THE EFFECT OF LYOPHILIZED PLATELET RICH PLASMA IN A THIRD MOLAR EXTRACTION SOCKET AND ITS SURROUNDING TISSUES

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2019

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RESEARCH REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF CLINICAL DENTISTRY (ORAL AND MAXILLOFACIAL SURGERY)

FACULTY OF DENTISTRY
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KUALA LUMPUR

2019

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ORIGINAL LITERARY WORK DECLARATION

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Matric No: DGJ 160004

Name of Degree: MASTERS OF CLINICAL SCIENCE (ORAL AND MAXILLOFACIAL SURGERY)

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

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Field of Study: ORAL AND MAXILLOFACIAL SURGERY

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THE EFFECT OF LYOPHILIZED PLATELET RICH PLASMA IN A THIRD MOLAR EXTRACTION SOCKET AND ITS SURROUNDING TISSUES

ABSTRACT

Lyophilized platelet rich plasma (LPRP) is centrifuged platelet cells that have been freeze-dried into powder form, available for reconstitution for use at any point of time. α-granules in platelets contain mitogenic, chemotactic growth factors and associated healing molecules. Since the socket healing is dynamic in nature, this study tried to determine if repeated placement of LPRP helps in the healing process of the associated soft and hard tissue. **Methods:** Commercially prepared LPRP was randomized to be placed/injected into fresh sockets using a third molar surgical model. The control contralateral sockets of the same patient did not receive anything. The application was done intraoperatively, at 1 month and 2 months postoperatively. The endpoints measurement was post-operative pain, swelling, trismus, pocket depth at mid distal adjacent second molar and bone formation in extraction socket; the last was assessed radiographically. **Results:** Fifteen healthy young adults were recruited into this study. They received standard bilateral wisdom tooth surgery under general anaesthesia, with the LPRP prepared commercially at least 1 month ahead. There was no significant difference in post-operative pain, size of swelling, trismus and bony healing within their specific timeline of study. There was no early significant difference with regards to pocket depth at mid distal second molar, however, the LPRP group showed significant reduction in pocket depth at 2 months post-operative. **Conclusion:** LPRP seems to improve soft tissue healing at the adjacent tooth, suggesting that the application of LPRP may be beneficial to periodontal health.

Word count: 239 words

Key words: Lyophilized platelet rich plasma, Repeated LPRP, third molar surgery

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IMPAK PLASMA KAYA PLATELET YANG MELALUI PROSES PEMBEKUAN KERING DALAM SOCKET CABUTAN GIGI GERAHAM BONGSU

ABSTRAK

Plasma kaya platelet yang melalui proses pembekuan kering (LPRP) adalah sel platelet yang diproses secara pembekuan kering dimana produk akhir adalah serbuk platelet. Sebuk platelet yang dihasilkan boleh digunakan untuk proses regenerasi tisu dan dapat digunakan berulang kali. α-granul yang terdapat dalam platelet mengandungi fungsi mitogenik, dan faktor pertumbuhan chemotactic, yang berkaitan. Memandangkan proses penyembuhan soket adalah dinamik, kajian ini cuba menguji aplikasi berulang LPRP yang membantu dalam proses penyembuhan gusi dan tulang sekeliling soket cabutan gigi geraham bongsu. **Kaedah**: LPRP yang disediakan secara komersial diletakkan dan disuntik ke dalam soket cabutan gigi geraham bongsu secara rawak.. Soket cabutan gigi geraham bongsu yang bertentangan belah berfungsi sebagai soket kawalan dimana tiada sebarang intervensi dilakukan. Intervensi LPRP dibuat semasa operasi gigi geraham bongsu, pada bulan pertama dan yang, kedua selepas operasi gigi geraham bongsu. Parameter yang diukur adalah skala kesakitan selepas pembedahan, pembengkakkan, trismus, kedalaman poket gusi bersebelahan gigi molar kedua dan pembentukan tulang di soket pengekstrakan; pembentukan tulang dinilai menggunakan bantuan x-ray. Lima belas orang dewasa muda yang sihat telah dimasukan ke dalam kajian ini. Mereka menerima pembedahan gigi geraham bongsu di bawah anestesia umum, dengan LPRP disiapkan secara komersil sekurang-kurangnya 1 bulan dari tarikh operasi gigi geraham bongsu. **Keputusan**: Tidak ada perbezaan yang ketara dalam skala kesakitan selepas pembedahan, saiz pembengkakan, trismus dan penyembuhan tulang belakang. Tidak terdapat perbezaan yang ketara dari segi kedalaman poket pada molar kedua tengah distal pada bulan

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pertama, walau bagaimanapun soket cabutan dengan intervensi LPRP menunjukkan pengurangan ketara dalam kedalaman poket pada 2 bulan selepas operasi. **Kesimpulan:** LPRP membantu dalam penyembuhan poket gusi dan secara langsung menunjukkan bahawa penggunaan LPRP dapat memberi manfaat kepada kesihatan periodontal secara amnya.

Bilangan perkataan: 275

Kata Kunci :Plasma kaya platelet, aplikasi berulang, pembedahan gigi geraham bongsu

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AKNOWLEDGMENT

To my wife and two beautiful daughters for giving me the space and time to work on this study, I owe it all to you guys.

My teachers, Prof Dr Ngeow Wei Cheong and Associate Prof. Norliza Binti Ibrahim, for the continuous support during this study. Thank you for your patience, motivation, and immense knowledge shared during the process of this study. I could not have imagined having a better advisor and mentor.

A very special gratitude goes out to our partner StemTECH International Sdn. Bhd, Malaysia for giving us the opportunity to explore new clinical paradigm in Malaysia. Special thanks to Mr James Tien, Mr Ooi Ghee Chien and all their staff for the best technical support during the process of this study.

And finally, last but by no means least, to all lecturers, colleagues and staffs of the Department of Oro-Maxillofacial Surgical & Medical Sciences for the continuous support and mentoring during my master's programme.

Thank you for all your encouragement!

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LIST OF ABBREVIATIONS

BMP	: Bone morphogenic proteins
C	: Control
CEJ	: Cementoenamal junction
COX	: Cyclooxygenase enzymes
CPDA	: Citrate phosphate dextrose adenine
DMS	: Demarcation membrane system
DPT	: Digital panoramic radiograph
EGF	: Epidermal growth factor
FBC	: Full blood count
FGF	: Fibroblast growth factors
GF	: Growth factors
HGF	: Hepatocyte growth factors
HIV	: Human immunodeficiency virus
IGF-1	: Insulin like growth factors 1
IOPA	: Intraoral Periapical radiograph
L-PRF	: leucocyte and platelet rich fibrin concentrates
LPRP	: Lyophilized platelet rich plasma
NSAIDS	: Nonsteroidal anti-inflammatory drugs
P-PRF	: Leucocyte poor/pure platelet rich fibrin
PDAF	: Platelet derived angiogenesis factor
PDGF	: Platelet derived growth factors
PIS	: Patient information sheet
PO1M	: Post- operative one month
PO2M	: Post-operative two month
PO3M	: Post-operative three month
POD1	: Post-operative day one
POD2	: Post -operative day two
POD7	: Post -operative day seven
PRF	: Platelet rich fibrin
PRP	: Platelet rich plasma
RBC	: Red Blood Cell
TGF	: Transforming growth factors
VEGF	: Vascular endothelial growth factor

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CHAPTER 1: INTRODUCTION

1.1 Introduction

The loss of hard tissue in the oral cavity due to extraction, trauma and chronic periodontitis has been the leading cause of osseous deformity over the alveolar ridge. Deformity over the alveolar ridge presents a clinical challenge to the clinician trying to rehabilitate the oral cavity. The preservation and reconstruction of the alveolar ridge has become the holy grail of the research worldwide.

In view of the dynamic nature of post extraction healing, there has been a spectrum of method described and advocated in the literature ranging from the simple to complex techniques for alveolar ridge preservation. These methods have their unique pearls and pitfalls. In this study we investigated the effect of Lyophilized Platelet Rich Plasma (LPRP), a method used to preserve platelet rich plasma (PRP) on soft and hard tissue healing. A recent systematic review by Dragonas (Dragonas, Schiavo, Avila-Ortiz, Palaiologou, & Katsaros, 2019) stated that there is limited evidence regarding the effect of PRP in intraoral bone grafting procedure, and they suggested further research is needed to fully identify its indication and effectiveness in patients.

Generally, PRP is derived from the centrifugation of fresh blood taken via venepuncture, and processed chairside prior to placement into extraction socket. This method is simple and convenient. However, the quantity of the platelet used is not quantified prior to placement. This limitation can be addressed by modification into LPRP. LPRP is essentially platelet cells in plasma that has been freeze-dried into powder form with a minimum of 2 billion platelet cells per vial. Since the socket healing is dynamic in nature, with a process that takes up a few months to completely

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heal, we would like to see if repeated placement of LPRP helps in the healing process of the soft and hard tissue, at the same time determine if any symptoms are present in association with the surgical extraction sites studied.

Studies in literature has largely focused on chairside processed PRP for alveolar ridge preservation. However, these studies used multiple protocols to obtain PRP. Therefore, the aim of this study is to determine the effect of giving a repeat dose of quantified PRP that is available in lyophilized form on soft and hard tissue healing following surgical removal of third molars. In addition, its potential ability to reduce post-operative sequelae, namely swelling, pain, soft tissue healing and trismus was determined.

1.2 Research Purpose and Question

The main purpose of this study was to answer our clinical research question: Does repeated dose of quantified LPRP induce faster bone and soft tissue healing as well as reducing symptoms associated with surgical extraction? We hope our study using a third molar surgical model will help us to better understand the potential role of LPRP in the healing process of surgical extraction socket.

To achieve this aim, we are guided by these objective questions:

1. Does LPRP have effect on soft tissue healing?
2. Does LPRP have effect on post extraction bone healing?
3. Does LPRP have effect in post-operative pain?
4. Does LPRP have effect on post-operative swelling?
5. Does LPRP have effect on post-operative trismus?
6. Does LPRP have effect on periodontal pocket healing?

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All the parameters for the above questions will be further elaborated in the *Research Methodology* chapter. The next chapter discusses recent literatures relevant to this study.

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CHAPTER 2: LITERATURE REVIEW

2.1 History of Platelet

Platelet or as it was originally known as “plate” was discovered by Italian pathologist Giulio Bizzozero in 1881 during his *in vitro* flow chamber study. Giulio Bizzozero described the role of platelets during haemostasis, thrombosis and the discovery of bone marrow as the site of production (Bizzozero, 1881). James Homer Wright in 1906 further described platelets as detached portions of megakaryocytes (Wright, 1906).

2.2 Platelet Formation

Megakaryocytes are widely accepted as the precursor cells that produce and release platelets into the blood circulation. Megakaryocytes originate from pluripotent stem cells, which then go through multiple DNA replications with no cell division, which is called endomitosis. Once endomitosis is complete, the polyploid megakaryocytes will start rapid cytoplasmic expansion to form the demarcation membrane system (DMS), together with the accumulation of cytoplasmic protein and granules. At this stage there are three proposed models of platelet formation; (1) the megakaryocyte cytoplasm will form beaded cytoplasmic extension described as proplatelets or platelet, (2) via platelet budding and finally (3) via cytoplasmic fragmentation through the DMS (Michelson, 2007).

2.3 Physiology of platelets

Platelets are an anucleate cells, measuring approximately 2 μm - 5 μm in diameter, 0.5 μm in thickness with a volume of 6-10 femtolitres. Being discoid in shaped with secretory granules, they have the smallest density of any blood cell. They

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have a life span of 7-10 days (Blair & Flaumenhaft, 2009; Michelson, 2007). In three dimensions, the platelets have an appearance similar to gyri and sulcus of the brain. At any given time, there are around 150,000 to 400,000 platelets per μL. Besides their prominent role in thrombosis and haemostasis, the role of platelets in atherosclerosis, wound healing, host defence, malignancy and angiogenesis has been shown to play an important role in recent studies.

