COMBINATION OF PLATELET-RICH FIBRIN AND CALCIUM SULFATE IN SOCKET AUGMENTATION

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FACULTY OF DENTISTRY
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COMBINATION OF PLATELET-RICH FIBRIN AND CALCIUM SULFATE IN SOCKET AUGMENTATION

ABSTRACT

Objectives: The aim of this clinical study was to evaluate and compare vertical, horizontal and volumetric dimensional changes of alveolar ridge using combinations of platelet-rich fibrin and calcium sulfate (PRF-CS) and platelet-rich fibrin and xenograft (PRF-X) in socket augmentation procedure. **Materials and Methods:** Ten subjects requiring single maxillary premolar tooth extraction were included. Five sockets received PRF-CS grafts and five sockets received PRF-X grafts. Stone cast models were used to assess the changes of soft tissue level. Cone beam computerized tomography (CBCT) images were produced to measure horizontal and vertical dimensions. The CBCT images were then exported into MIMICS software to analyze the bone volume. All measurements were recorded at baseline (before extraction) and at 5 months post-extraction. **Results:** Significant reduction in vertical and horizontal dimensions was observed in both the groups at 5 months post-extraction except for distal bone height (D_BH) (p=0.094) and palatal bone height (P_BH) (p=0.065) in PRF-X group. PRF-CS group demonstrated 12.2 % horizontal resorption as compared with 14.84% in PRF-X group (p>0.05). For PRF-CS group, resorption for mesial bone height (M_BH=19.58%), buccal bone height (B_BH=18.87%) and palatal bone height (P_BH=15.66%) was more than resorption for PRF-X (M_BH=8.48%, B_BH=7.26% and P_BH=4.33%) (p>0.05). The bone volume reduction of 11.43% and 7.74% was observed in PRF-CS group and PRF-X group respectively (p>0.05). **Conclusion:** PRF-CS can be used as a graft material for socket augmentation procedure.

Keywords: Extraction socket, socket augmentation, platelet-rich fibrin, calcium sulfate, xenograft

GABUNGAN FIBRIN KAYA PLATELET DAN KALSIUM SULFAT DALAM AUGMENTASI SOKET

ABSTRAK

Tujuan: Tujuan kajian klinikal ini adalah untuk menilai dan membandingkan perubahan dimensi menegak, mendatar dan volumetrik rabung alveolar dengan menggunakan gabungan fibrin kaya platelet dan kalsium sulfat (PRF-CS) dan fibrin kaya platelet dan xenograft (PRF-X) dalam prosedur augmentasi soket. **Bahan dan Kaedah:** Kajian ini melibatkan 10 subjek yang memerlukan cabutan tunggal gigi premolar di rahang maksila. Lima soket menerima graf PRF-CS dan lima soket menerima graf PRF-X. Model acuan batu digunakan untuk menilai perubahan tisu lembut. Imej Cone Beam CT (CBCT) dihasilkan untuk mengukur dimensi mendatar dan menegak. Kemudian, imej CBCT ini dimasukkan ke dalam perisian MIMICS telah digunakan untuk menganalisa isipadu tulang. Semua ukuran telah direkodkan pada garis dasar (sebelum cabutan gigi) dan pada 5 bulan selapas cabutan gigi. **Keputusan:** Pengurangan ketara dalam dimensi menegak dan mendatar telah diperhatikan dalam kedua-dua kumpulan pada 5 bulan selepas cabutan gigi kecuali ketinggian tulang distal (D_BH) ($p = 0.094$) dan ketinggian tulang palatal (P_BH) ($p = 0.065$) dalam kumpulan PRF-X. Kumpulan PRF-CS menunjukkan resorpsi mendatar 12.2% berbanding 14.84% dalam kumpulan PRF-X ($p > 0.05$). Bagi kumpulan PRF-CS, resorpsi untuk ketinggian tulang mesial (M_BH = 19.58%), ketinggian tulang buccal (B_BH = 18.87%) dan ketinggian tulang palatal (P_BH = 15.66%) adalah lebih daripada resorpsi untuk PRF-X (M_BH = 8.48% , B_BH = 7.26% dan P_BH = 4.33%) ($p > 0.05$). Pengurangan isipadu tulang sebanyak 11.43% dan 7.74% diperhatikan dalam kumpulan PRF-CS dan kumpulan PRF-X masing-masing ($p > 0.05$). **Kesimpulan:** PRF-CS mempunyai kesan dalam prosedur augmentasi soket.

Kata kunci: soket pengekstrakan, augmentasi soket, fibrin kaya platelet, kalsium sulfat, xenograf

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

B_BH	:	Buccal bone height
B_SH	:	Buccal soft tissue height
BMP	:	Bone morphogenetic protein
β-TCP	:	Beta tricalcium phosphate
CBCT	:	Cone beam computed tomography
CS	:	Calcium sulfate
CT	:	Computed tomography
DFDBA	:	Demineralized freeze dried bone allograft
D_BH	:	Distal bone height
D_SH	:	Distal soft tissue height
EGF	:	Epithelial growth factor
ePTFE	:	Expanded polytetrafluoroethylene
FDBA	:	Freeze dried bone allograft
FGF	:	Fibroblast growth factor
FOV	:	Field of view
HA	:	Hydroxyapatite
HU	:	Hounsfield
IBD	:	Intrabony defects
IGF	:	Insulin like growth factor
IL	:	Interleukin
M_BH	:	Mesial bone height
M_SH	:	Mesial soft tissue height
Mg	:	Milligram
MGCSH	:	Medical grade calcium sulfate hemihydrate

MIMICS	:	Materialise's interactive medical image control system
mm	:	Millimeter
mm^3	:	Cubicmeter
µm	:	Micrometer
OPG	:	Orthopantomogram
PDGF	:	Platelet derived growth factor
PD	:	Periodontal defects
P_BH	:	Palatal bone height
P_SH	:	Palatal soft tissue height
PRF	:	Platelet-rich fibrin
PoC	:	Percentage of changes
PRP	:	Platelet-rich plasma
PRF-CS	:	Platelet rich fibrin and calcium sulfate
PRF-X	:	Platelet-rich fibrin and xenograft
SH	:	Socket height
TCP	:	Tricalcium phosphate
TGF	:	Transforming growth factor
TMJ	:	Temporomandibular joint
TNF-α	:	Tumor necrosis factor alpha
VEGF	:	Vascular endothelial growth factor
W	:	Horizontal bone width
X	:	Xenograft

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Tooth extraction/exodontia

Extraction is a process of removing tooth from its socket in the alveolar bone and is a most widely performed procedure in dentistry. Extraction of a tooth is advised when a tooth cannot be sustained in an adequate condition for longstanding health, esthetics and function (Avila-Ortiz et al., 2014). Depending on the location of tooth loss, it causes functional impairment such as on chewing and esthetics (Gerritsen et al., 2010). After tooth extraction, the alveolar bone experiences a remodelling process that results in a pronounced diminution of alveolar ridge. A definitive preclinical study with a canine model reported severe resorption of the buccal plate in the first 8 weeks coming from marked osteoclastic activity (Araújo & Lindhe, 2005). More distinct resorption on the crestal level of the buccal wall to the lingual wall was documented in the study. There are two distinct phases to define the pattern of resorption; the bundle bone resorption and replacement with the woven bone, and both buccal and lingual wall resorption from the outer surfaces. This resorption pattern occurs because of the thinner buccal plate when compared to the lingual plate of alveolar bone. The blood supply of the bundle bone is discontinued after tooth extraction, resulting in less nutrient supply and significant resorption of the buccal plate (Araújo & Lindhe, 2005).

1.2 Rationale of exodontia

Endodontic lesion, severe periodontitis and trauma are the basic and most common reasons of exodontia (Checchi et al., 2011). The most common and well known causative agent which leads to exodontia in all age groups are dental caries (Ainamo et al., 1984). However, after 40 years of age, periodontitis also plays a key role in permanent tooth loss (Kay & Blinkhorn, 1986). In addition, other categories of extractions are also described by Kay and Blinkhorn (1986) such as; pre-prosthetic tooth extraction for a better

prosthetic restoration, orthodontic tooth extraction to avert or correct malocclusion and wisdom tooth extraction. Other frequently occurring causes of extractions are pulp necrosis, cracked teeth, malposition teeth, and supernumerary teeth, teeth associated with pathologic lesions and patients who are to receive radiation therapy for head, neck and oral cancer.

1.3 Alveolar ridge resorption

The whole anatomy of the alveolar ridge is determined by the eruption axis of teeth, their prospective inclinations and tooth form (Araújo & Lindhe, 2005). Subsequent to tooth extraction, the fundic portion of the bony extraction socket will be filled by connective tissue and bone whereas alveolar crest resorbs (Pietrokovski & Massler, 1967). The basic phenomenon is that the crestal part of alveolar bone is solely comprised of bundle bone. Following extraction, a marked osteoclastic activity occurs that results in the resorption and replacement of the bundle bone of crestal region to woven bone on the buccal side. Hence, the crest of the buccal bone shifts at a greater extent compared to its original palatal or lingual site, resulting in considerable vertical reduction. It has been documented in various studies that after extraction, bone resorption is more noticeable at the buccal aspect as it is generally thinner in comparison to lingual or palatal aspects (Johnson, 1969; Lekovic et al., 1998; Pietrokovski & Massler, 1967; Schropp et al., 2003). In addition, greater resorption occurs in width averaging 2.6 and 4.6 mm rather than the height with average of 0.4 and 3.9 mm of alveolar ridge in natural healing (Ten Heggeler et al., 2011). Furthermore, both human and animal studies showed that the marginal 1/3 of the sockets exhibits most of the hard tissue loss (Araújo & Lindhe, 2005; Misawa et al., 2016). The amount of bone resorption is greatest within the initial 3-6 months of extraction and it continues after this period (Schropp et al., 2003; Tan et al., 2012). Moreover, alveolar bone loss is very critical for dental restorations, as adequate amount of bone is essential for reconstruction.

1.4 Extraction socket augmentation

Extraction socket augmentation procedure appears to rescue more bone after tooth removal (Zhang et al., 2018). The augmented extraction sockets have less bone resorption and maintained hard and soft tissues in comparison to non-augmented sockets (Barone et al., 2012; Hauser. et al., 2013). Darby et al. (2009) in a systematic review have concluded that the socket augmentation approach may reduce the resorption of alveolar bone width and height and may slow the process of alveolar bone resorption. Furthermore, several studies have reported that socket augmentation procedures prevent the soft and hard tissue collapse and can minimize the requirement for future augmentation procedures (Cardaropoli & Cardaropoli, 2008; Fickl et al., 2008; Fickl. et al., 2008a). Hence, preservation of the alveolus by socket augmentation at the time of extraction is crucial to maintain adequate quantity of alveolar bone for optimal dental restorations.

1.5 Effectiveness of extraction socket augmentation

Current systematic reviews have shown the effectiveness and confirmed the efficiency of socket augmentation and its potential to inhibit alveolar bone resorption. Vignoletti et al. (2012a) reported that non-grafted site had more alveolar ridge width and height loss when compared to the sites that received bone graft. Authors of this study reported that these sites reduced 1.83mm and 1.47mm in width and height respectively. Avila-Ortiz et al. (2014) reported that the socket augmentation sites with the use of xenograft or allograft reduced in buccal height and ridge width for about 2.07mm and 1.89mm respectively compared to natural healing. In addition, the study also concluded that the use of a membrane with xenograft or allograft and flapped approach showed beneficial effects. Nevertheless, a previous study by Fickl. et al. (2008b) confirmed that if the flapped approach is applied in extraction surgery, the opened flap blocks the blood supply of the buccal bone wall and further enhancing the resorption of buccal bone wall. Araujo et al. (2015) reported that the flapless tooth extraction causes reduction in the dimensional

changes of the hard tissues after socket augmentation. A recent study also reported that the flapless approach in socket augmentation procedure favored the maintenance of ridge dimensions, decreased the loss of buccal bone crest and enhanced bone formation (de Barros et al., 2017).

Several ridge preservation procedures have been explained, including socket augmentation with autografts, xenografts, allografts and alloplasts material either combined with resorbable or non-resorbable membranes. Recently, Jambhekar et al. (2015) reported xenografts as an effective material followed by allografts and alloplasts in socket augmentation when compared to extraction alone. In addition, the remnants of graft material was highest for allografts followed by xenografts and alloplasts (Jambhekar et al., 2015). The usage of resorbable or non-resorbable membranes with these bone substitutes showed loss of soft tissue thickness and reduced ridge width, ultimately compromising the final outcome (Iasella et al., 2003). The high concentration of bone substitutes remnants may decrease the final osseointegration between bone and implant (Anwandter et al., 2016). Autografts are considered as gold standard but frequently include limited availability and donor site morbidity. Overall, socket augmentation techniques limit the extent of ridge alterations, but do not completely prevent it.

Innovative biological procedures have been proposed to overcome the disadvantages of autografts and non-vital bone substitutes (Simon et al., 2011). The latter involves first and second-generation platelet concentrates. The first group includes platelet-rich plasma (PRP). In the preparation of PRPs, artificial additives (e.g. thrombin, anticoagulants or calcium chloride) are used to operate the coagulation process. Concentration of platelets and a fine fibrin mesh are richly present in PRPs (Dohan Ehrenfest et al., 2010).

The second generation platelet concentrates contain leukocytes and platelet-rich fibrin (PRF). It was initially introduced in France by Choukroun et al. (2001). PRF is prepared

by a centrifugation process from patients' own blood without the inclusion of any coagulation or anticoagulants factor. The naturally manufactured coagulation in PRF preparation results in a fibrin clot in which 97% of circulating platelets and 50% of leukocytes are present (Dohan. et al., 2006b). The fibrin clot is detached from the red components and the supernatant acellular plasma. Depending on clinical needs, the fibrin clot can be shaped by light compression to form either a membrane or a plug (Dohan. et al., 2006a). These membranes comprise of highly dense cross-linked fibrin mesh in three dimensions containing viable platelets. Multiple growth factors, anti-inflammatory cytokines and adhesion molecules are released by this biologically active PRF for up to 1 week (D. M. Dohan et al., 2006; Dohan. et al., 2006a). With the existence of growth factors, adhesion molecules and cytokines, PRF can increase the effectiveness of angiogenesis, tissue regeneration and neovascularization, regulate reparative inflammatory response and decrease postoperative edema and pain. These characteristics of PRF make it a biologically adequate graft for socket augmentation. In comparison with non-bioactive graft materials, PRF may enhance the tissue regeneration once applied in the extraction sockets (Anwandter et al., 2016).

Calcium sulfate (CS) is a type of alloplastic material and has been employed since many years in clinical use compared to recent biomaterials. It is widely accepted as a socket grafting material and undergoes rapid and complete resorption. In the presence of bone, mostly it becomes osteogenic (Guarnieri et al., 2004). Crespi et al. (2009) reported rapid resorption of CS and improved bone formation compared to magnesium-enriched hydroxyapatite (MHA) in socket augmentation procedure at 3 months of histologic evaluation. CS provides a mechanism to increase new bone growth. Strocchi et al. (2002) reported in a study that CS creates an osteoconductive lattice that stimulates bone ingrowth into a defect.

Xenografts are the graft biomaterials extracted from animal origin (bovine or equine) (Lang & Lindhe, 2015). They have osteoconductive properties and biocompatible to the human recipients. The trabecular architecture with the presence of interconnecting pores in xenograft permits optimum in-growth of new vascularity. MinerOss®, is a commercially available biocompatible an organic porous bovine derived bone mineral for use in periodontal, oral and maxillofacial surgery. MinerOss® is formed by elimination of all organic elements from bovine bone. It is available in cancellous and cortical forms. A recent study was conducted by Serrano Mendez et al. (2017) to compare the healing by applying allografts and xenografts for extraction socket augmentation. Ten patients received demineralized freeze-dried cortical bone allograft and ten patients received deproteinized cancellous bovine bone xenograft. On histological analysis after 6 months of extraction, new bone formation was higher (35.3 ± 16.8%) at the xenograft sites when compared with allograft augmented sites (25.5 ± 10.1%). The study concluded that both grafting materials are suitable for alveolar ridge preservation.

1.6 Problem statement

In the abovementioned studies when individually employed grafting materials including PRF, CS and xenografts were utilized, the alveolar bone healing characteristics were significantly improved (Aimetti et al., 2009; Barone et al., 2008; Hauser. et al., 2013). Moreover, recent studies employed the combination of various grafting materials (such as, PRP and CS, PRP and xenograft, PRF and xenograft etc.) and reported the improved healing with minimal ridge resorption (Cheah et al., 2014; Kutkut et al., 2012; Potres et al., 2016; Yilmaz et al., 2013). In aforementioned combinations, PRF-X showed superior results. However, xenograft is derived from different species and is expensive thus may not be acceptable to all patients. In search for an alternative grafting material, combination of PRF-CS was proposed to be used for socket augmentation in this study.

1.7 Aim of the study

The aim of this study is to evaluate and compare dimensional (vertical and horizontal) and volumetric changes of alveolar ridge when using combination of platelet-rich fibrin with calcium sulfate (PRF-CS) as compared to PRF-xenograft (MinerOss®X) (PRF-X) in socket augmentation procedure.

1.8 Research objectives

The study will focus on the following research objectives:

1. To compare the vertical alveolar bone resorption of extraction sockets that have been augmented with PRF-CS and PRF-X.
2. To compare the horizontal alveolar bone resorption of sockets that have been augmented with PRF-CS and PRF-X.
3. To investigate and compare the changes in soft tissue levels of extraction sockets that have been augmented using PRF-CS and PRF-X.
4. To investigate and compare the volumetric changes of extraction sockets that have been augmented with PRF-CS and PRF-X.

1.9 Null hypothesis

The null hypothesis of this study is that there is no significant difference in dimensional (vertical and horizontal) and volumetric changes of alveolar ridge between PRF-CS augmented and PRF-X augmented extraction sockets.

1.9.1 Research questions

1. Is there any difference in vertical alveolar bone resorption of sockets that have been augmented with PRF-CS and PRF-X?
2. Is there any difference in horizontal alveolar bone resorption of sockets that have been augmented with PRF-CS and PRF-X?

3. Is there any difference in soft tissue levels of sockets that have been augmented with PRF-CS and PRF-X?
4. Is there any difference in volumetric changes of extraction sockets that have been augmented with PRF-CS and PRF-X?

CHAPTER 2: LITERATURE REVIEW

2.1 Events of socket healing following tooth extraction

A human study by Amler (1969) has been reported to describe detailed and accurate time sequence for tissue healing in human extraction socket wounds. The study explained the episodes of human extraction socket healing in uninterrupted extraction wounds histologically. The author concluded the five different stages in osseous socket healing after tooth extraction as shown in Figure 2.1 (Amler, 1969).

Figure 2.1: Summary of the duration of extraction socket healing in humans

2.2 Factors affecting post-extraction healing

Healing following tooth extractions depends on various factors. Generally, they can be characterized into local and systemic factors.

2.2.1 Local factors

The following are some local factors that prolong the process of healing after tooth extraction for example effects of multiple extractions or size of the extraction sockets, traumatic extractions or extractions achieved by surgical process, impacts of tissue biotype on healing, variation between sites in the mouth or dental arch differences, oxygenation and pre and post-extraction infection at the site of wound area.

2.2.1.1 Effects of multiple extractions

Wide socket area which is commonly observed in multiple and traumatic extractions which cause large defects that ultimately affects the healing process and require more time to heal (Amler, 1969). The most common post-extraction complication in multiple extractions are pain, trismus and infection (dry socket) (Capuzzi et al., 1994). There is also pronounced apicoronal changes observed at multiple extraction sites as compared to single extraction site (Johnson, 1969).

2.2.1.2 Traumatic extractions

There is a marked alteration of tissue contours and architecture of alveolar ridge following tooth extraction. It has already been studied that the coronal portion of the buccal bone wall is comprised entirely of bundle bone. After extraction, this bundle bone resorbed gradually because of the osteoclastic activity that ultimately results in significant reduction of horizontal and vertical buccal crest (Araújo & Lindhe, 2005). Surgical technique with flap elevation approach for extraction of tooth and clearing the buccal bone may cause additional harmful reactions on healing and resorption process (Hauser. et al., 2013; Wilderman, 1963; Wood et al., 1972). Furthermore, flap elevation may

increase the chances of gingival recession and hard tissue alterations (Fickl. et al., 2008a). Tarakji et al. (2015) reported that vigorous and traumatic extraction techniques alter the extraction socket and release direct tissue activators that convert plasminogen to plasmin, dissolves the fibrin clot and resulting the most common post-operative complications of pain and foul smell termed as alveolar osteitis (dry socket).

2.2.1.3 Resorption in relation to dental arches

The mandibular arch has less blood supply and vascular blood flow as compared to maxillary arch. This leads to a more resorption of the mandibular arch after tooth extraction compared to maxilla (Atwood & Coy, 1971; Schropp et al., 2003). A study by Pietrokovski and Massler (1967) reported that both the maxillary and mandibular arches have thin and frail buccal plates and cause more resorption compared to palatal and lingual plates. Therefore, due to the thin buccal plates, these arches usually experience distortion during tooth removal and may result in pronounced resorption.

2.2.1.4 Impact of tissue biotype on healing

A study by Ochsenbein and Ross (1969) reported that the gingival architecture mimics the underlying bone and defines the gingival outlines as scalloped or flat. Seibert and Lindhe (1989) used the word periodontal biotype and classified the gingiva towards 'thin-scalloped' or 'thick-flat'. A gingival thickness of less than 1.5 mm is considered as thin biotype whereas a gingival thickness more than 2 mm is defined as thick biotype (Abraham et al., 2014). Thin scalloped biotype occurs in thin underlying alveolar bone and accompanied with more gingival recession with great magnitude at the buccal aspect. Cook et al. (2011) described that there is an important association among the gingival biotype and thickness of the buccal plates in such a way that thin biotypes are associated with thin buccal bone. Thick gingival tissue has an improved blood supply which will promote the revascularization of bone grafts, accelerate graft incorporation and healing.

