vs posterior regions; Increased prevalence anterior region: (RR 1.34; 95% CI 1.07, 1.69)	

6.1.3 PERI-IMPLANTITIS DEFECTS

An attempt to characterise peri-implantitis defects was first made following a comparison of naturally occurring defects in 24 patients undergoing surgery to address peri-implant infections with ligature-induced peri-implantitis lesions in five beagle dogs (Schwarz et al., 2007). Defects were first characterised into two basic types: Class I defects (where bone loss had an intrabony aspect) and Class II defects (where bone loss was suprabony in nature, characterised by consistent, horizontal bone loss, with implant components consequently exposed above the alveolar level). Class I defects were further sub-classified into five subtypes based on the location of bone loss; all can occur when the implant body is located either within or beyond the bony envelope. These authors noted that defects usually featured a combined defect (Class I and Class II components present), and the most common intrabony component was a circumferential pattern of bone loss (Class Ie); this accounted for more than half of the lesions seen (Schwarz et al., 2007). In a later study, the same team indicated that defect morphology might affect clinical outcomes, with circumferential defects appearing to offer more favourable treatment response than where a dehiscence component is present at the defect site (Schwarz et al., 2010).

While subsequent studies also noted that Class Ie defects were the most frequent defect type seen, researchers have found that pre-operative radiographic assessment struggles to accurately describe defects, tending to underestimate the extent of bone loss compared to peri-implant bone levels measured intra-surgically, often by 1 mm or more (García-García et al., 2016, Serino et al., 2017). Zhang and colleagues proposed a radiographic classification of peri-implant defects but found challenges in implementing this even at intra-observer level with a single operator (Zhang et al., 2014). For radiographic accuracy and reliability, intra-oral periapicals represent an optimal approach for radiographic assessment of the marginal bone level at implants (De Smet et al., 2002). However, Zhang and colleagues highlight that obtaining periapical images is often difficult and uncomfortable for this patient cohort, and these images may often fail to capture the apical aspect

of the implants (Zhang et al., 2014). Notably, De Smet and colleagues also found that panoramic images had an acceptable level of accuracy for radiographic assessment of marginal bone levels; this may represent a practical and pragmatic alternative in cases with significant resorption or in cases where implant restorations remain in-situ (De Smet et al., 2002). It is evident that assessment of the peri-implant region presents challenges, especially since the presence of the prosthetic construction also hampers the accuracy of clinical measurement. Serino and colleagues noted that probing pocket depth measurements taken at the same site before vs after removal of reconstructions differed by a margin of ≥2 mm at approximately 1 in every 4 sites (Serino et al., 2013).

6.1.4 PATHOGENESIS

Peri-implantitis is often considered to be analogous to periodontal disease but there are some unique considerations of this disease process that can affect diagnosis and management.

6.1.4.1 Microbiology

Findings in the early literature reported that while paper-point sampling indicated that the microbiota at healthy implant sites was similar to that at healthy teeth, the same could not be said for diseased implant sites compared to periodontitis sites. Putative periodontopathogens (including *A.actinomycetemcomitans, P.gingivalis* and *P. intermedia*) were found in the majority of cases and opportunistic microorganisms including staphylococcal species, *Candida* and enterics were also found (Leonhardt et al., 1999). When early colonisation was evaluated (from immediately after insertion of dental implants and up to 12 weeks, findings revealed that contamination of the implant surface was already evident at 30 minutes post-insertion and the colonising microbes included opportunistic species like *S. aureus* (Fürst et al., 2007). However, all implants remained clinically healthy at 1 year (Salvi et al.,

2008), with bacterial counts remaining higher at adjacent tooth surfaces than at the implant surfaces themselves. A contemporaneous systematic review conducted around this time noted that the available literature was generally descriptive in nature and possessed limited ability to make firm conclusions. Nevertheless, it appeared that both increased surface roughness and increased surface free energy favoured biofilm formation. While implant-abutment design and surface chemistry were also noted to have a potential effect on colonisation (Subramani et al., 2009).

Renvert characterised 40 bacterial species using DNA-DNA hybridisation from microbial sampling of more than 200 patients (>900 implants) which had been in function for at least 10 years. Few bacteria differed between teeth and implants, based either on whether patients were dentate or not, or on the status of the peri-implant tissues (Renvert et al., 2007). While a later study noted the presence of common periodontopathogens (including *A. actinomycetemcomitans, P. gingivalis* and *T. forsythia*) at implants in 30 partially edentulous patients with implants, these species were present at both healthy and diseased sites, and the authors concluded that the detection of pathogenic species was not an automatic indicator of disease (Casado et al., 2011).

More recently, a systematic review noted that a complex and variable microbiologic profile exists in peri-implantitis, with Gram-negative anaerobic periodontopathogens and opportunistic microbes present in almost equal amounts. This differs from the relationship seen in periodontitis. The authors noted that since the qualitative composition of the microbiota is similar in healthy tissues and sites with peri-implantitis, quantitative characteristics of the microflora cohabitants must represent the key determinant of disease (Rakic et al., 2016).

The micro gap at the implant abutment-connection (IAC) constitutes a reservoir for plaque biofilm, which may subsequently contaminate the surrounding tissues through leakage and negatively affect peri-implant tissues

health. A recent systematic review found that this relationship appears to exist regardless of connection type, as all connections evaluated presented some micro-gap and associated bacterial micro-leakage, although some connection types (e.g. conical) may perform better (Candotto et al., 2019).

6.1.4.2 Histopathology

The lesions of periodontitis and peri-implantitis also have some histopathologic differences that may explain some of the differences in onset and progression of these disease processes. In a systematic review conducted for the Seventh European workshop in periodontology, Berglundh and colleagues compared the histopathological characteristics of peri-implant and periodontal lesions, utilising studies on human biopsy material, and experimental animals. In human biopsies, the apical extent of the inflammatory infiltrate was greater than in periodontitis, and frequently located apical to the pocket epithelium. While plasma cells and lymphocytes were the dominant cells in both lesions, peri-implant lesions showed greater proportions of neutrophils and macrophages. In experimental animal studies, following ligature removal, periodontal lesions showed self-imitation of the inflammatory infiltrate, whereas the infiltrate in peri-implantitis continued to enlarge and often extended to the crestal level (Berglundh et al., 2011). Indeed, in a later study Carcuac collected soft tissue biopsies from 40 patients each with severe periodontitis and severe peri-implantitis. Diseased sites selected for a biopsy had interproximal probing depth ≥7 mm with bleeding on probing (BOP). Histology demonstrated that peri-implantitis lesions were more than twice as large as periodontal lesions (3.5 mm^2 vs 1.5 mm^2). In addition, they extended apical to the pocket epithelium to a point not surrounded by non-infiltrated connective tissue. The surrounding connective tissue showed an increase in vascular engorgement and lesions had an increased proportion of inflammatory cells relative to those seen in periodontal lesions. In conclusion, the lesions of periodontitis and peri-implantitis do have histopathologic differences that may explain some of the dissimilarities in onset and progression between the two diseases (Carcuac and Berglundh, 2014).

The impact of implant surfaces has been questioned in recent years. A systematic review concluded that the available clinical studies do not support the idea that the incidence of peri-implantitis differs among the various types of implant surface. There was inadequate information available in the literature to make conclusions about the impact of implant material on the incidence/progression of peri-implantitis (Stavropoulos et al., 2021). These results were considered and accepted by the consensus of the European Academy of Osseointegration (EAO). This consensus also noted some evidence that restricted access for oral hygiene and an over-contoured, emergence profile at the implant – abutment junction may be associated with an increased risk for peri-implantitis (Schwarz et al., 2021).

6.1.5 RISK FACTORS

A discussion of the risk factors for peri-implantitis is challenged not only by heterogeneity in the definition of peri-implant diseases, but also frequently by definitions of the items under investigation, for example, what constitutes a periodontitis subject or a smoker patient. It is also clear that evidence suggesting that a factor is contributory to implant failure does not automatically imply it has a role in peri-implant diseases. Nevertheless, a series of studies have shown significantly higher odds for patients with periodontitis to have peri-implantitis, based on assessments using cross-sectional (Roos-Jansåker et al., 2006), retrospective (Derks et al., 2016) and prospective studies (Roccuzzo et al., 2012). A commissioned review for the 2017 World Workshop on periodontology concluded there is strong evidence from longitudinal and cross-sectional studies that a history of periodontitis constitutes a risk factor/indicator for peri-implantitis (Schwarz et al., 2018).

Conversely, while the association of smoking with implant failure appears clear (Strietzel et al., 2007, Moraschini and Barboza, 2016); evidence linking it to peri-implantitis is less clear. A review for the 2017 World Workshop noted that while some studies have suggested a strong association between smoking and peri-implantitis, the majority of studies have not done so. This may relate to how studies have categorised smoking and non-smoking, as well as the reliance on self-reporting of smoking status by patients. In addition, it is highly possible that the influence of smoking is confounded by other factors such as a history of periodontitis. The authors concluded that there is currently an absence of conclusive evidence that smoking constitutes a risk factor/indicator for peri-implantitis (Schwarz et al., 2018)

6.1.6 CLINICAL TREATMENT APPROACHES TO PERI-IMPLANT DISEASE

A variety of clinical treatment approaches have been used in the management of peri-implant disease (Chan et al., 2023, Monje et al., 2024). Given the previous variations in disease criteria used to define peri-implantitis (PI) in the existing literature, it is perhaps unsurprising that there is no consensus among dental professionals regarding a preferred treatment protocol to treat peri-implantitis and significant empiricism exists in the approaches selected (Renvert and Polyzois, 2018). It appears that while non-surgical treatment is effective in treating peri-implant mucositis, its effect on the management of peri-implantitis is more questionable (Suárez-López Del Amo et al., 2016). Many surgical approaches to PI have been advocated but the rate of treatment success may also be relatively poor (de Waal et al., 2016). Nevertheless, surgical treatment can offer long-term effectiveness (Berglundh et al., 2018).

6.1.6.1 EFP Clinical Practice Guideline on the prevention and treatment of peri-implant diseases

The 18th European Workshop on Periodontology convened to develop an S3-level Clinical Practice Guideline (CPG) on the prevention and treatment of peri-implant diseases (Herrera et al., 2023). Thirteen systematic reviews were commissioned to support the guideline development using the format of a structured consensus development conference. The CPG presents a standardised conceptual construct for management of peri-implant diseases that is analogous to and founded on the principles outlined for the management of periodontitis in a previous clinical practice guideline (Sanz et al., 2020). Consequently, treatment follows a sequential, step-wise approach that allows for incorporation of individual contributory factors and treatment response. This treatment approach aims to arrest inflammation within the peri-implant tissues and to control contributory local and systemic risk factors, with disruption of local biofilm accumulation a key component (Herrera et al., 2023) .