2.4 Functional platelet and their growth factors

There are three types of secretory granules in a platelet; they are α-granules, dense granule and lysosomes. α-granules make up the most of secretory granules (Blair & Flaumenhaft, 2009). Platelet α-granules contains mitogenic, chemotactic growth factors (GF) and associated healing molecules. These associated healing molecules which are present in an inactive form are Platelet derived growth factors (PDGF), Platelet derived angiogenesis factor (PDAF), Insulin like growth factors 1 (IGF-1), Transforming growth factors -β1, -β2 and -β3 (TGF-β1, TGF-β2, TGF-β3), Epidermal growth factor (EGF), Epithelial cell grow factor (ECGF) and a host of other cytokines. Besides that, the plasma that holds platelets also contains active proteins like Insulin like growth factor (IGF-1) and hepatocyte growth factors (HGF) (Lubkowska, Dolegowska, & Banfi, 2012). Table 2.1 shows the α-granules content and their functional categories.

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Table 2.1 : Listing α-granules content and their functional categories (Marx et al., 1998; Michelson, 2007)

Category	Term	Biological activities
Adhesive proteins	VWF + pro-peptide Fg, Fn, Vn TSP-1 Laminin-8	Cell contact Interactions, clotting, extracellular matrix composition
Clotting factors and associated proteins	Factor V/Va, Factor XI, multimerin, gas6, protein S, high molecular weight kininogen, antithrombin tissue factor pathway inhibitor (TFP1)	Thrombin production and its regulation angiogenesis
Fibrinolytic factors and associated proteins	Plasminogen, PAI-I, u-PA, Osteonectin, α2-antiplasmin, histidine-rich glycoprotein, TAFI, α2-macroglobulin	Plasmin production and vascular modelling
Proteases and anti-proteases	Tissue inhibitor of metalloprotease -4(TIMP-4) Metalloprotease-4, platelet inhibitor of FIX, protease nexin-2, C1 inhibitor , α1-antitrypsin	Angiogenesis, vascular modelling, regulation of coagulation, regulation of cellular behaviour
Growth factors cytokines and chemokines	PDGF, TGF-β-1 and EGF, IGF-1, VEGF (A and C), bFGF and FGF-2, hepatocyte growth factor, RANTES, IL-8, MIP-1α, growth -regulated oncogene-α, ENA-78, MCP-3, angiopoietin-1, IL-1β, IGF BP-3, neutrophil chemotactive protein	Chemotaxis, cell proliferation and differentiation angiogenesis
Basic protein and others	PF4, β-thromboglobulin, platelet basic protein, connective -tissue-activating peptide III, neutrophil-activating- peptide-2, endostatins	Regulation of angiogenesis, vascular modelling, cellular interactions
Others	Chondroitin 4-sulfate, albumin, immunoglobulins	Diverse
Membrane glycoproteins	αIIbβ3, αvβ3, GPIb, PECAM-1, most plasma membrane constituents, receptors for primary agonist,CD40L, tissue factor, P-selectin	Platelet aggregation and adhesion, endocytosis of proteins, inflammation, thrombin generation, platelet-leukocyte interaction.

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2.5 The application of Platelet Rich Plasma (PRP) in Oral and Maxillofacial Surgery

Platelet rich plasma (PRP) is also known as platelet concentrate, Matrix Platelet Rich Fibrin (PRF), platelet rich growth factors (GF's), and Platelet rich fibrin (PRF). The term PRP was first coined by haematologists in 1970 to indicate thrombocytopenia (Alves & Grimalt, 2018). In the maxillofacial region, the use of PRP was first introduced by Whitman (Whitman, Berry, & Green, 1997) to repair cleft, mandibular reconstruction and implant placement. Marx (Marx et al., 1998) claimed that the true clinical value of PRP lies in its speeding effect on autograft bone healing (enhanced density and maturity), although he also found soft tissue improvement at the donor site of split skin grafts in a different study (Marx, 2004).

With regards to studies that determined soft and hard tissue healing, Mozzati (Mozzati M, 2007) observed only enhanced soft tissue healing, while Ogundipe (Ogundipe, Ugboko, & Owotade, 2011) reported no beneficial effects on both soft and hard tissue healing. For studies that reported enhanced healing on hard tissue healing, all reported favourable early bone formation. However, the long term beneficial effect of hard tissue healing was variable. For example, Célio-Mariano (Celio-Mariano, de Melo, & Carneiro-Avelino, 2012) reported significantly faster bone formation in sockets treated with PRP and found significant bone formation was observed in the first 3 months for this group. However, no statistical differences were observed on the sixth month of investigation. Vivek (Vivek & Sripathi Rao, 2009) found similar outcome, with no difference in bone density being observed even earlier at 4 months. In contrast, Nathani (Nathani, Sequeira, & Rao, 2015) reported higher radiological bone density even at 4 months at the PRP treated sites. Antonello (Antonello Gde et al., 2013) later reported that the healing of PRP treated third molar sockets was

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significantly different from that of control sockets which did not receive any substance at 1, 3, and 6 month follow up. Although studies on the use of bone graft impregnated with autologous platelet–rich plasma has shown remarkably positive, the results obtained is not consistent results. The addition of collagen or biomaterial scaffolds did not seem to alter the variable outcome in long term results. Sammartino (Sammartino, Tia, Gentile, Marenzi, & Claudio, 2009) reported that the association of PRP to resorbable collagen membrane of porcine origin showed earlier signs of bone maturation histologically but not a higher grade of bone regeneration. Arenaz-Búa (Arenaz-Bua et al., 2010) reported that there was no further acceleration in bone formation at 6 months, even in those cases where PRP was mixed with autologous bone or other biomaterials. Although studies that look into the results on the use of bone graft impregnated with autologous platelet–rich plasma has shown remarkably positive results. In addition, a number of clinical studies that evaluated the use of autologous PRP for sinus floor augmentation to improve the height of posterior maxilla have reported no statistically significant benefit (Butterfield, Bennett, Gronowicz, & Adams, 2005; Kassolis, Rosen, & Reynolds, 2000; Maiorana, Sommariva, Brivio, Sigurta, & Santoro, 2003). A systematic review in 2014 reported that the scientific evidence for the use of PRP to promote third molar socket healing was poor (Barona-Dorado, Gonzalez-Regueiro, Martin-Ares, Arias-Irimia, & Martinez-Gonzalez, 2014). Anitua (Anitua, Andia, Ardanza, Nurden, & Nurden, 2004) has studied PRP extensively in many disciplines of medicine and concluded that it does promote significant bone healing. However, current clinical results are not reproducible due to various techniques used. At least 19 clinical studies had tried to determine the healing effect of PRP, as shown in Table 2.2. Of these, 12 assessed both soft and hard tissue healing, 2 assessed soft tissue healing alone, and 5 assessed hard

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tissue healing alone. Table 2.2 summarizes pertinent literatures related to the outcome of PRP application in extraction sockets

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Table 2.2 : Summary of the outcome of PRP on extraction sockets

Authors	Subject/Research methods	Outcome	PRP production technique
(Simon, Manuel, Geetha, & Naik, 2004) n = 14 SOFT TISSUE: √ HARD TISSUE: √	Individual design i.e. 7 PRP & 7 non-treated control underwent third molar extraction. Assessed over 1,3,5,7,9,12, and 16 weeks post operatively for sequalae of surgery and soft tissue healing.	Reduced pain and better mouth opening when topical PRP gel was used. Soft tissue healing differed significantly between the two groups, with the test group exhibiting better results. Radiographic evidence of bone formation was visible as early as 1 week in PRP group.	Not explained.
(Sammartino et al., 2005) n = 18 SOFT TISSUE: √ HARD TISSUE: √	Split mouth design i.e. 18 PRP with 18 contralateral non-treated control underwent third molar extraction. Gingival recession was assessed at 12 and 18 weeks. Osseous biopsy was performed at the PRP treated sites after 12 weeks	Notable reduction in the probing depth and improvement in the probing attachment level PRP group at 12 weeks postoperative Histology confirms formation of new bone tissue in the bone defect in 94.49% of PRP treated sites.	Vacutainer tubes used containing 10% trisodium citrate ($Na3C_6H_5O_7$). PRP was collected together with 1–2 mm of the RBC fraction to form gel.
(Mozzati M, 2007) n = 5 SOFT TISSUE: √ HARD TISSUE: X	Split mouth design i.e. 5 PRP with 5 contralateral non-treated control underwent third molar extraction. Periodontal healing was assessed at 2 months, radiographic examination done at 1 week, 1 month & 2 months.	The PRP treated site did not show reduced swelling but a reduction in the pain was reported. Periodontal healing showed improvement in the PRP treated sites. No radiographic evidence of bone formation visible in both treated group at 2 months.	PRP was produced after extraction mixing the platelet concentrate (10cc) with autologous trombone (1-1.5cc) (taken from the RBC's fraction) then activated with calcium gluconate (0.8cc)in a no-eparined becker.

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Table 2.2 : Continued

Authors	Subject/Research methods	Outcome	PRP production technique
(Sammartino et al., 2009) n = 18 SOFT TISSUE: √ HARD TISSUE: √	Split mouth design i.e. 18 PRP + resorbable collagen membrane of porcine origin with 18 contralateral PRP non-treated control underwent third molar extraction. Periodontal healing was assessed at 12 and 18 weeks. Osseous biopsy was performed at the PRP treated sites after 12 weeks	Both sides showed comparable results. The association of PRP to resorbable collagen membrane of porcine origin showed earlier signs of bone maturation, histologically but not a higher grade of bone regeneration.	Vacutainer tubes used containing 10% trisodium citrate ($Na3C_6H_5O_7$). PRP was collected together with 1–2 mm of the RBC fraction to form gel.
(Gawande & Halli, 2009) n = 20 SOFT TISSUE: √ HARD TISSUE: √	Split mouth design i.e. 20 PRP with 20 contralateral non-treated control underwent third molar extraction. IOPA radiographs and DPT were taken on 2nd postoperative day and subsequent1st, 3rd and 6th month. Bone density was evaluated radiographically using grey level histogram.	There was significantly less postoperative swelling on the PRP treated side. Good soft tissue healing response in PRP treated sites as compared to the other site. Radiographically there was rapid bone regeneration in site treated with PRP when compared to control.	Centrifugation tubes contain CPDA. Autologous thrombin was recovered from a portion of PRP and was mixed with PRP in the 1:4 ratio to form a coagulate.
(Vivek & Sripathi Rao, 2009) n = 10 SOFT TISSUE: √ HARD TISSUE: √	Split mouth design i.e. 10 PRP with 10 contralateral non-treated control underwent third molar extraction. IOPA was taken preoperatively, and at 8 weeks, 12 weeks, 16 weeks postoperatively with assessment of the extraction site done at 4 months to evaluate the change in bone density.	There was no difference in pain scores. Enhanced soft tissue healing and increased rate of bone formation was observed in the PRP treated sites. However, no difference in bone density was observed at 4 months.	PRP of patients was prepared by taking 10ml of blood and centrifuged in laboratory and PRP was separated from blood.

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Table 2.2 : Continued

Authors	Subject/Research methods	Outcome	PRP production technique
(Arenaz-Bua et al., 2010) n = 82 SOFT TISSUE: -- HARD TISSUE: √	Patients were divided into 5 groups. In two groups (groups 1 and 2) they compared the control socket (non-treated) with the study socket (2 types of PRP). The remaining 3 groups were PRP + autologous bone, PRP + synthetic calcium hydroxyapatite, and PRP + Allogeneic demineralized bone matrix	Bone gain observed at 3rd postoperative month compared to the immediate postoperative period was higher in groups 1, 2 and 3 (PRP and autologous bone) and lower in the PRP + synthetic calcium hydroxyapatite, and PRP + Allogeneic demineralized bone matrix groups. However, there was no further acceleration in bone formation at 6 months, either in cases in which PRP was used alone, nor in those cases where PRP was mixed with autologous bone or other biomaterials.	PRP was obtained in two different services of haematology. Both methods used a double spin system, but with different parameters and different methods for extracting the supernatant (one used a laminar flow hood and another by pipetting).
(Rutkowski, Johnson, Radio, & Fennell, 2010) n = 6 SOFT TISSUE: -- HARD TISSUE: √	Split mouth design i.e. 6 PRP with 6 contralateral non-treated control underwent third molar extraction. Observer evaluations plus digital radiographs were done at the 3 days post-operative plus weeks 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24.	Early increase in bone density at the PRP treated sites noted. It required 6 weeks for control extraction sites to reach comparable bone density that PRP treated sites achieved at week 1. In all there was significant increase in bone density in the socket treated with PRP over 25 week period	Whole blood was drawn using two 4.5 mL BD Vacutainer tubes containing 0.45 mL of the anticoagulant trisodium citrate (9 : 1). "Buffy Coat'' technique was used to obtain PRP. Gelfoam was placed into both sockets.