These thick gingival tissues are also capable of achieving and sustaining primary closure during healing (Abraham et al., 2014).

2.2.1.5 Role of oxygenation

Oxygen, carried by blood is an important factor for all cell activities, cell metabolism and healing of wounds. Many metabolically active cells consume high amounts of oxygen. Following extraction, there is vascular disruption. This causes oxygen depletion in the micro-environment of early wounds and finally causes hypoxia (Guo & DiPietro, 2010). In addition, numerous systemic conditions like diabetes and advancing age impairs vascular flow, consequently setting the level for deficient tissue oxygenation. A study by Bishop (2008) reported the importance of oxygen as it upgrades angiogenesis, differentiation of keratinocytes, migration and re-epithelialization, increases fibroblast proliferation and synthesis of collagen and ultimately encourages wound contraction. After injury, temporary hypoxia initiates wound healing but if hypoxia persists, it causes delayed wound healing (Guo & DiPietro, 2010). Hypoxia also actuates wound healing by activating endothelium and platelets, and by encouraging cytokines released from monocytes, platelets and parenchymal cells [e.g. tumor necrosis factor (TNF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factors (VEGF)] (Schreml et al., 2010). In wound healing, these cytokines and growth factors are important supporters for cell proliferation, chemotaxis and migration and angiogenesis.

2.2.1.6 Infection

Bacteria present in dental biofilms comprise of compound communities of aggregated microorganisms placed within a self-produced extracellular polysaccharide matrix (Edwards & Harding, 2004). The mature biofilms develop defensive micro-environments which are resistant to common antibiotics. *Staphylococcus aureus (S. aureus)*, *β-*

hemolytic streptococci and *Pseudomonas aeruginosa (P. aeruginosa)* are common bacteria in infected wounds (Edwards & Harding, 2004). Once a surface is injured, microorganisms obtain entrance to the underlying tissues. Contamination is the process of non-replicating micro-organisms, colonization is the appearance of replicating organisms with tissue damage and local infection is the initiation of organisms with local tissue response. Invasive infection is the existence of organisms with successive host injury (Guo & DiPietro, 2010).

Chronic inflammation with damage of tooth supporting tissues is primarily caused by micro-organisms derived from infected root canals. Periapical lesions and apical periodontitis are critically poly-microbial infections produced by opportunistic and anaerobic bacteria. The presence of periapical and periodontal lesion may also affect the healing sequence of wounds by enhancing the inflammatory process. In a human study by Ferrus et al. (2010), immediate implants placement in periodontitis sites exhibited a mean vertical defect fill of 60% when compared to 83% defect fill in non-periodontitis sites at 16 weeks after second stage surgery.

Dental treatments are considered high risk for medically compromised patients and it is recommended that prophylaxis be given to patients before gingival manipulation or periapical region of teeth and prior to perforate oral mucosa for dental procedures. Moreno-Drada and García-Perdomo (2016) reported in a systematic review that the incidence of localized infections in patients undergoing tooth extraction can be decreased with the use of prophylactic antibiotics. However, it is controversial whether to use preoperative prophylactic antibiotics. This decision would be based on whether the patient has any medical risk factors which could influence the patient's defense mechanism such as; uncontrolled diabetes, immunosuppressive diseases, kidney diseases

and patients' taking chemotherapeutic agents or immunosuppressive drugs (Moreno-Drada & García-Perdomo, 2016).

2.2.2 Systemic factors

The following are some systemic factors that may affect the process of healing after tooth extraction such as; diabetes mellitus, obesity, alcohol consumption, smoking, influence of nutrition on healing, role of medications, effects of aging on healing, sex hormones, conflicts caused by stress.

2.2.2.1 Diabetes mellitus in healing

Diabetes mellitus is a disorder which influences millions of people worldwide. It is characterized as a metabolic disease in which impairment in insulin secretion causes hyperglycemia. Type 1 diabetes mellitus is a complete deficiency of insulin-secreting beta cells of pancreas while type 2 diabetes mellitus is characterized by impaired insulin secretion. Retzepi and Donos (2010) reported in a review study that both types of diabetes mellitus impair osseous wound healing and cause bone loss in osseointegration of implants. In patients with diabetes mellitus, during the initial osseous healing period, reduced cellularity and diminished osteoid matrix production were observed (Weiss & Reddi, 1980). The impaired healing was occurred due to hypoxia, impaired neovascularization and angiogenesis, fibroblasts dysfunction and epidermal cells growth and increased levels of metalloproteases.

Diabetes mellitus is also associated with prolonged healing time because of the delayed osteoblasts differentiation and genesis of cell proliferation. Younis et al. in 2013 conducted an animal study to assess the healing pattern of tooth sockets in diabetic rabbits. They observed the expressions of transforming growth factor (TGF) beta-3, insulin like growth factor (IGF) -1R, vascular endothelial growth factors (VEGFs) and bone morphogenetic protein-4 in sockets immunohistochemically. The untreated diabetic

group showed an increase in IGF-1R and prolonged healing time while the group treated with insulin represented high levels of TGF beta-3 and less IGF-1R and accelerated and enhanced socket healing (Younis et al., 2013).

2.2.2.2 Smoking

The negative impacts of smoking on healing have been observed for a long-time (Guo & DiPietro, 2010). Tobacco contains nicotine which influences oral wound healing by decreasing capillary blood flow. Substances used in tobacco particularly cotinine, carbon monoxide and nicotine are cytotoxic to wound healing. Nicotine stimulates sympathetic nervous activity, releases adrenaline which causes decreased tissue perfusion and peripheral vasoconstriction (Guo & DiPietro, 2010). Kasat and Ladda (2012) had reported that nicotine decreases fibrinolytic activity and increases platelet adhesiveness, which ultimately increases blood viscosity. In addition, nicotine enhances platelet adhesiveness, increasing micro vascular occlusion and tissue ischemia and also induces catecholamine release which results in vasoconstriction and reduced tissue perfusion (Al-Belasy, 2004). Patients who smokes post-operatively, showed a hindrance in healing of wound and increase in complications like infection, anastomotic leakage, wound rupture, flap necrosis and decreased tensile strength of wounds (Guo & DiPietro, 2010).

2.2.2.3 Alcohol consumption

Acute alcohol exposure causes disturbances in wound healing patterns, increases the post-injury infections by diminishing recruitment of neutrophil and phagocytic function (Greiffenstein & Molina, 2008). A significant delay was observed in the formation of reparative bone in alcoholic rats' leading to prolonged alveolar wound healing (Bombonato-Prado et al., 2004).

2.2.2.4 Influence of nutrition in healing

Improper diet or malnutrition has a profound impact on wound healing. The amount of carbohydrate, proteins, fat and vitamins have a major role on the healing process (Arnold & Barbul, 2006). Patients with unhealed or chronic wounds should to have a history of nutritional deficiencies (Guo & DiPietro, 2010).

Carbohydrates have been well known as the main origin of energy for all functions of cells. A catabolic state induced by injury or wounds will increase the demands of energy. The metabolism of carbohydrates richly produce glucose which later on converts into cellular adenosine triphosphate (ATP) followed by energy production for angiogenesis and newly formed tissue deposition (Shepherd, 2003).

Protein is an important factor affecting healing of wounds. Its deficiency can impair fibroblast proliferation, capillary formation, collagen and proteoglycan synthesis and finally compromise wound remodeling. Moreover, protein malnutrition also affects the immunity with increased susceptibility to infections. Arginine is classified as a semi-essential amino acid and plays a major role in wound healing, immune system, hormone secretion, vascular and endothelial functions (Guo & DiPietro, 2010). Glutamine, another class of proteins stimulates inflammatory immune response which is required in early wound healing (Arnold & Barbul, 2006).

Fatty acids and vitamins provide nutritional support and building blocks for tissue repair and wound healing. A class of fatty acid, omega-3 has the ability to enhance systematic immune function and decrease infectious complications (Arnold & Barbul, 2006). Vitamins C and E are considered to have potent anti-inflammatory and anti-oxidant properties. Vitamin C plays various roles in healing of wounds. Vitamin C deficiency causes many problems such as compromised immune system, diminished collagen synthesis, decreased fibroblast proliferation thus reducing angiogenesis (Guo &

DiPietro, 2010). Vitamin E possesses anti-inflammatory effects and reduces excess scar formation in chronic wounds.

2.2.2.5 Role of medications in healing

Some medications have an important influence on healing like non-steroidal anti-inflammatory drugs (NSAIDs), antihypertensive drugs, chemotherapeutic drugs and glucocorticoid steroids. NSAIDs and glucocorticoids are used as an anti-inflammatory drug. They are considered to be among the most commonly used drugs in the treatment of acute or chronic pain. The mechanism of action of NSAIDs is to reduce the prostaglandin synthesis by inhibiting the cyclooxygenase (COX) activity. Prostaglandins in association with cytokines and other growth factors play a key role for dynamic and complex processes of bone healing. Similarly, NSAIDs have been shown to decrease the healing process in animal and human studies (Gajraj, 2003; Radi & Khan, 2005). They are associated with reduced bone area, bone density and decreased bone-implant contact causing delayed peri-implant bone healing (Fu et al., 2012).

Usage of oral contraceptives was reported to be associated with a substantial increase in the incidence of dry socket. Increased level of estrogen by oral contraceptive causes local increase of fibrinolytic activity which leads to large amount of plasmin production that digests the fibrin clot inside an extraction socket and results in infection and delayed healing (Catellani et al., 1980).

Chemotherapeutic drugs also impair normal healing process by inhibiting cellular activity, decreasing migration of cell to the wound site and matrix formation, diminished fibroblasts proliferation and wounds contraction (Franz et al., 2007).

Bisphosphonates are generally used in the management of Paget's disease, osteoporosis, multiple myeloma and some metastatic tumors. The bone in the oral cavity

differs from other bones of the body. Jaw bone developed from intramembranous ossification consists of fatty marrow which is highly exposed to microorganisms. In addition, oral cavity bones have vulnerability to osteonecrosis of the jaw (ONJ) when treated with bisphosphonates (Allen, 2011). The effects of bisphosphonates are transient in relation to production of bone in the healing socket. Studies showed that when longer durations (14-70 days) of healing were observed, no difference was found between controls and bisphosphonate treated group and similar blood vessel number, area and similar bone volume was noted (Allen, 2011). Anti-hypertensive drugs also have great impact on soft tissue changes. Most commonly used are calcium channel blockers which have been shown to cause gingival enlargements in some cases (Sharma & Sharma, 2012).

2.2.2.6 Sex hormones

Sex hormones have a pronounced effect on healing. A study reported that female estrogens, male androgens and their steroid progenitor dehydroepiandrosterone (DHEA) seem to possess important roles on wound healing in both men and women (Gilliver et al., 2007). Female sex hormones cause early healing as compared to male hormones in acute injuries (Guo & DiPietro, 2010). Similarly, estrogen regulates numerous genes that are associated with regeneration, epidermal function, matrix production, inflammation and protease inhibition (Guo & DiPietro, 2010).

2.2.2.7 Stress

Stress possesses great influence on social behavior and human health. It is related to poorer wound healing. The mechanism by which stress affects wound healing is through the hypothalamic-pituitary gland which modulates the release of pituitary and adrenal hormones. These hormones contain prolactin, cortisol, adrenocorticotrophic hormones (ACTH), epinephrine and nor-epinephrine. At the wound site, stress reduces the level of

pro-inflammatory cytokines IL-1β, IL-6 and TNF-α by up-regulating glucocorticoids (Guo & DiPietro, 2010). Stress also decreases IL-1α and IL-8, which serves as chemo-attractants for early inflammatory phase in the process of wound healing (Godbout & Glaser, 2006). In addition, glucocorticoids control immune cells by regulating gene transcription, arresting proliferation and differentiation and decreasing cell adhesion molecules expression. The glucocorticoid cortisol functions as an anti-inflammatory agent and modulates type 1 helper cells (Th1) mediated immune response that is important for the initial healing phases. Moreover, the level of glucocorticoid cortisol increases during stress which alters immune response consequently leading to the impaired wound healing (Guo & DiPietro, 2010). Therefore, psychological stress disturbs cell mediated immunity resulting in remarkable delay in healing (Godbout & Glaser, 2006).

2.3 Dimensional changes of alveolar ridge after extraction

The initial attempts to study the phenomenon of alveolar ridge resorption started in the early 1960s (Amler et al., 1960). A classic preclinical study by Araújo and Lindhe (2005) reported an enormous resorption at the buccal plate of a canine model within 2 months after extraction characterized by pronounced osteoclastic activity. They observed marked resorption at the crestal surface of the buccal wall when related to the lingual wall. The study also documented loss in height of both bony walls of alveolar ridge, though the buccal wall crest was constantly more apical to the lingual wall crest.

The magnitude of the dimensional changes of alveolar ridge following tooth extraction was investigated by Schropp et al. (2003) using study casts. The authors observed that the alveolar ridge dimensions altered over the 12 months study period. The alveolar ridge width had decreased by around 50% with the loss of alveolar ridge height being less considerable. Although, the changes came over the 12 months study period, around 2/3rds

of the ridge dimensional changes appeared in the initial 3 months after tooth extraction. Systematic reviews reported that alveolar ridge dimensional changes following extraction in humans were roughly 3.5mm of ridge width reduction and 1.5mm of ridge height reduction after tooth extraction (Van der Weijden et al., 2009; Vignoletti et al., 2012a).

One of the variables that may also effect the healing of the extraction sockets is the surgical method utilized. The choice of extractions between a flapless and a flapped approach procedure may disturb the ridge dimensions. After a full-thickness flap reflection, 0.6mm loss of crestal height was reported in humans (Wood et al., 1972). A study in a canine model found 0.5mm to 0.7mm of additional volumetric shrinkage when using flap elevation compared to flapless extractions at 4 months of healing (Fickl. et al., 2008b). Interestingly, a study using canine teeth had observed no changes in volume in a flapless or flapped method after 6 months post-extraction (Araujo & Lindhe., 2009).

To avoid or restrict the alveolar ridge modification, numerous preservation procedures have been narrated, including socket augmentation with autografts, allografts, xenografts or alloplastic materials, used in combination with resorbable and non-resorbable membranes (Anwandter et al., 2016).

2.4 Concept of bone regeneration

Extraction socket augmentation helps to reduce ridge atrophy, reduce bone resorption and maintain soft and hard tissues that provides primary stability to future prosthesis and implants and also minimize the necessity of additional bone grafting procedures. Studies have also proved that at the time of extraction, the augmented extraction sockets have less resorption of bone and maintain hard and soft tissues in comparison to non-augmented sockets (Barone et al., 2012; Fickl. et al., 2008a; Hauser. et al., 2013). Basically the concept of socket augmentation or alveolar ridge preservation has been introduced since 1980's (Casey & Lauciello, 1980; Von Wowern & Winther, 1981).

2.5 Socket grafting and its types

Socket grafting is a procedure attempted to minimizes the resorption of bone after tooth extraction. Socket grafting provides a predictable, easy way to save the buccolingual and mesiodistal dimensions of extraction sockets and alveolar ridge for future dental restorations (Jackson & Morcos, 2007). It also permits the regeneration of bone where the alveolar ridge is compromised and resorbed. Evidence directs that effective grafting relies on the presence of cells in the graft, mainly of the subset of stem cells and progenitors that have the ability to create new tissues (Muschler & Midura, 2002). Therefore, selection of a source which can efficiently enhance and differentiate into bone tissues is the most important and first step for bone regeneration. Grafting materials are used for various clinical applications due to their osteogenic, osteoinductive and osteoconductive properties (Lang & Lindhe, 2015). McAllister and Haghighat (2007) in a review study have explained these terms as; osteogenesis has been narrated as the direct transfer of vital cells to the area that will regenerate new bone. Osteoinduction represents the principle of converting pluripotential, mesenchymal-derived cells along an osteoblast pathway with the subsequent formation of bone. Osteoconduction embraces the concept of giving the space and a substratum for the cellular and biochemical events which continue to bone formation (McAllister & Haghighat, 2007). There are four types of graft materials namely, autografts, allografts, xenografts, and alloplasts. All these materials are briefly discussed in subsequent sections.

2.5.1 Autogenous graft

Autograft is a graft harvested and transferred from extraoral or intraoral sites of the same individual (Lang & Lindhe, 2015). Autografts are the most predictable organic graft which possesses highly osteogenic properties for osseous tissue regeneration. The common sites for harvesting autografts are the mandible (mandibular ramus, chin and mandibular corpus), the maxilla (crista zygomatico-alveolaris, spina nasalis and tuber),

the calvaria, the tibia and the iliac crest (pelvic rim). The morbidity ratio is less with intraoral donor sites in comparison to extraoral sites (Misch, 1997).

Autografts can be of cortical or cancellous nature or a composite of both. Due to the soft and porous structure of cancellous grafts, it has the ability to revascularize shortly, after the fifth day of transplantation when compared to cortical grafts. Cortical grafts show great initial strength that reduces over time. After 6 months of implantation, cortical grafts have been found to be 50%-60% weaker in strength in comparison to normal bone (Wilk, 2004). Conversely, cancellous autografts continue to gain strength over a period of time to achieve maximum dimensional stability (Sheikh et al., 2013).

Autografts are currently considered as "gold standard" for bone regeneration due to its osteoconduction, osteoinduction and osteogenesis-inducing properties (Fernandez et al., 2015). Nevertheless, autografts infrequently have significant disadvantages such as increased postoperative pain and morbidity, requirement for an additional surgery, limited graft volume and absence of appropriate bone mass on the donor site. Interestingly, a study reported patients' preferences for bone grafts utilized in dentistry (Fernandez et al., 2015). The lowest refusal rates for grafts were the autografts (3 %) followed by alloplasts (2 %) while the highest rates of refusal from patients were for allografts and xenografts.

2.5.2 Allografts

Allografts are bone grafts harvested from cadaver donors and processed by either freezing alone or demineralization and freezing (Lang & Lindhe, 2015). These grafts are then sterilized and supplied by specially licensed tissue banks as bone particles or large blocks. Bone allografts are harvested from genetically different members of same species mostly human cadavers. Allografts can be of cortical or cancellous in nature or both in combination. They are used in sinus augmentation, alveolar ridge preservation and socket augmentation after tooth extraction (Clark et al., 2018; Kolerman et al., 2017). The

resorption rate of allografts is very slow in socket augmentation applications. The demineralized forms of allografts are resorbed more rapidly than calcium and phosphorus salts (freeze dried bone allograft) containing allografts (Beck & Mealey, 2010).

2.5.2.1 Freeze-Dried Bone Allografts (FDBAs)

Freeze-dried bone allografts (FDBAs) are obtained from cortical bone within 12 hours after death of the donor, defatted, cut into small pieces and after that cleansed in alcohol and then deep frozen. It acts as a scaffold and is considered as an osteoconductive material, space maintaining for new bone regeneration and can be applied in combination with autografts to increase osteogenic potentials of grafting materials. In addition, it is also available in cancellous forms. Cortical FDBAs enhance volume of bone matrix that increases the resorption time of graft and contains less antigenicity. FDBAs with its property of osteoconduction have been proved to be a successful grafting material in periodontal osseous defects assessed both in animals and humans (Mellonig, 1991). In socket augmentation, FDBAs were used to minimize the resorption of ridge width after 5 months healing period for placement of implants (Wallace & Gellin, 2010). A recent study by Clark et al. (2018) conducted a study on socket augmentation using advanced PRF and FDBA in 40 patients and histologically compared the effectiveness after 15 weeks of healing. Authors reported increased bone mineral density (551 ± 58 mg/cm^3) in the FDBA grafted sockets compared to non-grafted sockets (487 ± 64 mg/cm^3).

2.5.2.2 Demineralized Freeze-Dried Bone Allografts (DFDBAs)

Freeze dried bone allografts can be demineralized in cold temperature. It is ground into small particles, sieved and is then diluted in hydrochloric acid. Hydrochloric acid exposes its bone matrix components. It is then finally freeze dried which known as demineralized freeze-dried bone allografts (DFDBAs). The bone matrix of DFDBAs is accompanied with collagen fibrils which has osteoinductive potential and has been termed as bone

morphogenetic proteins (BMPs). These bone grafts have shown osteoconductive as well as osteoinductive properties due to the release of BMPs during the demineralization process (Lang & Lindhe, 2015).

DFDBAs are well known due to its highly osteogenic potential. It has a rapid tendency to resorb followed by new bone regeneration. A study by Bansal and Bharti (2013) compared the effectiveness of DFDBAs alone or in combination with platelet-rich fibrin (PRF) in periodontal intra-bony defects. In this study of 10 patients after 6 months of surgery, it was concluded that DFDBAs in combination with PRF demonstrated more reduction of probing depth and gain of clinical attachment level in comparison to using DFDBAs alone in the treatment of intrabony defects. In socket augmentation procedures, DFDBAs have shown remarkable effects. Wang and Tsao (2008) performed a pilot study on socket augmentation. Their study comprised of 5 patients in which mineralized allografts were followed by placement of bioabsorbable collagen membrane over the sockets as a wound dressing. After 5-6 months of healing, low level of remaining graft particles was observed histologically and showed newly formed bone. In a study by Wood and Mealey (2012) on socket augmentation using FDBA and DFDBA, they histologically compared the effectiveness after 19 weeks of extraction. Authors reported the greater percentage of vital bone in the sockets grafted with DFDBAs (38.42%), compared to the sockets grafted with FDBA (24.63%). Moreover, the study showed less remnants of graft particles for DFDBAs (8.88%) compared to FDBAs (25.42%). Furthermore, the sockets grafted with DFDBAs had significantly shown greater new bone formation. Another study using combination of PRF-DFDBAs as an interventional group versus DFDBAs alone as a control group in socket augmentation procedure reported less resorption in ridge width in the PRF-DFDBAs group. However, no statistically significant difference was found in resorption of ridge height between the two groups (Thakkar et al., 2016).