The CPG recognises peri-implant mucositis as a reversible inflammatory condition that occurs in response to biofilm accumulation and which may be managed with

professional mechanical plaque removal (PMPR) in combination with personal biofilm removal. The outcomes of this treatment may be evaluated after 2-3 months. The desired 'endpoints' of care at implant level are the absence of suppuration and the absence of profuse (≤ 1 site) or any (≤ 2 sites) bleeding on probing at the implant (Herrera et al., 2023).

In turn, peri-implantitis is recognised as an irreversible condition with variable treatment options due to the range of site, local and patient factors that may contribute to disease (Herrera et al., 2023). The CPG highlights that following a diagnosis of peri-implantitis the clinician must first decide if the affected implant is treatable. If so, an initial non-surgical step that includes sub-marginal instrumentation is conducted (Herrera et al., 2023). This step also incorporates oral hygiene instruction, risk factor control and cleaning, or removal and adjustment of any implant prosthesis, where necessary and/or practical (Cosgarea et al., 2023, de Waal et al., 2023, Liñares et al., 2023). Supra-marginal and sub-marginal instrumentation are delivered, with simultaneous provision of periodontal therapy, if indicated (Cosgarea et al., 2023, de Waal et al., 2023, Liñares et al., 2023). Based on the low volume and quality of research, the CPG recommends that sub-marginal instrumentation is achieved through the use of mechanical instrumentation with curettes, sonic or ultrasonic instruments (Herrera et al., 2023). Guidance from the commissioned systematic reviews indicates that evidence does not presently support the use of lasers or air polishing for sub-marginal instrumentation (Cosgarea et al., 2023), nor the use of photodynamic therapy or antiseptics as sole or adjunctive agents (de Waal et al., 2023). Based on a systematic review by Liñares and colleagues (Liñares et al., 2023) and considerations around antimicrobial stewardship, consensus does not support the use of probiotics, local or systemic antimicrobials in non-surgical peri-implantitis treatment (Herrera et al., 2023).

Following sub-marginal instrumentation, re-evaluation at 6-12 weeks is recommended; the desired clinical endpoints at implant level are residual pocket depths ≤5mm, the absence of suppuration and no more than one site that exhibits bleeding on probing (Herrera et al., 2023). Where these endpoints are reached, the patient may be enrolled in a supportive peri-implant care (SPIC) programme. However, failure to achieve these endpoints indicates additional treatment, and

surgery may be recommended in these circumstances (Herrera et al., 2023). The surgical step provides access to the implant surface to facilitate decontamination and will therefore always include additional sub-marginal instrumentation following flap elevation. Before proceeding to surgical management, the patient must be able to perform effective self-performed oral hygiene. Where suprastructures or prostheses do not facilitate access for self-performed oral hygiene, they should be removed and adjusted prior to surgery. Similarly, restoration removal at the time of surgery is conducted where possible to facilitate surgical access and effective treatment (Herrera et al., 2023).

When selecting an approach for the surgical step of treatment, access flaps with or without reconstructive procedures may be considered. Access flaps provide clinically superior outcomes relative to non-surgical peri-implant therapy alone (Karlsson et al., 2023). The use of an access flap and resective surgery where indicated was associated with reductions in mean peri-implant probing depth and bleeding on probing. Reconstructive surgery appears to result in similar levels of probing depth reduction and a small but statistically significant additional benefit in bone gain, amounting to approximately 0.75 mm (95% CI 0.11-1.39 mm), compared to access flap and resective surgery (Donos et al., 2023). However, these authors noted some inconsistency in the findings of the individual studies that compared surgical approaches, and no single technique demonstrated clear superiority. Furthermore, Donos and colleagues highlighted that the rate of disease recurrence and progression across included studies was high (Donos et al., 2023) Consequently, the EFP CPG recommends reconstructive surgery primarily where bony defects ≥3 mm deep are present at the peri-implant site. When providing reconstructive surgery, bone grafts - with or without membranes - may be considered. The literature does not confirm whether submerged or transmucosal healing is necessary (Herrera et al., 2023). Based on a systematic review by Ramanauskaite and colleagues (Ramanauskaite et al., 2023) , the CPG notes that use of titanium brushes may be considered as an alternative or adjunct to surface decontamination but there is insufficient evidence to make clear recommendations regarding the use of implantoplasty. There is also limited evidence on which to base recommendations for the use of aids such as photodynamic therapy, chlorhexidine or local or systemic antimicrobial agents adjunctive to surgery (Wilensky et al., 2023, Teughels et al., 2023) and the consensus of the CPG does not currently support their use.

6.1.6.2 Surgical Regenerative Treatment of peri-implantitis and the possibility of re-osseointegration

A systematic review on the surgical regenerative treatment of PI in human clinical studies concluded that this therapy, appears to offer a predictable approach to management of PI but there was insufficient evidence to support the superiority of regenerative versus non-regenerative surgical treatment (Daugela et al., 2016). This conclusion is supported by the recent Clinical Practice Guideline issued by the European Federation of Periodontology (Herrera et al., 2023). While animal research has demonstrated that osseointegration is possible to achieve on a previously contaminated implant surface (Kolonidis et al., 2003, Alhag et al., 2008, Mohamed et al., 2010), human studies have offered limited evidence to support the possibility this can be routinely achieved in clinical practice (Renvert and Polyzois, 2018).

The question as to whether treatment of PI can result in true re-osseointegration has been controversial in the literature and is primarily restricted to animal studies in order to be able to carry out histological analysis (Persson et al., 1999, Persson et al., 2001, Sennerby et al., 2005, Schwarz et al., 2006, Almohandes et al., 2019). In one human case series, peri-implantitis lesions were treated surgically using porous titanium granules following decontamination of the implant surface. Following 12 months of healing, one implant and surrounding structures were excised from only one site and histological and micro CT analysis were carried out which indicated that that re-osseointegration is possible in the human model (Wohlfahrt et al., 2011).

A systematic review by Renvert et al., (2009) investigated the evidence for re-osseointegration following PI treatment at contaminated implant surfaces. It revealed that access surgery with closed healing, positively affected the rate of re-osseointegration compared to non-surgical therapy. Additionally, surgical therapy and the use of adjunctive regenerative materials resulted in variable levels of re-osseointegration. The authors concluded that while re-osseointegration is possible, the extent to which it can be achieved is influenced by different implant surface characteristics and that surface decontamination alone will not achieve a substantial level of re-osseointegration. Furthermore, the literature failed to identify a predictable

method of achieving re-osseointegration in a previously contaminated site, with the various techniques yielding a wide range of re-osseointegration from 1-84% (Schwarz et al., 2006, Renvert et al., 2009). The application of such studies to the human model is difficult due to obvious ethical restrictions; hence, it has not been comprehensively studied.

The application of growth factors like enamel matrix derivatives, concentrated growth factors and platelet-rich fibrin membranes (PRF) to the regeneration of periodontal and peri-implant defects is a new regenerative approach and the goal is to enhance wound healing events. PRF products, in the form of Leukocyte and platelet rich fibrin (L-PRF), were used extensively in the study herein. Hamzacebi et al. conducted the first human study to compare the effectiveness of L-PRF application and conventional flap surgery on the treatment of peri-implant bone defects. Nineteen patients with 38 implants were diagnosed with peri-implantitis, with diagnostic criteria including probing depths of ≥5 mm and radiographic bone loss of ≥ 2mm with BOP. The subjects were randomly assigned to either receive open flap debridement with adjunctive L-PRF membranes or open flap debridement alone. At 6-month follow up, regardless of the defect configuration, the L-PRF group demonstrated significantly higher clinical attachment level gain and mean probing depth reductions than the control group. Furthermore, the amount of keratinised tissue for the L-PRF group also increased significantly (Hamzacebi et al., 2015).

With limited human histological evidence, conclusions regarding the potential of surgical procedures to obtain re-osseointegration on the implant surface are difficult. At this moment in time, the clinician must rely on surrogate indicators of re-osseointegration, such as radiological evidence of bone-implant contacts and increasing implant stability over time following treatment, using technologies such as Magnetic resonance frequency analysis (MRFA). Current available evidence regarding the use of MRFA in assessing peri-implant disease treatment outcomes is relatively scarce. The relationship between ISQ values and changes in peri-implant parameters following non-surgical and surgical intervention, such as plaque levels, BOP, probing depths or radiographic bone level changes and defect fill, remains largely unexplored.

6.1.7 STUDY AIMS

In the context of the discussion above, the aims of the present study were:

- To investigate the impact of peri-implantitis treatment upon clinical parameters and implant stability quotient values in the medium term
- To ascertain if MRFA can be used as a tool to demonstrate post-operative healing following treatment of peri-implantitis
- To assess whether surgical peri-implantitis treatment approaches involving L-PRF materials provide favourable treatment responses at 2-3 years

6.2 MATERIAL AND METHODS

Study participants were recruited from patients referred for treatment at a dedicated peri-implantitis clinic at Dublin Dental University Hospital, Dublin, Ireland.

Following initial assessment, consecutive patients aged >18 years were enrolled for treatment of peri-implantitis. This study protocol received approval from the relevant local research ethics committee (St James' Hospital/ AMNCH Research Ethics committee, Dublin, Ireland Ref: 2017-05 list 19 (2) and 2018-04 Chairman's action; Appendix I). Treatment of these cases involved different clinical approaches, based on the variety of clinical presentations of disease among this cohort. The present reporting considered the checklist items as proposed in the STROBE statement (von Elm et al., 2014).

6.2.1 CHARACTERISTICS OF TREATED SITES

Inclusion Criteria

All participants demonstrated the following features:

(a) Presence of bleeding and/or suppuration on gentle probing, increased probing depth compared to previous examination and presence of bone loss beyond what would be viewed as initial bone remodelling (Berglundh et al., 2018, Schwarz et al., 2018).

(b) In the absence of previous examination data: at least one functioning, non-mobile, osseointegrated implant with probing pocket depths ≥6 mm and the presence of bleeding and/or suppuration on gentle probing

or evidence of progressive peri-implant bone loss/ bone loss of ≥3 mm detected radiographically (standard intraoral or panoramic) (Berglundh et al., 2018, Schwarz et al., 2018).