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Table 2.2 : Continued

Authors	Subject/Research methods	Outcome	PRP production technique
(Ogundipe et al., 2011) n = 60 SOFT TISSUE: X HARD TISSUE: X	Individual design i.e. 30 PRP & 30 non-treated control underwent third molar extraction. Patients were recalled at 1-, 3-, 5-, 7-, & 14-day postoperative for clinical outcome. Patients were also recalled at the 4th, 10th, and 16th week postoperatively for radiographic assessment.	The mean postoperative pain score was lower for the PRP group at all time points when compared with the control. The mean bone scores for overall density and trabecular pattern was not different between both groups.	Centrifugation tubes contain citrate phosphate dextrose. Activation of PRP was performed with a mixture of 0.5 mL 10% calcium chloride and 1,000 U bovine thrombin.
(Celio-Mariano et al., 2012) n = 15 SOFT TISSUE: -- HARD TISSUE: √	Split mouth design i.e. 6 PRP with 6 contralateral non-treated control underwent third molar extraction. IOPA was used to evaluate healing bone density at 7 days, 1, 2, 3, and 6 months postoperatively.	Faster bone formation was reported at the first three months, but no difference was observed at 6 months. There were higher means of radiographic bone density in PRP treated sockets.	Autologous blood collected was kept into 5-mL Vacutainer tubes containing 3.2% sodium citrate ($Na3C_6H_5O_7$). 50µL of 10% $CaCl_2$ was added for 1.0 mL of PRP.
(Kaul, Godhi, & Singh, 2012) n = 25 SOFT TISSUE: √ HARD TISSUE: √	Split mouth design i.e. 25 PRP with 25 contralateral non-treated control underwent third molar extraction. IOPA was used to evaluate healing bone density & alveolar bone level after 1st, 2nd & 7th day and 3rd & 6th month post-operative.	PRP treated sockets showed significantly less dehiscence. It has greater reduction in probing depth from initial period to 3 and 6 months. The decrease in alveolar bone level was highly significant in PRP grafted sockets in 3rd and 6th month post operatively.	Centrifugation tubes contain citrate phosphate dextrose adenine (CPDA). 2.5 ml of PRP is mixed with 0.08 ml of $CaCl_2$ form thrombin. 6 ml PRP + 1 ml autologous thrombin form PRP gel.

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Table 2.2 : Continued

Authors	Subject/Research methods	Outcome	PRP production technique
(Antonello Gde et al., 2013) n = 25 SOFT TISSUE: -- HARD TISSUE: √	Split mouth design i.e. 20 PRP with 20 contralateral non-treated control. Patients underwent two separate surgical procedures, with a minimum interval of 15 days between them. Radiographic assessment for bone healing was done immediately after extraction and at 1, 3, and 5 months postoperatively.	PRP treated sockets in the mandible and the maxilla showed significantly increased healing from that of control sockets at 1-, 3-, and 6-month follow up. Greater differences were detected in the maxilla.	Centrifugation tubes contain 0.5 mL sodium citrate 3.8% solution. A 1-mL aliquot of autogenous thrombin was added to every 4 mL of the plasma fraction obtained to induce formation of a dense clot.
(Dutta, Singh, Passi, & Patter, 2015) n = 60 SOFT TISSUE: √ HARD TISSUE: √	Individual design i.e. 30 PRP & 30 control underwent third molar extraction. Patients were assessed on day 3, 7 and 14 for dry socket and soft tissue healing. Radiographic assessment for bone healing was done at 3rd week, 2nd month and 4th month	There was less postoperative discomfort on the PRP treated sites. Soft tissue healing was significantly better in PRP treated site. There was significant rapid bone regeneration in PRP treated sockets.	Centrifugation tubes contain 0.4ml CPDA. PRP was activated with $CaCl_2$ to form PRP gel.
(Nathani et al., 2015) n = 10 SOFT TISSUE: √ HARD TISSUE: √	Split mouth design i.e. 10 PRP with 10 contralateral Hydroxyapatite + Bioactive glass (bioactive ceramics HA/BG)]-treated control. Patients were assessed for postoperative pain and soft tissue healing. Radiological assessment was done at 8, 12 and 16 weeks post-operative	PRP treated sites reported less pain, and better soft tissue healing for the first 3 post- operative day. Radiological assessment at 4 months showed higher bone density at PRP treated sites.	Intravenous blood was transferred to plastic tubes containing 1 ml of 3.2% sodium citrate. PRP was mixed with 0.5–1 cc of 10% $CaCl_2$ to produce gel form

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Table 2.2 : Continued

Authors	Subject/Research methods	Outcome	PRP production technique
(Doiphode et al., 2016) n = 30 SOFT TISSUE: √ HARD TISSUE: --	Individual design i.e. 15 untreated control, 15 PRP /15 PRF (the latter group underwent split mouth design) underwent third molar extraction.	Reduction of periodontal pocket depth was more PRF>PRP>control	Centrifugation tubes contain CPDA. 2 ml of PRP was treated with 60 µl of citrate inhibitor sterile 10% $CaCl_2$. Autologous thrombin rich plasma was mixed with PRP in the 1:4 ratios to form PRP gel. To produce PRF, the anti-coagulant free vacutainers were placed in the centrifuge at 3000 rpm for 10 min
(Gandevivala et al., 2017) n = 50 SOFT TISSUE: √ HARD TISSUE: --	Individual design i.e. 25 PRP & 25 control underwent third molar extraction. Healing was evaluated by visual control and cautious exploration of a periodontal probe on the 1st, 3rd, 7th , and 60th day post-operative	PRP treated groups showed significant percentage reduction in facial swelling at day 3. Also significantly less PRP treated patients had wound dehiscence. Probing depth shown significant reduction in PRP treated patients.	Centrifugation tubes contain 1 ml CPDA. Calcium gluconate alone was mixed with PRP to form an autologous platelet gel
(Bhujbal et al., 2018) n = 20 SOFT TISSUE: √ HARD TISSUE: √	Split mouth design i.e. 20 PRP with 20 contralateral non-treated control underwent third molar extraction. Healing was evaluated by visual control. Changes in bone density was assessed using digital panoramic radiograph (DPT) 3 and 6 months post-operative	Swelling was significantly less at the PRP treated sides. Soft tissue healing was better in the PRP sockets. Similarly, mean bone density at the 3rd and 6th postoperative months was significantly higher.	Centrifugation tubes contain CPDA. 0.5–1 mL of 10% calcium chloride was added to the PRP, leading to the formation of PRP gel.

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Table 2.2 : Continued

Authors	Subject/Research methods	Outcome	PRP production technique
(Gurbuzer et al., 2010) N = 14 SOFT TISSUE : - HARD TISSUE : √	Split mouth design – 14 PRF with 14 contralateral non treated control underwent surgical extraction. All patients had soft tissue impacted third molars. Changes in bone healing was assessed with technetium-99m methylene diphosphonate uptake as an indication of enhanced bone healing.	PRF might not lead to enhanced bone healing in soft tissue impacted mandibular third molar extraction sockets 4 weeks after surgery	10 mL of venous blood was collected in a sterilized dry, neutral glass tube without an anticoagulant. After immediate centrifugation at 400g (2,030 rpm) for 10 minutes, the platelet-poor plasma was discarded. PRF was dissected approximately 2 mm below its connection to the red corpuscle beneath to include remaining platelets, which have been proposed to localize below the junction between PRF and the red corpuscle.

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2.6 Difference between Platelet rich plasma and platelet rich fibrin

Platelet rich plasma (PRP) adds bovine thrombin and calcium chloride to the peripherally drawn blood, which is then centrifuged two times. The first spin is done at 1300 rpm for 10 minutes and the second spin is done at 2000 rpm for 10 minutes. Fibrin polymerization is directly dependent on the amount of bovine thrombin and calcium chloride used. Microscopically, it forms bilateral junctions with thickening of the fibrin polymers, thus producing a rigid network which is unfavourable for cytokines and cellular migration (Kumar & Shubhashini, 2013). PRP gives an immediate release of growth factors into its surrounding tissue. However, there are some concerns with the use in commercial bovine thrombin in the market, as it has been associated with the development of antibodies to clotting factors V, XI and thrombin which may lead to life threatening coagulopathies (Bansal, Garg, Khurana, & Chhabra, 2017). The benefit of using PRP is that it is free from blood borne disease such as hepatitis and human immunodeficiency virus (HIV). Furthermore, the presence of platelets helps to attract cytokines and growth factors towards the injured site, which was not achievable with fibrin glue.

Platelet rich fibrin (PRF) does not use any anticoagulants and obtained with a single spin of 3000rpm for 10 minutes. Platelet undergoes natural polymerization once they come in contact with glass particles of the test tube resulting in a physiologic thrombin concentrate. Microscopically, it forms equilateral junctions with fine and flexible fibrin network to support cytokines and cellular migration. This gives great elasticity to the fibrin matrix. Better still, growth factors are released over a period of 7 days or more (Bansal et al., 2017). The benefit of using PRF is similar to PRP, with added benefit of slow growth factor release during the healing process. PRF membrane should be prepared just before surgical procedures as it is known to shrink due to

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dehydration. PRF cannot be stored for a long time due to the risk of bacterial contamination (Bansal et al., 2017).

2.7 Types of Platelet Rich Fibrin

PRF are then further divided into two types, leucocyte poor/pure platelet rich fibrin (P-PRF) or (L-PRF or Choukroun's PRF). P-PRF undergoes 2 step processes where the blood is first centrifuged to separate the buffy coat and platelet poor plasma, which is then transferred to a tube containing calcium chloride ($CaCl_2$). The buffy coat, platelet poor plasma and calcium chloride is centrifuged for 15 minutes. Very low amount of leucocytes are collected due to the use of separator gels. Special blood tube has to be purchased to be able to get pure platelet rich fibrin. The efficiency of this method has yet to be published (Kumar & Shubhashini, 2013).

L-PRF/Choukron's PRF on the other hand is a simple and free technique. Blood is collected into an anticoagulant free tube. The tube is centrifuged at 2500 rpm for 10 minutes as per Choukroun protocol (Choukroun et al., 2006). Due to the absence of anticoagulants, the coagulation cascade begins within a few minutes. The result is a fibrin clot containing platelets left in the middle of the tube, between blood cells and the acellular plasma. The fibrin clot is placed on a grid that produces autologous fibrin membrane (Kumar & Shubhashini, 2013).

2.8 Lyophilized Platelets

Lyophilization literally means to freeze dry a substance. In 1935, Flosdorf was experimenting in preserving biological material for delayed use when he developed this technique. Lyophilization later became a life saver during World War II where preserved blood plasma was used as resuscitation fluid. Brinkhous and Read (Brinkhous & Read, 1978) in 1978 suggested freeze drying protocol to extend the shelf

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life of fixed platelets. They concluded that the fixed freezed platelets can be used for a year. However in 1989, when Read and Bode evaluated the earlier protocol, it was apparent that the platelets were incapable of normal platelet function. It was Klein et al. (1955) who reported the first experiment using a freeze dried preparation of extracted platelet suspension. This freeze dried preparation was infused into a leukaemia patient to help restore haemostasis, unfortunately this approach was not reproducible (Bode & Fischer, 2007).

In a recent study by Silva (da Silva et al., 2018), platelet rich plasma lyophilization enables growth factor preservation and functionality when compared with fresh PRP, with the latter being unable to show normal aggregation. In PRP, the platelets may have been used up during the process of activation. However the growth factors which are the essence of PRP were maintained. Nevertheless, for the lyophilized platelet, there was a decrease in platelet count of around 57%. However, both fresh and lyophilized PRP showed no significant difference growth factor concentration (VEGF, EGF, PDGF, TGF-β). Cell proliferation analysed in fibroblast culture showed an increase of proliferation in the first 24 hours for both fresh PRP and LPRP, as compared to 48 hours for the control group (da Silva et al., 2018). Muraglia's (Muraglia et al., 2014) study found that freeze dried PRP regenerated at 5% concentration, enhanced proliferation rate of osteoblast, fibroblast and bone marrow. They also pointed that the rate of proliferation is not proportionate with the increase of concentration. Their study found that the increase in concentration can become toxic to the cell.

