A Quasi-experimental Study Comparing the Effects of L-PRF and White Porous Titanium Granules on the Preservation of the Buccal Bone Following Immediate Implant Placement

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Author's Declaration

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Summary

The major advantage of placing an implant at the time of extraction is that it reduces the number of surgical procedures and reduces the overall treatment time. It is now well established that immediate implant placement does not prevent the bony modelling and remodelling that occurs following tooth extraction, as once originally thought. As a result of this, several surgical techniques have been attempted / introduced to minimise this inevitable bone resorption.

The aim of this quasi-experimental study was to compare the effects of leucocyte and platelet-rich fibrin (L-PRF) and white porous titanium granules (WPTG) on the preservation of the buccal bone plate following immediate implant placement. It also attempted to identify the possible variables affecting this remodelling.

35 implants were placed in the anterior maxilla immediately following tooth extraction. Following implant placement clinical measurements were made to determine the dimensions of the alveolar ridge and the void between the implant and the buccal bone. Photographs were also taken to compliment the clinical measurements. The voids were then grafted with either L-PRF or WPTG. The clinical and photographic measurements were repeated at 2nd stage surgery following 4 months of submerged healing.

The results of this study reaffirm those already described in the literature. Immediate implant placement with simultaneous bone regeneration does not prevent bone remodelling following tooth extraction. Both grafting protocols

resulted in clinically acceptable results and a 100% survival rate, at least in the short term. WPTG was superior to L-PRF resulting in greater buccal bone thickness and buccal bone height at 4 months, although the differences were not statistically significant.

As shown in previous studies, statistical analysis determined the gap width, the buccal bone thickness and the horizontal buccal bone dimension were the main indicators for the ridge width at re-entry. It was also demonstrated that specific socket characteristics were key determinants of the magnitude of alveolar ridge remodelling, in particular gap width and gap depth.

In conclusion, the grafting protocols employed in this study can be considered successful following immediate implant placement in the anterior maxilla. However, WPTG appears to be superior to L-PRF in preservation of the buccal bone plate.

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BBHD: Buccal bone horizontal dimension; measured by combining BBW-C and GW

BBW-C: Buccal bone width measured 1mm apical to the crest

BBW-M: Buccal bone width measured half-way to the apical end of the socket

BBW-PO: Buccal bone width post-op measured 1mm apical to the crest at 2nd stage

BIC: Bone-to-implant contact

BSD: Buccal socket depth

BWR: Buccal width reduction

CBCT: Cone-beam computed tomography

CIP: Conventional implant placement

DBBM-C: Demineralised bovine bone mineral with 10% collagen

First BIC: first bone-to-implant contact measured from the rim of the implant to the most coronal buccal bone

GBR: Guided bone regeneration

GDB: Gap depth measured in contact with the bone

GDI: Gap depth measured in contact with the implant surface

GW: Gap width

L-PRF: Leucocyte and platelet-rich fibrin

LSD: Lingual socket depth

MRF: Midfacial recession

PH: Papilla height

PPP: Platelet-poor plasma

PRP: Platelet-rich plasma

PWC: Palatal bone width coronally

RBC: Red blood cells

RW-PO: Ridge width post-op at 2nd stage

RW: Ridge width

RWR: Ridge width reduction

WPTG: White porous titanium granules

1 Introduction

The placement of implants into fresh extraction sockets was first documented in Germany in the late 1970's (Schulte et al., 1978). The proposed advantages of placing implants into fresh extraction sockets were a reduction in treatment time and the preservation of the bony walls of the socket (Lazzara, 1989, Paolantonio et al., 2001). It wasn't until the ITI consensus statement in 2004 that the timing of implant placement was first extensively scrutinised (Hammerle et al., 2004). This statement outlined definitions for the timing of implant placement and their advantages and disadvantages based on the evidence available at that time.

These definitions are as follows:

- Type 1 / Immediate: the implant is placed immediately following tooth extraction and as part of the same surgical procedure
- Type 2 / Immediate delayed: the implant is placed 4-8 weeks after tooth extraction to allow complete soft tissue coverage of the socket
- Type 3 / Immediate delayed: the implant is placed 12-16 weeks after tooth extraction to allow substantial clinical / radiographic bone fill of the socket
- Type 4 / Delayed: the implant is placed >16 weeks after tooth extraction when the socket has fully healed

This terminology has since been simplified (Chen, 2008). The two immediate delayed categories have been merged to be called 'Early implant placement'. Therefore the accepted terms are immediate, early and delayed.

The major advantage of placing an implant at the time of extraction is that it reduces the number of surgical procedures and reduces the overall treatment time (Gotfredsen et al., 1993, Hammerle et al., 2004). It was also originally proposed that this technique minimises bone resorption around the extraction socket following extraction (Paolantonio et al., 2001) but this concept/theory has since been disproved (Araujo et al., 2005, Araujo et al., 2006b, Botticelli et al., 2004). It is now well established that immediate implant placement does not prevent the bony modelling and remodelling that occurs following tooth extraction (Lee et al., 2014) and as a result of this, several surgical techniques have been attempted / introduced to minimise this inevitable bone resorption. Following tooth extraction and immediate implant placement, vertical and horizontal defects are created between the implant surface and inner bone walls. Several studies have shown that the placement of a grafting material in these gaps, and in particular, the buccal gap, reduce the amount of vertical and horizontal bone loss (Araujo et al., 2011, Chen et al., 2007, Sanz et al., 2016). Studies have also attempted to identify the socket characteristics that may influence the bony remodelling after implant placement (Ferrus et al., 2010, Tomasi et al., 2010), as well implant position in the socket (Caneva et al., 2010c) and implant shape / macrodesign (Sanz et al., 2010).

As a result of this inevitable bony remodelling following tooth extraction, immediate implants have been associated with some aesthetic complications, in particular midfacial recession (Chen and Buser, 2014). This systematic review estimated a frequency of midfacial recession of >1mm at a median of 26% of sites, 1-3 years after immediate implant placement. Although, they acknowledged there are a

limited number of studies with long term follow up (>5 years). One such study reported a high incidence of aesthetic complications with 8/22 patients developing >1mm of midfacial recession over a 5 year period (Cosyn et al., 2016).

It has been recommended to use a grafting material with a low substitution rate to minimise bone alterations following immediate implant placement (Buser et al., 2017). The majority of studies have used deproteinised bovine-derived bone mineral to graft the buccal gap (Chen et al., 2007, Cosyn et al., 2016, Sanz et al., 2016) exhibiting good results. The biocompatibility and osetoconductivity of bovine bone has been previously demonstrated in several preclinical studies (Hammerle et al., 1997, Schmid et al., 1997). However, whether deproteinised bovine bone mineral is bioresorbable still remains unclear (Berglundh and Lindhe, 1997, Fugazzotto, 2003). A clinical trial found particles of deproteinised bovine-bone unchanged in the bone 11 years after sinus augmentation (Mordenfeld et al., 2010). It is this lack of resorption that makes deproteinised bovine-bone suitable for minimising bone loss around immediate implants.

A non-resorbable biomaterial has recently been developed for use a bone substitute in periodontal and implant regenerative procedures (Natix™, Tigran Technologies AB: Malmo, Sweden). It is a porous material made of commercially pure titanium and is considered osteoconductive, acting as a scaffold for osseous ingrowth (Wohlfahrt et al., 2010). In contrast a biomaterial with a high substitution rate, called platelet-rich fibrin (PRF), has also been developed for use in oral tissue regeneration (Choukroun J, 2001). PRF is an autogenous source of platelets and growth factors, trapped in a fibrin mesh and has been described as an optimised

blood clot (Dohan Ehrenfest et al., 2010a). PRF has been shown to release growth factors for up to 10 days (Dohan et al., 2006b) and has the ability to stimulate proliferation and differentiation of human oral bone mesenchymal stem cells (Dohan Ehrenfest et al., 2009b).

This study aims to compare the effects of leucocyte and platelet-rich fibrin and white porous titanium granules on the preservation of the buccal bone plate following immediate implant placement. It also aims to collect data regarding specific bone characteristics of the alveolar socket and ridge prior to an immediate implant placement and to correlate them to the thickness of the buccal bone plate as well the thickness of the alveolar ridge at the second stage surgery, after four months of healing.

2 Literature review

2.1 Survival rates of Immediate Implants

The criteria for success of osseointegrated endosseous implants was outlined by Smith and Zarb in 1989 when they suggested a success-rate of 85% at 5-years and 80% at 10-years of observation (Smith and Zarb, 1989). A Cochrane review was published in 2010 which looked at how the timing of implant placement can effect the treatment outcome (Esposito et al., 2010). Due to the strict inclusion criteria there were only 7 randomised trials that met their requirements. There was an overall high risk of bias amongst the studies, which were also considered underpowered. It was concluded that immediate and immediate-delayed implants may be at higher risk of failure and complications than delayed implants but aesthetic outcomes may be superior when placing the implants immediately.

A recent systematic review aimed to evaluate the success and survival rate of implants placed immediately into fresh extraction sockets (Lang et al., 2012). A total of 46 prospective studies with 2908 implants placed and a mean follow-up time of 2.08 years met the inclusion criteria. There was an annual failure rate of 0.82% (95% CI: 0.48-1.39%) and a 2-year survival rate of 98.4% (97.3-99%). A total of nine studies with a follow-up period of 3 years or longer were analysed separately and yielded a 4-year implant survival rate of 97.5% (95.2-98.8%). Several factors were analysed to determine their influence on the survival rate. The use of antibiotics was the only factor to have statistically significant results. The annual failure rate was

lower after a 5-7 day post-operative course of antibiotics (0.51%) compared to a single dose of pre-operative antibiotics (1.87%) (P=0.002).

Subsequently another systematic review and meta-analysis compared dental implants inserted in fresh extraction sockets versus healed sites (Chrcanovic et al., 2015a). Their search yielded 73 publications with 8,241 implants in fresh extraction sockets, with a failure rate of 4%. This was in comparison to a 3.09% failure rate for 19,410 implants placed in healed sites. This difference was significant for the studies with implant supported single crowns but not for those with full-arch prostheses. Therefore it was suggested the placement of implants in fresh extraction sockets increases the risk for failure by roughly 1.5 times (RR 1.58, 95% CI 1.27-1.95, P<0.0001).

Another systematic review from the same author considered the outcome of implants placed into infected sites (Chrcanovic et al., 2015b). This review suggested that implants placed in sites with endodontic or periodontal lesions can successfully osseointegrate provided appropriate clinical procedures are performed prior to implant installation. These procedures include complete removal of granulation tissue / alveolar debridement and meticulous cleaning of the socket. The authors acknowledged the results should be interpreted with caution due to the heterogeneity and short-term follow-up of the studies included.

2.2 Healing of Extraction Sockets

2.2.1 Histological events

There have been numerous animal studies carried out in the past aiming to characterise the changes that occur following the extraction of a tooth (Cardaropoli et al., 2003, Araujo and Lindhe, 2005). These models have allowed examination of the whole extraction socket with surrounding soft and mineralised tissue, for up to 180 days (Cardaropoli et al., 2003). In the first stage, blood immediately fills the socket forming a coagulum comprised mainly of erythrocytes and platelets trapped in a fibrin matrix. In the second stage the coagulum is replaced by a richly vascularised granulation tissue over a period of up to 7 days. Following this a connective tissue matrix slowly replaces the granulation tissue and is rich in blood vessels and inflammatory cells. At 14 days the inflammatory cell infiltrate is limited to the coronal third of the socket with mineralised bone dominating the apical two-thirds. The fourth stage begins at 7-10 days with appearance of osteoid at the base of the socket. Woven bone can be observed extending from the socket walls to the centre of the wound by day 14. In 4-6 weeks most of the socket is filled with newly formed bone and the presence of osteoclasts indicates that the process of remodelling is ongoing. A hard tissue bridge forms to separate the marginal mucosa from the extraction socket and is comprised of lamellar bone deposited on top of woven bone. As remodelling progresses bone marrow occupies the greatest proportion of the socket with few remaining inflammatory cells and only small amounts of mineralised bone. The fifth stage is characterised by the epithelialisation of the socket margins. At 14 days the epithelialisation is characterised by a connective tissue rich in vessels and inflammatory cells at the margins of the socket.

This process is usually complete by day 30 when the connective tissue is lined with keratinised epithelium and accompanied by a reduction in inflammatory infiltrate in the coronal aspect of the socket (Cardaropoli et al., 2003).

A study of the healing in human extraction sockets demonstrated there is great variability in the remodelling process (Trombelli et al., 2008). A provisional connective tissue predictably forms within the first 2-4 weeks. However, the process by which woven bone and subsequently lamellar bone is laid down is much less predictable. There was high heterogeneity in the composition of specimens at each time interval and the process of remodelling was not completed at 24 weeks after tooth extraction. The results from the animal studies appear homogenous in comparison (Cardaropoli et al., 2003).

2.2.2 Ridge dimensional changes

The shape and volume of the alveolar process can be determined by several factors including; the number of teeth present, the anatomy of the roots and the direction the teeth follow during eruption (Tallgren, 1972, Marks and Schroeder, 1996). Following tooth extraction there is complete loss of cementum, periodontal ligament and bundle bone and as a result the alveolar process undergoes atrophy (Cardaropoli et al., 2003) (Araujo and Lindhe, 2005). In response to the changes in the alveolar process there are dimensional changes in the overlying soft tissues (Schropp et al., 2003).

2.2.2.1 Experimental studies

Several experimental studies have documented the dimensional and structural changes following tooth extraction in mandibular premolars of dogs (Cardaropoli et al., 2003) (Araujo and Lindhe, 2005). Bone modelling can be defined as a change in the shape and architecture of the bone, whereas bone remodelling is change without concomitant change in shape and architecture of the bone (Araujo et al., 2015b). A study by Araujo and Lindhe in 2005 measured the alterations of the height and width of the bone crest following tooth extraction over an 8-week period. They demonstrated a vertical bone loss of about 2.2mm on the buccal aspect, using the lingual crest as a reference. Some weeks after extraction osteoclasts were present on the outer and inner aspects of the buccal and lingual crests. The greater buccal bone loss (compared to lingual bone) was attributed to the thinner buccal bone plate comprised mostly of bundle bone. Bundle bone is considered a tooth-dependent structure and hence it's resorption following tooth extraction. The lingual wall is comprised of both bundle bone and lamellar bone. The inner portion of the socket is known as bundle bone, which is the tissue in which the extrinsic collagen fibre bundles of the periodontal ligament are embedded (Lindhe J, 2008). Bone modelling in humans is about two-thirds complete within 3 months (Schropp et al., 2003), unlike bone remodelling, which may take substantially longer (Trombelli et al., 2008).

Attempts to counteract this modelling process following tooth extraction have utilised approaches such as socket grafting (Araujo and Lindhe, 2009b) and immediate implant placement (Araujo et al., 2005, Araujo et al., 2006a, Araujo et al.,

2006b). These approaches along with other factors influencing post-extraction dimensional alterations will be discussed below.

2.2.2.2 Clinical studies

The dimensional changes of the alveolar ridge following tooth extraction have been of interest for several decades (Carlsson and Persson, 1967). With the advent of ridge preservation / socket grafting techniques (Ten Heggeler et al., 2011) it is becoming an increasingly popular area of research. In recent years, three systematic reviews have reported on the dimensional changes occurring following tooth extraction in humans (Van der Weijden et al., 2009, Ten Heggeler et al., 2011, Tan et al., 2012). All three had similar eligibility criteria for which studies would be included; randomised-controlled clinical trials, controlled clinical trials, prospective clinical studies and case series documenting the natural healing post-extraction dimensional changes relative to a fixed reference point over a specific time period. Most of the data extracted from these papers is from control groups of studies evaluating socket preservation. There was however some studies designed to evaluate dimensional alterations (Carlsson and Persson, 1967, Schropp et al., 2003, Moya-Villaescusa and Sanchez-Perez, 2010, Rodd et al., 2007). Combining the three systematic reviews there were twenty papers included for analysis and they employed three different methods of measuring the post-extraction dimensional changes; direct clinical measurements in surgical re-entry procedures, imaging techniques using linear measurements or subtraction radiography and measurements made on sequential study models.

One such study used study models and subtraction radiography to measure the clinical and radiographic changes with 12 months follow-up (Schropp et al., 2003). It was demonstrated that most of the bone fill and the loss of height of the alveolar ridge took place during the first 3 months. In addition the width of the ridge was reduced by 50%, two-thirds of which occurred in the first 3 months. Beyond 3 months there was little change in the dimension of the sockets. The bone levels on the mesial and distal surfaces of the adjacent teeth were largely unchanged at 12 months, with a loss of 0.1mm, however, the bone levels corresponding to the mesial and distal of the extracted tooth reduced by 0.3mm (Schropp et al., 2003).

Similar to Schropp et al 2003, two re-entry studies using acrylic stents to measure direct bony changes, demonstrated the bone loss at the mesial and distal aspects of a tooth is less than that observed on the buccal and / or lingual surfaces (Barone et al., 2008, Aimetti et al., 2009). This can be explained by the presence of adjacent teeth to the extraction sites. They also demonstrated that the buccal vertical resorption is consistently greater than the lingual vertical resorption; 0.9-3.6mm versus 0.4-3.0mm at 3-7 months (Barone et al., 2008, Aimetti et al., 2009, Iasella et al., 2003). The difference in height between the buccal and lingual walls appears to be less than that in the experimental studies (Araujo and Lindhe, 2005). It has been suggested the buccal and lingual walls are equally susceptible to resorption in humans (Van der Weijden et al., 2009). Tan et al (2012) in a systematic review calculated the overall percentage change in height of the buccal bone wall over a 6 month period as between 11-22% post-extraction. This review also calculated the hard tissue horizontal dimensional changes, as measured from the crest of the alveolar ridge, using the data from five re-entry studies (Lekovic et al., 1997, Lekovic et al., 1998,

Camargo et al., 2000, Iasella et al., 2003, Pelegrine et al., 2010). This resorption ranged from 2.46-4.56mm with a weighted mean resorption of 3.79mm at 6 months. It has been demonstrated that the horizontal ridge resorption decreases as the distance from the alveolar crest increases i.e. moving in an apical direction (Kerr et al., 2008, Farmer and Darby, 2014). The overall horizontal ridge reduction was calculated as a 32% reduction at 3 months and a 29-63% reduction at 6 months (Tan et al., 2012). Despite all of the above, the heterogeneity of the studies included in the systematic reviews was high and therefore weighted mean values should be interpreted with caution.

The use of cone beam computed-tomography is now considered an important diagnostic tool in implant planning (Tyndall et al., 2012) More recently it has been utilised as a non-invasive method of measuring the dimensional changes following tooth extraction and other interventions (Chappuis et al., 2013, Araujo et al., 2015a). The dimensional changes observed in these studies are similar to those described in the clinical studies above. A randomised clinical trial compared the effects of Bio-Oss Collagen to non-grafted controls on ridge alterations following tooth extraction in the maxilla (Araujo et al., 2015a). Using CBCT to measure the dimensional changes at 4 months it was observed that sites allowed to heal naturally had an overall reduction in cross-sectional area of the edentulous site of 25%. Another study by the same group then analysed the dimensional changes of similar edentulous sites with 12 months of follow-up (Misawa et al., 2016). At 12 months the overall cross-sectional area of the edentulous sites reduced by 34%. The majority of the resorption occurred in the marginal region (>60%) and interestingly there was significant resorption in the more apical regions with >40% 5mm below the crest

and >30% 7mm below the crest. This resulted in a triangular ridge configuration (Misawa et al., 2016). This apical resorption was not observed in the experimental studies. (Cardaropoli et al., 2003, Araujo and Lindhe, 2005). A prospective study of 39 patients, again utilising CBCT, measured dimensional changes following flapless tooth extraction at 8 weeks in the aesthetic zone (Chappuis et al., 2013). They identified a critical buccal bone thickness of <1mm, at which pronounced bone resorption occurred in the central portion of the buccal wall, with a median of 7.5mm. When the buccal bone was >1mm bone resorption was only 1.1mm.

2.2.3 Factors influencing socket healing

Several possible factors have been identified which may influence the dimensional changes following tooth extraction. It has generally been accepted that raising a full mucoperiosteal flap will cause resorption of bone due to the disruption of the vascular supply to the periodontium (Wood et al., 1972, Yaffe et al., 1994). In the dog model it was demonstrated that there was less bone resorption following extraction of a tooth without raising a mucoperiosteal flap (Fickl et al., 2008a). In contrast, another study in the dog did not demonstrate a difference in bone resorption following tooth extraction with and without flap elevation at 6 months (Araujo and Lindhe, 2009a). It has been suggested the resorptive changes following extraction can be limited with the use of minimally invasive techniques and instruments (Muska et al., 2013, Araujo et al., 2015b).

The location of teeth in the arch has not been shown to influence the degree of vertical dimensional change post-extraction (Moya-Villaescusa and Sanchez-Perez, 2010). There was no statistically significant difference between the vertical loss of bone in single-rooted teeth (4.16mm) and multi-rooted teeth (4.48mm).

Smoking was found to result in a greater amount of vertical bone loss at 6 months post-extraction; 1.5mm in smokers versus 1.0mm in non-smokers (Saldanha et al., 2006).

Rinsing with chlorhexidine for 1-month post-extraction was shown to maintain crestal bone levels at 6 months compared to a loss of 1mm bone height when rinsing with a placebo solution (Bragger et al., 1994).

The wearing of immediate dentures post-extraction was shown to cause greater resorption in the short term, but the difference in conventional and immediate denture wearing at 2 years was insignificant (Carlsson and Persson, 1967).

The extent of bone loss following tooth extraction may be influenced by the thickness of the buccal bone (Chappuis et al., 2013). It was shown there is up to 3.5 times more vertical resorption when the buccal bone is of <1mm thickness as opposed to sites of >1mm thickness. The buccal bone thickness in the anterior maxilla has been identified using CBCT as <1mm in roughly 90% of sites (Braut et al., 2011) and <0.5mm in almost 50% of sites (Januario et al., 2011). The high frequency of the thin buccal plates in this region likely contributes to the extent on bone loss following extraction.

2.3 Socket Preservation

As previously discussed following tooth extraction there is loss of ridge dimensions and contour (Schropp et al., 2003). A recent systematic review demonstrated the alveolar ridge undergoes a mean horizontal ridge width reduction of 3.8mm and a mean vertical ridge height reduction of 1.24mm following 6 months of undisturbed healing (Tan et al., 2012). These ridge alterations may inhibit the ability to place a dental implant and may also result in compromised aesthetic outcomes. As a result, socket / ridge preservation procedures have been recommended to counteract these changes.

Ridge preservation was recently defined as "preserving the ridge volume within the envelope existing at the time of extraction" (Hammerle et al., 2012). Many different techniques have been suggested for ridge preservation including, atraumatic flapless extraction (Fickl et al., 2008a), the immediate placement of dental implants (Paolantonio et al., 2001) and the filling of the socket with different grafting materials, with or without barrier membranes (Araujo and Lindhe, 2009b). According to the Osteology Consensus report from 2012 (Hammerle et al., 2012) the indications for ridge preservation procedures include the maintenance of a stable ridge volume for optimising functional and aesthetic outcomes, generation of good hard and soft tissue volume for implant placement, immediate or early implant placement is not possible, contouring the ridge for conventional prosthetic treatment and to reduce the need for sinus floor elevation. Many different materials have been employed in ridge preservation techniques including but not limited to; autogenous bone grafts, xenografts, mineralised and demineralised freeze-dried

bone allografts, synthetic materials, resorbable and non-resorbable membranes and soft tissue autografts. It is not within the remit of this review to discuss the outcomes of the different materials used in ridge preservation however, in a recent systematic review no significant differences were found between the various materials (Vignoletti et al., 2012).

2.3.1 Experimental studies

As previously discussed, it has been demonstrated in animal studies that there is significant resorption of the alveolar ridge following tooth extraction. The majority of this occurs due to resorption of the buccal bone plate (Araujo and Lindhe, 2005). Subsequently there were attempts to modify this resorption using several different socket-grafting techniques. An experimental study in beagle dogs compared the ridge alterations following tooth extraction with (flap) and without (flapless) raising a full mucoperiosteal flap (Fickl et al., 2008a). The study showed that removing a tooth without raising a flap i.e. flapless, resulted in lower resorption rates. In contrast, a similarly designed study, again in beagle dogs, did not find any significant differences in resorption rates of the alveolar ridge following flap and flapless tooth extractions (Araujo and Lindhe, 2009a). The different outcomes may be a result of the slightly different surgical techniques employed. The Fickl (2008) study used two vertical releasing incisions in their flap design, whereas Araujo & Lindhe (2009) used a more conservative intrasulcular incision, raising the flap to beyond the mucogingival junction.

Following on from this, xenogenic graft materials were tested in extraction sockets to see if they can influence ridge preservation. Two such studies compared the ridge dimensional changes 3 and 6 months following tooth extraction with and without the placement of Bio-Oss Collagen in the socket (Araujo et al., 2008, Araujo and Lindhe, 2009b). It was clearly demonstrated that the grafted sites better preserved the alveolar ridge profile compared to non-grafted sites. Comparable results were obtained in another study with a similar experimental design (Fickl et al., 2008b). The differences between the 3 month (Araujo et al., 2008) and 6 month (Araujo and Lindhe, 2009b) results in these studies are interesting. Between 3 and 6 months there was partial replacement of the woven bone with lamellar bone at the crest of the ridge i.e. cortical bone formation. This indicates that complete replacement of woven bone may require many more months or years. After 3 months of healing about 12% of the socket was occupied with Bio-Oss, indicating Bio-Oss did not enhance new bone formation but behaved as a scaffold for tissue ingrowth. Furthermore, Bio-Oss particles were not observed to be actively resorbed. Therefore the elimination of this biomaterial appears to be very slow. In another experimental study from the same research group, sockets were grafted with autologous chips of bone harvested from the buccal bone plate (Araujo and Lindhe, 2011). The autogenous bone failed both to stimulate new bone formation and failed to prevent ridge resorption following tooth extraction. The sockets exhibited similar dimensional and histological healing to that of non-grafted sites from their previous studies (Araujo and Lindhe, 2005, Araujo et al., 2008).

2.3.2 Clinical studies

There have been several systematic reviews on the outcomes of alveolar ridge preservation procedures. For example, a review and meta-analysis from 2012, including 14 studies with a minimum follow-up period of 3 months calculated the weighted mean differences in alveolar ridge dimension changes following ridge preservation and natural healing (Vignoletti et al., 2012). It found there was less ridge reduction following ridge preservation procedures compared to controls; 1.47mm (95% CI 1.98, 0.95) in terms of height and 1.83mm (95% CI -2.95, 073) in terms of width. A slightly more recent meta-analysis found similar trends with less reduction in bone height of 0.91 to 1.12mm and less reduction in bone width of 1.31 to 1.54mm following ridge preservation (Willenbacher et al., 2016). A Cochrane Review, which included only 8 randomised controlled trials, was published on the topic in 2015 (Atieh et al., 2015). It also had results favouring ridge preservation with less reduction in bone height of 2.6mm (95% CI 3.34 to 1.76) and less reduction in bone width of 1.97mm (95% CI 2.48 to 1.46). Lastly, a systematic review was conducted on ridge preservation techniques limited to non-molar teeth (Ten Heggeler et al., 2011). It concluded that ridge preservation is helpful in limiting the bone dimensional changes after tooth extraction. The primary outcome measures in most of the studies on ridge preservation is the change in height and width of the alveolar ridge, as measured on study casts, radiographs, computed tomography or by direct clinical re-entry measurements. Therefore, the systematic reviews have similar primary outcome measures. As a consequence, there is very limited data on whether these ridge preservation techniques allow subsequent implant placement without the need for additional augmentation and the long-term

success / survival rates of these implants. Willenbacher (2016) did calculate that implants could be placed in the desired position without further augmentation in 90% of sites that were preserved, compared to 79% of the control sockets. Based on the results of the above systematic reviews and meta-analysis ridge preservation does not prevent resorption of the alveolar ridge but it does reduce the ridge dimensional changes and allow for future implant placement. Despite similar trends in the outcomes of ridge preservation techniques the results should be interpreted with caution as even systematic reviews and meta-analysis have their limitations. A quality assessment of systematic reviews on alveolar ridge preservation was recently published (Moraschini and Barboza Edos, 2016). Some of the reviews were considered of good methodological quality but overall there was a large variation in the structure and methodology of the 12 systematic reviews included. None of the reviews obtained the maximum score using the AMSTAR tool or the checklist from Glenny et al 2003 (Glenny et al., 2003).

More recent studies have utilised cone beam computed tomography to assess the ridge dimensional changes following extraction with or without ridge preservation (Araujo et al., 2015a, Jung et al., 2013). In an randomised controlled trial, maxillary premolars, canines and incisors planned for extraction were assigned to receive a socket graft of Bio-Oss Collagen or left to heal spontaneously (Araujo et al., 2015a). A CBCT was taken immediately post-operatively and again 4 months later. While the socket grafting procedure failed to prevent resorption of the buccal and palatal walls, it did limit the resorption significantly compared to the control group. The cross-sectional area of the alveolar ridge was reduced by only 3% for the socket graft compared to 25% for the natural healing. Similar trends were observed in Jung

et al 2013. In another randomised controlled trial CBCT was obtained after 6 months of healing. The best results were achieved with Bio-Oss Collagen combined with a collagen membrane or an autogenous soft-tissue graft. The ridge width reduction 1mm below the crest was limited to 1.2mm and 1.4mm respectively.

As previously stated one of the main functions of alveolar ridge preservation is to maintain the existing hard and soft tissue envelope and to allow for future implant placement (Hammerle et al., 2012). There is however, a lack of data on the nature and quality of the tissue formed following these procedures and the influence that may have on the success of implant therapy. A recent systematic review and meta-analysis attempted to analyse the histological outcomes of alveolar ridge preservation techniques (De Risi et al., 2015). The review included 38 studies; randomised controlled trials, controlled clinical trials, prospective / retrospective clinical trials and case series with a minimum of 4 biopsies analysed per group. Meta-analysis was performed on the variations in mean percentage of bone, connective tissue and residual graft material. Overall there were no histological and histomorphometrical statistical differences found between all materials used or when compared to natural healing. Allograft procedures at 3 months produced the highest bone percentages of 54.4%, while xenografts at 5 months produced the lowest of 23.6%. Allograft procedures also produced the lowest percentage of residual graft material of 12.4-21.11%, while xenografts and alloplasts had their lowest percentages at 7 months (37.14 and 37.23%). Due to the fact there were no statistical differences between the ridge preservation procedures and natural healing in terms of bone and connective tissue percentages, the authors questioned the need to wait longer periods prior to implant placement. There was however

great heterogeneity in the included studies in terms of extraction sites, socket characteristics, surgical techniques, re-entry times and biopsy retrieval techniques.

2.4 Immediate implants

2.4.1 History

The placement of implants into fresh extraction sockets was first documented in Germany in the late 1970's (Schulte et al., 1978). However, it wasn't until the early 1990's that we began to see more research emerge on this topic. The early-proposed advantages of implants into fresh extraction sockets were a reduction in treatment time and the preservation of the bony walls of the socket (Lazzara, 1989, Paolantonio et al., 2001). Throughout this period there were several clinical case series and case reports on the various surgical approaches for bone regeneration around immediate implants (Lazzara, 1989, Becker and Becker, 1990, Gelb, 1993, Lang et al., 1994, Bragger et al., 1996, Schwartz-Arad and Chaushu, 1997, Grunder et al., 1999). Several studies used ePTFE non-resorbable membranes and membrane exposure was a common complication (Gelb, 1993, Lang et al., 1994, Bragger et al., 1996). Soon after this the use of resorbable membranes became more popular due to their lower complication rates and the fact they do not need a second procedure to remove them. It was recognised the terminology used up until this point was inconsistent across authors. This led to the publication of a classification of the timing of implant placement (Hammerle et al., 2004) which was subsequently simplified (Chen, 2008). As a result, the accepted terminology today is immediate, early and late implant placement and will be used in this thesis.

2.4.2 Experimental studies

The first experimental studies on implants in fresh extraction sockets aimed to determine if osseointegration occurred in a similar process to that of standard implant placement in a healed alveolus. The roots of specific teeth were extracted and machined-copied to a titanium analogue. This analogue was then implanted into the extraction socket either on the same day, 2 weeks later or implanted in the contra-lateral sockets. These experiments demonstrated that osseointegration did indeed occur on immediate implants with a high level of predictability (Lundgren et al., 1992, Kohal et al., 1997). However, they did not describe the physiological process of bone healing and dimensional changes that occur after implant placement into extraction sockets. This was subsequently investigated in a series of studies by Araujo et al (Araujo et al., 2005, Araujo et al., 2006a, Araujo et al., 2006b).

The histological process of healing around titanium implants i.e. osseointegration, was first described by Berglundh in 2003 using the wound-chamber model (Berglundh et al., 2003). Implants with a sandblasted and acid-etched surface were placed in the mandible of dogs and the healing process was analysed over a period from 2 hours to 12 weeks. The basic process consisted of the initial formation of a coagulum, replacement with granulation tissue, a provisional matrix of connective tissue, newly formed woven bone and subsequent lamellar bone formation. Bone formation could be recognised during the first week, in contact with the parent bone

(distance osteogenesis) and also de novo bone formation on the implant surface (contact osteogenesis) (Berglundh et al., 2003). In a similar wound-chamber model, osseointegration was analysed from 4 hours to 8 weeks after implant placement into fresh extraction sockets (Vignoletti et al., 2009b). The histological bone healing process described is similar to that observed after tooth extraction (Cardaropoli et al., 2003) and after implant installation in a healed ridge (Berglundh et al., 2003, Abrahamsson et al., 2004). There were some subtle differences between the two processes. Woven bone formation was not observed until the 2nd week in immediate implants as opposed to the 1st week in a healed ridge. There was however, evidence of bone formation in contact with the parent bone and new bone formation directly on the implant surface during protocols. Osteoclastic activity was greater around implants in fresh extraction sockets, being observed in both the wound chamber and the marginal regions at 1 week. The bone-to-implant contact (BIC) decreased from day 0 to 1 week, reaching around 5% and thereafter gradually increased (Vignoletti et al., 2009b). In contrast, the BIC at implants placed in healed ridges ranged from 14-25% at 1 week (Abrahamsson et al., 2004). It has been suggested the differences identified between the two processes is a result of the superimposition of the early remodelling of the socket with the normal process of osseointegration of the implant (Salvi et al., 2015).

As previously mentioned a series of studies investigated the dimensional changes of the alveolar ridge following implant placement into fresh extraction sockets (Araujo et al., 2005, Araujo et al., 2006a, Araujo et al., 2006b). Prior to these studies it was demonstrated that following tooth extraction a vertical bone loss of 2.2mm occurred on the buccal crest, relative to the lingual crest (Araujo and Lindhe, 2005). Using a

similar experimental model in beagle dogs, implants were placed into fresh extraction sockets with the SLA-coated surface flush or slightly apical to the buccal bone crest and allowed to heal for 12 weeks (Araujo et al., 2006a, Araujo et al., 2006b). The results suggest that implants in fresh extraction sockets do not prevent the modelling and remodelling processes following tooth extraction. There was a vertical bone loss of 0.7 ± 0.5mm and 2.1 ± 0.4mm on the buccal aspect after 4 and 12 weeks of healing respectively, with only 0.4 ± 0.4mm on the lingual aspect at 12 weeks (Araujo et al., 2006a). This suggests the majority of the crestal resorption occurred between 1 and 3 months. In addition to this there was a marked reduction in the thickness of the buccal bone walls between 4 and 12 weeks with relatively minor changes of the thickness of the lingual walls (Araujo et al., 2006b). In a similarly designed experimental study in beagle dogs, different results were obtained (Vignoletti et al., 2009a). The vertical bone loss on the buccal aspect following implant placement into fresh extraction sockets amounted to 0.73 ± 0.28mm after 8 weeks of healing. In addition the majority of this resorption occurred in the first week, in contrast to the Araujo et al 2006a study. However, these studies did use implants with different surface topography and characteristics, different implant diameters and employ different healing periods. Both of these beagle dog studies did show similar trends in the healing of the gap between the implant surface and the inner socket wall. Araujo 2006b compared the marginal defects around premolars and molars while Vignoletti et al 2009 compared the defects around the 3rd and 4th premolars (Araujo et al., 2006b, Vignoletti et al., 2009a). It was found the wider the combined defect and bone wall dimension the more coronal the bone-to-implant contact. It is not clear whether the crestal bone wall thickness or the gap size is more important but its has been suggested that due

to the reduced resorption observed on the lingual aspect and the relatively greater amount of bundle bone in the buccal wall, the thickness of the crestal bone wall plays the more important role (Vignoletti and Sanz, 2014).

2.4.2.1 Implant surface

The influence of implant macrodesign on the modelling of the buccal bone following immediate implant placement was investigated (de Sanctis et al., 2009). Four different commercially available implant systems were placed in the distal socket of mandibular premolars of beagle dogs and allowed to heal for 6 weeks. All systems healed with a predictable osseointegration with the mean BIC% ranging from 58.5% and 72.1%. There was no statistically significant difference in the amount of buccal bone resorption observed between the systems. Another study from the same group also failed to show a difference between two difference implant surfaces (Vignoletti et al., 2009b). A dual acid-etched surface (DAE, Osseotitie®, Biomet 3i) was compared to a surface modified by the deposition of discrete crystals of CaP (DCD nano-particles Nanotite™, Biomet 3i) both yielding similar BIC% of 45.7% and 42.4%, respectively.