(c) Compliance with efforts at oral hygiene and willing to adhere to a peri-implant maintenance schedule over a 12-month period

Exclusion criteria

Patients were excluded from participation in the investigation in the following circumstances:

(a) An uncontrolled medical condition

(b) Pregnant or lactating mothers

(c) Implants that had received previous non-surgical/surgical treatment for peri-implantitis

(d) Implants restored with prosthetic supra-structures that could not be removed easily in order to facilitate multiple examinations/maintenance and treatment

(e) Current smokers.

6.2.2 INITIAL NON-SURGICAL TREATMENT

Prior to surgical treatment, all participants received non-surgical therapy to reduce signs of clinical inflammation. This consisted of mechanical debridement of the affected implants, as well as instruction and motivation in self-performed oral hygiene methods. Where possible, modification of the involved restorations to facilitate access for appropriate oral hygiene aids was conducted in conjunction with treatment. No surgery had been performed before non-surgical therapy and the patient had demonstrated evidence of satisfactory compliance with oral hygiene methods (≤ 30% full mouth plaque score). Re-evaluation was performed at 6 weeks.

6.2.3 TREATMENT GROUPS

Four treatment groups were included in this study:

1) Non-surgical therapy as a monotherapy.

2) Non-surgical therapy followed by open-flap debridement approach

3) Non-surgical therapy followed by open flap debridement and the application of L-PRF only (plugs and/or membranes)

4) Non-surgical therapy followed by open flap debridement and the application of a hard tissue regenerative material with or without L-PRF.

6.2.4 TREATMENT APPROACH

For the non-surgical treatment approach, the implant surface was debrided with titanium implant curettes (Wingrove, Paradise Dental Technologies, Missoula, MT, USA) and subsequently decontaminated chemically with 3% hydrogen peroxide applied for one minute before being washed with sterile saline solution for 1 minute. The non-surgical treatment was the same for all four groups.

The surgical approach used in each case, and the use of biomaterials (if any), was based on the clinical judgment of the surgeons involved. In patients where more than one implant was affected by peri-implantitis, all sites in the involved arch were treated simultaneously and with the same treatment method.

All surgical procedures were performed under local anaesthesia. Prior to surgery, the prosthetic superstructure was removed to facilitate surgical access to the affected implant sites. A crestal incision connected affected sites and was extended to allow sufficient access to the associated bone defects. A full-thickness mucoperiosteal flap was elevated to expose the alveolar crest beyond the apical border of the peri-implant bone defects and granulation tissue was removed. The implant surface was debrided with titanium implant curettes (Wingrove, Paradise Dental Technologies, Missoula, MT, USA) and subsequently decontaminated chemically with 3% hydrogen peroxide applied for one minute before being washed with sterile saline solution for 1 minute.

In sites where reconstruction was attempted, the lingual and buccal mucoperiosteal flaps were repositioned and stabilised using tension-free closure with a combination

of horizontal mattress and single interrupted sutures. The prosthetic superstructure was cleaned and repositioned at the end of the surgical procedure.

In all groups, patients were prescribed systemic antimicrobial therapy (Amoxicillin 500mg + metronidazole 200mg each three times daily for 5 days) and were instructed to rinse with 0.2% chlorhexidine digluconate mouthrinse (Corsodyl, GlaxoSmithKline Consumer Healthcare) twice daily for 14 days. Each patient received personalised instruction in oral hygiene methods according to their individual needs and supra-structure design.

Patients were recalled at 3, 6, 12, and 24-36 months after surgery for peri-implant maintenance appointments; these consisted of evaluation of oral hygiene, re-instruction and motivation in oral hygiene techniques and supra-gingival instrumentation when required. During these appointments, all supra-structures were removed. Clinical and implant stability measurements were taken at 6, 12, and 24-36 months (Table 34).

6.2.5 CLINICAL AND RADIOGRAPHIC EVALUATION

Clinical evaluation included measurement of MRFA using an Osstell® ISQ device (Stampgatan, Göteborg, Sweden), probing pocket depths, measurements of mucosal recession, presence of bleeding on gentle probing and presence of plaque. All parameters were recorded at six sites per implant. At the end, the width of keratinised tissue was recorded.

As all patients represented inter-clinic referrals to the peri-implantitis clinic, patients had undergone recent radiographic exposure – most commonly orthopantomograms – prior to their attendance at our clinic. Additional images in the form of intraoral periapical films (long cone technique) were requested where the radiographic angulation or diagnostic quality of the provided images was not sufficiently satisfactory for surgical planning. Care was taken to minimise additional radiographs, following the principle of keeping patient exposure *as low as reasonably achievable*. Post-operative radiographs were taken at the 12-month and 24-36 recall to evaluate

the outcomes of treatment and/or to assess for additional treatment needs. The distance from the implant platform to the most coronal aspect of the bone was measured as the defect depth. This was compared between baseline 12-month and 24-36 months' time-points. All radiographs taken were digital images recorded within the patient management system of the Hospital. The radiographic software used (Romexis, Planmeca, Helsinki, Finland) offers an inbuilt, calibrated tool for making dimensional measurements. As radiographs used were not standardised, marginal bone level changes were reported but were not included in the regression analysis.

6.2.6 STATISTICAL ANALYSIS

A unique tabulated data collection sheet was used to collect data for each implant at each time point (Appendices J). All data were then transferred to Microsoft Excel® spreadsheets and following cleaning of the data, input into SPSS® software (Version 27; IBM®, USA) for statistical analysis. No calculation of sample size was performed, as there were no studies available with similar design that could be used for the calculations.

All data components were statistically analysed at implant level using SPSS® software. Statistical significance and mean values were determined for normally distributed data using One-Way ANOVA analysis at the 0.05 significance level and a Tukey post-test was performed when $p < 0.05$. A Kruskal-Wallis H test was performed for non-parametric data at the 0.05 significance level and Dunn post-test was performed when $p < 0.05$. Sub-group analysis was performed to determine the effects of the various treatments provided in the study.

A regression model to determine factors affecting Osstell® (ISQ) measurements over time (dependent variable) with respect to four selected independent variables was developed using SAS v 9.4 PROC MIXED. The independent variables included in the model were treatment group (4), implant location (maxilla/mandible), average BOP over time and average PPD over time. In addition to looking at the above –named predictor variables, interactions with treatment group and implant location were also

considered. All predictors and two-factor interactions were included in the initial model. A manual backwards model selection method was then applied to arrive at the final, most optimal model. A QQ-plot of the standardized residuals illustrated a linear pattern, thus indicating that residuals assumptions were satisfied (Figure 21).

Finally, in order to explore the nature of the relationship between ISQ measurement, average BOP and average PPD, the linear correlation between ISQ measurements and both BOP and average PPD, at all four time-points was evaluated.

Table 34: Timeline of research measurements

	Baseline	3 Months	6 Months	12 Months	24/36 Months
Clinical Measurements	✓		✓	✓	✓
MRFA (Osstell ™)	✓		✓	✓	✓
Treatment	✓				
Radiographs	✓				✓
Removal of Prosthesis and OH instructions	✓	✓	✓	✓	✓

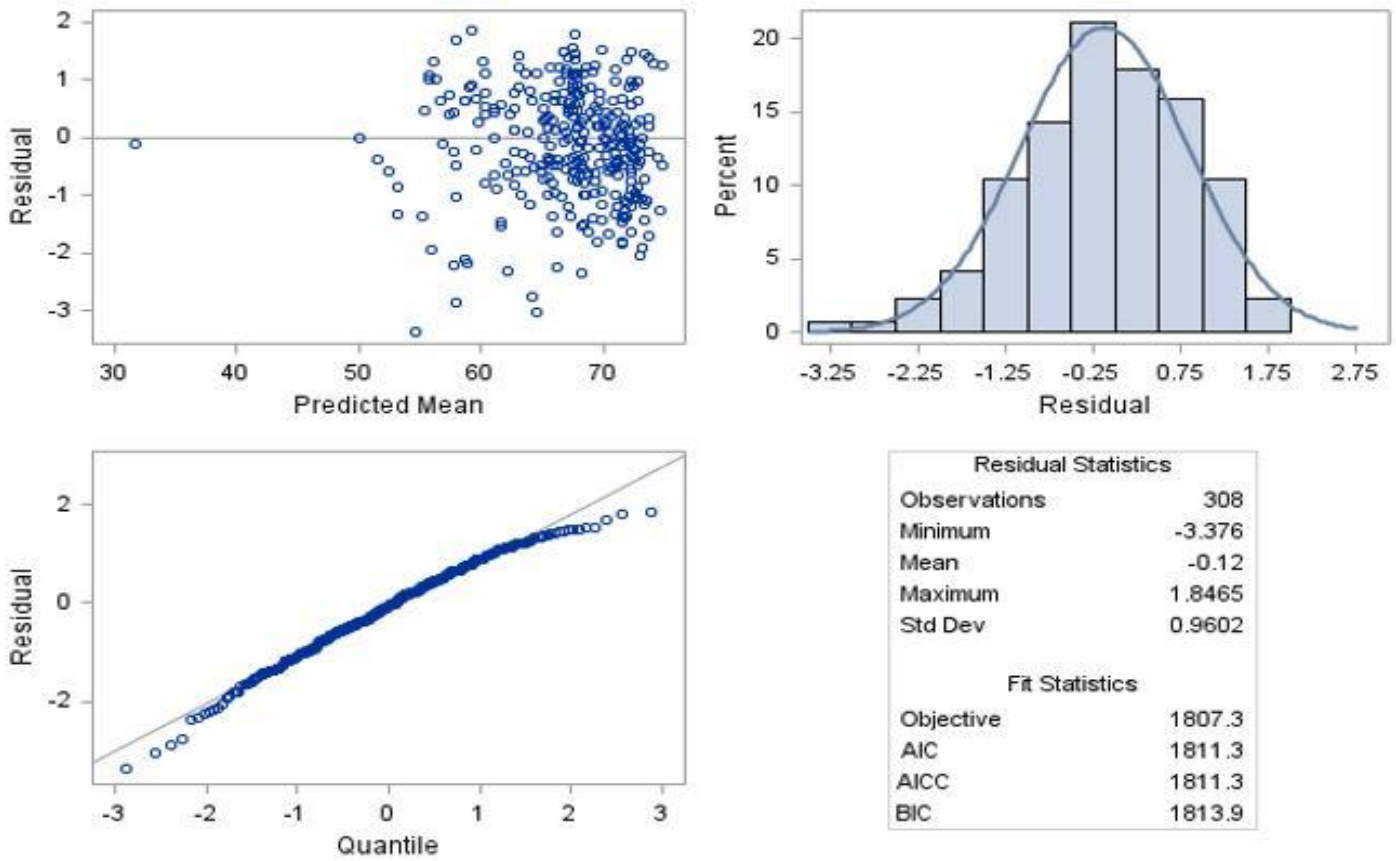

Figure 21: QQ plot and residual diagnostics for the regression model

6.3 RESULTS

Twenty-six patients with 86 implants (aged 41–76 years) prospectively agreed to participate. Following non-surgical treatment, three implants in three different patients were deemed hopeless and were removed. Finally, 25 patients (17 female, 8 male) with 83 implants were included in the study. The vast majority of implants were either Brånemark® (n = 31) (Nobel Biocare AB, Gothenberg, Sweden), Nobel Replace® (Nobel Biocare AB, Kloten, Switzerland) or Biomet 3i® (n = 47; Palm Beach Gardens, Florida, USA). Additionally, seven Ankylos® implants (Dentsply Sirona, York, Pennsylvania, USA), and one Straumann® (Straumann Institute AG, Waldenburg, Switzerland) were present. Out of all implants diagnosed with peri-implantitis, 50 implants were supporting full-arch fixed prostheses. Fourteen implants retained implant-supported overdentures by means of a bar, while 7 did so by means of locator attachments. Three implants supported fixed partial dentures, while nine implants supported single unit implant restorations. In total, 45 implants were located in the maxilla and 38 in the mandible.