2.5.3 Xenografts

Xenografts are graft biomaterials of animal origin (Lang & Lindhe, 2015). The bone source for xenografts, which is commonly used in clinical practice are from various source such as, bovine (cow), porcine (pig) or equine (horse) origin. In 1889, xenografts were first reported as a bone grafting material in aseptic bone cavities (Senn, 1889). These graft materials were deproteinized in order to completely remove the organic component and thus avoid any immunogenic reaction. This low heat and chemical process preserves the original architecture and the inorganic mineral composition (Sheikh et al., 2013). Xenografts possess osteoconductive properties and are biocompatible to human recipients. The trabecular architecture with interconnecting pores in xenografts allows for optimal in-growth of new vascularity and osteoconduction (Lang & Lindhe, 2015). It can act as a lattice or framework for cell growth, allowing osteoblast from the wound margin to infiltrate the defect and to migrate across the graft. This brings a population of osteoblasts into the graft site.

2.5.3.1 Bovine derived xenografts

Clinically applicable xenografts are mostly derived from bovine (cow) origin. Commercially available xenografts, such as BioOss® (Geistlich Pharma AB, Wolhusen, Switzerland) is the most widely used graft material (Barone et al., 2013; Tomasi et al., 2018). It is considered to be an osteoconductive, porous mineral of bone matrix which is derived from cortical or cancellous bone. In BioOss®, porosity and trabecular anatomy is maintained but the organic components are removed for successful results. They also have some osteogenic potential as their physical features allow stabilization of clot and revascularization which may enhance and allow migration of osteoblasts (McAllister & Haghighat, 2007).

Cardaropoli et al. (2012) conducted a clinical study to augment extraction sockets by using bovine bone mineral (BioOss®) followed by collagen membrane. Authors reported significantly less resorption of horizontal ridge width in BioOss® group (1.04 ± 1.08 mm) compared to non-grafted sites (4.48 ± 0.65 mm). Additionally, resorption in ridge height was 0.46 ± 0.46 mm and 1.54 ± 0.33 mm in BioOss® group and non-grafted sites respectively at 4 months of healing. Moreover, grafted sockets have shown different levels of bone maturation and bone formation without any inflammatory response ultimately minimized the amount of vertical and horizontal resorption of alveolar bone. Barone et al. (2013) conducted a study on the augmentation of 62 human extraction sockets by using two commercially available bovine bone xenografts, Endobon® (test) and BioOss® (control). At 6 months of healing, the two treatment groups were similar in histologic outcomes, supporting the efficacy of bovine bone xenograft for socket augmentation when subsequent implant placement is required. A study by Xuan et al. (2014) compared the constructive effects in sinus bone grafting between platelet-rich fibrin mixed with BioOss® and commercial fibrin (Tisseel®) mixed with BioOss® on animal canine sinus models. The results of this study concluded that PRF-mixed BioOss®, grafted particles were mostly surrounded by a layer of newly formed bone and connective tissues.

A recent study was conducted by Serrano Mendez et al. (2017) to compare the healing by applying allografts and xenografts for extraction socket augmentation. Ten patients received demineralized freeze-dried cortical bone allograft and ten patients received deproteinized cancellous bovine bone xenograft. On histological analysis after 6 months of extraction, new bone formation was higher (35.3 ± 16.8%) at the xenograft sites when compared with allograft augmented sites (25.5 ± 10.1%).

MinerOss® is another commercially available biocompatible porous bovine derived bone mineral for use in periodontal, oral and maxillofacial surgery. MinerOss® is a natural, porous bone mineral matrix, produced by removal of all organic components from bovine bone. It is available in cancellous and cortical forms. Potres et al. (2016), recently conducted an animal study to evaluate the effectiveness of PRF and MinerOss® in vertical bone regeneration and wound healing with immediate implant placement by performing histologic analysis. The authors concluded the enhancement of vertical bone augmentation and healing when a combination of PRF-MinerOss® was used. In another recent study by Guarnieri et al. (2017) reported lower vertical and horizontal bone changes after 3 months, when using MinerOss® and collagen membrane in extraction socket augmentation in 20 patients as compared to untreated sites of 10 patients.

2.5.3.2 Equine derived bone graft material

Equine derived bone graft is considered to be a new grafting material extracted from the long bone of horse. Nevins et al. (2012) evaluated the effects of equine derived bone graft by combining with human platelet derived growth factor for regeneration of intrabony periodontal defects. After 3 months, animals were sacrificed, and clinical and histologic analysis was performed. The results revealed higher amount of newly formed bones (Nevins et al., 2012). Another study by Park et al. (2010) was performed to preserve extraction socket using equine derived bone mineral. After 6 months of healing, clinical and histological analysis was obtained on total 4 patients. Through osteoconductive activities of this grafting material, new bone formation with maintained ridge dimensions were observed in this study.

2.5.3.3 Coral derived materials

There are two different types of coral derived materials used: natural coral material (calcium carbonate) and synthetic coralline hydroxyapatite. Both of these materials are

biocompatible. Coralline calcium carbonate induces bone formation without requiring surface transformation to carbonate. Due to its biocompatibility, safety and osteoconduction potentials, it is used as an alternative biomaterial of bone in clinical applications (Sheikh et al., 2015). Coralline hydroxyapatite has been used as a carrier system for delivery of growth factors to increase osteointegration in peri-implant osseous tissue (Damien & Revell, 2003). Coral derived materials were associated with a pronounced gain in periodontal defect fill, gain in clinical attachment and reduced probing depths during periodontal regeneration applications (Sheikh et al., 2015).

2.5.4 Alloplasts

Alloplasts are synthetic bone substitutes that involve different combinations of calcium phosphates. These substitutes are fabricated under different sintering conditions to yield different physical properties and resorption rates (Lang & Lindhe, 2015). Alloplastic bone grafts have lower morbidity rates affiliated with the augmentation procedure and facilitates bone regeneration techniques (Mayer et al., 2016). They are mainly osteoconductive and are relatively safe and cost effective. The major advantage of alloplasts is their abundant availability relative to the other graft materials. Alloplastic bone grafts are not osteoinductive in itself, but in the presence of periosteum or bone, they carry osteoinductive growth factors and osteogenic cell populations and almost become osteogenic (Guarnieri et al., 2004).

2.5.4.1 Calcium Phosphate

Calcium phosphate compounds have been utilized for bone augmentation due to their high solubility and physiological pH. These compounds support partial osteogenesis as they result in the generation of fibro-vascular tissue and woven bone. Kesmas et al. (2010) assessed effectiveness of calcium phosphate and collagen membrane in socket augmentation procedure. In 8 patients with labial wall defect, calcium phosphate was

placed in the socket followed by resorbable collagen membrane. After 4 months of healing, radiographical and histologic analysis revealed the presence of osteoblast-like cells and new bone formation which minimized the alveolar ridge resorption.

Tricalcium phosphate (TCP) is considered to be a porous form of calcium phosphate biomaterial with good tissue compatibility. TCP is divided into two crystallographic forms, alpha-TCP and beta-TCP. β-TCP is commonly used in clinical practice (Sheikh et al., 2013). Due to the porous nature of tricalcium phosphate, it enhances the stabilization of clot during the healing phase. An animal study assessed the effectiveness of porous β-TCP in bone augmentation procedure (Yang et al., 2015). Histologically, faster bone growth, uniform distribution, maturity and activity of mandibular bone was observed and concluded that β-TCP scaffolds showed beneficial results to promote bone augmentation and volume. Mayer et al. (2016) employed combination of β-TCP and biphasic calcium phosphate (BCS) for their test group compared to non-grafted control in socket augmentation procedure. Authors reported remarkable reduction in horizontal width in the control group whereas β-TCP with BCS group resulted greater stability in horizontal dimensions after 4 months. Das et al. (2016) determined the effectiveness of β-TCP with collagen membrane as a control group while only PRF was used as a test group in socket augmentation procedure. No significant difference was found and both groups showed similar results to preserve extraction sockets.

2.5.4.2 Bioactive glasses

Bioactives are defined as materials that sustain specific surface reactions when achieving contact with tissue fluids after implantation into a body, leading to genesis of hydroxyapatite layer which is responsible for osteoblast attraction and formation of bonds with soft and hard tissues, ultimately resulting in new bone formation. They are composed of sodium oxide (24.5%), silicon dioxide (45%), phosphorus pentoxide (6%) and calcium

oxide (24.5%). Bioactive glass has shown better prognosis in the treatment of periodontal intrabony defects in terms of increased clinical attachment level and decreased probing depth, mobility and gingival index (Grover et al., 2013). Successful results using bioactive glass and autogenous cortical bone was also observed for the treatment of intrabony periodontal defects whereby increased alveolar bone height and reduced periodontal pocket depths after 6 months was reported (Sumer et al., 2013).

2.5.4.3 Calcium Sulfate

Calcium sulfate (CS) has been utilized as a graft material in dentistry and orthopedics for more than 100 years (Toloue et al., 2012). It is largely available with low cost and has the ability to be sterilized. It is considered as a biodegradable and osteoconductive graft material that may enhance osteogenesis. CS has been shown to construct an osteoconductive lattice or framework that activates bone ingrowth within the defect (Strocchi et al., 2002). The addition of CS to graft materials appears to accelerate the rate of vital bone formation. Walsh et al. (2003) deliberated that the degradation of CS creates a localized fall in pH, which causes demineralization of adjacent socket walls thus, releasing growth factors such as platelet derived growth factor (PDGF), transforming growth factor beta (TGF-β), bone morphogenetic proteins (BMP) 2 and 7.

CS can be considered a valuable option in periodontal regeneration. Pandit et al. (2015) reported a study using CS in the treatment of intrabony defects on 45 sites having moderate to advanced periodontitis. At 6 months and 12 months postoperatively, clinical and radiographic evaluation showed the enhanced gain in clinical attachment level (CAL). In the reduction of intrabony defects and in sinus augmentation procedures, CS appeared to be an effective bone graft (Leonardis & Pecora, 2000; Orsini et al., 2001).

The most commonly used form of CS for extraction socket augmentation and bone regeneration is medical grade calcium sulfate hemihydrate (MGCSH). Bagoff et al.

(2013) reported the effectiveness of MGCSH alone and with combination of other allografts such as mineralized irradiated cancellous bone allograft (MICBA) for the regeneration of alveolar bone in the extraction socket and sinus. After 4 months, the grafted sites showed keratinized soft tissue formation and after 6 months, thin cortical plate with densely formed trabecular bone was observed. The study also reported that the MGCSH can improve handling characteristics and graft particle containment of particle-based bone grafts. MGCSH is a cost-effective option, prevents soft tissue ingrowth and can assist in bone regeneration (Bagoff et al., 2013).

Guarnieri et al. (2005) conducted a study on 10 patients to evaluate the influence of MGCSH on histo-pathological pattern of intra-socket regenerated bone. Authors reported that after 3 months, no MGCSH was present and completely resorbed at the grafted site. Moreover, there was no difference between the coronal, medial and apical areas and new bone formation with lamellar arrangements was recognized. In another study by Aimetti et al. (2009), socket augmentation was performed using CS in a test group and no graft in the control group. In addition, after 3 months of healing, histologic analysis was performed and showed greater trabecular bone and increased lamellar bone in the sockets grafted with CS.

Kutkut et al. (2012) have performed an extraction socket augmentation study using combination of platelet-rich plasma (PRP) with CS and they assessed the ridge clinically and histologically following augmentation. These sites showed rapid bone healing and greater vital bone after 3 months of extraction socket augmentation. Cheah et al. (2014) have conducted a comparative study using a combination of PRP with CS versus CS alone for socket augmentation. Furthermore, at 4 months of healing, sockets grafted with PRP-CS showed higher mineralized bone content as compared to CS group.

2.6 Growth regulatory factors

Growth factors are responsible to speed up the regeneration of soft and hard tissues (Yun et al., 2012). Moreover, they are present in all tissues and are recognized to empower cellular growth, migration, proliferation and differentiation. They are mostly stored in extracellular matrix, but they are released on injury and contribute in the process of bone rebuilding. During bone rebuilding process, bone marrow stromal cells, fibroblasts, osteoblasts, endothelial cells and inflammatory cells trigger growth factors (Yun et al., 2012). Several studies have shown that the recombinant growth factors have been proven beneficial for bone regeneration (Bowler & Dym, 2015; Sanz-Sanchez et al., 2015). However, the use of such growth factors suffer from two major limitations such as, high cost, and supra-physiologic doses (Kobayashi et al., 2016). Thus, to overcome these limitations of recombinant growth factors, recent studies have shown the robustness and several significant pros of using the autologous growth factors in bone regeneration (Del Fabbro et al., 2014). From the last decade, two popular autologous growth factors have been reported beneficial for bone regeneration namely, platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) (Cheah et al., 2014; Dohan et al., 2006; Marx et al., 1998; Zhang et al., 2018). Thus, this section discusses the details of PRP and PRF in the light of recent literature and their usage in dentistry.

2.6.1 Platelet-rich plasma

Platelet-rich plasma (PRP) belongs to autologous growth factors. It is extracted from patient blood centrifuged to obtain natural concentrations (Del Corso et al., 2012; Simonpieri et al., 2012). It was announced as “fibrin glue” in 1970 and gained recognition in medicine and dentistry for bone regeneration (Kobayashi et al., 2016). Several periodontists and oral surgeons utilized PRP for bone regeneration and have reported its benefits in variety of dental regenerative procedures (Marx, 2004; Marx et al., 1998; Panda et al., 2016). Moreover, several studies have reported that PRP can also be

combined with numerous other bone grafting materials such as calcium sulfate, beta-tricalcium phosphate to show better results in bone regeneration (Cheah et al., 2014; Kutkut et al., 2012; Ozdemir & Okte, 2012). However, its limitation is that it comprises of anticoagulants that intervenes with the process of natural healing process despite having various growth factors for bone regeneration (Del Corso et al., 2012; Simonpieri et al., 2012). Therefore, to overcome this drawback of PRP, researchers initiated the research investigation on a platelet concentrate created from blood without utilization of coagulants to improve bone regeneration (Choukroun. et al., 2006; Dohan. et al., 2006a, 2006b). The result of such research investigation is platelet-rich fibrin (PRF). It involves the utilization of a fibrin clot that may be used as a membrane comprised of autologous growth factors for bone regeneration (Dohan Ehrenfest et al., 2010). The further detail of PRF is discussed in Section 2.6.2.

2.6.2 Platelet-rich fibrin

Platelet-rich fibrin (PRF) is considered as an autogenous biomaterial and classified as a second-generation platelet derivative of platelet concentrates. In oral and maxillofacial surgery, Joseph Choukroun from France with his associates were the innovators for utilizing PRF protocol to enhance bone healings for further prosthesis or implant placement (Gupta et al., 2011). The PRF protocol is quite simple as; collection of blood sample without anticoagulants in 10ml tubes and then immediately centrifuged at 3000 rpm for 10 minutes (Dohan. et al., 2006a). This results in a formation of three layers with the upper layer being acellular plasma, the middle layer is the fibrin clot and the bottom layer is red blood cells (RBCs) (Dohan Ehrenfest et al., 2010). Later on, the middle fibrin clot, charged with serum and platelets is separated and can be compressed to form PRF membrane and plug (Dohan Ehrenfest et al., 2010).

It also acts like an osteoinductive and osteoconductive graft materials (Dohan Ehrenfest et al., 2010). PRF matrix associated with bone morphogenetic proteins (BMPs) possesses hemostatic, angiotrophic and osseous conductive properties and are able to induce bone (Choukroun et al., 2006). It consists of leukocyte, platelet rich fibrin biomaterials, many growth factors; platelet derived growth factors (PDGF), vascular endothelial growth factor (VEGF), living cells and platelet cytokines. There is no risk of disease transmission. Since the PRF is acquired from autologous blood sample in low quantities therefore only little volume of PRF can be used.

Several studies concluded that PRF is promising in reducing dimensional changes and limiting alveolar bone resorption mainly due to its innate bioactive capacity as it favors tissue regeneration (Anwandter et al., 2016; Castro et al., 2017a, 2017b; Temmerman et al., 2016; Zhang et al., 2018). Bansal and Bharti (2013) employed demineralized freeze-dried bone allograft (DFDBA) in group 1 (10 patients) and combination of PRF and DFDBA in group 2 (10 patients) in the treatment of periodontal intra-bony defects. At 6 months of healing, authors reported reduction in pocket depth of 4 mm and 3.1 mm for group 1 and for group 2 respectively. Moreover, clinical attachment gain was 2.3 mm for group 1 and 3.4 mm for group 2. The study concluded beneficial effects of combination of PRF and DFDBA compared to DFDBA alone. Gassling et al. (2013) carried out comparison of PRF with the generally used collagen membrane Bio-Gide™ as a framework for seeding of human osteoblast cell for bone tissue engineering. The authors reported that the extracellular bone matrix with entrapped cytokines due to Bio-Gide™ and polymerizing nature of PRF have a significant impact in angiogenesis that was found to be beneficial for tissue engineering. A study by Xuan et al. (2014) compared the constructive effects in sinus bone grafting between PRF mixed with BioOss® and commercial fibrin (Tisseel®) mixed with BioOss® on animal canine sinus models. After 6 months of healing, authors evaluated the new bone formation rate was 41.8 ± 5.9% and

31.3 ± 6.4% in the PRF-BioOss® composite sites and Tisseel®-BioOss® composite sites respectively. In addition, the osseointegration rate was 43.5 ± 12.4% in the PRF-BioOss® composite sites and 30.7 ± 7.9% in the Tisseel®-BioOss® composite sites. The study findings suggested the combination of PRF and BioOss® for bone regeneration in maxillary sinus. Recently, Miron et al. (2017) conducted a systematic review utilizing PRF for soft tissue regeneration, augmentation and wound healing. The results of the review highlighted the positive effects of using PRF for various soft tissue defect management in the field of medicine and dentistry.

2.6.2.1 Comparison of PRF with other platelet concentrates

Platelet concentrates are generally classified as Platelet-rich plasma (PRP), Plasma-rich in growth factors (PRGF) (Anitua, 2001) and Platelet-rich fibrin (PRF) classified on the principles of Platelet-rich preparations (Dohan et al., 2006). Platelet-rich preparations enhance and accelerate healing at initial time points to diminish discomfort and possibilities of adverse outcomes, including poor wound closure, infection and delays in the process of bone formation for subsequent procedures (implant) (Davis et al., 2014). Dohan Ehrenfest et al. (2009) reported technological classification of platelet preparations based on fibrin density, content of leukocyte and standardization degree of the procedure as ; pure platelet-rich plasma (P-PRP), such as Vivostat PRF or Anitua's PRGF, cell separator PRP; leukocyte and platelet-rich plasma (L-PRP), such as Curasan, Regen, Plateltex, SmartPReP etc; pure platelet-rich fibrin (P-PRF), such as Fibrinet; and leukocyte and platelet-rich fibrin (L-PRF), such as Choukroun's PRF. Nevertheless, PRF has high density of fibrin clot, supporting cell migration, cytokine release and can serve as a biological healing matrix developing the range of its potential applications prominently (Dohan Ehrenfest et al., 2009).

Platelet-rich fibrin (PRF) is unique with other platelet concentrates as it contains no anti-coagulants. For the preparation of PRP and PRGF, biochemical blood handling is essential while for PRF no handling is required and also clotting achieved in PRF is completely physiologic. In addition, in a study by Dohan Ehrenfest et al. (2009), it was reported that in P-PRP and L-PRP preparations, the network of fibrin is immature and comprises of small diameter fibrillae because of simple polymerization. This immature small diameter fibrin network is supportive for platelet application during surgery, however it dissolves quickly. Conversely, in P-PRF and L-PRF, fibrin network is mature and thick because of multiple fibre assembly which create a resistant matrix. In PRP, release of growth factors are immediate at very time points whereas in PRF, growth factors and Thrombospondin-1 are released slowly, more than 7 days due to its stable growth factor released from PRF (Dohan et al., 2009). Therefore, PRF encourages its environment for a considerable duration during its remodelling.

Growth factors play a predominant role in wound healing. These growth factors are only released by platelet concentrates once clotting has completed. In PRF, platelets and leukocytes growth factors are richly embedded. Moreover, for attaining a clot with enhanced platelets, immediate fibrin polymerization and increased number of growth factors, it was suggested to avoid anticoagulants (Dohan Ehrenfest et al., 2009; Dohan Ehrenfest et al., 2017). The most common growth factors present in fibrin clots are; fibroblast growth factors (FGF), platelet-derived growth factors (PDGF), insulin like growth factors (IGF), transforming growth factors beta-1 (TGFβ-1), bone morphogenetic proteins 2 (BMP-2) and vascular endothelial growth factors (VEGF).

Leukocytes are richly trapped in fibrin matrix and have a key anti-infectious role in surgical platelet concentrates as an immune regulation and for direct action. PRF has shown to have highest leukocytes counts (50%) as compared to PRP (0-50%) and PRGF

(0%). Leukocytes, besides its anti-infectious role, also produces large amounts of growth factors such as, TGFβ-1 and VEGF which stimulate the healing process and also promote angiogenesis (Dohan et al., 2009). When the VEGF or TGFβ-1 level becomes very low, leukocytes produced new molecules to maintain the growth factors. Moreover, leukocytes present in the PRF can cause gradual release of cytokines (Dohan et al., 2009). In most of the protocols for platelet concentrate preparations, platelets are richly activated and there is no pronounced fibrin matrix to assist cell migration and growth factor release. However, in Choukroun's PRF, leukocytes and growth factors are massively activated and released over the 7 days after application on the surgical site (Dohan et al., 2009). With these properties of PRF, it could be a useful healing biomaterial with antihemorrhagic agent on clinical applications.