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2.9 Proposed mechanism of action PRP in mucosal and bone healing

The skeleton is a very robust micro system where it undergoes constant remodelling cycle to main homeostasis. Growth factors and cytokines play a major role in this homeostasis. This cycle entails certain cell population being identified and differentiates to become cells in helping with both resorption and formation of bone. Platelets derived growth factors (PDGF) plays a key mediator role by acting as a chemoattractant and mitogen that aid angiogenesis and tissue repair (Hollinger, Hart, Hirsch, Lynch, & Friedlaender, 2008).

When an injury happens secondary to surgical assault, the first respond is the activation of the coagulation cascade followed by formation of stable blood clot over the injured site. Prior to that, platelet cells aggregate over the injured area and release cytokines together with PDGF from the α-granules. Then, neutrophils and macrophages are attracted and activated with the help of PDGF. Granulation tissue is formed with the help of PDGF and other growth factors. Mesenchymal cells are attracted, with the help of PDGF to the site of injury via chemotaxis followed by mitogenesis where fibroblast, osteoblast and chondrocytes also contribute to the healing process. Beside PDGF, TGF-β also helps in chemotaxis and healing. Bone morphogenic proteins (BMP), the morphogenetic signal which decides tissue modelling all over the body seems to help FGF in bone healing regulation. Figure 2.1 below helps explain this concept (Hollinger et al., 2008).

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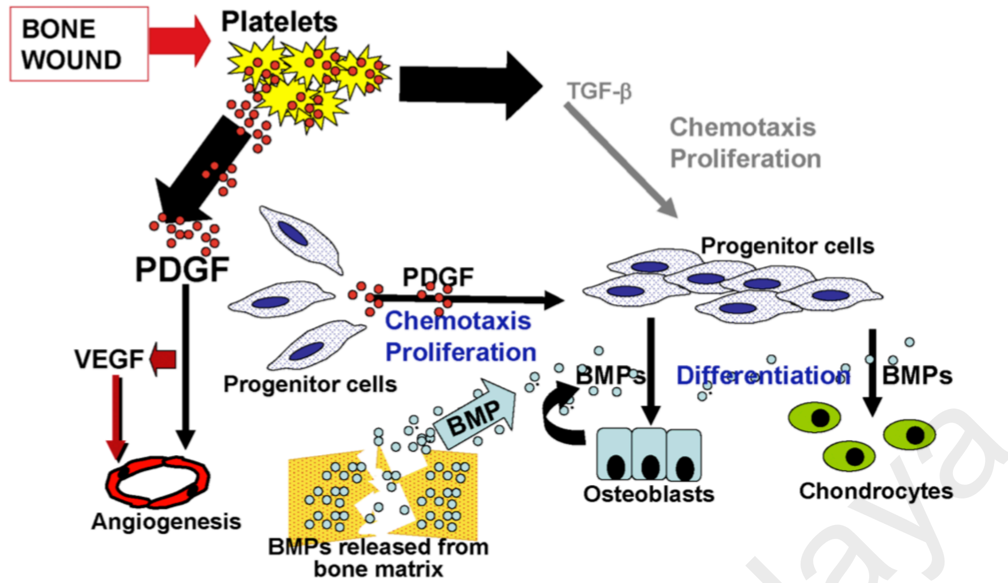

Figure 2.1: Mechanism of action and bone regeneration (Ayoub, 2009)

Lindeboom (Lindeboom et al., 2007) suggested that the microcirculation is an essential part of healing of the oral mucosa. Lindeboom assessed the changes at the mucosa capillaries once growth factors have been added. They found a statistically significant increase of capillary density as compared to placebo for 14 days. However, after 14 days they noted that the density of the capillary has equalised. This shows an increase in early microcirculatory angiogenesis, hence improves mucosa repair for immediate repair. Vascular Endothelial growth factor (VEGF) has been identified as the link stimulating the endothelial cell migration into the injured site, but the exact mechanism of control is unclear (Lindeboom et al., 2007).

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2.10 Extraction Socket

2.10.1 Dimensional changes to the alveolar process post extraction

Tooth extraction, trauma and odontogenic infection/tumour causes significant bone resorption followed by a period of internal bone reorganization. This reorganization happens in accordance to Wolff's law which dictates that functional changes of bone will be followed by changes to the internal architecture and its external shape (Lam, 1960). The amount of bone loss varies widely from one clinical condition to another. These may be affected by both systemic and local factors such as tooth position in the arch, bone disease, gingival biotype, number of tooth extracted, type of arch, periodontal disease, immediate prosthesis used and smoking. The presence of dehiscence and/or fenestration will worsen bone resorption (Bhujbal et al., 2018).

Craddock as early as in 1951 discovered that post extraction resorption happens in 2 stages. The early stage is an acute resorption that happens during the healing stage. For the late stage, the resorption phase is slow and goes on indefinitely. Lam (Lam, 1960) showed that changes of the contour will reach its peak activity in 3 to 4 weeks post extraction. The resorption thereafter is less, but it continues up to a period of four to five months. The extraction socket will have little to no changes when it is one year old. Ackerman (Ackermann, 2009) suggested that reduction of height and width of the extraction socket is largely due to loss of bundle bone. Bundle bone is the histological term given to the bone over the alveolar process that encapsulates teeth. This is in fact a cortical bone where the collagen fibres of periodontal ligament are embedded. In a dog model, the extraction socket was filled with woven bone with significant loss of bundle bone. This loss of bundle bone has also been associated with significant loss of bone around the extraction socket.

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Amler (Amler, 1969) presented a time line associated of tooth socket healing in his paper. Table 2.3 summarises his findings.

Table 2.3: Timeline and histological changes to extraction socket

Stage	Events	Duration
First	Cessation of haemorrhage forms an initial blood clot	Same day after extraction
Second	Transformation of blood clot to granulation tissue with cords of endothelial cells associated to budding capillaries. There is evidence of epithelization.	4-5 days
Third	Connective tissue gradually replaces the granulation tissue. Appearance of osteoid at base of socket.	14-16 days
Fourth	Complete epithelial closure of the socket. Initiation of calcification process via osteoid formation at periphery of socket.	3-6 weeks
Fifth	Completion of bone fill and reduced osteogenic activity by the 16th week.	5-10 weeks

It has been shown that 2-4mm or 50% of the vertical height and 4 to 5mm width is lost during the first 4-6 months.

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2.10.2 Consideration for socket preservation

Socket preservation is important if the ridge is to be restored by removable/fixed prosthesis or implants. Its indication can be broadly divided into functional and aesthetics purpose. Aesthetics is important when we need to successfully keep the interproximal gingival contour and height of the interproximal papilla. This is paramount when we are dealing with the loss of anterior tooth. Besides the dimension of the ridge, soft tissue colour, contour and consistency is also important. Patients with a high lip line and thin biotype are susceptible to recession over time (Darby, Chen, & Buser, 2009).

As for the functional indication, these involve sockets where there is less than 1.5-2 mm of buccal plate with predominantly anterior aesthetic zone involvement. Besides that, in areas like posterior maxilla or mandible, the adjacent structures like the sinus and the inferior alveolar nerve can be exposed if socket preservation is not carried out. Finally for patients with multiple teeth extractions, socket preservation is essential to help maintain adequate ridge for oral rehabilitation (Darby et al., 2009).

2.10.3 Socket preservation modalities

Generally the technique is categorized into preservation with natural tooth, implants designed as roots, guided tissue regeneration and guided bone regeneration techniques. As for the materials for grafting, they include autogenous, allogenous, xenogeneic and alloplastic bone graft with osteoconductive material. Ideal graft material for socket preservation should prevent loss of volume of the socket by remaining in the socket until bone formation occurs. The materials used can be categorized based on the inert properties of osteoconductivity, osteogenicity and osteoconductivity. Osteoconductive material provides a passive porous scaffold for attachment osteoblast and osteoprogenitor cells and also allows vessel formation.

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Osteogenicity is the cellular quality of graft which allows adaptation to the native bone. Osteoinductivity is the ability of the graft to change stem cells into osteogenic cells (Laurencin, Khan, & El-Amin, 2006).

Two systematic reviews (Darby et al., 2009; Laurencin et al., 2006) showed that current treatment modalities include packing of extraction socket with:

1. bovine bone particles with complete flap closure
2. mix of bovine bone particles with porcine collagen and complete flap closure
3. cortico-cancellous porcine bone particles with complete flap closure
4. allograft particles with complete closure
5. alloplastic material with or without complete flap closure
6. autologous blood derived products, cell therapy, recombinant morphogenic protein 2 and primary flap closure.

They concluded that there is a strong evidence that the ridge is preserved in both height and width with only a slight difference regardless of grafting material used.

Darby (Darby et al., 2009) also investigated the need for primary closure following grafting, and concluded that primary closure is not always necessary and success does not depend on the closure techniques used. They also concluded that ridge preservation will be useful in reducing vertical and horizontal ridge alteration. In conclusion there is no evidence that one technique is better than the other in ridge preservation.

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2.11 Post third molar surgery sequalae

2.11.1 Oedema

Oedema is defined as an excess of plasma proteins in the interstitial space. Every surgical procedure will present with a variable degree of oedema. When there is surgical injury, inflammatory mediators like prostaglandin, leukotrienes and bradykinin will be released at the surgical site. These inflammatory mediators will cause vascular dilatation and increase permeability, thus resulting in oedema over the surgical site. This inflammatory response is a defence mechanism to help repair tissues that are damaged during surgery. Yaedu (Renato Yassutaka Faria Yaedu, 2018) showed there were risk factors that contribute to increased facial oedema. These risk factors are summarised in Table 2.4.

Facial oedema is one of the sequalae of dental extraction, usually affects the mandibular third molars more than any other sites of surgical extraction. Facial measurement for oedema can be performed by using facial bow method, ultrasound, stereophotographic, cuboid element, tape measurement, sonographic, photo, face scanning and taking a three dimensional mould. Oedema control can be done with cryotheraphy, hilotheraphy, low power laser to control inflammatory process, manual lymphatic drainage (gentle pressure following lymphatic system and kinesio taping (Renato Yassutaka Faria Yaedu, 2018). Besides the above methods, oedema control can also be achieved with medication. Literature has suggested usage of corticosteroid, analgesia (NSAIDS) and hyaluronic acid for oedema control. There has been some evidence of oedema reduction, however there is no proper study/protocol available in any clinical research to support this (Renato Yassutaka Faria Yaedu, 2018).

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Table 2.4: Risk factors associated with increased facial oedema

RISK FACTORS	CLINICAL FINDINGS
Body mass index (BMI)	People with higher BMI, develop greater oedema, however the reduction rate is faster compared to normal BMI
Type of surgery & Surgical trauma	Distal and horizonal impaction result in greater oedema due to osteotomy that causes greater surgical trauma
Surgeons experience	The surgeons experience is difficult to measure but it is associated with reduced surgical time, minimal trauma and minimal blood loss. All this factors in combination does reduce the inflammatory process
Blood Loss	The amount of blood loss during surgery has a liner correlation with postoperative oedema
Induced Hypotension	Hypotensive anaesthesia has been shown to improve development and reduce the amount of oedema.
Age	Older patients have prolonged inflammatory process thus leading to reduction of oedema.
Gender	High density bone and stronger muscles are associated with more oedema in males.
Vomiting	The link of nausea and vomiting with oedema has not been shown in literature but clinically when there is increased effort during the motion of vomiting, there is increase of facial oedema.
Postoperative rest	The placement of patient at head propped up 30 degrees helps to reduce the pressure over the blood vessels of the face thus reducing bleeding and facial oedema.