Following treatment, there was an overall mean radiographic bone-level gain of 0.27mm and a reduction in mean implant plaque scores from 76.89% at baseline to 44.58% at 2–3years. The deepest mean probing depth at the treated implants reduced by 1.55 mm while the average probing depth reduced by 1.43 mm. There was an average clinical attachment gain of 1.25 mm at the treated implants and a reduction in mean bleeding on probing scores of 25.42%. Differences from baseline remained statistically significant across all three different time-points. (Table 34). There were no statistically significant changes in keratinised tissue levels. Finally, ISQ scores marginally increased from a mean baseline value of 65.09 to 66.69 at 2–3years post-treatment, however, this was not statistically significant (p = 0.4684). (Table 35, Figure 22).

Table 35: Clinical and RFA outcomes at treated implants (all modalities). Statistically significant differences from baseline in red

VARIABLE	BASELINE	6 MONTHS	12 MONTHS	24/36 MONTHS	
MARGINAL BONE LEVEL (mm)	76		80	75	**N**
	3.97 (1.90)		3.53 (1.91)	3.70 (1.71)	**Mean (SD)**
	3.72		3.12	3.40	**Median**
	0		0	0.05	**Min**
	10.65		8.60	8.15	**Max**
					Sig. p=0.311
DEEPEST PROBING DEPTH (mm)	83	81	83	77	**N**
	5.84 (2.46)	3.85 (1.47)	3.92 (1.71)	4.29 (2.01)	**Mean (SD)**
	6	4	4	4	**Median**
	2	2	1	2	**Min**
	17	8	10	10	**Max**
					Sig. $p < 0.0001$*
AVERAGE PROBING DEPTH (mm)	83	81	83	77	**N**
	4.88 (2.30)	3.18 (1.29)	3.29 (1.62)	3.45 (1.76)	**Mean (SD)**
	4.66	2.83	2.83	3.16	**Median**
	1.50	1.33	1.00	1.16	**Min**
	16.83	8.00	9.33	9.50	**Max**
					Sig. $p < 0.0001$*
AVERAGE CLINICAL ATTACHMENT LOSS (mm)	83	81	83	77	**N**
	5.38 (2.35)	3.73 (1.56)	3.92 (1.81)	4.13 (1.95)	**Mean (SD)**
	5.16	3.33	3.33	3.66	**Median**
	1.50	1.33	1	1.33	**Min**
	16.83	9.66	10	10.50	**Max**
					Sig. $p < 0.0001$*
BLEEDING ON PROBING (%)	83	81	83	77	**N**
	57.02 (43.95)	30.04 (33.06)	31.72 (36.49)	31.60 (37.71)	**Mean (SD)**
	66.70	16.67	16.67	16.67	**Median**
	0	0	0	0	**Min**
	100	100	100	100	**Max**
					Sig. $p < 0.01$*
PLAQUE SCORE (%)	83	81	83	77	**N**
	76.89 (40.98)	41.97 (43.06)	49.99 (46.99)	44.58	**Mean (SD)**
	100	33.33	50	16.67	**Median**
	0	0	0	0	**Min**
	100	100	100	100	**Max**
					Sig. $p < 0.0001$*
KERATINIZED TISSUE (mm)	83	81	81	77	**N**
	2 (1.91)	2 (1.64)	1.92 (1.70)	2.14 (1.73)	**Mean (SD)**
	2	2	2	2	**Median**
	0	0	0	0	**Min**
	7	6	7	7	**Max**
					Sig. p=0.8368*
ISQ SCORE (1-100)	81	80	82	65	**N**
	65.09 (8.06)	66.43 (8.13)	66.67 (8.32)	66.69 (9.11)	**Mean (SD)**
	66	68	66.50	68	**Median**
	31	38	42	31	**Min**
	80	83	83	82	**Max**
					Sig. p=0.4684*

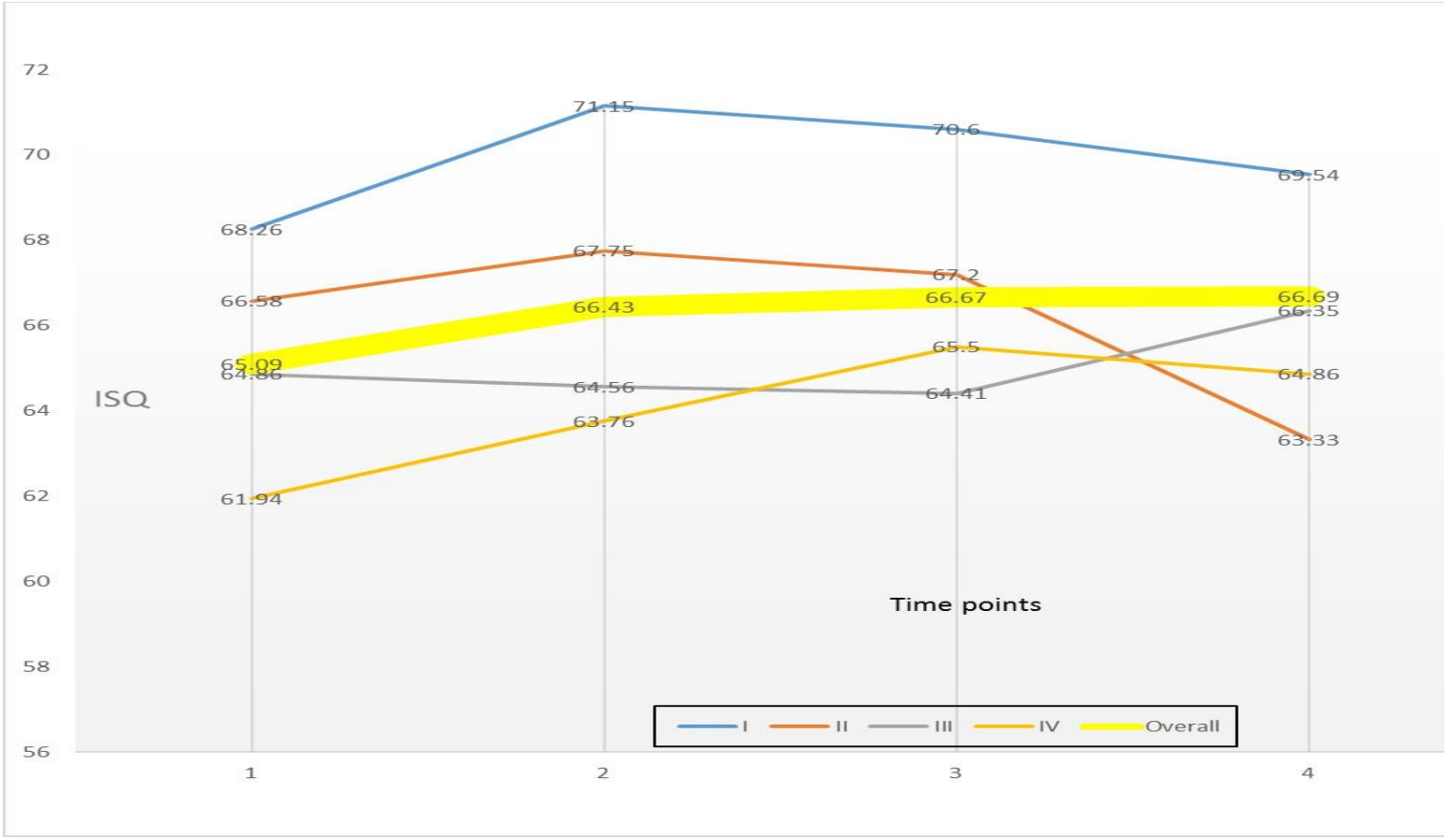

Figure 22: Mean ISQ values for all time points and for the four treatment modalities

Table 36: Treatment subgroup analysis for all clinical and RFA outcomes at each time point. Statistically significant differences from baseline in red. Bold values are statistically significant. *Kruskal-Wallis test completed for non- normally distributed data at the 0.05 significance level + Dunn post-test when p < 0.05 **One way ANOVA test completed for normally distributed data at 0.05 significance level+ Tukey post-test when p<0.05