A centrifuge is a device in which a small amount of blood is inserted and rotated at high speed on a circular path to separate plasma, fibrin, and red blood cells from blood (Dohan Ehrenfest et al., 2017). This device usually operates on a certain centrifugation protocols such as, speed of centrifugation, vibrations of centrifuges, vibration shocks in the acceleration phase and resonance of vibrations (Dohan Ehrenfest et al., 2017). The speed refers to the rotator speed of the centrifuge and it is usually measured in revolutions per minute (RPM). The vibration of centrifuges refers to the repetitive variations on an equilibrium point of centrifuge device. The vibration shock of the centrifuge device refers to a sudden acceleration which creates an impulse on centrifuge device. The vibration resonance is a condition of the centrifuge device in which an object is subjected to repetitive variating force having a frequency close to its own natural frequency.

Aforementioned parameters make a difference in the cell content, weight, volume and fibrin architecture of PRF. For instance, when the speed of centrifugation is low, it produces more vibrations and exceeds the resonance threshold limit of 1. This increased

resonance threshold causes difficulty in separation of blood, as there is damage and destruction of the cell content (Dohan Ehrenfest et al., 2017). Indeed, it was proven by Dohan Ehrenfest et al. (2017) that leukocytes rich platelet-rich fibrin (L-PRF) produced from 9 ml of blood by using Intraspin centrifugation machine possess clots and membranes with heavy weights, dense architectures and large sizes whereas the second best PRF produced was prepared using 1500 rpm speed from 10 ml of blood, which is almost identical to the current study protocol. Moreover, the study proposed that increase in time duration and greater g force for centrifugation usually provides more time for fibrin clot polymerization. An adequate fibrin clot polymerization triggers slow and prolonged delivery of growth factors to the application sites. The centrifugation time was not needed to last longer than 12 minutes (Dohan Ehrenfest et al., 2017).

2.6.2.2 Benefits of PRF

Naik et al. (2013) in a review study, described the advantages of using PRF in wound healing as;

1. Absence of biochemical handling of blood.
2. Use of anticoagulants and bovine thrombin are not required.
3. Easy, less time consuming and cost-effective process of PRF preparation
4. Advantageous healing due to slow polymerization of PRF over a period of 10 days due to its cytokines and growth factors.
5. Immediate centrifugation of blood in PRF preparation further minimizes activation of coagulation cascade.
6. More effective cell proliferation and migration because of the fibrous structure of PRF which retains a greater number of growth factors and cytokines in three dimensional fibrin scaffold.
7. PRF has more efficacy on immune system because of its leukocyte content.

8. PRF assists in hemostasis.

2.7 Radiographic assessment

Radiographic assessment of alveolar bone is important for appropriate presurgical treatment planning. Specific indications, radiation dose and magnification rate are to be considered when determining the type of radiographic modality (Amarnath et al., 2015). Various radiographic techniques can be used to evaluate the alveolar ridge dimensional changes after extraction or prior to implant placements. Previously, alveolar ridge dimensional changes were measured clinically by taking photographs, study models and tracings drawn according to the study casts (Pietrokovski & Massler, 1967). Radio-opaque pastes were applied on the palate to measure the radiographic dimensional changes 2 weeks after extraction to avoid errors caused by soft tissue changes (Johnson, 1969). Apart from periapical, panoramic and cephalometric radiographs, cone beam computerized tomography (CBCT) images were also used to ensure accurate measurements (Araújo & Lindhe, 2005; Tyndall & Brooks, 2000).

The imaging modality for radiographic assessment should possesses the following characteristics (Frederiksen, 1995);

1. It should give cross-sectional views of both dental arches for visualizations of alveolar process and dimensional relationship of anatomic structures.
2. Image plane of a technique must be flat for precise measurements.
3. Images should permit the assessment of cortical bone density (bone quality) and trabecular bone.
4. The distortion should be minimized. Image contrast and brightness should be at an optimal level and free from artifacts.
5. If more than one technique is used, it should be financially affordable to patients.

2.8 Radiographic methods

The common radiographic methods that were employed in implant dentistry are discussed in subsequent sections.

2.8.1 Intraoral radiography

Intraoral radiograph is easily available, inexpensive, well tolerated by patients and generates high resolution images. These images possess excellent dimension and contrast resolution with less distortions. The images taking with film-holding devices permit segmental visualizations of anteroposterior bounds, vertical of remaining alveolar ridges and locations of adjacent anatomical structures (Tyndall et al., 2012). Therefore, it is most commonly used as a first line radiograph for implant site evaluation. However, it has a few drawbacks such as, distortions of images and absence of cross-sectional information (Sewerin, 1990).

2.8.2 Panoramic radiography

Panoramic radiographs are considered as a good standard of care for pre-operative analysis and for long-term assessment of implant success (Tyndall & Brooks, 2000). The advantages include low cost, easy availability and many anatomic features can be visualized on one image. The disadvantages of panoramic radiographs are the possibility of positioning errors, absence of cross-sectional information and non-uniform horizontal magnification (Tyndall & Brooks, 2000). Moreover, the images are horizontally distorted in relation to its vertical plane where the lingual structures are not properly positioned in the focal through superiorly projected in comparison to its actual location. Panoramic radiographs produce inherent magnification distortion of 20%-30%. In addition, longitudinal evaluations are not possible using these machines due to the difficulty in reproducing original patient position (Tyndall & Brooks, 2000).

2.8.3 Cone beam computed tomography (CBCT)

The term “cone beam” refers to conical form of beam. The x-ray source and detector move synchronously in a circular path around the vertical axis of the patient’s head. Cone beam machines were first commercially introduced as New Tom DVT 9000 (QR srl, Verona, Italy) in Europe in 1999. This system scans the patient in supine position. Previously larger units were used which was replaced by smaller units with better scanner quality to fit in dental office (Amarnath et al., 2015).

CBCT images are used in implant therapy as well as complex endodontic and orthodontic cases. The 3D high resolution images are very useful in assessing various pathologic and abnormal conditions of the maxillofacial region (Miracle & Mukherji, 2009). CBCT is recommended when panoramic radiographs fail to provide adequate information for the bucco-lingually located structures of the maxilla (Amarnath et al., 2015). The volumetric datasets of CBCT can be transferred in DICOM (Digital Imaging and Communications in Medicine) format (Tyndall et al., 2012). The cross sectional and 3D images creation, simulated implant placement and computer-guided surgeries can be performed using a third-party software. Volumetric datasets give additional information for more refined analysis and broadened treatment planning options for achieving adequate prosthetic results (Tyndall et al., 2012). The additional costs of CBCT compared with panoramic techniques are higher. However, the utilization of CBCT is still limited in comparison to panoramic radiograph.

2.8.4 Materialise’s Interactive Medical Image Control System (MIMICS)

MIMICS (Materialise Interactive Medical Image Control System) (Belgian company Materialize NV) is a 3D image analysis software that can use to reconstruct and remodel DICOM images from different imaging modalities such as; CBCT, magnetic resonance imaging (MRI), micro-CT and medical computed tomography (CT). MIMICS with the

help of “Image segmentation module” calculates the volume and creates 3D models by stacking 2D image data. This software produces images in XZ (coronal) and YZ (sagittal) direction. Region of interest (ROI) can be segmented and converted into a 3D surface model (Al-Maqtari et al., 2014). An accurate 3D surface model can be created in stereolithography (STL) format using ‘adaptive marching cubes’ algorithm.

MIMICS software allows a direct link of the 3D models with computer-aided design (CAD), rapid prototyping (RP) and computer aided engineering (CAE) for surgical simulation and advanced engineering analysis. These analysis are useful for assessment and treatment planning for maxillofacial surgery, neurosurgery, craniofacial surgery and orthopedics research (Mavili et al., 2007).

MIMICS software is reliable in determining volume and densities of newly regenerated bone. The software has successfully assessed the density, volume and dimensions of the grafts (Yavuz et al., 2009) and distribution of stress on the peri-implant bone (Martini et al., 2013). The simulation of sinus floor augmentation including symphysis bone graft was successfully performed using the software (Buyukkurt et al., 2010). In addition, the accuracy of this software in reconstructing 3D models has been described for volumetric evaluation (Li et al., 2013).

MIMICS has been used in many previous studies. For instance, Pang et al. (2014) performed a study of socket augmentation procedure in 30 patients. After 3 and 6 months of socket augmentation, bone volume, ridge width and height were analyzed by using MIMICS software. Recently, Saruhan and Ertas (2018) conducted a study for the alveolar cleft treatment using corticocancellous iliac bone graft and platelet-rich fibrin. The author calculated the alveolar cleft volume by using MIMICS software preoperatively and 6 months postoperatively.

MIMICS has been used as an evolutionary software in the assessment of alveolar ridge dimensional changes. Aimetti et al. (2018) performed extraction sockets augmentation after randomly allocated 30 patients into test (bovine derived bone) and control (natural healing) groups. The volume of bone has been calculated using MIMICS software at baseline and at 12 months post-extraction. Manavella et al. (2018) have also performed sockets augmentation in 11 patients using bovine derived xenografts with collagen membrane. Through MIMICS software, volumetric changes of sockets were analyzed at baseline and at 12 months post-extraction.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

This study design was a randomized clinical trial. Ethical approval was obtained from the Ethics Committee, Faculty of Dentistry, University of Malaya, Kuala Lumpur [DF RD 1621/0076(L)]. An information sheet explaining the nature of the study, available in both English and Bahasa Malaysia was given to each patient. Additionally, the details of the study as well as the procedures involved were explained verbally in either English or Bahasa Malaysia. Written consent was then obtained from all the patients who agreed to participate in this study. All patients were recruited from the outpatient department and oral and maxillofacial surgery department.

3.2 Sample selection

The patients who fulfilled the following criteria were included in the study. Inclusion criteria for the patients were;

1. Maxillary premolar teeth with neighbouring mesial and distal sound/restored teeth.
2. Alveolar bone level (measured from periapical radiographs) more than 50% of the root length.
3. Age ranges from 25-55 years.

Exclusion criteria for these patients were;

1. Bony fenestration of the socket wall confirmed from CBCT scan.
2. Acute signs of infection.
3. Periodontally compromised teeth.
4. Absence of buccal plates.

5. Any systemic disease which will hinder the healing process e.g., diabetes, hypertension, bleeding disorders like thrombocytopenia, thalassemia, asthma, patients on long term steroids, osteoporosis.
6. Smokers.
7. Pregnant and lactating women.
8. History of malignancy or radiotherapy and chemotherapy.

3.2.1 Sample size calculation

The sample size for this study was calculated using *t* tests of means difference between two independent groups, based on a power of 80% and a detectable difference between the two comparative groups of 0.05. Additionally, the mean differences for socket depth were 13.73 mm and 9.50 mm with standard deviations of 3.00 and 3.08 respectively (Das et al., 2016). The sample size was calculated as 20 subjects (10 subjects in each group). All the patients were randomly allocated to either PRF-CS (test) or PRF-xenograft (MinerOss® X syringe, Bio Horizons, Alabama, United States) (PRF-X) (control) group using coin-toss method (Section 5.1 explains the reasons of using this method).

3.3 Pre-extraction preparation

A day before extraction, clinical photographs and impressions of the jaw were performed. An acrylic stent (A) for each patient was prepared using light cure material (Figure 3.1 a). It was designed to include the teeth on the mesial and distal to the tooth scheduled for extraction. Three holes which were situated 3mm below the cervical margin were prepared on the buccal aspect of the tooth, while 1 hole was prepared on the mid palatal aspect, 3mm below the cervical margin. The holes were prepared using 1mm fissure bur and filled with gutta-percha as shown in Figure 3.1 a.

Stent A was then placed over the planned extraction site. A cone beam computerized tomography (CBCT) scan was performed using Kodak 9000C® (Carestream, United

States) software. The selected scanning parameters were 70 kV; 10 mA; voxel size = 76µm; acquisition time = 10.8s. The gutta-percha filled holes in the stent (Figure 3.6), served as fixed reference points to help identify the same CBCT slice for measurement at baseline and 5 months post-extraction. The parameters measured from the CBCT scan at baseline were mesial bone height (M_BH), distal bone height (D_BH), socket bone height (SH), buccal bone height (B_BH), palatal bone height (P_BH), horizontal bone width (W) and volume of bone.

To measure the soft tissue profile, another occlusal acrylic stent (stent B) (Figure 3.1 b) was fabricated using light cure acrylic. It was designed to include the teeth on mesial and distal aspects of the tooth scheduled for extraction. The material covered only half of the crown height. Three grooves were prepared on mesial, mid and distal on the buccal aspect of the tooth scheduled for extraction. One groove was prepared on the mid-palatal aspect. These grooves served as fixed points for the periodontal probe (546/1 Williams periodontal probe, QS® dental supply, Malaysia) when measurements were completed at baseline and 5 months post-extraction as shown in Figure 3.1 (b). The parameters measured for clinical measurements were mesial soft tissue height (M_SH), distal soft tissue height (D_SH), buccal soft tissue height (B_SH), and palatal soft tissue height (P_SH).

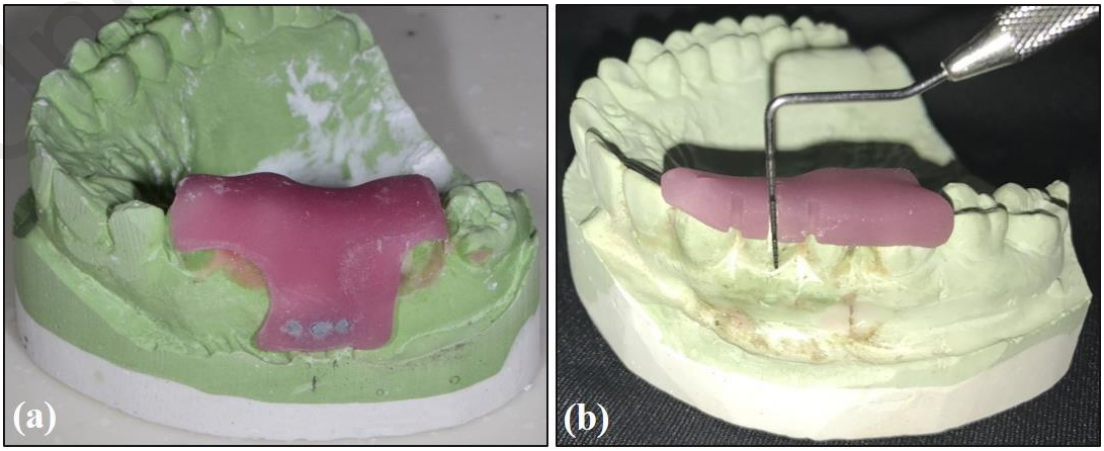

Figure 3.1: Pre-extraction preparation. An acrylic template for CBCT scan (stent A) (a) and an occlusal acrylic stent with demarcations (stent B) (b).

3.4 Extraction procedures

The same operator treated all patients under the supervision of his supervisors. The preparation of PRF, extraction socket augmentation and follow up visits were performed in the postgraduate periodontology clinic, University of Malaya.

3.4.1 Procurement of PRF

The protocol for platelet-rich fibrin (PRF) preparation was established by Choukroun in 2001 in France. After the provision of patients' written informed consent, 20ml of venous blood was collected using winged infusion set from cubital vein in two sterile vacutainer tubes (10ml in each tube), prior to tooth extraction. The test tubes were devoid of any anticoagulants. The venous blood was quickly spun in the centrifugation machine at 1300 revolutions per minute (rpm) for 8 minutes (Figure 3.2) and kept separate for 3-5 minutes. After the completion of centrifugation, the tube consisted of three layers; red blood cells (RBCs) at the bottom layer, the middle layer of fresh fibrin clot and the straw colored acellular plasma at the upper layer (Figure 3.3 a). The fibrin clot was picked up using a tweezer while a pair of sterile scissors was used to separate the RBCs layer (Figure 3.3 b and Figure 3.3 c). Finally, the fibrin clot was placed in the PRF box (PRF processing, Nice, France) and was compressed lightly for 15 seconds under low pressure. The remaining acellular plasma went down at the lower part of the PRF box due to the pressure over it. The fibrin clot from first tube was compressed to form the PRF membrane whereas the fibrin clot from second tube was compressed to form the PRF plug (Figure 3.3 d). Once prepared, the PRF membrane was cut into small pieces and was mixed with either CS or xenograft (Figure 3.3 e). CS powder was prepared prior to the day of extraction. Zero point three nine (0.39) grams of CS powder (Asia Plaster company limited, Bangkok, Thailand) was measured using Sartorius Cubis® Analytical Balances system (Sartorius AG Göttingen, Germany) and placed into autoclave pouches for

sterilization in a vacuum autoclave (Little Sister SES 225B Vacuum Autoclave, Eschmann® West Sussex, United Kingdom).

Figure 3.2 Centrifugation machine for the preparation of PRF

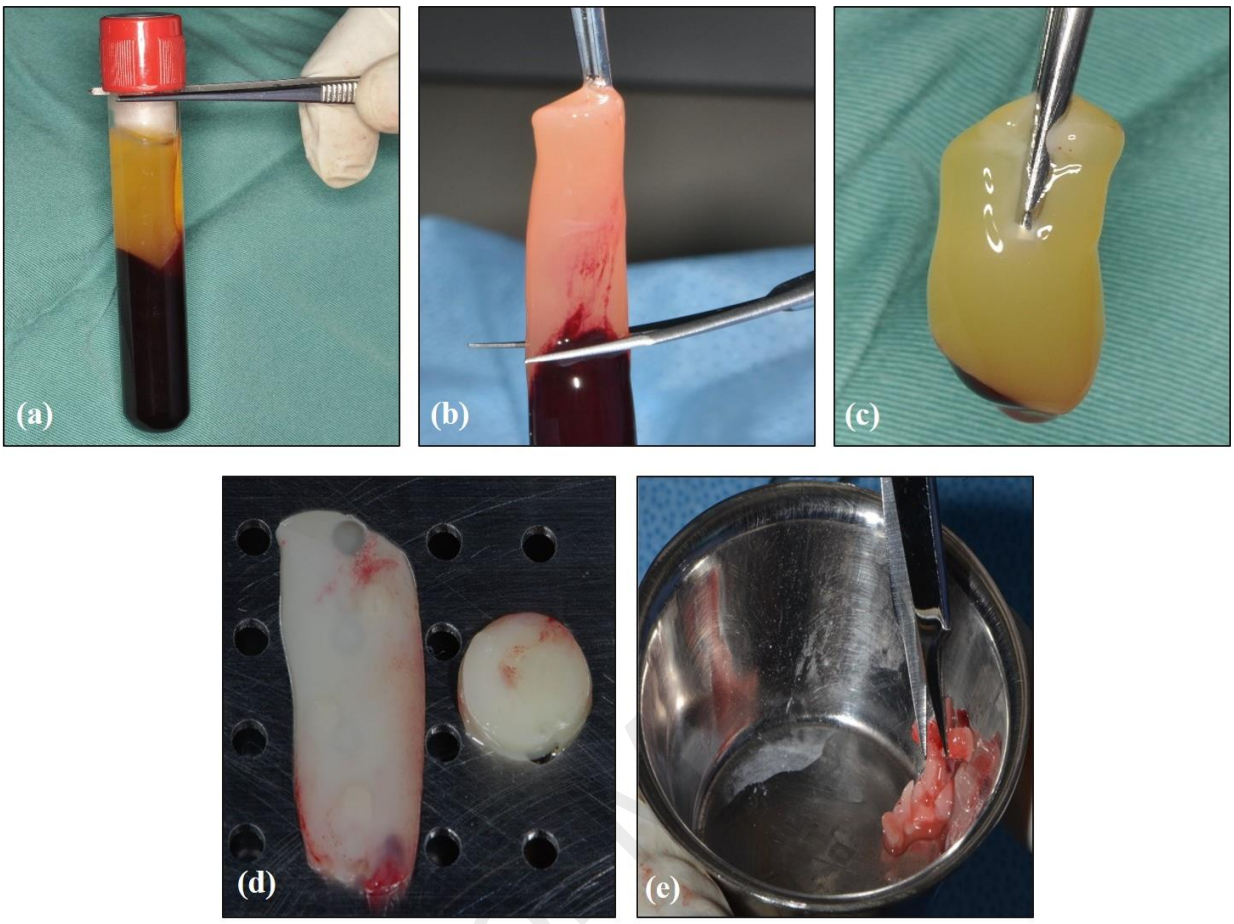

Figure 3.3: Procurement of PRF. After centrifugation three layers of blood (a), separation of fibrin clot (b), fibrin clot (c), prepared PRF membrane (left) and PRF plug (right) (d) and cutting PRF membrane into small pieces to mix with graft (e).

3.4.2 Surgical technique

After administering local anesthesia utilizing 2% lignocaine with 1:100,000 concentration of epinephrine, atraumatic tooth extraction was achieved. Periotome (Hu-Friedy® Manufacturing, Co., Chicago, United States) was used to detach the periodontal fiber appendage followed by using premolar extraction forceps (#35A, 12.035.01Z, B. Braun Aesculap®, United States). Great care was taken to maintain the buccal plate. Next the extraction socket was carefully curetted and was washed with normal saline as shown in Figure 3.4 (a).

In the control group, xenograft (MinerOss®X, BioHorizons®, United States) (size 250-1000µ, 0.5cc) was mixed with PRF membrane pieces and placed inside the socket.