2.4.2.2 Implant position

Another research group, again using a similar methodology to the Araujo et al studies, investigated the influence of the implant position in the extraction socket on osseointegration (Caneva et al., 2010c). Control implants were placed in the centre

of the socket with the margin of the rough surface flush to the buccal bone and the test implants were placed 0.8mm deeper and in contact with the lingual wall of the socket. Implants of 3.3mm diameter were used. There were similar degrees of resorption in absolute values in test and control sites but as a result of the deeper placement in test sites, there was a more coronal bone-to-implant contact in the buccal aspect. In a similar study from the same group, implants of different diameters, 3.3mm versus 5mm, were placed in fresh extraction sockets and allowed to heal for 4 months (Caneva et al., 2010d). Neither implant prevented resorption of the alveolar crest but there was statistically significant more resorption at the buccal aspect of the wider implants, 2.7 ± 0.4mm versus 1.5 ± 0.6mm.

2.4.2.3 Surgical protocol

There are two available studies comparing the influence of surgical protocol on the bony changes following implant placement in fresh extraction sockets (Blanco et al., 2008, Caneva et al., 2010b). Both compared immediate implant placement in mandibular premolars of dogs with and without raising a full mucoperiosteal flap and exposure of crestal bone. The extent of bone resorption following these two approaches has been analysed following tooth extraction in experimental studies with contrasting results (Fickl et al., 2008a, Araujo and Lindhe, 2009a). Similarly, these two studies had conflicting results. Blanco et al 2008 found the mean distance from the peri-implant mucosa margin to the first BIC on the buccal aspect was statistically significantly greater in the flap group; 3.02mm versus 3.69mm, therefore favouring the flapless group. In contrast Caneva et al 2010 found similar

levels of bone resorption occurred whether the procedure was performed with a flap or flapless; 1.7mm versus 1.5mm respectively.

2.4.2.4 Bone regeneration

Following the establishment that implant placement into fresh extraction sockets does not prevent the resorption of the surrounding alveolar bone crest, studies were conducted to test whether the use of bone-regeneration techniques could negate this inevitable bone loss. One such study compared immediate implant placement in the mandible of Labrador dogs with and without collagen-resorbable membranes with a fully submerged healing for 4 months (Caneva et al., 2010a). At both test and control sites bone resorption occurred but the presence of the collagen membrane resulted in less buccal bone resorption compared to the control site; 1.7mm of bone loss versus 2.2mm. Following on from their previous studies Araujo & Lindhe assessed the effects of a xenograft (Bio-Oss® Collagen) in the buccal gap of immediate implants in the beagle dog (Araujo et al., 2011). They used the same experimental design as before (Araujo et al., 2006a, Araujo et al., 2006b) but used implants of a narrower diameter (3.3mm versus 4.1mm) and the implants were placed in contact with the lingual wall of the extraction socket. The defects were roughly 1-2mm wide and 3mm deep. It was demonstrated at 6 months that the placement of the xenograft in the buccal gap reduced the amount of hard tissue loss compared to non-grafted controls. Grafted sites had a higher bone-to-implant contact on the buccal aspect as measured from the SLA surface; 0.1 ± 0.5mm versus 1.3 ± 0.7mm. In addition the thickness of the buccal bone was greater at all levels

from the SLA to 3mm apically. Histologically the bone that formed adjacent to the implant surface was comprised of woven bone and parallel fibered bone. Similarly positive results were obtained in a beagle dog study using porcine collagenated bone (MP3®, Osteobiol) to fill the buccal gap with the addition of a collagen membrane (Evolution®, Osteobiol) to cover the area of GBR (Barone et al., 2011). In contrast, another similar experimental study in labradors assessed the effects of deproteinised bovine bone mineral and a collagen membrane of porcine origin around immediate implants, with different results (Caneva et al., 2012). The grafted sites did have improved bone-to-implant contact over non-grafted controls however, both test and control sites had similar reductions in the buccal bone height; 1.8 ± 1.1mm and 2.1 ± 1mm, respectively. The conflicting results in this study may be explained by the different surgical protocols employed. The extraction socket of the mesial roots of the 3rd mandibular premolar was used compared to the distal socket of the 4th mandibular premolar (Araujo et al., 2011, Barone et al., 2011). This resulted in buccal marginal defects of reduced dimensions with defects being about 0.6mm wide and 3.1mm deep. It has been suggested the presence of a larger marginal gap may contribute to improved bone-to-implant contact levels (Araujo et al., 2006b).

2.4.3 Hard tissue changes

Several clinical studies have examined the changes in the alveolar ridge that occur following immediate implant placement in humans using direct bony measurements (Botticelli et al., 2004, Sanz et al., 2010, Sanz et al., 2016). This is usually conducted

by raising a full thickness flap at the time of implant placement and again at second stage surgery and comparing the dimensions of the alveolar ridge. These human studies have shown the same general results as the experimental studies described above, with significant resorption of the bone walls in a horizontal and vertical dimension. Similarly most of these changes occur on the buccal aspect.

One of the first such clinical studies compared the bone dimensional changes occurring after 21 implants were placed in extraction sockets and allowed to heal with a submerged protocol (Botticelli et al., 2004). No grafting material or membrane was used to fill the defects around the implants at the time of implant installation. Following 4 months of healing marked horizontal resorption of the buccal and palatal bone walls had occurred. The distance between the implant and the outer bone walls reduced from 3.4mm to 1.5mm (56%) on the buccal aspect and from 3.0mm to 2.2mm on the palatal aspect (30%). It was also observed that the marginal gaps around the implants were mostly resolved at 4 months, even if the gap were >3mm wide. Another clinical study from the same year also demonstrated clinical infill of the peri-implant gaps following immediate implant placement with a submerged protocol and without barrier membranes and / or grafting materials (Covani et al., 2004). A slightly more recent clinical trial compared cylindrical and conical implants placed into fresh extraction sockets with similar results (Sanz et al., 2010). Implant placement did not prevent ridge alterations following tooth extraction and there was marked horizontal reduction of both the buccal and palatal bone walls. The buccal wall reduced by 36%, which was roughly twice that of the palatal wall reduction of 14%. Similar to Botticelli et al (2004) this study reported infill of the marginal gaps around the implants between 4 months and baseline.

There was a horizontal reduction of the buccal gap by 71% and vertical reduction of about 60-70% (buccal and palatal). The dimensional changes were not significantly different between the two implant configurations. Using the same cohort of patients further analysis was done to identity the factors that may influence ridge alterations occurring at the buccal aspect of the extraction site following immediate implant placement (Ferrus et al., 2010, Tomasi et al., 2010). The thickness of the buccal bone wall significantly influenced the horizontal bone resorptive changes while the implant position influenced the amount of vertical bone change.

2.4.3.1 Bone regeneration

In a bid to counteract these resorptive changes following immediate implant placement the use of bone grafting materials and barrier membranes was suggested. A prospective randomised clinical trial evaluated the bony changes of non-submerged immediate implants in the anterior and premolar region in the maxilla using transmucosal implants with a sand-blasted and acid-etched surface (Chen et al., 2007). Following the placement of 30 immediate implants the sites were randomly assigned to receive deproteinised bovine bone mineral only, the combination of deproteinised bovine bone mineral and a resorbable collagen membrane or left unfilled (control group). There were no significant differences between the groups for vertical bone resorption. Of significance, the control group showed greater horizontal resorption (48.3 ± 9.5%) that the two grafted groups (15.8 ± 16.9% and 20 ± 21.9%), representing roughly a 25% decrease in horizontal buccal bone resorption. A larger and multi-centred randomised controlled trial also

evaluated the effects of grafting the buccal gap in immediate implant sites on the dimensional bone changes (Sanz et al., 2016). 86 implants were placed in non-molar sites in the maxilla and grafted with either deproteinised bovine bone mineral mixed with collagen 10% or left unfilled. Healing abutments were placed with a semi-submerged healing for 16 weeks prior to re-entry. The results showed that in the grafted sites there was less bone resorption on the buccal aspect compared to non-grafted controls; 1.1mm versus 1.6mm respectively. The benefits of grafting was more evident in sites with thin buccal walls of <1mm and in anterior areas. In sites with thin buccal bone of <1mm, the reduction in the crest dimension, as measured at the marginal region and 1mm apical to the crest, was significantly less pronounced in the grafted sites than in the non-grafted controls; 0.4mm versus 2.7mm and 0.7mm versus 2.3mm respectively. There was also significantly smaller reductions in the horizontal crest dimension in the grafted than non-grafted sites; 1.0mm versus 1.9mm. In contrast, when the buccal gap size was >2mm, the difference in the horizontal and vertical reductions between grafted and non-grafted sites did not reach statistical significance. Similar to other studies, gap closure exhibited a high degree of infill of 60-70% horizontally and 90% vertically. However, grafting did not improve gap closure nor did it affect the amount of vertical resorption. Based on the above two studies the placement of a bone graft appears to reduce the horizontal bone changes on the buccal aspect after implant placement in fresh extraction sockets.

A systematic review and meta-analysis has been carried out on the alterations of the bone dimension following immediate implant placement in humans (Lee et al., 2014). Six studies met the inclusion criteria reporting on the mean horizontal and

vertical dimension reductions with 4-12 months follow-up (Botticelli et al., 2004, Chen et al., 2007, Sanz et al., 2010, Degidi et al., 2013, Roe et al., 2012, Rossi et al., 2013). The weighted mean buccal horizontal bone dimension reduction (BHDr) was 1.07mm and the buccal vertical bone dimensional reduction (BVDr) was 0.78mm. However, there was considerable heterogeneity amongst the studies, with some using bone regenerative techniques, different surgical protocols and different methods of measurements. Subgroup analysis compared grafting and no grafting with a weighted mean BHDr difference of 0.53mm (0.79mm versus 1.32mm respectively).

More recently several studies have used cone beam computed tomography (CBCT) to assess the bony dimensions around immediate implants, avoiding the need to raise a flap and perform direct clinical measurements (Miyamoto and Obama, 2011, Roe et al., 2012, Degidi et al., 2013). Miyamoto et al 2011 compared the buccal bone thickness following immediate and delayed (in combination with GBR) implant placement. CBCTs were taken on average 28.2 months post operatively. Immediate implant placement was significantly associated with the least amount of buccal bone (0.48 ± 0.67mm) compared to delayed placement in conjunction with a non-resorbable membrane (2.22 ± 0.81mm) and a resorbable membrane (1.15 ± 0.82mm). Similarly, the amount of vertical bone loss (measured from the implant platform) was significantly greater for immediate implants (3.25 ± 4.68mm) compared to the delayed approaches (0.13 ± 0.36mm and 0.70 ± 1.02mm). Two studies with similar methodology but from different research groups analysed the bone changes around immediate implants grafted with bovine bone graft material and with immediate provisional restorations, using CBCT immediately post

operatively and again at 12 months (Roe et al., 2012, Degidi et al., 2013). Degidi et al 2012 placed 60 implants while Roe et al 2012 placed 21 implants. Both studies have very similar results. The vertical bone loss on the buccal aspect was 0.76 ± 0.96mm (Degidi et al., 2013) and 0.82 ± 0.64mm (Roe et al., 2012). The horizontal bone loss on the buccal aspect was 0.88 ± 0.51mm or 29% (Degidi et al., 2013) and 0.64 ± 0.55mm (measured 1mm below the implant platform) (Roe et al., 2012). As a result, the remaining buccal bone thickness at 12 months was 2.12 ± 0.92mm (Degidi et al., 2013) and 2.02 ± 1.17mm (Roe et al., 2012). The vertical and horizontal bone loss in these two studies is much reduced compared to that of Miyamoto 2011. This is likely due to the different surgical approaches employed, with better outcomes associated with flapless technique, immediate provisionalisation and the use of a slowly resorbing grafting material (Degidi et al., 2013, Roe et al., 2012). The benefit of grafting the buccal gap is again evident from the results of another study using CBCT to measure the bone around immediate implants placed without any grafting material (Rossi et al., 2013). At 4 months, the vertical and horizontal bone loss on the buccal aspect was 1mm and 1.9mm respectively, measured 1mm below the implant platform. In comparison, a mean buccal bone thickness of 2.2mm at 5-9 years is reported following early implant placement with contour augmentation (Buser et al., 2013). The above studies on immediate implants are of short-term follow up and therefore the long-term outcome of buccal bone stability remains unknown.

2.4.4 Soft tissue healing and Aesthetic outcomes

One of the frequently cited complications with immediate implants is the development of gingival recession on the mid-facial aspect. The systematic review by Lang (2012) reported that 20% of patients developed mid-facial recession of ≥1mm in studies with observation periods of 3 years or more (Lang et al., 2012). Similarly Chen et al (2007) reported 33.3% of sites had ≥1mm mid-facial recession at 6 months (n=10). However, this was significantly associated with a buccal position of the implant shoulder and recession occurred in only 16.7% of lingually positioned implants (Chen et al., 2007). In addition, the primary aim of that systematic review was to determine the survival rate of immediate implants, rather than assessing soft tissue changes. At that time there was a lack of primary studies investigating the soft tissue changes and / or aesthetic outcomes of immediate implants.

Since then there have been more reviews published regarding soft tissue response and aesthetic changes (Cosyn et al., 2012, Chen and Buser, 2014, Slagter et al., 2014, Khzam et al., 2015, Lee et al., 2016). There is however only one meta-analysis on the overall soft tissue changes around immediate implants and the effects of different surgical factors on the soft tissue levels (Kinaia et al., 2017). This review included 12 studies with at least 12 months of follow-up after functional loading of rough-surface immediate implants and reporting on mid-facial recession (MFR) or papilla height (PH) changes compared to conventional implant placement (CIP), in native/healed bone. MFR was less in CIP than IIP but the result was not statistically significant (mean difference -0.064mm; P=0.687). There was also better PH

maintenance in CIP but with only the distal papilla height showing statistical significance (mean difference -0.765, P>0.001). MFR was reduced in IIP with a thick tissue biotype but the difference was not statistically significant. When IIP with an immediate provisional was analysed, there was less MFR (mean difference 0.253, P=0.384), although not significant but there was significantly better PH maintenance versus conventional restoration (mean difference-0.519, P=0.028). This review acknowledged there were a limited number of studies included and a high level of heterogeneity.

The systematic review by Slagter et al (2014) used pooled analysis to determine soft tissue changes following immediate implant placement and found a greater mean MFR of -0.54 ± 0.39mm. In contrast to other reviews, a gain in mid-facial gingival level (0.07mm, 95% CI: -0.44 to 0.59: P=0.12) was found by a systematic review of immediate implant placement with simultaneous connective tissue grafting (Lee et al., 2016).

At the same time the classification for the timing of implant placement was published it was recognised there was a lack of criteria to evaluate the aesthetic outcomes following implant placement (Belser et al., 2004). In the period since then there has been a gradual increase in the reporting of aesthetic parameters and outcomes (Benic et al., 2012b, Annibali et al., 2012). A systematic review on the topic compared aesthetic outcomes following immediate and early implant placement in the anterior maxilla (Chen and Buser, 2014). There was considerable heterogeneity amongst included studies but the aesthetic outcome was mostly determined used the pink aesthetic score and positional changes in the peri-implant

mucosa. It concluded that aesthetic outcomes can be achieved with both immediate and early implant placement for single-tooth implants but that immediate implants are at higher risk of mid-facial recession. There was a higher frequency of MFR of >1mm (median 26% of sites, 1-3 years after placement) with immediate implants compared to early implant placement (no sites of >1mm recession). There are a limited number of studies with longer-term follow-up (≥5 years) and these studies appear to use the change in gingival margin position to determine aesthetic outcomes (Mura, 2012, de Carvalho et al., 2013, Ross et al., 2014, Cooper et al., 2014). There is one prospective study with 5-year follow-up evaluating the aesthetic outcomes of single immediate implants in the aesthetic zone (Cosyn et al., 2016). Patients considered a low aesthetic risk received an immediate implant with simultaneous grafting of the buccal gap and an immediate provisional restoration. Aesthetic outcomes were evaluated by mesial and distal papillary recession, mid-facial recession and pink aesthetic scores (PES). Aesthetic complications were high, with 5 out of 22 patients requiring additional soft tissue grafting due to advanced mid-facial recession 3 months after implant placement. At 5 years, 3 more implants demonstrated advanced mid-facial recession (≥1mm). Therefore at 5 years, a total of 8/17 carefully selected patients had aesthetic complications. Papilla height increased over the 5-year period ($p \leq 0.007$). The mean mid-facial recession was 0.53mm at 5 years and did not reach statistical significance ($p=0.072$). Interestingly, at 5 years, the cases treated with additional soft tissue grafting had similar levels of recession to those that did not receive grafting (0.5mm and 0.63mm respectively). The PES on both the mesial and distal papilla improved significantly however, the overall PES deteriorated during the 5 years from 12.15 to 11.18 ($p=0.03$).

2.4.5 Biological complications

A recent systematic review evaluating the survival of implants placed immediately into fresh extraction sockets (Lang et al., 2012) identified there was scarce reporting on the biological complications regarding this surgical approach. Regarding aesthetic complications, suboptimal aesthetic outcomes were reported in about 20% of patients due to increased buccal soft tissue recession, in studies with >3 years follow-up, of which there were two (Bianchi and Sanfilippo, 2004, Botticelli et al., 2008). Only one study had a follow-up of 10-years, reporting an overall survival rate of 91.8% for single-tooth immediate implants placed with a submerged healing protocol (Covani et al., 2012). GBR was performed when the gap between the implant and bone wall was >2mm but there was no statistically significant difference between the GBR and non-GBR groups for early or late implant failure rates over 10 years ($p>0.05$). However, the GBR group did experience less mean mid-buccal soft tissue recession compared to the non-GBR (-0.7± 0.4mm versus -1.1 ± 0.7mm) with the difference being statistically significant ($p<0.05$). Aesthetic and soft tissue complications have been discussed above in more detail.

In their study, Bianchi and Sanfilippo (2004), compared the outcomes of single tooth immediate implants with and without connective tissue grafts over a 9-year period. Overall the scores for bleeding on probing (BOP) appeared similar for both groups. The group without soft tissue grafts demonstrated about 70% of the sites had a BOP score of 0, 20% had score 1 and 9% had score 2. In comparison the soft tissue graft group about 69% of the sites had a BOP score of 0, 26% had score 1 and 4% had score 2. Regarding probing depths, both groups had a small increase in PD from

2.5mm to 3.5mm during the first year. However, the mean PD was significantly lower in the group that received the soft tissue graft during the 6-9 years interval, with the other group having mean values of about 4mm. The group that had soft tissue grafting presented with 27% of sites with >3mm PD compared to 45% in the group without grafting.

More recently, a 5-year prospective study compared the biological complications in immediate implants (group II) to those of delayed implants (DI) in the same patients (Rodrigo et al., 2012). There were no significant differences for plaque index, bleeding on probing, probing depths or radiographic changes at the end of the study. There was a slight tendency for more biological complications in the immediate implant group; with mucositis present at six (17.6%) of implants and peri-implantitis at three (8.8%) implants. The values for the delayed group were 20.5% and 2.9% respectively (Rodrigo et al., 2012). A more recent randomised controlled trial also compared the complications in patients receiving 124 implants, either at the time of extraction or 12 weeks after (Tonetti et al., 2017). Wound failure (wound dehiscence, oedema and suppuration) occurred in 26% of immediate implants and 5.3% of delayed implants ($p = 0.02$). At 1 year, immediate implants had deeper probing depths of 4.1 ± 1.2mm compared to 3.3 ± 1.1mm for delayed implants ($p < 0.01$). There was also a trend for greater radiographic bone loss at immediate implants over the initial 3-year period (Tonetti et al., 2017).

The studies that have used direct bony measurements to measure the dimensional alterations around immediate implants have consistently demonstrated a vertical bone loss on the buccal aspect (Botticelli et al., 2004, Sanz et al., 2016). The vertical

bone loss has been shown to occur to a greater extent in anterior sites and when the buccal bone plate is thin (Sanz et al., 2010, Ferrus et al., 2010). The long-term effects of this bone loss remain unclear. The influence of residual bone marginal dehiscence-type defects after guided bone regeneration on peri-implant health has been investigated (Schwarz et al., 2012). Implants with residual dehiscence defects of >1mm were at higher risk of presenting with bleeding on probing and mucosal recession at 4 years. Whether there is a similar problem with immediate implants remains unclear.

The paucity of data on biological complications is likely due to the lack of long-term studies on immediate implants and because peri-implantitis often occurs after 5 years or more (Zitzmann and Berglundh, 2008). Although more recent evidence suggests the onset of peri-implantitis usually occurs before 5 years and as early as 2-3 years (Derks et al., 2016).

2.5 Porous Titanium Granules

Titanium has been used as an implant material in orthopaedics and dentistry due to its biocompatibility and direct bone-to-implant contact (Branemark, 1983). Titanium is typically used in a dense form in which the surface topography can be modified. Recently, porous titanium granules were developed for use a bone substitute material in periodontal and implant regenerative procedures (Natix™, Tigran Technologies AB: Malmo, Sweden). Prior to this they have been applied

successfully in orthopaedic surgery (Alffram et al., 2007) and have also demonstrated osseointegration in an experimental model (Turner et al., 2007).

A porous biomaterial with a three-dimensional structure would in theory provide a suitable scaffold for the ingrowth of osteogenic cells into the pores (Pilliar et al., 1986). A regular titanium granule is 500-1000μm in diameter but due to its porosity the total surface area is up to $2cm^2$ according to the manufacturer (Natix®, Tigran Technologies AB, Malmo, Sweden). It is likely this large surface area / porosity that makes the titanium granules an attractive substrate for promoting osteogenic activity. It was demonstrated that human cultures grown on porous Ti had increased osteogenic cell proliferation compared to cultures on dense Ti (Rosa et al., 2009). Furthermore, titanium itself seems to promote the formation of a blood clot (Hong et al., 1999).

There are two available forms of the porous titanium granules. Metallic porous titanium granules (PTG) and oxidised (white) porous titanium granules (WPTG; Natix®, Tigran Technologies AB, Malmo, Sweden). They are both non-resorbable materials of porous, commercially pure titanium. According to the manufacturer the PTG is 80% porous and the WPTG is 56% porous. PTG and WPTG are osteoconductive graft materials acting as scaffolds for osseous ingrowth (Wohlfahrt et al., 2010).

Porous titanium granules osteoconductive activity has been demonstrated in several experimental studies. Both PTG and WPTG were shown to promote bone formation and new bone growth in osseous defects adjacent to titanium implants in rabbits

(Wohlfahrt et al., 2010). Another experimental study using mini-pigs demonstrated that implants successfully osseointegrated in extraction sites previously grafted with PTG and WPTG (Verket et al., 2014). When PTG was used to preserve extraction sockets in beagle dogs, there was less vertical resorption of the buccal bone crest compared to sockets left empty, however the difference was not statistically significant (Bashara et al., 2012). PTG has also recently been studied for grafting degree II furcation lesions in mini-pigs. PTG outperformed deproteinised bovine bone mineral in terms of vertical and horizontal defect fill and supported osseous regrowth in the defects (Wohlfahrt et al., 2012a).

Several clinical studies have documented the performance of PTG in various different applications. One of the first ever clinical trials to evaluate PTG used it in sinus augmentation procedures prior to or in conjunction with dental implant placement (Bystedt and Rasmusson, 2009). However, two out of five implants placed in a staged protocol did not osseointegrate and were removed at abutment connection. There was an overall survival rate of 87% after 36 months of prosthetic loading. Subsequently a multicentre trial investigated PTG for sinus augmentation with simultaneous implant placement (Lyngstadaas et al., 2015). At 5 centres, 40 patients had a total of 70 implants installed. At 12 months only one implant failed to osseointegrate. A study from the same group analysed the histological appearance of PTG used as a sinus graft 6 months post augmentation (Verket et al., 2013). PTG alone occupied 26% of the total mean area. There was a mean area of new bone formation of 16% and the newly formed bone consisted of woven bone in close contact with the granules.

PTG has also been investigated in the treatment of peri-implant defects. A prospective randomised clinical trial compared open flap debridement and surface contamination with EDTA (n=16) to the same approach with the addition of PTG (n=16) (Wohlfahrt et al., 2012c). At 12 months the PTG group had significantly greater radiographic peri-implant defect fill compared to the control. Clinical parameters improved in both groups with no differences detected between them. The results of this study were mirrored in a large multicentre randomised trial comparing OFD alone to OFD with the addition of PTG. Hydrogen peroxide was instead used as a surface decontaminant. Again the PTG group had superior radiographic peri-implant defect fill at 12 months. Both groups had similar improvements in clinical parameters. Another randomised trial compared the use of PTG with bovine bone mineral with a collagen membrane in the regenerative treatment of peri-implant defects (Arab et al., 2016). There were no statistically significant differences between the two protocols in terms of radiographic bone fill or clinical parameters at 6 months.

PTG has also been used in the surgical treatment of mandibular class II furcation defects in a series of 10 consecutive cases (Wohlfahrt et al., 2012b). It was found to be safe to be used in close proximity to root surfaces but there were no improvements in the vertical and horizontal furcation attachment levels. Probing pocket depth was the only clinical parameter that showed improvement at 12 months.

2.6 Leucocyte and platelet-rich fibrin

2.6.1 History and background

Wound healing is considered a dynamic process made up of four key overlapping phases; haemostasis, inflammation, proliferation and maturation (Gosain and DiPietro, 2004). Different cell types and populations dominate each phase. The initial phase begins immediately after injury and is characterised by vasoconstriction and fibrin clot formation. Pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF) are released. The inflammatory phase begins with the migration of neutrophils and macrophages (chemotaxis) to the wound area. Subsequently lymphocytes migrate into wound areas and persist throughout the inflammatory and proliferative phases. The proliferative phase is characterised by re-epithelialisation and extra-cellular matrix formation (ECM). Finally the maturation phases consists of remodelling of the ECM and a return to that of normal tissue (Gosain and DiPietro, 2004) (Guo and Dipietro, 2010). It is clear that wound healing is a precise yet complex process.

Since the concept of guided tissue regeneration was introduced in the 1980's (Gottlow et al., 1984, Karring et al., 1993) a plethora of approaches have been tested and advocated for the regeneration of tissues in the oral cavity. The materials used for regeneration come in many forms and typically consist of barrier membranes, bone-grafting materials and recombinant growth factors (McAllister and Haghighat, 2007). These materials can be derived from the same individual receiving the graft

(autograft), a different individual of the same species (allograft), a different species to that of the individual receiving the graft (xenograft) or synthetic / inorganic variants (alloplast). While all of the above biomaterials have been shown to possess the ability to stimulate regeneration of oral tissues, they rely on the existing blood supply to the damaged area as a source of nutrients for regeneration and do not improve / promote the blood supply themselves (Benic and Hammerle, 2014).

Platelets play a key role in the first / haemostasis phase of wound healing and have been shown to secrete a multitude of growth factors, chemokines and cytokines from their α-granules (Nurden, 2011). These include platelet-derived growth factor (PDGF) and vascular endothelial growth factors (VEGF), which play roles in angiogenesis, vascular modelling, bone formation and chemotaxis of other cells involved in wound healing including fibroblasts, neutrophils and macrophages (Nurden, 2011). It is not surprising that these modulators of wound healing have been adapted for use in oral tissue regeneration. A recombinant form of platelet-derived growth factor (rhPDGF-BB) has been used successfully in the regeneration of periodontal defects (Lynch et al., 1989, Nevins et al., 2013). Another strategy was developed to obtain physiological solutions containing platelets in high concentrations via centrifugation (Marx, 2004). Over the last 10 years platelet concentrates have dramatically increased in popularity since the development of platelet-rich fibrin in 2001 (Choukroun J, 2001).

Platelet concentrates have been used in medicine since the 1970's when they were developed for use as fibrin glues (Matras, 1970) for the prevention of haemorrhage, particularly during surgery. They have been used successfully in cardiothoracic and

vascular surgery as well as orthopaedic surgery. They are still in use today and one such product is Tisseel from Baxter International Inc. They were one of the first biological surgical adjuvants developed and utilised the fibrin matrix from blood as their active ingredient (Gibble and Ness, 1990).

The use of platelet concentrates in oral and maxillofacial surgery became extremely popular in the late 1990's following the publications of Whitman (1997) and Marx (1998) (Whitman et al., 1997, Marx et al., 1998). The products in these publications were termed Platelet-Rich Plasma, PRP. At this time they were using PRP as a surgical adjunctive and enhancement for bone grafts.

Not long after this, a new form of platelet concentrate was developed in France and called Platelet-Rich Fibrin, PRF (Choukroun J, 2001, Dohan et al., 2006a). It was termed a "second-generation" platelet concentrate, as it was clearly different from the PRPs. In subsequent years it was identified the platelet concentrates were associated with various forms of growth factors and cells, particularly leukocytes (Dohan et al., 2006b, Dohan et al., 2006c, Everts et al., 2006).

Over the last 20 years a myriad of publications on the use of platelet concentrates in OMFS has resulted in confusion in the field. Due to the fact there are several techniques available for the development of platelet concentrates, each methods leads to a unique product with different biology and potential indications. The lack of clear terminology led to the formation of the first classification system in 2009 (Dohan Ehrenfest et al., 2009c).

2.6.2 Classification of platelet concentrates

A classification system was proposed in 2009 with the aim of providing objective parameters upon which further research can be based and appraised (Dohan Ehrenfest et al., 2009c).

The difficultly in developing a classification system for platelet concentrates is derived from the fact there are many PRP / PRF systems available on the market each producing a slightly different product, each with different biology.

Definition of parameters for classification

Dohan Ehrenfest et al (2009) identified 3 main sets of parameters necessary to be able to classify platelet concentrates. A detailed description can be found in Table 1.

A. Relates to preparation kits and centrifuges used
B. Relates to the content of the concentrate (platelets and leucocytes)
C. Relates to the fibrin network

Key parameters	Subparameters	Definition
A: Preparation kits and centrifuge (for processing of 50 mL of whole blood)	A1: Size and weight of the centrifuge type required for the method	• Heavy (and cumbersome) • Light (and compact) • Heavy but potentially light (i.e. a commercialized system is heavy, but technique could be performed with a smaller centrifuge)
	A2: Duration of procedure (from blood harvest to surgical application)	• Quick (less than 20 min) • Long (between 20 and 60 min) • Very long (more than 1 h)
	A3: Cost (initial cost of equipment and repeated costs for reagents and kits)	• Very inexpensive, less than 5 euros • Inexpensive, between 5 and 50 euros • Expensive, more than 50 euros
	A4: Ergonomy of the kit (including required manipulations) and complexity of procedure	• Very simple (+ +) • Simple (+) • Complex (−) • Very complex (− −)
B: Platelets and leucocytes	B1: Final volume of platelet gel material (relative to initial blood harvest)	• Large, more than 25% of the blood sample • Small, less than 25%, • Variable, if additional fibrin-rich PPP can be preserved to increase volume above 25%
	B2: Platelet collection efficiency B3: Leucocyte collection efficiency	• Excellent, more than 80% • Good, between 40 and 80% • Low, less than 40% • Sometimes unknown • No leucocytes, when technique eliminates most leucocytes
	B4: Preservation of the platelets and leucocytes	• Healthy • Damaged • Unknown • Activated, when coagulation is induced during the centrifugation process
C: Fibrin	C1: Fibrinogen concentration and fibrin density	• High density • Low density
	C2: Fibrin polymerization type	• Strong, mainly trimolecular or equilateral junctions • Weak, mainly tetramolecular or bilateral junctions

Table 1: Key parameters to be evaluated in each platelet concentrate protocol (Dohan Ehrenfest 2009c)

This classification divides the platelet concentrates into four categories based on their leucocyte and fibrin content. A detailed description can be found in Table 2.

- Pure platelet-rich plasma (P-PRP)
- Leucocyte and platelet-rich plasma (L-PRP)
- Pure platelet-rich fibrin (P-PRF)
- Leucocyte and platelet-rich fibrin (L-PRF)

PC class	Method (and relevant Refs)		Main characteristics									
			A: Process				B: Content				C: Fibrin	
			A1: Centrifuge type	A2: Duration	A3: Cost	A4: Ergonomy	B1: Volume	B2: Platelet collection	B3: Leucocyte collection	B4: Preservation	C1: Density	C2: Polymer-ization
P-PRP	AP	Cell separator PRP	Heavy	Very long	Expensive	– –	Small	Excellent	No leucocytes	Damaged	Low	Weak
		Vivostat PRF	Heavy	Long	Expensive	+	Small	Low	No leucocytes	Damaged	Low	Weak
	MP	Anitua's PRGF Nahita PRP	Heavy but potentially light	Long	Inexpensive	–	Variable	Low	No leucocytes	Unknown	Low	Weak
L-PRP	AP	PCCS PRP SmartPReP PRP Magellan PRP GPS PRP	Heavy	Long	Expensive	+	Variable	Good	Good	Unknown	Low	Weak
	MP	Friadent PRP Curasan PRP Regen PRP Plateltex PRP Ace PRP	Heavy but potentially light	Long	Expensive	–	Variable	Good	Good	Unknown	Low	Weak
P-PRF	MP	Fibrinet PRFM	Heavy but potentially light	Long	Expensive	+	Large	Good	No leucocytes	Healthy, activated	High	Strong
L-PRF	MP	Choukroun's PRI	Light	Quick	Very inexpensive	+ +	Large	Excellent	Good	Healthy, activated	High	Strong

Table 2: Characteristics and classification of the main platelet concentrates protocols available (Dohan Ehrenfest 2009c)

2.6.3 PRP vs PRF

2.6.3.1 Platelet-rich Plasma

Collection Process: Two-step centrifugation (Figure 1)

- Blood collected with anticoagulant just before or during surgery and immediately processed by centrifugation (low forces / soft-spin)
- Creation of 3 layers: red bloods cells (RBCs) at the bottom, acellular plasma (PPP, platelet-poor plasma) is the supernatant and a "buffy coat" layer in the middle, in which the platelets are concentrated
- The PPP and the "buffy coat" layer are transferred to another tube and subjected to more centrifugation (high forces / hard-spin). The PPP layer is eliminated

- The obtained platelet concentrate is rich in platelets suspended in fibrin-rich plasma (P-PRP)
- It is applied to the surgical site with a syringe, together with thrombin and/or calcium chloride to trigger platelet activation and fibrin polymerisation

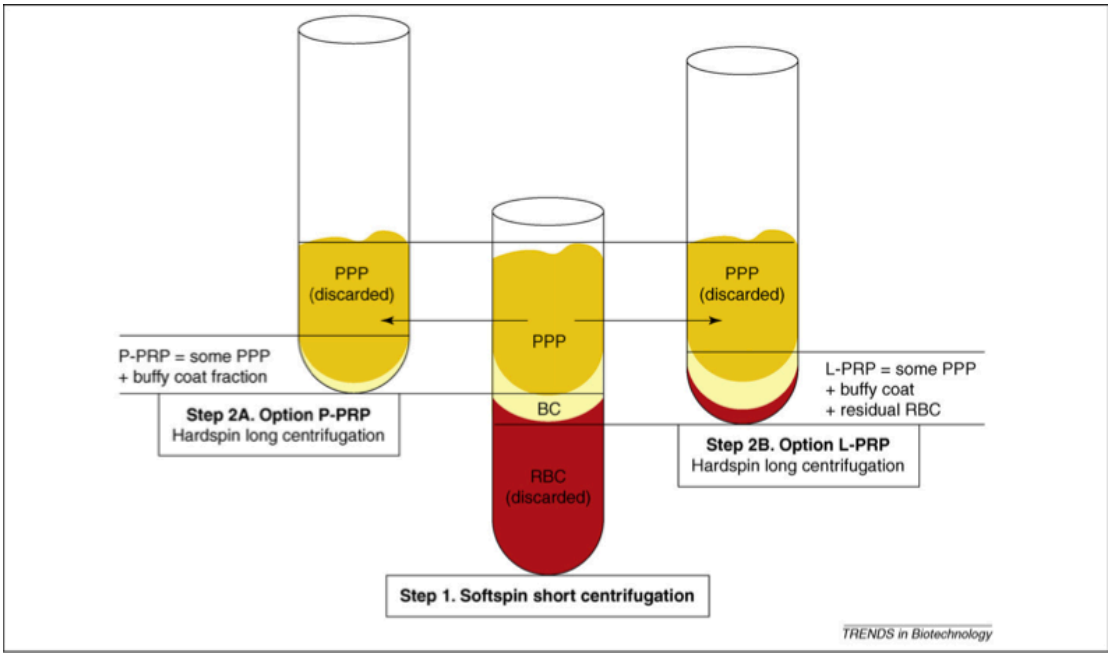

Figure 1: Classical manual platelet-rich plasma (PRP) protocol using a two-step centrifugation procedure (Dohan Ehrenfest 2009c)

Disadvantages of PRP

- The collection process is lengthy, ranging in time from 30 minutes to 60 minutes
- Requires the addition of bovine thrombin and calcium chloride, both of which are known inhibitors of wound healing

- Liquid nature – therefore it was necessary to combine it with other biomaterials
- Short period of activity – PRP has been shown to quickly release a surge of growth factors once activated but this release is not sustained for a prolonged period of time (Kobayashi et al., 2016)

2.6.3.2 PRF

Platelet-Rich Fibrin

- Blood is collected without any anticoagulant and immediately centrifuged, coagulation starts quickly
- Blood is separated into 3 components with the formation of a strong fibrin clot in the middle, RBCs at the bottom and PPP at the top (Figure 2)
- The clot acts as a plug that traps the platelets and leucocytes and eventually results in the formation of a dense leucocyte-rich PRF (L-PRF)

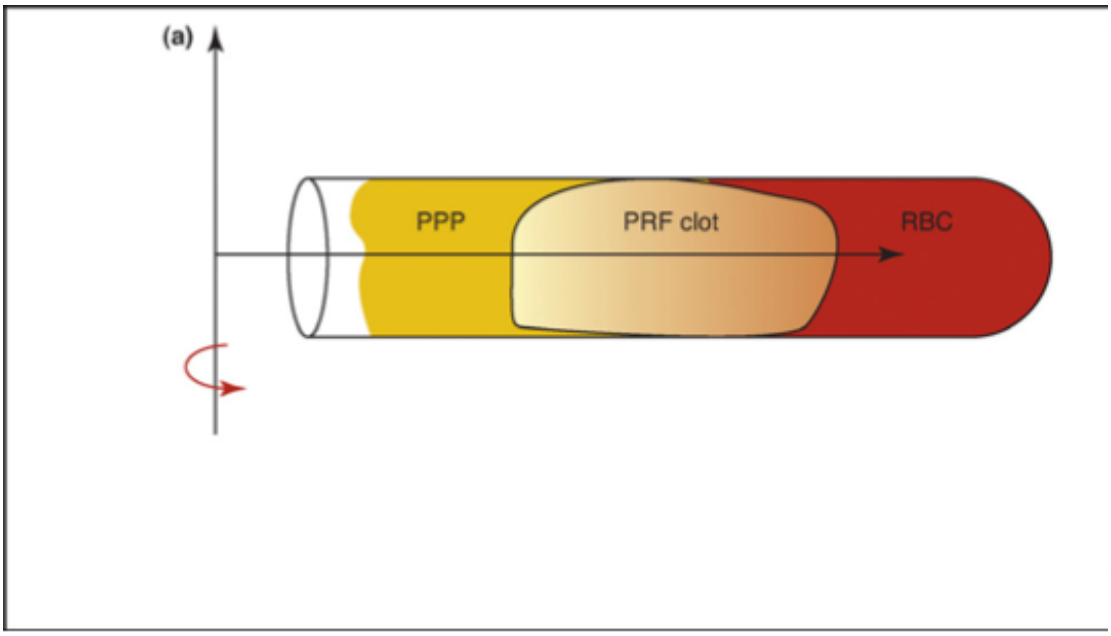

Figure 2: Choukroun's platelet-rich fibrin (PRF) method (Dohan Ehrenfest 2009c)

2.6.4 Centrifuge characteristics

As stated before there are many systems available on the market for producing PRP and/or PRF. Assuming that all these systems are slightly different in design, the products will also be different, with different biologies and therefore different methods of action and indications. Making an evidence based decision as to which system to use is almost impossible as these companies have not published the impact of their systems on the cells, growth factors and fibrin architechture produced.