Variable	Modality	Baseline	6 Months	12 Months	24/36 Months	Diff Baseline-24/36	*Sig.*
Marginal Bone Level (mm)	I Mean (SD)	3.17 (1.01)		2.68 (1.44)	2.70 (1.12)	+0.47mm	*p=0.1177**
	II	2.79 (2.17)		3.03 (1.79)	3.85 (1.37)	-1.06 mm	*p=0.5041***
	III	4.46 (1.66)		4.28 (1.68)	4.48 (1.50)	-0.02 mm	*p=0.9460**
	IV	4.62 (2.19)		3.75 (2.24)	3.75 (2.03)	+0.87 mm	*p=0.2618***
Deepest Probing Depth (mm)	I Mean (SD)	4.85 (1.42)	3.71 (0.95)	3.42 (1.02)	3.90 (2.14)	+ 0.95 mm	***p<0.0001****
	II	4.41 (1.50)	2.75 (0.75)	3.08 (1.24)	3.44 (1.23)	+0.97 mm	***p=0.0302****
	III	6.25 (2.42)	3.95 (1.71)	4.50 (2.20)	4.72 (2.25)	+1.53 mm	***p=0.049****
	IV	6.92 (2.91)	4.48 (1.58)	4.11 (1.65)	4.56 (1.85)	+2.36 mm	***p=0.0001****
Average Probing Depth (mm)	I Mean (SD)	3.92 (1.28)	2.90 (0.84)	2.97 (0.88)	3.05 (1.73)	+0.87 mm	***p=0.0010****
	II	3.48 (1.40)	2.38 (0.68)	2.51(1.05)	2.74 (1.08)	+0.74 mm	*p=0.0785***
	III	5.35 (2.16)	3.31(1.46)	3.83 (2.20)	3.97 (2.08)	+1.38 mm	***p=0.0052****
	IV	5.87 (2.82)	3.69 (1.47)	3.40 (1.55)	3.58 (1.61)	+2.29 mm	***p=0.0001****
Average Clinical Attachment Level (mm)	I Mean (SD)	4.06 (1.23)	2.97 (0.90)	3.12 (0.88)	3.19 (1.66)	+0.87 mm	***p=0.0161****
	II	4.16 (1.84)	3.19 (1.53)	3.54 (1.86)	4.14 (1.83)	+0.02 mm	*p=0.4903***
	III	5.82 (2.03)	4.07 (1.76)	4.70 (2.16)	4.93 (1.91)	+0.89 mm	***p=0.0062****
	IV	6.59 (2.78)	4.33 (1.56)	4.02 (1.78)	4.21 (2.036)	+2.38 mm	***p=0.0002****
Bleeding on Probing (%)	I Mean (SD)	42% (45)	20% (26)	24% (33)	19% (29)	-23%	*p=0.4080**
	II	80% (22)	20% (21)	43% (43)	46% (46)	-34%	***p=0.0095****
	III	47% (51)	40% (40)	35% (38)	37% (43)	-10%	*p=0.9399**
	IV	64% (39)	36% (35)	30% (35)	28% (35)	-36%	***p=0.0076****
Plaque Score (%)	I Mean (SD)	48% (27)	18% (27)	52% (51)	29% (40)	-19%	*p=0.1296**
	II	88% (29)	55% (47)	62% (43)	87% (33)	-1%	*p=0.1626**
	III	78% (39)	55% (41)	54% (45)	50% (48)	-28%	*p=0.1253**
	IV	92% (25)	43% (46)	38% (46)	36% (46)	-56%	***p=0.0001****
Keratinized Tissue (mm)	I Mean (SD)	2.57 (1.72)	2.85 (1.62)	2.76 (1.72)	2.80 (1.75)	+0.23 mm	*p=0.9006**
	II	0.33 (0.44)	0.91 (1.08)	0.75 (1.05)	0,66 (1.11)	+0.33 mm	*p=0.7403**
	III	2.18 (1.89)	1.95 (1.77)	1.87 (1.72)	2.15 (1.84)	-0.03 mm	*p=0.9342**
	IV	2.16 (2.15)	1.86 (1.48)	1.83 (1.63)	2.12 (1.53)	-0.04 mm	*p=0.9158**
ISQ score (1-100)	I Mean (SD)	68.26 (4.81)	71.15 (5.91)	70.60 (6.65)	69.54 (7.89)	+1.28	*p=0.4932***
	II	66.58 (7.44)	67.75 (4.94)	67.20 (7.02)	63.33 (4.27)	-3.25	*p=0.3482**
	III	64.86 (7.24)	64.56 (7.05)	64.41 (7.33)	66.35 (7.20)	+1.49	*p=0.8134***
	IV	61.94 (10.19)	63.76 (10.15)	65.50 (10.03)	64.86 (12.58)	+2.92	*p=0.3676**

Table 36 outlines the treatment subgroup analysis for all clinical and RFA outcomes at each time point.

Table 37 reports the number implants included in the analysis for each modality and

at each time point. Non-surgical therapy yielded statistically significant reductions in mean and deepest probing depths as well as gain in clinical attachment, which were largely maintained for 2–3 years.

An open flap approach, which encompassed surface decontamination and open flap debridement, resulted in a modest reduction of average probing depths (- 0.74 mm), even though there was a very significant reduction in mean bleeding on probing levels.

The greatest reduction in deepest PPD and gain in CAL was observed in the treatment modality IV group; a 2.29 mm average PPD reduction and a 2.38 mm gain in CAL at 2–3years were found. In addition, there was also a continued improvement in bleeding and plaque levels at all time-points.

In tandem with changes in ISQ values, no statistically significant changes were detected. Overall, there was a minimal increase in the ISQ mean value at 6 months (+ 1.34). The implant stability remained stable at 12 and 24–36 months. The greatest increase in ISQ levels was observed at implants that received the regenerative treatment modality IV (+ 2.92) but this increase was not a statistically significant ($p = 0.4932$). (Table 36, Figure 22).

Table 37: Number of implants included in the analysis for each modality and at each time point by variable measured

VARIABLE	MODALITY	BASELINE	6 MONTHS	12 MONTHS	24/36 MONTHS
Marginal bone level	I	20		20	19
	II	9		12	7
	III	22		23	23
	IV	25		25	26
Deepest probing depth, Average probing depth, Average clinical attachment level, Bleeding on probing, Plaque score	I	21	21	21	21
	II	12	12	12	9
	III	24	23	24	22
	IV	26	25	26	25
Keratinised tissue	I	21	21	21	21
	II	12	12	12	9
	III	24	23	24	22
	IV	26	25	24	25
ISQ score (1-100)	I	21	20	20	21
	II	12	12	12	6
	III	23	23	24	20
	IV	25	25	26	18

For the regression analysis, all predictors selected (treatment groups, implant location, BOP and PPD) and two-factor interactions were included in the initial model. A manual backwards model selection method was then applied to arrive at the final, most optimal model. Based on the Type 3 tests shown in Table 38, visit and mean PPD both had a statistically significant effect on ISQ measurements ($p < 0.05$). The [treatment group-visit], [treatment group- average PPD] and [visit-implant location] all also had a statistically significant effect on ISQ measurements (Table 38).

Results from Pearson correlation between ISQ measurements and PPD indicates a strong negative linear relationship between these two variables at all-time points. As ISQ measurements increase, PPD linearly decreases (Table 39). Although significant, the correlation is weak between ISQ measurements and BOP.

Table 38: Type 3 tests of fixed effects

Effect	Num DF	Den DF	F value	Pr > F
Visit	3	71	4.33	0.0074
Location (max/mand)	1	26	0.51	0.4829
Treatment modality	3	10	0.37	0.7763
Average PPD	1	258	75.13	<0.0001
Visit – Location	3	71	4.38	0.0069
Visit – Tx modality	9	26	3.08	0.0119
Av PPD – Tx Modality	3	258	3.55	0.0151

Table 39: Pearson Correlation Coefficients between ISQ measurement, BOP and Average PPD at all 4 time-points.

	Baseline	6 months	12 months	24/36 months
Av PPD/ISQ	r = -0.618 p <0.0001	r = -0.551 p <0.0001	r = -0.591 p <0.0001	r = -0.604 p <0.0001
BOP/ISQ	r = -0.22 p <0.043	r = -0.324 p <0.0030	r = -0.443 p <0.0001	r = -0.255 p <0.0005

6.4 DISCUSSION

The aim of the study was to investigate the medium-term impact of peri-implantitis treatment upon clinical parameters and implant stability quotient values.

Overall, clinical and radiographic parameters improved over the study duration. There was a significant reduction in average and deepest probing depths at 6 months following treatment but a slight relapse was seen at 2 - 3 years. Similar observations were made for plaque scores and bleeding scores. Other studies have reported similar findings, i.e. initial improvements following treatment and a degree of relapse in the subsequent follow-up duration. Compliance with maintenance therapy and decreased standards of oral hygiene are often cited as critical factors (Monje et al., 2016, Heitz-Mayfield et al., 2018, Lin et al., 2019). Rinke et al., demonstrated that patients who had received implants and did not comply with regular SPT visits had an 11-fold increased risk of developing PI, while Ferreira et al., presented similar findings (Rinke et al., 2011, Ferreira et al., 2006). However, one should consider that these two studies refer to the importance of SPT in preventing the development of peri-implantitis following the placement of implants and not of recurrence of peri-implantitis following treatment.

Our results regarding non-surgical therapy are in line with the findings from a recent systematic review (Cosgarea et al., 2023). On the contrary, results regarding PD reductions and gain in clinical attachment following the open flap approach were not in agreement with what has been reported in previous studies. Additionally, and in agreement with previous observations, relapse was common (Karlsson et al., 2023).

Enhanced clinical improvements were observed in the 2 regenerative treatment groups, which is line with the scientific literature to date. Some studies like the ones by Roccuzzo and colleagues have demonstrated greater improvements in clinical parameters following regenerative interventions (Roccuzzo et al., 2011, Roccuzzo et al., 2017). These authors treated single intrabony PI defects with a xenograft around TPS and SLA implants. The subjects were then followed up for 7-years. TPS surface

implants underwent a mean PPD reduction of 2.4 mm at twelve months and to 3.8 mm at 7-years. SLA implants meanwhile, had a mean PPD reduction of 3.2 mm at 12 months and 3.4 mm at 7-years. In addition, there was a 35 per cent reduction in BOP at twelve months and 60 per cent at 7-years for the TPS group and 62.5 per cent at twelve months and up to 67.5 per cent at 7-years for the SLA group (Roccuzzo et al., 2011, Roccuzzo et al., 2017). Overall, available evidence is limited as there is large heterogeneity in the existing literature with inconsistent inclusion criteria and types of biomaterials used.

When considering the application of L-PRF membranes to the treatment of PI defects, Hamzacebi and co-workers demonstrated a mean PPD reduction of 2.82 mm for implants treated with open flap debridement and an adjunctive L-PRF membrane, compared to a 2.05 mm decrease in the control group, which received access flap alone, at 6-months (Hamzacebi et al., 2015). This is consistent with changes in our study, where the incorporation of L-PRF membrane to the defect, yielded a reduction in average PPD of 2.04 mm at 6-months, compared to a mean 1.1 mm reduction for the open flap debridement group.