In the test group, calcium sulfate (CS) was mixed with PRF membrane pieces and placed inside the socket. The graft materials were placed up to the level of crest of the extraction socket. After that the socket was covered with the PRF plug. Simple interrupted sutures (black silk 4-0, Ethilon® Mersilk, Cornelia, Georgia) were applied on the mesial, middle and distal areas as shown in Figure 3.4 (b).

An antibiotic, Amoxicillin 500mg (capsule) every 8 hours for 5 days and an analgesic, Paracetamol 1g (tablet) every 8 hours for 3 days were then prescribed. The patients were advised to rinse with 0.12% chlorhexidine mouth rinse twice daily for a week. Patients were recalled after a week for suture removal followed by another recall 5 months post-extraction (Figure 3.4 c).

At the 5 months post-extraction review, CBCT scans were performed with stent A to assess the area for planning of implant placement. Dimensions of the healed socket were recorded as shown in Figure 3.5 (b) and in Figure 3.6 (b). Impressions were taken to prepare stone (study) models and the soft tissue profile change was then measured by using stent B. All the parameters previously mentioned were measured and recorded on CBCT and the cast models.

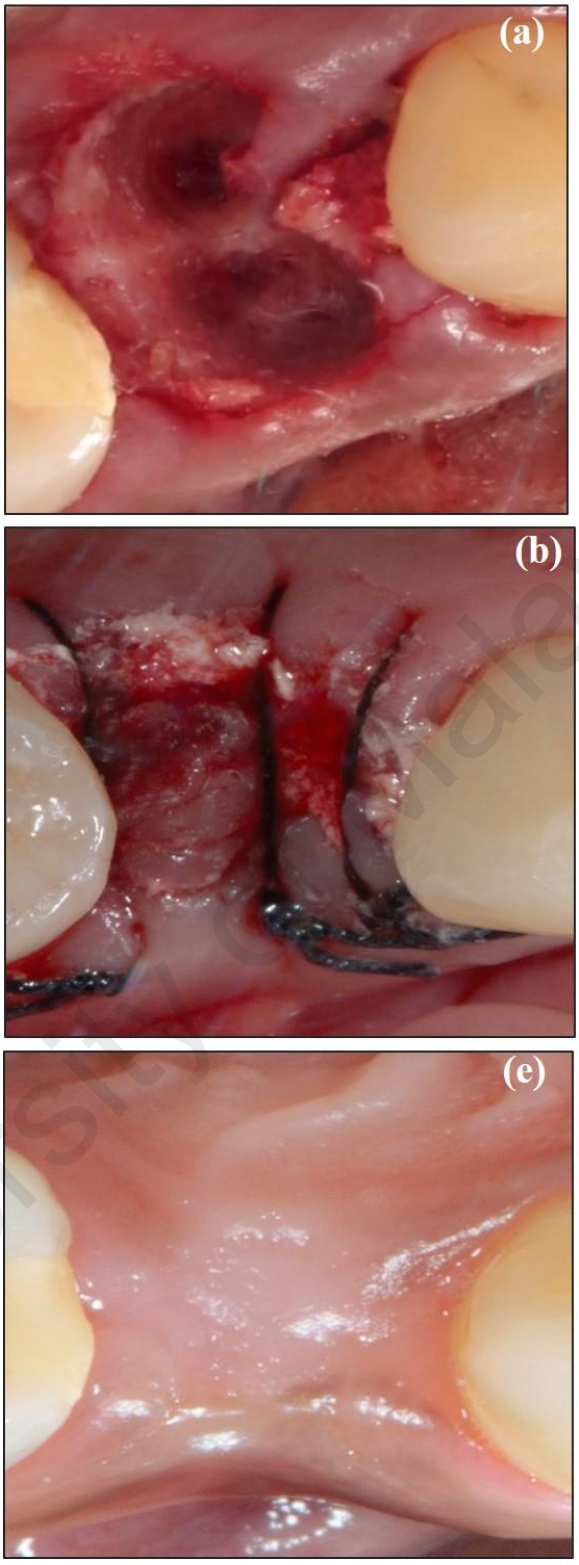

Figure 3.4: The premolar socket after atraumatic extraction (a), the socket with PRF-CS graft materials followed by PRF plug over the socket (b) and the socket area at 5 months post-extraction (c).

3.4.3 Measurements of M_BH, D_BH and SH

Mesial bone height (M_BH), distal bone height (D_BH) and socket height (SH) were analyzed using KODAK 9000C®, Carestream imaging 7.0 software. A panoramic view was created using curved slicing method. An auxiliary line (X1) was drawn in relation to the cemento-enamel junctions of the adjacent teeth and three perpendicular lines (M, D and S) were drawn to measure the M_BH, D_BH and SH as shown in Figure 3.5 (a) and (b).

1. M_BH: distance measured from the auxiliary line to the mesial bone crest.
2. D_BH: distance measured from the auxiliary line to distal bone crest.
3. SH: distance measured from the auxiliary line to the apex of the scheduled extracted tooth.

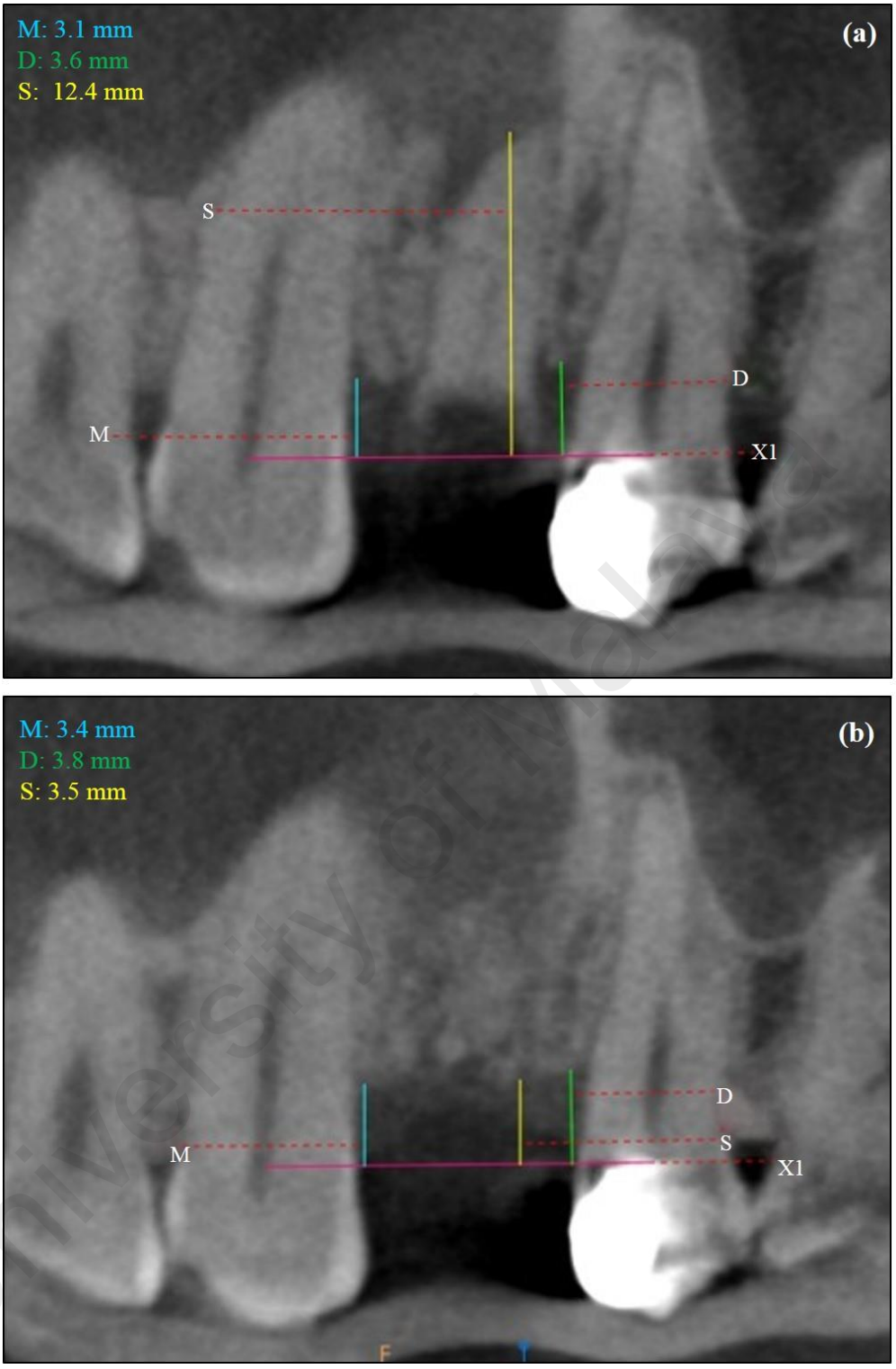

Figure 3.5: Measurements of M_BH, D_BH and SH. All lines were drawn on the panoramic view at baseline (a) and at 5 months post-extraction (b).

- M_BH
- D_BH
- SH

3.4.4 Measurements of B_BH, P_BH and W

Buccal bone height (B_BH), palatal bone height (P_BH) and horizontal bone width (W) measurements were performed by drawing an auxiliary line (X2) from the lower border of hard palate on a coronal view. Two perpendicular lines (B and P) were drawn to measure the B_BH and P_BH. Second auxiliary line (X3) was drawn from the upper edges of the stent to measure the W as shown in Figure 3.6 (a) and (b).

1. B_BH: distance measured from the first auxiliary line to the buccal bone crest.
2. P_BH: distance measured from the first auxiliary line to palatal bone crest.
3. W: distance measured bucco-palatally on the second auxiliary line.

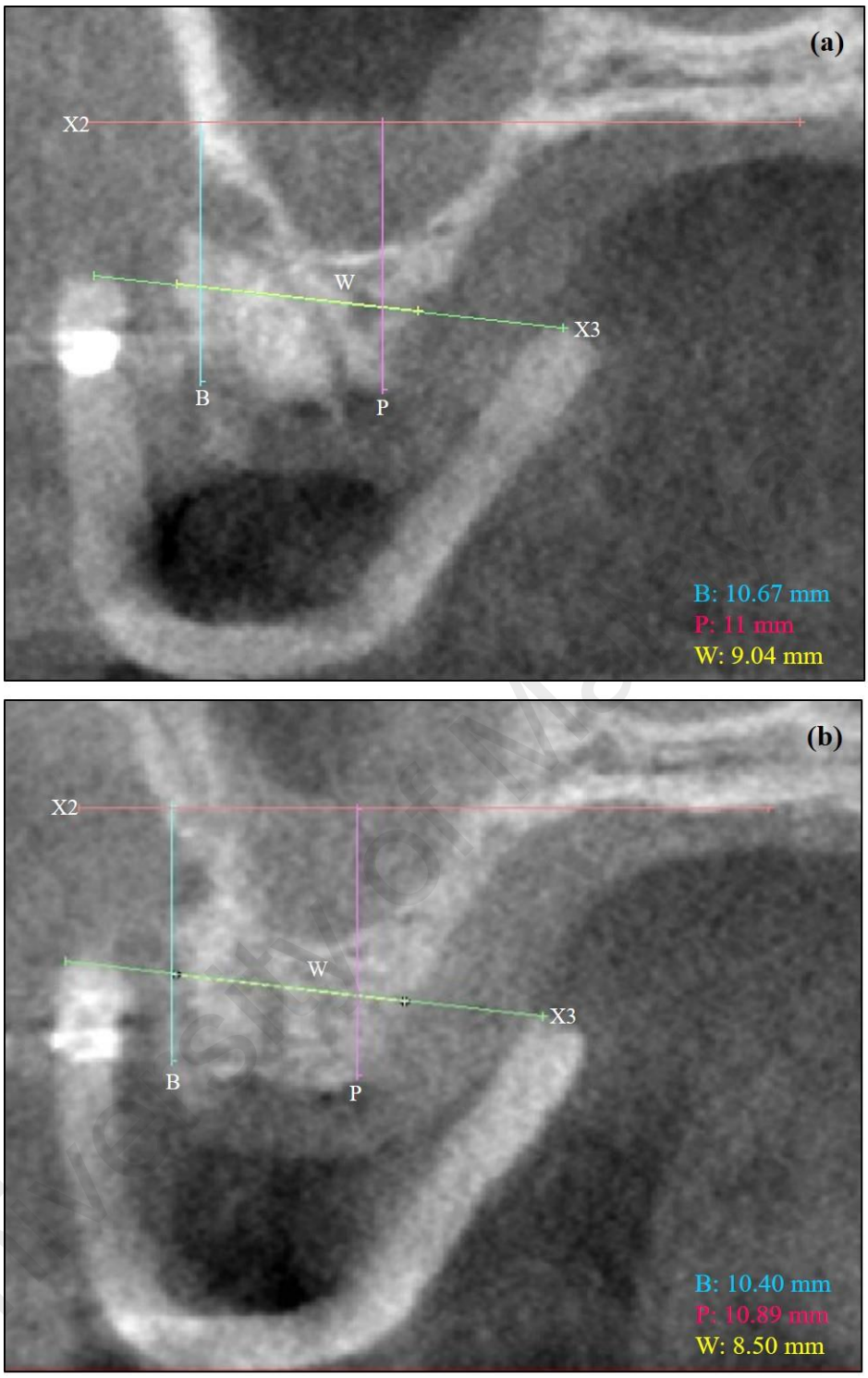

Figure 3.6: Measurements of B_BH, P_BH and W. All lines were drawn on the coronal view at baseline (a) and at 5 months post-extraction (b).

B_BH

P_BH

W

3.4.5 Bone volumetric assessment

MIMICS® software (Materialise NV, Belgium, version 16.0) was used for the analysis of bone volume at baseline and at 5 months post-extraction. The CBCT images were exported in the image analysis software in DICOM format. Greyscale threshold values (1250-4025) were set to create a new mask of the region of interest (ROI). The ROI selection was made using axial, coronal and sagittal slice which covers one tooth mesial and one tooth distal to the socket. For the axial slice, the top selected slice was 1-3 mm above the apical ends and the most bottom axial slice was at the cemento-enamel junctions level (Figure 3.7). The ROI was then segmented using the selected threshold value as shown in Figure 3.8 a.

Additionally, a multiple slice editing technique was manually performed to separate bone from the surrounding structures (Figure 3.8 b). The final 3-D model of the ROI was generated to allow the volumetric assessment of the resorbed bone. To assess the bone resorption, the baseline model (Figure 3.9 a) and 5 months post-extraction model (Figure 3.9 b) were superimposed (Figure 3.9 c). Dimensional changes were also assessed in axial view at different levels to demonstrate the collapse of bone ridge as shown in

Figure 3.10. The measurements were performed by one observer and repeated after 2 weeks.

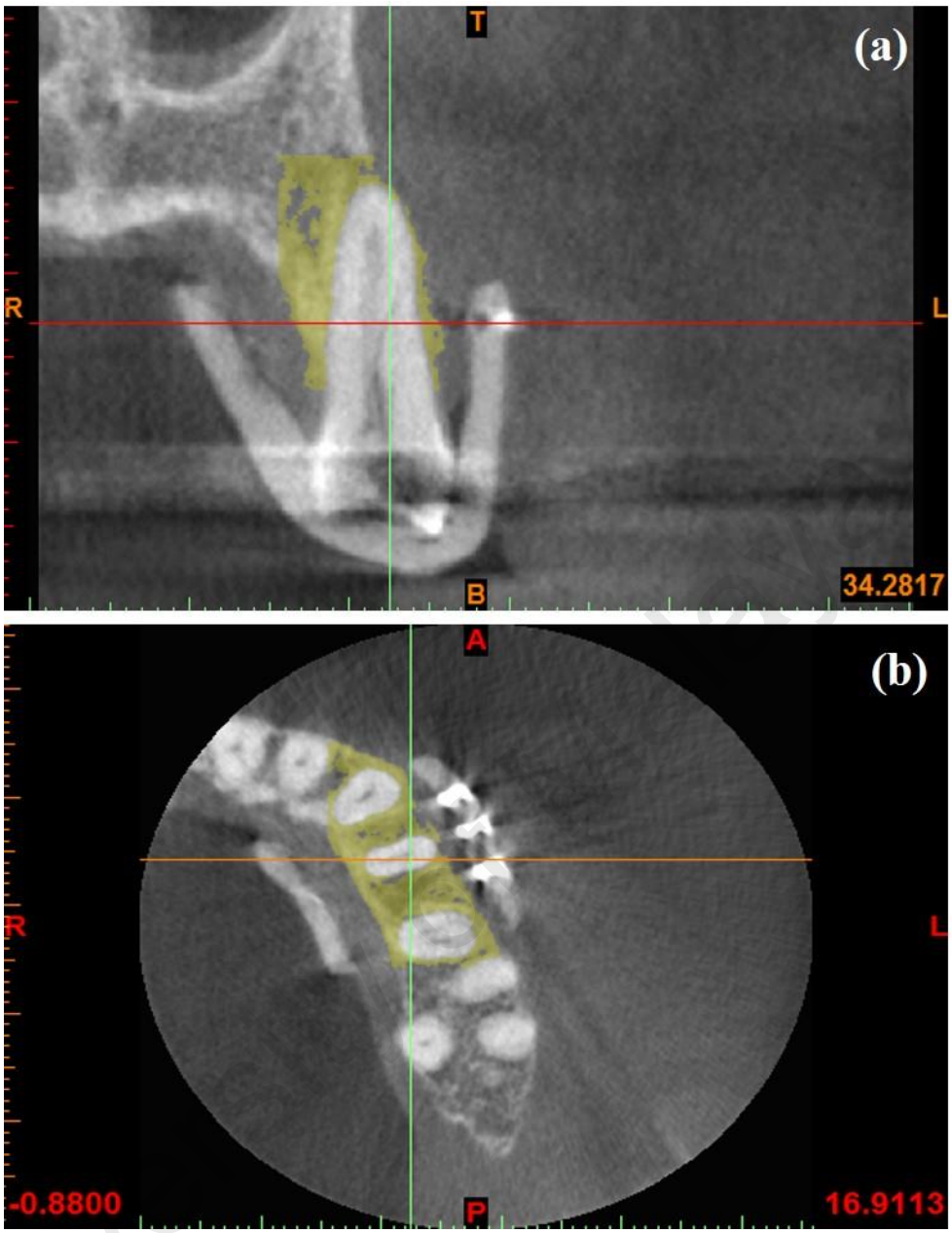

Figure 3.7: Region of interest (ROI) on coronal (a) and axial (b) views.

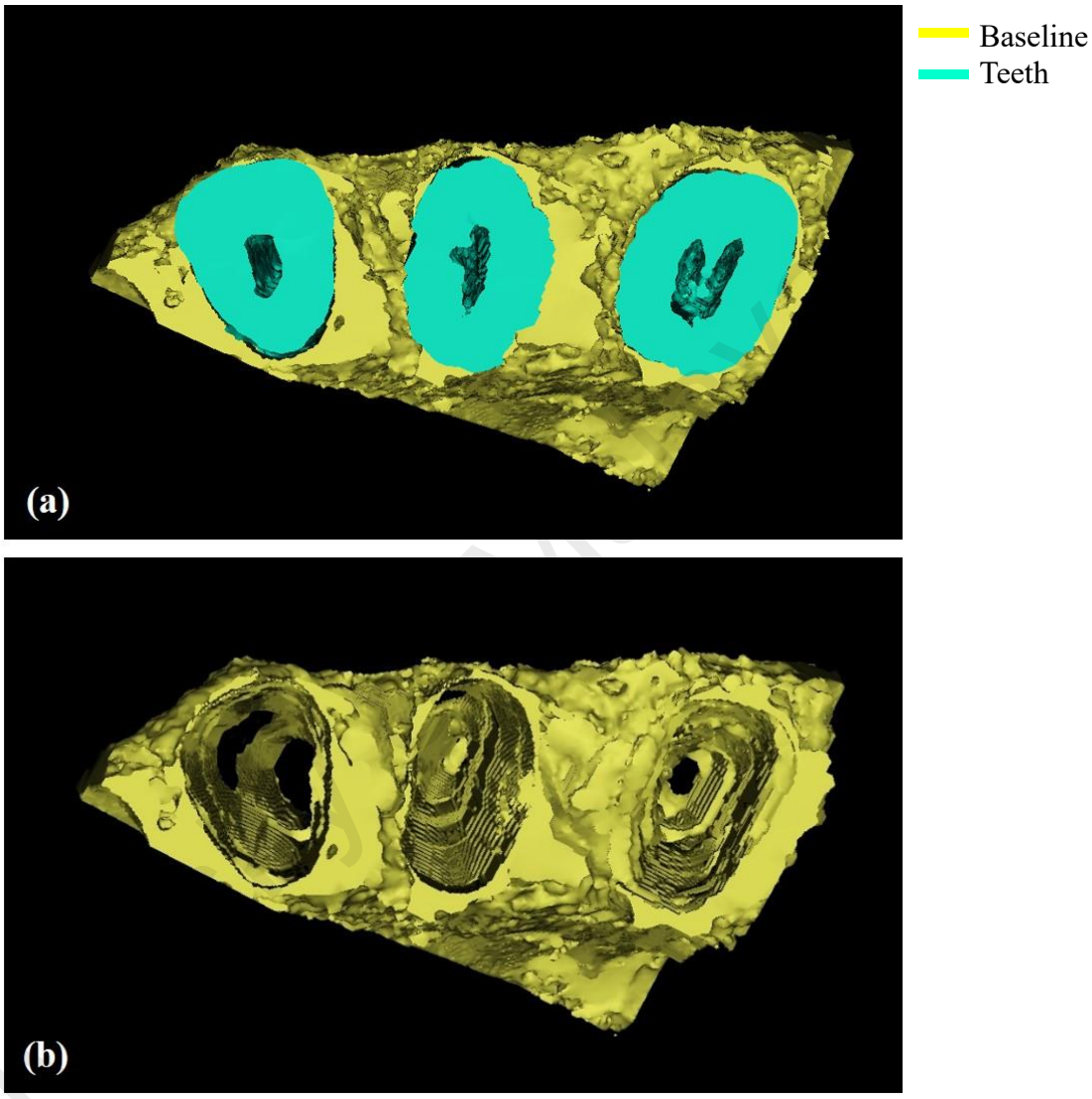

Figure 3.8: 3-dimensional images of region of interest (ROI) at baseline. (a) ROI with bone (yellow) and teeth (sea green), (b) the ROI after segmentation of the teeth in MIMICS software.