To the best of my knowledge there is only one system available that is FDA-aproved and CE-marked: Intra-Spin L-PRF (Intra-Lock Inc., Boca Raton, FL, USA) (Dohan Ehrenfest DM, 2014b)

The POSEIDO journal; Periodontology, Oral Surgery, Esthetic and Implant Dentistry Open journal is an non-profit international scientific consortium with an open access journal. They have published a series of three articles on the impact of the centrifuge characteristics and centrifguation protocols of 4 models of table centrifuges commercially available for producing L-PRF (Dohan Ehrenfest DM, 2014b, Pinto NR, 2014, Dohan Ehrenfest DM, 2014a) . The models tested were the Intra-Spin L-PRF centrifuge (Intra-Lock International, Boca-Raton, FL, USA), centrifuge A-PRF 12 (Advanced PRF, Process for PRF, Nice, France), centrifuge LW-UPD8 (LW Secintific, Lawreneceville, GA, USA) and centrifuge Salvin 1310 (Salvin Dental Specialties, Charlotte, NC, USA).

The first of these studies evaluated the levels of vibrations produced by the 4 models of table centrifuges (Dohan Ehrenfest DM, 2014b). Each centrifuge was tested at half and full tube loads and various rotational speeds. One centrifuge had only one available rotational speed (3,400 rpm). Firstly, it was clear that all centrifuges had an increasing level of vibrations when rotational speed was increasing. Secondly, there were significant differences in the level of vibrations at each rotational speed for the 4 machines. The Intra-Spin machine had the lowest level of vibrations at all tested roational speeds and the increase in vibrations remained limited as speed was increasing. At the classical speed of production of L-PRF (2,700 rpm), the level of vibration on the Intra-Spin centrifuge was 4.5 to 6 times lower than the other centrifgues. The Intra-Spin machine presented the most stable of the centrifuges tested.

The second article describes the macroscopic and microscopic characteristics and cell composition of the L-PRF clots and membranes produced with the 4 different machines (Pinto NR, 2014). 8 healthy volunteers (no history of blood dyscrasia or recent aspirin intake) provided blood samples. The centrifuges were standardised so 400g force was used to allow isolation of only the vibration parameter. The membranes were prepared for Scanning Electron Microscopy (SEM) anaylsis and light / photonic microscopy. Macroscopic analysis revealed the Intra-Spin clot / membrane performed the best in most categories. It produced the heavist clot and largest quantity of exudate. The Intra-Spin and Salvin produced membranes of similar weight with the other machine's membranes being significantly lighter. The length and width of the clots and membranes from Intra-Spin and Salvin were similar and again the other two machines were significantly shorter and narrower. Light microscopy analysis showed that most of the cell bodies were concentrated in the proximal (head-face) area of each membrane. Three quarters of the cell bodies in Intra-Spin, A-PRF and Salvin were in the proximal area and the last quarter were in the centre. In the LW centrifuge the cell bodies appeared more equally distributed over the membrane (40% proximal, 48% centre and 12% distal). SEM microscopic evaluation showed the Intra-Spin L-PRF membrane produced a strongly polymerised thick fibrin network with the presence of a large cell population. All the observed cells appeared alive and with a normal shape. The PRF membranes produced with A-PRF, Salvin and LW all had a lightly polymerised, thinner and disorganised fibrin network. The observed cells appeared damaged, shrunk or squashed. In this study the blood collection material, tubes, protocol and centrifugation force (400g) were all strictly controlled, meaning the meachanical vibrations would be responsible for the differences in the products. The authors

concluded, that given the destruction and / or damage of cells within the A-PRF, Salvin and LW membranes, these clots / membranes cannot be classified as L-PRF and probably fit the Pure Platelet-Rich Fibrin (P-PRF) criterion more closely. The centrifuge characteristics and particularly the vibrations impact the architecture and cell content of a L-PRF clot.

The third and final study in this series compared the growth factor content and slow release between the original L-PRF and the modified A-PRF (Advanced Platelet-Rich Fibrin) membranes (Dohan Ehrenfest DM, 2014a). The rationale for this experiment was to evaluate how the changes of the L-PRF protocol may influence its biological features, independently from the characteristics of the centrifuge. As per the original protocol blood was collected in Intra-Spin 9ml glass-coated plastic tubes and A-PRF 10ml glass tubes. Tubes were immediately centrifuged at 2,700 rpm (around 400g) for 12 minsutes to produce L-PRF or at 1,500 rpm for 14 minutes to produce A-PRF. Both processes used the original L-PRF centrifuge (Intra-Spin, Intra-Lock). The membranes were used to quantify the release of Transforming Growth Factor ß-1 (TGFß-1), Platelet Derived Growth Factor AB (PDGH-AB), Vascular Endothelial Growth Factor (VEGF) and Bone Morphogenetic Protein 2 (BMP-2) at 7 experimental times: 20 minutes, 1 hour, 4 hours, 24 hours, 72 hours, 120 hours and 168 hours using ELISA kits. The results demonstrated the slow release of 3 tested growth factors (TGFß-1, PDGF-AB and VEGF) from L-PRF membranes was significantly stronger (more than twice as strong; $p<0.001$) at all experimental times than the release from A-PRF membranes. BMP-2 was released from the L-PRF membranes for the 7 experimental days but was not detected at all from the A-PRF membranes. The A-PRF membranes dissolved after 3 days while the L-PRF

remained intact during the 7 day experimental period. The authors concluded that A-PRF has a much weaker biological signature than the original L-PRF.

A different research group have also published on the effects of the centrifugation process on the production of PRF (Ghanaati et al., 2014, Kobayashi et al., 2016, Fujioka-Kobayashi et al., 2017). They were able to demonstrate that altering the centrifugation protcol i.e. the time and speed, can lead to a different distrtibution pattern of cells within the fibrin clot (Ghanaati et al., 2014). They compared the original centrifugation protcol of 2700 rpm for 12 minutes (S-PRF) to 1500 rpm for 14 minutes (Advanced PRF, A-PRF). Most cell types, including lymphocytes and monocytes, had a similar distribution pattern with no statistically significant differences between the two groups. The cells were located within the first 25-30% of the total clot length from the buffy coat. However, platelets were more evenly distributed throughout the entire clot. Of significance, in the A-PRF group, the distribution of neutrophilic granulocytes was increased to 68 ± 24% of the length of the clot compared to 25 ± 12% in the S-PRF group. Following on from this they investigated and compared more centrfugation protocols on the release of the fibrin matrix development and release of growth factors (Fujioka-Kobayashi et al., 2017). They compared protocols of 2700 rpm for 12 minutes (L-PRF), 1300 rpm for 14 minutes (A-PRF) and 1300 rpm for 8 minutes (A-PRF+). All 3 protocols produced platelet formulations with excellent cell biocompatibility and with high numbers of living cells. A-PRF+ produced the significantly highest amount of total growth factor release while L-PRF was significantly the lowest. This trend continued with A-PRF and A-PRF+ exerting a 300% increase in HGF (human gingival fibroblast) activity compared to a 200% increase for L-PRF.

It is clear that centrifugation protocols influence the composition of the three-dimensional fibrin matrix, the cell distribution within the scaffold and the subsequent growth factor release. This conept may allow the development of scaffolds tailored for specific clinical indicaitions. However, the influence of these different protocols on clinical outcomes is yet to be investigated.

2.6.5 Cellular content / Mode of action of PRF

Platelet-rich fibrin has been defined as an optimised blood clot (Dohan Ehrenfest et al., 2010a) providing the natural ingredients for wound healing:

1. Cells (platelets and leucocytes)
2. Growth factors (released from the platelets)
3. Scaffold (fibrin matrix).

2.6.5.1 Cellular Composition of L-PRF

A study by Dohan Ehrenfest described the three-dimensional architecture of a L-PRF clot (Dohan Ehrenfest et al., 2010a). The number of leukocytes, red blood cells and platelets in whole blood (control) was compared to that in the residual RBC base after collection of the PRF membrane (test groups). It was found that there were very few platelets (3%) left behind in the RBC layer, the PPP or the exudate from compressing the PRF clot, suggesting that most of the platelets (97%) from the

whole-blood sample were contained within the PRF membranes (P<0.01). 50% of leukocytes present in whole-blood were transferred to and were captured within the PRF membrane (P<0.01). There were increased proportions of neutrophilic leukocytes left behind in the tests groups, indicating that lymphocytes were the mostly likely to be trapped in the PRF membrane. The large proportion of lymphocytes was confirmed by the SEM examination. Light microscopy showed the distribution of the platelets and leukocytes was uneven throughout the membrane. These cells were clustered in a layer between the RBCs and the fibrin clot, which has been termed the 'buffy-coat' layer. The concentration of cells decreased significantly as one moved away from the RBC end, with no cells found in the second half of the membrane.

2.6.5.2 Release of growth factors

The release of growth factors from platelet concentrates has been demonstrated by in vitro studies. When compared to whole blood, PRP has been shown to produce increased levels of PDGF-AB, PDGF-BB, TGF-B1, VEGF and EGF (El-Sharkawy et al., 2007). Similarly, PRF also releases numerous growth factors from its fibrin matrix including PDGF-AB, TGF-B1, VEGF, EGF and IGF-1 (Su et al., 2009, Dohan et al., 2006b). Whilst these studies demonstrated the ability of these platelet concentrates to secrete growth factors, they did not compare them nor did they evaluate the release of growth factors over a prolonged period of time. Therefore a study was designed to compare the release of growth factors over time from PRP, PRF and a slower centrifugation protocol called A-PRF (Kobayashi et al., 2016). Five growth

factors; PDGF, TGF-B1, VEGF, EGF and IGF were assessed for release at 15 minutes, 60 minutes, 8 hours, 1 day, 3 days and 10 days using ELISA assays. PRP was found to release the highest amount of growths factors at the early time points compared to PRF and A-PRF. Using PDGF-AA as an example, after 15 minutes, PRP had release significantly higher levels compared to PRF and A-PRF but by 60 minutes significantly lower levels were released. This demonstrates the initial surge in release of growth factors by PRP. However, PRF and A-PRF were shown to release a greater overall number of growth factors over the 10 day period when compared to PRP. In addition their growth factors were released more gradually up to the 10 days. It has been hypothesised the structure of the fibrin matrix contributes to this more gradual release (Dohan Ehrenfest et al., 2009a). Interestingly, PDGF-AA was released from all platelet concentrates at 6-10 time's higher concentration compared to PDGF-AB and PDGF-BB. EGF and IGF were released in far lower quantities compared to all other growths factors tested. In another similar study L-PRF was again shown to be superior to PRP in releasing a larger number of growth factors and over a longer period of time (Schar et al., 2015).

When the platelet α-granules degranulate they release a large number of growth factors including transforming growth factor beta-1 (TGF-β1), platelet-derived growth factors (PDGF), vascular endothelial growth factors (VEGF), epidermal growth factor (EGF) and insulin-like growth factor (IFGF) (Nurden, 2011). Their basic roles are described below.

TGF-β1: TGF-β1 is the most commonly produced isoform of the TGF-β family. It is known as a fibrosis agent (Border and Noble, 1994). It induces synthesis of matrix

molecules like collagen 1 and fibronectin, via osteoblasts or fibroblasts. Through its capacity to induce fibrosis it can be considered an inflammatory regulator (Border and Noble, 1994).

PDGF: Regulate the migration, proliferation and survival of mesenchymal cell linages (Rosenkranz and Kazlauskas, 1999). They are able to both stimulate and inhibit mesenchymal cells, depending on the distribution of their receptors (Heldin, 1997). They play an important role in the process of wound healing.

VEGF: Is the most potent promoter of angiogenesis. It controls the migration and proliferation of endothelial cells and therefore controls the formation of new blood vessels (Ruhrberg, 2003).

2.6.5.3 Cell behaviour in response to L-PRF

The in vitro effects of PRF on human primary cultures of gingival fibroblasts and maxillofacial osteoblasts has been investigated (Dohan Ehrenfest et al., 2009b). Tissue specimens and blood were harvested from the same patient. Gingival fibroblasts were harvested from the alveolar ridge and the osteoblasts from a mandibular bone harvest. These cells were cultivated with and without a PRF membrane and cell counts were performed at 3, 7, 14 and 21 days and also 28 days for osteoblasts. Osteoblasts were also cultured in differentiation conditions, again with and without PRF. The results showed that PRF stimulated the proliferation of all cell types. There was a particularly significant increase in osteoblasts, with a peak

growth of 5.5 times that of the control group at 7 days. The proliferative effect on osteoblasts was dose-dependent, with the application of 2 PRF membranes per culture, having a greater increase compared to 1 membrane ($P<0.01$). The effect on fibroblasts was not dose-dependent but there was still a significant increase in the number of gingival fibroblasts in culture at all experimental times ($P<0.01$). By day 3, there was 3 times the number of gingival fibroblasts in the PRF group compared to the control group. PRF was also demonstrated to activate a strong differentiation in the osteoblasts. SEM analysis revealed a mineralisation process within the PRF membrane itself after 14 days and leukocytes appeared to interact with the osteoblasts during this process. The results of the above study were repeated when the in vitro effects of PRF on human bone mesenchymal stem cells (BMSC), harvested from the posterior maxilla, were investigated (Dohan Ehrenfest et al., 2010b). Therefore PRF appears to have the ability to stimulate both proliferation and differentiation of oral BMSC. In contrast, inconsistent results have been obtained when platelet-rich plasma (PRP) has been tested in vitro for proliferation and differentiation of human primary cultures (Cenni et al., 2005, Graziani et al., 2006). The absence of cytotoxicity of PRF during culture was demonstrated in the former study (Dohan Ehrenfest et al., 2009b) and in a previous study by the same author (O'Connell, 2007).

Other research groups have also demonstrated the ability of L-PRF to induce strong migration and proliferation of mesenchymal stem cells (Schar et al., 2015) and human gingival fibroblasts (Fujioka-Kobayashi et al., 2017) in vitro. In addition, the newly cultured gingival fibroblasts demonstrated a local increase in mRNA levels of

PDGF and TGF-β and collagen1 mRNA at either 3 or 7 days (Fujioka-Kobayashi et al., 2017).

2.6.5.4 Three-dimensional fibrin network

The three-dimensional matrix that is PRF is made up of fibrin (Dohan et al., 2006a). Fibrin is the activated form of a plasmatic molecule called fibrinogen and is present in plasma and in platelet α-granules (Mosesson et al., 2001). Fibrinogen exists as a soluble protein and is transformed into insoluble fibrin by thrombin (Clark, 2001). PRF is created by the slow and natural polymerisation of fibrinogen by circulating thrombin during centrifugation (Dohan et al., 2006a). The centrifugation concentrates the fibrin clot in the middle of the tube and prevents the diffuse polymerisation of the fibrinogen. A fine, flexible and elastic fibrin network is established and is able to entrap a massive amount of platelets and cytokines (Dohan et al., 2006b). It is this fibrin matrix that results in the slow and gradual release of growth factors over time. It also acts as a scaffold for the recruitment of additional cells involved in the wound healing process.

2.6.6 L-PRF in Periodontology and Implantology

Since the introduction of L-PRF (Choukroun 2001)(Dohan et al., 2006a) its use has become popular in the fields of periodontology and implantology. It has been used as a regenerative material alone or in combination with other materials.

A recent systematic review has analysed the benefits of L-PRF when used as a regenerative material in periodontal surgery, namely intra-bony defects, furcation defects and periodontal plastic surgery (Castro et al., 2017a). This review included only randomised controlled trials using an L-PRF protocol of 2700 rpm / 12 mins or 3000 rpm / 10mins, as a sole biomaterial or in combination with other materials in periodontal surgery. A total of 24 articles met the inclusion criteria, 13 for the treatment of intra-bony defects, 2 for furcation defects and 9 for periodontal plastic surgery. Meta-analysis was performed for all three subgroups. Overall L-PRF showed promising results. When L-PRF was compared to open flap debridement (OFD) for the treatment of intra-bony defects it had significantly better pocket depth reduction (1.1 ± 0.5mm. $p<0.001$), better clinical attachment gain (1.2 ± 0.6mm, $p<0.001$) and better bone fill (1.7 ± 0.7mm, $p<0.001$). Similarly for furcation defects, L-PRF had significantly better pocket depth reduction (1.9 ± 1.5mm, $p=0.01$), better clinical attachment gain (1.3 ± 0.4mm, $p<0.001$) and better bone fill (1.5 ± 0.3mm, $p<0.001$). When comparing a coronally advanced flap (CAF) alone to CAF + L-PRF there was no statistically significant differences in outcome measures found. However, trends suggested L-PRF was superior. When comparing a CAF with L-PRF versus with a connective tissue graft (CTG) no statistical significant differences were found. In this review a mean root coverage of 86.5% at 6 months was found for CAF + L-PRF. This is lower than the mean root coverage of 90.3% at 6 months for CAF + CTG reported in another systematic review (Cairo et al., 2008).

Another systematic review from the same group reported on the use of L-PRF on bone regeneration procedures and osseointegration (Castro et al., 2017b). Similarly, randomised controlled trials only, using L-PRF protocols of 2700 rpm / 12 mins or

3000 rpm / 10mins, as a sole biomaterial or in combination with other materials applied in bone regeneration and implant surgery were included. A total of 14 articles met the inclusion criteria but a meta-analysis was not possible due to the high heterogeneity amongst studies. Three subgroups were again identified depending on the application of the L-PRF: sinus floor elevation (SFE) (3 studies), alveolar ridge preservation (8 studies) and implant therapy (3 studies). In the three studies on SFR, L-PRF was always combined with a xenograft and compared to the xenograft alone. Superior histological healing was observed in the L-PRF groups. No study used L-PRF as the sole filling material in sinus floor elevation. Good results have been obtained in case series published using L-PRF as the sole grafting material with simultaneous implant placement, using both the lateral window approach (Mazor et al., 2009, Simonpieri et al., 2011) and the transalveolar approach (Diss et al., 2008). L-PRF improved alveolar ridge preservation outcomes compared to natural healing with reduced buccal bone resorption. However, five of the 8 studies included reported on the extraction of wisdom teeth and most of the studies used only 1 L-PRF membrane in the extraction socket. The results cannot be compared to other systematic reviews on alveolar ridge preservation, especially those in analysing extraction sites in the aesthetic zone (Ten Heggeler et al., 2011). Subsequent to this systematic review a split-mouth randomised controlled trial on alveolar ridge preservation using L-PRF was published (Temmerman et al., 2016). L-PRF showed superior results to natural healing in both vertical and horizontal dimensions at 3 months. Total ridge width reduction was 22.84% for L-PRF compared to that of 51.92% for natural healing ($p<0.005$). When L-PRF was applied to implants at the time of placement it resulted in better implant stability over time and reduced marginal bone loss.

Despite the promising results presented in both of these systematic reviews the majority of studies included were at a moderate to high risk of bias. The studies included were limited to those using specific L-PRF protocols. However, it was acknowledged that not all studies used enough of the L-PRF membranes, which is considered crucial for optimal results (Castro et al., 2017b).

2.7 3i T3 Implant

The minimum criteria for success were proposed by Albrektsson et al (Albrektsson et al., 1986).

- Immobile implant at uncover
- Absence of peri-implant radiolucency
- After the first year in function, radiographic vertical bone loss <0.2mm per annum
- Absence of signs and symptoms, such as pain or infections
- A success rate of 85% at the end of a 5-year observation and 80% at the end of a 10-year observation period

Similar criteria were outlined by Smith and Zarb in 1989 (Smith and Zarb, 1989). The above criteria do not address the amount of crestal bone loss occurring in the first year. It was subsequently suggested that for optimal implant health <2mm of bone loss would occur from the initial surgery (Misch et al., 2008).

The Osseotite implant by 3i was introduced in 1995. It has a hybrid surface; the coronal portion being smooth while from the third thread to the apex is dual acid-etched (hydrochloric acid/sulphuric acid) (Biomet, 2018b). The etching process removes a small amount of material to create irregularities and increase the surface area (Wennerberg and Albrektsson, 2009). The Osseotite surface is able to entrap fibrin strands between the peaks (1-3 microns) created by the etching process, initiating a blood clot (Park and Davies, 2000). Compared to a machined surface, the Osseotite surface has been shown to exhibit increased red blood cell agglomeration and platelet adhesion (Park and Davies, 2000).

The etched surface of the Osseotite implant has been compared with machined surfaces in animal studies. The acid-etched surfaces were shown to require four times higher torque values for removal compared to the machined surfaces (Klokkevold et al., 1997). A study comparing machined surface implants (Sa = 0.53μm) to the Osseotite's rougher surface implant (Sa = 0.94μm), found significantly higher BIC% for the Osseotite implants (Abrahamsson et al., 2001).

There are human histologic studies demonstrating the superior BIC% associated with the Osseotite surface when compared to a machined implant surface (Lazzara et al., 1999, Trisi et al., 2002). The mean BIC value for the Osseotite surface (72.96 ± 25.13%) was statistically significantly higher ($p<0.05$) than the mean value for the machined surface (33.98 ± 31.04%)(Lazzara et al., 1999) and similar trends were observed in Trisi et al (2002).

The long-term survival of these implants has been well documented. In a prospective multicentre study the 5-year cumulative survival rate of 1,583 3i implants (including Osseotite) was 96.5% (Davarpanah et al., 2002). The pooling of data from the multicentre studies using 3i implants allowed analysis to be carried out comparing machined-surface and Osseotite implants. A meta-analysis compared the survival of 2,614 machined-surface and 2,288 Osseotitie implants in poor-quality bone (Stach and Kohles, 2003). Machined-surface implants placed in good quality bone had a 4-year survival rate of 93.6% compared to 88.2% for those placed in poor quality bone ($p<0.05$). In contrast, the Osseotite implants had survival rates of 98.4% and 98.1% in good and poor quality bone respectively. In another analysis of data from these multicentre studies, Osseotite implants again demonstrated their superior survival rate compared to machined-surfaced implants (Feldman et al., 2004). The risk of failure of short-length (10mm or less) implants was compared between Osseotite implants and machined-surfaced implants. Overall there was a 2.2% difference in the 5-year cumulative survival rates for machined-surfaced short implants and standard implants, which was statistically significant ($p<0.05$). In contrast, the difference between the short and standard Osseotite implants was insignificant at 0.7%.

The Zimmer Biomet T3 Implant™ (Biomet 3i implant innovations, Palm Beach Gardens, Florida, USA) was introduced in 2007. It is described by its manufacturers as having a contemporary hybrid surface provided by complex multi-surface topography. The implant's coronal aspect has decreased roughness of the dual acid etching (1-3 microns), while the apical surface roughness increases. The apical

surface has three distinct layers of topography. Submicron topography with deposition of 10 to 100nm of hydroxyapatite covering approximately 50% of the surface (discrete crystalline deposition (DCD) of calcium phosphate), micron topography with dual acid etching of 1-3 μm of pitting and lastly, a hybrid coarse micron topography via resorbable calcium phosphate media blast (Biomet, 2018a). The surface has been shown to have a mean surface roughness value of 1.4 μm (Gubbi P, 2012).

The patterns of bone modelling and remodelling around this implant surface (Nevins et al., 2012) are consistent with those of previous descriptions (Berglundh et al., 2003, Abrahamsson et al., 2004). BIC levels of roughly 70% have been demonstrated (Nevins et al., 2012). The discrete crystalline deposition (DCD) has been shown to enhance osseointegration compared to the same surface but without DCD, in pre-clinical (Mendes V, 2011) and clinical studies (Orsini et al., 2007). The 3i T3 implant utilises the Osseotite® surface at the coronal aspect of the implant and in a study with 5-years of follow up, this dual acid-etched surface, showed no increased risk of peri-implant disease compared to a machined surface (Zetterqvist et al., 2010).

Primary implant stability is considered one of most the important factors in achieving osseointegration of dental implants (Meredith, 1998, Lioubavina-Hack et al., 2006). There are several methods for improving primary stability of dental implants, such as altering the implant macrodesign. Tapered implants have been shown to increase implant primary stability compared to standard straight screw type implants (O'Sullivan et al., 2004, Friberg et al., 2003). This ability to improve

primary stability is achieved by controlling the final bone preparation process and may be particularly important in areas of poorer bone quality (Moon et al., 2010). It has been demonstrated when placing implants in fresh extraction sockets good primary stability is essential to their success (Meltzer, 2012). The T3 tapered implant design has been shown to be able to achieve high levels of insertion torque (53Ncm) at implant placement (Ostman et al., 2013). The T3 tapered implants have also been placed in fresh extraction sockets of molar teeth with high levels of insertion torque (Block, 2011).

Tapered implants have also been used for immediate implant placement aiming to reduce or minimise the dimensions of the resulting voids between the implant surface and the socket walls following installation (Lang et al., 2007, Sanz et al., 2010). These studies compared immediate placement of tapered and cylindrical implant designs in fresh extraction sockets. Results demonstrated that both implant designs exhibit similar primary stability and an equal need for bone augmentation at the time of placement (Lang et al., 2007). In addition, there was no benefit of one design over the other in term of bony remodelling, with the buccal bone crest located at a similar level at both implant designs (Sanz et al., 2010).

3 Materials and Methods

3.1 Study design

As a standalone study this is an observational clinical case series on a cohort of patients referred to the department of Periodontology in the Dublin Dental University Hospital for immediate implant placement. It was designed to observe the effect of L-PRF on the preservation of the buccal bone plate following immediate implant placement. This study was also part of a larger quasi-experimental study comparing the effect of L-PRF to that of porous titanium granules on the preservation of the buccal bone plate following immediate implant placement.

3.2 Ethical approval

Ethical approval was sought through the SJH/AMNCH Research Ethics committee. Along with the application, copies of the patient information leaflet and informed consent forms were submitted. Ethical approval was obtained in March 2016. The obtained ethical approval form is attached in the appendix (Appendix A) as is the patient information leaflet (Appendix B) and informed consent form (Appendix C).

3.3 Study sample

A convenient sample was recruited from patients referred to the department of Periodontology requiring a dental implant for one or more failing teeth in the anterior maxilla (between tooth locations 1.5 and 2.5). Patients were invited to attend a screening appointment to confirm they were suitable for implant treatment and that they meet the inclusion criteria. Once the inclusion criteria had been met and the treatment options discussed with the patient, the information leaflet was given to potential candidates. This leaflet explained the aims of the study, outlining the subsequent benefits and risks of participation and ensuring confidentiality. The principal investigator's contact details were also included in case further queries or clarifications were needed. Patients were given a minimum of 7 days to consider their options before attending a second appointment at which they decided upon their treatment plan. Patients who did not wish for an implant were referred on to the appropriate department for further treatment. Those wishing to participate signed the informed consent form.

3.4 Inclusion criteria

1. Adult (<18 years of age) male or female patients
2. Presence of one or more non-restorable teeth in the anterior maxilla (incisors, canines and premolars), which can be replaced by an implant. The teeth had to be abutted by two adjacent teeth, had to be free of any active pathology/infection and the sites had to be suitable for immediate placement.

3. Patients had to be periodontally healthy with FMPS ≤20% (O'Leary Plaque control record, 1972). In the event of generalised or localised periodontal disease, successful control of the disease was required prior to the inclusion of the patient in the study.
4. Patients had to be in good general health and be able to tolerate minor dental surgeries
5. Patients have to be able to have blood drawn to allow fabrication of the L-PRF. The preferred site for blood draw is the antecubital fossa and in cases where this is not possible, the dorsum of the hand will be used.
6. Patients had to be able to give consent to participate in this study and sign a consent form approved by the Research Ethic Committee of the Faculty of Health Sciences, Trinity College Dublin.
7. Patients should be able to attend all the required appointments
8. The presence of an intact extraction socket following tooth removal

3.5 Exclusion criteria

1. Pregnant females
2. Heavy smokers (>10 cigarettes a day)
3. Active drug-addiction patients
4. Patients on chemo/radiation therapy
5. Patients with uncontrolled Diabetes Mellitus
6. Patients taking bisphosphonates
7. A missing or absent buccal bone plate following tooth extraction or the presence of a buccal fenestration >3mm from the marginal bone crest

8. In general patients who do not fulfil the inclusion criteria

3.6 Study timetable

This study first ran from 2013 to 2015 with two operators, G.G and I.P. The first cohort of patients enrolled received a combination of Natix® titanium granules (PTG White) and a resorbable collagen membrane (Osseoguard® Biomet 3i) as the materials to graft the buccal gap following immediate implant placement. A total of 19 patients received 21 implants in this first cohort. At this time, the effects of WPTG on the preservation of the buccal bone plate following immediate implant placement were evaluated.

The second part of the study ran from 2016 to 2018, again with two operators, (although with one operator changing), M.M and I.P. Leucocyte and platelet-rich fibrin (L-PRF) was used to graft the buccal gap following immediate implant placement. A total of 14 patients received 14 implants in the second cohort. The effects of L-PRF were then evaluated in a similar fashion as the first cohort. In total, 35 implants were placed in 33 patients, which allowed comparisons to be made between the effects of WPTG and L-PRF. See Figure 3 for study timeline.

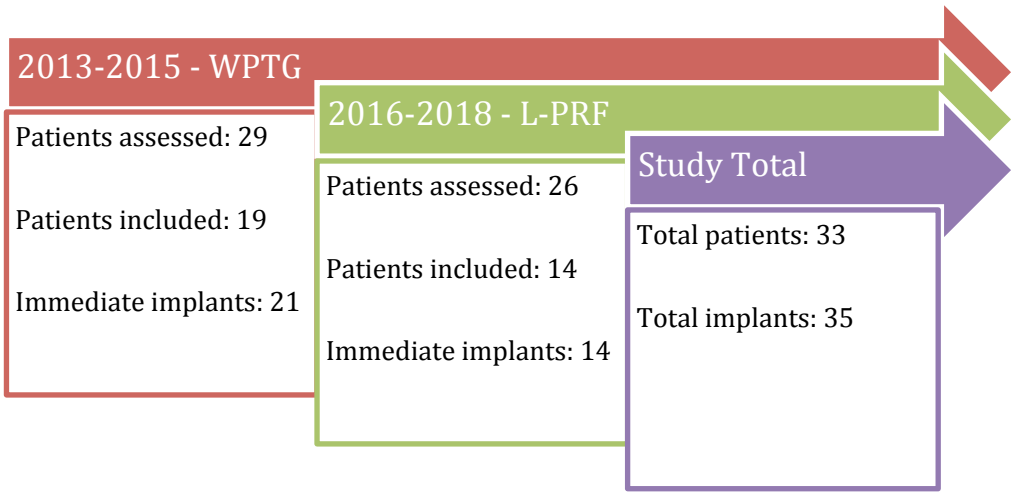

Figure 3: Flowchart of number of patients and implants included in this study

All participants were asked to attend 4 appointments. The first appointment was a screening appointment with clinical and radiographic examination to determine if the patient met the inclusion criteria and to discuss their treatment options. Those patients who did not meet the inclusion criteria were referred on to the appropriate department for further treatment, Patients who met the inclusion criteria were given an information leaflet and reappointed no sooner than 7 days later. At the second appointment, they confirmed their participation and signed the informed consent form. For those patients who did not wish to participate, alternative treatment was arranged and/or provided. The third appointment consisted of the extraction, implant surgery and the necessary clinical and photographic measurements. Following a four-month healing period each participant returned for the 2nd stage implant surgery in which further clinical measurements and photographs were taken.

3.7 Treatment protocol

Patients received a loading dose of antibiotic one hour pre-operatively (3g Amoxicillin or 600mg Clindamycin in patients allergic to Penicillin).

Selected teeth were extracted atraumatically with the use of a periotome (Figures 4, 13, 21, 29). Following this the extraction socket was assessed to ensure the buccal wall was intact and meeting the criteria for immediate implant placement. Provided the criteria were met a full thickness mucoperiosteal flap was raised buccally and palatally exposing the coronal and middle parts of the alveolar ridge (Figures 5, 14, 22, 30). The dimensions of the bony socket and alveolar ridge were recorded prior to implant placement. In all sites, a 4.1mm diameter Zimmer Biomet T3™ implant (Biomet 3i implant innovations, Palm Beach Gardens, Florida, USA) (Figures 6, 15, 23, 31) was placed with good primary stability. Implants were placed 0.5-1.0mm submerged relative to the palatal bone crest. Implants were placed with a view to having a screw-retained restoration and in contact with the palatal wall. After implant placement the dimensions of the void were measured clinically and a photograph was recorded in an angle parallel to the long axis of the implant. In all implants a cover screw was placed and submerged healing protocol used.

3.7.1 WPTG protocol

In this first cohort of patients Natix® titanium granules (PTG White) were used to graft the gap between the buccal bone and the buccal aspect of the implant, irrespective of the gap size (Figures 32, 34). The site was then covered with a single

layer resorbable collagen (Osseoguard® Biomet 3i) (Figure 33). The flaps were sutured using 4.0 Coated Vicryl™ sutures leaving in most cases (17 out of 21) the collagen membrane exposed (Figure 35).

3.7.2 L-PRF protocol

Prior to the extraction of the tooth blood was drawn for the centrifugation process and fabrication of the L-PRF clots. The antecubital fossa was the preferred site for blood draw and in cases where this was not possible, the dorsum of the hand was used. The L-PRF clots were prepared according to a protocol described by the Department of Oral Health Sciences, Periodontology, Catholic University of Leuven, Belgium (Appendix D). Depending on the quantity of blood flow at the time of blood draw, between two and six tubes (Red Cap Intraspin™ 9ml Blood Collection Tubes) were obtained. With the aid of an assistant, the tubes were transferred to the centrifuge as quickly as possible. When drawing more than two tubes, the first two tubes were spun in the centrifuge for 60 seconds while the remaining tubes were being collected. Once all tubes had been collected, they were placed in the centrifuge and spun for 12 minutes at 2,700 rpm.

Using the Xpression™ Box the L-PRF clots were compressed to create at least one membrane and one socket plug each (Figure 7). The socket plug was used to graft the void/gap between the buccal bone and the buccal aspect of the implant, irrespective of the gap size (Figures 8, 16, 24). The site was then covered with as many L-PRF membranes that could fit over the implant and the flaps were sutured

using 4-0 Coated Vicryl™ sutures leaving the L-PRF membrane exposed in all cases (Figures 9, 17, 25).