No statistically significant changes in bone levels from baseline to 12-months were revealed in any of the treatment groups. When applied to the clinical scenario in everyday practice, a gain of 1 mm in peri-implant bone levels following regenerative surgery would usually be clinically significant, particularly when combined with significant reductions in probing depths and inflammation (Herrera et al., 2023).

There were no significant changes in keratinized tissue levels over the 2/3 year follow-up period in any of the 4 treatment modalities, despite the addition of L-PRF membrane in treatment modality III or hard tissue grafting combined with L-PRF in treatment modality IV. Following conventional treatment modalities, a gain in keratinized mucosa width would not usually be anticipated. However, the use of L-PRF in specific cases alters such expectations. While the systematic review by Castro et al., focused on the gain in keratinized mucosa width around teeth, a mean difference of only 0.3 mm was found between the L-PRF and connective tissue grafting groups, when combined with a coronally advanced flap at 6-months (Castro

et al., 2017b). If these findings were applied to the implants in our study that received an L-PRF membrane, one may have predicted a greater gain in keratinized mucosa width for this cohort.

None of the 4 treatment modalities resulted in statistically significant changes in ISQ levels at any time point. However, for the non-surgical, and the two regenerative groups, there was a modest overall increase in ISQ levels from baseline to the end of the observation period. The open flap group was the only one that demonstrated a mild decrease in stability 2-3 years following treatment. An interesting observation about the regenerative treatment group III which employed L- PRF products as the sole grafting material, was that the pattern of the implant stability progression was very different to the other groups and the implants demonstrated increasing stability between 12 months and 2/3 years (Figure 22). It has been demonstrated that platelet concentrates and fibrin exudates are rich in growth factors and serum proteins promoting cell adhesion and migration into the fibrin clot. They also have mild antibacterial properties. These effects, seem to have a positive impact on osseointegration so one could suggest that they might have a similar effect on re-osseointegration following peri-implantitis treatment (Öncü et al., 2016, Schuldt et al., 2021).

In order to determine the true applicability of these results to clinical implant practice, the study limitations must be considered. Despite the treatment of 85 implants in total, the most significant limitation in the study is the small number. This limitation is further emphasized when considering the 85 implants were spread across four treatment groups. Implant level analysis must be mentioned as another limitation of the study herein. Finally, marginal bone level changes should be interpreted with caution, as radiographs used were not standardized. As a result, possible correlations between bone level changes and ISQ values could not be explored. Finally the Osstell® transducers where tightened by hand and not with a torque controlled device and this might have affected the reliability of the ISQ measurements (Naughton et al. 2023).

6.5 CONCLUSIONS

Over the 2-3 years follow-up period, all four treatment modalities resulted in improved clinical and radiographic peri-implant parameters

However, mplant stability post-treatment, as indicated by the recorded ISQ scores remained stable. As a result, using MRFA as an adjunct to the traditionally used periodontal and radiographic tools for the evaluation of post-operative implant stability following the treatment of peri-implant disease cannot be recommended.

- Treatment of peri-implantitis defects resulted in improved peri-implant parameters up to 2–3 years of follow-up in all treatment groups.
- Addition of L-PRF to access flap surgery (Open flap debridement; OFD) resulted in reduction of mean probing depth versus OFD alone. Surgical treatment of peri-implantitis utilising L-PRF materials may provide clinical outcomes equivalent to other regenerative materials and may be considered for clinical use in peri-implantitis treatment
- However, implant stability quotient (ISQ) values did not demonstrate statistically significant changes following treatment and it appears that use of magnetic resonance frequency analysis (MRFA) cannot be recommended as an adjunct to clinical/ radiographic assessment to evaluate implant stability following peri-implantitis treatment

CHAPTER 7: GENERAL DISCUSSION & CONCLUSIONS

7.1 GENERAL DISCUSSION

While the use of L-PRF and its variants has been studied extensively in recent years for a wide variety of clinical indications, the investigations undertaken in the course of the present research highlight some areas for further investigation.

Alveolar ridge preservation

Our systematic review that compared the use of L-PRF alone in comparison to the use of L-PRF in composite form with a bone substitute material for the purposes of alveolar ridge preservation uncovered only four research studies. Given this paucity of information, additional research investigations are merited. Our randomised controlled trial compared L-PRF alone versus L-PRF Block in non-molar extraction sockets. While the study failed to demonstrate statistically significant differences in clinical, radiographic or patient-reported outcomes, in light of the observed limitations of this study and the lack of comparative studies in the literature, additional randomised clinical trials are undoubtedly required to assist clinicians and researchers with their decision-making on this topic. Given that some dimensional outcomes appeared to trend in favour of L-PRF Block, an increasingly powered comparative randomised controlled trial is suggested to assist towards this aim. Furthermore, our study was limited by the fact that a sizeable proportion of enrolled patients did not progress to dental implant placement; this reduced our ability to assess horizontal changes at bone-level and to compare the implant stability quotient of dental implants placed in both types of grafted site. Since these parameters are undoubtedly of value to treating clinicians, further studies may consequently be advised to require participation of enrolled subjects up to and including implant surgery in order to incorporate such measurements.

Histologic information on bone quality would also be of interest in determining the healing response of extraction sites treated by L-PRF versus L-PRF Block. This would undoubtedly complement the research on post-extraction healing at naturally healed or grafted sites by Araújo and colleagues, as presented in Chapter 2 of this thesis. The presently available studies have generally used animal models or a limited number of human volunteers to determine the healing processes at play during the early period of healing. However, collection of histologic information - in the form of trephine core biopsies from the socket site at the time of implant first stage surgery - would dramatically enhance our knowledge on the nature of healing that takes place with these materials in a representative sample of the adult population following alveolar ridge preservation. This would undoubtedly add context for decision-making on dimensional changes observed. Such a proposal should not create ethical concerns once informed consent is obtained from participants, since the trephine biopsy collection could be incorporated within the implant preparation/drilling protocol. However, histologic analysis would require additional expertise and accrue additional cost for any study, and while we did consider the concept at the time of our study design, we determined it was not feasible for inclusion in this PhD. If considered in nay subsequent studies, this would still necessitate funding and a research collaboration to achieve it.

There is a notable trend in the literature for using CBCT at multiple time-points to record dimensional changes following extraction and/or alveolar ridge preservation. This method overcomes many of the challenges of clinical assessment but undoubtedly results in additional radiation exposure for the patient in comparison to local intra-oral imaging of the extraction sites. Our research design did not incorporate CBCT for this reason, as local ethical approval would not have been granted for use of multiple CBCTs in each patient. However, with improvement in the available equipment/facilities to incorporate CBCT imaging with limited field of view and high resolution, this

approach may be viable at our site in future research studies, and would simplify further research studies undertaken by our group on this topic.

Finally, as acknowledged in Chapter 1, interpretation of PRF research has been challenged by heterogeneity in approaches used to prepare PRF. A variety of centrifugation protocols have been used to prepare collected blood samples, resulting in a large array of liquid and solids PRF products. Differences in centrifuge design, centrifugation angulation and speed and blood collection tubes, among other factors, has further complicated interpretation and comparison of studies. Much of the recent PRF research has revolved around putative benefits of horizontal centrifugation (H-PRF products) in comparison to the more established protocols using fixed-angle centrifugation (including L-PRF products). Notwithstanding commercial interests, it would appear that the horizontal centrifugation approach has momentum; this has arguably been assisted by research published from this research team that questions the blood collection tubes used in past studies using fixed-angle centrifuges, and their safety going forward. As such, if one clear set of preparation protocols becomes established as a "preferred" protocol, much of the exiting research results may be questioned, or at least require repetition or further elaboration in future. Pending this, conducting a similar randomised controlled trial using horizontal centrifugation with the specified glass blood-collection tubes may be advised.

Laboratory study of membrane degradation

In chapter 5, we compared degradation of L-PRF and A-PRF membranes in an enzymatic solution of plasmin in DMEM. L-PRF membranes were initially larger but demonstrated greater reduction in mean membrane weight over the study period compared to A-PRF; findings were statistically significant. Furthermore, while the majority of individual membranes visually maintained

their integrity up to 14 days in solution, L-PRF membranes were more likely to demonstrate visual changes over the study period. While the limitations of this study were discussed in Chapter 5, these may inform considerations around future research.

The enzyme plasmin was used to promote PRF degradation as a simulated model of fibrin degradation of blood clots in the wound environment. The enzyme concentration selected was based on a simple pilot study, and the main study reinforced that membranes placed in this solution demonstrated greater proportional mean reduction in membrane weight than membranes placed in DMEM alone. This was evident for both A-PRF and L-PRF membranes, indicating that plasmin was active in solution. However, while our study establishes this in principle, further research wherein the study media are changed regularly throughout the study would be of value. The half-life of plasmin in a clinical situation is brief and the manufacturers cannot verify its likely duration of action in solution. It is possible that the rate of degradation and/or physical signs of membrane change could be increased by replacing solution, either at day 7, or more often, to expose membranes to fresh plasmin. This would also help to allay questions of whether membrane degradation products may actually delay the degradation process. This approach was not incorporated in our design based on the pilot study, wherein membranes in plasmin continued to degrade faster than those in DMEM alone. The high cost of plasmin and time restrictions involved in working at a shared sterile cabinet site also informed this decision. Looking forward, the time-heavy nature of obtaining membrane measurements while maintaining sterility somewhat restricts the performance of further similar studies unless a less-used sterile cabinet site could be accessed. If so, further studies might incorporate some of the principles highlighted below. Nevertheless, I believe our study establishes a way forward for future research studies on PRF membrane degradation.

The present study compared A-PRF and L-PRF protocols as described in the literature (rpm and time) using a single centrifuge design. This resulted in an A-PRF preparation protocol that has a RCF different to the one that would be obtained using the commercially marketed A-PRF centrifuge. Therefore, to more truly compare A-PRF and L-PRF, future studies may prepare PRF variants in the centrifuges used when initially describing the protocol, or more practically, may use a single centrifuge but modify rpm values to more accurately correspond to the RCF values described in the literature for each preparation protocol. On a similar note, comparison of H-PRF degradation with that of the membranes assessed in the present study (A-PRF or L-PRF) may offer clarity on whether horizontal centrifugation protocols enhance the durability/resistance to degradation of PRF membranes. This would be of value in determining which membrane preparation protocol is most likely to provide a clinically useful membrane that maintains its barrier function at the wound site.