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2.11.2 Post-operative pain

Surgical procedure causes local damage to tissue which results in the release of prostaglandin, histamine, serotonin, bradykinin, substance P to activate the nociceptors. These nociceptors will transmit pain signals to the brain via ascending pathways (stimulating) and descending pathway (inhibition). The ascending pathway starts from the peripheral tissues and ends in the contralateral somatosensory cerebral cortex. It goes through a series of integration known as the 3 order of neurons. They are transmitted by the Aδ and C fibres of the primary afferent neurons. Whereas the descending pathways starts from the thalamus, descends down to the dorsolateral funiculus and synapses into the dorsal horn which will release serotonin, noradrenaline and enkephalins (Use dental pain pathway which is different). The body's endogenous opioids system works at the periaqueductal grey area and nucleus raphe magnum, where it travels down through the descending pathways to the spinal level inhibitory interneurons. Opioid μ receptors are found mainly in the ascending pathways (1st order efferent presynaptic membrane and 2nd order afferent post synaptic membrane). Tramadol works at the descending pathways where it inhibits the release of serotonin and noradrenaline. Non-steroidal Anti- Inflammatory drug (NSAID) works by inhibiting the activity of cyclooxygenase enzymes (COX1 or COX2) which are involved in the synthesis of mediators such as prostaglandin.

Besides that, reduction of pH oxygen tension and the increase in lactate concentration surrounding the surgical site will persist for several days. This will lead to peripheral sensitization via the muscles C-fibres.

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2.11.3 Trismus

Trismus or trismos, is a prolonged tetanic spasm of the masticatory muscle system. The temporary stiffness of the jaw starts postoperatively and peaks on the second day of surgery. The swelling usually resolves in one week or less depending on the type of procedure. Besides surgery being the common cause of trismus during impacted third molar removal, low grade infection, multiple puncture during the inferior alveolar block and elevation of flap pass the external oblique ridge has been recorded as the cause of trismus during third molar surgery. Balakrishan (Balakrishnan, Narendar, Kavin, Venkataraman, & Gokulanathan, 2017) concluded that trismus in impacted third molar removal is multifactorial in nature. There should be improvement of mouth opening in 7-10 days post-surgery.

2.11.4 Effects of PRP on the clinical sequala of third molar surgery

Eleven of the studies listed in Table 2.2 also determined the effect of PRP on clinical sequelae of third molar surgery (Arenaz-Bua et al., 2010; Bhujbal et al., 2018; Celio-Mariano et al., 2012; Gandevivala et al., 2017; Gawande & Halli, 2009; Mozzati M, 2007; Nathani et al., 2015; Ogundipe et al., 2011; Rutkowski et al., 2010; Simon et al., 2004; Vivek & Sripathi Rao, 2009). Similar to the study on soft and hard tissue healing, the results obtained were variable. Pain reduction was reported in 4 studies (Mozzati M, 2007; Nathani et al., 2015; Ogundipe et al., 2011; Simon et al., 2004), while another 5 studies did not reported any difference with the non-treated control (Arenaz-Bua et al., 2010; Bhujbal et al., 2018; Gawande & Halli, 2009; Rutkowski et al., 2010; Vivek & Sripathi Rao, 2009). Four studies reported reduction in swelling (Bhujbal et al., 2018; Gandevivala et al., 2017; Gawande & Halli, 2009; Rutkowski et al., 2010) but another 3 studies reported no improvement (Arenaz-Bua et al., 2010; Mozzati M, 2007; Ogundipe et al., 2011). Lastly, only Simon (Simon et al., 2004)

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reported improvement of trismus, while Arenaz-Búa (Arenaz-Bua et al., 2010) and Ogundipe (Ogundipe et al., 2011) did not find any difference with control.

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CHAPTER 3: METHODOLOGY

3.1 Research Methods

This is a prospective randomized study using the impacted third molar surgery model on outpatients attending to the Oral and Maxillofacial Surgery Clinic of the Faculty of Dentistry of the University of Malaya. This study investigated soft and hard tissue healing, and symptoms associated with third molar extraction in LPRP treated and non LPRP treated socket sites. Ethical approval for the study was obtained from the Medical Ethics Committee, Faculty of Dentistry, University Malaya (No: DF OS1801/0002(P)) (Appendix E), prior to starting the study.

3.2 Subjects

A sample of 15 patients with clinical indications for extraction of two lower impacted third molars with similar orientation, depth, and root morphology were identified from the pool of patients presenting at Oral and Maxillofacial Clinic, Dental Faculty, University Malaya. These samples consisted of healthy American Society of Anaesthesiologist classification of Class 1 (ASA1) male or female patients aged 18 to 35 years. Excluded patients are those presented with any blood dyscrasia, patient taking anti-platelet, patient with chronic alcoholism, or those suffering from myasthenia gravis, sleep apnoea, severe respiratory failure, severe hepatic impairment, narrow angle glaucoma, or females with pregnancy or lactating infants. Clinically indicated patients who fulfilled inclusion criteria were invited into the study, with relevant information provided in the Patient Information Sheet (PIS) (Appendix A). Patient were then given time to understand the nature of the study and were encouraged to ask any question

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relevant to their enrolment and/or surgery. Patients who were not keen to participate in the study were given an appointment for surgical removal as per routine clinic protocol.

During the first visit, interested patients were informed of their diagnosis and the need for surgical intervention, with an outline of the treatment plan together with their rights and responsibilities. Patients were sent for blood investigation, namely baseline Full Blood Count (FBC) to ensure that their haemoglobin and platelet levels were within normal range. During the second visit, these patients were provided with a written informed consent entailing the title of the study, possible risk and complications that may arise from the surgery (Appendix B). Once consented, patient's pre-blood donation vital signs (blood pressure, oxygen saturation, pulse rate, and temperature) were recorded into a form designed for this study (Appendix C). After making sure that their vitals were within normal range, blood collection was carried out using the blood donations kit provided by StemTECH International the collaborator of this research. The blood collection kit contains one JMS® single blood transfer bag with anticoagulant (Citrate-Phosphate-Dextrose-Adenine), 2 BD Vacutainer® SST (contains silica and polymer gel) and a consent booklet for blood taking procedure and screening of infectious diseases (Figure 3.1). Around 300 -350 ml of their periphery blood were collected according to strict protocol. Around 10 ml of blood was collected into 2 BD Vacutainer® SST for screening of infectious disease. All blood products were labelled with patient's name, national identification numbers and sealed accordingly (Figure 3.2). They were monitored for around 1 hours post blood transfusion with regular vital sign monitoring before discharged.

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The blood products were transported with a tracking device to local partner laboratory, StemTECH International at least 1 month before the planned surgical procedure. Personal information, tentative operative dates, tentative postoperative follow-up dates, vital sign monitoring data were recorded on a specifically designed form.

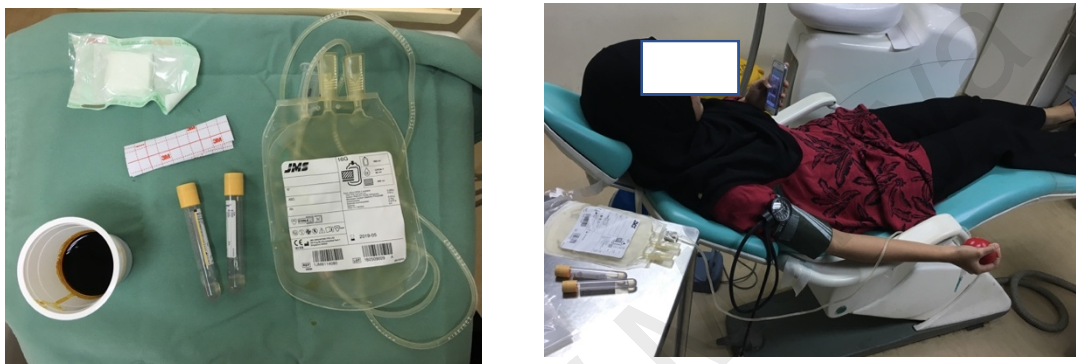

Figure 3.1 : Preparation for blood donation (left) and patients position during blood donation (right)

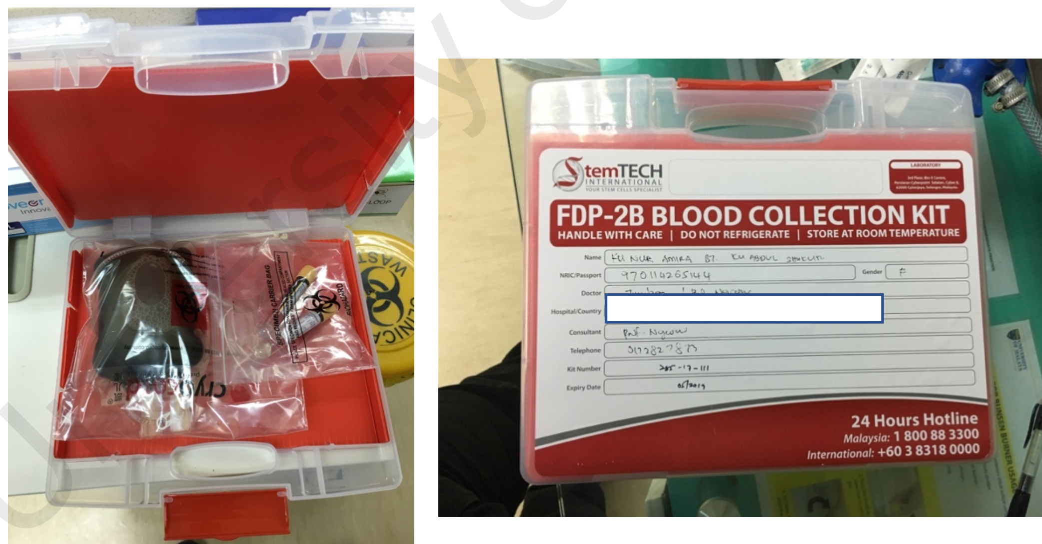

Figure 3.2 : All blood products were labelled with patient's details and sealed

3.3 LPRP preparation

The blood products were tested via serology and nucleic acid test for potential blood borne infection such as Syphilis, Hepatitis B, Hepatitis C, and HIV. Once cleared, they were centrifuged according to the protocol to prepare lysate PRP set by StemTECH International. The details of the processing procedure remains a trade secret as it is dependent on StemTECH International proprietary activation products used. In summary, the collected blood was mixed with an anticoagulant (i.e., ascorbic acid or 10% calcium citrate) to prevent premature platelet rupture throughout the remaining steps. The mixture of whole blood and anticoagulant was then transferred to a disposable collection chamber or bucket and was placed in the automated centrifuge. Once the mixture has been properly spun down within the centrifuge, the plasma was meticulously pipetted. Automatic pipettes with sterile, disposable tips were used to avoid turbulence and mixing of fractions.

The first 0.5 mL (fraction 1) of plasma has platelets content similar to that of peripheral blood. The next plasma fraction (fraction 2) has a higher platelet concentration than fraction 1. The third fraction contains twice the concentration of platelets, whereas fraction 4, the 0.5 mL immediately above the erythrocyte fraction, has the highest platelet and GF content four to six times that of circulating blood. This was the fraction used in PRP processing, where it was drawn off and placed in a sterile delivery system. It was mixed with 5000 IU topical bovine thrombin and 10% calcium citrate to activate the platelets to release concentrated growth factors for use (Note: Companies like StemTECH International have their own proprietary activation products, which was used in the current study instead of those mentioned in the literature) The entire process

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of preparing the autologous platelet-rich plasma usually takes between 15 and 30 minutes. It was then converted to the lysate form, available to the researchers in at least 5 vials per patient (Figure 3.3). In all cases, the processed LPRP contains 2 billion platelets/vial. All prepared vial will be tested for bacterial and fungal prior to release from StemTECH International. Should the PRP obtained from a certain patient proved unsatisfactory, the patient would be excluded from the study, but they continue to receive the needed third molar surgery.