3.7.3 Post-operative care

Patients were advised to avoid brushing the surgical site in the immediate post-operative period and were asked not to rinse with chlorhexidine for the first 12 hours. In addition, all patients were prescribed antibiotics, analgesics and anti-inflammatories. Ten to fourteen days after implant placement, the patient was reviewed and sutures removed. When required for aesthetic reasons, as Essix retainer was fitted as a tooth-supported provisional restoration. No implant-supported provisional restorations were used during the first 4 months post-implant placement.

3.7.4 2nd stage surgery

A minimum of 16 weeks healing was allowed before the patients returned for re-entry second stage implant surgery (Figures 10, 18, 26, 36). Full thickness buccal and palatal flaps were raised in a minimal fashion, allowing visualisation of the head of the implant and the crest of the alveolar ridge (Figures 11, 19, 27, 37). Soft tissue was gently removed from around the implant and the cover screw was removed. Bony measurements of the buccal bone and ridge dimensions were recorded as well as a photograph, again with an angle parallel to the long axis of the implant. A

healing abutment was placed and the flap closed with 5-0 or 4-0 Coated Vicryl ™ sutures (Figures 12, 20, 28, 38). Patients returned 7-10 days later for review and suture removal.

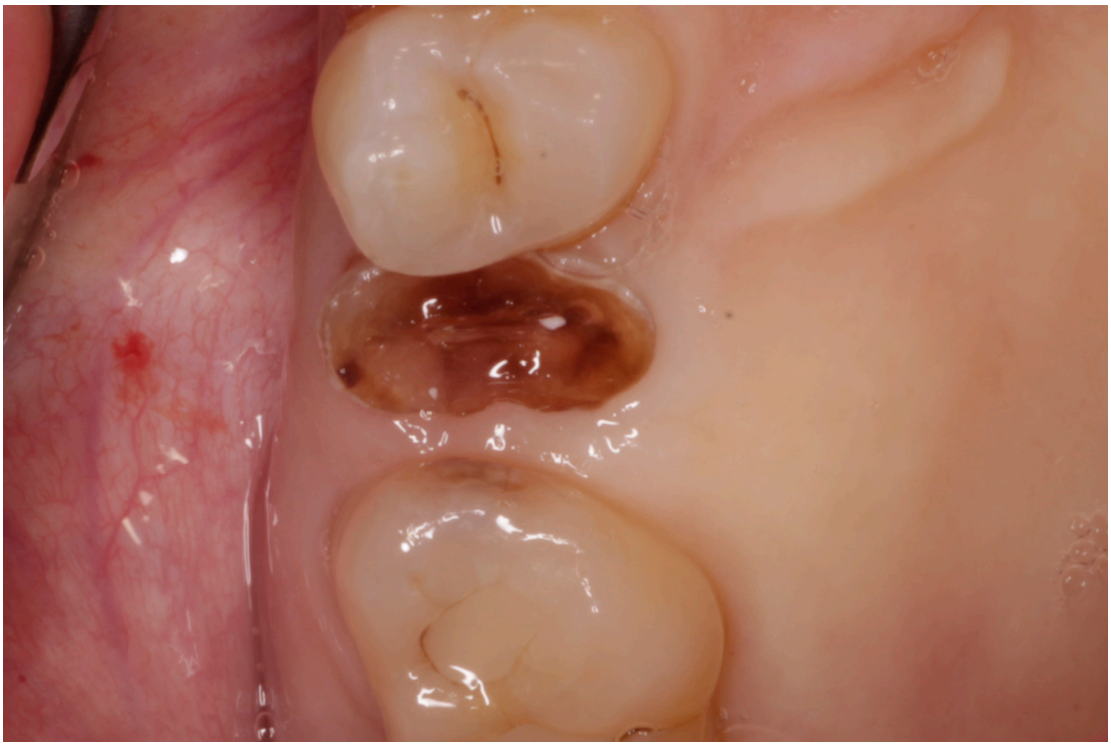

Figure 4: Tooth for extraction

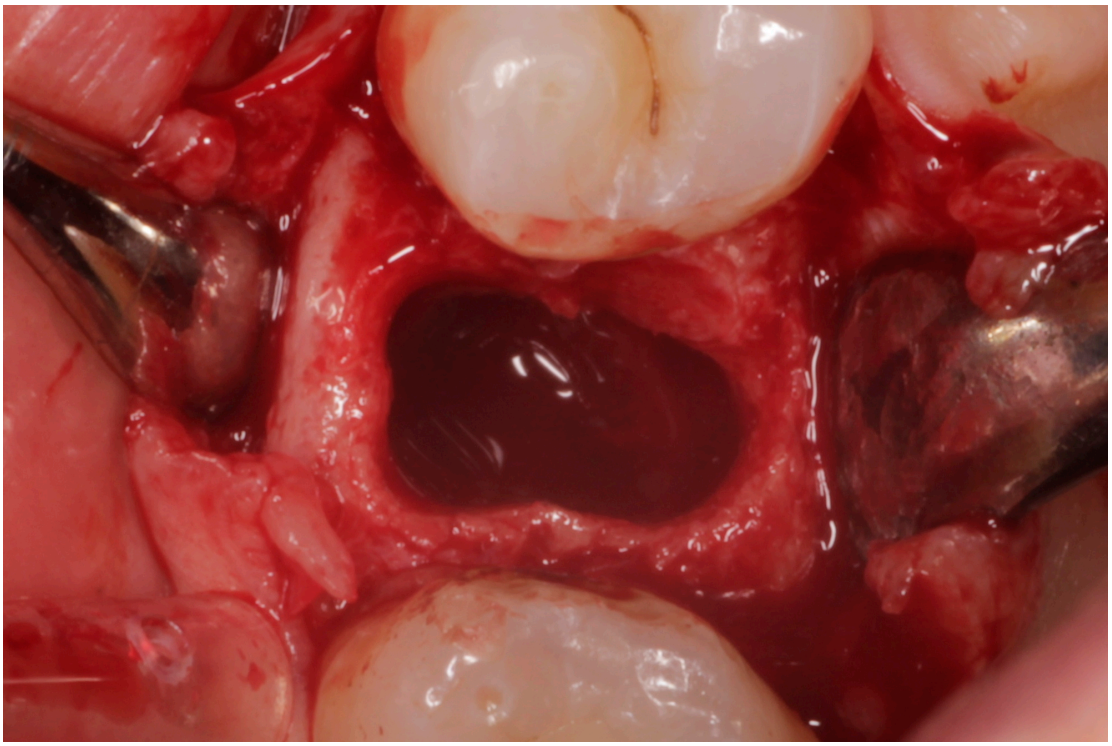

Figure 5: Socket walls, buccal and palatal full thickness flaps raised

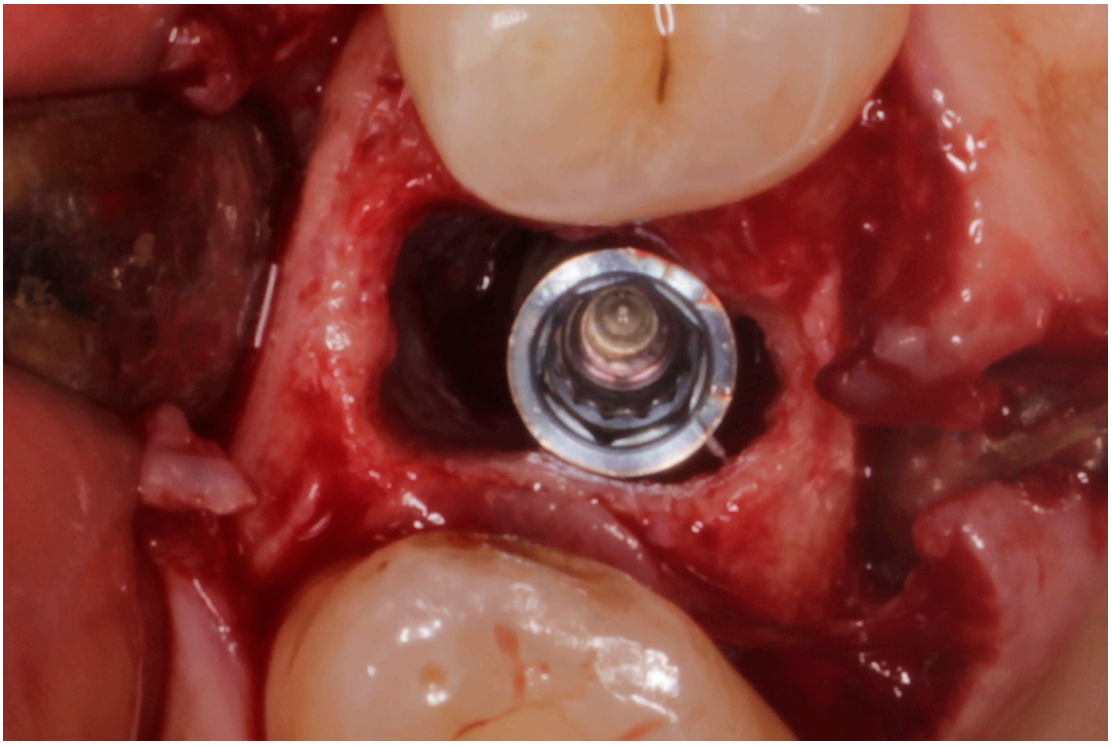

Figure 6: Implant installed

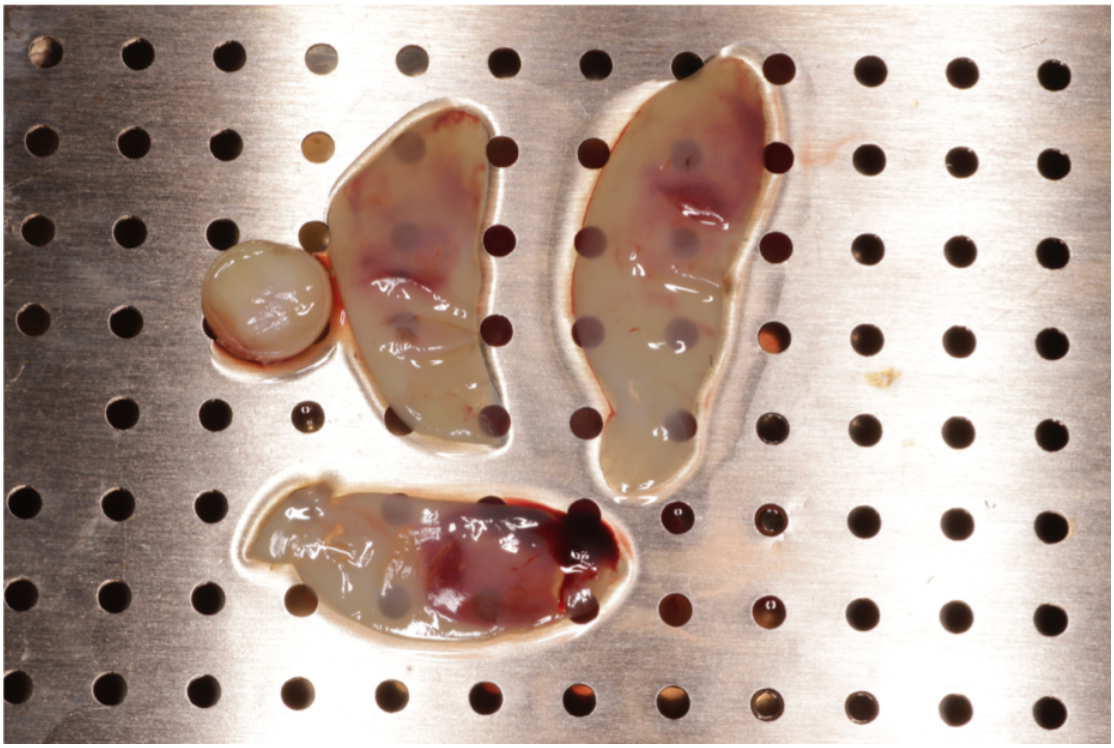

Figure 7: L-PRF plug and membranes

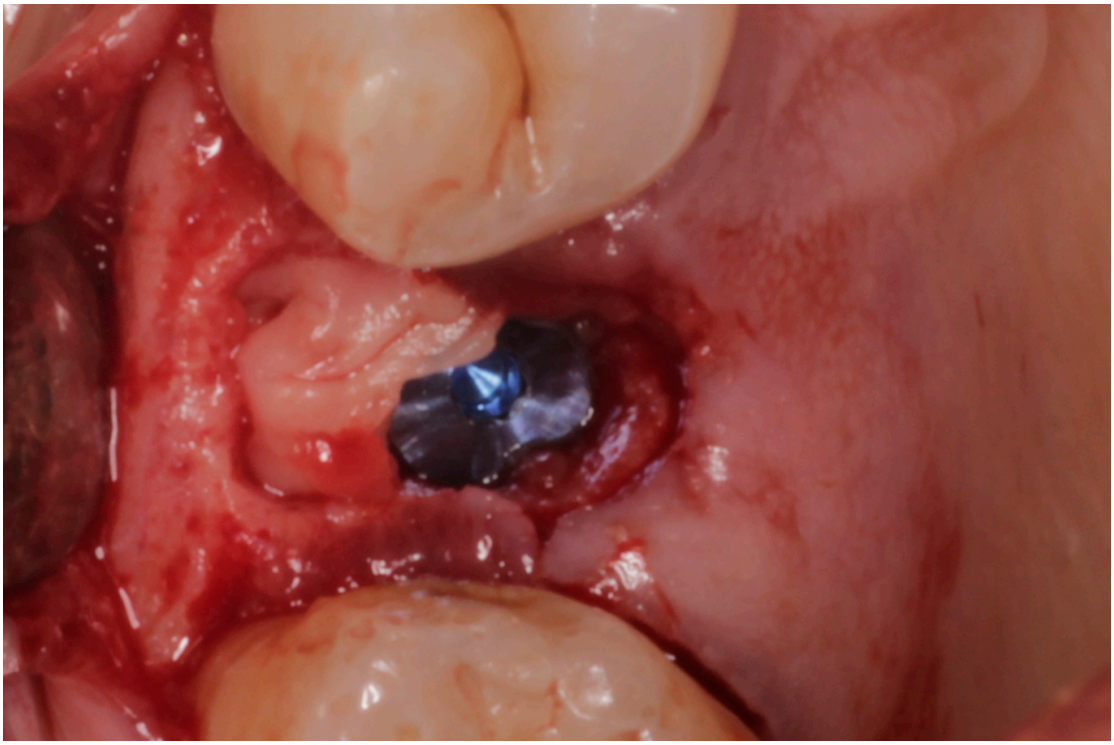

Figure 8: Buccal void grafted with L-PRF plug

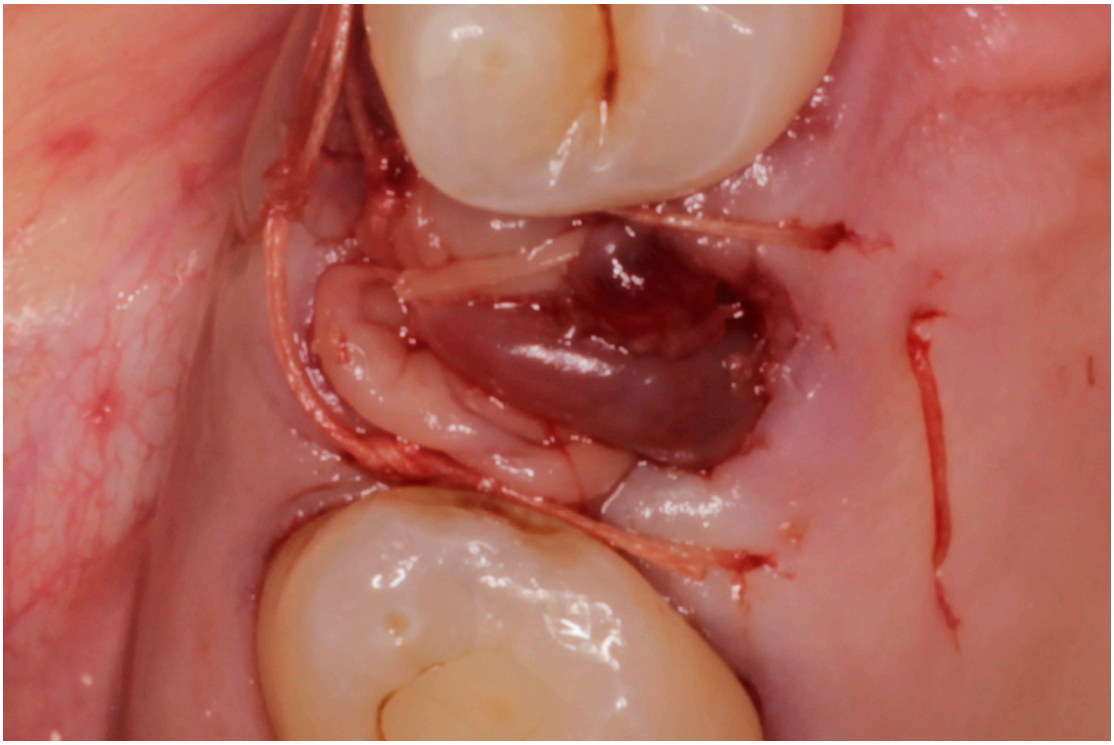

Figure 9: L-PRF membranes over the implant and held in place with sutures

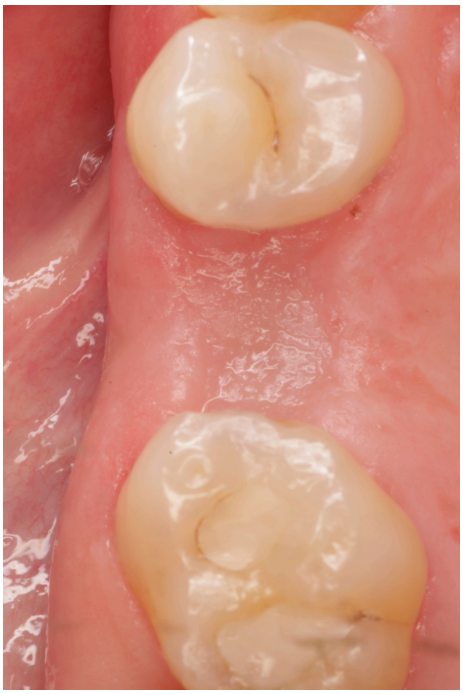

Figure 10: Implant site 4 months post-surgery

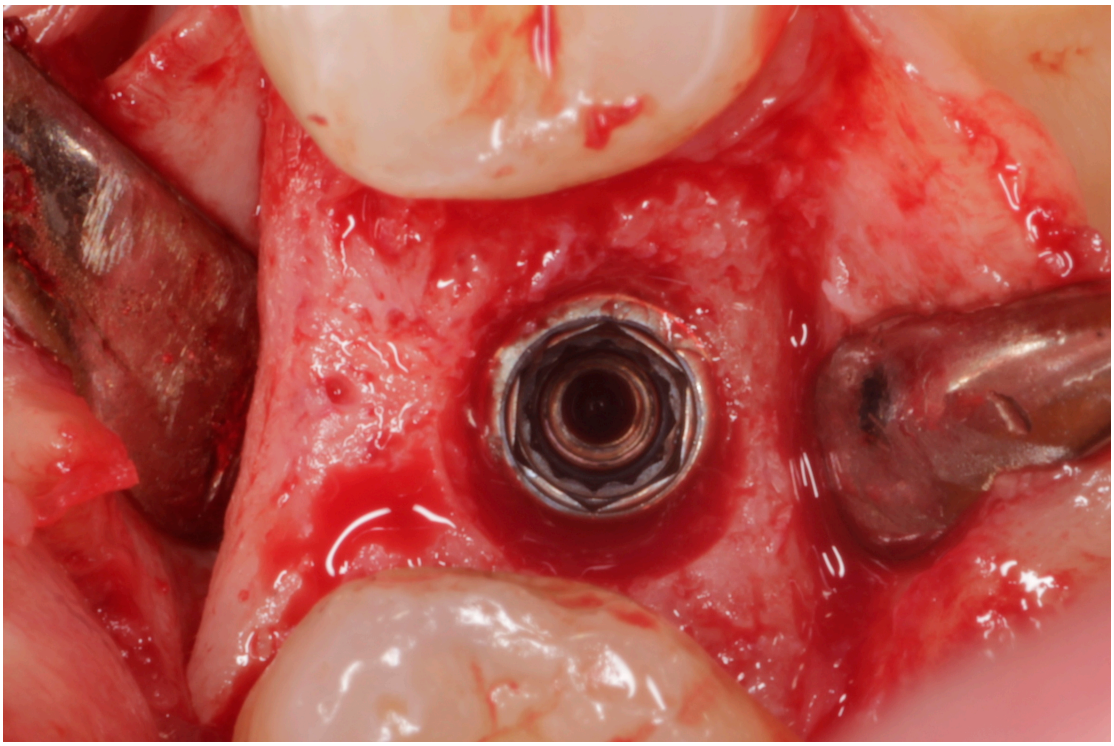

Figure 11: Exposure of the implant

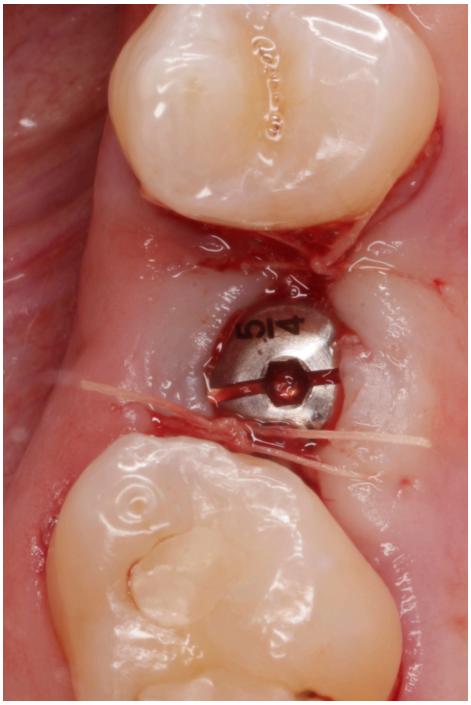

Figure 12: Healing abutment

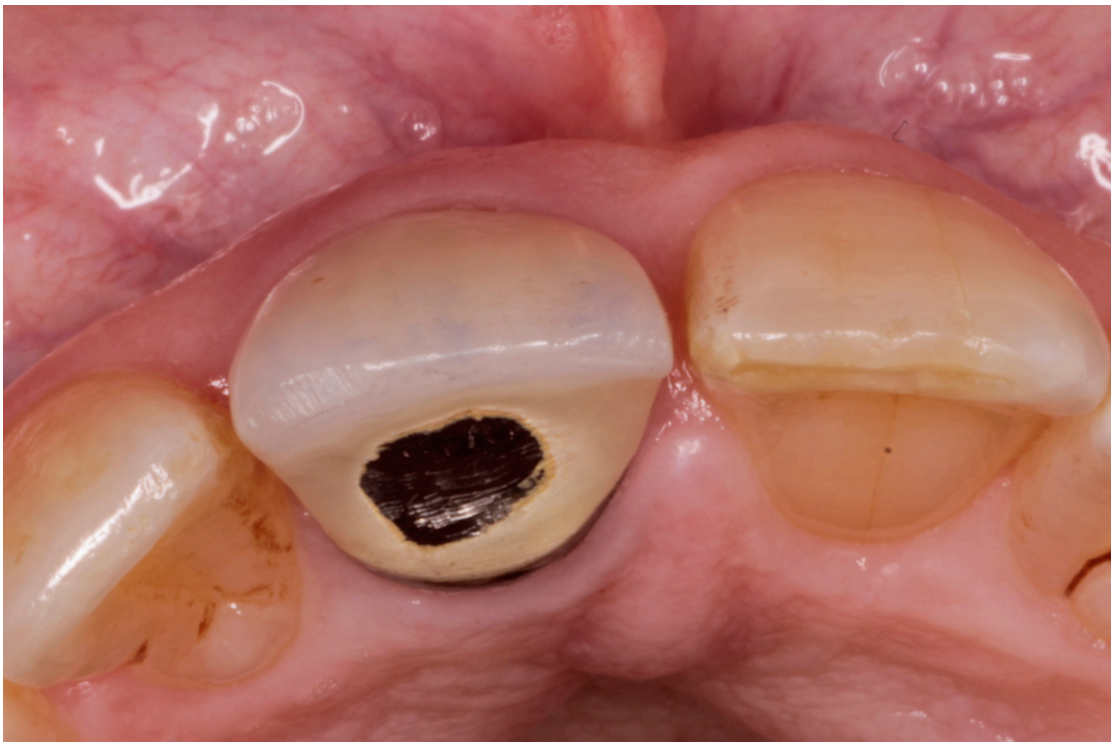

Figure 13 Tooth for extraction

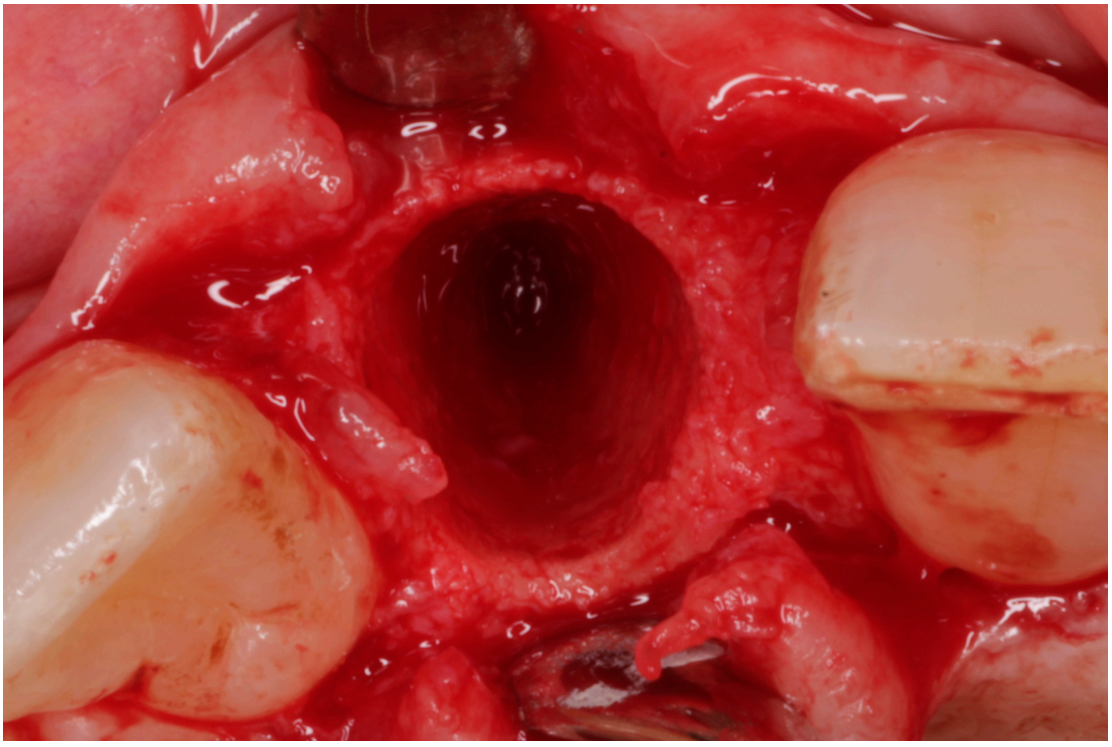

Figure 14: Socket walls, buccal and palatal full thickness flaps raised

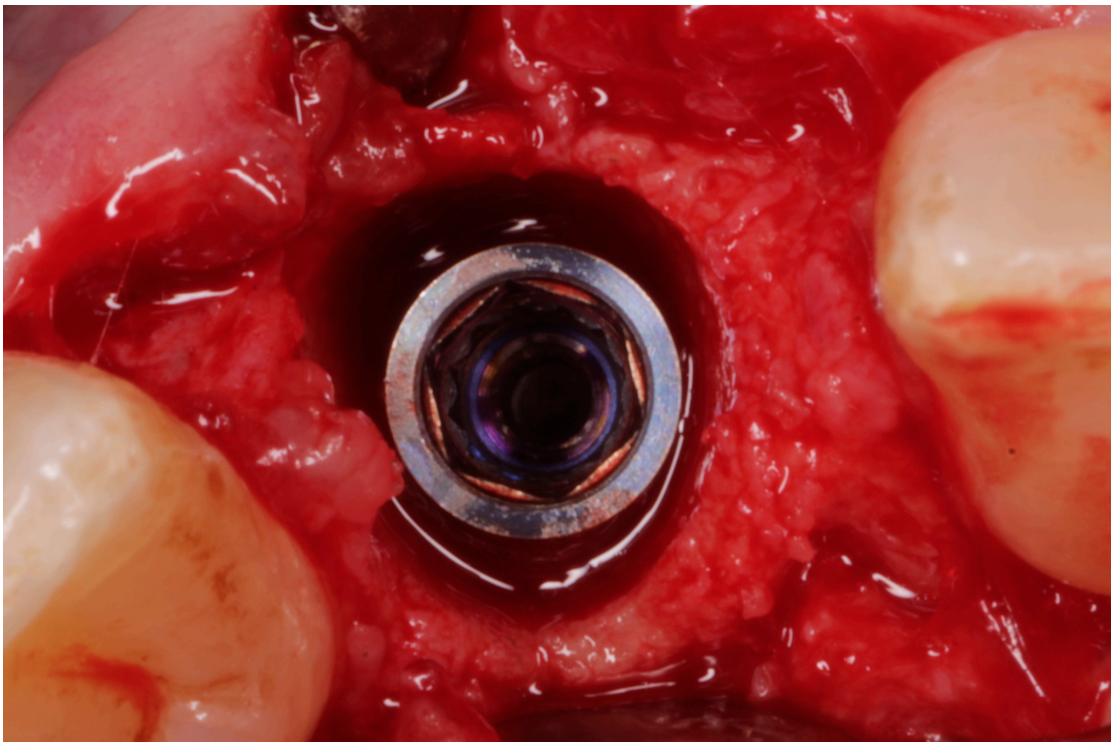

Figure 15: Implant installed

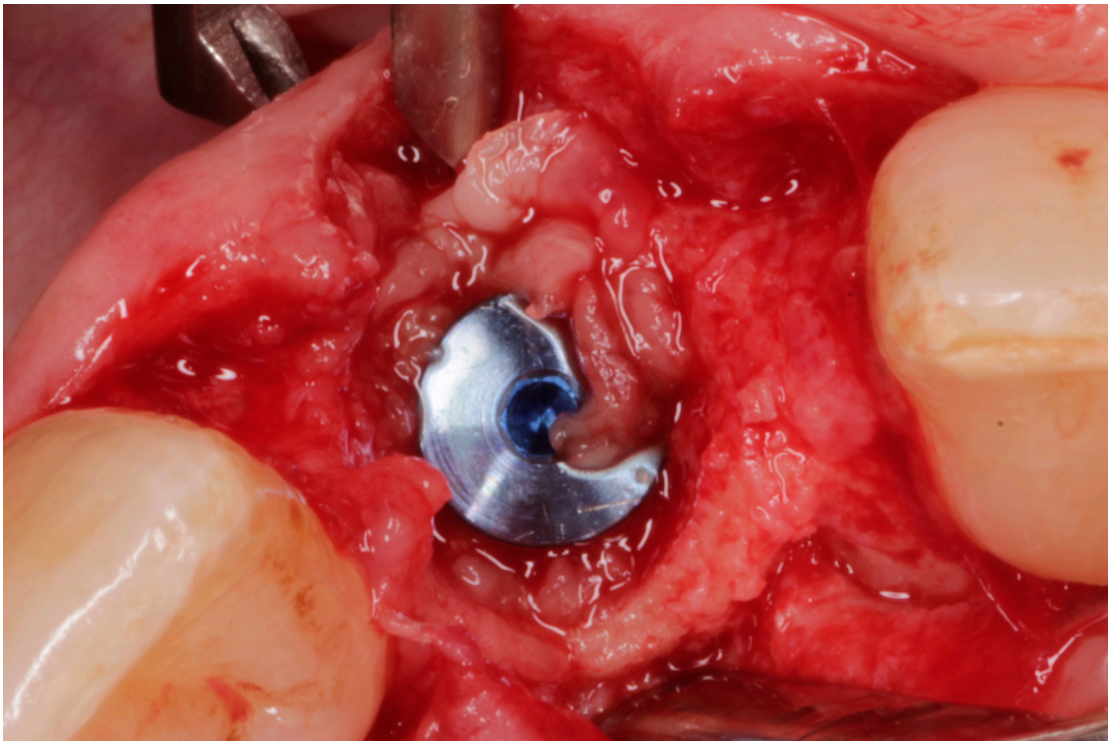

Figure 16: Buccal void grafted with L-PRF plug

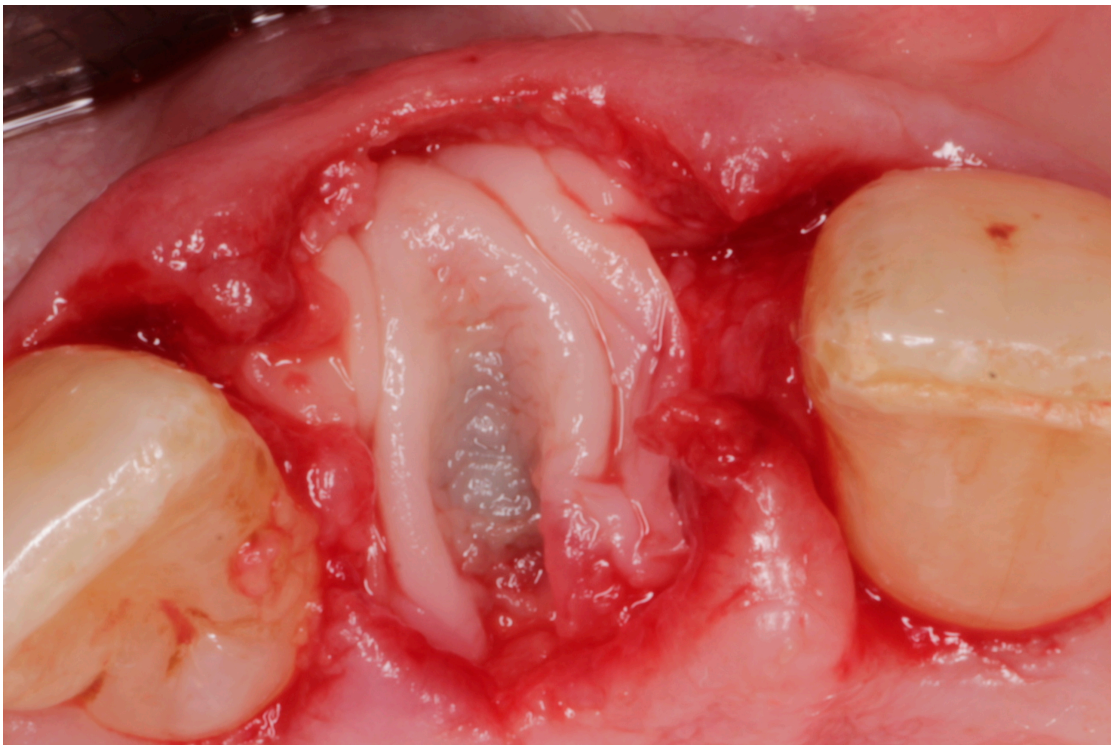

Figure 17: L-PRF membranes over the implant

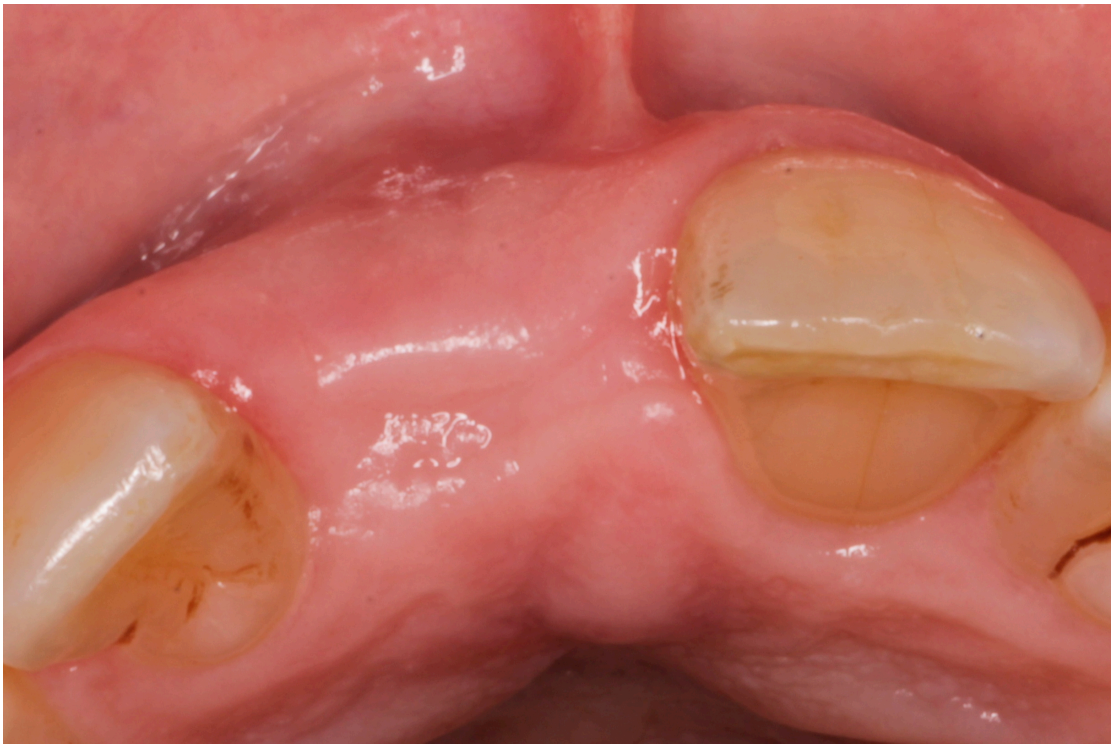

Figure 18: Implant site 4 months post-surgery

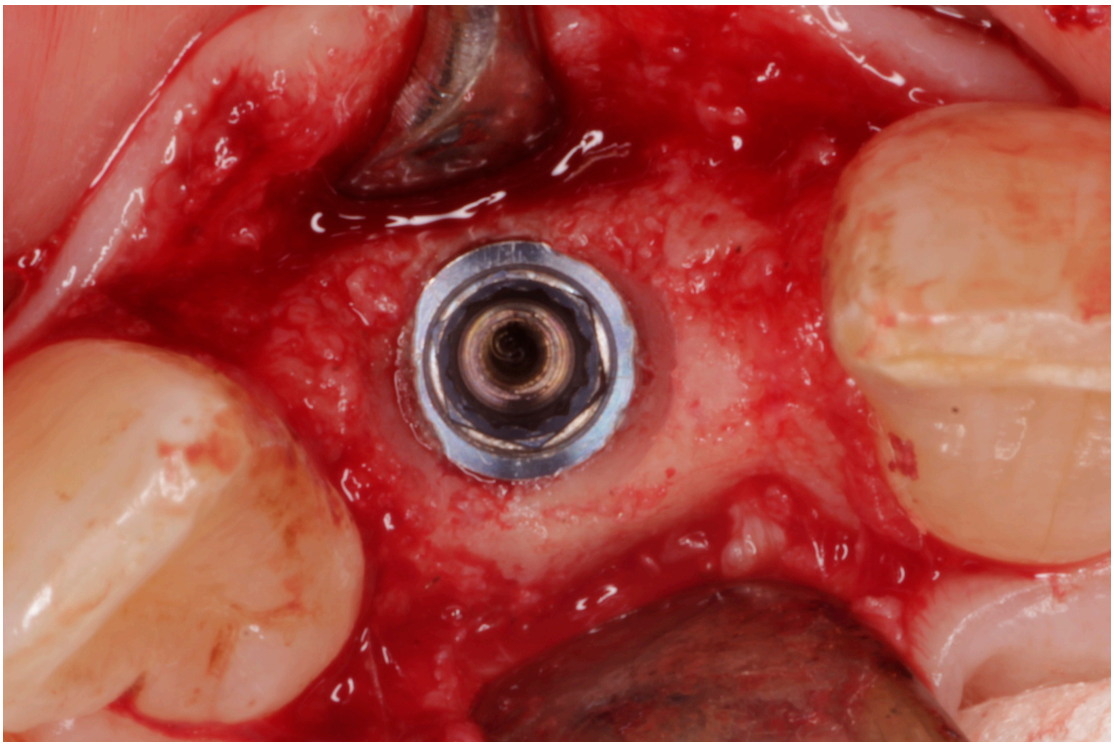

Figure 19: Exposure of the implant

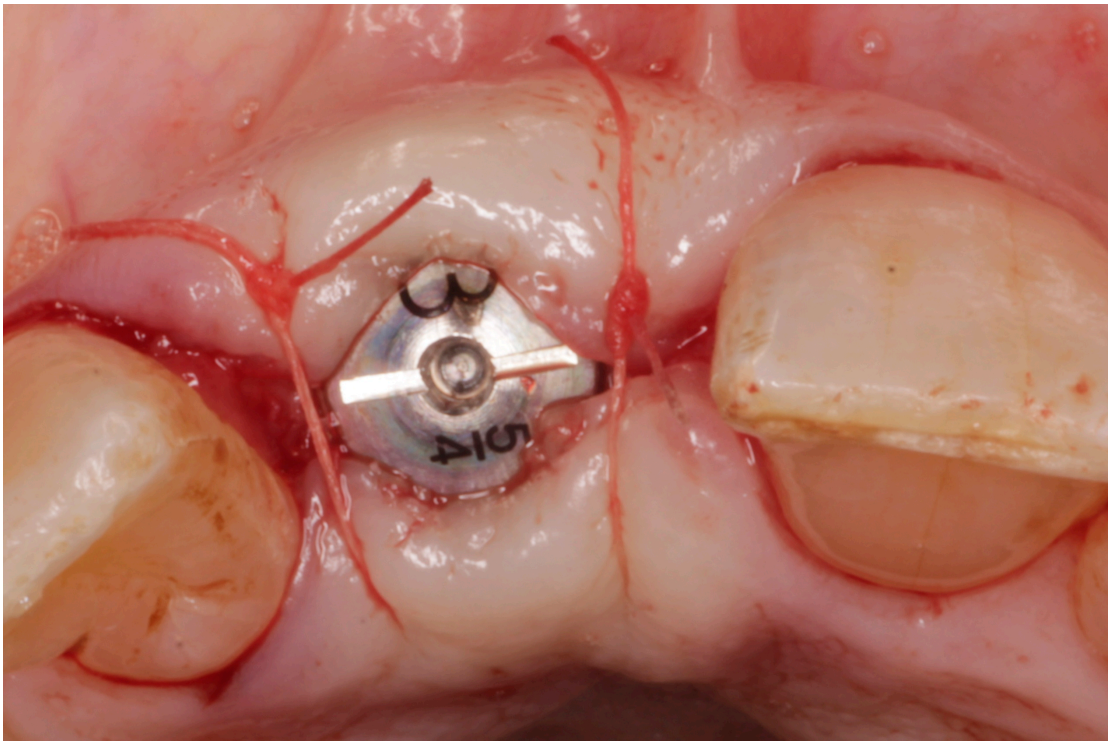

Figure 20: Healing abutment

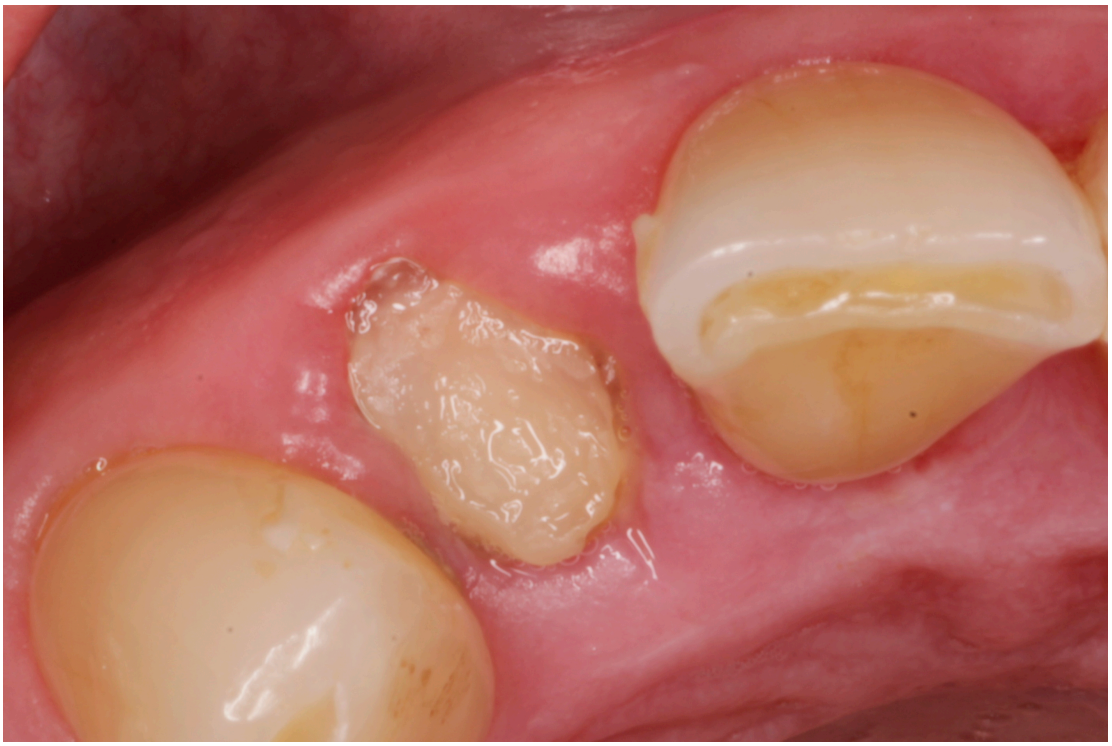

Figure 21: Tooth for extraction

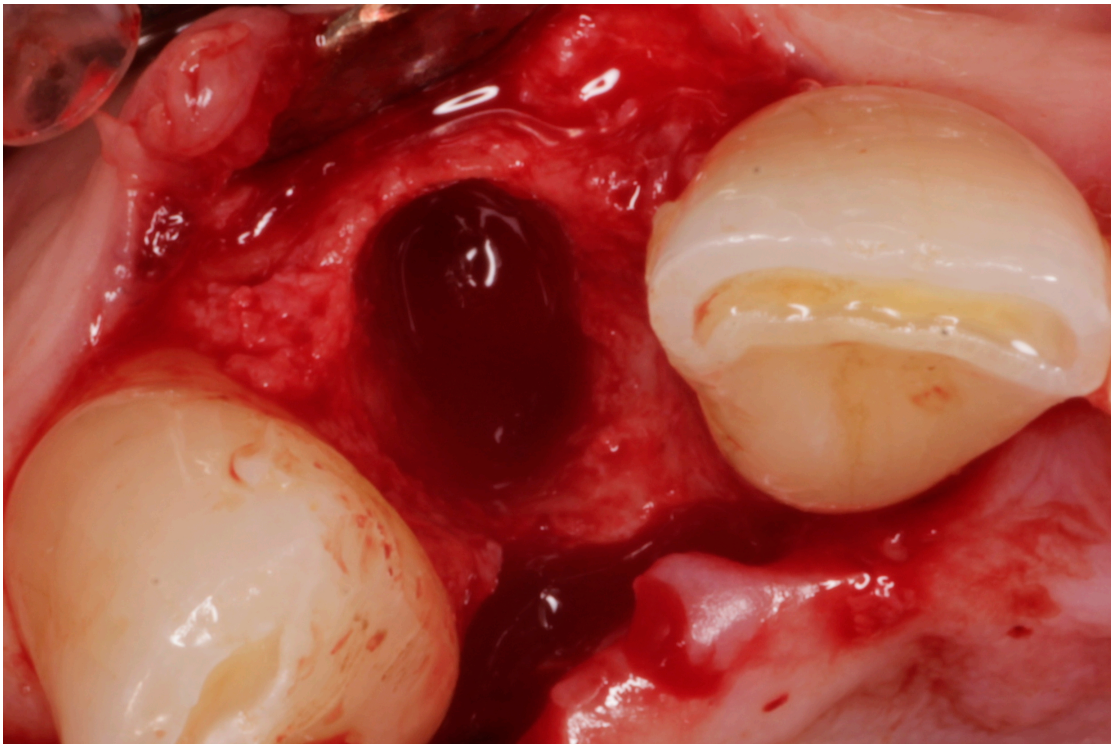

Figure 22: Socket walls, buccal and palatal full thickness flaps raised

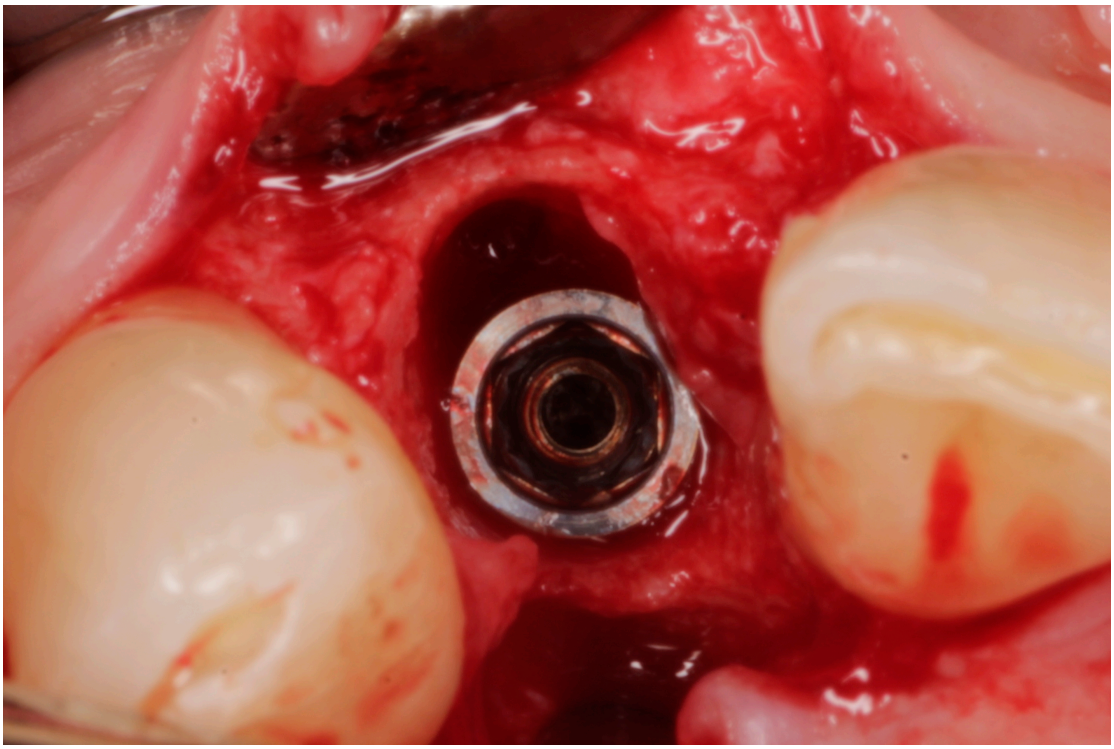

Figure 23: Implant installed

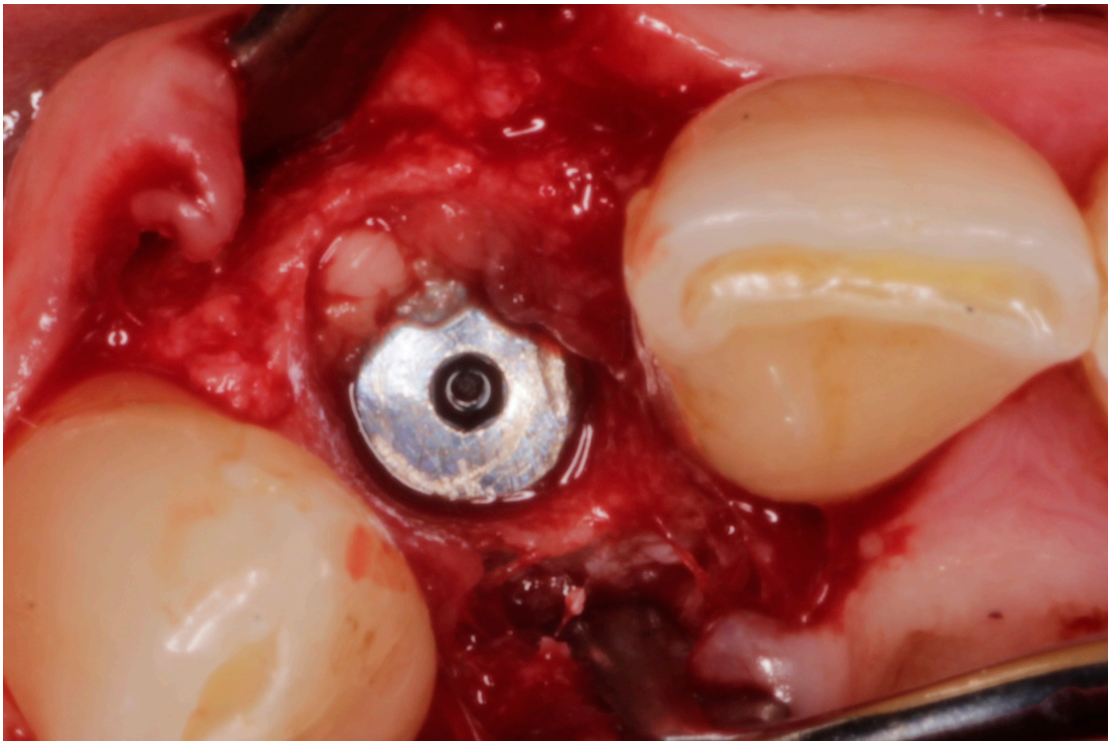

Figure 24: Buccal void grafted with L-PRF plug

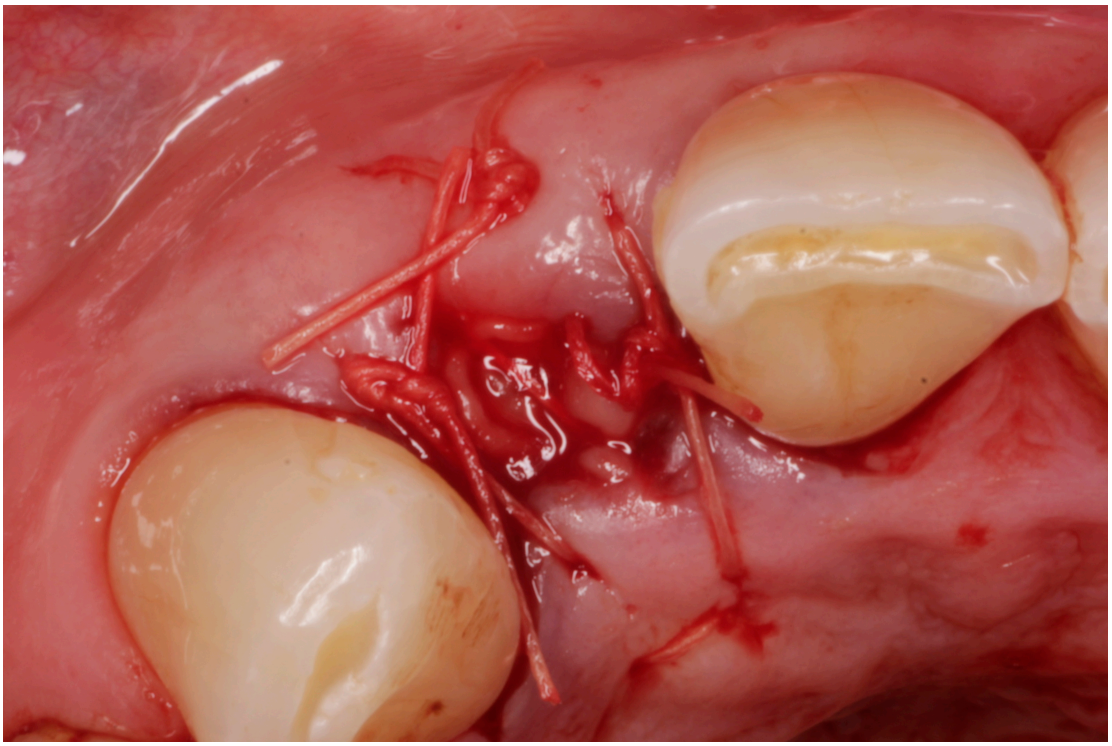

Figure 25: L-PRF membranes over the implant and held in placed with sutures

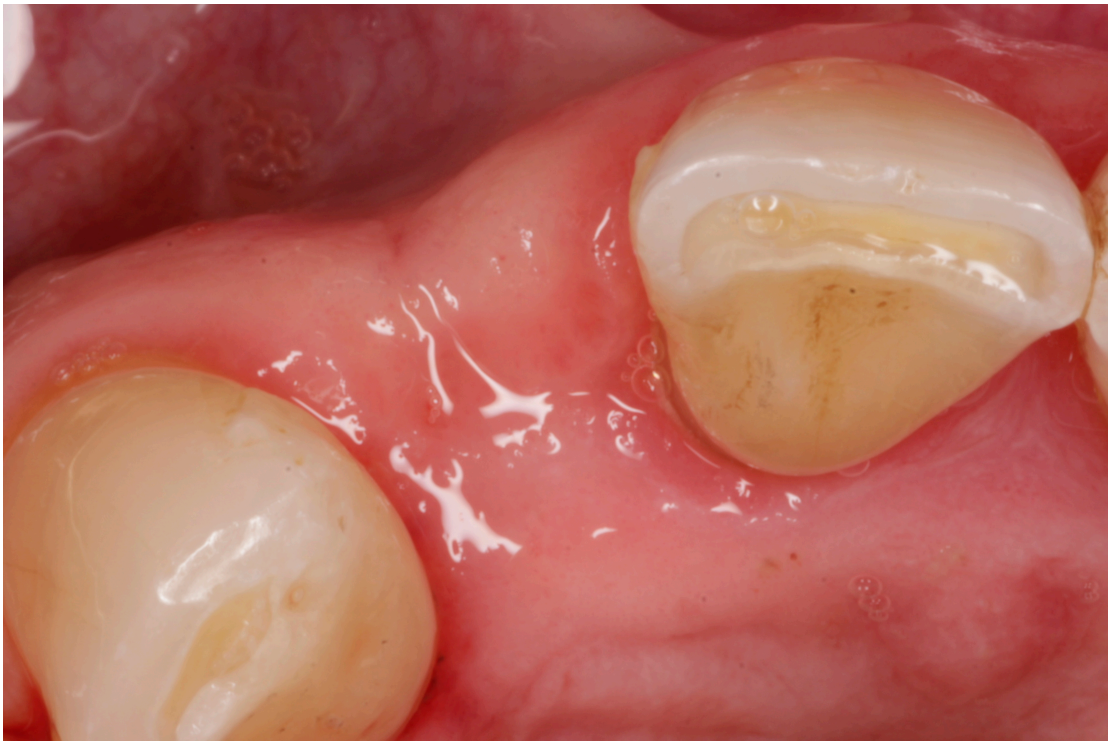

Figure 26: Implant site 4 months post-surgery

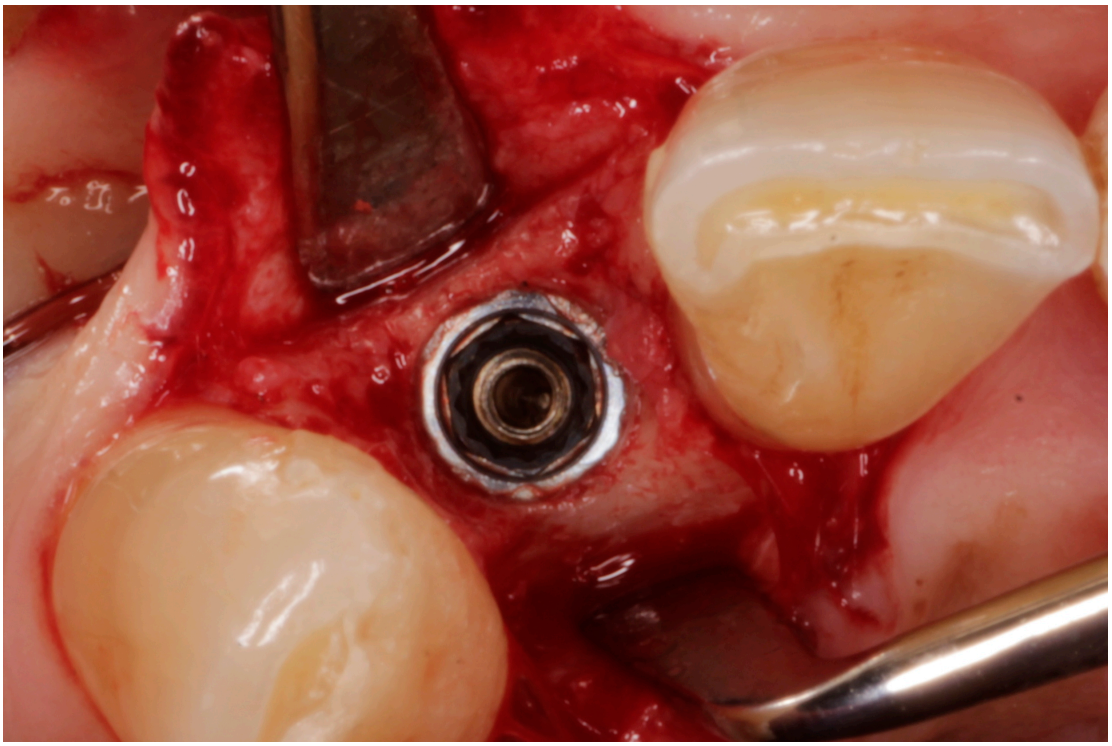

Figure 27: Exposure of the implant

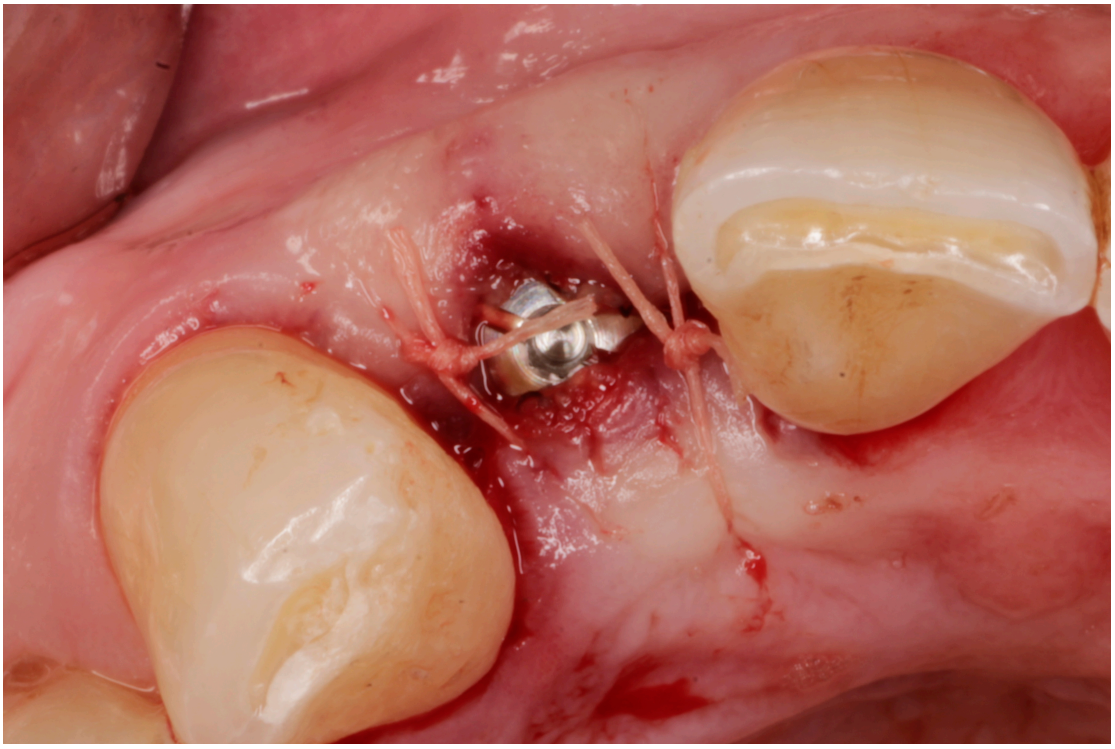

Figure 28: Healing abutment

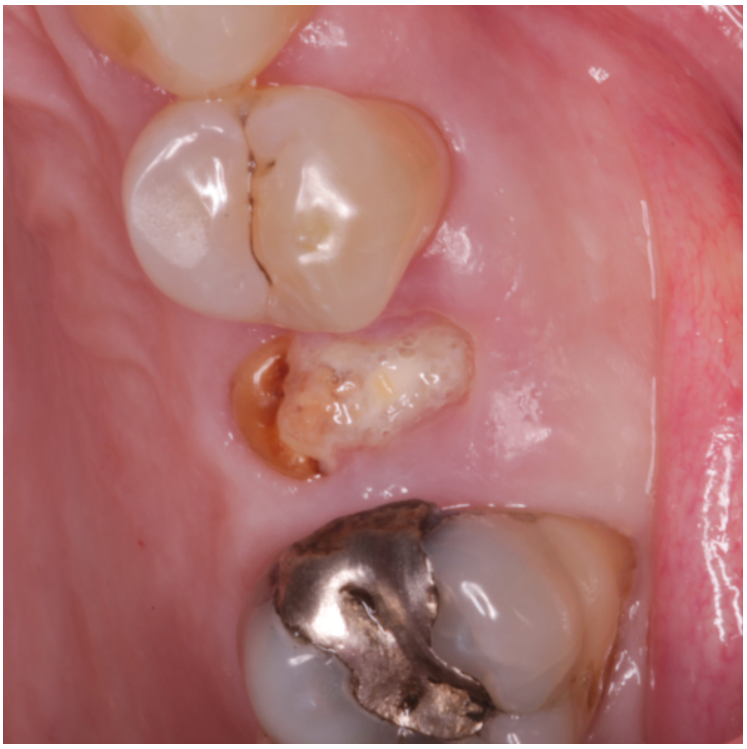

Figure 29: Tooth for extraction

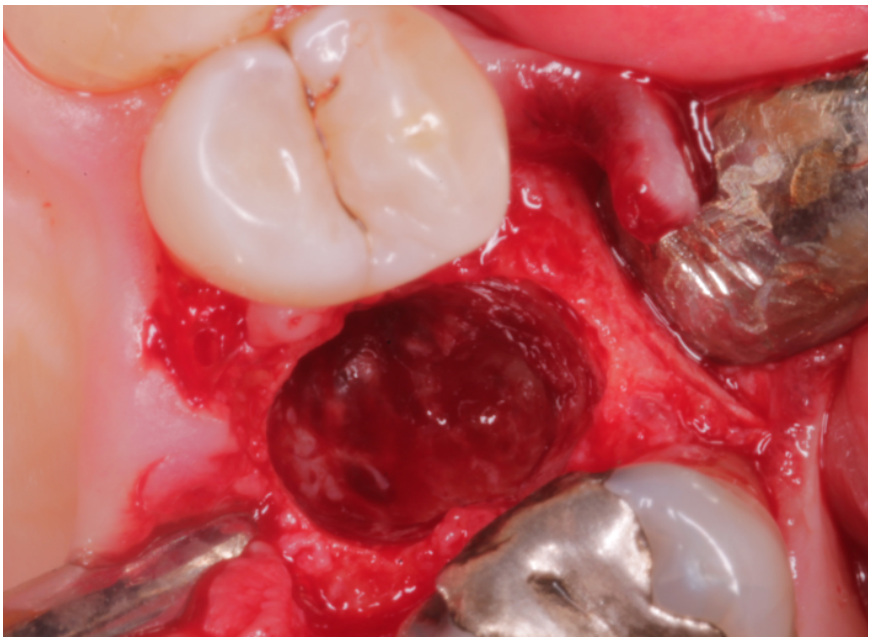

Figure 30: Socket walls, buccal and palatal full thickness flaps raised

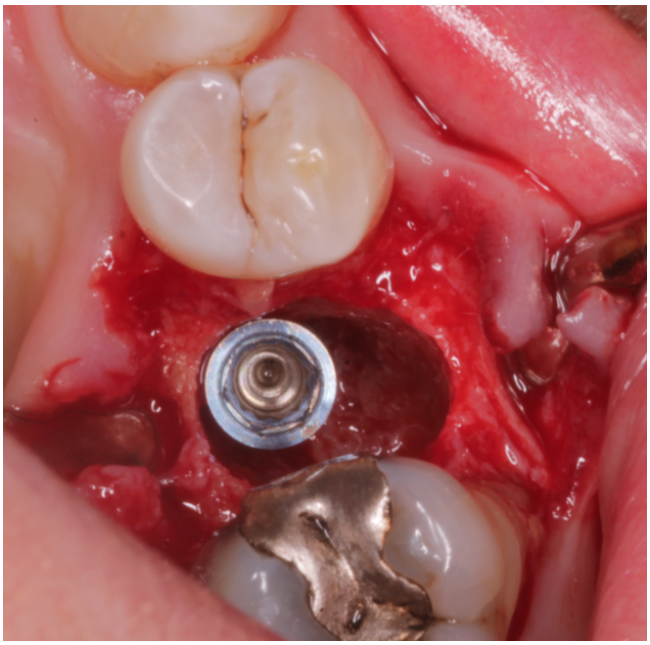

Figure 31: Implant installed

Figure 32: Natix™ titanium granules (PTG White)

Figure 33: Osseoguard™ Biomet 3i collagen membrane

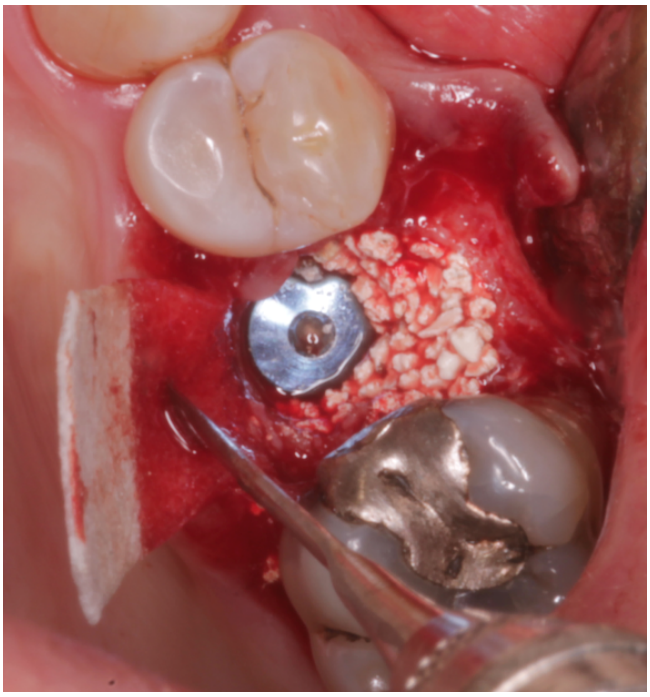

Figure 34: Buccal void grafted titanium granules and covered with a single layer resorbable membrane

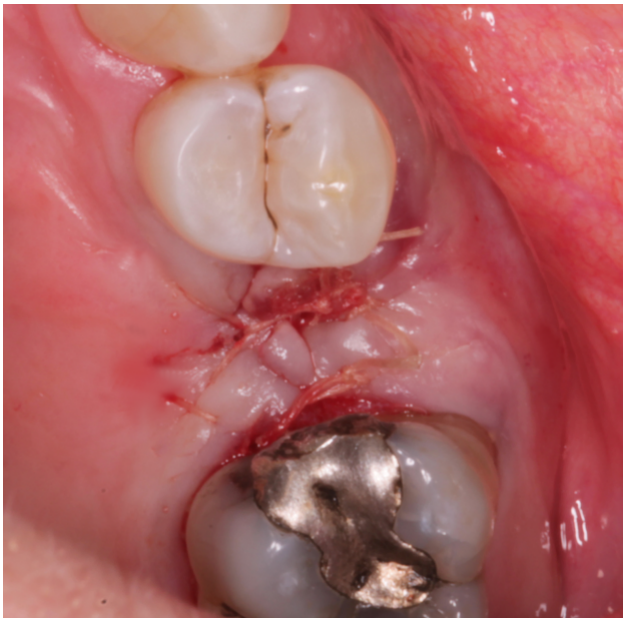

Figure 35: Implant site sutured

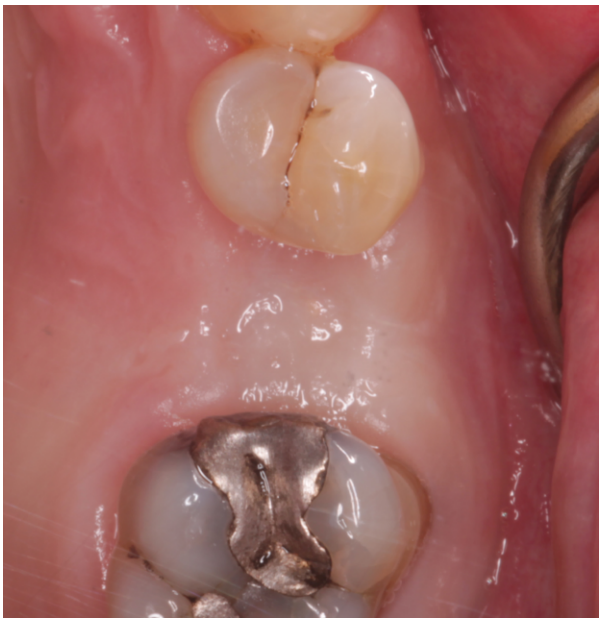

Figure 36: Implant site 4 months post-surgery

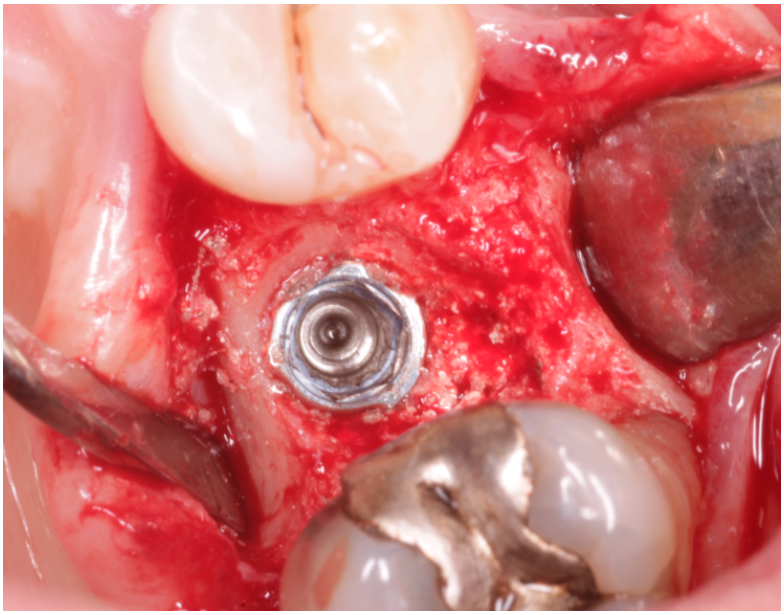

Figure 37: Exposure of the implant

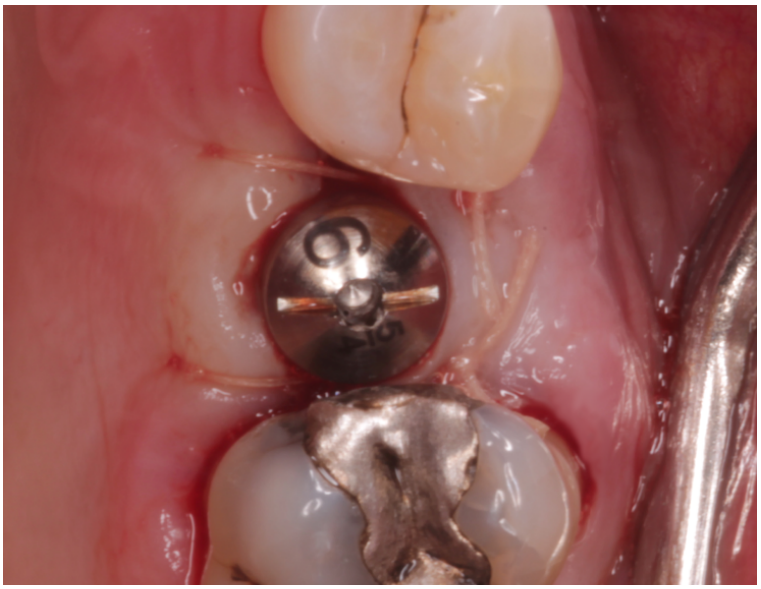

Figure 38: Healing abutment

3.8 Clinical measurements

The first 21 extractions and immediate implants (WPTG) were performed by two operators (G.G and I.P) and the next 14 (L-PRF) were performed by one different operator and one of the previous operators (M.M and I.P). All clinical measurements were taken and confirmed by both sets of clinicians. A UNC15 Hu-Friedy Chicago, USA periodontal probe (Figure 39) and a Hu-Friedy 40mm Straight Castroviejo Caliper (Figure 39) were used for all the measurements. All measurements were to the nearest half millimetre.

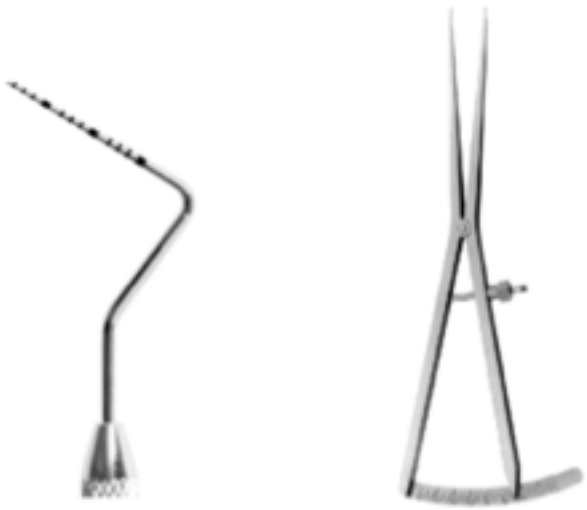

Figure 39: UNC15 Hu-Friedy Chicago, USA periodontal probe and a Hu-Friedy 40mm Straight Castroviejo Caliper

The clinical measurements recorded at first stage surgery were:

1. B to A, the depth of the buccal socket wall
2. P to A, the depth of the lingual socket wall
3. B to P, the alveolar ridge width (width of the socket?)
4. OB to IBC, the buccal bone width in the coronal part (1mm apical to the crest of the ridge)
5. M1 to M2, the buccal bone width in the middle third of the socket
6. OPC to IPC, the palatal bone width at the crestal level
7. S to R, the distance between the buccal bone crest and the most buccal surface of the implant (void/gap)

8. R to D, the depth of the void/gap measured in contact with the implant surface
9. B to D, the depth of the void/gap measured in contact with the buccal bone surface
10. B to S, the submersion of the implant

Figure 40 demonstrates the landmarks used for the measurements recorded at first stage

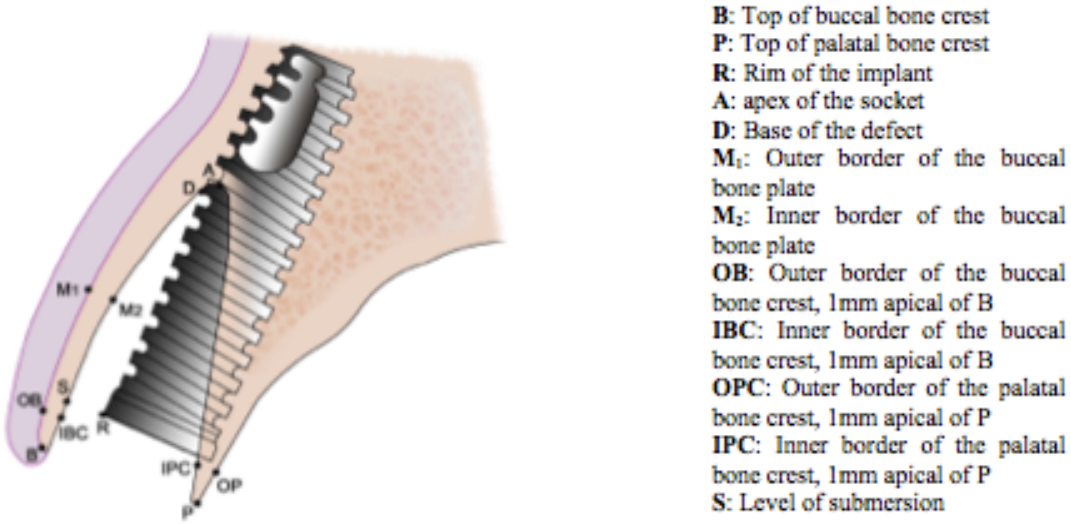

Figure 40: Landmarks used for the measurements recorded at 1st stage

The clinical measurements recorded at second stage were:

1. BIC to OBC1, the distance of the first bone-implant contact buccally to the ridge crest
2. R to BIC, the vertical buccal loss of osseointegration (if existing)
3. B1 to P1, the alveolar ridge width

Figure 41 demonstrates the landmarks used for the measurements recorded at second stage

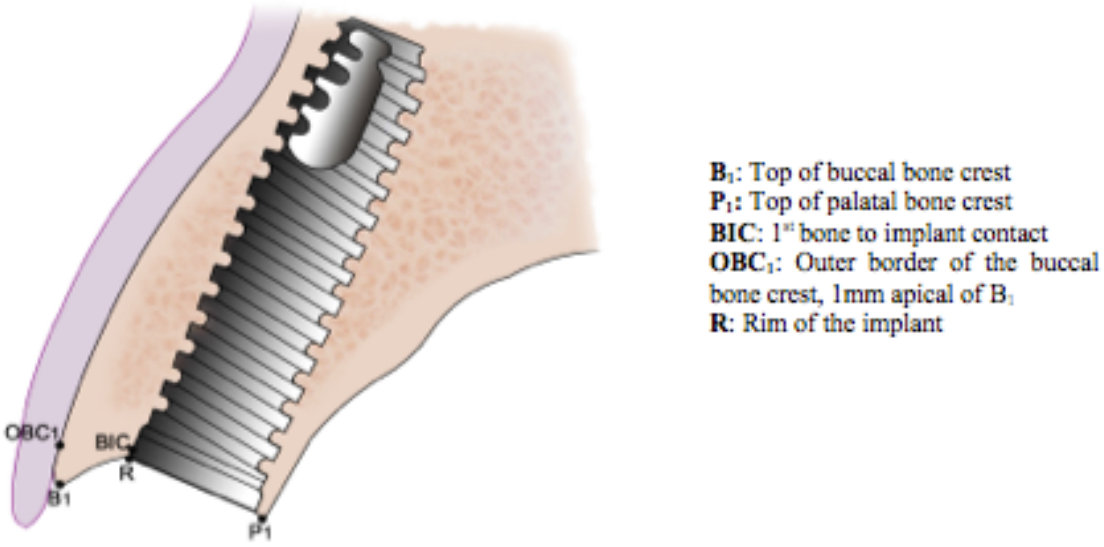

Figure 41: Landmarks used for the measurements recorded at 2nd stage

3.9 Photographic measurements

As stated above, the clinical measurements were performed using a UNC15 Hu-Friedy Chicago, USA periodontal probe (Figure 39) and a Hu-Friedy 40mm Straight Castroviejo Caliper (Figure 39). These measurements were performed to the nearest half millimetre. To increase the reliability of the clinical measurements it was decided to combine / reinforce them with photographic measurements. A photograph was recorded in an angle parallel to the long axis of the implant, immediately after implant installation and again at the re-entry procedure. An estimated average of the clinical and photographic measurements was calculated and their mean score was used for the statistical analysis. Photographic measurements were made only for gap width (S-R), buccal bone width 1mm apical to crest (OB-IBC), ridge width at 1st stage (B-P), buccal bone width plus the buccal gap i.e. buccal bone horizontal dimension (OB-R), buccal bone width at 2nd stage (BIC-OBC1) and the ridge width at 1st stage (B1-P1).

The photographs recorded at first and second stage implant surgeries were analysed using ImageJ software (Version 1.51s; National Institutes of Health, USA). The known implant diameter of 4.1mm was used to calibrate the measurements performed using the software (Figure 42). This was possible as all implants had the same diameter and the photographs were taken in an angle parallel to the long axis of the implant.

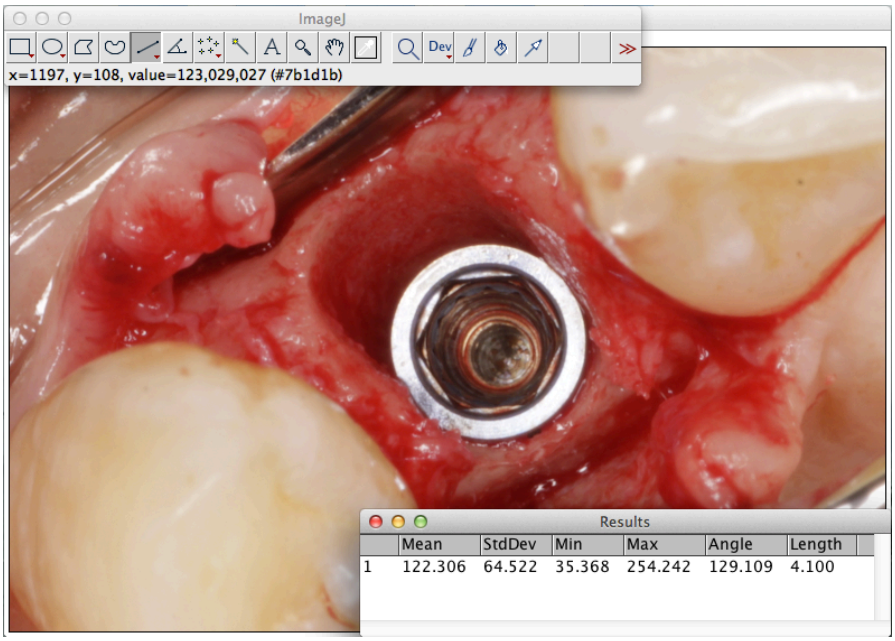

Figure 42: ImageJ Software

Following 1st stage surgery measurements were made for each patient; ridge width, gap width, buccal bone width and buccal bone horizontal dimension. Then using the photographs taken at 2nd stage, the ridge width and buccal bone width were measured (Figure 43).

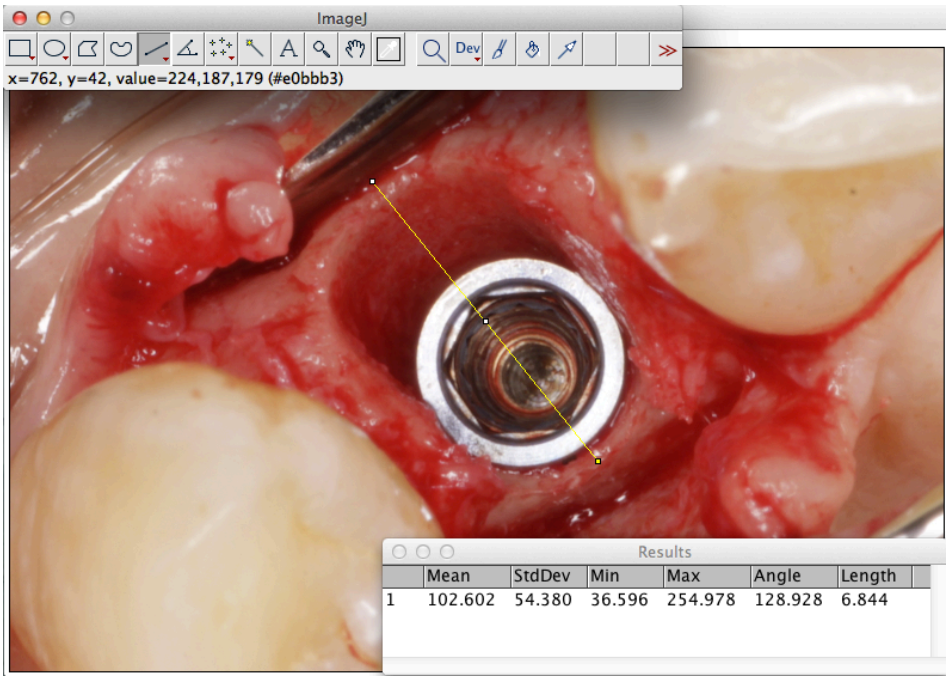

Figure 43: Known implant diameter was used to calibrate measurements

3.10 Statistical analysis

The null hypothesis states that no difference exists in the crestal alveolar bone changes occurring following immediate implant placement with simultaneous grafting, with either white porous titanium granules or leucocyte and platelet-rich fibrin, in the anterior maxilla.

All data was entered into an Excel spread sheet. Data analysis was performed using IBM SPSS Statistics 25.0 for Windows. Demographics and other baseline characteristics were presented by means of descriptive statistics. Continuous variable were presented by means of the number of observations (N), mean and standard deviations (SD) and discrete variables by frequency and percentage. Intergroup comparisons were made by means of paired t-tests and Wilcoxon's rank

sum tests. A two-sided P-value of ≤0.05 was considered to be statistically significant. Statistical correlations were performed to see how strongly specific socket characteristics were related to the dimensional changes. The correlations between the different variables were determined using Pearson and Spearman's correlation tests. A stepwise multiple regression analysis was performed to determine possible predictors for the buccal bone width at 2nd stage and the ridge width at 2nd stage following 4 months of healing.

4 Results

4.1 Demographic and Categorical Results

Demographic and baseline characteristics are presented by means of descriptive statistics in Table 3.

To allow for comparison with a previous research study carried out in this institution some of their results will also be presented in this section. That study followed the exact same methodology as this one, except for the material used to graft the buccal gap. Instead of L-PRF, WPTG were used in conjunction with a collagen membrane. Figure 44 describes the timeline of patient recruitment.

Of the 26 patients assessed in the L-PRF study, 14 were included in the study. The remaining 12 patients were excluded for either not meeting the inclusion criteria, financial restraints or opting for alternative treatment. Three patients were excluded following tooth extraction, 2 due to a lack of buccal bone for immediate implant placement and 1 who required a narrow diameter implant in a lateral incisor site. In comparison, for the WPTG study, 29 patients were assessed and 19 included.

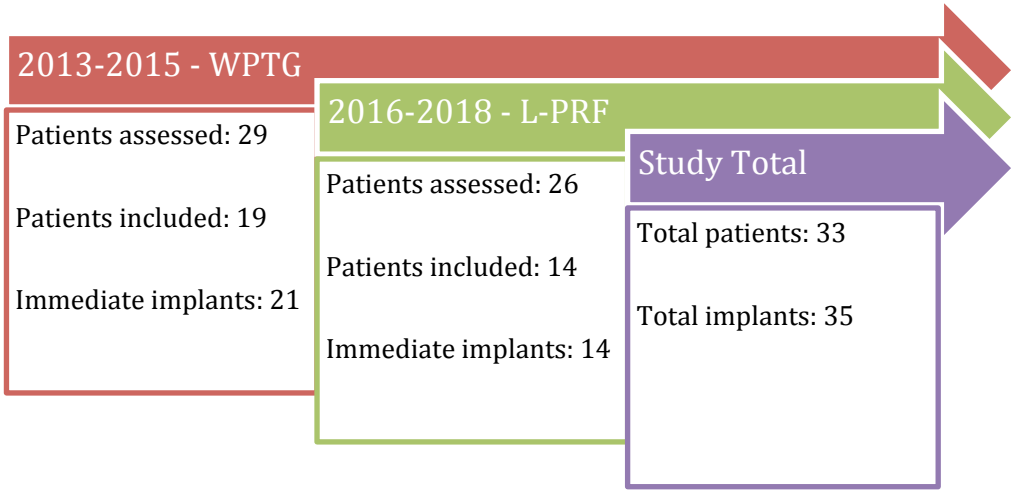

Figure 44: Flowchart of number of patients and implants included in this study

In the 14 patients who agreed to participate in the L-PRF study, a total of 14 implants were placed, 1 per patient. 13 of the implants were placed in females and only 1 in a male. The mean age was 49.36 ± 12.42 years. One of the implants was installed in a patient who was a light smoker (<10 cigarettes/day). In the WPTG study, a total number of 21 implants were placed in the 19 patients as two of the participants presented with 2 teeth suitable for replacement with immediate implant. Six implants were placed in males and 15 in females. The mean age was 53.14 ± 16.16 years. Two of the 21 implants were placed in light smokers (<10 cigarettes/day).

All of the teeth in the L-PRF study were extracted because they were unrestorable and none for periodontal reasons. Three of the implants were placed in central incisor sites, 4 in lateral incisors sites and the remaining 7 in premolar sites. In contrast, in the WPTG study, 2 of the 21 teeth were extracted for periodontal

reasons and the remainder because they were unrestorable. 6 implants were placed in central incisors, 6 in lateral incisors, 2 in canines and 7 in premolars.

All implant sites healed without complications in both study groups. It was observed that the soft tissue healing at 10-14 days was excellent in the L-PRF group. No significant inflammatory signs were noted in the 16-week healing period between 1st stage and the re-entry surgery.

Gender (n and % of sites)		**L-PRF**		**WPTG**	
	Total	14	100%	21	100%
	Male	1	7.1%	6	29%
	Female	13	92.9%	15	71%
Gender (n and % of participants)					
	Male	1	7.1%	5	26.31%
	Female	13	92.9%	14	73.69%
Age (years)					
	Mean +/- SD	49.36 ± 12.42		53.14 ± 16.16	
	Range	33-76		20-77	
Smoking during healing period					
	Yes	1	7.1%	2	9.5%
	No	13	92.9%	19	90.5%
Teeth extracted (n and % of sites)					
	Maxillary Central Incisors	3	21.4%	6	28.55%
	Maxillary Lateral Incisors	4	28.6%	6	28.55%
	Maxillary Canines	0	0%	2	9.5%
	Maxillary Premolars	7	50%	7	33.4%
Reason for extraction (n and % of sites)					
	Periodontitis	0	0%	2	9.5%
	Unrestorable	14	100%	19	90.5%

Table 3: Demographic and Categorical data

4.2 Baseline Measurements

4.2.1 Clinical measurements

The baseline clinical measurements were made using a periodontal probe (UNC15 Hu-Friedy Chicago, USA periodontal probe) and callipers (Hu-Friedy 40mm Straight Castroviejo Caliper). Following tooth extraction, the dimensions of the alveolar ridge and socket walls / dimensions were measured. Following implant placement (and prior to grafting) the depth and the width of the resulting void was also measured. These 1st stage measurements are presented in Tables 4 and 5 for L-PRF and WPTG respectively.

The mean baseline clinical measurements of interest at 1st stage for the L-PRF and WPTG are as follows: The mean buccal gap widths were 2.07 ± 0.51mm (L-PRF) and 2.33 ± 0.87mm (WPTG). The mean buccal bone widths were 1.11 ± 0.53mm (L-PRF) and 0.95 ± 0.35mm (WPTG). The mean ridge widths were 7.39 ± 1.00mm (L-PRF) and 8.11 ± 1.02mm (WPTG).

Implants were allowed to heal with a submerged protocol for 16 weeks prior to re-entry. At this 2nd stage procedure, the width of the buccal bone plate (BIC-OBC1), the width of the alveolar ridge (B1-P1) and the buccal bone height (R-BIC) were measured. These results are presented in Tables 4 and 5.

The mean baseline clinical measurements of interest at 2nd stage for the L-PRF and WPTG groups are as follows: The mean buccal bone widths were 1.25 ± 0.96mm (L-PRF) and 2.14 ± 0.88mm (WPTG). The mean ridge widths were 6.61 ± 1.11mm (L-PRF) and 7.07 ± 0.89mm (WPTG).

4.2.2 Photographic measurements

A photograph was recorded in an angle parallel to the long axis of the implant, immediately after implant installation and again at the re-entry procedure. The images were imported into ImageJ software (Version 1.51s; National Institutes of Health, USA) and measurements were made for comparison with their equivalent clinical measurements. At 1st stage the gap width, buccal bone width (measured 1mm apical to the crest) and the ridge width were measured. At 2nd stage the buccal bone width and the ridge width were measured. These measurements are presented in Tables 4 and 5.

		Clinical				Photographic			
1st Stage	**Variable**	**Min**	**Max**	**Mean**	**Std. D**	**Min**	**Max**	**Mean**	**Std. D**
	BSD	5.00	14.00	11.10	2.33				
	LSD	7.00	14.00	11.5	1.99				
	GDB	3.00	13.00	8.93	3.09				
	GDI	4.00	11.50	8.77	2.29				
	PWC	0.50	2.00	1.32	0.54	0.39	1.81	1.12	0.41
	GW	1.00	3.00	2.07	0.51	1.10	2.50	1.71	0.45
	BBW-C	0.50	2.00	1.11	0.53	0.50	2.00	0.98	0.47
	BBW-M	0.50	3.00	1.61	0.79				
	BBHD	2.50	4.50	3.18	0.64	1.85	4.01	2.71	0.59
	RW	6.00	9.00	7.39	1.00	6.40	8.80	7.51	0.75
	SM	-2.00	0.00	-0.68	0.64				
2nd Stage									
	BBW-PO	0.00	4.00	1.25	0.96	0.00	3.52	1.13	0.87
	RW-PO	5.00	9.50	6.61	1.11	4.67	8.89	6.15	1.05
	First BIC	-4.00	1.00	0.86	1.49				

Table 4: Baseline clinical and photographic measurements: L-PRF

(BSD, buccal socket depth; LSD, lingual socket depth; GDB, gap depth measured in contact with the bone; GDI, gap depth measured in contact with the implant surface; PWC, palatal bone width coronally; GW, gap width; BBW-C, buccal bone width measured 1mm apical to the crest; BBW-M, buccal bone width measured half-way to the apical end of the socket; BBHD, buccal bone horizontal dimension measured by combining BBW-C with gap width; RW, ridge width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; RW-PO, ridge width post-op, First BIC, first bone-to-implant contact measured from the rim of the implant to the most coronal buccal bone)

		Clinical				Photographic			
1st Stage	**Variable**	**Min**	**Max**	**Mean**	**Std. D**	**Min**	**Max**	**Mean**	**Std. D**
	BSD	2.00	15.00	10.90	3.23				
	LSD	8.00	15.00	11.07	2.27				
	GDB	2.00	15.00	9.66	2.86				
	GDI	6.00	15.00	9.33	2.15				
	PWC	0.50	2.50	1.54	0.54	0.70	2.40	1.55	0.53
	GW	1.00	4.00	2.33	0.87	1.20	3.90	2.42	0.80
	BBW-C	0.50	1.50	0.95	0.35	0.40	2.20	1.25	0.46
	BBW-M	1.00	3.00	1.62	0.58				
	BBHD	1.50	4.50	3.23	0.94	1.80	5.40	3.37	0.97
	RW	6.00	10.00	8.11	1.02	6.10	10.10	8.20	1.05
	SM	0.05	3.00	1.02	0.62				
2nd Stage									
	BBW-PO	0.50	4.00	2.14	0.88	0.30	4.20	2.11	0.88
	RW-PO	6.00	9.00	7.07	0.89	5.80	9.20	7.08	0.89
	First BIC	0.00	1.00	0.16	0.36				

Table 5: Baseline clinical and photographic measurements: WPTG

(BSD, buccal socket depth; LSD, lingual socket depth; GDB, gap depth measured in contact with the bone; GDI, gap depth measured in contact with the implant surface; PWC, palatal bone width coronally; GW, gap width; BBW-C, buccal bone width measured 1mm apical to the crest; BBW-M, buccal bone width measured half-way to the apical end of the socket; BBHD, buccal bone horizontal dimension measured by combining BBW-C with gap width; RW, ridge width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; RW-PO, ridge width post-op, First BIC, first bone-to-implant contact measured from the rim of the implant to the most coronal buccal bone)

4.2.3 Combining the clinical and photographic measurements

To increase the reliability of the clinical measurements an estimated average of the clinical and photographic measurements was calculated and their mean score was used for the statistical analysis. The mean values of the clinical and photographic measurements are presented in Tables 6 and 7 for L-PRF and WPTG respectively. Any measurement that does not have a photographic counterpart is a clinical measurement only. It was these averaged measurements that were used for any further calculations and statistics.

Paired samples t-tests and Wilcoxon signed rank tests were used to determine if there were statistically significant differences between the clinical and photographic measurements for both L-PRF and WPTG groups (paired t-tests unless otherwise stated). In the L-PRF group there were statistically significant differences between the buccal gap widths (0.36 ± 0.21mm: p=0.0005), the buccal bone horizontal dimension (0.47 ± 0.35mm: p=0.0005) and the ridge widths post-op (0.45 ± 0.46mm: p=0.003). In the WPTG group there were statistically significant differences between the ridge widths at 1st stage (0.085 ± 0.17mm. p = 0.038), the buccal gap widths (0.9 ± 0.15mm, p = 0.013) and the buccal bone width at 1st stage (0.29 ± 0.44mm, p = 0.01, Wilcoxon test).

In the L-PRF group the mean gap width was 1.89 ± 0.47mm. The mean buccal bone width was 1.04 ± 0.47mm (measured 1mm apical to the crest) and 1.61 ± 0.79mm (measured half-way to the most apical end of the socket). The mean ridge width at 1st stage was 7.45 ± 0.85mm and at re-entry it reduced to 6.37 ± 1.06mm. In the

WPTG group the mean gap width was 2.37 ± 0.83mm. The mean buccal bone width was 1.10 ± 0.37mm (1mm apical to the crest) and 1.62 ± 0.58mm (half-way to the most apical end of the socket). The mean ridge width at 1st stage was 8.16 ± 1.03mm and at re-entry it reduced to 7.70 ± 0.89mm.

In the L-PRF group the buccal bone width and gap width measurements were combined to calculate the overall mean buccal bone horizontal dimension of 2.94 ± 0.59mm. At 2nd stage the, mean value for the buccal bone width had reduced to 1.19 ± 0.90mm. Similarly in the WPTG the mean buccal bone horizontal dimension was calculated to be 3.49 ± 0.99mm. At 2nd stage, the mean value for the buccal bone width had reduced to 2.12 ± 0.87mm.

Average Clinical and Photographic Measurements: L-PRF					
		Min	**Max**	**Mean**	**Std. D**
1st Stage	**Variable**				
	BSD	5.00	14.00	11.10	2.33
	LSD	7.00	14.00	11.5	1.99
	GDB	3.00	13.00	8.93	3.09
	GDI	4.00	11.50	8.77	2.29
	PWC	0.45	1.91	1.22	0.47
	GW	1.06	2.75	1.89	0.47
	BBW-C	0.60	2.08	1.04	0.47
	BBW-M	0.50	3.00	1.61	0.79
	BBHD	2.29	4.25	2.94	0.59
	RW	6.20	8.90	7.45	0.85
	SM	-2.00	0.00	-0.68	0.64
2nd Stage					
	BBW-PO	0.00	3.76	1.19	0.90
	RW-PO	4.83	9.24	6.37	1.05
	First BIC	-4.00	1.00	0.86	1.49

Table 6: Combined clinical and photographic measurements: L-PRF

(BSD, buccal socket depth; LSD, lingual socket depth; GDB, gap depth measured in contact with the bone; GDI, gap depth measured in contact with the implant surface; PWC, palatal bone width coronally; GW, gap width; BBW-C, buccal bone width measured 1mm apical to the crest; BBW-M, buccal bone width measured half-way to the apical end of the socket; BBHD, buccal bone horizontal dimension measured by combining BBW-C with gap width; RW, ridge width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; RW-PO, ridge width post-op, First BIC, first bone-to-implant contact measured from the rim of the implant to the most coronal buccal bone)

Average Clinical and Photographic Measurements: WPTG					
		Min	**Max**	**Mean**	**Std. D**
1st Stage	**Variable**				
	BSD	2.00	15.00	10.90	3.23
	LSD	8.00	15.00	11.07	2.27
	GDB	2.00	15.00	9.66	2.86
	GDI	6.00	15.00	9.33	2.15
	PWC	0.60	2.45	1.55	0.53
	GW	1.10	3.95	2.37	0.83
	BBW-C	0.45	1.85	1.10	0.37
	BBW-M	1.00	3.00	1.62	0.58
	BBHD	1.65	4.95	3.49	0.99
	RW	6.05	10.05	8.16	1.03
	SM	0.05	3.00	-1.02	0.62
2nd Stage					
	BBW-PO	0.40	4.10	2.12	0.87
	RW-PO	5.90	9.10	7.07	0.89
	First BIC	0.00	1.00	0.16	0.36

Table 7: Combined clinical and photographic measurements: WPTG

(BSD, buccal socket depth; LSD, lingual socket depth; GDB, gap depth measured in contact with the bone; GDI, gap depth measured in contact with the implant surface; PWC, palatal bone width coronally; GW, gap width; BBW-C, buccal bone width measured 1mm apical to the crest; BBW-M, buccal bone width measured half-way to the apical end of the socket; BBHD, buccal bone horizontal dimension measured by combining BBW-C with gap width; RW, ridge width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; RW-PO, ridge width post-op, First BIC, first bone-to-implant contact measured from the rim of the implant to the most coronal buccal bone)

4.3 Dimensional Changes

The dimensional changes were calculated by comparing the mean values obtained at 1st and 2nd stage.

4.3.1 Buccal horizontal changes

The buccal bone plate and gap width measurements were combined to calculate the overall mean buccal bone horizontal dimension at 1st stage of 2.94 ± 0.59mm for sites grafted with L-PRF and 3.49 ± 0.99mm for sites grafted with WPTG. At 2nd stage the, mean value for the buccal bone width was 1.19 ± 0.90mm for L-PRF and 2.12 ± 0.87mm for WPTG. Therefore the mean buccal bone horizontal dimension reduction was 1.75 ± 0.66mm or 61.36% ($p<0.001$, paired t-test) for L-PRF sites and 1.37 ± 0.86 or 37.97% ($p<0.0001$, paired t-test) for WPTG sites. These results are presented in Table 8.

The buccal bone horizontal dimension reduction was then calculated according to sites that had initial buccal bone width of <1mm and >1mm at 1st stage surgery in Table 9 and 10, for L-PRF and WPTG respectively. In the L-PRF group when the buccal bone width was <1mm at 1st stage, the buccal bone horizontal dimension reduced by 68.1%, compared to a 49.2% reduction when the initial buccal bone width was >1mm. In the WPTG group when the buccal bone width was <1mm at 1st stage, the buccal bone horizontal dimension reduction was 35.35%, compared to a 38.07% reduction was initial buccal bone width was >1mm.

The position of the implant in the maxillary arch also appears to influence the buccal width resorption. Implants placed in anterior sites had greater resorption when grafted with L-PRF of 1.88 ± 0.63mm (n = 7) compared to that of WPTG 1.31 ± 0.63mm (n = 14). However, the choice of grafting material appeared to have less influence on buccal width resorption of premolar sites, with 1.62 ± 0.70mm BWR for L-PRF (n = 7) and 1.48 ± 1.26mm for WPTG (n = 7).

The buccal width reduction for sites grafted with L-PRF was compared to those grafted with WPTG. The mean difference in BWR was 0.38 ± 0.27mm, which was not statistically significant (p = 0.166, unpaired t-test).

Buccal Width Reduction												
	L-PRF						**WPTG**					
Tooth position	**N**	**Min**	**Max**	**Mean**	**Std. D**	**%**	**N**	**Min**	**Max**	**Mean**	**Std. D**	**%**
Anterior teeth	7	1.10	2.75	1.88	0.63		14	0.40	2.25	1.31	0.63	
Premolar teeth	7	0.49	2.65	1.62	0.70		7	-0.60	3.70	1.48	1.26	
Total	14	0.49	2.75	1.75	0.66	61.36	21	-0.60	3.70	1.37	0.86	37.97

Table 8: Buccal Width Reduction for L-PRF and WPTG

Buccal Width Reduction – according to baseline buccal bone width: L-PRF					
	N	**BBHD Baseline**	**BBW-PO**	**Reduction in mm**	**Reduction %**
Overall	14	2.94 ± 0.59	1.19 ± 0.90	1.75 ± 0.66	61.36
Sites of initial buccal bone: <1mm	9	2.84 ± 0.47	0.91 ± 0.56	1.93 ± 0.62	68.10
Sites of initial buccal bone: >1mm	5	3.11 ± 0.80	1.70 ± 1.24	1.42 ± 0.65	49.20

Table 9: Buccal Width Reduction – according to baseline buccal bone width: L-PRF

(BBHD, buccal bone horizontal dimension measured by combining BBW-C with gap width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; Reduction, buccal bone width reduction between 1st and 2nd stage)

Buccal Width Reduction – according to baseline buccal bone width: WPTG					
	N	**BBW Baseline**	**BBW-PO**	**Reduction in mm**	**Reduction %**
Mean	21	3.49 ± 0.99	2.12 ± 0.88	1.37 ± 0.86	37.97
Sites of initial buccal bone: <1mm	8	3.03 ± 0.82	1.90 ± 0.71	1.04 ± 0.62	35.35
Sites of initial buccal bone: >1mm	13	3.78 ± 1.00	2.27 ± 0.97	1.51 ± 1.01	38.07

Table 10: Buccal Width Reduction – according to baseline buccal bone width: WPTG

(BBHD, buccal bone horizontal dimension measured by combining BBW-C with gap width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; Reduction, buccal bone width reduction between 1st and 2nd stage)

4.3.2 Buccal vertical changes

Loss of buccal bone height was calculated using the rim of the implant as a reference point. The level of submersion of the rim of the implant was measured at 1st stage (B-S) and the first bone-to-implant contact was measured at 2nd stage (R-BIC), allowing the loss of vertical buccal bone height to be calculated.

The mean value was 0.86 ± 1.5mm apical to the rim of the implant for L-PRF. In 2 implants the BIC at 2nd stage was 1mm coronal to the rim of the implant. For comparison the mean value when using WPTG was 0.16 ± 0.36mm.

4.3.3 Ridge width changes

In the L-PRF group the mean ridge width at 1st stage was 7.45 ± 0.85mm and at re-entry it reduced to 6.37 ± 1.06mm. Therefore the mean ridge width reduction using L-PRF was 1.07 ± 0.96mm or 14%, ($p<0.001$, paired t-test). In the WPTG group the mean ridge width at 1st stage was 8.16 ± 1.03mm and at re-entry it reduced to 7.70 ± 0.89mm. Therefore the mean ridge width reduction was 1.08 ± 0.78mm or 12.81% ($p<0.001$, paired t-test) for WPTG. The ridge width changes for L-PRF and WPTG are presented in Table 11.

The position of the implant in the maxillary arch appears to influence the amount of ridge width resorption. When grafting with L-PRF the ridge width reduction was greater in anterior teeth (1.14 ± 0.82mm) compared to premolar teeth (0.99 ± 1.14mm). In contrast when grafting with WTPG the premolar teeth had greater ridge width reduction (1.33 ± 0.99mm) compared to anterior teeth (0.96 ± 0.66mm).

Ridge Width Reduction												
	L-PRF						WPTG					
Tooth position	**N**	**Min**	**Max**	**Mean**	**Std. D**	**%**	**N**	**Min**	**Max**	**Mean**	**Std. D**	**%**
Anterior teeth	7	-0.07	2.26	1.14	0.82		14	0.05	2.10	0.96	0.66	
Premolar teeth	7	-0.48	2.11	0.99	1.14		7	0.00	3.00	1.33	0.99	
Total	14	-0.48	2.26	1.07	0.96	14	21	0.00	3.00	1.08	0.78	12.81

Table 11: Ridge Width Reduction for L-PRF and WPTG

The ridge width reduction for sites grafted with L-PRF was compared to those grafted with WPTG. The mean difference in ridge width reduction was -0.018 ± 0.29mm, which was not statistically significant (p = 0.950, unpaired t-test).

4.4 Statistical Correlations

Statistical correlations were performed to see how strongly specific socket characteristics were related to the dimensional changes. Correlations were investigated with Pearson and Spearman's coefficient depending on the distribution of the data.

4.4.1 L-PRF correlations

None of the correlations were statistically significant for the L-PRF group. A summary of the correlations for L-PRF is presented in Table 12.

Predictor variable	Outcome variable	Test	r	P-value
GW	BBW-PO	Spearman	**0.57**	0.846
GW	RW-PO	Pearson	0.33	0.912
GW	BWR	Pearson	0.314	0.275
BBW-C	BBW-PO	Spearman	**0.515**	0.60
BBW-C	RW-PO	Spearman	0.227	0.436
BBW-C	BWR	Spearman	-0.200	0.492
BBHD	BBW-PO	Spearman	0.405	0.151
BBHD	RW-PO	Pearson	0.433	0.122
BBHD	BWR	Pearson	-0.40	0.891
RW	RW-PO	Pearson	**0.525**	0.54
RW	RWR	Pearson	0.317	0.269

Table 12: Correlations for L-PRF

(GW, gap width; BBW-C, buccal bone width measured 1mm apical to the crest; BBHD, buccal bone horizontal dimension measured by combining BBW-C with GW; RW, ridge width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; RW-PO, ridge width post-op; BWR, buccal width reduction is the difference between the mean BBHD at 1st stage and the mean BBW-PO; RWR, ridge width reduction is the difference between the mean ridge width at 1st and 2nd stages)

Scatter plots for the three strongest correlations are presented below. Figure 45 demonstrates the correlation between gap width at 1st stage and the buccal bone width at 2nd stage. Spearman correlation was r = 0.57, indicating a positive correlation (p = 0.846).

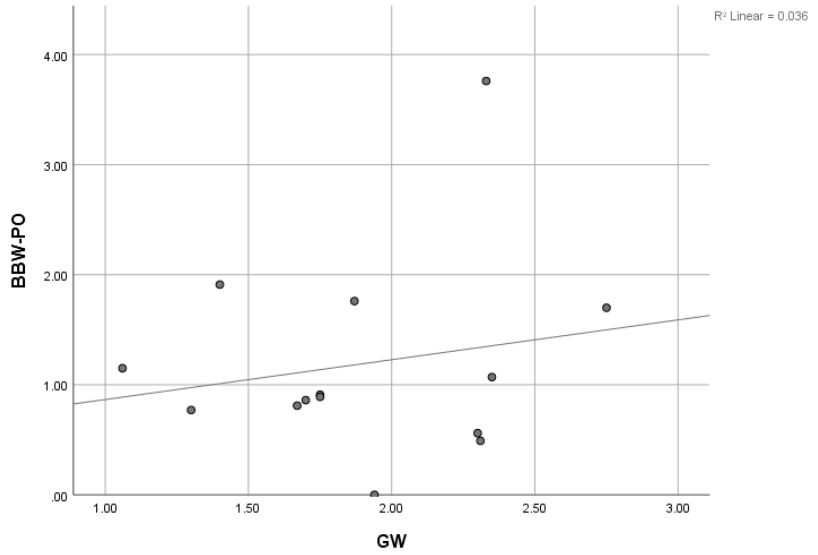

Figure 45: Correlation between gap width at 1st stage and the buccal bone width at 2nd stage.

Figure 46 demonstrates the scatter plot for the correlation between buccal bone width at 1st stage and buccal bone width at 2nd stage. Spearman correlation was r = 0.515, again indicating a positive correlation (p = 0.60).

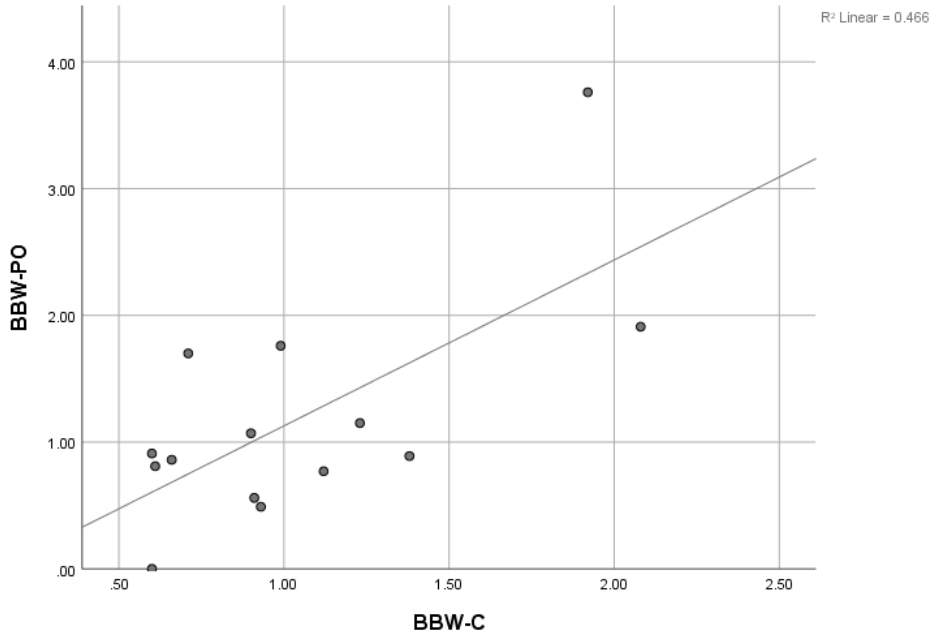

Figure 46: Correlation between buccal bone width at 1st stage and buccal bone width at 2nd stage

Figure 47 demonstrates the correlation between ridge width measured at 1st stage and ridge width measured at 2nd stage. The Pearson correlation was r = 0.525, indicating a positive correlation (p = 0.54).

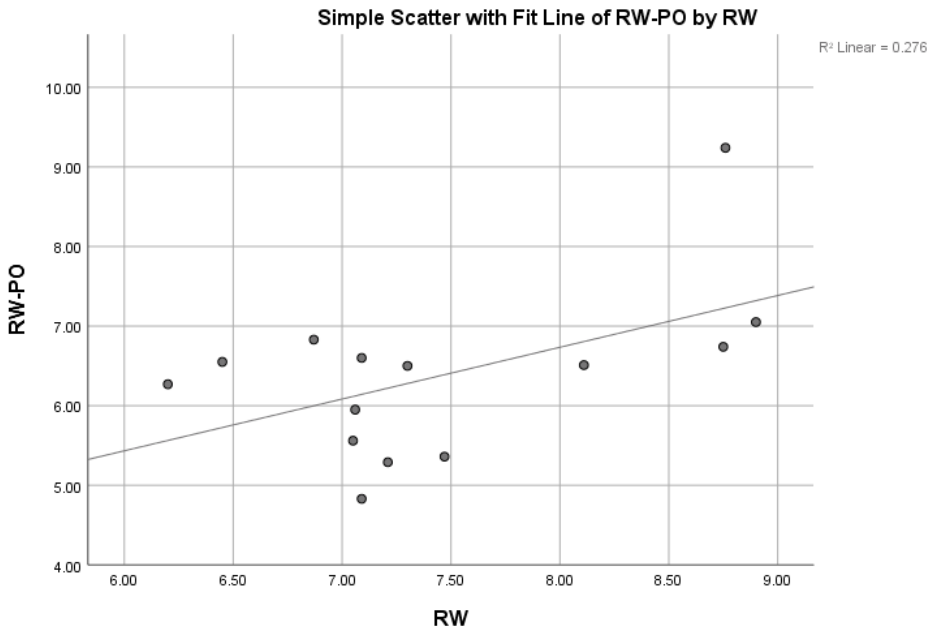

Figure 47: Correlation between ridge width measured at 1st stage and ridge width measured at 2nd stage

4.4.2 WPTG correlations

Statistical correlations were also performed for the WPTG group. In contrast to the L-PRF group, there were several correlations with statistical significance, which are presented below in Table 13.

Predictor variable	**Outcome variable**	**Test**	**r**	**P-value**
GW	BBW-PO	Pearson	0.51	**0.017**
GW	RW-PO	Pearson	**0.63**	**0.002**
GW	BWR	Spearman	**0.61**	**0.005**
BBW-C	RW-PO	Pearson	0.48	**0.045**
BBHD	BBW-PO	Spearman	0.51	**0.016**
BBHD	RW-PO	Spearman	**0.68**	**0.001**
RW	RWR	Spearman	0.48	**0.026**

Table 13: Correlations for WPTG

(GW, gap width; BBW-C, buccal bone width measured 1mm apical to the crest; BBHD, buccal bone horizontal dimension measured by combining BBW-C with GW; RW, ridge width; BBW-PO, buccal bone width post-op measured 1mm apical to the crest at 2nd stage; RW-PO, ridge width post-op; BWR, buccal width reduction is the difference between the mean BBHD at 1st stage and the mean BBW-PO; RWR, ridge width reduction is the difference between the mean ridge width at 1st and 2nd stages)

A strong correlation between gap width values measured at 1st stage and ridge width values measured at 2nd stage was demonstrated r = 0.63 (p = 0.002, Pearson).

Using Spearman's test the gap width values at 1st stage were correlated with the buccal width resorption, r = 0.61 (p = 0.005). This positive correlation indicates that the wider the gap width is following implant placement, the more marked the buccal width resorption will be.

Lastly, the buccal bone horizontal dimension values appear to be strongly positively correlated to the ridge width values at 2nd stage, r = 0.68 (p = 0.001, Spearman's).

4.5 Multiple Regression Analysis

A multiple regression analysis was conducted to determine possible predictors for the buccal bone width at 2nd stage and the ridge width at 2nd stage following 4 months of healing in the L-PRF group.

4.5.1 Buccal bone width 2nd stage

The first regression was conducted to predict the buccal bone width at 2nd stage in the L-PRF group from ridge width, buccal socket depth, lingual socket depth, palatal width, gap depth bone, gap depth implant, gap width, buccal bone width coronally, buccal bone width midroot and buccal bone horizontal dimension.

The linear combination of these predictors was not statistically significantly related to the buccal bone width at 2nd stage: F(10, 2) = 11.293, p=0.084. The sample multiple correlation coefficient was 0.99, indicating that 98% of the variance of the buccal bone width at 2nd stage can be accounted for by the linear combination of the predictors.

A similar regression model was performed for the WPTG group. Two predictors contributed with statistical significance. It can be inferred that for every 1mm increase in gap depth in contact with the bone (GDB), a 0.31mm increase in buccal bone width at 2nd stage can be expected ($p<0.01$). In addition, for every 1mm increase in gap depth measured in contact with the implant surface (GDI), a 0.4mm decrease in buccal bone width at 2nd stage can be expected ($p<0.05$). These independent variables explain approximately only 27% of the variance in buccal bone width at 2nd stage.

4.5.2 Ridge width 2nd stage

The second regression was conducted to predict ridge width at 2nd stage for the L-PRF group from ridge width, buccal socket depth, lingual socket depth, palatal width, gap depth bone, gap depth implant, gap width, buccal bone width coronally, buccal bone width midroot and buccal bone horizontal dimension.

The linear combination of these predictors was not statistically significantly related to the ridge width at 2nd stage: F(10, 2) = 15.033, p=0.064. The sample multiple

correlation coefficient was 0.99, indicating that 99% of the variance of the ridge width at 2nd stage can be accounted for by the linear combination of the predictors.

Of the predictors only gap depth measured in contact with the implant (GDI) contributed to the prediction with statistical significance p=0.032, suggesting for every 1mm increase in gap depth measured in contact with the implant, a 0.7mm decrease in ridge width at 2nd stage would be expected.

Again a similar regression model was performed for the WPTG group. The following can be inferred: for every 1mm increase in buccal gap width, a 0.45mm increase in ridge width at 2nd stage can be expected (p=0.03) and for every 1mm increase in ridge width at 1st stage, a 0.43mm increase in ridge width at 2nd stage can be expected (p=0.01). Lastly, for every 1mm increase in gap depth in contact with the implant surface, a 0.11mm decrease in ridge width at 2nd stage can be expected, although this did not reach statistical significance (p=0.09).

5 Discussion

The aim of this study was to measure the alveolar ridge alterations in the anterior maxilla following extraction and immediate implant placement. The gap created between the implant and the buccal bone was simultaneously grafted with either a permanent / non-resorbable grafting material in WPTG and a collagen membrane (n = 21) or an autogenous source of L-PRF plugs and membranes (n = 14), a second-generation blood concentrate. In an attempt to outline the variables influencing bone dimensional changes, as well as the protocol's efficacy, both the baseline dimensions of all extraction sockets and the amount of bone resorption after 4 months were measured clinically.

All 35 implants installed, healed uneventfully. No short-term failures or significant post-operative complications were observed. All implants were subsequently restored and no additional treatment or interventions were required. On a subjective level, both the operators and the patients were satisfied with the aesthetic outcome of the implant restorations. Therefore, based on the evidence presented in this study, it can be suggested that in the short-term, extraction of single teeth with simultaneous implant placement and grafting of the buccal gap with either WPTG and a single layer collagen membrane or L-PRF can be accepted as a successful protocol for replacing teeth in the anterior maxilla.

The null hypothesis cannot be rejected, as there was not a statistically significant difference in the crestal alveolar bone changes following immediate implant

placement and simultaneous grafting, with either WPTG or L-PRF, in the anterior maxilla.

The buccal bone horizontal reduction (BWR) was 1.37 ± 0.86mm for WPTG and 1.75 ± 0.66mm for L-PRF. The mean difference in BWR of 0.38 ± 0.27mm was not statistically significant ($p = 0.166$, unpaired t-test). The overall ridge width reduction (RWR) was 1.08 ± 0.78mm for WPTG and 1.07 ± 0.96mm for L-PRF. The mean difference in RWR of 0.018 ± 0.29mm was not statistically significant ($p = 0.950$, unpaired t-test). However, in both measurements WPTG underwent less resorption in terms of ridge width and buccal width reductions.

Following immediate implant installation and simultaneous grafting, the implants healed with a submerged protocol for 4 months. At re-entry the amount of bone present on the buccal aspect of the implant was measured. This amounted to 2.12 ± 0.87mm for sites grafted with WPTG and 1.19 ± 0.90mm for L-PRF. This again demonstrated the superior preservation of buccal bone of WPTG compared to L-PRF.

At the time of implant installation the implants were submerged just below the palatal bone crest. At re-entry bone loss was measured on the buccal aspect from the rim of the implant to the first BIC. The mean value was 0.86 ± 1.5mm for the L-PRF group and 0.16 ± 0.36mm for the WPTG group. WPTG appears to result in a more coronal BIC compared to L-PRF.

This trial was designed to determine the effects of L-PRF and WPTG on the preservation of the buccal bone plate following immediate implant placement. This objective was achieved using direct clinical measurements, a methodology which has been used in several previous studies (Botticelli et al., 2004, Chen et al., 2007, Sanz et al., 2010, Sanz et al., 2016). This study was carried out in a single-centre University setting. There were a total of two operators (G.G and M.M), one for each phase of the study and there was one supervisor (I.P), who was consistent throughout. The single-centre setting allowed for a homogenous cohort of patients and good control of the patient's appointments and attendance. Having only two operators was advantageous as it reduced the variability between operators and both operators were of a similar level of experience.

All implants placed in this study were of the same diameter, design and had the same surface characteristics, the Zimmer Biomet T3 Implant™ (Biomet 3i implant innovations, Palm Beach Gardens, Florida, USA). This eliminated the potential for different implant designs and surfaces to elicit different bone responses following implant placement. In addition all implants were placed engaging the palatal wall of the extraction socket and about 1mm below the palatal crest of bone. The position of the implant in the extraction socket has been shown to influence the bone resorption occurring following immediate implant placement (Caneva et al., 2010c, Tomasi et al., 2010).

This study was a prospective quasi-experimental clinical study. Given that two different grafting materials were employed in this study a randomised controlled

trial would have been preferable. This would have allowed a better analysis of the difference in the effects of the two materials on the preservation of the buccal bone plate. However, the largest randomised controlled trial on immediate implants to date determined from a power calculation that each group required 50 patients (Sanz et al., 2016) and this study was a multicentre study carried out in four different universities. Therefore I do not feel a randomised controlled trial would have been feasible given the time allocated to complete this study and the large number of patients required. Despite this, it must be acknowledged that the sample size in this study is small and the results should be interpreted accordingly.

The primary outcome measure in this study was the dimensional changes occurring in the alveolar ridge following immediate implant placement and simultaneous grafting. As only two out of the 35 patients received 2 implants, the analysis performed was done only on an implant-level and did not include any patient-level analysis. Patient-level analysis has not been employed very often in studies on immediate implants (Tomasi et al., 2010). Another potential limitation of this study is the raising of a mucoperiosteal flap at both 1st and 2nd stage surgery, as this has been associated with increased bone loss due to the disruption of the vascular supply to the periodontium (Wood et al., 1972, Yaffe et al., 1994). However, there is limited evidence to say there is greater bone loss following immediate implants placed with raising a mucoperiosteal flap compared to those placed flapless (Raes et al., 2011). As mentioned already, direct clinical measurements have been used in other studies with acceptable clinical results. Alternatively, CBCT could be used to measure the bony changes around immediate implants but the accuracy of this has

been questioned (Razavi et al., 2010) and its use may not adhere to local policies governing dental radiation protection.

Another limitation of this study was the fact only one outcome measure was considered / assumed. There are several variables relevant to immediate implants which could have been included such as; radiographic bone levels, soft tissue response, mucosal level changes, papilla height, aesthetics (PES and WES), biological complications and patient related outcomes.

As already mentioned this study used direct clinical measurements in a similar methodological approach to previous studies. The baseline measurements of the socket characteristics and gap depth and gap width in this study were similar to those reported in these other studies. The data from this study is compared to other clinical studies in Table 14.

The mean ridge width at 1st stage was 7.45 ± 0.85mm for the L-PRF group and 8.16 ± 1.03mm for the WPTG group. It is also similar to other studies utilising clinical measurements such as Botticelli et al 2004 (7.3 ± 1.1mm) and Covani et al 2004 (10 ± 1.52mm). Some studies only report the dimensional changes that occur as opposed to the pre and postoperative measurements (Sanz et al., 2010, Sanz et al., 2016).

The mean buccal bone width (measured 1mm apical to the bone crest) at 1st stage was 1.04 ± 0.47mm for the L-PRF group and 1.10 ± 0.37mm for the WPTG group. These measurements are similar to those reported in earlier clinical studies, such as 1.4 ± 0.4mm (Botticelli et al., 2004) and 1.0 ± 0.5mm (Sanz et al., 2010). Studies

using CBCT to measure the buccal bone thickness around teeth in the anterior maxilla have found roughly 90% of sites to be of <1mm (Braut et al., 2011) and <0.5mm in almost 50% of sites (Januario et al., 2011).

The mean buccal gap width in this study was 1.89 ± 0.47 for the L-PRF group and 2.37 ± 0.83 for the WPTG group. This is again comparable to measurements reported in other studies ranging from 1.8 ± 0.7mm to 2.24 ± 0.83mm (Botticelli et al., 2004, Sanz et al., 2010, Chen et al., 2007, Rossi et al., 2013, Kuchler et al., 2016). The buccal gap width is influenced by the diameter of the implant placed and by the bucco-linugal positioning of the implant. It has been demonstrated that wider implants that fill the entire extraction socket result in more buccal bone resorption than narrower implants which leave a gap between the implant surface and inner buccal bone wall (Caneva et al., 2010d). In another experimental study it was demonstrated that both small and larger buccal gaps completely fill with bone after 3 months of healing (Araujo et al., 2006b). Despite the fact that larger gap widths results in greater bone infill, the degree of bone fill, as measured by percentage of horizontal defect resolution, has been shown to be more pronounced in smaller defects (Ferrus et al., 2010).

The buccal bone horizontal dimension (OBC-R) was calculated by combining the buccal width and the gap width. It was measured to be 2.94 ± 0.59mm for the L-PRF group and 3.30 ± 0.95mm for the WPTG group. In general these values are similar to those reported in other studies (Botticelli et al., 2004, Chen et al., 2007, Sanz et al., 2010, Degidi et al., 2012, Roe et al., 2012, Rossi et al., 2013).

The gap depth measured in contact with the implant surface (R-D) is a dimension of interest in other studies. In this study the gap depth in contact with the implant surface was 8.77 ± 2.29mm for the L-PRF group and 9.33 ± 2.15mm for the WPTG group. These measurements are similar to those reported in two other studies, which also used direct clinical measurements (Botticelli et al., 2004, Chen et al., 2007). In contrast, studies that have used CBCT to measure the defects around immediate implants have recorded lower values for the gap depth in contact with the implant surface. Degidi et al (2012) reported 4.41 ± 2.45mm and Rossi et al (2013) had values of roughly 5mm. However, there are also studies using direct clinical measurements and similar methodology to this study reporting slightly lower values for gap depth in contact with the implant surface. For example, Sanz et al (2010) reported a measurement of 7.5 ± 3.4mm and Kuchler et al (2016) reported 7mm. It could be speculated these smaller measurements are due to the use of larger diameter implants or implants of different configurations, but it is more likely due to the implants not being in contact with the lingual bone wall, resulting in a more buccal position of the implant.

Lastly, the mean level of implant submersion relative to the buccal bone crest (B-S) at implant placement in this study was 0.68 ± 0.64mm for the L-PRF group and 1.02 ± 0.62mm for the WPTG group. Similar levels of submersion have been reported in other clinical studies, ranging from 0.95 ± 0.63mm to 2mm (Botticelli et al., 2004, Chen et al., 2007, Kuchler et al., 2016). The level of submersion in the Degidi et al (2012 study) was slightly higher at 2.97 ± 1.2mm.

It has been demonstrated in animal experimental studies that when implants are placed closer to the buccal wall, a reduced gap or no gap exists between the implant surface and the buccal bone wall (Araujo et al., 2006b). As a result of this implant position, greater buccal bone loss was observed compared to implants placed closer to the lingual / palatal wall (Caneva et al., 2010d). Another study from the same research group then demonstrated implants placed in contact with the lingual / palatal wall (and 1mm apical to the buccal bone) reduced the exposure of the implant surface above the alveolar crest (Caneva et al., 2010c).

Therefore it has been recommended that when implants are placed in fresh extraction sockets they contact the lingual / palatal wall rather than the centre of the socket, as this creates a gap / space between the implant surface and buccal bone for the formation of a blood clot and subsequently bone formation. In addition and as previously discussed, grafting of this buccal gap reduces the amount of buccal bone loss compared to sites allowed with a blood clot (Araujo et al., 2011, Sanz et al., 2016). In this study all implants were placed in contact with the palatal wall of the extraction socket. This allowed the creation of a buccal gap and placement of grafting material.

The majority of clinical studies on immediate implants describe the placement of the implant in a palatal position relative to the extraction socket (Weigl and Strangio, 2016). As a result of this there is very limited information available on the effect of implants placed closer to the buccal bone wall. Some studies have reported an increase of cases with advanced midfacial recession of >1mm when implants were placed more buccally than palatally; 16.67% vs 58.33% (Chen et al., 2007) and

28.13% vs 80% (Evans and Chen, 2008). Another study showed less mean marginal level change from baseline for palatally positioned (2.6%) than buccally positioned implants (6.9%) (Chen et al., 2009). Interestingly, these three studies used tissue-level implants, which cannot be placed as deep as bone-level implants and also have wider platform diameter than the implant body. Aesthetic outcomes may be more difficult to achieve with tissue-level implants but this was not the case in a trial comparing bone-level and tissue-level designs at early implant placement with a 5-9 year follow up (Chappuis et al., 2016). There were no statistically significant differences in terms of soft tissue parameters or PES scores between the two designs. A recent randomised controlled trial compared the aesthetic outcome of immediate implants (bone-level) placed either in a slightly palatal position or in the natural 'central' position where the tooth would have been (Esposito et al., 2018). One year after loading the mean PES score was not statistically significantly different between the two groups, however, the sample size was small with only 15 implants per group and they did not directly record the change in the marginal mucosal level.

	Variable	L-PRF	WPTG	Botticelli et al 2004	Chen et al 2007	Degidi et al 2012	Sanz et al 2010	Sanz et al 2016
1st stage	GDB	8.93 ± 3.09	9.66 ± 2.86	N/A				
	GDI	8.77 ± 2.29	9.33 ± 2.15	8.2 ± 2.1	9.6 ± 2.2	4.41 ± 2.45	7.5 ± 3.4	
	GW	1.89 ± 0.47	2.37 ± 0.83	2.0 ± 0.7	1.8 ± 0.7	2.24 ± 0.83	2.21 ± 1.1	
	BBW	1.04 ± 0.47	1.10 ± 0.37	1.4 ± 0.4			1.0 ± 0.5	
	BBDH	2.94 ± 0.59	3.30 ± 0.95	3.4 ± 0.7	2.3 ± 0.7	3.0 ± 0.86	3.0 ± 1.1	
	RW	7.45 ± 0.85	8.16 ± 1.03	7.3 ± 1.1				
	SM	0.68 ± 0.64	1.02 ± 0.62	1.6 ± 0.9		2.97 ± 1.2	0.3 ± 0.1	
2nd stage	BBW-PO	1.19 ± 0.90	2.12 ± 0.87	1.5 ± 0.9		2.12 ± 0.92	1.9 ± 1.2	
	RW-PO	6.37 ± 1.05	7.07 ± 0.89	N/A				
	BWR	1.75 ± 0.66	1.17 ± 0.90	1.9 ± 0.9		0.88 ± 0.51	1.1 ± 1.0	1.07 ± 1.10
	RWR	1.07 ± 0.96	1.08 ± 0.78	2.7			1.6	2.19 ± 2.10

Table 14: Mean baseline measurements compared with previous studies

As it can be seen from Table 14, not all studies record the same measurements, which can make it difficult to compare studies. The variations observed amongst studies can be attributed to the different surgical protocols employed in addition to the different methods for carrying out the measurements. In this study, all measurements were performed clinically, at implant placement and again at the re-entry procedure, necessitating the elevation of a mucoperiosteal flap at both time points. This approach has been used in several studies (Botticelli et al., 2004, Chen et al., 2007, Sanz et al., 2010, Sanz et al., 2016), however other studies have used three-dimensional imaging to calculate bony measurements around implants (Degidi et al., 2013, Rossi et al., 2013).

In this study, all clinical measurements were taken and confirmed by two clinicians for each study phase. G.G and I.P for the first phase with WPTG and M.M and I.P for the second phase with L-PRF. The measurements were performed with a UNC15 Hu-Friedy Chicago, USA periodontal probe and a Hu-Friedy 40mm Straight Castroviejo Bone Calliper. A similar approach was employed in three other studies (Botticelli et al., 2004, Sanz et al., 2010, Sanz et al., 2016), however instead of a straight calliper, an Iwanson calliper (Hu-Friedy) was used, which has a curved tip. The decision to use straight calliper originates back to the initial study performed using WPTG as the grafting material. The straight calliper was chosen following a number of pilot / test measurements performed before the commencement of the study. It was observed that although the Iwanson calliper was performing well in the measurement of the bone around wide sockets, due to its bulbosity it was very difficult to make accurate measurements around narrow sockets. In addition, when

measuring the palatal socket wall, the palatal soft tissues had to be lifted more than necessary to allow for access and proper positioning of the Iwanson calliper. These problems were not encountered when the Castroviejo bone calliper was used. Additionally, ImageJ software was used to confirm and increase the reliability of our clinical measurements. ImageJ has been utilised in another study, as a means to make bony measurements around implants on CBCT scans 12 months post immediate implant placement (Rossi et al., 2013).

At the time of immediate implant placement the mean distance from the outer buccal bone crest to the implant surface (OBC-R) was 3.49 ± 0.99mm in the WPTG. After 4 months of healing, at the re-entry procedure, the same measurement was repeated resulting in 2.12 ± 0.87mm. This buccal width reduction (BWR) of 1.37 ± 0.86mm for the WPTG is somewhat similar to the overall ridge width resorption (RWR) value of 1.08 ± 0.78mm. This result reinforces the concept that the majority of the bone remodelling occurs on buccal aspect of the ridge, regardless of grafting of the buccal gap (Schropp et al., 2003, Araujo and Lindhe, 2005, Araujo et al., 2011).

The same trend is apparent in the L-PRF group, although the BWR of 1.75 ± 0.66mm is slightly greater than the overall ridge width resorption of 1.07 ± 0.96mm. We could speculate that this could be attributed to the regenerative capacity of the L-PRF membrane as a significant amount was packed over the coronal and palatal part of the implant. This indicates that grafting the buccal gap with L-PRF did not preserve the horizontal buccal bone dimension as well as WPTG (61.35% vs 33.68%). The reduction in the buccal bone dimension of 61.36% for L-PRF is similar to that of the Botticelli 2004 study of 56%, in which no grafting material was used.

This is almost double the amount of reduction seen with the WPTG. Despite this, the mean difference in BWR between sites grafted with WPTG and L-PRF of 0.38 ± 0.27mm was not statistically significant (p = 0.166, unpaired t-test). To achieve a statistically significant difference between these two grafting approaches would likely require a larger sample size. For example in the Sanz (2016) multicentre study where they compared the effect of a bone graft in the gap at immediate implants to that of no graft, they calculated 50 patients would be required per group.

In a study by Sanz et al (2010) immediate implants were placed without grafting the buccal gap. Their baseline buccal bone dimension was 3 ± 1.1mm and reduced to 1.9 ± 1.2mm after 4 months of healing. Therefore their buccal width resorption of 36% without grafting is comparable to that seen in our research when grafting with WPTG. However, their overall ridge width resorption was higher at 25%, suggesting the palatal bone sites in their study exhibited increased resorption. This may be due to the fact the implants in their study were placed in a more central position in the socket, which we could suggest may increase the percentage of palatal bone resorption. It has previously been suggested it is beneficial to place immediate implants more lingually / palatally and deeper into the extraction socket to prevent exposure of the implant surface above the alveolar crest (Caneva et al., 2010c).

Subsequently, Sanz et al (2016) compared immediate implant placement with (DBBM-C) and without grafting the buccal gap. The results obtained for their grafted sites are similar to those for WPTG in this study. Their buccal width reduction of 1.1mm (29%) for DBBM-C is comparable to that of 1.37 ± 0.86mm (38%) for WPTG. Similar results were obtained in another study using bovine bone and a collagen

membrane to graft the buccal gap (Chen et al., 2007). Buccal width reduction was 0.6 ± 0.7mm (20 ± 21.9%) for the grafted sites and 1.1 ± 0.3mm (48.3 ± 9.5%) for the non-grafted sites.

When comparing the results of this study with others of similar methodology that have used direct clinical measurements at implant placement and again at a re-entry procedure, the results are all quite similar (Botticelli et al., 2004, Chen et al., 2007, Sanz et al., 2010, Sanz et al., 2016). The benefits of grafting the buccal gap are clear in reducing the amount of horizontal buccal bone loss. Immediate implants that did not receive any grafting had reductions of 36% (Sanz et al., 2010) and 56% (Botticelli et al., 2004) while those that were grafted had reductions of 25% (Chen et al., 2007), 29% (Sanz et al., 2016) and 38% in this study with WPTG. In contrast, sites grafted with L-PRF exhibited horizontal buccal bone loss of 61%. A recent systematic review and meta-analysis calculated the weighted mean horizontal bone loss following immediate implant placement to be 1.07mm (Lee et al., 2014). Subgroup analysis showed in terms of horizontal buccal bone reduction the weighted mean difference between grafting and non-grafting was 0.53mm (0.79mm versus 1.32mm, respectively). This again highlights the benefits of grafting, which appears to be in the region of 0.5mm (Lee et al., 2014, Sanz et al., 2016).

Several studies have utilised cone beam computed tomography (CBCT) to assess the bone dimensional changes following immediate implant placement. Using CBCT instead of direct clinical measurements offers some advantages. It allows the clinician to avoid raising a full mucoperiosteal flap i.e. flapless procedure, as flapped procedures have been associated increased bone loss (Wood et al., 1972, Fickl et al.,

2008a, Blanco et al., 2008). Degidi et al (2013) placed immediate implants, grafted the buccal gap with DBBM-C and also placed immediate provisional crowns. CBCT scans were taken immediately post-operatively and again at 12 months. The horizontal buccal bone resorption was measured to be 0.88 ± 0.51mm, which was a 29.3% reduction (Degidi et al., 2013). Similar results were found by Roe et al (2012), with 0.64 ± 0.55mm (24%) horizontal buccal bone resorption. The results from these studies are similar to those using direct clinical measurements to measure the dimensional changes and in conjunction with bovine bone to graft the buccal gap (Chen et al., 2007, Sanz et al., 2016).

The use of CBCT to perform bony measurements around dental implants has come into question. Studies have compared the accuracy of the bony measurements obtained using CBCT with that of digital intra-oral radiographs and with histological ground sections following implant placement in dogs (Ritter et al., 2014). In general the measurements of CBCT correlated well with histomorphology of the buccal and lingual bone thickness (all p-values <0.05). The mean difference between CBCT and histomorphology ranged from 0.06 to 2.61mm. Another study compared the accuracy of CBCT to light microscopy in measuring the cortical bone thickness adjacent to dental implants in prepared bovine ribs (Razavi et al., 2010). CBCT measurements closely approximated those of the ground sections, except when cortical bone thickness was <0.8mm. Human studies have assessed the accuracy and precision of CBCT using periapical radiographs (PA) as a reference to evaluate interproximal bone levels around dental implants (Raes et al., 2013). Accuracy of CBCT was low as the mean bone level was 0.70mm on PA and 0.23mm on CBCT. In general, metallic artefacts limit the visualisation quality of bone around dental

implants. In contrast, the bone thickness assessment around natural teeth using CBCT is considered to be highly diagnostically accurate (Fu et al., 2010, Cook et al., 2011). This is in keeping with our chosen methodology, as clinical measurements do not have these limitations.

All implants in this study were placed below the level of the palatal bone crest. The level of submersion was measured from the buccal bone crest to the rim of the implant (B-S). The mean level of submersion in this study was 1.02 ± 0.62mm in the WPTG group and 0.68 ± 0.64mm in the L-PRF group. At re-entry the vertical bone loss was measured from the rim of the implant to the first bone-to-implant contact (R-BIC). This amounted to 0.16 ± 0.36mm for WPTG and 0.86 ± 1.49mm for L-PRF. The first BIC in other similar studies, but without any grafting of the buccal gap, has been documented as 2.0 ± 0.8mm (Botticelli et al., 2004) and 0.7 ± 1.9mm (Sanz et al., 2010). The results from our study are comparable to those of similar design and it could be suggested that grafting with WPTG resulted in a more coronal BIC compared to L-PRF and to those without grafting.

Studies that have also used direct clinical measurements have demonstrated that immediate implant placement with simultaneous grafting does not prevent vertical buccal bone resorption. Buccal vertical bone resorption at ungrafted sites have been reported as 0.3 ± 0.6mm (Botticelli et al., 2004), 1.3 ± 0.9mm (Chen et al., 2007) and 1mm (Sanz et al., 2010). Interestingly, when grafting protocols have been employed, the buccal vertical bone loss has not been significantly different to the non-grafted controls. In the study by Chen et al (2007), there was not a statistically significant difference between sites grafted with bovine bone and a collagen membrane

compared to non-grafted sites. Similarly, in the study by Sanz et al (2016), both grafted and non-grafted sites underwent 0.3mm of buccal vertical bone loss.

Caneva et al (2010) investigated the effect of placing implants apical / deeper to the bone crest, to compensate for this bone loss. As expected, the same amount of bone loss occurred regardless of depth of placement, but implants placed more apically resulted in a more coronal bone-to-implant contact (Caneva et al., 2010c). It has been recommended that immediate implants be placed 3mm apical to the future free gingival margin of the final restoration (Kan et al., 2018).

Studies using CBCT measurements have also reported similar reductions in buccal vertical bone following immediate implant placement. Degidi et al (2013) reported a mean vertical buccal bone loss of 0.76 ± 0.96mm (25.6%) after 12 months. Similarly, Roe et al (2012) also demonstrated a buccal vertical bone loss of 0.82 ± 0.64mm at 12 months. In the latter study, despite the fact implants were placed on average 0.95 ± 0.73mm below the buccal bone crest, 8 out of the 21 implants demonstrated BIC levels apical to the rim of the implant at 12 months.

A recent systematic review and meta-analysis, which included several of the studies discussed above, calculated the weighted mean vertical bone loss following immediate implant placement to be 0.78mm (Lee et al., 2014). These studies had a follow-up of between 4-12 months. In longer-term studies following immediate implants, greater vertical bone loss has been reported that in the above studies. A retrospective study (Miyamoto and Obama, 2011) with a mean follow-up of 47 months, CBCT measurements demonstrated a vertical bone loss of 3.25 ± 4.68mm.

Another study (Benic et al., 2012a) using CBCT analysed bone changes around immediate implants after 7 years in function and reported buccal vertical bone loss of 3.1 ± 4.6mm. The latter two studies included cases with compromised buccal plates and had small sample sizes, with 7 and 14 implants, respectively.

The overall ridge width resorption (RWR) in this study was calculated to be 1.07 ± 0.96mm or 14% for the L-PRF group and 1.08 ± 0.78mm or 12.81% for the WPTG group. These reductions were statistically significant ($p<0.001$, paired t-tests). However, the difference in RWR of 0.018 ± 0.29mm between L-PRF and WPTG groups did not prove to be statistically significant ($p=0.950$, unpaired t-test).

In earlier clinical studies where the immediate implants were placed without any grafting material to fill the voids, the RWR was reported to be 2.7mm or 40% (Botticelli et al., 2004) in one such study and 1.9mm or 19% in another (Covani et al., 2004). Similarly another study that placed immediate implants without grafting the buccal gap reported an overall RWR of 1.6mm or 25% (Sanz et al., 2010). In the study by Sanz et al (2016), the buccal gaps were grafted with demineralised bovine bone mineral with 10% collagen (DBBM-C) and compared to non-grafted controls. The difference in ridge width reduction between the two groups was not statistically significant with 11% for grafted sites and 16% for non-grafted sites.

Some of the aforementioned studies did not graft the gaps / voids present around the immediate implants and therefore it can be speculated; the improved ridge width reductions observed in our study groups (14% and 12.81%), can be attributed to grafting the buccal gap with either L-PRF, or WPTG (and covering the

site with a resorbable collagen membrane or multiple L-PRF membranes), reducing the amount of bone resorption or even promoting new bone formation.

The above results are also evident when considering the effectiveness of ridge preservation procedures. There are many reviews on the effectiveness of ridge preservation procedures. One such recent meta-analysis determined ridge preservation procedures reduce the horizontal ridge resorption by 1.31-1.54mm compared to non-grafted controls (Willenbacher et al., 2016).

Statistical correlations were performed to see how strongly specific socket characteristics were related to the dimensional changes. None of the correlations were statistically significant for the L-PRF group but there were several significant correlations for WPTG.

In this study the gap width values for the WPTG group at 1st stage had a moderately positive correlation with the buccal bone width at 2nd stage ($r = 0.51$, $p = 0.017$). This suggests, the wider the initial buccal gap width at implant placement, the greater the amount of newly formed bone and the thicker the buccal bone at the 4-month re-entry. This finding is in agreement with similar earlier studies (Botticelli et al., 2004, Ferrus et al., 2010), which showed sites with a gap width >1mm had a greater amount of gap fill. The L-PRF group also showed a moderately positive correlation for this but it was not statistically significant ($r = 0.57$, $p = 0.846$).

Gap width in the WPTG group was also strongly positively correlated with the amount of horizontal buccal bone resorption ($r = 0.61$, $p = 0.005$) and the ridge

width at re-entry (r = 0.63, p = 0.002). Unfortunately there is limited evidence available on the effect of the gap width on ridge dimensional changes (Lee et al., 2014) and the results from our study provide conflicting comparisons with Ferrus et al (2010).

In the present study, in the WPTG group, buccal bone width at 1st stage was moderately positively correlated with the ridge width at re-entry (r = 0.48, p = 0.045). This implies a thicker buccal bone width will result in a wider ridge width at re-entry. This finding is in agreement with earlier studies. Ferrus et al (2010) found significantly greater horizontal buccal bone loss at sites with thin buccal bone <1mm (43%) compared to sites with thick buccal bone of >1mm (21%) (p < 0.01). Similarly, Tomasi et al (2010) found sites with thick (>1mm) buccal bone width had less horizontal buccal bone resorption (0.45mm, p < 0.05). This trend was apparent in the L-PRF group, where an increase in buccal bone width at 1st stage was associated with an increased in buccal bone width at 2nd stage, although it was not statistically significant (r = 0.515, p = 0.60).

In this study a multiple regression analysis was conducted to determine possible predictors for the buccal bone width at 2nd stage and the ridge width at 2nd stage following 4 months of healing in the both the WPTG and the L-PRF groups.

The regression analysis revealed that gap depth measured in contact with the implant (GDI) was a predictor with statistical significant for buccal bone width at 2nd stage for WPTG and for ridge width at 2nd stage for both WPTG and L-PRF. For every 1mm increase in GDI, a 0.4mm decrease in buccal bone width at 2nd stage can be

expected when using WPTG. For every 1mm increase in GDI, a 0.11mm decrease and 0.7mm decrease in ridge width at 2nd stage could be expected for WPTG and L-PRF respectively. Therefore a greater depth of bone measured in contact with the implant is associated with a decrease in buccal bone width and ridge width post-operatively. This finding shows that buccal bone width and ridge width preservation are dependent on the size of the initial buccal void created after immediate implant installation and more specifically its depth.

Interestingly, this is first study to date to document a relationship between the GDI and the buccal bone width and ridge widths at 2nd stage. Previous studies have described how the thickness of the buccal bone and the horizontal buccal gap width influence the buccal width resorption and the position of the implant influences the buccal vertical resorption (Ferrus et al., 2010, Tomasi et al., 2010).

One of the limitations of my study is that it did not allow for aesthetic evaluation of the immediate implants. However, on a subjective level both the operators and the patients were satisfied with the aesthetic outcome of the implant restorations. Maximising the bone present on the buccal aspect of an implant has been shown to be a critical factor in determining the stability of the peri-implant mucosa and aesthetic outcome (Chen and Buser, 2014).

There are a limited number of studies that have evaluated the aesthetic outcome of immediate implants with regard to the peri-implant bone levels. Benic et al (2012) carried out CBCT evaluation of 14 immediate implants 7 years after placement reported a mean buccal vertical bone loss of 3.1 ± 4.6mm. Interestingly, one third of

the implants (n = 5) had almost no buccal bone present and the gingival margin of these implants was located only 1mm more apically compared to the implants with intact buccal bone. Of these 5 implants, 4 of them underwent GBR at the time of immediate implant placement. The fact there was only 1mm of difference in the gingival margin level between these two groups suggests other factors, besides buccal bone have a role to play in soft tissue stability around implants. In contrast to this study, Kuchler et al (2016) did not find a lack of buccal bone was associated with a more apical gingival margin position, despite the fact they also found one quarter of implants had significant loss of buccal bone on CBCT, 10 years following immediate implant placement. Another study using CBCT calculated a mean vertical buccal bone loss of 3.25 ± 4.68mm 47 months following immediate implant placement (n = 7) (Miyamoto and Obama, 2011). This vertical bone loss was strongly positively correlated with a mean gingival recession of 0.82 ± 0.75mm ($r = 0.784$, $p< 0.001$). Another study with 10-year follow up compared immediate implants with and without GBR procedures (Covani et al., 2012) and found the gingival margin was statistically significantly more apical in the non-GBR group (1.1 ± 0.7mm) compared to GBR group (0.7 ± 0.4mm) ($p<0.05$). This study did not perform any bony measurements at the time of implant placement or at re-entry and therefore these mucosal differences cannot be correlated to the peri-implant bone levels.

Immediate implants are often placed and restored simultaneously in the aesthetic zone. The major advantage of this approach is that the patient has better immediate aesthetics and the provisional restoration preserves the soft tissue morphology of the extraction site (Kan et al., 2003, De Rouck et al., 2009). A recent systematic

review assessed the impact of immediately placed and restored single-tooth implants in the anterior maxilla on the peri-implant soft tissue changes (Weigl and Strangio, 2016). It included 17 studies with 626 implants. Roughly two thirds of the implants were placed flapless and roughly two thirds were also grafted. The mean interproximal mucosa level changes were <1mm compared to baseline and the mean midfacial gingival recession was 0.95mm. There was no difference for thick and thin gingival biotypes.

Evidence that immediate provisional crowns preserve the soft tissues also stems from a randomised controlled trial comparing immediate implants with immediate provisional crowns to that of a submerged healing protocol (De Rouck et al., 2009). Following permanent restorations there was a mean difference of 0.75mm in midfacial recession favouring the immediate restoration group at 1 year ($p = 0.005$). Midfacial recession was 2.5 to 3 times higher with the submerged protocol. Another study using immediate provisional restorations with mean follow up of 4 years reported a mean midfacial recession of 1.13 ± 0.87mm (Kan et al., 2011). This is one of the few studies that have identified a statistically significant different midfacial gingival recession in thick and thin gingival biotypes with 0.56 ± 0.46mm and 1.50 ± 0.88mm, respectively ($p = 0.0008$).

The majority of studies on immediate implants with immediate provisional restorations are of short-term follow up (<12 months) (Weigl and Strangio, 2016). A recent randomised controlled trial with 3-years of follow-up also compared immediate provisional restorations to a submerged protocol in terms of aesthetic outcomes (Arora and Ivanovski, 2018). Aesthetic outcomes were determined using

PES and WES. The mean PES (10.7 ± 2.16) and WES (8.1 ± 1.31) scores were not statistically significantly different between both groups. The only PES variable that provided a significant difference was the distal papilla, favouring the immediate provisional restoration group (mean 1.7 vs 1.25; p = 0.006). The PES and WES scores reported in this study are in accordance with other similar studies (Slagter et al., 2015, Rieder et al., 2016). This study was unable to report on midfacial recession due to its retrospective nature.

The implants used in this study were platform matched (PM) i.e. non-platform switched. Platform switching (PS) moves the implant-abutment interface medially, away from the outer edge of the implant platform and has demonstrated reduced radiographic bone loss around implants (Lazzara and Porter, 2006). A randomised controlled trial demonstrated that after 21 months there was greater bone loss of 1.49 ± 0.54mm in PM implants than PS, 0.56 ± 0.31mm (Canullo et al., 2010). A recent meta-analysis of randomised and prospective controlled trials demonstrated a clear reduction in mean marginal bone levels around PS implants (0.49mm) compared to PM implants (1.01mm) ($P<0.0001$) (Strietzel et al., 2015).

The data on the effect of PS on immediate implants is lacking in comparison to conventional implant placement as well as their effect on soft tissue responses. The majority of studies using PS for immediate implants are case series of short-term follow up (Lin et al., 2014). There are two randomised controlled trials assessing the soft tissue response of PS and PM immediate implants (Canullo et al., 2009, Pieri et al., 2011). Pieri et al (2011) did not find any statistically significant differences in recession of the midfacial gingiva or papilla at 12 months. However, only patients

with thick biotypes were included in that study. In contrast, Canullo et al (2009) included patients with both thick and thin biotypes. PS improved midfacial gingival recession by almost 1mm in the thin biotype group, while the difference in the thick biotype was only 0.4mm. Therefore PS may be of particular importance in patients with thin gingival biotypes.

The implants used in this study were the Zimmer Biomet T3 Implant™ (Biomet 3i implant innovations, Palm Beach Gardens, Florida, USA). This implant is of a tapered design and the majority of studies published on immediate implants also use tapered implants (Weigl and Strangio, 2016). It is believed tapered implants can be placed with increased primary stability over straight screw implants (O'Sullivan et al., 2004, Moon et al., 2010). This may be of particular importance in fresh extraction sockets as there is limited bone availability for primary stability compared to a fully healed site. Primary stability is achieved by utilising the bone palatal and apical to the extraction socket. Despite this, two randomised controlled trials have demonstrated that both straight screw and tapered implant designs have been placed in fresh extraction sockets with no difference in success or survival rates between them (Lang et al., 2007, Sanz et al., 2010).

All the implants in this study were judged to have good primary stability at placement and were allowed to heal with a submerged protocol. The T3 tapered implant design has been shown to be able to achieve high levels of insertion torque (mean 53Ncm) at implant placement in fully healed sites (Ostman et al., 2013) and they have also been placed in fresh extraction sockets of molar teeth with high levels of insertion torque (Block, 2011).

The Zimmer Biomet T3 Implant™ used in the study has a hybrid surface where the coronal aspect has decreased roughness of the dual acid etching (1-3 microns) and the apical surface roughness is increased in comparison. The apical surface is treated with discrete crystalline deposition (DCD) which, has been shown to enhance osseointegration compared to the same surface but without DCD, in pre-clinical (Mendes V, 2011) and clinical studies (Orsini et al., 2007). The surface has been shown to have a mean surface roughness value of 1.4 μm (Gubbi P, 2012) which would make it a moderately rough surface (Albrektsson and Wennerberg, 2004). Almost all the studies on single-tooth immediate implants in the aesthetic zone have used implants with a moderately rough surface (Weigl and Strangio, 2016). This is presumably because roughened surface implants have been shown to promote the rate and degree of osseointegration (Abrahamsson et al., 2004, Wennerberg and Albrektsson, 2009).

6 Conclusion

The placement of 35 Zimmer Biomet T3 Implants™ (Biomet 3i implant innovations, Palm Beach Gardens, Florida, USA) in 33 patients resulted in a 100% survival rate. This allows us to say this implant system can be used with confidence in the placement of implants in fresh extraction sockets.

This study reaffirms the results of previous studies that immediate implants combined with bone regeneration techniques do not prevent the remodelling of the alveolar ridge following tooth extraction.

To the best of our knowledge, this is the first study that has quantified the bony remodelling occurring around immediate implants when the void is filled with either WTPG or L-PRF. This study demonstrated the surgical protocols employed were successful, at least in the short-term.

Grafting the void with WPTG and L-PRF preserved the buccal bone plate with clinically acceptable results. WPTG was superior to L-PRF is its ability to preserve bone in both the horizontal and vertical dimensions following immediate implant placement. WPTG performed similarly to other grafting materials described in the literature.

Within the limits of this study, the gap width, the buccal bone thickness and the horizontal buccal bone dimension were the main indicators for the ridge width at re-entry. The gap width and in particular the gap depth after implant placement were

shown to key determinants of the magnitude of alveolar ridge remodelling, as well as the buccal bone width and height. As has been shown in other studies, these specific characteristics appear to influence the amount of gap infill and buccal bone thickness 4 months after immediate implant placement.

7 Bibliography

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8 Appendices

Appendix A: Ethical approval letter

THIS NOTEPAPER MUST NOT BE USED FOR PRESCRIPTIONS OR INVOICING PURPOSES

SJH/AMNCH Research Ethics Committee Secretariat
Claire Hartin Ph: 4142199
email: claire.hartin@amnch.ie

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
INCORPORATING
THE NATIONAL CHILDREN'S HOSPITAL

TALLAGHT, DUBLIN 24, IRELAND
TELEPHONE +353 1 4142000

Dr. Mark McLaughlin
Dept. of Restorative Dentistry and Periodontology
Trinity College Dublin
Dublin Dental University Hospital
Lincoln Place
Dublin 2

23rd February 2016

Re: The effect of leucocyte and platelet rich fibrin (L-PRF) on the preservation of the buccal bone plate following immediate implant placement

REC Reference: 2016/02/05
(Please quote reference on all correspondence)

Dear Dr. McLaughlin,

The Committee decided to give ethical approval to this study subject to the following conditions:

- The PIL overall needs to be rewritten in a much more patient friendly language.
- The consent form should include the option to withdraw from the study
- The application form advises that 3 visits will take place before removal of teeth occurs on the 3rd visit. The PIL advises that removal of teeth will occur at visit no. 2. This must be clarified.
- Part K2 (c) of the application is incomplete
- The consent form should be more specific with regard to potential use of photographs and radiographs for future studies

Yours sincerely,

pp

Dr. Peter Lavin
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 00486

Appendix B: Patient information letter

PARTICIPANT INFORMATION LEAFLET

The Effect of Leucocyte and Platelet Rich Fibrin (L-PRF) on the Preservation of the Buccal Bone Plate Following Immediate Implant Placement

You are being invited to participate in a research study:

In order to make an informed judgement on whether you want to be part of this research study, you should understand its potential risks and benefits. This is called informed consent. This leaflet gives you information about the research study, which will be discussed with you. Ask us if there is anything that is not clear or if you would like more information. Once you understand the study, you will be asked to sign a consent form if you wish to participate. Should you not wish to participate, simply do not return the consent form

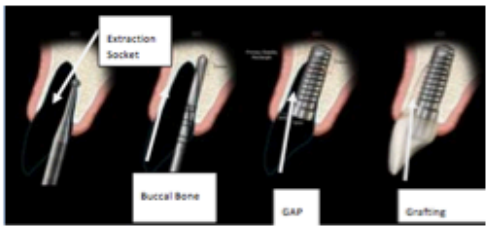
Study Summary:

This study will collect information on the healing of a bone socket after extraction and the immediate placement of a dental implant. A membrane made from your own blood cells will be used to aid healing of bone around the implant. We will correlate the bone dimensions of the bony socket after extraction with the quantity of bone remaining at the area after 4 months of healing.

Background information:

Each tooth is surrounded by a bony socket. When a tooth is extracted, some of the jawbone at the socket site is lost through a process known as resorption. This reduction of bone dimensions can affect the options available to the dentist for restoring (replacing) the gap left by the missing tooth and can also affect the aesthetics (cosmetic appearance) of these restorations.

One of the options available for replacing a missing tooth is a dental implant. A dental implant can be described as an "artificial root" made of titanium, which is placed surgically inside the jawbone and is used to support various types of restorations, such as a crown, a bridge or a denture. After their placement, implants start to integrate with bone achieving long-term stability. As dental implants are placed in the bone, preserving the tooth socket and surrounding bone after extractions is very important.



When implants first became available, the protocol suggested waiting up to 6 months following tooth extraction before implant placement. This approach may result in bone loss due to post extraction resorption, often leading to difficulties in implant placement and aesthetics. Placing an implant at the time of tooth extraction (Immediate placement) is appealing to both patients and dentists, since it significantly reduces the treatment time. Additionally, it better preserves the profile of the bone, helping the dentist to achieve better aesthetic results

As tooth roots vary in shape and size when implants are placed at the time of tooth extraction, there is often lack of adaptation between the walls of the socket bone and the implant (See picture). This gap is usually widest near the opening of the socket. Having a gap between the implant and bone can affect the success of the implant, as it may not integrate well with the bone. In an attempt to reduce this risk a variety of different grafting materials have been used to fill the gap present between the implant and bone. The standard procedure used at the Dublin Dental Hospital is to place a bone graft material, derived from chemically treated cowbone, in the gap. The graft and the implant is then covered with a membrane (protective covering, also derived from cow tissue) and the implant is buried beneath the gum to allow healing.

Study Overview:

This study aims to collect information about characteristics of the bony socket and surrounding bone after tooth extraction in the front area of the mouth and before placement of an immediate dental implant. The thickness and height of the bone will again be measured following 4 months of healing. Buccal bone plate preservation (thickness and height) is very important for the long-term stability of a dental implant as well as for achieving a good aesthetic result. Information gathered from this study could allow us to predict possible functional and aesthetic complications that could arise when placing immediate implants.

This study will use the T3 implant, an implant used routinely in the Dublin Dental Hospital. Instead of using a bone graft and membrane from cowbone, we will create a membrane from your own blood cells (L-PRF membrane). This involves taking a blood sample from your arm (approximately 4-8 teaspoons of blood). The blood is placed in a special machine, which spins the blood so that the blood forms a strong blood clot; this clot can then be used to fill the gap between implant and bony socket. It can also serve as the membrane to cover the socket and support the gum tissues covering the implant.

Are you suitable for this study?

To be included in the study you must fulfil the criteria below:

- Be 18 years old or over.
- Have a tooth planned for extraction (removal) and replacement with a dental implant. The tooth must have a neighbouring tooth on either side and must be free from any active infection.
- Be in good general health and able to tolerate minor dental surgeries
- Be able to give consent to participate in this study and sign a consent form approved by the Research Ethics Committee of the Faculty of Health sciences, Trinity College Dublin.
- Be able to attend all the appointments described below

If you are initially included in the study but you fail to complete all stages of the treatment due to poor attendance, you will have to be excluded from the study.

Study Appointments

Screening appointment: You will be invited to attend an initial screening appointment to confirm you are suitable for implant treatment and that you fit the admission criteria for the study. If you are suitable you will be invited to participate. You will be given a detailed description of the study and some written information to take home.

1. Study visit 1: Pre-surgery An informed consent form will be signed to show you understand and agree to participate. If necessary, impressions and radiographs will be taken at this time. (this is the normal procedure for any implant placement)
2. Study visit 2: At this appointment, the extraction of the non-restorable tooth and the immediate implant placement will take place (this is the normal procedure for immediate implant placement). In addition we will record the necessary bone measurements and take some clinical photographs to document the appearance of the bone and gum tissues and placement of the membrane.
3. Study visit 3: Following a four-month healing period you will need to attend the "2nd stage surgery". At this stage the implant is exposed to check if it has been successfully integrated and to connect the restoration to the implant. We will record the necessary bone measurements and some clinical photographs.

The surgery procedures described above represent the normal process followed when an implant is placed immediately following extraction of a tooth. Cells from your own blood will be used to aid healing, instead of a graft made from cowbone. The additional research component is the bony measurements. Overall, we expect that these bone measurements will increase the surgical time by about 5 min in the first study appointment and 2 minutes in the final appointment.

**After the surgery appointments, stitches will be placed, which will have to be removed approximately 7 days later.

The types of dental treatments provided in this study are an alternative to:

- No treatment – removing the tooth and leaving a gap.
- Replacing the gap with a fixed or removable option, such as a bridge or partial denture.
- Implant placement using grafting materials (bone graft and protective membrane covering) derived from a bovine (cow) source to fill the gap.

Dental treatment is associated with risk. The risks associated with this type of treatment include:

- pain following treatment
- bleeding following surgery
- swelling of the gums after the surgery
- bruising of the face following the operations
- infection of the area of your mouth where the implant is placed
- failure of the grafting material
- failure of the implant to integrate with your own bone, which will result in loss of the implant

Other information of relevance to the study:

- Participants will have to pay for their treatment. However, implants will be provided at a reduced rate. The grafting material (L-PRF membrane) will be provided for free.
- Other dental treatments such as fillings cannot be provided in the study.
- If for any reason we have to exclude you from the study but you would like to continue with your treatment in the Dublin Dental Hospital, you can.
- Your identity will remain confidential. Your name will not be published.
- If you decide to participate in this study, you may withdraw at any time. If you decide not to participate, or withdraw, you will not give up benefits that you had before entering the study.
- You understand that the investigators may withdraw your participation in the study at any time without your consent.

Contact details

If you have any questions please contact the principal researcher, Dr. Mark McLaughlin

Email: mark.mclaughlin@dental.tcd.ie

Appendix C: Informed consent form

Informed Consent Form

The Effect of Leucocyte and Platelet Rich Fibrin (L-PRF) on the Preservation of the Buccal Bone Plate Following Immediate Implant Placement

Staff Conducting Research

Dr. Mark McLaughlin

Dr. Ioannis Polyzois

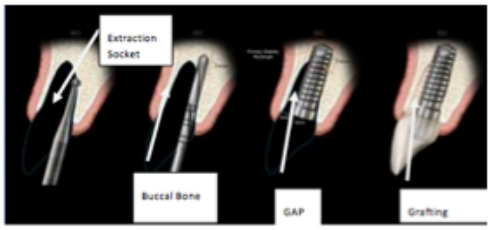
Study Summary:

This study will collect information on the healing of a bone socket after extraction and the immediate placement of a dental implant. A membrane made from your own blood cells will be used to aid healing of bone around the implant. We will correlate the bone dimensions of the bony socket after extraction with the quantity of bone remaining at the area after 4 months of healing.

Background information:

When dental implants first became available the protocol suggested waiting up to 6 months following tooth extraction before implant placement. This approach may result in bone loss due to post extraction resorption, often leading to difficulties in implant placement and aesthetics. Placing an implant at the time of tooth extraction (Immediate placement) is appealing to both patients and operators, since it significantly reduces the treatment time. Additionally, it better preserves the profile of the bone, helping the operator to achieve better aesthetic results.

As tooth roots vary in shape and size when implants are placed at the time of tooth extraction, there is often lack of adaptation between the walls of the socket bone and the implant (See picture). This gap is usually widest near the opening of the socket. Having a gap between the implant and bone can affect the success of the implant, as it may not integrate well with the bone. In an attempt to reduce this risk a variety of different grafting materials have been used to fill the gap present between the implant and bone. The standard procedure used at the Dublin Dental Hospital is to place a bone graft material, derived from chemically treated cowbone, in the gap. The graft and the implant is then covered with a membrane (protective covering, also derived from cow tissue) and the implant is buried beneath the gum to allow healing.



This study will use the T3 implant, an implant used routinely in the Dublin Dental Hospital. Instead of using a bone graft and membrane from cowbone, we will create a membrane from your own blood cells (L-PRF membrane). This involves taking a blood sample from your arm (approximately 4-8 teaspoons of blood). The blood is placed in a special machine, which spins the blood so that the blood forms a strong blood clot; this clot can then be used to fill the gap between implant and bony socket. It can also serve as the membrane to cover the socket and support the gum tissues covering the implant.

The aim of the study:

To correlate post-extraction socket and alveolar ridge dimensions with the quantity of the buccal bone and alveolar ridge after 4 months of healing.

Confidentiality:

Your name will not be used to identify the sample and no-one outside the study group will have access to your details to ensure your confidentiality.

Consent:

Having read the participant information leaflet, if you wish to participate please sign the declaration below. Should you not wish to take part in this study, it will have no impact on the care you receive from the hospital and do not sign the declaration below.

Declaration:

I have read, or had read to me, the information leaflet for this project and I understand the contents. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, without prejudice to my legal and ethical rights. I understand that I may withdraw from my study at any time and I have received a copy of this agreement.

_____I consent to my records including radiographs or photographs being used for research purposes.
_____I agree to the further use of data (e.g. radiographs and photographs) collected during this study in possible future studies, without the need for my additional consent.
_____I agree to the possible publication of results from this study
_____I understand that any of the research or educational purposes referred to above will preserve my anonymity and my name will not be linked to any of these activities or publications.
_____I understand that I may withdraw from this study at any time I wish

Participant's name:.....

Contact details:.....

Participant's signature: .....

Date:.....

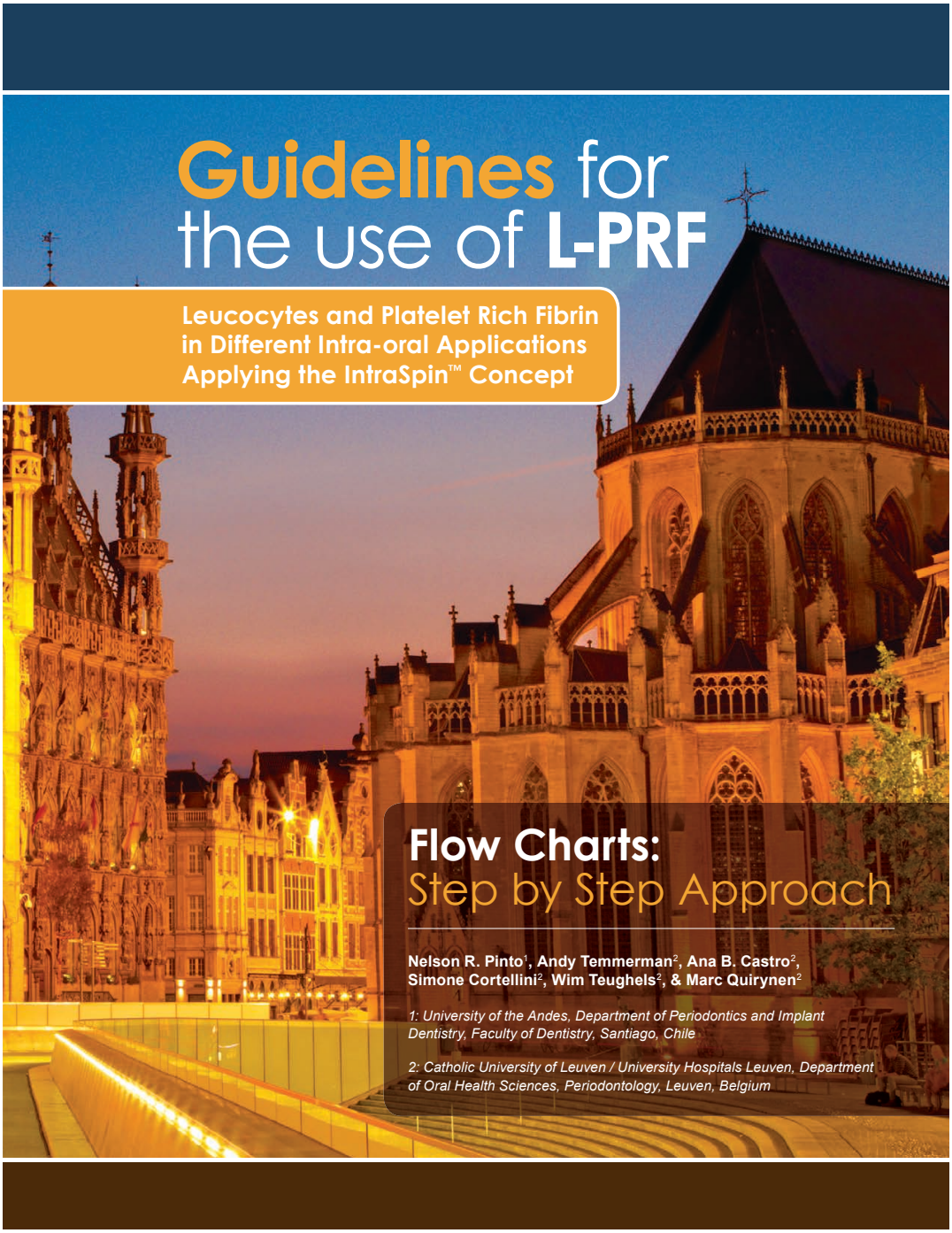
I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered such questions. I believe the participant understands my explanation and has freely given informed consent.

Investigator's signature: .....

Date:.....

OSPIDÉAL DÉADACH BHAILE ÁTHA CLIATH
DUBLIN DENTAL HOSPITAL

Appendix D: L-PRF protocol



Guidelines for the use of L-PRF

Leucocytes and Platelet Rich Fibrin in Different Intra-oral Applications Applying the IntraSpin™ Concept

Universidad de los Andes

KU LEUVEN

Flow Charts: Step by Step Approach

Favorable wound healing has always been a major quest in dental surgery. It is a concern in healthy as well as compromised patients. In an effort to improve and accelerate healing of both hard and soft tissues, substitutes including growth factors and bio-materials have been traditionally employed. Membranes were also introduced to separate tissues.

Recent research clearly indicates that L-PRF (Leukocyte -Platelet Rich Fibrin, a second generation of platelet concentrates) significantly enhances wound healing in both soft and hard tissues. Evidence now supports the assertion that this has the potential to replace the above mentioned substitutes in many situations.

Clinical procedures benefit from recent advancements with platelet concentrate protocols including, but not limited to: soft tissue healing, plastic periodontal surgery, gingiva enlargement, MRONJ, regeneration of infra-bony defects, ridge preservation, sinus augmentation, immediate implant placement and implant osseointegration itself. An added benefit is that these platelet concentrate protocols offer significantly lower cost treatment solutions to our patients, due to the fact of their ease of use and inexpensive preparation.

Flow Chart **One**

Step by step approach for the preparation of L-PRF
(simple chair-side procedure)

Protocol for preparation of L-PRF clots:

- Venipuncture: With a 21G butterfly needle collect up to 8 9ml red cap tubes of blood.
- After the first two tubes of blood are collected, immediately place them into the IntraSpin™ centrifuge, opposite to each other to ensure the centrifuge is properly balanced. Close the cover and set the timer to "1" minute. Press START and allow the centrifuge to run for one minute, after one minute the centrifuge will come to a full stop and the cover will pop open. While it is spinning for 1 minute collect the third and forth tubes of blood from patient, and repeat prodcedure for the other tubes.
- Centrifugation should be at 408g (2700 rpm using the IntraSpin™ centrifuge, for at least 12 minutes (start timing after loading the centrifuge with last 2 tubes).
- After ≥ 12 minutes centrifugation (for patient taking anti-coagulant medication up to 18 minutes are recommended) L-PRF clots are ready.
- Take the fibrin clots out of the tubes and separate them from the red blood cells.

Protocol for preparation of L-PRF membranes:

- Place fibrin clots in Xpression™ box for gentle compression by gravity (e.g. with light metal plate, Figure 2).
- 5 minutes later the L-PRF membranes are ready for use.
- 2.5 to 3 hours is the viability for expressed membranes, as long as they are re-hydrated with exudate.

Protocol for preparation of L-PRF plugs:

- Place fibrin clots in the small white cylinder of the Xpression™ box.
- Use the piston to carefully compress the clot, until holder is level to cylinder.
- 2.5 to 3 hours is the viability for expressed plugs, as long as they are re-hydrated with exudate.

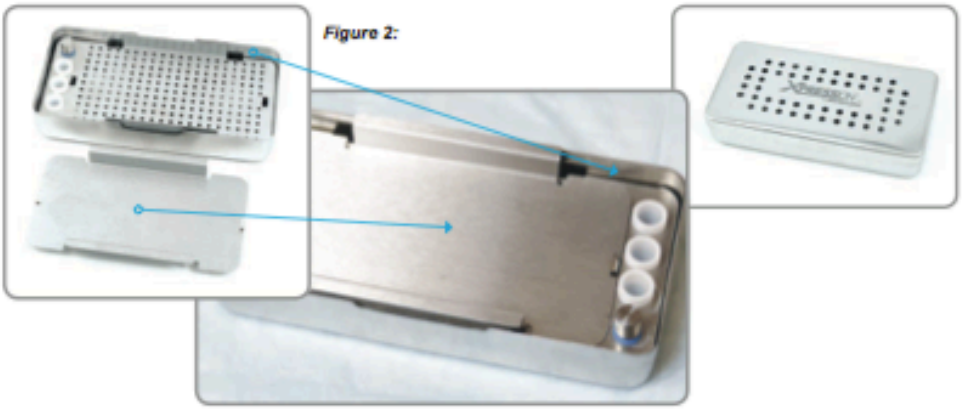


Figure 2:

Flow Chart **One**

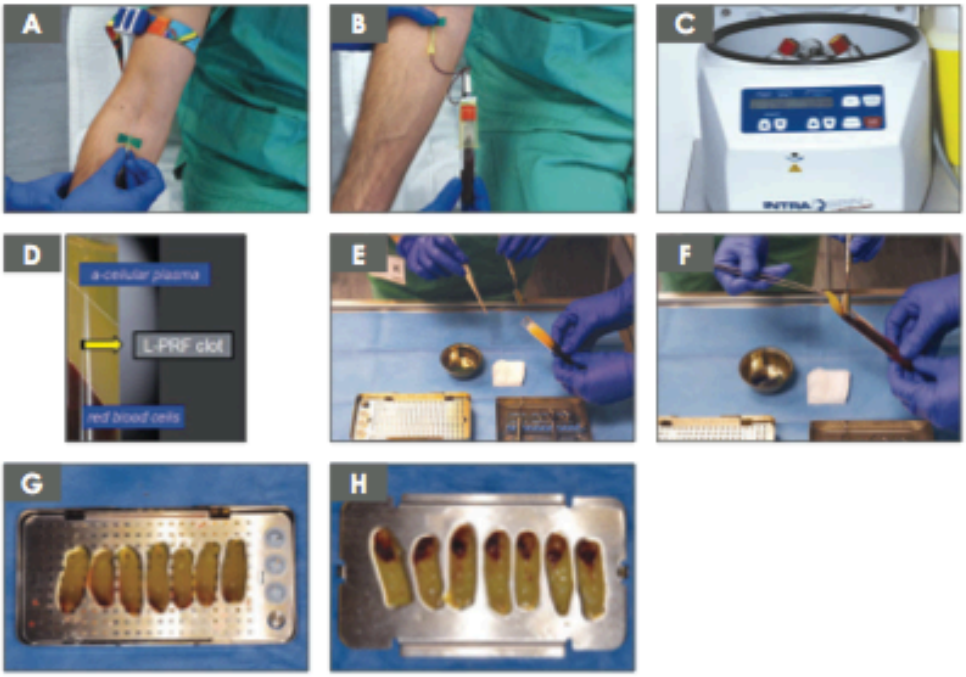


Figure 3:

Process of preparing L-PRF clots and membranes.

A&B: Venipuncture and blood collection using 21G butterfly needle and 9 ml red cap tubes.

C: Centrifugation at 408g RCF, (2700 rpm) with IntraSpin™ centrifuge.

D: L-PRF clot in tube; clear separation: red blood corpuscles (RBCs) at the bottom, PPP (platelet poor plasma) on the top, and L-PRF fibrin clot in the middle.

E&F: Remove clot from tube and separate clot from red blood cells.

G: Specially designed kit (Xpression™ box) to compress L-PRF clots into L-PRF membranes with a consistent thickness of 1 mm. A piston and cylinder assembly (right site) can be used for the creation of L-PRF plugs, suitable for filling extraction sockets.

H: L-PRF membranes after gentle compression; the red area of the membrane represents the face side, where most leucocytes, platelets and stem cells are concentrated.