While plasmin appears to be a viable and practical enzymatic model to assess degradation of autologous fibrin products, given the aforementioned cost and uncertainties about its duration of action, alternative enzyme models may be considered in future studies. However, it must be noted that while trypsin has been used in a previous study of PRF membranes, it stimulated a rate of degradation dramatically faster than what would be expected clinically, while research using artificial saliva solutions appears to offer reduced value to simulate a covered oral wound site.

Regenerative treatment of peri-implant defects

In our study evaluating 2-3 year follow-up of peri-implantitis defects treated with either non-surgical, resective or regenerative surgical approaches, L-PRF and L-PRF Block were among several approaches used for regeneration, with

material used depending on the clinical situation and operator perception of the most appropriate clinical approach. All four studied treatment modalities, including regenerative treatment approaches, resulted in improved clinical and radiographic peri-implant parameters but the study failed to disclose additional value from using MRFA as an adjunctive tool for measuring treatment response in addition to clinical and radiographic parameters.

The area of peri-implantitis treatment is an ongoing research track at DDUH. While MRFA does not appear to offer promise in measuring outcomes following peri-implantitis treatment, this research did disclose that L- PRF and L-PRF Block may be associated with clinical and radiographic outcomes equivalent to other regenerative materials and may be considered suitable materials for clinical use in the absence of any gold standard regenerative material. Given the variation in peri-implant defects that present naturally in sequentially attending patients, it appears appropriate to continue to treat according to clinical judgment of the surgical operator. Opportunity exists to evaluate the present patient cohort at a later time-point to provide longer-term data (e.g. 5 years) but this must be taken in context of non-standardised supportive peri--implant care that may have occurred in the interim, as patients were discharged to the care of their own general dental practitioners.

For new research avenues, a more restrictive set of inclusion criteria that allows greater potential to obtain more homogeneous peri-implant defects and that focuses on outcomes following regenerative use of PRF variants may provide increased observational data to support the appropriateness of including these materials as a more “routine” part of the armoury of regenerative materials at peri-implantitis sites.

Reflecting further on the lack of clear gold standard materials for regenerative treatment of sockets and peri-implantitis defect sites, patient-reported outcomes offer potential value in assisting decision-making. Patient-reported

outcome measures will therefore be incorporated in any future regenerative trials conducted by our group. It must be recognised that existing tools are limited and frequently have not been extensively validated. Collaborative research to enhance the meaningfulness of such tools therefore represents an avenue for future research.

The antimicrobial potential of L-PRF is an area where further research appears indicated. A recent systematic review highlighted that although the literature on this subject remains scarce, the existing evidence suggests the PRF materials may have a significant antimicrobial effect (Moraschini et al., 2023). It is notable that while *in vitro* studies of periodontopathogens have been conducted (Badade et al., 2016, Castro et al., 2019b), there may be additional opportunity to evaluate the antimicrobial capacity of PRF materials against species such as *Staphylococcus aureus* that are frequently isolated from sub-optimally healing or chronic wound sites(Serra et al., 2015). Among the medical indications for L-PRF is its use at sub-optimally healing or chronic wound sites (Cieslik-Bielecka et al., 2012a), and although antimicrobial activity against *S. aureus* has been highlighted for PRP (Edelblute et al., 2015), the activity of L-PRF against *S. aureus* species has yet to be addressed in detail. Polak and co-authors conducted an *in vitro* study wherein various antibiotics were incorporated into PRF via addition to whole blood prior to centrifugation and anti-bacterial activity against S. aureus assayed up to 4 days The anti-bacterial action of these modified PRF materials was statistically significant compared to PRF alone. These authors concluded that such modified PRF preparations may offer promise to enhance the antimicrobial effect of PRF at intra-oral wound sites and help to minimise the use of systemic antimicrobials in future (Polak et al., 2019). However, further studies appear desirable to fully elucidate a possible role for L-PRF in enhancing wound healing at surgical sites intra-orally, particularly those at dental implants, where *S. aureus* has been observed to colonise even from the earliest stages of healing (Fürst et al., 2007).

7.2 CONCLUSIONS OF INDIVIDUAL RESEARCH STUDIES

The conclusions of each of the four studies that contributed to this research thesis are listed in the following paragraphs.

A comparison of the outcomes of alveolar ridge preservation with Platelet-rich fibrin (PRF) alone versus PRF used in combination with other biomaterials: A systematic review

- Based on systematic review of the literature, there is insufficient evidence on which to evaluate the outcomes of alveolar ridge preservation (ARP) using platelet rich fibrin (PRF) materials alone in comparison to hybrid biomaterials where PRF is used in combination with another graft material
- The combination of PRF with another graft material for ARP did not yield consistent evidence of a statistically significant benefit when compared to PRF used alone, with respect to dimensional changes or bone density findings.
- Only one study addressed pain and soft tissue healing when comparing these materials. Data suggested some additional benefits may be obtained when a hybrid material is used versus using PRF alone.
- Additional studies are warranted to elaborate further on the comparison of PRF used alone versus used in combination with other materials for ARP.

A comparative study investigating the effects of L-PRF and L-PRF Block on the regenerative treatment of non-molar extraction sockets

- Alveolar ridge preservation (ARP) at single, non-molar sites using L-PRF Block was not associated with statistically significant differences compared to the use of L-PRF alone, with regard to clinical or radiographic changes observed at 4-6 months
- Dental implant placement was feasible in all subjects who progressed to surgery, while only one subject required additional grafting to accommodate restoratively- driven placement
- Soft tissue healing was favourable following both ARP protocols
- Post-operative pain levels (as measured by VAS reported by patients)were generally low and did not demonstrate statistically significant differences by study protocol
- Based on the dimensional changes observed, both L-PRF and L-PRF Block appear to be acceptable material options for ARP.

An in vitro comparison of the degradation of L-PRF and A-PRF membranes in plasmin

- L-PRF membranes were larger than A-PRF membranes at baseline
- Mean membrane weight decreased up to 14d and was increased in test medium and for L - PRF
- Approximately 1 in 4 membranes demonstrated visual signs of degradation with only 4/64 membranes showing complete degradation at 14d

PRF preparation protocol does appear to influence the degradation profile of membranes produced

A clinical & radiographic evaluation of the use of L-PRF and L-PRF Block in the regenerative treatment of peri-implant defects

- Treatment of peri-implantitis defects resulted in improved peri-implant parameters up to 2–3 years of follow-up in all treatment groups.
- Addition of L-PRF to access flap surgery (Open flap debridement; OFD) resulted in reduction of mean probing depth versus OFD alone. Surgical treatment of peri-implantitis utilising L-PRF materials may provide clinical outcomes equivalent to other regenerative materials and may be considered for clinical use in peri-implantitis treatment
- However, implant stability quotient (ISQ) values did not demonstrate statistically significant changes following treatment and it appears that use of magnetic resonance frequency analysis (MRFA) cannot be recommended as an adjunct to clinical/ radiographic assessment to evaluate implant stability following peri-implantitis treatment

7.3 OVERALL CONCLUSIONS OF PHD

In the context of this investigation of L_PRF overall conclusions of this research thesis may be summarised as follows

- Based on systematic review of the literature, there is insufficient evidence on which to compare platelet rich fibrin (PRF) materials alone in comparison to hybrid biomaterials where PRF is used in combination with another graft material for the purposes of alveolar ridge preservation. Further studies are warranted.
- Alveolar ridge preservation (ARP) at single, non-molar sites using L-PRF Block was not associated with statistically significant differences compared to the use of L-PRF alone, with regard to early soft tissue healing and post-operative pain, or clinical or radiographic dimensional changes observed at 4-6 months. Both materials appear to be suitable materials for ARP.
- PRF preparation protocol appears to influence the degradation profile of membranes in enzymatic solution.
- Surgical treatment of peri-implantitis utilising L-PRF materials may provide clinical outcomes equivalent to other regenerative materials and may be considered for clinical use in peri-implantitis treatment

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APPENDICES

ID	Title	Status
CRD42023434015	A comparison of the outcomes of ridge preservation following tooth extraction with platelet-rich fibrin (PRF) alone versus PRF used in combination with other biomaterials	Registered

THIS NOTEPAPER MUST NOT BE USED FOR PRESCRIPTIONS OR INVOICING PURPOSES

SJH/AMNCH Research Ethics Committee Secretariat
Claire Hanlon Ph: 4142109
email: claire.hanlon@amnch.ie

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
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Dr Ioannis Polyzois
Associate Professor of Periodontology
Dublin Dental University Hospital,
Trinity College Dublin
Dublin Dental University Hospital
Lincoln Place
Dublin 2.

20th April 2018

Re: A clinical & biochemical investigation of the use of L-PRF in the regeneration of periodontal and peri-implant defects

REC Reference: 2018/04/04
(Please quote reference on all correspondence)

Dear Dr. Polyzois,

The Committee decided to give ethical approval to this study subject to the following conditions:

- C2.3 (b): 'A minimum interval of seven (7) days will be provided.' The committee note that a seven-day interval is no longer a legal obligation and appears excessive. The committee recommend that allowing potential participants 'ample' time is sufficient.
- E2.3 (a): 'Anonymized'. As the data will be linked by code this should read as: 'Coded'.
- PIL: The committee recommend that the PIL is designed in a more lay friendly and less condensed manner.
- PIL: The PIL should be explicit that, in the event that the patient declines to participate, that the chosen treatment (e.g. L-PRF or L-PRF Block (or other)) will be dictated by the treating dental practitioner (if this is the case).
- PIL: The PIL states that 'the dental treatments provided in this study are an alternative to no treatment'. This implies that no treatment is an alternative. However, the application states that patients requiring a graft are to be recruited. Therefore, the PIL should be clear that 'no treatment' with regard to the alternative types of treatment for a damaged tooth would not be a treatment pathway that a dental practitioner would recommend.

Yours sincerely,

p.p.

Prof. Richard Deane
Chairman, SJH/AMNCH Research Ethics Committee

The SJH/AMNCH Joint Research and Ethics Committee operates in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.

NSV Code: WPA 00436

INFORMED CONSENT FORM

Title of Study:

A comparative study investigating the effects of L-PRF and L-PRF Block on the regenerative treatment of extraction sockets

Staff Conducting Research:

Research Investigator: Dr Peter Harrison
Research Supervisor: Dr Ioannis Polyzois

Confidentiality:

Your name will not be used to identify your study records and nobody outside of the study group will have access to your details to ensure your confidentiality.

Consent:

Having read the Patient Information Leaflet and having had the opportunity to ask any questions you may have about the study, please signed the declaration below if you wish to participate.
If you decide not to take part in this study, this will have no impact on the care you receive from the Dublin Dental University Hospital.

Study Summary:

This study will collect clinical and radiographic (X-ray) information on the healing that occurs at the tooth socket (bony housing that supports the tooth) following extraction of a tooth and placing a graft material to fill the socket cavity.

The graft material will be formed by making a strong blood clot from a sample of your own blood - this material is referred to by the scientific name L-PRF. In half of the study participants, this material alone will be used to fill the socket, whereas in the other half of patients, it will be combined with a bone graft material (derived from cow bone) – this graft material is called "L-PRF Block". In all cases, a membrane (protective covering) made from the L-PRF material will be used to cover the socket cavity to protect the site during healing.

When a tooth is removed, the normal healing process results in some loss of height and thickness of the remaining bone at that area. Placing a graft material can help to reduce this bone loss, making future placement of a dental implant at the site easier. We will measure the amount of change in the bone dimensions that occurs following 6 months of healing at the socket site. We will compare the healing results between the two graft techniques to learn which approach is most helpful in grafting extraction sockets. You will also be asked to record any pain medication you take or symptoms you feel during the first week after surgery, so that we can learn more about how these surgeries affect patients.

Declaration:

- I have read, or had read to me, the Patient Information Leaflet for this study and I understand the contents. I have been given a copy of this information leaflet to keep.
- The research study has been fully explained to me in terms I can understand. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. The research team has explained why the research is being done and any foreseeable risks or consequences involved. I understand that no guarantees can be given about possible results of treatment.
- I freely and voluntarily agree to be part of this research study, without prejudice to my legal and ethical rights. I have received a copy of this agreement.

_____ I give permission for my medical records to be looked at and information taken from them to be analysed in strict confidence by the study investigators or by people from organisations supervising the conduct of their research.

_____ I agree to the possible publication of results from this study

_____ I consent to my records, including radiographs and photographs, being used for research purposes, including possible use in research publications or scientific presentations on the outcomes of this research.

_____ I understand that in any of the research or educational purposes referred to above, my anonymity will be preserved and my name will not be linked to any of these records

_____ I have been told that all medical information/data relating to me will be protected by the principles of confidentiality and both national and EU legislation
I understand that I may withdraw from this study at any time I wish

Participant's name: _____

Contact details: _____

Participant's signature: _____

Date: _____

I have explained the nature and purpose of this research study, the procedure is to be undertaken and any risks that may be involved. I have offered to answer any questions the participants may have and fully answered such questions. I believe the participant understands my explanation and has freely given informed consent.

Investigators signature: _____

Date: _____

Socket Preservation Study

Date:

Subject No:

Reason for extraction:

Bone dehiscence present?

Tooth Distal

Probing depth at mid-col

Gingival margin position

Tooth Mesial

Probing depth at mid-col

Gingival margin position

Socket Measurements

Depth (mm)

External width (mm)

Internal width (mm) Buccal-Lingual

Internal width (mm) Mesio-Distal

At (Crest – 1mm)

Width of buccal bone wall

Width of lingual bone wall

Width of ridge at mid-buccal (external)

Socket Preservation Study – Data Collection End of Study

Date:

Subject No:

Tooth Distal to site

Probing depth at mid-col

Gingival margin position

Tooth Mesial to site

Probing depth at mid-col

Gingival margin position

Extraction site Measurements - At (Crest – 1mm)

Width of ridge at mid-buccal (soft tissue included) (mm)

Surgical data collection

External width of ridge (bone only) (mm)

Height to mid-crest from adjacent tooth CEJ

Bone density at site

Adequate bone to place ideally positioned implant?	YES	NO	N/A
Additional grafting needed to allow implant placement?	YES	NO	N/A

Osstell reading:

Patient Diary of Symptoms

Today's Date:

Directions: Answer the following questions in terms of how you felt in the past 24 hours.

1. How much did having your graft placed interfere with your ability to do each of the following in the past 24 hours? (Circle one number for each statement that applies to you).

Function	None	A Little	Some	Quite a bit	Lots
Chew the foods you want	1	2	3	4	5
Open your mouth wide	1	2	3	4	5
Talk and carry on conversations	1	2	3	4	5
Sleep	1	2	3	4	5
Go to work or school	1	2	3	4	5
Perform your regular daily routine	1	2	3	4	5
Carry on your regular social life	1	2	3	4	5
Participate in your favourite recreation activity	1	2	3	4	5

2. ***At its worst…***
   How would you rate your pain during the past 24 hours? Place an X on the line to indicate the severity of the pain at its worst

No Pain ——————————————— Worst pain Imaginable

3. **On the average…**
   How intense was your pain in the past 24 hours? Place an X on the line to indicate the average intensity of pain

No Pain ——————————————— Worst pain Imaginable

4. How much did your pain interfere with your routine activities in the past 24 hours? Place an X on the line to indicate the amount of interference.

No Interference	———————————————	Unable to carry on any activities

5. How much did you experience any of the following in the past 24 hours? Circle one number for each condition that applies to you.

Symptoms	None	A little	Some	Quite a bit	Lots
Bleeding	1	2	3	4	5
Bruising	1	2	3	4	5
Swelling	1	2	3	4	5
Nausea	1	2	3	4	5
Bad taste/Bad breath	1	2	3	4	5
Food collecting at the site of your surgery	1	2	3	4	5

6. If you took any medication today to relieve pain or swelling…
Write in the type of medication, the strength, and the number of pills you took today

Name of medication	Strength (such as mgs)	Number of pills taken in last 24 hr

Please store this sheet carefully and bring to your next dental appointment.

Post-Operative Assessment

Study ID:

Date: **(Circle one)** 7 Day 14 Day

Operator 1 initials: **Operator 2 initials:**

Directions: Assess presence/absence of the following features based on your clinical assessment.

1. Post-operative swelling (Operator 1 & 2 – Consensus)
   Please make an independent assessment of the extent of swelling at the surgical site using the scale below. Discuss with your fellow operator and agree on a decision of severity. Circle the answer you feel best quantifies the extent of swelling at the surgical site.

No swelling	Mild swelling	Moderate swelling	Severe swelling
0	1	2	3

2. Wound Healing (Operator 1 only)
   For each of the clinical signs below, evaluate whether that sign is present or absent at the surgical site today. Circle the appropriate answer.

Clinical Feature	Score 0	Score 1
Bleeding (Spontaneous/on palpation)	Yes	No
Suppuration	Yes	No
Bruising	Yes	No
Wound dehiscence	Yes	No
Flap closure maintained	Yes	No
Tissue colour	Redder or whiter than opposite side tissue	Like opposite side tissue

APPENDIX H – LETTER OF ETHICAL APPROVAL - MEMBRANE DEGRADATION STUDY (CHAPTER 5)

DUBLIN DENTAL
UNIVERSITY HOSPITAL
OSPIDÉAL DEACHAIDH
OLLSCOILE ÁTHA CLIATH

Lincoln Place, Dublin 2,
Ireland, D02 F859.
Plás Lincoln, Baile Átha Cliath 2,
Éire, D02 F859.

Secretary of the Ethics Committee,
Faculty of Health Sciences Research Ethics Committee
Trinity College, Dublin 2.

30th September 2021

Re: An in vitro comparison of the degradation of L-PRF and A-PRF membranes in plasmin.
(FHS REC Ref 210208)
Investigator - Peter Harrison
TCD Student: 96503351

To whom it concerns,

This letter serves as certification that conduct of the above-named study has been approved by Dublin Dental University Hospital (DDUH).

The study in question will be conducted by Dr Peter Harrison, under the supervision of his research supervisor Dr Ioannis Polyzois, in part-contribution towards his PhD in periodontology at DDUH, entitled *"A clinical & biochemical investigation of the use of L-PRF in the regeneration of periodontal and peri-implant defects"*. The study will include collection and centrifugation of a blood sample from eight adult volunteers. This will be conducted on-site at DDUH and will involve a single blood collection, after which participants' involvement in the study will be complete.
This will be followed by laboratory work to analyse the degradation of the collected blood clots, in conjunction with collaborators at the Royal College of Surgeons in Ireland (RCSI). All samples will be anonymised on collection and will be fully disposed of at the end of the study period (14 days).

Prior to his submission for ethical approval via your committee, Dr Harrison sought preliminary feedback from the local ethics committee (School of Dental Science Ethics Committee). Based on his plan to collect a blood sample from each participant, this was beyond the scope of our school committee and referral to the Faculty of Health Science Research Ethics Committee (FHS REC) was indicated. He advises me that this study has now received conditional approval from the FHS REC, pending the furnishing of a letter from DDUH. I am satisfied to confirm that he has approval from the School and Hospital to conduct the study. Please let me know if you require any further information.

Yours sincerely,

M. Thérèse Garvey

Dr Thérèse Garvey
Clinical Director
Dublin Dental University Hospital,
Trinity College Dublin, Dublin D02 F858
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THIS NOTEPAPER MUST NOT BE USED FOR PRESCRIPTIONS OR INVOICING PURPOSES

SJH/AMNCH Research Ethics Committee Secretariat
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Dr. Ed Madeley
Dublin Dental University Hospital
Trinity College Dublin
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Dublin 2

18th May 2017

Re: Longitudinal analysis of treatment of peri-implant disease utilising magnetic resonance frequency analysis

REC: 2017-05 list 19 (2)
Reference: (Please quote reference on all correspondence)

Dear Dr. Madeley,

Thank you for your correspondence in which you responded to the concerns of the SJH/AMNCH Research and Ethics Committee.

The Chairman has reviewed this correspondence, is happy with the responses therein and has given ethical approval for this study to proceed.

Yours sincerely,

Claire Hartin
Secretary
SJH/AMNCH Research Ethics Committee

The SJH/AMNCH Joint Research and Ethics Committee operates in compliance with and is constituted in accordance with the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004 & ICH GCP guidelines.

APPENDIX J – DATA COLLECTION SHEET - PERI-IMPLANTITIS STUDY (CHAPTER 6)

2-3-Year Data Sheet – Peri-implant Study

Patient Name	
Chart	
Implant Location	
Restoration Type	
SmartPeg Required	
Treatment provided at baseline	

Time point		Baseline (Day of Treatment)			12-month Follow-up			2-3 year Follow-up		
Date										
Lingual	Plaque									
	BOP									
	Rec									
	PD									
Buccal	PD									
	Rec									
	BOP									
	Plaque									
Time point		Baseline			12-month Follow-up			2-3 year follow-up		
ISQ Osstell Readings		1	2	Mean	1	2	Mean	1	2	Mean
KT Width										