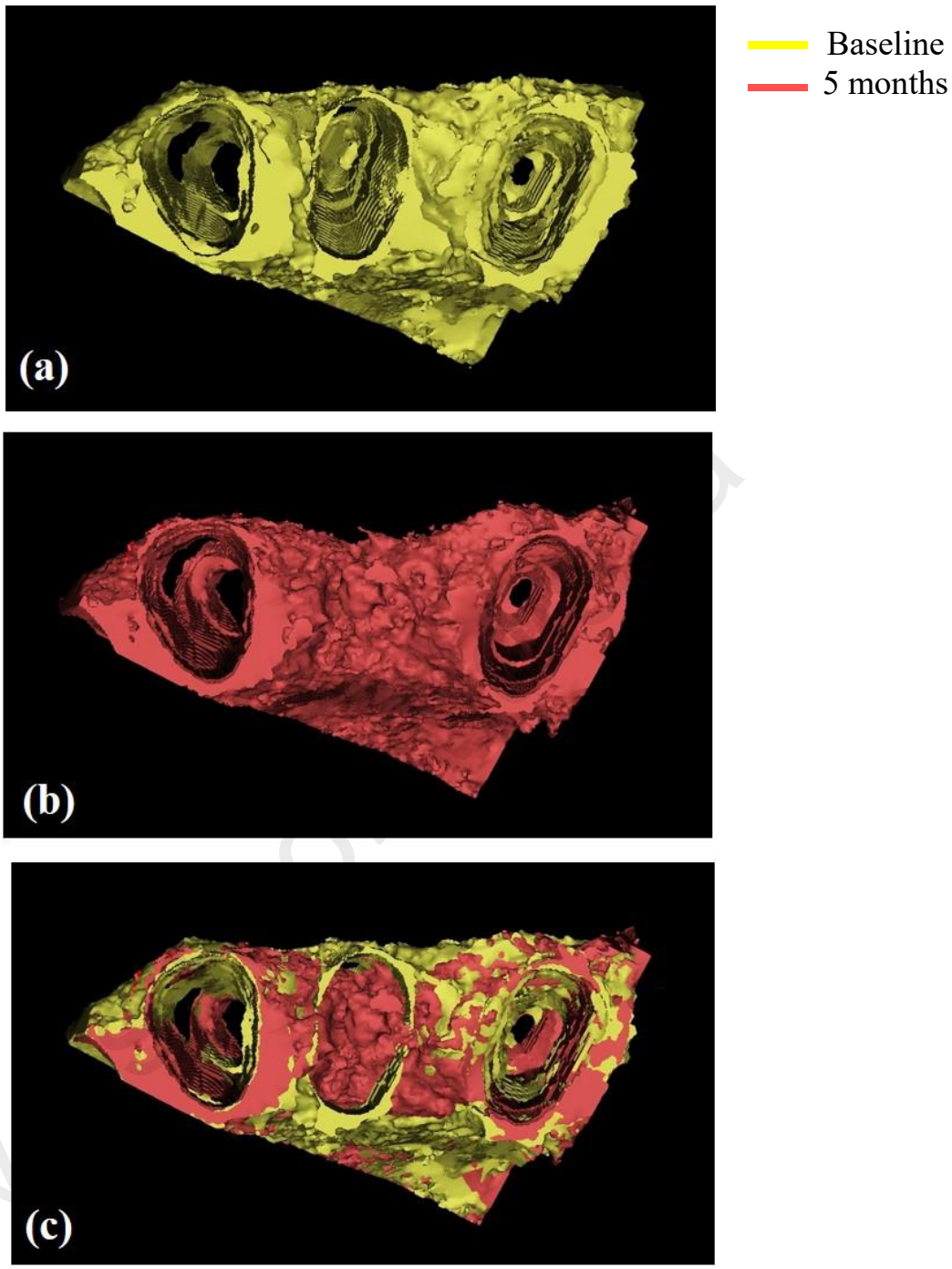

Figure 3.9: Segmentation and superimposition of the ROIs. (a) 3D image of bone at baseline (yellow), (b) at 5 months post-extraction (dark orange) and (c) superimposition of bone at baseline and 5 months.

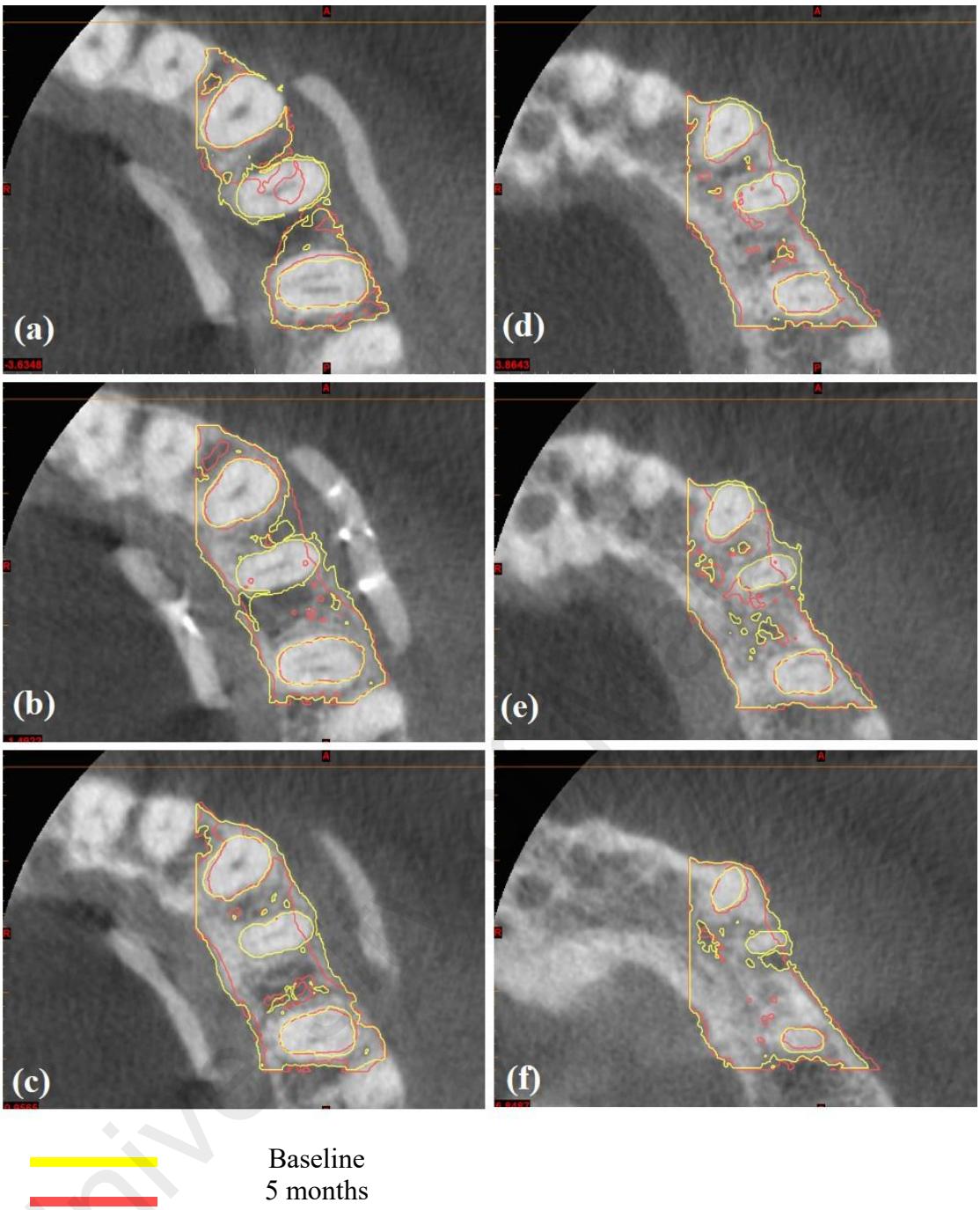

Figure 3.10: Superimposed images were on axial slice of bone at baseline (yellow) and at 5 months (dark orange). Coronal part (a), coronal 1/3rd of the ridge (b), coronal 2/3rd of the ridge (c), middle of the ridge (d) and apical region of the ridge (e, f).

3.4.6 Soft tissue level assessment

At the 5 months post-extraction review, study casts models were prepared again and with the same acrylic stent B and periodontal probe, soft tissue levels were measured (Figure 3.1 b). The parameters were mesial soft tissue height (M_SH), distal soft tissue height (D_SH), buccal soft tissue height (B_SH) and palatal soft tissue height (P_SH).

3.5 Flow chart of the methodology

The complete flowchart of methodology is shown in Figure 3.11.

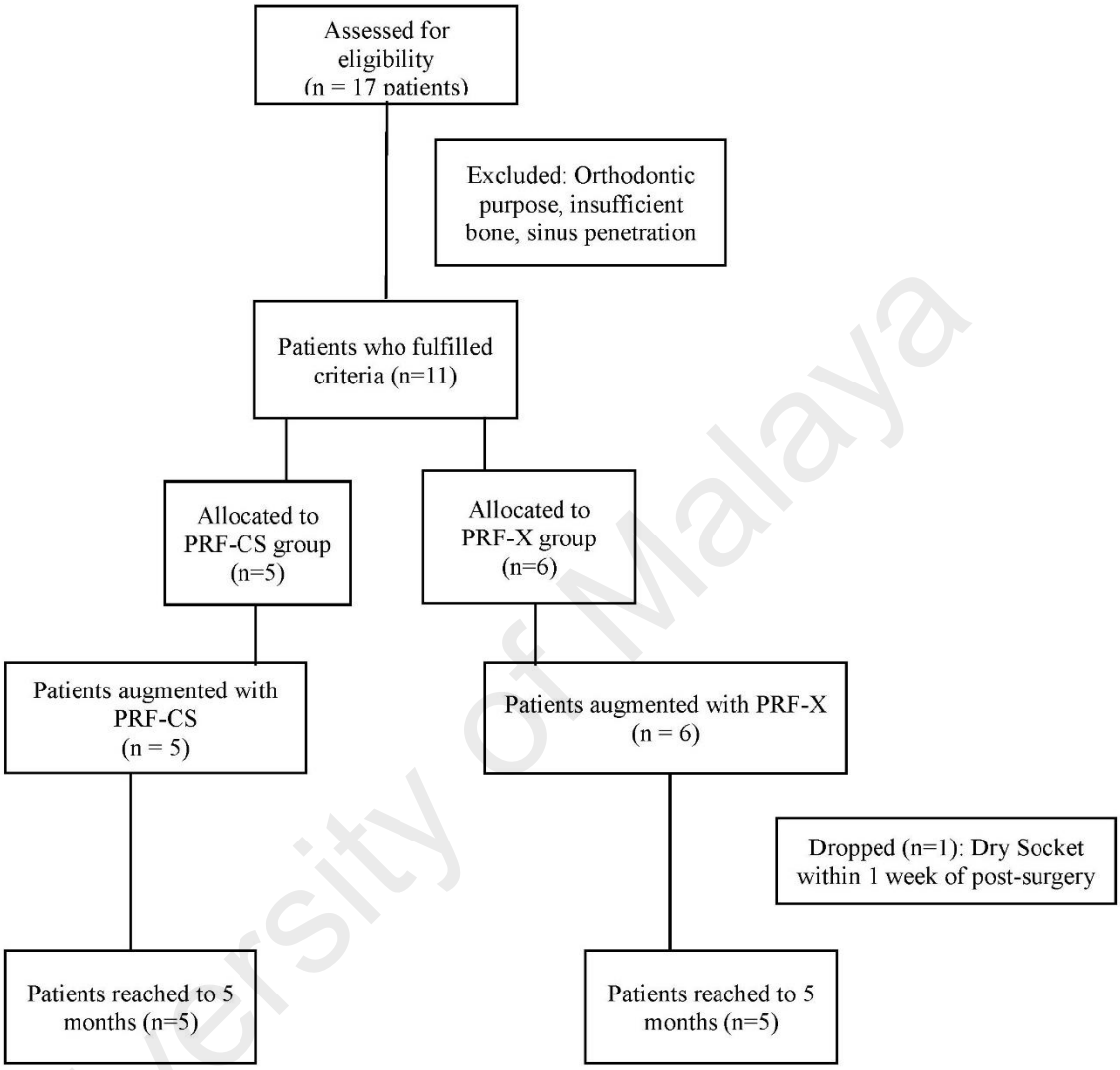

Figure 3.11: Flowchart of the methodology

3.5.1 Reproducibility study

For any clinical trial or experiment reliability, validity and responsiveness at acceptable levels are the essential factors to make it clinically meaningful. Within a study population, standardization with decreased inherent variability is necessary to be achieved.

All the assessments were obtained by the same examiner (author) in this study. The three experienced research supervisors provided the didactic training to the examiner throughout the research. To achieve the standardization process, specific and complete details for methods of assessment and measurements of parameters were explained. The methods and location points for measurement recordings were calibrated during this study.

Because of the involvement of only one examiner in the study, intra-examiner reliability was fulfilled to validate the ability of the examiner. The intraclass correlation coefficient (ICC) analysis was used to persistently reproduce quantitative results of the measurements of parameters under uniform experimental conditions. Intra-examiner reproducibility (R) was achieved by performing assessment on initial 4 patients. CBCT images were imported into the KODAK 9000® and MIMICS (version 16.0) software.

The assessments on CBCT images and MIMICS software were performed on two different time intervals with a gap of three to four hours and finally the mean reading value for the day was recorded. The same process was repeated on the second week and thus two readings were compared for intra-examiner reliability. The analysis was performed using IBM, SPSS Statistics version 22.

3.5.2 Outcomes of the reproducibility study

The intraclass correlation coefficient (ICC) analysis with 95% confidence interval was used to describe the output of the reproducibility study. In addition, absolute agreement analysis type was employed to calculate the ICC values. The detailed methodology for study reproducibility is discussed in Section 3.5.1. Table 3.5.1 shows the study reproducibility results including ICC values and upper and lower bound range values using 95% confidence interval. As shown in Table 3.5.1, the vertical alveolar ridge assessments including the MBH (R=0.947), DBH (R=0.954), SH (R=0.936), BBH

(R=0.972), and PBH (R=0.952) are highly correlated. Moreover, the horizontal ridge width assessment (R=0.962) and volumetric assessment (0.931) are also highly correlated. The results demonstrated that the value of reproducibility of the study were excellent (R > 0.9).

Table 3.5.1: Output of the reproducibility study

Parameters	Intra-class correlation coefficient (ICC)	95% confidence interval	
		Lower bound	Upper bound
M_BH	0.947	0.739	0.988
D_BH	0.954	0.845	0.988
SH	0.936	0.915	0.994
B_BH	0.972	0.870	0.993
P_BH	0.952	0.872	0.988
W	0.962	0.879	0.991
Volume	0.931	0.915	0.987

CHAPTER 4: RESULTS

4.1 Demographic characteristics of subjects

Error! Reference source not found. shows the demographic background of the s ubjects in this study. From the PRF-X group, one subject was dropped out due to dry socket. The remaining 10 subjects were treated and completed the 5 months re-evaluation period. The 10 sockets were augmented with PRF-CS (n=5) and with PRF-X (n=5). The age ranges of the subjects were from 25 to 55 years. The mean age was as 38.00 ± 10.05 years old for PRF-CS group and 38.60 ± 8.82 years old for PRF-X group. For PRF-CS group, 3 right upper first premolars, 1 right upper second premolar and 1 left upper first premolar teeth were extracted. For PRF-X group, 2 left upper first premolars, 2 right upper second premolars and 1 right upper first premolar teeth were extracted.

Table 4.1.1 Demographic characteristics of subjects augmented with PRF-CS or PRF-X

	PRF-CS (n=5 %)	PRF-X (n=5 %)
GENDER		
Male	1 (20)	2 (40)
Female	4 (80)	3 (60)
ETHINICITY		
Malay	3 (60)	3 (60)
Chinese	1 (20)	-
Indian	1 (20)	2 (40)
AGE GROUP		
25-40	2 (40)	3 (60)
40-55	3 (60)	2 (40)

4.2 Radiographic assessment of bone level at baseline and at 5 months post-extraction

The radiographic measurements of bone levels for the PRF-CS group and PRF-X group were completed at baseline and at 5 months post-extraction. Mean and standard

deviations were recorded to demonstrate average bone resorption and percentage of changes (PoC). The PoC were used to calculate the percentage of difference in mean values from baseline to 5 months within and between groups.

4.2.1 Comparison of mesial bone height (M_BH) within and between groups

Comparison of the mesial bone height (M_BH) from baseline to 5 months post-extraction within each group showed in Table 4.2.1. When comparisons were made by using mean and standard deviations, statistically significant difference was observed within the PRF-CS (p=0.008*) group as well as PRF-X (p=0.013*) group. This indicates that the mesial bone height was significantly reduced at 5 months post-extraction for both groups. The percentage of changes (PoC) in the PRF-X (8.48%) group were closely 3 times lower than that of the PRF-CS (19.58%) group between baseline and at 5 months post-extraction. However, statistically there was no significant difference observed between the two groups (p=0.064).

Table 4.2.1 Changes in mesial bone height (M_BH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

M_BH	Baseline	5 months	Difference		*p* value*
	Mean ± SD	Mean ± SD	Changes,%	Mean ± SD	
PRF-CS	2.86 ± 0.90	3.42 ± 0.70	-19.58	0.56 ± 0.25	0.008*
PRF-X	3.30 ± 0.58	3.58 ± 0.44	-8.48	0.28 ± 0.14	0.013*
p* value			0.064		

Significance level was set at 0.05.

Mean values are in millimeter.

* Paired samples *t* test was used to compare changes within group.

** Independent samples *t* test was used to compare changes between groups.

4.2.2 Comparison of distal bone height (D_BH) within and between groups

As shown in Table 4.2.2, the changes in distal bone height (D_BH) was statistically significant difference at 5 months post-extraction within the PRF-CS (p=0.015*) group but not in the PRF-X (p=0.094) group. This shows that there was significant reduction of the distal bone height in PRF-CS group while the reduction in the PRF-X group was not significant. The percentage of changes (PoC) of distal bone height (D_BH) for the PRF-CS

(13%) group was 0.58% more than the PoC for PRF-X (12.42%) group. No statistically significant difference was observed between the two groups (p=0.725).

Table 4.2.2 Changes in distal bone height (D_BH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

D_BH	Baseline	5 months	Difference		*p* value*
	Mean ± SD	Mean ± SD	Changes,%	Mean ± SD	
PRF-CS	2.78 ± 0.68	3.14 ± 0.68	-13	0.36 ± 0.19	0.015*
PRF-X	3.54 ± 0.88	4.00 ± 0.72	-12.42	0.44 ± 0.45	0.094
p* value			0.725		

Significance level was set at 0.05.

Mean values are in millimeter.

* Paired samples *t* test was used to compare changes within group.

** Independent samples *t* test was used to compare changes between groups.

4.2.3 Comparison of bone socket height (SH) within and between groups

Table 4.2.3 shows that there was a significant difference in bone socket height (SH) within the PRF-CS ($p<0.001$*) group as well as PRF-X ($p<0.001$*) group. This indicates that there was a significant bony in-fill from baseline of the study to 5 months post-extraction for both groups. The percentage of changes (PoC) for socket height (SH), in the PRF-X (69.0%) group were 1-2% lower than that of the PRF-CS (70.85%) group at 5 months post-extraction. There was no significant difference found when comparison was made between the two groups (p=0.497).

Table 4.2.3 Changes in bone socket height (SH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

SH	Baseline	5 months	Difference		*p* value*
	Mean ± SD	Mean ± SD	Changes,%	Mean ± SD	
PRF-CS	13.38 ± 0.93	3.90 ± 0.80	70.85	-9.48 ± 0.40	<0.001*
PRF-X	13.06 ± 1.04	4.06 ± 0.91	69.0	-9.00 ± 1.45	<0.001*
p* value			0.497		

Significance level was set at 0.05.

Mean values are in millimeter.

* Paired samples *t* test was used to compare changes within group.

** Independent samples *t* test was used to compare changes between groups.

4.2.4 Comparison of buccal bone height (B_BH) within and between groups

Table 4.2.4 shows a comparison of buccal bone height (B_BH) from baseline to 5 months post-extraction within each group. There were statistically significant difference in B_BH when measured after 5 months within the PRF-CS (p=0.016*) group and in the PRF-X (p=0.022*) group. This indicates that the buccal bone height was significantly reduced at 5 months post-extraction for both groups. Buccal bone height (B_BH), percentage of changes (PoC) in the PRF-X (7.26%) were 3 times lower than that of the PRF-CS (18.87%) between baseline and at 5 months post-extraction. Although, no statistical significant difference was observed between the two groups (p=0.056).

Table 4.2.4 Changes in buccal bone height (B_BH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

B_BH	Baseline	5 months	Difference		*p* value*
	Mean ± SD	Mean ± SD	Changes,%	Mean ± SD	
PRF-CS	8.57 ± 2.61	6.95 ± 2.82	-18.87	-1.62 ± 0.91	0.016*
PRF-X	8.65 ± 2.18	8.02 ± 2.28	-7.26	-0.63 ± 0.39	0.022*
p* value			0.056		

Significance level was set at 0.05.