Figure 3.3: LPRP vials available to researchers

3.4 Methods

Pre-operative baseline facial measurements and the width of mouth opening was taken immediately before surgery. A preoperative digital panoramic radiograph was available prior to each surgery. Each digital panoramic radiograph was obtained no more than 2 months preoperatively. All radiographs were obtained by the same technician, using the same technique and standardized exposure times, kVp settings and processing methods. All patients were draped

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with a lead apron (0.25 mm lead equivalence) for the radiographic procedure. Standard third molar surgery was performed by a single operator with the sockets randomised to be control or as PRP study site. The lysate PRP returned from StemTECH International at least 3 days before the day of surgery and at -80 degrees. The height of the distal exposed root to the cementoenamal junction (CEJ) of the adjacent second molar was measured using a periodontal probe at immediate post-operation. Using the split mouth approach, sockets on one side would receive PRP, whereas no PRP was applied on the contralateral sockets (control). Following randomisation, LPRP was placed topically to the extraction sockets of impacted third molars with the aid of the Mitchell's osseous trimmer and a plastic instrument (Figure 3.5). All bony surfaces were completely lined with LPRP, regardless of their size, and shortly thereafter closed with simple interrupted sutures, using 4/0 Vicryl (Prolene®; Johnson & Johnson, USA) on a 20-mm cutting needle after a clot has form over it. The remaining of lysate PRP was reconstituted using 2 millilitre of normal saline (Figure 3.4) and injected into the submucosa as per protocol used to inject steroids into the submucosal region done at our centre (Figure 3.5) (Lim & Ngeow, 2017).

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Figures 3.4: Reconstituted LPRP with 2 ml of normal saline (left) and powder form of LPRP prior to topical placement in the extraction socket (right)

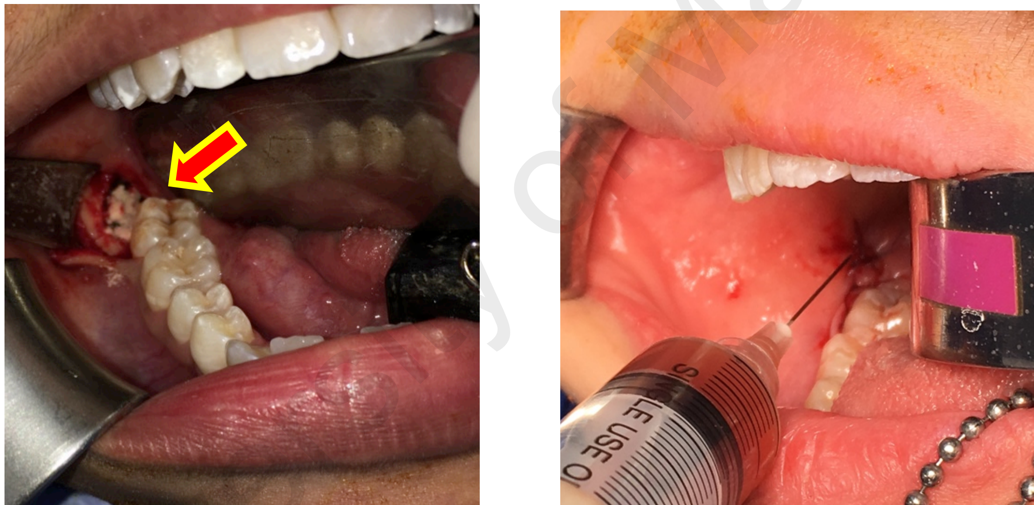

Figure 3.5: Topical placement of LPRP into extraction socket (left) and submucosal injection of reconstituted LPRP

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All patients underwent three months of clinical and radiographic follow-up. Reviews were done on post-operative day one, two and seven for assessment of healing at structures adjacent to the surgical site. Facial swelling measurements were taken as the sum of length of two lines along the pre-determined facial reference points from the outer corner of the eye to angle of mandible and tragus of the ear to corner of the mouth. Facial measurement was measured using a tape measure. The percentage

of facial swelling was then calculated based on the differences between baseline measurements with measurements taken on the three days of the study period. Trismus was measured as the changes in the width of mouth opening (maximum interincisal distance) between pre-operative and post-operative day one, two and seven. This distance was measured using a metal ruler. Pain will be evaluated and recorded on post-operative day one, two and seven using a 10 cm visual analogue scale (VAS). The amount of analgesic consumed throughout the same period of time (sodium diclofenac, with rescue drug of paracetamol when necessary) was also recorded. The state of soft tissue healing was assessed on post-operative day seven, with periodontal pocketing distal to the second molar measured using a periodontal probe to the CEJ at post-operative day seven, post-operative one and two months. Healing was considered as satisfactory if the socket was pain-free and healed by primary intention, or, the socket must be self-cleansing and did not require any occlusive dressing when secondary healing was anticipated. There should be no signs of infection. The healing index of Landry et al. and Gonshor (Gonshor, 2002; R.G Landry, 1988) was used to complement these measurements. This entails assessing colour of tissues, epithelialization of wound margins, presence of bleeding on palpation, granulation and suppuration. The index used is summarized as below:

Healing Index 1: Very poor (has 2 or more of the following)

- Tissue colour : C50 % of gingiva red
- Response to palpation: bleeding
- Granulation tissue: present
- Incision margin: not epithelialized, with loss of epithelium beyond incision margin
- Suppuration present

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Healing Index 2: Poor

- Tissue colour: C50 % of gingiva red
- Response to palpation: bleeding
- Granulation tissue: present
- Incision margin: not epithelialized, with connective tissue exposed

Healing Index 3: Good

- Tissue colour: C25 and 50 % of gingiva red
- Response to palpation: no bleeding
- Granulation tissue: none
- Incision margin: no connective tissue exposed

Healing Index 4: Very good

- Tissue colour: 25 % of gingiva red
- Response to palpation: no bleeding
- Granulation tissue: none
- Incision margin: no connective tissue exposed

Healing Index 5: Excellent

- Tissue colour: all tissues pink
- Response to palpation: no bleeding
- Granulation tissue: none
- Incision margin: no connective tissue exposed

During the first and second months of review, another two doses of constituted lysate PRP were injected into the submucosa adjacent to the socket. Prior to the injections, periodontal pocketing middle distal to the second molar was measured using a periodontal probe to the CEJ and recorded.

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The changes in 3D contour around the extraction sockets were determined by visually comparing the contour at 3 months post-operative using a study model . A visual scale was created with a score of 1 to 4. The description for each score is as the followings :

- Score 1 : represents severe depression at apex of the ridge
- Score 2 : represents moderate depression at the apex of the ridge
- Score 3 : represents mild depression over the apex of the ridge
- Score 4 : has no depression over the apex of the ridge.

Bone repair was assessed by using an intraoral periapical (IOPA) radiograph obtained at 3 months postoperatively. All radiographs were obtained by the same technician, using the same technique and standardized exposure times, kVp settings and processing method. As before, patients were draped with a lead apron (0.25mm lead equivalence) for this purpose. Changes in bone repair was assessed by image histogram analysis using ImageJ 1.52a. The histogram indicates how many pixel of a selected area share the same grey spectrum (0 = pure black and 255 = pure white). Image J 1.52 produces a graft with the x-axis showing grey levels (0 to 255) and the y-axis shows their frequency in the selected area. The radiographic densities of the extraction sockets were compared to the densities of the basal bone and interradicular space of the adjacent teeth, with the difference between both served to distinguish changes in the LPRP and control sides (Figure 3.6). All the above data will be recorded in patients data collection form (Appendix D).

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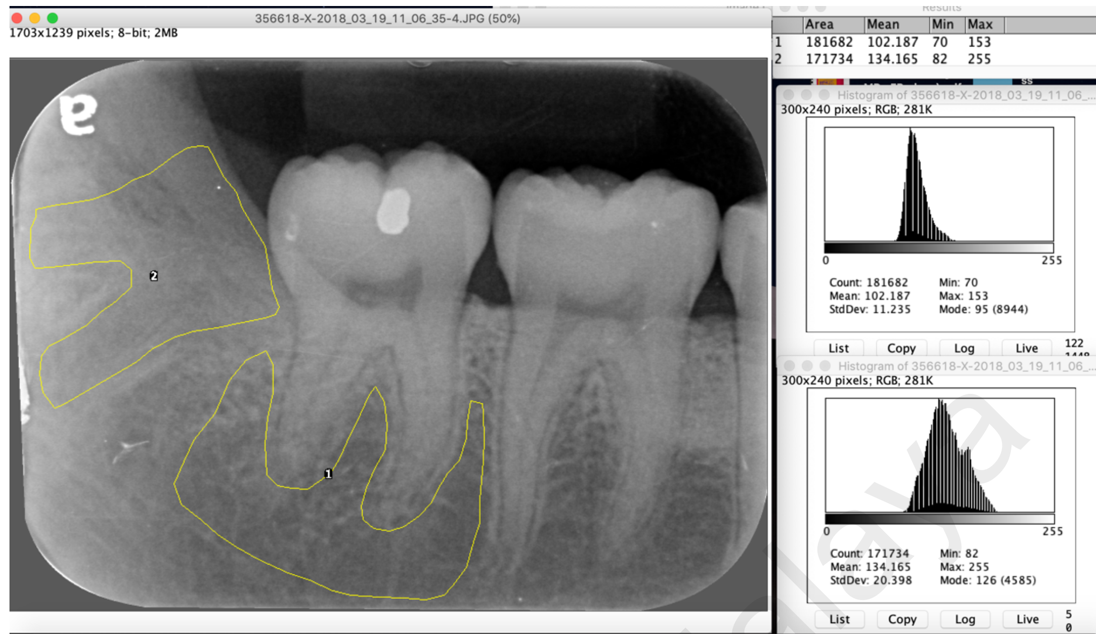

Figure 3.6: Histogram analysis using ImageJ 1.52a

The difference between the two histograms taken 3 months post-surgery ("histogram difference", HD) was calculated. HD is inversely proportional to new bone formation; that is, the lower the difference, the greater is the degree of tissue repair. Therefore, the extraction socket histogram was always compared with the same landmark on the radiograph, in order to compensate for any differences in tone from one film to another.

Histogram data from each case was recorded on a specific table containing information on each extracted tooth. In keeping with the split-mouth design of the study to minimize variability, HDs of both sides of the same patient was compared as well. The median HD was calculated for each radiographic assessment (at 3-month follow-up) to show the progress of bone healing in the PRP side as compared with the control side.

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Below is a timeline (Figure 3.7) to better understand the sequence for each patient during this study.

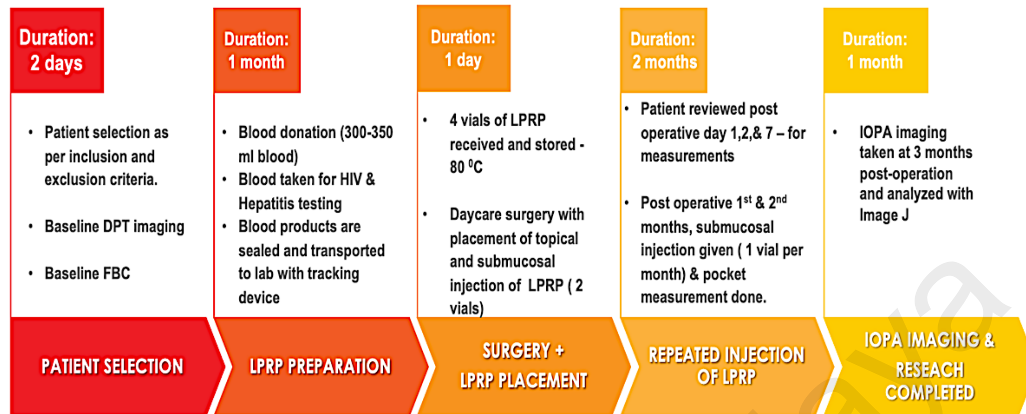

Figure 3.7: Timeline for each LPRP patient during this study

3.5 Data Analysis

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) software version 12.0. Findings from the two study sites was analysed using Levenes's test to show equal homogenous assumption prior to independent sample t-test test with regards to the histogram finding, pain, soft tissue healing and swelling. Followed by Independent sample t-test was done for the paired groups to evaluate changes in pain, histogram, soft tissue healing and swelling. As for the mouth opening the mean was calculated to compare to baseline. The significant value was set at p <0.05.

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CHAPTER 4: RESULTS

4.1 Results

The subjects of this study consisted of 15 patients, 4 male and 11 female with a mean age of 25 years (range: 21- 33 years). All subjects had ASA Class I medical status, and all of them had bilateral removal of their mandibular third molars under general anaesthesia a day care case. All blood taken from patient tested negative for syphilis, hepatitis B, Hepatitis C and HIV. All LPRP vial tested negative for bacterial and fungal. All subjects completed the study with no adverse reactions or surgical complications.

4.2 Soft tissue healing and 3D contour of extraction socket treated with LPRP in comparison to control group

Soft tissue healing in both groups at post-operative day 7 (POD7) were recorded as healing index score 3 for all patients. There was no difference between control and LPRP treated socket. As for the 3D contour, the mean for LPRP and control group was the same mean score of 3.44. This suggest that there is no difference in soft tissue healing and 3D contour in LPRP treated socket when compared to control.

4.3 Pain score of extraction sockets treated with LPRP in comparison to control group

Post-operative pain scores on day one (POD1), day two (POD2) and day seven (POD7) collected were grouped into control (C) group and LRPR group. Levenes' Test carried out showed equal homogenous assumption prior to independent sample t-test. A summary of pain scores obtained is shown in Table 4.1. In all, there is no

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difference in pain scores reported between the group treated with LPRP versus control. However, the group treated with LPRP recorded slightly higher pain score.

Table 4.1: Comparison of pain score between LPRP treated and control group at different time

VAS Pain score (SD) [Range]			*P*-value
Post-operative	LPRP	Control	
Day 1			
At 9 am	2.33 (1.84) [0-7.0]	1.0 (1.69) [0-5.0]	0. 346
At 6 pm	2.47 (1.25) [0-5.0]	2.0 (1.41) [0-5.0]	0. 609
Day 2			
At 9 am	2.67 (1.88) [0-7.0]	2.60 (2.13) [0-7.0]	0.324
At 6 pm	3.13 (2.17) [0-9.0]	2.33 (2.19) [0-7.0]	0.928
Day 7			
At 9 am	1.27 (1.49) [0-6.0]	1.20 (1.47) [0-4.0]	0.910
At 6 pm	1.60 (1.40) [0-5.0]	1.53 (1.77) [0-7.0]	0.903

4.4 Size of facial swelling at extraction socket sites treated with LPRP in comparison to control group

The size of facial swelling measured at day one (POD1), day two (POD2) and day seven (POD7) were grouped into control (C) group and LRPR group. This exercise involved the measurement of distance of tragus to the corner of the mouth and the distance from the outer canthus to the mandible angle. Levenes' test showed equal homogenous assumption prior to independent sample t-test. A summary of the size of facial swelling obtained is shown in Table 4.2. In all, there is no difference in size of facial swelling reported between the group treated with LPRP versus control. However, the group treated with LPRP recorded slightly bigger swelling.

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Table 4.2: Comparison of the distance from tragus to the corner of the mouth between LPRP treated and control group at different time

The distance from tragus to the corner of the mouth (SD) [Range]			*P*-value
Post-operative	LPRP	Control	
Baseline	114.8 (8.1) mm [104-130 mm]	114.2 (8.0) mm [103-125 mm]	0.840
Day 1	121.1 (9.0) mm [105-135 mm]	117.9 (7.0) mm [109-130 mm]	0.296
Day 2	121.8 (9.1) mm [108-140 mm]	118.1(5.5) mm [112-127 mm]	0.193
Day 7	116.5 (7.0) mm [104-129 mm]	115.7 (7.9) mm [104-130 mm]	0.754
The distance from the outer canthus to the mandible angle (SD) [Range]			*P*-value
Post-operative	LPRP	Control	
Baseline	102.8 (6.1) mm [94-115 mm]	105.0 (6.5) mm [95-118 mm]	0.363
Day 1	119.0 (11.3) mm [104-140 mm]	116.0 (9.3) mm [103-135 mm]	0.434
Day 2	115.7 (10.7) mm [100-135 mm]	114.7 (8.3) mm [100-135 mm]	0.777
Day 7	111.9 (11.6) mm [98-135 mm]	113.4 (9.5) mm [98-135 mm]	0.696

4.5 Mouth opening assessment at baseline, POD1, POD2 and POD7

Interincisal mouth opening was assessed during the first week post-surgery. Mean interincisal baseline was 43.75. There was a significant reduction of interincisal mouth opening at POD1 (17.13 mm) and at POD2 (19.80 mm). The size of mouth opening significantly improved to 27.20 mm at POD7, as compared to the size at POD1 (P<0.00). There was one patient with a mouth opening of 45 mm at POD7. A summary of the post-operative mean size of mouth opening compared to baseline is shown in Table 4.3.

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Table 4.3 : Post-operative mean size of mouth opening as compared to baseline

Mean size of mouth opening (SD) [Range]		*P*-value
Baseline	43.73 (2.84) mm [41.0-50.0 mm]	
POD1	17.13 (4.98) mm [8.0-28.0 mm]	<0.00
POD2	19.80 (5.21) mm [13.0-31.0 mm]	<0.00
POD7	27.20 (7.29) mm [17.0-45.0 mm]	<0.00

4.6 Pocket depth at extraction sockets treated with LPRP in comparison to control group

The pocket depth at mid distal tooth of second molar of LPRP treated socket and control were measured at post-operative day seven (POD7), and at 1 month (PO1M) and 2 months (PO2M) post-surgery. The results are summarized in Table 4.4. In all there is no significant difference in pocket depth between the LPRP treated group in comparison to control throughout the first month of study. However, by the second month, there was significant improvement i.e. reduction in pocket depth in the LPRP treated group.

Table 4.4: Pocket depth measurement at mid distal second molar

Pocket depth (in mm) (SD) [Range]			*P*-value
Post-operative	LPRP	Control	
POD7	5.67 (0.82) [5.0-7.0]	5.73 (0.88) [4.0-7.0]	0.832
PO1M	4.53 (0.64) [4.0-6.0]	4.87 (0.83) [4.0-6.0]	0.230
PO2M	3.53 (0.52) [3.0-4.0]	4.40 (0.63) [3.0-5.0]	<0.000

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4.7 Image J bone analysis of extraction sockets treated with LPRP in comparison to control group

Image J software was used to analyse differences in bone density as obtained using IOPA radiograph. It provides digital data of the scanned bone over the control extraction socket and its surrounding normal bone. Similar method was used for the socket treated with LPRP. Figure 4.2 shows the summary of analysed Image J data for individual patients in this study. Levenes's test showed equal homogenous assumption prior to independent sample t-test for the control extraction socket as compared with the socket that was treated with LPRP. This finding is shown in Table 4.5.

Table 4.5: Image J analysis of sockets treated with LPRP and control

IMAGE J DATA (SD)			*P*-value
Post-operative	LPRP	Control	
Bone	87.24 (19.7)	94.76 (18.2)	0.287
Socket	86.71 (26.2)	90.20 (22.1)	0.696

The derived histogram of control bone and socket is 94.76 (SD 18.2) and 90.20 (SD 22.1), respectively. In contrast the derived histogram of LPRP treated bone and socket is slightly lower at 87.24 (SD 19.7) and 86.71 (SD 26.2), respectively (Figure 4.1).

Independent sample t-test showed no significant difference between control socket and its surrounding normal bone (P = 0.542). Similarly, no significant difference was found between the extraction socket treated with LPRP and its surrounding normal bone (P=0.950). A comparison between the control extraction socket and the socket treated with LPRP yielded no significant difference (Independent t-test; P= 0.287 at bone, P= 0.696 at socket), suggesting no beneficial benefit of a repeat application of LPRP.

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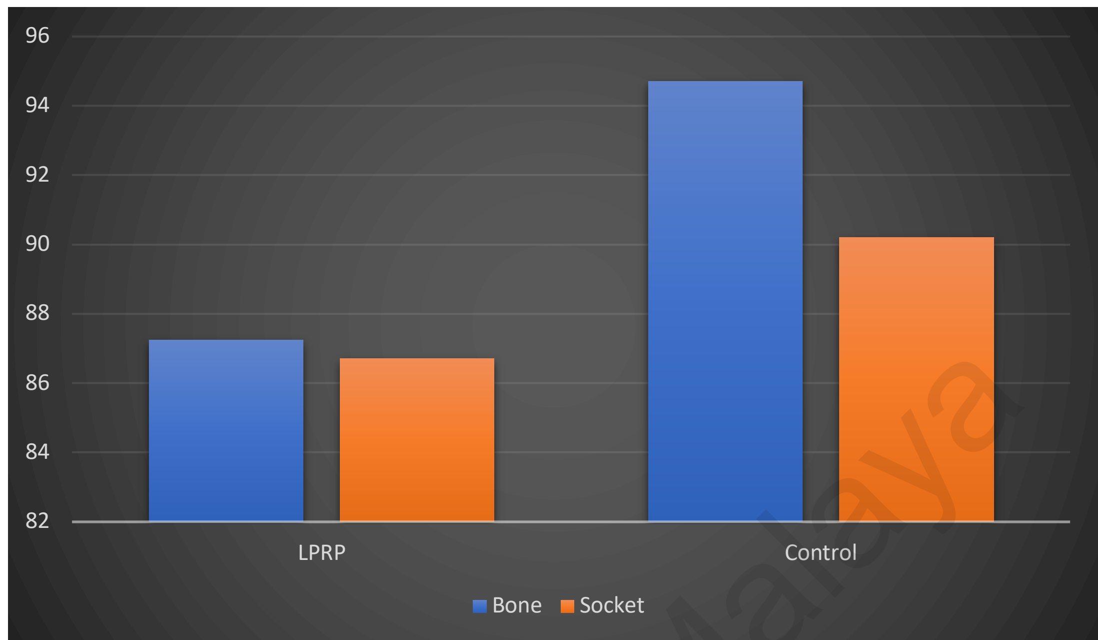

Figure 4.1: Image J analysis of sockets treated with LPRP and control

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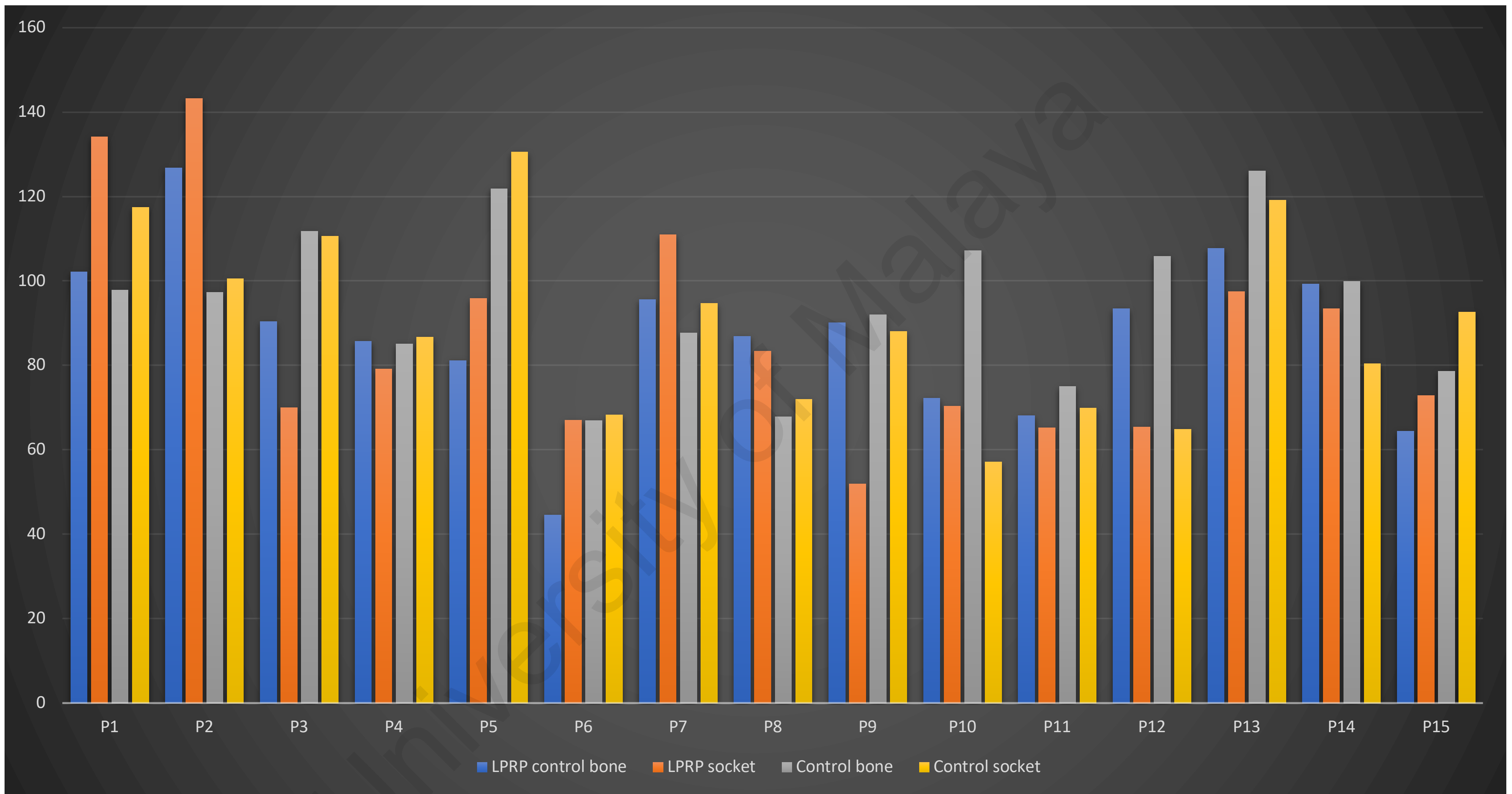

Figure 4.2 : Image J analysis for individual patients

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CHAPTER 5: DISCUSSION

5.1 Discussion

Platelets are very important in the wound healing. They are the first line to arrive at wound site to trigger the coagulation process. Platelets also release multiple wound healing growth factors and cytokines with includes PDGF, TGF-β1 and -β2, VEGF, PDEGF, IL-1, basic fibroblast growth factor (bFGF), and platelet activating factor-4 (PAF-4)(Dutta et al., 2015). These growth factors are responsible for increasing cell mitosis, increasing collagen production, recruiting other cells to the site of injury, initiating vascular growth, and inducing cell differentiation. Because of this, platelet concentrates have been used to promote healing. PRP and its variant, PRF is used widely in medicine (Marx et al., 1998), but its application in dentistry is comparatively limited. It has been tested in dentoalveolar (socket preservation, socket healing, sinus grafting & implant placement) (Antonello Gde et al., 2013; Butterfield et al., 2005; Celio-Mariano et al., 2012; Kassolis et al., 2000; Maiorana et al., 2003; Whitman et al., 1997) and maxillofacial surgery (repair of cleft, bone grafting and mandibular reconstruction) (Marx, 2004; Marx et al., 1998; Whitman et al., 1997). A systematic review in 2014 reported that the scientific evidence for the use of PRP to promote third molar socket healing was poor (Barona-Dorado et al., 2014).

Various systematic reviews have indicated the reason for variation in results were due to the difference of PRP preparation protocol and unquantifiable platelet counts/concentration (Antonello Gde et al., 2013). Besides, the concentration of PDGF over the surgical socket tends to reduce over time, not allowing the growth factors to induce maximal effect. In an attempt to reduce limitation in this study, the number of LPRP was quantified in each vial, a standard preparation protocol was

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implemented and the healing sockets received two additional submucosal injection of LPRP over a period of 2 months. All this effort was to standardize our protocol in order for any clinicians to replicate the results in future.

5.2 The effect on LPRP on periodontal pocket healing and soft tissue healing

The current study however, only found enhanced periodontal pocket healing by the second month. Statistically significant reduction in periodontal pocket depth, is in agreement with several authors (Doiphode et al., 2016; Gandevivala et al., 2017; Kaul et al., 2012; Mozzati M, 2007; Sammartino et al., 2009). This may be related the presence of PDGF and EGF which are the main growth factors involved in the migration, attachment, proliferation, and differentiation of periodontal progenitor cells (Giannobile, 1996). Two repetitions of submucosal injection of LPRP over a period of 2 months might have helped to increase/maintain the level of PDGF over the healing extraction socket. Increased concentrations of these growth factors are likely the reason for the accelerated soft tissue wound healing, which is suggested to be at least 2-3 times faster than that of normal (Anitua et al., 2004). However for the general clinical appearance of soft tissue which was scored via Landry Healing Index (R.G Landry, 1988) over the extraction socket at POD7 was clinically similar for both control and socket treated with LPRP. There was no episode of alveolar osteitis or infection recorded in all patients.

Linderboom (Lindeboom et al., 2007) showed that PRP packed extraction socket had a strong stimulant effect of capillary regeneration as compared to control group. The increase in proliferative activity of microvessels is quite significant during the early stages of wound healing which leads to good soft tissue repair. Raghoebar (Raghoebar et al., 2005), reported similar results to this study as there was better soft tissue healing in patient treated with PRP and there was no significant increase in bone

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formation. Alissa (Alissa, Esposito, Horner, & Oliver, 2010), described that PRP had improved soft tissue healing over extraction sockets, but limited evidence that PRP promotes bone healing. Dutta (Dutta et al., 2015) in a study using the model similar to the current study, reported that PRP is biocompatible and has significant improved soft tissue healing, bone regeneration and increase in bone density in extraction sockets. However, their study was not done on a split mouth model. It is unsure if the difference in group sampling resulted in a better outcome as compared to the current finding, as there is a patient related factor that cannot be ruled out. In addition, the criteria for bone healing and the scoring system for radiographic assessment was based on modification of the Kelley's method as described by Ogundipe (Ogundipe et al., 2011). Marx (Marx et al., 1998) claimed that the true clinical value of PRP lies in its speeding effect on autograft bone healing (enhanced density and maturity), although he also found soft tissue improvement at the donor site of split skin grafts in a different study (Marx, 2004).

5.3 The effect of LPRP on post-operative pain

The VAS pain score measured in all our patients shows a slightly higher pain score for the site treated with LPRP. However, there is no statistical difference between control and LPRP. Besides this there were 5 patients who reported significant pain (VAS > 5) during and after the injection of LPRP . The pain in average lasted around 3-5 minutes before reducing in intensity. At this point of time there is no possible explanation for the increased incidence of pain in our patients. However, Thanasas (Thanasas, Papadimitriou, Charalambidis, Paraskevopoulos, & Papanikolaou, 2011) experienced the same event in patients treated with PRP for Chronic lateral elbow epicondylitis, hypothesized that the increase in the presence of white blood cells may have caused intense inflammation response thus leading to pain.

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A meta-analysis undertaken by He et al. for third molar surgery treated with PRF up to 2016 (He, Chen, Huang, Pan, & Nie, 2017) reported that it significantly relieved pain, reduced 3-day postoperative swelling as well as reducing the incidence of alveolar osteitis. However, they found no significant differences between PRF and non-PRF groups with respect to 1-day post-operative swelling and osteoblastic activity. In contrast to the current study, Haraji (Haraji, Lassemi, Motamedi, Alavi, & Adibnejad, 2012) had reported that postoperative pain was significantly less in sockets treated with PRP as compared to control. Besides finding no significant difference with regards to pain, swelling and sequalae, the current study had found that that the LPRP group exhibited slightly higher post-operative pain and swelling. The difference in this finding may be the result of using different versions of platelet concentrates. In addition, the anti-coagulants in LPRP used in this study might induce mild inflammatory effects at the wound site.

5.4 The effects of LPRP on post-operative swelling

There was no statistical difference in the size of facial swelling between LPRP versus control sites. However we noted that the LRPR site recorded a slightly larger swelling as compared to control. This increase was only noticeable for the first two days post-operative, at POD7 the swelling for the control and LPRP group were similar. In contrary, Rutkowski (Rutkowski et al., 2010), showed that there was significantly less facial oedema over the site that received PRP. Besides that many literature which studied facial swelling with PRP did not specify the size of changes as compared to baseline and post-operative days.

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In our study we hypnotized that the increase in swelling might be caused by the same mechanism that is contributing to the increase in pain over the LPRP site such as anti-coagulants or higher concentration of platelets used.

5.5 The effect of LPRP on bone density

There was no statistical significance for socket treated with LPRP when compared to adjacent healthy bone and contralateral control socket. This finding is similar to Gurbuzer (Gurbuzer et al., 2010) that reported no significant difference between PRF and non PRF treated sockets. Besides that, Aghaloo (Aghaloo, Moy, & Freymiller, 2002), in an animal model, did not show a significant increase in bone formation in PRP added socket in both histomorphometric and radiographic assessments. Raghoebar (Raghoebar et al., 2005), also echoed the same finding where there was no significant increase in bone formation by adding PRP. They went on to propose that PRP has no additional value in promoting healing. Jakse (Jakse et al., 2003), in an animal study showed that regenerative capacity of PRP exhibited a very low potency in bone formation. Butterfield (Butterfield et al., 2005), based on a rabbit model suggested besides the theoretical benefit of PRP, the results did not show an increase in histologic total bone, bone formation rate or bone density. They further suggested that the lifespan of platelets where there is direct influence with growth factors is only 5 days.

On the flip side, Mariano (Celio-Mariano et al., 2012), showed that the radiographic density difference was significant at 1,2,3 month, but there were no statistical significance when comparing PRP group to control group at 6 months. They suggested that PRP can be used to induce and accelerate bone healing in periodontal

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defects and distal root of mandibular second molar after third molar extraction. They noted that men presented with a higher radiographic bone density.

Antonello (Antonello Gde et al., 2013) found that PRP have beneficial effects on bony repair , most significant during the early stages by mitogenesis, angiogenesis and chemotaxis. Rutkowski (Rutkowski et al., 2010), IOPA radiographs displayed an increase in bone density in PRP treated socket suggesting a greater volume of new bone formation. They reported that the control side required 16 weeks to reach the same radiographic density as the PRP treated socket which was achieved in 8 weeks.

There is no clear evidence for the lack of bone formation in our study , but this may be attributed to the optimal amount and concentration of PPR needed for significant bone healing. The risk of over saturating the socket with platelets cannot be ruled out.

5.6 The effects of LPRP on post-operative trismus

Trismus was measured post operatively for 3 days, but the results is unreliable as this is a split mouth model. It will be difficult to objectively state that there is improvement in trismus secondary to LPRP as the point of measurement is at midline of both LPRP treated socket and control. In comparison to baseline there is significant limitation of mouth opening over the first two days of post-surgery. The mouth opening limitation tends to improve at POD7. Eventually at the end of 1 week, the mouth opening improves significantly.

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CHAPTER 6: CONCLUSION

6.1 Conclusion

In this study the aims were to assess the effect of repeated LPRP placement on bone healing , soft tissue healing, pain, swelling, trismus and periodontal pocket healing. Therefore the study was carried out in three parts. The preclinical part is diagnosis and blood donation to help with obtaining 4 vials of quantified platelet concentration. The second part of the study involved the clinical aspect, where the surgical removal of bilateral impacted third molar was performed and placement of LPRP powder into the socket followed by an injection of LPRP. The third part involves repeating LPRP injection over the surgical site and then data collection. Within the limitations of this study, the following conclusions may be drawn:

1. This study suggests that there is no difference in soft tissue healing in both groups.
2. This study shows that there is no significant difference in post extraction bone healing (bone density) when compared to adjacent normal bone and contralateral control extraction socket.
3. This study shows no significant correlation between LPRP and pain over the operative site. There was an increase incidence in pain over the LPRP site after surgery and during repeated injections of LPRP in 5 patients.
4. This study shows that there is no statistical difference between LPRP and the size of post-operative swelling.
5. This study shows that trismus cannot be objectively assessed in this split mouth technique. Limited mouth opening were noted for the first two days and then gradually improved at postoperative day seven.

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6. This study suggests that LPRP can improve the periodontal pocket healing at mid distal of the second lower molar region.

6.2 Limitation of study

Limited sample size in this study was attributed to the cost involved in preparing the LPRP from fresh blood. Besides that, even though the platelets were quantified, the growth factor concentration in each vial was not quantified.

6.3 Study Recommendation

We propose that future studies should be conducted using a bigger sample size to help in reducing bias. Besides that, each vial of platelet should also have their respective growth factors quantified (growth factor concentration) as this is the main bioactive component spearheading the healing process in all PRP studies. Micro CT should be used to asses postoperative bone healing as this method provides high resolution images of bone healing in an extraction socket. Specially designed high definition intraoral camera to take pictures of impacted molar socket healing over a period of 1 week is essential to reduce bias. The platelets provided for this study was in powder form leading to difficulties in introducing it into the tight space of the third molar socket. A modification/reengineering of the powder into block or gel form will help in application of the platelets during clinical phase of the study.

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