Mean values are in millimeter.

* Paired samples *t* test was used to compare changes within group.

** Independent samples *t* test was used to compare changes between groups.

4.2.5 Comparison of palatal bone height (P_BH) within and between groups

As shown in Table 4.2.5, the palatal bone height (P_BH) was significantly different when measured at baseline and at 5 months post-extraction within the PRF-CS (p=0.023*) group but not in the PRF-X (p=0.065) group. This indicates that the reduction of palatal bone height was significant in PRF-CS group whereas the reduction in the PRF-X was not significant. On comparison between the two groups, percentage of changes (PoC) for palatal bone height (P_BH) in the PRF-X (4.33%) were 3 times lower than that

of the PRF-CS (15.66%) between baseline and at 5 months post-extraction. However, both the groups showed no statistical significant difference (p=0.059).

Table 4.2.5 Changes in palatal bone height (P_BH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

P_BH	Baseline	5 months	Difference		*p* value*
	Mean ± SD	Mean ± SD	Changes,%	Mean ± SD	
PRF-CS	8.86 ± 1.80	7.47 ± 2.22	-15.66	-1.39 ± 0.87	0.023*
PRF-X	8.86 ± 2.50	8.48 ± 2.41	-4.33	-0.39 ± 0.34	0.065
p* value			0.059		

Significance level was set at 0.05.

Mean values are in millimeter.

* Paired samples *t* test was used to compare changes within group.

** Independent samples *t* test was used to compare changes between groups.

4.2.6 Comparison of horizontal bone width (W) within and between groups

Table 4.2.6 shows that there was a significant difference in horizontal bone width (W) within the PRF-CS (*p*=0.026*) group as well as in the PRF-X (*p*=0.021*) group. This demonstrates that the horizontal bone width was significantly reduced from baseline of the study to 5 months post-extraction for PRF-CS and PRF-X group. For horizontal bone width (W), percentage of changes (PoC) in PRF-X group was 14.84% while PRF-CS group was 12.2%. No significant difference was found between the groups (*p*=0.803).

Table 4.2.6 Changes in horizontal bone width (W) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

W	Baseline	5 months	Difference		*p* value*
	Mean ± SD	Mean ± SD	Changes,%	Mean ± SD	
PRF-CS	10.39 ± 1.22	9.13 ± 1.50	-12.2	-1.27 ± 0.82	0.026*
PRF-X	9.46 ± 1.20	8.05 ± 1.65	-14.84	-1.40 ± 0.85	0.021*
p* value			0.803		

Significance level was set at 0.05.

Mean values are in millimeter.

* Paired samples *t* test was used to compare changes within group.

** Independent samples *t* test was used to compare changes between groups.

4.2.7 Comparison of volume of bone within and between groups

As shown in Table 4.2.7, there was statistically significant difference within the PRF-CS (p=0.004*) group as well as in the PRF-X (p=0.002*) group. This indicates that there was a significant decrease in bone volume from baseline to 5 months post-extraction for both the PRF-CS and PRF-X groups. As for the volume of bone, percentage of changes (PoC) for PRF-X (7.74%) was 3-4 % less in comparison to the volume for PRF-CS (11.43%) group. Comparison between the groups showed no significant difference (p=0.087).

Table 4.2.7 Changes in volume of bone from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

Volume	Baseline	5 months	Difference		*p* value*
	Mean ± SD	Mean ± SD	Changes,%	Mean ± SD	
PRF-CS	1472.89 ± 410.16	1304.56 ± 415.64	-11.43	168.33 ± 63.68	0.004*
PRF-X	1328.46 ± 298.97	1225.58 ± 293.11	-7.74	102.88 ± 32.93	0.002*
p* value		0.087			

Significance level was set at 0.05.

Mean values are in millimeter.

* Paired samples *t* test was used to compare changes within group.

** Independent samples *t* test was used to compare changes between groups.

4.3 Clinical assessment of soft tissue level at baseline and at 5 months post-extraction

The clinical measurements of soft tissue levels for the PRF-CS and PRF-X were performed on cast models at baseline and at 5 months post-extraction. Mean with standard deviations were calculated to report the percentage of change (PoC). Though, due to the altered distribution of soft tissue resorption at every site, median values with interquartile range were also reported. The PoC were used to calculate the % of difference in mean values from baseline to 5 months within groups and to compare between both groups.

4.3.1 Comparison of mesial soft tissue height (M_SH) within and between groups

As shown in Table 4.3.1, when comparisons were made between the medians at baseline and at 5 months post-extraction within each group, statistically significant difference was observed within the PRF-CS (p=0.025*) group as well as PRF-X (p=0.025*) group. This indicates that the mesial soft tissue height (M_SH) was significantly reduced after 5 months for PRF-CS and PRF-X groups. In the PRF-CS group, percentage of changes (PoC) between the two time intervals, in the mesial soft tissue height (M_SH) (26.31%), was 2-3% more than the M_SH (23.80%) for the PRF-X group. No significant difference noted between the groups (p=1.000).

Table 4.3.1 Changes in mesial soft tissue height (M_SH) from baseline to 5 months post-extrction within and between the PRF-CS and PRF-X group

M_SH	**Baseline** Median (IQR)	**5 months** Median (IQR)	**Difference** Changes,%	Median (IQR)	***p* value***
PRF-CS	3.00 (2.00)	4.00 (2.00)	-26.31	1.00 (0.75)	0.025*
PRF-X	4.00 (0.50)	5.00 (0.50)	-23.80	1.00 (0.75)	0.025*
p* value			1.000		

Significance level was set at 0.05.

IQR, interquartile range.

* Non-parametric Wilcoxon signed rank test was used for within (Intragroup) comparison.

** Non-parametric Mann-Whitney U test was used for between (Intergroup) comparisons.

4.3.2 Comparison of distal soft tissue height (D_SH) within and between groups

Comparison of the distal soft tissue height (D_SH) from baseline to 5 months post-extraction within each group and between the groups was shown in Table 4.3.2. The D_SH was significantly different after 5 months in PRF-X (p=0.034*) group but not in the PRF-CS (p=0.083) group. This showed significant reduction in distal soft tissue height of PRF-X group while the reduction in the PRF-CS group was not significant. For distal soft tissue height (D_SH), the percentage of changes (PoC) for PRF-X (28.57%) are nearly two times more than the PRF-CS (15%). Nonetheless, comparison between the groups showed no significant difference (p=0.222).

Table 4.3.2 Changes in distal soft tissue height (D_SH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

D_SH	Baseline Median (IQR)	5 months Median (IQR)	Difference Changes,%	Difference Median (IQR)	*p* value*
PRF-CS	3.00 (2.50)	4.00 (1.50)	-15.0	1.00 (1.00)	0.083
PRF-X	4.00 (1.50)	5.00 (1.00)	-28.57	1.00 (0.50)	0.034*
p* value			0.222		

Significance level was set at 0.05.

IQR, interquartile range.

* Non-parametric Wilcoxon signed rank test was used for within (Intragroup) comparison.

** Non-parametric Mann-Whitney U test was used for between (Intergroup) comparisons.

4.3.3 Comparison of buccal soft tissue height (B_SH) within and between groups

A comparison of buccal soft tissue height (B_SH) from baseline to 5 months post-extraction within each group and between both the groups was shown in

Table 4.3.3. There was no significant difference within the PRF-CS (p=0.066) group as well as PRF-X (p=0.059) group. This indicates that the soft tissue height at the buccal region was not significantly changed from baseline to 5 months post-extraction for both groups. In the PRF-CS group, the percentage of changes (PoC) for B_SH (34.78%), was 12% more than the B_SH (23.07%) for the PRF-X group. No significant difference was found on comparison between the groups (p=0.548).

Table 4.3.3 Changes in buccal soft tissue height (B_SH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

B_SH	Baseline Median (IQR)	5 months Median (IQR)	Difference Changes,%	Difference Median (IQR)	*p* value*
PRF-CS	4.00 (2.50)	6.00 (0.50)	34.78	2.00 (2.00)	0.066
PRF-X	6.00 (2.00)	6.00 (1.00)	23.07	1.00 (1.50)	0.059
p* value			0.548		

Significance level was set at 0.05.

IQR, interquartile range.

* Non-parametric Wilcoxon signed rank test was used for within (Intragroup) comparison.

** Non-parametric Mann-Whitney U test was used for between (Intergroup) comparisons.

4.3.4 Comparison of palatal soft tissue height (P_SH) within and between groups

Table 4.3.4 showed a comparison of palatal soft tissue height (P_SH) from baseline to 5 months post-extraction within and between the groups. No statistically significant difference was found within the PRF-CS (p=0.083) group as well as PRF-X (p=0.059) group. This indicates that the soft tissue height of the palatal region was not significantly changed from baseline to 5 months post-extraction for both groups. The palatal soft tissue height (P_SH) is reduced 28% in PRF-X group, closely equal in comparison to that of PRF-CS group (27.27%). In addition, no significant difference was detected between both groups (p=0.841).

Table 4.3.4 Changes in palatal soft tissue height (P_SH) from baseline to 5 months post-extraction within and between the PRF-CS and PRF-X group

P_SH	**Baseline** Median (IQR)	**5 months** Median (IQR)	**Difference** Changes,%	Median (IQR)	***p* value***
PRF-CS	4.00 (3.00)	6.00 (1.00)	27.27	2.00 (2.00)	0.083
PRF-X	5.00 (2.00)	6.00 (2.00)	27.95	2.00 (1.50)	0.059
p* value			0.841		

Significance level was set at 0.05.

IQR, interquartile range.

* Non-parametric Wilcoxon signed rank test was used for within (Intragroup) comparison.

** Non-parametric Mann-Whitney U test was used for between (Intergroup) comparisons.

CHAPTER 5: DISCUSSION

5.1 Study design and subject recruitment

This randomized clinical trial was designed to evaluate and compare the effects of platelet-rich fibrin (PRF) with calcium sulfate (CS) as a test group or PRF with xenograft (X) as a control group in; (1) reducing horizontal alveolar bone resorption, (2) reducing vertical alveolar bone resorption, (3) soft tissue level changes and (4) volumetric changes in extraction socket augmentation procedure.

In clinical trials, a control group is necessary to validate that an intervention is exceptional, cost effective, or affiliated with less complications in comparison to conventional practice. Several studies reported that conventional practices of tooth extraction where no grafting material was used in the control group, often results in more bone resorption (Barone et al., 2008; Barone et al., 2012; Eshghpour et al., 2014; Hauser et al., 2013; Iasella et al., 2003; Lekovic et al., 1998). Weijden. et al. (2009) reported in a review that the amount of alveolar ridge width loss is about 3 to 4mm and alveolar ridge height loss is about 1.5 to 2 mm after 3-7 months of tooth extraction. Tan et al. (2012) performed a systematic review on post-extraction alveolar hard tissue dimensional changes in humans. This review reported that horizontal bone loss of 29-63% and vertical bone loss of 11-12% after 6 months of tooth extraction where no grafting material was used. When compared to non-grafted controls, there was about 1-2 mm less ridge height reduction and 1.5-2 mm less ridge width reduction when using a bone replacements graft (Avila-Ortiz et al., 2014; Iocca et al., 2017). Therefore, to overcome such resorption consequences, the extraction sockets were augmented through various grafting materials. Since it has already been established that leaving a socket non-grafted would result in more bone loss than a grafted site, thus in the current study, the control group was not left ungrafted.

For control group, PRF-X was used as a grafting material because several recent studies have reported that PRF-X showed less bone resorption compared to natural healing, and other grafting materials such as, PRF, CS or xenograft alone (Barone et al., 2015; Kalash et al., 2017). For instance, Barone et al. (2015) employed PRF-X in extraction sockets in 33 patients and reported the effective results for bone regeneration and increased soft tissue level. Kalash et al. (2017) conducted a study of immediate implants placement in fresh extraction sockets of 18 patients. In addition, the peri-implant gap was filled using combination of advanced-PRF (A-PRF) and xenograft compared to using xenograft alone and assessed their effectiveness in probing depth, marginal bone height and bone density. Authors reported that the combination of PRF and xenograft showed more reduction in probing depth (2.57 mm) compared to xenograft (2.89 mm) at 6 months. Moreover, the combination of PRF and xenograft showed less reduction in marginal bone height (1.91 mm) compared to xenograft (1.79 mm) at 9 months.

For the test group, the combination of PRF and CS was employed. Intini et al. (2002) reported that the precipitation of CS causes an exothermic reaction which activates the platelets present within the platelet concentrates. In addition, CS can be a potential carrier for platelet derived growth factor (PDGF). Finally, it may enhance the osseous tissue regeneration by increasing cell proliferation and supporting cell growth.

Several existing studies employed the combination of PRP (previous generation of PRF) and CS and reported the effectiveness of their combination in test group. For instance, Kutkut et al. (2012) have performed a study using the combination of PRP-CS in socket augmentation and histologically found the presence of 100% living, well-mineralized trabecular bone, rapid bone healing enhancement and greater vital bone after 3 months. Cheah et al. (2014) have also reported that the sockets augmented with PRP-CS showed higher mineralized bone content as compared to CS alone.

Hauser. et al. (2013) conducted a randomized clinical study of 23 patients and evaluated the effectiveness of PRF in socket augmentation procedure. Moreover, authors reported that the PRF is an autogenous biomaterial of blood origin and it does not leave any residual particles in the augmented sites. In addition, the effectiveness of PRF for the improvement of the microarchitecture and intrinsic bone tissue quality of the healing socket after 2 months was reported. Additionally, more effective cell proliferation and migration using PRF have been reported because of its fibrous structure and PRF retains a greater number of growth factors and cytokines in three-dimensional fibrin scaffold (Naik et al., 2013). Henceforth, in the current study, it was hypothesized that the combination of PRF-CS may produce better results in socket augmentation compared to PRP-CS.

In this study, randomization was performed by using coin-toss method. This method is widely employed in human clinical trials (Iasella et al., 2003). Additionally, it avoids selection bias (Calasans-Maia et al., 2014). Similarly, previous studies used randomization into treatment and control groups to evaluate the effectiveness of their interventions (Clark et al., 2018; Fiorellini et al., 2005). Carrying out randomization in relatively small sample size may be problematic as it may result in an imbalanced number of participants among groups (Suresh, 2011). However, in this study, since the sample size was small we were unable to assess if the subjects were equally distributed.

Sample size can affect the outcome of a study and the strength of the conclusion (Faber & Fonseca, 2014; Kumar, 2014). In the current study, sample size was calculated as 20 subjects (10 subjects per group). However, because of the difficulty in acquiring suitable number of patients that fulfilled the criteria and lack of time availability, the number of subjects recruited was initially 11. The underlying choice of generally low-risk patients with strict criteria can remove the influence of specific confounding factors of any study

and can provide a study model with a reduced variability. Furthermore, extraction sockets, presented with fenestrations or trauma after extraction had to be excluded from the study. One of the patients was dropped out due to the development of infection within first week of socket augmentation. Thus, the sample size was reduced to10 (5 subjects per group).

Resorption pattern can be influenced by site of extraction, different arches and number of teeth extracted (Al-Hamoudi et al., 2015; Araujo et al., 2015; Schropp et al., 2003). Buccal or labial plates have been reported to be thin in the maxilla which leads to alveolar bone resorption being much faster than in the palatal plates (Pietrokovski & Massler, 1967) (Tomasi et al., 2010). In this study, single tooth extraction was performed only at maxillary premolar region to ensure homogeneity to minimize bias. Similarly, a recent study has also reported to only consider maxillary teeth for socket augmentation (Hassan et al., 2017).

5.2 Surgical procedure

The reasons for tooth extraction were advanced caries lesions, endodontic treatment failures and root fractures. A minimally traumatic procedure for tooth extraction without raising mucosal flap has appeared to be advantageous for the preservation of hard tissues and also promote the effects of grafting material after socket filling (Hauser et al., 2013). Even in a minimal full thickness flap, superficial layer of the exposed alveolar bone may possess osteoclastic activity and cause resorption of bone (Lekovic et al., 1997). Furthermore, flap elevation may increase the chances of gingival recession and this has been reported in a study by Fickl. et al. (2008a), where they have demonstrated 0.7 mm additional shrinkage of the coronal aspects on the buccal side compared with a non-flap procedure. Traumatic or vigorous extraction technique leads to distortion of socket walls resulting the most common post-operative complications of pain and foul smell termed as alveolar osteitis (dry socket) caused by complete or partial disintegration of blood clot

inside the socket (Tarakji et al., 2015). Therefore, in the current study, non-flap atraumatic extractions were performed. Following extraction, a thorough and gentle curettage of the sockets were achieved before graft placement (Froum et al., 2002) and to facilitate the bleeding inside the sockets for the initial stages of healing (Cardaropoli et al., 2003).

It was distinctly proven that the centrifugation protocols and centrifuge characteristics produce significant influence on cell functions, growth factors and the fibrin architecture (Dohan Ehrenfest et al., 2017). The speed of centrifugation, vibrations of centrifuges, vibration shocks in the acceleration phase and resonance of vibrations are the key parameters which may make a difference in the cell content, weight, volume and fibrin architecture of PRF (Dohan Ehrenfest et al., 2017). Thus, in this study, PRF was prepared according to the protocol described by Choukroun in 2001. Blood sample was taken and centrifuged at 1300 rpm for 8 minutes (using PRF Process, Nice France). For every patient, 20 ml of blood sample was taken in glass tubes. It was previously reported that the PRF membranes and clots production did not appear to be disturbed by the usage of glass-coated plastic tubes or glass tubes (Dohan Ehrenfest et al., 2010).

In the related literature, various platelet concentrate preparations have been produced by different preparatory protocols (such as, lab-based techniques, centrifugation, plasmapheresis) to form different materials with dissimilar biological and potential usage (Dohan Ehrenfest et al., 2009). The time utilized to prepare platelet concentrate varies but is mostly completed in an hour. Previously, platelet concentrate (pure PRP) was manufactured using plasmapheresis in which cell separator was used either from a harvested blood bag with anticoagulants or from intermittent blood flow set up. This procedure obtained around 40 ml of PRP from 450 ml of blood (Dohan Ehrenfest et al., 2009). Moreover, Anitua (1999) proposed platelet concentrate preparation with richly present growth factors (PRGF). The PRGF prepared by discarding acellular plasma from

upper layer after centrifugation using several pipetting steps with eyeballing as a measuring tool and followed by addition of 10% calcium chloride for fibrin polymerization. In addition, leukocytes rich platelet-rich plasma (L-PRP) was manufactured using manual (Curasan method) and automated method (Platelet concentrate collection system). However, the leukocytes counts are very low along with the usage of separator gel and calcium chloride was used to activate platelets and fibrin polymerization. Unlike other platelet concentrates, Choukroun's PRF does not dissolve quickly after application and results in slow remodeling due to solid consistency of its fibrin matrix (Clark, 2001). Furthermore, leukocytes and platelets are collected with their potential efficacies and preserved throughout. Moreover, in PRF preparations, fibrin fibers are very thick because of the multiple fiber assembly that establish a resistant matrix which may be appraised as a fibrin biomaterial (Dohan Ehrenfest et al., 2009). Another remarkable advantage in this method of PRF preparation are their low cost and significant ease of procedure that permits many concentrates production excluding the anticoagulants materials and within short spans of time. Therefore, in this study, the centrifugation protocol proposed by Choukroun in 2001 was used to prepare PRF instead of other lab-based techniques.

In the current study we were not able to assess the amount of growth factors present in the PRF used for our subjects. However previous studies have reported that the quantity of platelet concentrates are positively correlated to the release of growth factors (Eppley et al., 2004). In general, the activated platelets release growth factors that are capable of initiating and regulating the wound healing in hard and soft tissues. Eppley et al. (2004) performed a study on the quantification of platelet and growth factor analysis from platelet rich plasma (PRP). From 10 healthy patients, whole blood was drawn and concentrated into PRP. The author found that platelets were increased by approximately 8-fold in PRP compared to whole blood. The study concluded that the concentration of

growth factors increases with increased number of platelets. However, growth factor concentration varied from individual to individual. The authors also found that 0.01ng of isoforms of PDGF-BB were released per 1 million of platelets. The author also reported that 6-ml of PRP sample contained approximately 9.6 x 109 platelets. Although PRP showed increased growth factors, several studies found that PRF modulates more growth factors compared to PRP (Dohan Ehrenfest et al., 2017). Thus, in this study, PRF has been employed for socket augmentation.

The quality of PRF clot can be affected by age as increasing age interferes with the platelets and white blood cells (WBCs) and can alter the fibrin network pattern and architecture. Yajamanya et al. (2016) reported that there were variations in the number of platelets and WBCs along with dense and loose type of fibrin. In addition, it was also reported that the platelets and WBCs were reduced in terms of quality and quantity in older patients. As the age increases, i.e. from 20-50 years and above, fibrin network patterning becomes further loose and inconsistent in organization and fibrin becoming thinner with reduced entrapment of WBCs and platelets (Yajamanya et al., 2016). In the current study, 60 % subjects were between 40-55 years of age in the PRF-CS group and 60% of subjects were between 25-40 years of age in PRF-X group. Hence, there is a possibility that PRF dissolved early in the PRF-CS group and might cause more bone resorption when compared to PRF-X group.

To retard the resorption process and provide sufficient time for new osseous tissue replacement, placement of the graft in the extraction socket was achieved in layers (as stratification method) and densely packed up to the crestal level (Cheah et al., 2014; Thakkar et al., 2016; Zhao et al., 2011). Usually, open extraction sockets can cause most of the bone graft loss. Hence, PRF membrane and plug was used to support and retain the bone graft material inside the socket walls (Thakkar et al., 2016). As PRF is a fibrin clot,

it stimulates the arrest of bleeding as well, thus reducing graft and wound stabilization. Following graft placement, 4-0 black silk sutures were placed across the opening of the socket. All efforts were made to avoid additional pressure and force from the suture application to preserve the gingival architecture. The socket was intentionally left exposed to the oral cavity and allowed to heal by secondary intention.

One subject developed dry socket within 1 week and was subsequently dropped out from the study. The patient was on oral contraceptive pills that caused increased estrogen levels which may have caused increased plasminogen fibrinolytic activity that digests fibrin clot and results in a dry socket (Catellani et al., 1980). All other extraction sites showed no infection or excessive inflammatory response and had uneventful healing.

5.3 Post-operative management

Post-operative complications such as pain, trismus and infections are the common sequalae after tooth extraction (Tong et al., 2014). In order to prevent the risks of post-operative infections, due to the placement of graft inside the socket, antibiotics (amoxicillin 500mg tds for 5 days) and mouth rinse (0.12% chlorhexidine twice daily for a week) were prescribed to all patients of both groups (Cheah et al., 2014). An analgesic, (paracetamol 1g) was prescribed to control post-operative pain (Anwandter et al., 2016). In all patients, no hypersensitivity reactions were observed.

5.4 Measurements and records

Numerous tools have been employed to assess the soft tissue alterations after socket augmentation such as, cast models and fabricated stents (Kutkut et al., 2012; Schropp et al., 2003). In existing literature, the most widely used tool is fabricated stent to serve as a fixed reference guide for vertical measurements (Iasella et al., 2003). Thus, this study also utilized fabricated stents for the soft tissue assessments.

Several image analysis tools have been used to minimize the diagnostic error and to improve the treatment planning in dentistry (Shah et al., 2014). Studies reported that cone beam computerized tomography (CBCT) minimizes the superimposition of the images and provides finest accuracy compared to orthopantomography (OPG). Moreover, it is recommended in pre-surgical planning of buccolingual locations of maxilla which cannot be obtained from OPG (Amarnath et al., 2015), and dentoalveolar regions in the precise measurement of bone dimensions and densities (Pauwels et al., 2012). Therefore, this study employed CBCT to assess hard tissue dimensional and volumetric alterations. CBCT scanning was performed before tooth extraction to assess the anatomy of the root of the tooth to be extracted and the surrounding bone support in 3 dimensions. In addition, in this present study, Kodak 9000C® (Carestream, United States) software is used to focus only at the implant site and to measure the bone volume and dimensions.

Recent studies argued that accurate volumetric assessments are essential after tooth extraction (Aimetti et al., 2018; Manavella et al., 2018; Pang et al., 2014). To achieve this, studies employed image analysis software such as, MIMICS (discussed in Section 2.8.4) as an evolutionary software for volumetric analysis of extraction sockets and alveolar bone (Aimetti et al., 2018; Manavella et al., 2018; Pang et al., 2014). Thus, in this study, volumetric analysis was performed using MIMICS software. This software has provided the benefit of altering the segmentation method and also has the capacity to perform volumetric examination in different dimensions (coronal, sagittal, axial and 3D). It also allows the examiner to include or exclude mask at the region of concern where it is unable to demarcate mechanically. Moreover, it also allows its users to scroll through the entire volume with simultaneous viewing in all planes (coronal, sagittal and axial).

5.5 Changes in horizontal dimensions

Systematic reviews on alveolar ridge dimensional changes following extraction in humans reported approximately 3.5 mm to 3.8 mm (29% to 63%) of ridge width reduction after tooth extraction (Tan et al., 2012; Van der Weijden et al., 2009; Vignoletti et al., 2012a). Thus, to limit the ridge width reduction, several studies employed grafting material and showed better results compared to natural healing in socket augmentation procedures. For instance, Zhang et al. (2018) employed PRF on test groups of 14 patients and employed no grafting material on 14 patients of control group. After 3 months of healing, the authors reported the width reduction of 1.0 ± 0.70 mm for the PRF group whereas 2.05 ± 0.77 mm for the non-grafted sites. Manavella et al. (2018) also conducted a study using xenograft in 11 patients for socket augmentation. They used CBCT and reported the mean width reduction of 1.37 ± 1.29 mm after 12 months of extraction. Aimetti et al. (2018) performed socket augmentation using xenograft and reported mean horizontal width reduction of 1.97 ± 1.55 mm for treated and 3.83 ± 1.49 mm for untreated sockets after 12 months of socket augmentation. Recently, Clark et al. (2018) reported 1.7±1.2 mm ridge width reduction when using the combination of A-PRF and FDBA in non-molar teeth of 10 patients.

In the present study, horizontal ridge width reduction from baseline to 5 months post-extraction in the PRF-CS group was 12.2% (1.27 ± 0.82 mm) and 14.84% (1.40 ± 0.85 mm) for the PRF-X group. The difference in horizontal width reduction between the groups was however not significant. As can be seen here, the width reduction results of the current study are better than Manavella et al. (2018), Aimetti et al. (2018) and Clark et al. (2018). This might be due to the differences in either methodology or tools used for dimensional assessment or patients' follow up period (as discussed in Section 2.8.4). However, data from our present study showed more width reduction compared to Zhang et al. (2018). This disagreement maybe explained by the inclusion of maxillary and

mandibular molars in their study because the amount and pattern of resorption are different in between maxilla and mandible and in between anterior and posterior teeth (Pietrokovski & Massler, 1967; Schropp et al., 2003). In addition, this may be due, somewhat to the types of tooth investigated. A systematic review on the effects of ridge preservation by Avila-Ortiz et al. (2014), reported a 1.89 mm clinical advantage on ridge width preservation when compared to tooth extraction alone. This result was based on 6 studies of non-molar tooth extraction. Another systematic review comprised of 9 studies, of which, 2 studies used molar tooth extraction (Vignoletti et al., 2012b). The review reported a 1.83 mm of clinical benefit on alveolar ridge width preservation when compared to extraction alone. Our data from the present study found less ridge width reduction for either the PRF-CS or the PRF-X group. On this ground, it can be suggested that for horizontal augmentation, PRF-CS is the material of choice.

5.6 Changes in vertical dimensions

Araújo and Lindhe (2005) concluded that more pronounced and most of the resorption takes place in the buccal region among all vertical parameters. The buccal plate is usually thinner and experiences a greater magnitude of resorption than the lingual plate. This causes more ridge resorption in a lingual and apical direction (Araujo & Lindhe, 2009; Lekovic et al., 1998). Systematic reviews on alveolar ridge dimensional changes following extraction in humans reported roughly 0.4 mm to 3.9 mm of ridge height reduction in naturally healed sockets (Ten Heggeler et al., 2011). Therefore, to reduce the alveolar ridge resorption magnitude, application of socket augmentation procedures using graft materials have been advocated. When compared to non-grafted controls, there was about 1-2 mm less ridge height reduction when using a bone replacement graft (Avila-Ortiz et al., 2014; Iocca et al., 2017). The present study showed three times more resorption for mesial bone height (M_BH), buccal bone height (B_BH) and palatal bone height (P_BH) in PRF-CS group compared to PRF-X group between the two time intervals

(see Table 4.2.1, Table 4.2.4 and Table 4.2.5). Moreover, resorption for distal bone height (D_BH) and socket height (SH) in both the groups were nearly equal. Nonetheless, these differences in results for all vertical dimensions between the two groups were not statistically significant ($p > 0.05$). From our results, it is obvious that buccal bone significantly resorbed more than the palatal bone for both the PRF-CS and PRF-X groups.

Hauser. et al. (2013) reported mesial height resorption of 0.86 mm and distal height resorption of 2.15 mm using PRF in the socket augmentation procedure at 4 months post-extraction. Barone et al. (2015) conducted a study using the combination of PRF with xenograft in the treatment of buccal bone defects, performed extractions in the anterior maxillary regions followed by implant placement after 5 months and 1 year follow up. For mesial 0.8 ± 0.1 mm and for distal aspect 0.7 ± 0.1 mm resorption was calculated. The present study using PRF-CS, demonstrated mesial bone height (M_BH) resorption of 0.56 mm and distal bone height (D_BH) resorption of 0.36 mm at 5 months post-extraction. As can be noticed, the aforementioned studies showed more bone resorption compared to our study. This maybe because of the surgical technique used with the flap elevation for socket augmentation in the study by Hauser. et al. (2013). Indeed, Fickl. et al. (2008b) reported more dimensional resorption where flap surgery was performed. Moreover, the difference in the results of Barone et al. (2015) study maybe because the authors used only anterior teeth extraction which have thin buccal walls. However, it was reported that the hard tissue resorption tend to be more prominent at regions where thin bone walls are present (Tomasi et al., 2010).

In a study, Barone et al. (2012) performed socket augmentation on 40 patients. Authors employed cortico-cancellous porcine bone on test group and control group was not grafted. The test group showed the resorption of 1.6 mm in buccal height and resorption of 1.8 mm in palatal height. Additionally, in control group the resorption in buccal height

was 0.7 mm more than that of test group and resorption in palatal height was 0.3 mm more than that of test group. In the current study using PRF-CS, buccal bone height resorption (1.62 mm) and palatal bone height resorption (1.39 mm) were less than the results reported in Barone et al. (2012). Clark et al. (2018) conducted a randomized controlled clinical trial of socket augmentation on 40 patients using advanced-PRF (A-PRF) and freeze dried bone allograft (FDBA). The authors found vertical height resorption of 1.8 mm for A-PRF group and 2.2 mm for FDBA group at 15 weeks of healing. Zhang et al. (2018) conducted a clinical trial of socket augmentation on 28 patients (14 patients for test group and 14 patients for control group). Authors employed PRF membrane in test group and no grafting material in control group. Using CBCT, the authors reported buccal bone resorption of 1.60 mm for test group and 2.80 mm for control group after 3 months of extraction. Moreover, the lingual bone resorption was reported as 1.0 mm and 2.05 mm for test and control groups, respectively. Our study demonstrated similar buccal bone resorption for PRF-CS (1.62 mm) group but slightly increased palatal bone resorption of (1.39 mm) when compared to Zhang et al. (2018). However, palatal bone reduction of PRF-CS group was significantly lesser than that of control group of the study by Zhang et al. (2018). This disagreement maybe explained by the inclusion of maxillary and mandibular molars because the amount and pattern of resorption are different in between maxilla and mandible and in between anterior and posterior teeth (Pietrokovski & Massler, 1967). Moreover, Barone et al. (2012) raised mucosal flaps for graft placement and it was reported earlier that soft tissue reflection in flap surgeries may enhance the magnitude of bone resorption and cause additional dimensional changes (Hauser. et al., 2013; Wood et al., 1972)

When compared to untreated controls, a systematic review Avila-Ortiz et al. (2014) on non-molar tooth extraction, reported effects of ridge preservation as 2.07 mm clinically on the buccal bone height and 1.18 mm clinically on the lingual/palatal bone height.

Likewise, Vignoletti et al. (2012b) reported in a systematic review of 1.47 mm clinical benefit on alveolar ridge height preservation when compared to tooth extraction alone. The results from that review was based on 9 studies, out of which, only 2 studies included the molar teeth. Tan et al. (2012) conducted a systematic review on hard and soft tissues dimensional changes after socket augmentation. The author found vertical reduction (1.24 ± 0.11 mm on buccal, 0.84 ± 0.62 mm on mesial and 0.80 ± 0.71 mm on distal sites) at 6 months of socket augmentation procedure.

5.7 Changes in soft tissue dimensions

Soft tissue resorption in inevitable after tooth extraction. However, the resorption in soft tissue levels can be minimized by taking suitable measures of socket augmentation. The effect of alveolar ridge preservation is well recognized in the literature. Nevertheless, there is lack of studies that report the changes of soft tissue level on clinical results. In the PRF-CS group, the percentage of resorption between the two time intervals, in the mesial soft tissues height (M_SH) and buccal soft tissue height (BSH), was nearly 2-12% more than the M_SH and B_SH for the PRF-X group (see Table 4.3.1 and

Table 4.3.3). However, for distal soft tissue height (D_SH), the percentage of resorption for PRF-X are nearly two times more than the D_SH for PRF-CS group (see Table 4.3.2). The resorption for palatal soft tissue height (P_SH) in PRF-CS group was marginally lower than the PRF-X group (see Table 4.3.4). However, in both the groups, no statistical significant difference was observed ($p>0.05$).

Duggan (2001) performed socket augmentation to analyze soft tissue level on cast models. Authors employed fabricated acrylic stents to measure vertical and horizontal dimensions of ridge clinically and radiographically. Freeze-dried mineralized human bone allograft was utilized to preserve the sockets in 19 patients whereas control group comprises of 5 patients received no graft. At 3-4 months of extraction, using cast models

changes in mesial soft tissue height of 0.30 mm and distal soft tissue height of 0.65 mm were evaluated. Cardaropoli et al. (2012) performed extraction socket augmentation using bovine bone mineral and collagen membrane (test) and extraction alone (control) on 48 posterior teeth (41 patients). At 4 months of extraction, evaluation on cast models showed changes in buccal and lingual soft tissue height of 0.46 mm for test group and 1.54 mm for control group, respectively. The present study reported slight dissimilar range of buccal and palatal soft tissue changes of 2 mm for PRF-CS group at 5 months post-extraction. The difference in the aforementioned studies maybe because of the usage of mostly molar teeth of both the maxilla and mandible. It was reported previously that the percentage of reduction varies in between maxilla and mandible and in between premolar and molar regions (Schropp et al., 2003).

One of the short comings of the present study was that the gingival biotype was not recorded. Gingival tissue biotype is a crucial factor that dictates the outcome of dental treatment. The thickness of gingival tissue and bone affects the stability of osseous crest and soft tissue (Abraham et al., 2014). Gingivae can be categorized as “thick flat” and “thin scalloped” biotypes (Abraham et al., 2014). Thick gingival tissue comprises of broad zone of keratinized tissue with flat gingival tissue contour characteristic of thick bony architecture while thin scalloped biotype consists of narrow band of the keratinized tissue with scalloped gingival contour and thin bony structure. Extraction of tooth in thick biotypes result in less ridge atrophy, whereas surgical or traumatic extractions may cause fracture of the buccal/labial plates and excessive alveolar ridge resorption in thin bony plates (Abraham et al., 2014). In Asians, the gingival biotype has been reported to be of thin type (Lee et al., 2013). There is a possibility that the amount of bone resorption noted in the present study could have been influenced by the different gingival biotype.

5.8 Volumetric analysis

Recent studies argue that accurate volumetric assessments are essential for the accurate and reliable measurements of extraction sockets (Aimetti et al., 2018; Manavella et al., 2018; Pang et al., 2014). The percentage of changes for volume in the PRF-X (7.74%) group are slightly decreased about 3-4% in comparison to the volume for PRF-CS (11.43%) group. The bone volumetric reduction ranged from 1472.89 mm3 to 1304.56 mm3 for the PRF-CS group and bone volumetric reduction for the PRF-X ranged from 1328.46 mm3 to 1225.58 mm3 from baseline to 5 months post-extraction, showing a decrease in volume for the PRF-CS group but statistically, no significant difference was observed in between both groups ($p>0.05$). It has already been demonstrated that the volume of bone, especially after extraction plays a crucial role in the placement of implant. Pang et al. (2014) conducted a study to preserve alveolar ridge using deproteinized bovine bone graft and collagen membrane. After 6 months, they found the volume of preserved sites had resorbed 262.06 mm3 while the untreated sites resorbed by 342.32 mm3, it showed that the bone volume changes in the trial group was lower than the control group. No percent changes in alveolar bone volume were reported. This indicates that bone fill can be improved by socket augmentation. The current study showed a slightly lower changes in bone volume for the PRF-X (102.88 mm3) group when compared to the PRF-CS (168.33 mm3) group. Variance in the method used to determine the region of interest may explain the differences in numeric volumetric changes. In the present study, bone volume was calculated covering the 3 teeth whereas Pang et al. (2014) used only the extraction socket for bone volumetric analysis. Nevertheless, the condition of the extraction socket was not explained in their study and the amount of the volumetric changes maybe due to the loss of the buccal plate of alveolar bone. When the buccal plate is absent, the extraction socket most often fills with fibrous reparative tissues, which usually leads to a reduced net bone volume (Darby et al., 2009).

Manavella et al. (2018) utilized CBCT scans and MIMICS software to analyze volumetric changes over 12 months after tooth extraction with socket augmentation using bovine derived xenograft. Eleven patients with one hopeless tooth (maxilla) each for extraction due to severe periodontitis were included. They found bone volumetric reduction ranging from 206.92 mm3 to 191.14 mm3 from baseline to 12 months of socket augmentation. The mean difference was 15.78 mm3. Our data demonstrated significantly greater volumetric changes for the PRF-CS group. This maybe due to methodologic variances in determining volumetric changes. By utilizing CBCT scans and MIMICS software, they analyzed the extraction socket of the region of interest instead of the whole bone volume of the augmented area and the adjacent teeth area.

In another recent human study of socket augmentation, CBCT was utilized followed by using MIMICS software to compare ridge volumes after placement of a collagenated bovine derived bone graft and naturally healing extraction sockets (Aimetti et al., 2018). After 12 months, authors found the mean decrease in volume of 18.61 mm3 and 62.09 mm3 for the grafted versus non-grafted groups respectively. In our study, the PRF-CS group managed to have bone volume fill. This showed that socket augmentation can help in improving the bone fill in the extraction socket. Keeping in mind that these data (Aimetti et al., 2018; Manavella et al., 2018; Pang et al., 2014), are not directly comparable to the current study, it is obvious they are not in conflict.

In this present study, the test (PRF-CS) material did not show similar effects as the control (PRF-X) but the difference were not significant. Nevertheless, when compared to non-grafted sites, the amount of resorption was less.

5.9 Clinical significance

Adequate ridge bone and volume are an essential criterion for effectively functioning and aesthetically satisfying dental implant supported restorations. Dental implants have

developed an incredible long-standing treatment alternative to replace lost teeth with the survival rates of 93% to 95% (Hjalmarsson et al., 2016; Moraschini & Barboza, 2016). A lack of the buccal bone after tooth extraction may be cumbersome to place dental implants, by not permitting appropriate three-dimensional position of the implant, which may adversely affect the aesthetics of the ultimate restoration. Socket augmentation is needed to overcome these consequences following tooth extraction. The combination of PRF-CS in the current study has been shown to be as effective as PRF-X graft material to preserve the ridge dimensions and volume. PRF is an autogenous graft material, can enhance healing and CS is also readily available with osteoconductive properties. On the contrary, xenografts which are obtained from other species or allografts which are obtained from the dead cadavers, appear to be expensive and can limit the patients' affordability. Patients from certain ethnic groups do not prefer to use the allografts and xenografts. For instance, Fernandez et al. (2015) reported a survey study on usage of bone grafts in dentistry and patients' preferences. They found highest refusal rates for the allografts and xenografts among the patients. Furthermore, patients mostly preferred autologous and alloplastic bone grafts. Therefore, PRF-CS can be an alternative option to believers of certain religion, if they require grafting for the benefit of their dental health.

Other dental studies on socket augmentation have only focused on horizontal and vertical dimensions of the alveolar ridge. However, information on bone volume changes of the healing socket is still rarely obtainable in the literature (Das et al., 2016; Hauser. et al., 2013; Ten Heggeler et al., 2011). To the best of our knowledge, the current study is the first randomized clinical trial study to address the efficacy of PRF-CS in preserving alveolar ridge after socket augmentation.

CHAPTER 6: CONCLUSION

6.1 Introduction

The purpose of this randomized clinical study was to evaluate and compare the dimensional (vertical and horizontal) and volumetric changes of alveolar ridge when using combination of PRF and CS (PRF-CS) as compared to the combination of PRF and xenograft (PRF-X) in socket augmentation procedure.

6.2 Research outcomes

Within the limitations of this study the following can be concluded:

1. There was no significant difference in vertical alveolar bone resorption of sockets that have been augmented with PRF-CS and PRF-X.
2. There was no significant difference in horizontal alveolar bone resorption of sockets that have been augmented with PRF-CS and PRF-X.
3. There was no significant difference in soft tissue level of sockets that have been augmented with PRF-CS and PRF-X.
4. There was no significant difference in volumetric changes of sockets that have been augmented with PRF-CS and PRF-X.

6.3 Limitations and future work

The study has a few limitations, such as only premolar teeth were involved in the study and the small number of patients. Although the sample size was small, the study findings are clinically relevant. The protocol for PRF preparation in the current study utilized 1300 rpm for 8 minutes. As reported in a study by Dohan Ehrenfest et al. (2017), low centrifugation speed with time less than 12 minutes creates more vibration and kept the resonance threshold > 1 which become cumbersome to separate blood from fibrin clot. Additionally, it causes small clots and membranes formation and the membrane was dissolved within 3 days *in vitro*.

Future studies into this PRF-CS grafting material studies should include large number of patients with controlled groups to further assess the effectiveness of PRF-CS for socket augmentation procedure. Ideally, a split mouth design concept, with similar extraction sites should be applied in a clinical trial. However, it is closely impossible to obtain patients with the requirement of similar teeth to be extracted with healthy or restored neighbours. Moreover, future studies should also focus on implant survival rates and achievements in these grafted sites. In addition, trephined bone from these grafted sites should be collected just before implant placement for histomorphometric assessment to determine the bone composition and the fate of the graft particles after healing.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

1. **Sultan, T.**, Cheah, C. W., Vaithilingam, R. D. (2017). Platelet-rich fibrin-Calcium sulfate in socket preservation: A case report. Abstract presented at the 2017 International Association for Dental Research (IADR 2017). San Francisco, California, USA.
2. **Sultan, T.**, Cheah, C. W., Vaithilingam, R. D., Ibrahim, N. B. (2018). Effect of Platelet-rich fibrin with Calcium sulfate in socket augmentation. Abstract presented at the 2018 11th Postgraduate Conference, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia.