2013

# A Prospective Study Of Bone Augmentation With Growth Factors In Extraction Sockets

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# A PROSPECTIVE STUDY OF BONE AUGMENTATION WITH GROWTH FACTORS IN EXTRACTION SOCKETS

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A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Master of Science

BIRMINGHAM, ALABAMA
2013

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PERIODONTOLOGY

## ABSTRACT

Introduction:

Ridge preservation protocols reduce crestal remodeling after tooth extraction. Limited evidence supports the potential of different grafting materials to preserve the alveolar ridge. There is limited evidence to indicate advantage of bone replacement grafting in combination with platelet rich plasma (PRP), compared to grafting alone. A combination of recombinant human platelet derived growth factor (rhPDGF-BB) with beta tricalcium phosphate (β-TCP) has recently been approved to aid wound healing.

Aim: To evaluate early healing of grafted and non-grafted extraction sockets in the esthetic zone, with or without PRP and rhPDGF-BB.

Materials and Methods: Population consisted of 41 healthy adult patients whose treatment plan included extraction of anterior teeth and replacement by dental implants.

Participants were randomized into four groups. One site per subject was selected to receive trephine sampling. Teeth were extracted and following groups were formed:

Group 1: Saline irrigation (Control). Group 2: Freeze-dried bone allograft (FDBA)/TCP/collagen plug. Group 3: FDBA/TCP/PRP/collagen plug and Group 4: FDBA/TCP/ rhPDGF-BB /collagen plug. At 8 weeks, a core was harvested from the center of 41 sockets.

Cores were processed and histomorphometric analysis took place. Differences were analyzed using one-way analysis of variance (ANOVA) or chi-square tests for continuous and categorical data. If significant difference was determined, pairwise comparisons were tested using least squares means (LS-means). Spearman correlation coefficients were used to evaluate the relationship of bone growth with potential confounders. A p-value <0.05 was considered statistically significant.

Results: Analysis of variance did not indicate statistical significance in age gender, smoking, ethnicity or race distribution among groups. Significant differences in tissue distribution were identified between groups as well as between different thirds of harvested core. Overall, more new bone as well as soft tissue formation was noted in group 1 comparing to the groups where bone graft was used. Where growth factors were used, the amount of residual particles was less than group 2.

Conclusion: a)Inclusion of bone replacement graft suppressed new bone formation during early healing and b) Inclusion of PRP and rh-PDGFbb produced less residual bone graft particles, indicating more rapid turnover of bone graft but failed to induce significantly more new bone formation overall.

Keywords: Ridge preservation, platelet rich plasma (PRP), platelet –derived growth factor (rhPDGF-BB), Extraction socket, tricalcium phosphate (β-TCP), Freeze-dried bone allograft (FDBA).

Dedicated to Lillie

ACKNOWLEDGMENTS

I would like to thank all the members of my committee for supporting me in this effort and providing me with their valuable advice. It is humbling to have such names next to mine on this manuscript. I would particularly like to thank Dr. Geurs for <<initiating>> me in the mysteries of wound healing, feeding my curiosity and satisfying my understanding. This understanding allowed me to become a better scientist and a better surgeon. I would also like to thank him for trusting me with the opportunity to analyze this data. Special thanks go to Carrie Huisingh from the UAB Center for Clinical and Translational Science (CCTS) and Dr. Gerald McGwin from the Department of Epidemiology for all their support and the statistical analysis of the data. I am extremely grateful to them for their professionalism and promptness in analyzing the data and working with me during the preparation of this manuscript. I would also like to thank Mrs. Elizabeth Bolton for her support in coordinating the committee meetings and allowing me to focus solely on the task of preparing this manuscript.

TABLE OF CONTENTS

*Page*

ABSTRACT ..... iii

DEDICATION ..... v

ACKNOWLEDGMENTS ..... vi

LIST OF TABLES ..... ix

LIST OF FIGURES ..... x

1. INTRODUCTION ..... 1

1.1 Healing process of human alveolar extraction sockets ..... 1
1.2 Ridge preservation protocols ..... 3
1.3 The use of growth factors ..... 9

2. AIM OF THE PRESENT STUDY ..... 10

3. MATERIALS AND METHODS ..... 10

3.1 Inclusion Criteria ..... 10
3.2 Pre-surgical Procedures ..... 11
3.3 Surgical Procedures ..... 11
3.4 Randomization ..... 12
3.5 Surgical Extraction and Data Collection ..... 14
3.6 Post-Extraction Evaluations ..... 14
3.7 Drilling & Biopsy Technique ..... 15
3.8 Histologic and Histomorphometric Analysis ..... 16

4. OUTCOMES AND ANALYSES ..... 22

4.1 Data ..... 22
4.2 Statistical Analysis ..... 22

5. RESULTS…… ..... 23

6. DISCUSSION ..... 31

7. SUMMARY AND CONCLUSION ..... 41

8. REFERENCES .....43

APPENDIX

A IRB PROTOCOL APPROVAL FORM .....70

LIST OF TABLES


| *Tables* | | *Page* |
|---|---|---|
| 1 | Study Procedures Visits (1-6) | 15 |
| 2 | Demographic information of study population | 24 |
| 3 | Site distribution | 25 |
| 4 | Tissue distribution in core sections per group | 25 |
| 5 | Pairwise comparisons of apical 3rd tissue distribution | 28 |
| 6 | Pairwise comparisons of middle 3rd tissue distribution | 29 |
| 7 | Pairwise comparisons of coronal 3rd tissue distribution | 30 |
| 9 | Pairwise comparisons of entire core tissue distribution | 30 |
| 10 | Socket variables distribution among study groups | 31 |

LIST OF FIGURES

*Figure* *Page*

1 Histologic sample of a Group I socket (Control).....17

2 Processed histologic sample by pixel classifier.....18

3 Histologic sample of a Group III socket (FDBA/TCP/Platelet-Rich Plasma (PRP) + collaplug).....19

4 The same Group III sample processed by the pixel classifier.....19

5 Histologic sample of a Group IV socket (FDBA/TCP/Platelet-Derived Growth Factors (PDGF) + collaplug).....20

6 The same Group IV sample processed by the pixel classifier.....20

7 Control socket.....21

8 Control socket where the region of interest (ROI) includes the coronal portion...21

9 The entire socket analyzed by pixel classifier, after training.....22

# 1 INTRODUCTION

## 1.1 Healing process of human alveolar extraction sockets

A thin facial plate is noted in the majority of the teeth in the esthetic area. Hyuhn-Ba et al. 2010[1], showed that the facial plate thickness in 87% of the cases studied was less than 1 mm. This is in agreement with a recent Cone-beam computed tomography study by Januário et al[2], that showed that in more than 50% of the cases the thickness of the facial bone was less than or equal to 0.5 mm. Severe alterations take place after extraction of teeth[3, 4]. Amler[5] et al in a histological and histochemical evaluation of human sockets identified different phases that take place during undisturbed healing. Immediately after extraction, a blood clot formed of fibrin network and platelets, fills the socket. The clot remains present not more than 7 days, then is completely replaced by granulation tissue that is a well vascularized fibrous connective tissue. In 3 weeks, after the granulation tissue is replaced by a collagen network, woven bone begins to form. In 5 weeks, two thirds of the socket was covered with bone. In another study by Boyne[6], it was found that there was no bone formation before the first week post-extraction. New bone formation was identified under the lining of the socket periphery at 8 days. This is important since no new bone formation was identified in the socket space before 10 days. Another histologic study by Davon and Sloan[7] identified a displacement of the periodontal ligament in the center of the socket 2 weeks after the extraction. According to these findings, the human socket appears to collapse towards the center. In histologic

terms, the functional bundle bone remodels into woven bone. Different reasons might account for that. First, with the tooth extraction many functionally oriented fibers such as Sharpey's fibers are destroyed, and that stimulates bundle bone to resorb[3]. Another mechanism that may occur is that part of the bundle bone becomes exposed to the oral cavity. Exposure of bone to the oral environment leads to sloughing of that portion and subsequently exfoliation of the necrotic bone into the oral cavity[8]. Clinicians may identify this process when during periodontal surgery the thin lingual flap in the molar area is traumatized during periodontal procedures and as a result there is exposure of bone that becomes necrotic and disposed in the oral cavity after separation from the underlying bone through resorption. From the above mentioned studies[3, 5-7] it can be concluded that the first phase of the remodeling process is osteoclastic in nature which leads to resorption of a considerable part of the old socket while other areas are remodeled to participate in new bone formation. On a cellular level, the healing of a human socket follows the principles of intramembranous ossification and can be described in 4 phases[9]. 1)An ossification center appears in the fibrous connective tissue due to clustering of mesenchymal cells that differentiate into osteoblasts. 2) Osteoblasts secrete osteoid within the fibrous membrane that within a few days becomes mineralized. 3) Osteocytes are formed when osteoblasts are trapped in the bone matrix. Osteoid is comprised of fibers and ground substance. Fibers are mainly Type-I collagen while the main glycosaminoglycan that comprise ground substance is chondroitin sulfate[9]. 4) Woven bone has been shown to occupy part of the socket already within 2-4 weeks while maturation of bone with formation of lamellae is slower and takes place over 24 weeks post extraction[10]. Evian et al[11] examined the osteogenic activity of bone removed from

the healing extraction sites in humans utilizing cores removed from healing sites. According to the authors, cores removed from 8-12 week healing sockets contain a combination of proliferating osteogenic cells and relatively mature bone that can serve as a good source of autogenous bone graft. On a molecular level, using histo-immunology, Devlin and Sloan[7] were able to identify different cell populations that contribute to formation of osteoprogenitor cells, including periodontal ligament fibroblast populations. Bone resorption is more pronounced at the buccal plate, which is reduced both in height and width[3, 12, 13]. In a dog model study, Araujo et al[3] showed that dimensional changes take place in two phases over an 8-week period. In phase I, a pronounced reduction of the height of the facial palate occurs, which results in a 2 mm discrepancy between facial and lingual plates. In the second phase, reduction of the width of both plates takes place resulting in a collapse of the socket towards the center. According to a dry skull study by Pietrokovski[14], the width reduction seems to affect the facial plate more than its lingual counterpart resulting in the formation of a flattened surface that connects the alveoli of the adjacent teeth. Schropp et al[15] estimated a 50% reduction in the width of human premolar and molar sockets over a 12-month period post-extraction. Such dramatic changes of the hard and soft tissues may lead to a compromised site, where implant placement in a functionally and esthetically desirable position is compromised.

## 1.2 Ridge preservation protocols

According to the Osteology Consensus Group 2011[16], ridge preservation is a general term for interventions that aim to "preserve the ridge volume within the envelope existing at the time of extraction." The reasons for intervention were outlined and included[16, 17]: i) maintenance of the existing soft and hard tissue envelope ii)

maintenance of a stable ridge volume for optimizing functional and esthetic outcomes and iii) simplification of treatment procedures subsequent to the ridge preservation. Indications for ridge preservation include[16]: i) implant placement is planned at a time point later than tooth extraction ii)contouring of the ridge for conventional prosthetic treatment iii) provided the cost/benefit ratio is positive iv)reducing the need for elevation of sinus floor elevation. Contraindications include[16]: i) general contraindication against oral surgical interventions ii) infection at the site that cannot be managed at the time of procedure iii) planning for early implant placement[18]. A systematic review[19] outlined various interventions to prevent such changes from occurring during unimpeded socket healing.

Different bone or bone-substitutes grafts have been used for extraction socket grafting such as autografts, allografts, xenografts and synthetic materials[20-26]. These procedures are thought to retard resorption of the socket volume while providing some control over bone-fill – thereby preserving the integrity of the alveolar ridge for future reconstruction with dental implants. Becker et al[27] compared de-mineralized freeze-dried bone allograft (DFDBA) and autogenous bone graft in their ability to induce bone formation in human sockets. Their findings suggest that DFDBA failed to induce bone formation in the majority of cases while autologous graft use led to bone formation in all cases[27]. Their findings were in agreement with a study by Froum[28] et al where DFDBA showed limited new bone formation in extraction sites. Iasella et al[25] compared freeze-dried bone allograft with a resorbable membrane versus extraction alone. Although all sites were able to receive implants, there was superior bone fill and less ridge alterations noted where bone graft and membrane were used. It should be noted that in this particular

study there was no effort made to achieve primary closure over the membrane[25]. The results were in agreement with a study by Fowler et al [29] who used FDBA in conjunction with an acellular dermal matrix to preserve ridge dimensions. Subsequent studies comparing mineralized vs. de-mineralized bone allografts showed that in a 3-month healing period de-mineralized grafts induced more new bone versus mineralized grafts in human extraction sockets, but the ridge dimensions were not significantly different between the two groups[30]. Additionally, an extended healing period of 6 months did not provide more vital bone or less residual bone particles compared to a 3-month healing for sockets grafted with mineralized bone allografts[31]. In a recent study[32], Scheyer et al examined the outcomes of ridge preservation procedures with a syringable combination allograft and an extracellular matrix. The allograft was a combination of demineralized and mineralized bone particles. Encouraging results were noted at three different timeframes (6, 12 and 24 weeks). At 24 weeks, new woven bone was the dominant component of the histologic components harvested and the authors were able to place implants in all examined sites. Artzi et al[20] histologically and histomorphometrically evaluated 15 subjects, each with one extraction site, which were grafted with bovine bone. The Artzi study included only anterior and premolar teeth. Closure was achieved in primary fashion with a rotated pedicle from the palate. The apical portion of the sockets contained 82.3% lamellar bone and the coronal portion was primarily woven bone. The conclusion was that after 9 months, cancellous porous bovine bone mineral was still present, so future studies should evaluate the resorbability of this material. Artzi et al [21], in a second stage of that study, histochemically analyzed the same extraction sockets and demonstrated the biocompatibility of cancellous bovine bone mineral.

Recently Cardaropoli et al[33] showed favorable ridge preservation responses utilizing a composite bovine bone mineral and a bi-layer bovine collagen membrane. The bone graft consisted of a blend of deproteinized bovine bone granules and porcine type I collagen. The authors evaluated the dimensions of the ridge 4 months post grafting and found that the use of bone graft and membrane resulted in significantly less height and width loss compared to the non-grafted sockets. Histologically, the authors found bone regeneration as well as residual allograft particles in samples harvested at 4 months. Despite the well - researched biocompatibility of bovine bone mineral, there is a concern about slow resorbability of this type of bone graft[20, 21, 33-35]. In a canine study[36] implants were placed 3 months post extraction in sockets that had been grafted with bovine bone mineral. At re-entry, significant amount of residual particles were noted and osseointegration did not occur in the areas where residual particles remained. Evidence suggests an extension of the healing time before implant placement in extraction sites grafted with bovine xenografts[19, 34]. Froum et al[28] evaluated bioactive glass and demineralized freeze-dried bone allograft (DFDBA) compared to a control group, where sockets were left to heal naturally. 10 extraction sockets were randomized into each group, for a total of 30 sockets in the population. After extraction, primary closure was obtained by coronally advancing the flaps. Six to eight months after the extractions, the control group only had 34.7% vital bone; the bioactive glass group had 59.5% of vital bone, which was greater than sites grafted with DFDBA. However, no statistical significance was observed between the three groups regarding vital bone formation. Another study[37] looked at the use of medical grade calcium sulphate hemihydrate as a ridge preservation material without the use of a barrier membrane. Upon re-entry at 3 months, significant new bone

infill was noted with no residual particles, underlining the fast turnover of this material. According to the authors, calcium sulphate allowed for trabecular bone arrangement at 3 months[37]. Data from these studies support the potential of different bone grafting materials to preserve the alveolar ridge after extraction. Lindhe et al[38] used an alloplastic graft of high crystallinity (α-TCP core coated with nanocrystalline biomimetic hydroxyapatite, BPCAP- Collagen; Geistlich Pharma AG, Basel, Switzerland) to fill fresh canine premolar extraction sockets. The authors found that during 3 months α-TCP did not undergo marked resorption, although it allowed for bone fill and partially prevented ridge resorption. Mardas et al[39] in a subtractive radiographic study, found that alveolar ridge preservation with Straumann Bone Ceramic (Institut Straumann AG; Basel, Switzerland) or bovine bone mineral resulted in similar radiographic bone changes. Ridge preservation was also pursued by applying GBR principles without bone grafts[19].

Both resorbable and non-resorbable barriers have been used with encouraging results regarding bone fill[12, 40]. The use of non-resorbable membranes result in high percentage of exposures which have deleterious effects on bone fill[12]. The use of resorbable membranes allows for similar results in bone fill and eliminates the need for removal in case of premature exposure[40]. Currently the practice of using non-resorbable membranes in ridge preservation procedures is not favored. Resorbable membranes are commonly used, especially in sites where dehischencies or fenestrations are present[41]. Additionally, primary closure over resorbable barriers used for ridge preservation is not considered crucial for the future ability of placing implant in the site[19, 33, 41-43]. Healing of an intact socket is provided by 4- walls, therefore exposure of barrier membranes is of

less significance in ridge preservation compared to lateral or vertical ridge augmentation scenarios[44]. Interestingly, in many of the previously mentioned studies[19, 20, 25, 28, 32, 45], the outcome of ridge preservation was measured by the ability to place implants and no analytical approach to evaluate hard and soft tissue dimension changes was undertaken[17].

Titanium based materials have also been used as extraction socket fillers such as dental implants placed immediately at the time of tooth extraction. Experimental studies in the dog model have demonstrated that immediate implant placement does not prevent physiologic changes from taking place in an extraction socket[46, 47]. In a subsequent study, the same group utilized a xenograft to prevent such changes from occurring around immediate implants[48]. Despite that, immediate implants have demonstrated comparable survival rates with implants placed conventionally, they have been associated with a higher risk of developing marginal mucosal recession[49-52]. According to the Osteology consensus group review, immediate implants are not recommended in areas of esthetic importance and they should be utilized in premolar areas[53]. Titanium granules have also been used as fillers for extraction sockets[54]. In a canine study[54-56], porous titanium granules were found to have significant osteoconductive properties and to promote new vital bone formation. Ridge preservation with titanium granules (Natix granules; Tigran Technologies AB, Malmo, Sweden) was comparable with the control group where xenogenic material was used (BioOss, Geistlich Pharma AG, Wolhusen, Switzerland). Interestingly at 6 months, new bone was noted around and inside the titanium granules while newly formed bone was present around the xenogenic material particles without replacement of the xenogenic material by bone[54].

## 1.3 The use of growth factors

Growth factors have been added to bone grafts to improve their regenerative ability[57, 58]. Adding platelet rich plasma (PRP) to graft materials is a common clinical procedure that may aid in wound healing of both hard and soft tissues[59-64]. PRP is made of up human platelets that are centrifuged down from a small volume of plasma, forming and autologous structure. PRP contains growth factors from platelets and products of platelet degranulation, such as fibronectin, vitronectin and fibrin. Adult mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells, and epidermal cell lines are activated by the growth factors via receptors on the membranes of the aforementioned cells, resulting in the formation of PRP[64-66]. Anitua et al[58] reported on twenty patients who underwent tooth extractions due to periodontal disease or root fracture. One group received PRP, and to help prevent tissue collapse, five of the ten patients in this group received PRP mixed with autologous bone. The control group consisted of ten patients whose sockets were left to heal naturally. Sockets treated with PRP had completely epithelialized at the end of the study, which was not the case in sockets where PRP was not used. In addition, sockets treated with PRP had more mature bone. Despite the promising results, the study had significant limitations, such as the lack of any statistical analysis[58]. In 2004, Marx[64] published data to further support the addition of PRP to bone grafts. Despite its clinical acceptance, the scientific evidence for the use of PRP is based on promising case series and case reports. Unfortunately, there is little data available to indicate an evidence-based advantage of grafting plus PRP compared to grafting alone. In a multicenter study, Nevins[67]et al compared a controlled group of β-TCP alone versus two experimental groups of different concentrations of PDGFbb in conjuction with β-TCP. Their findings supported that inclusion of growth factor resulted in superior

periodontal clinical attachment gain. The group containing 0.3 mg/ ml of PDGFbb performed better than the 1 mg/ml group. Stefani et al[68], studied the effect of a combination of PDGF and insulin-like growth factor (IGF-1) on the wound healing around implants placed in extraction sockets. This canine study indicated the combination of PDGF/IGF-1 plays an active role in the early phases of wound healing (3 weeks)[68]. Chang et al[69] in a rat model study, showed that gene delivery of platelet-derived growth factor-BB (PDGF-BB) is comparable to human recombinant protein delivery of PDGF-BB for healing of alveolar bone defects and osseointegration.

# 2 AIM OF THE PRESENT STUDY

To evaluate the effect of different grafting materials and their combination with growth factors on early hard tissue healing of extraction sockets in the esthetic zone. It was hypothesized that inclusion of growth factors promotes new bone formation in grafted sockets.

# 3 MATERIALS AND METHODS

The population of this study consists of 41 healthy adult patients whose treatment plan included extraction of mandibular and/or maxillary pre-molars and/or maxillary anterior teeth and their replacement with root-form dental implants.

## 3.1 Inclusion Criteria

Participants were 19 years old or older with demonstrated ability to understand the proposed treatment recommendations and prognosis and be able to provide informed consent, in English, without the aid of ad hoc translation. Participants with a reported history of a previous malignant neoplasm, a known hypersensitivity to β-TCP or

rhPDGF-BB, a Titanium metal allergy, or any other health condition or medication regimen that, in the opinion of the investigators, may adversely affect bone healing were excluded. Also women who were pregnant or nursing at the time of recruiting were excluded. Participants were stratified for smoking status and randomized to one of four extraction groups; three experimental groups utilizing three different bone grafting applications and a control group where extraction sockets were allowed to heal naturally. A secondary randomization was conducted to select one tooth site per subject to receive trephine sampling for histology evaluation.

## 3.2 Pre-surgical Procedures

Impressions were made and a provisional Essix retainer was fabricated. The Essix retainer protected the healing tissue in an aesthetically pleasing manner but did not inhibit clinical observation of study sites. Prior to extraction surgery, participants were given a loading dose of antibiotics based on their medical history and concomitant medications. A suggested prophylactic regimen included: 2 gr of Amoxicillin 1 hour prior to the procedure followed by 500mg (TID) for 7 days. For patients with reported allergy to Amoxicillin, 600mg of Clindamycin was given 1 hour prior to procedure followed by 300mg (TID) for 7 days. Prior to beginning the surgical procedure, the patient rinsed with 0.12% chlorhexidine gluconate for 60 seconds and the lower third of the face was scrubbed with Chlorhexidine Gluconate foam. Prior to extractions, clinical photographs were taken from the buccal and occlusal aspects. Photographs were taken in a 1:1 ratio using a Nikon D-70 camera with a ring-flash.

## 3.3 Surgical Procedures

All surgeries were performed by qualified clinicians in an appropriately equipped surgical operatory, located in the Periodontal Clinic at UAB School of Dentistry.

Conscious sedation was used at the surgeon's discretion, to help manage patient anxiety. Local anesthesia with Lidocaine hydrochloride 2% with 1:100,000 epinephrine (Lignospan® Standard, Septodont, Lancaster, PA, USA) and Articaine HCl. 4% with 1:100,000 epinephrine (Septocaine®, Septodont, Lancaster, PA, USA) was used. The teeth were extracted with minimal trauma, without flap elevation, utilizing periotomes and the Easy X-TRAC® system (A-Titan Instruments, Inc. Hamburg, NY. USA). The integrity of the remaining socket walls was assessed and measurements recorded in clinical record forms. The sockets were thoroughly debrided with a socket curette.

## 3.4 Randomization

The following groups were formed:

Group 1 (n=9). Atraumatic extractions followed by saline irrigation and placement of collagen plug.(Control).

Group 2 (n=11). Atraumatic extractions followed by Freeze-Dried Bone Allograft (FDBA)/Tri-Calcium Phosphate (TCP) + collagen plug (Collaplug, Absorbable Collagen Wound Dressing, Zimmer Dental, Carlsbad, CA, USA).

Group 3 (n=12). Atraumatic extractions followed by FDBA/TCP/Platelet-Rich Plasma (PRP) +Collaplug.

Group 4 (n=9). Atraumatic extractions followed by FDBA/TCP/Platelet-Derived Growth Factors (PDGF) + Collaplug.

The grafting procedure for each group took place as follows:

Group-1: Each socket was irrigated with sterile saline and a collagen plug was placed and stabilized with a Vicryl 4.0 crossing mattress suture. (control group).

Group-2: Each socket was grafted with freeze dried bone allograft (FDBA) mixed with Tri-calcium phosphate (TCP) 8:2 ratio; reconstituted with sterile saline. Sockets were irrigated with sterile saline, composite graft was packed into each of the study sites using mild pressure achieving complete fill. A 3mm section of collaplug was trimmed and secured over the grafted areas with 4.0 Vicryl crossing mattress sutures.

Group-3: Each socket was grafted with an 8:2 ratio FDBA/TCP graft reconstituted with Platelet Rich Plasma (PRP). 9-18 cc of blood sample was collected from the patient via venipuncture and PRP was prepared following the Cascade Fibrinet System (MTF, NJ,USA). The composite graft of FDBA and TCP was mixed with PRP. Sockets were irrigated with sterile saline and composite graft was packed into each of the study sites using mild pressure achieving complete fill A 3mm section of collaplug was trimmed and secured over the grafted areas with 4.0 Vicryl crossing mattress sutures.

Group- 4: Each socket was grafted with an 8:2 ratio FDBA/TCP graft reconstituted with recombinant platelet derived growth factor (PDGF) as found in GEM-21(product insert Appendix B). Sockets were irrigated with sterile saline and composite graft was packed into each of the study sites using mild pressure achieving complete fill. A 3mm section of collaplug was trimmed and secured as described above. Once secured in place, the collaplug was soaked with PDGF. Participants returned for oral evaluations as close to 7-days after the extraction/grafting procedure as possible and again at 14 days; sutures were removed at the 14-day visit. An additional follow-up visit was scheduled approximately

1-month following extractions. Healing was closely monitored and appropriate data was recorded for 8 weeks.

## 3.5 Surgical Extraction and Data Collection

The following measurements were recorded after extraction:

1. Socket Measurements – the mesio-distal distance and the bucco-lingual distance of the socket were made with a UNC-15 periodontal probe and rounded to the nearest millimeter.
2. Dehiscence and Fenestration Defect Measurements – in relation to the crest, defect measurements were made with a UNC-15 periodontal probe and rounded to the nearest millimeter.
3. Wound Measurements – mesio-distal distance and bucco-lingual distance of the soft tissue wound margins after suturing were made with a UNC-15 periodontal probe and rounded to the nearest millimeter.

## 3.6 Post-Extraction Evaluations

Participants returned for oral evaluations as close to 7-days after the extraction/grafting procedure as possible and again at 14 days. Sutures were removed at the 14-day visit. An additional follow-up visit was scheduled approximately 1-month following extractions. Healing was closely monitored and appropriate data recorded on follow-up recording forms. At each visit, medical history was reviewed and any changes were documented. Information regarding adverse events was captured and recorded following IRB and Federal reporting guidelines. Eight weeks after the extractions, each study subject was appointed for a second CBCT scan, a bone-fill biopsy and surgical placement of implants.

Table 1. Study Procedures Visits (1-6).

| Procedures | V- (-1) Screening | V-1 Pre-Tx | V-2 EXT/G | V-3 Week 1 | V-4 Week 2 | V-5 Week 4 | V-6 Week 8 Implant/Trephine sample |
|---|---|---|---|---|---|---|---|
| Consent Discussion/Obtained | X | | | | | | |
| Medical History Review | | X | X | X | X | X | X |
| Tx Plan Review | | X | | | | | |
| Impressions | | X | | | | | |
| Essix Retainer | | X | | | | | |
| Randomized to Tx Groups | | X | | | | | |
| Biopsy site selection | | X | | | | | |
| Pre-Surg Antibiotics | | | X | | | | X |
| Pre-Surg Oral Rinse | | | X | | | | X |
| Tooth Extraction | | | X | | | | |
| Tx Group Procedure | | | X | | | | |
| Venipuncture (group 3 only) | | | X | | | | |
| Clinical Photographs | | | X | X | X | X | X |
| CBCT Scan | | | X | | | | X |
| Periapical Radiographs | | | | | | | X |
| Post-Surg Instructions/ Prescriptions | | | X | | | | X |
| Suture Removal | | | | X | | | |
| AE's recorded | | | X | X | X | X | X |
| Bone Biopsy | | | | | | | X |
| Implant Placement | | | | | | | X |

## 3.7 Drilling & Biopsy Technique

A standard drilling technique was utilized except for a modification in the depth of the osteotomy, and biopsy obtained from study sites (described below). Copious sterile saline was used during osseous drilling procedure. A 2mm diameter trephine drill was used first at the center of the study site, and then the osteotomy was increased with progressive drills to the appropriate size, based on final implant dimensions. The trephine containing tissue specimen was immediately placed in fixative for later

histological analysis. Vials were labeled with participant's ID and the date of biopsy; laboratory technicians were blinded and unaware of specimen group. Bone biopsies preserved in the trephines and stored in 10% neutral buffered formalin were transferred to UAB Center for Metabolic Bone Disease (CMBD) Core Laboratory. Trephine cores of 2x6mm were stained with paragon stain and processed for histologic and histomorphometric analysis.

### 3.8 Histologic and Histomorphometric Analysis

A Nikon eclipse 90i microscope was used to examine the samples under visible light. Magnification of 400x was used and digital photographs were taken and stitched together using the Nicon Elements Software version 3.20 (Nikon Corporation; Nikon Instruments; Melville NY, USA). Quantitative and qualitative analysis of the mineralized vs. the non-mineralized tissues was performed using the Nicon Elements Software. An Asus computer (ASUS Computer International, Fremont, CA, USA) with Microsoft Windows 7 (Microsoft Corporation, Redmond, WA, USA) operating system was used. The images were stored and analyzed in .TIFF format. In the Nikon Elements software, the pixel classifier tool was used to identify and differentiate between different phases (new bone, residual bone graft, soft tissue and artifact). The classifier allowed for segmentation of the image pixels according to different user-defined classes, and was based on different pixel features such as intensity values, RGB values (colors are specified in terms of the three primary colors: red, green, and blue), HIS values (hue, intensity and saturation) or RGB values ignoring intensity. The classifier enables data to be saved in separate files[70]. To avoid variability due to the staining process and outcome and since every specimen had different profile of RGB values, intensity or HIS values,

the authors opted for re-training the classifier for every specimen. Training of the classifier software took place for every specimen by the same author (A.N). Author A.N trained the classifier software by manually identifying different phases and assigning different pixel groups to represent new bone, residual graft, soft tissue or artifact/air. This process took place for every specimen. Training was done until the classifier software was able to identify all the different phases present (Figures 1-9). Once the classifier was able to identify all the different phases in the region of interest (ROI), the software was able to quantify the different tissues present (% of new bone fill, % of residual graft and % of soft tissue).

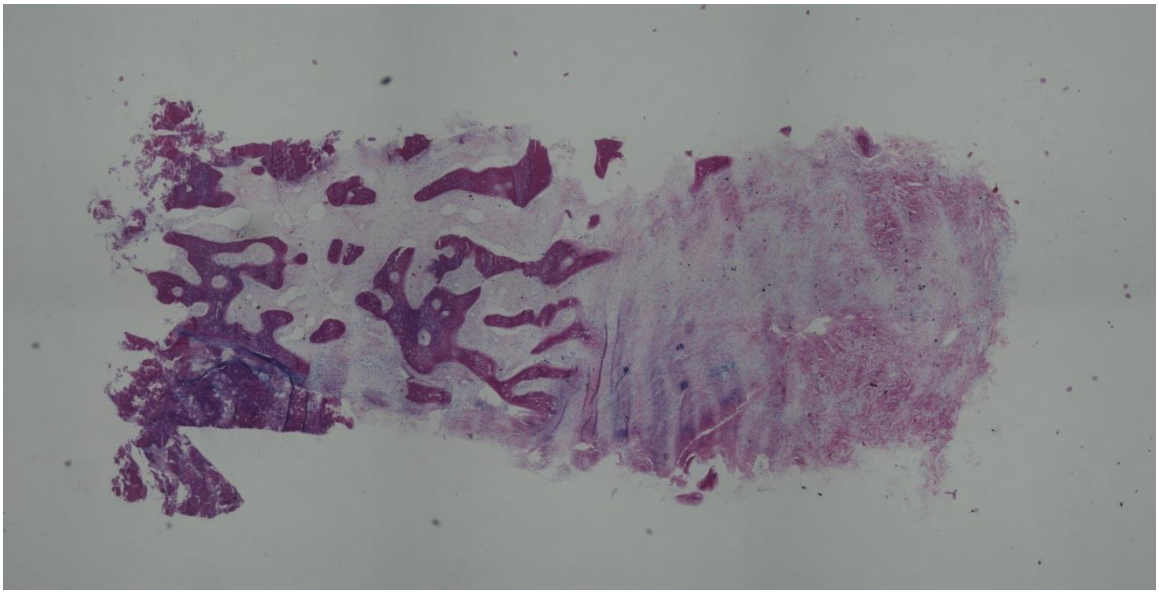

**Figure 1: Histologic sample of a Group I socket (Control).** Notice the woven bone formation (WB) as well as the soft tissue component which is dominant in this socket.

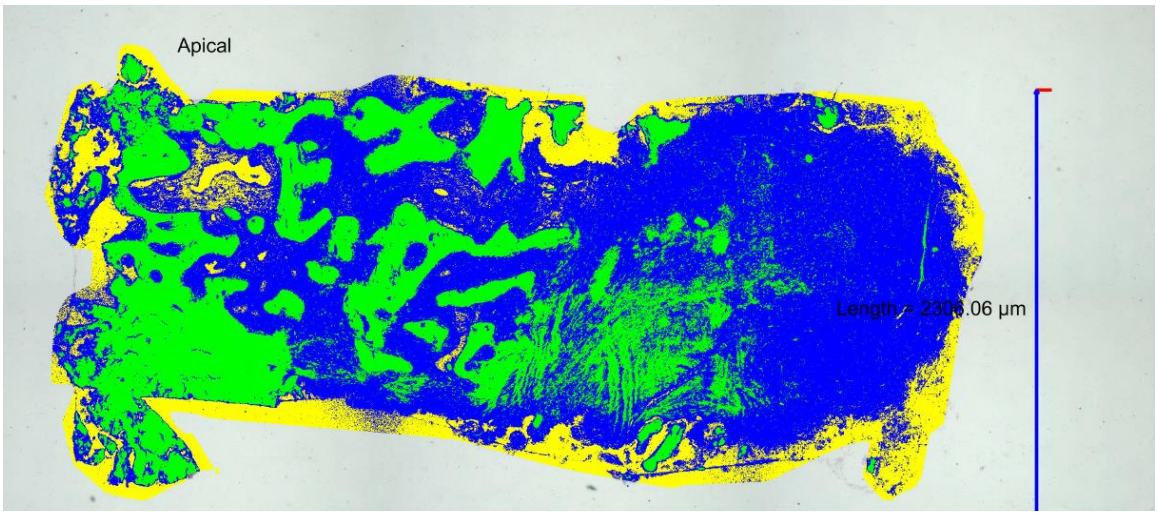


**Figure 2: Processed histologic sample by pixel classifier.** Soft tissue (blue), new bone (green) and artifact/air (yellow).

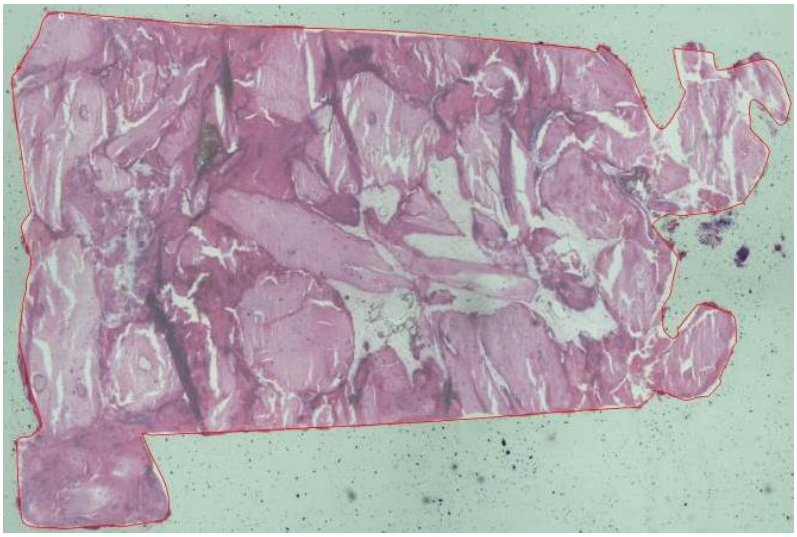

**Figure 3: Histologic sample of a Group III socket (FDBA/TCP/Platelet-Rich Plasma (PRP) + collaplug).** Residual bone particles can be noted as well as significant surface of artifact/air.

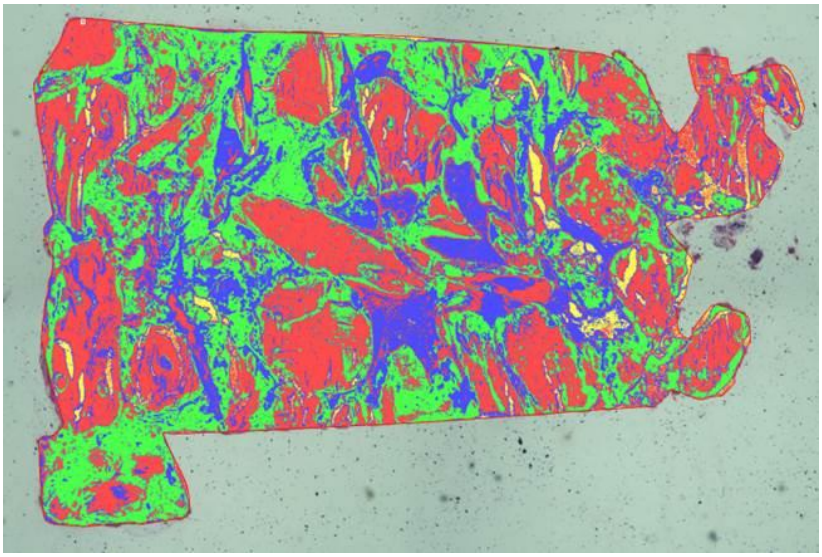

**Figure 4: The same Group III sample processed by the pixel classifier.** The region of interest (ROI) is identified by red outline. Bone graft particles (red), soft tissue (blue), new bone (green) and artifact/air (yellow).

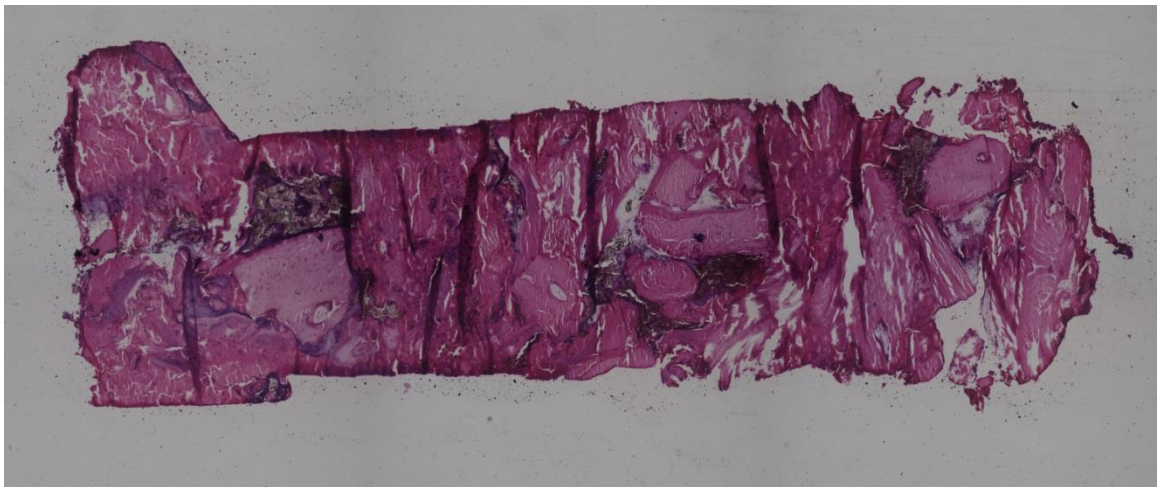

**Figure 5: Histologic sample of a Group IV socket (FDBA/TCP/Platelet-Derived Growth Factors (PDGF) + collaplug).** Notice the woven bone formation (WB) as well as the distinct residual bone graft particles.

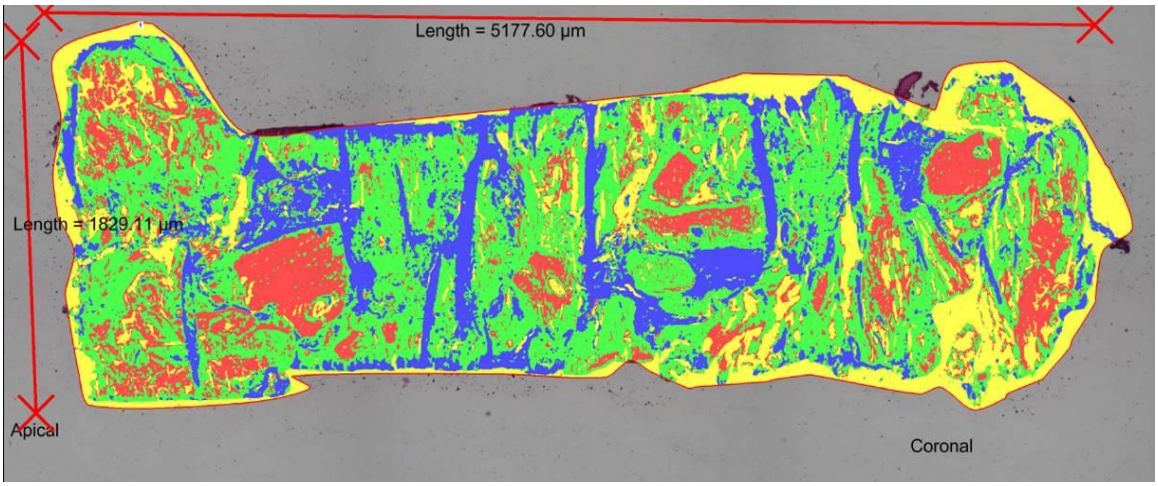


**Figure 6: The same Group IV sample processed by the pixel classifier.** The region of interest (ROI) is identified by red outline. Bone graft particles (red), soft tissue (blue), new bone (green), and artifact/air (yellow).

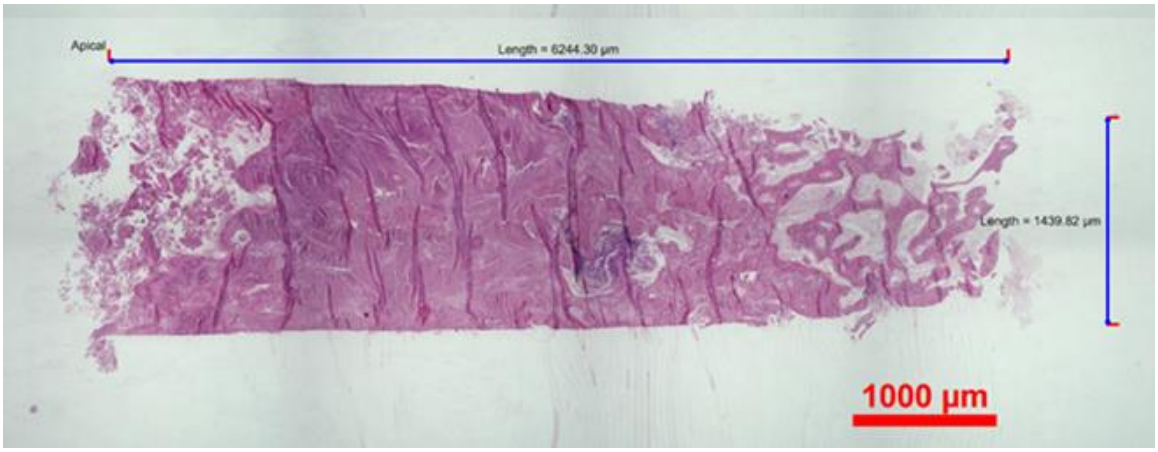


**Figure 7: Control socket.** Apical and middle portions significantly filled with new bone. At the coronal portion, formation of woven bone can be noted within a matrix of fibrous connective tissue.

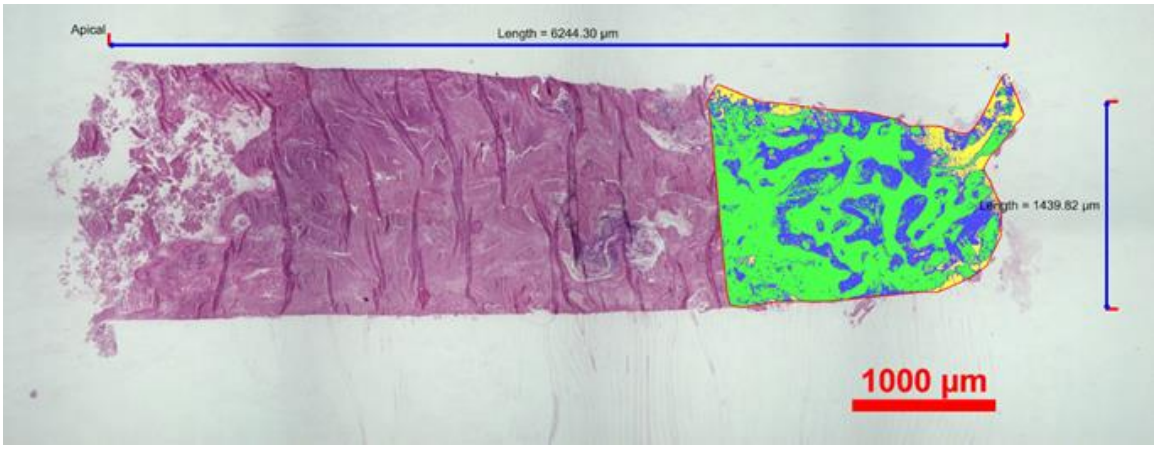


**Figure 8: Control socket where the region of interest (ROI) includes the coronal portion.** The pixel classifier after training was able to differentiate between new bone (green), soft tissue (blue) and artifact (yellow).

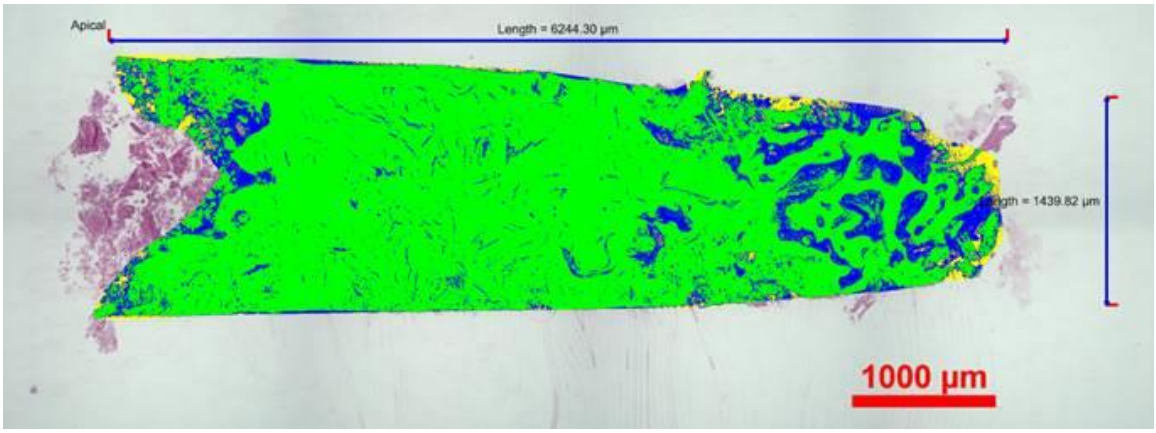


**Figure 9: The entire socket analyzed by pixel classifier, after training.** New bone (green), soft tissue (blue) and artifact (yellow).

# 4 OUTCOMES AND ANALYSES

## 4.1 Data

Demographic information (nationality, gender, age, race) as well as tooth to be extracted and smoking status were collected. Amount of bone fill and remaining bone graft (% of new bone fill, % of residual graft and % of soft tissue) material was determined and compared across groups.

## 4.2 Statistical Analysis

The general approach to statistical analysis of the study aims were based on mixed-model analysis of variance (ANOVA). Differences between groups were analyzed using one-way analysis of variance (ANOVA) or chi-square tests for continuous and categorical data, respectively. If a significant difference was determined from the ANOVA, all pairwise comparisons among the four groups were tested using least squares means (LS-means). Spearman correlation coefficients were used to evaluate the relationship of bone growth with potential confounders including age, ethnicity, gender, and smoking. A p-value $<0.05$ was considered statistically significant.

# 5 RESULTS

Sample population consisted of mainly Caucasians (90%) with a mean age of 52 years. 70% of the population were females. Analysis of variance did not indicate statistical significance in age distribution among groups (p=0.1862). No statistical significance in distribution of gender, smoking, ethnicity or race was identified between groups. The sample demographics are presented in Table 2a. The majority of the sites studied were premolars (n=26). Site distribution is presented in Table 2b. Tooth distribution among groups was also not significant ($\chi^2$=0.42). The tissue distribution per group is presented in Table 3 and graphically presented in Diagrams 1, 2, 3 and 4.

Significant differences in tissue distribution were identified between groups as well as between apical, middle and coronal third of the harvested core. Least square means comparisons revealed that in the apical 3$^{rd}$, significantly more new bone formation was noted in group 1 compared to group 2, while groups 3 and 4 did not differ significantly from group 1 or 2. There was significantly more soft tissue in groups 1 and 4. There was significantly less residual bone graft in groups 3 and 4 compared to group 2 (Table 4). In the middle 3$^{rd}$, significant differences were noted in the soft tissue component. More soft tissue was present in group 1 than any other group. Among groups where bone graft was used, group 4 presented the least amount of residual bone graft, while groups 2 and 3 did not differ significantly (Table 5). In the coronal 3$^{rd}$, group 1 presented significantly more soft tissue component. Significantly more graft particles were present in group 2 compared to 3 and 4 (Table 6).

In the entire core, more new bone as well as soft tissue formation was noted in group 1 compared to the groups were bone graft material was used. In these groups, residual particles comprised 16%-37% of the core. Higher concentrations were present in group 2. It is noticeable that in groups 3 and 4, where growth factors were used, the amount of residual particles was less than group 2, where bone graft was used without growth factor inclusion. In group 4, where PDGF was used, the amount of graft particles was the least from all groups that included grafting. Group 4 also included the highest percentage of artifact/air than other groups (Table 7).

Collected data included the number of socket walls, measurements of socket bucco-lingual and mesio-distal width and mesio-distal and bucco-lingual post -extraction wound width. In the study sample, 3 fenestrations were identified, one in each of groups 1, 2 and 3. The above mentioned independent variables are presented in Table 8. Distribution of these variables did not differ significantly among groups (Table 8).

Table 2a. Demographic information of study population.

| **Variables** | **Group 1 (n=9)** | **Group 2 (n=11)** | **Group 3 (n=12)** | **Group 4 (n=9)** | **Total (n=41)** | **p-value** |
|---|---|---|---|---|---|---|
| **Mean Age (SD)** | 53.6 (10) | 56.4(16) | 45 (8) | 53.5 (15.5) | | 0.18 |
| **Ethnicity** | | | | | | 0.47 |
| **Hispanic** | 0 (0%) | 0 (0%) | 1(8.33%) | 0 (0%) | 1(2%) | |
| **Non-Hispanic** | 9 (100%) | 11 (100%) | 11(91.6%) | 9 (100%) | 40 (98%) | |
| **Race** | | | | | | |
| **Whites** | 8 (19.5%) | 8 (19.5%) | 9 (22%) | 8 (19.5%) | 33 (80.4%) | |
| **Non-whites** | 1 (24%) | 3 (73%) | 3 (73%) | 1 (24%) | 8 (19.5%) | |
| **Gender** | | | | | | 0.93 |
| **Male** | 2 (22.2%) | 3 (27.3%) | 4 (33.3%) | 3 (33.3%) | 12 (29%) | |
| **Female** | 7 (77.8%) | 8 (72.7%) | 8 (66.7%) | 6 (66.7%) | 29 (71%) | |
| **Smoking** | | | | | | 0.14 |
| **Yes** | 1 (11%) | 0 (0%) | 4 (33.3%) | 1 (11%) | 6 (14.6%) | |
| **No** | 8 (88.9%) | 11 (100%) | 8 (66.7%) | 8 (88.9%) | 35 (86.4%) | |

Table 2b. Site distribution.

| Tooth Group (n=41) | Group 1 (n=9) | Group 2 (n=11) | Group 3 (n=12) | Group 4 (n=9) | Total (%) | p-value=0.4 |
|---|---|---|---|---|---|---|
| **Canine** | 1 (11%) | 3 (27%) | 1 (8.33%) | 0 (0%) | 5 (12%) | |
| **Central incisor** | 1 (11%) | 1 (9%) | 3 (25.00%) | 1 (11%) | 6 (14.6%) | |
| **Lateral incisor** | 0 (0%) | 0 (0%) | 2 (16.67%) | 2 (22%) | 4 (9%) | |
| **Premolar** | 7 (77.8%) | 7 (63.6%) | 6 (50.00%) | 6 (66.6%) | 26 (63%) | |

Table 3. Tissue distribution in core sections per group. *Indicates statistical significance.

| Apical section | Mean (SD) | Mean (SD) | Mean (SD) | Mean (SD) | p-value |
|---|---|---|---|---|---|
| **Bone graft** | 0 (0) | 0.35 (0.19) | 0.29 (0.13) | 0.17 (0.10) | <.0001* |
| **New bone** | 0.49 (0.25) | 0.26 (0.12) | 0.40 (0.16) | 0.36 (0.12) | 0.03* |
| **Soft tissue** | 0.38 (0.20) | 0.24 (0.15) | 0.17 (0.05) | 0.26 (0.13) | 0.01* |
| **Artifact** | 0.11 (0.11) | 0.14 (0.14) | 0.12 (0.11) | 0.2 (0.13) | 0.45 |
| **Middle section** | | | | | |
| **Bone graft** | 0 (0) | 0.33 (0.11) | 0.28 (0.14) | 0.18 (0.11) | <.0001* |
| **New bone** | 0.47 (0.28) | 0.30 (0.08) | 0.39 (0.19) | 0.37 (0.15) | 0.27 |
| **Soft tissue** | 0.44 (0.28) | 0.24 (0.1) | 0.19 (0.1) | 0.24 (0.14) | 0.01* |
| **Artifact** | 0.08 (0.09) | 0.10 (0.07) | 0.11 (0.09) | 0.19 (0.07) | 0.05* |
| **Coronal section** | | | | | |
| **Bone graft** | 0 (0) | 0.37 (0.15) | 0.23 (0.15) | 0.15 (0.11) | <.0001* |
| **New bone** | 0.33 (0.22) | 0.23 (0.10) | 0.26 (0.16) | 0.19 (0.15) | 0.33 |
| **Soft tissue** | 0.52 (0.21) | 0.25 (0.13) | 0.31 (0.21) | 0.30 (0.17) | 0.01* |
| **Artifact** | 0.13 (0.15) | 0.14 (0.08) | 0.19 (0.17) | 0.33 (0.16) | 0.01* |
| **Entire** | | | | | |

| Core | | | | | |
|---|---|---|---|---|---|
| **Bone graft** | 0 (0) | 0.35 (0.13) | 0.27 (0.13) | 0.17 (0.10) | <.0001* |
| **New bone** | 0.43 (0.24) | 0.27 (0.07) | 0.36 (0.15) | 0.28 (0.09) | 0.09 |
| **Soft tissue** | 0.45 (0.23) | 0.24 (0.10) | 0.22 (0.10) | 0.28 (0.12) | 0.005* |
| **Artifact** | 0.10 (0.1) | 0.12 (0.08) | 0.13 (0.11) | 0.25 (0.12) | 0.02* |

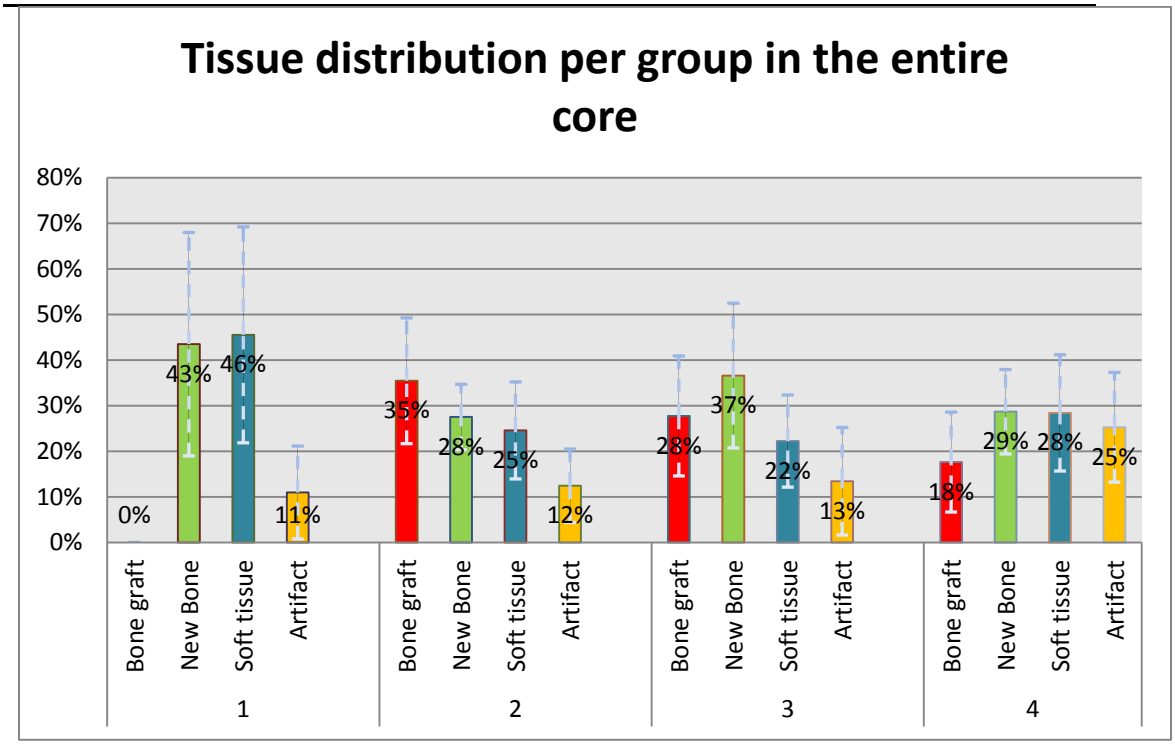


Diagram 1. Tissue distribution per group in the entire core. Note control sockets present with more new bone and soft tissue formation.

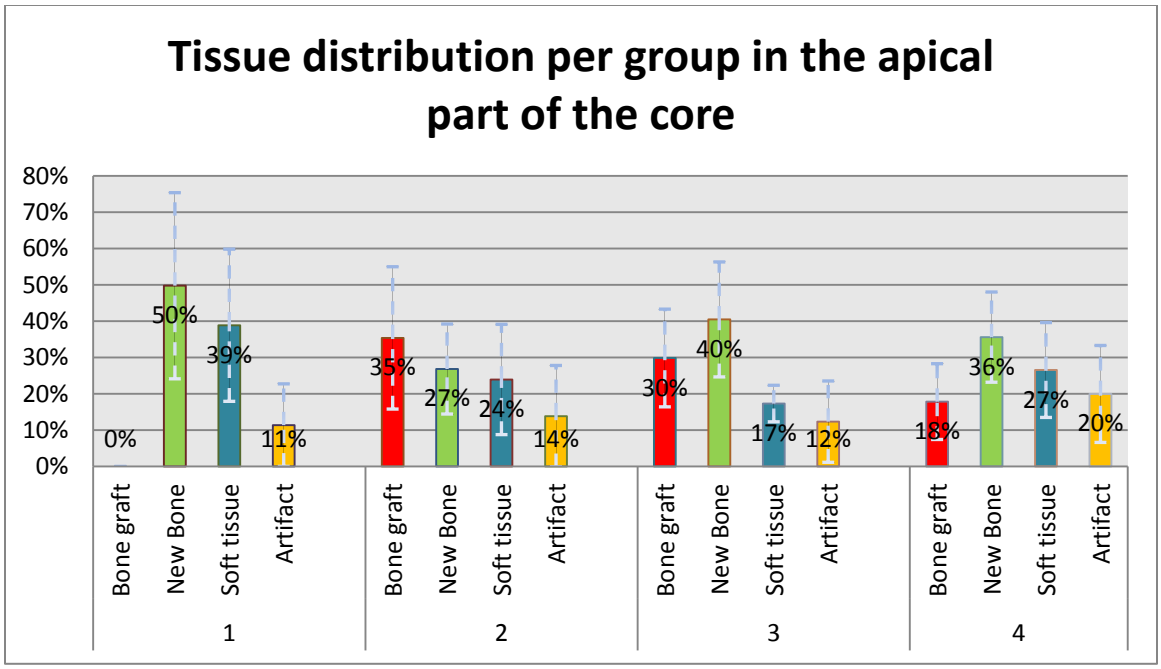


Diagram 2. Tissue distribution per group in the apical part of the core. Significant new bone formation is noted in all the groups.

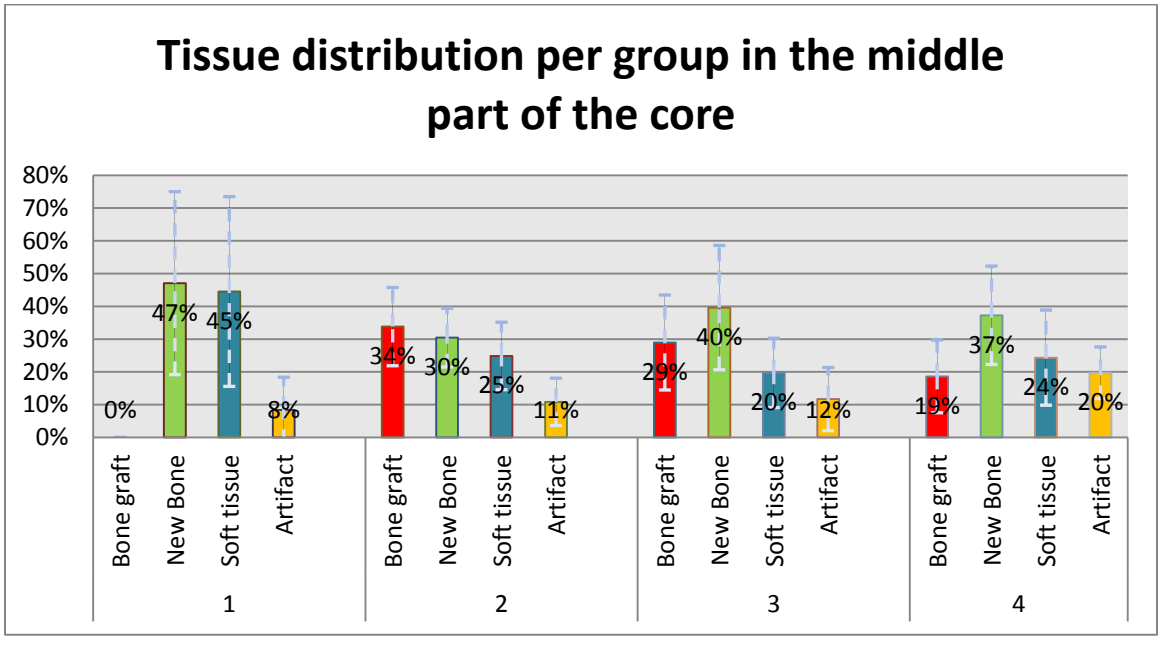


Diagram 3. Tissue distribution per group in the middle 3rd part of the core. The differences in new bone are not statistically significant, in contrast with the other tissues.

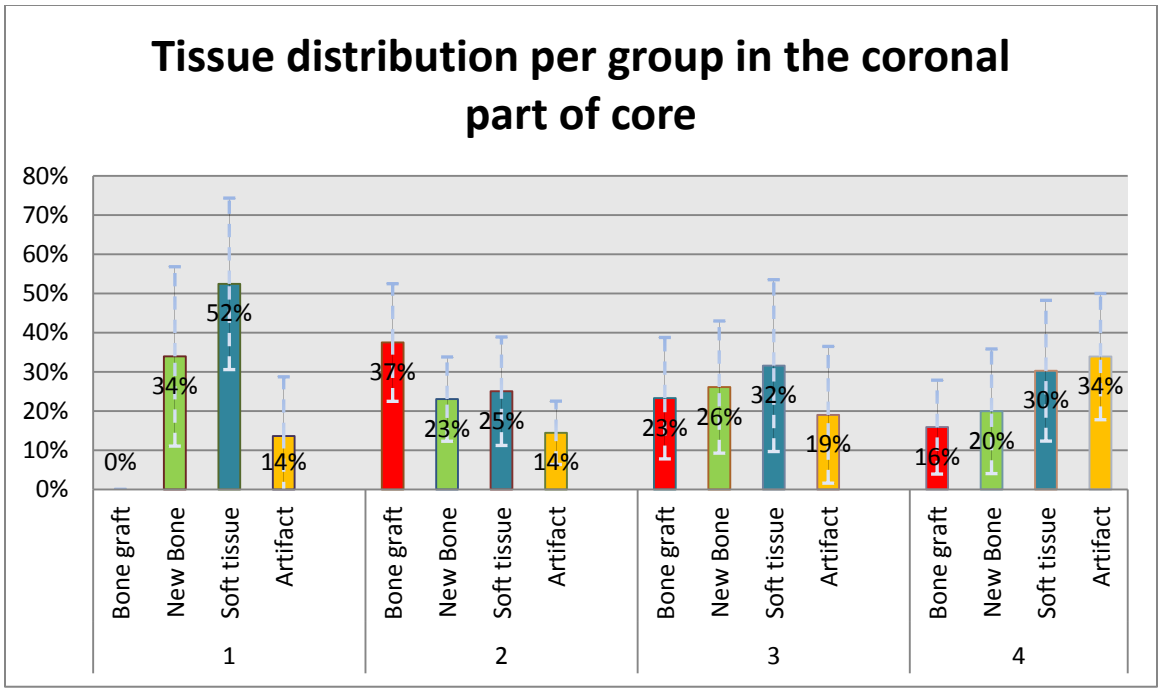


Diagram 4. Tissue distribution per group in the coronal 3rd part of the core. Compared to the apical and middle third, more soft tissue is present in the coronal part, especially in group 1. The differences in new bone are not statistically significant.

Table 4. Pairwise comparisons of apical 3rd tissue distribution. *Indicates statistical significance.

| i/j Bone graft | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| **Group 1** | | | | |
| **Group 2** | <.0001* | | | |
| **Group 3** | <.0001* | 0.33 | | |
| **Group 4** | 0.007* | 0.006* | 0.05* | |
| **i/j New bone** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | | | | |
| **Group 2** | 0.005* | | | |
| **Group 3** | 0.22 | 0.06 | | |
| **Group 4** | 0.08 | 0.25 | 0.52 | |
| **i/j Soft tissue** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | | | | |
| **Group 2** | 0.02* | | | |
| **Group 3** | 0.001* | 0.27 | | |
| **Group 4** | 0.07 | 0.68 | 0.14 | |

Table 5. Pairwise comparisons of middle 3rd tissue distribution.

Values for new bone were omitted because analysis of variance indicated non- significant difference between groups (p=0.27) and pairwise comparisons did not take place.

*Indicates statistical significance.

| **i/j Bone graft** | **Group 1** | **Group 2** | **Group 3** | **Group 4** |
|---|---|---|---|---|
| **Group 1** | | | | |
| **Group 2** | <.0001* | | | |
| **Group 3** | <.0001* | 0.3105 | | |
| **Group 4** | 0.0012* | 0.004* | 0.0449* | |
| **i/j New bone** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | NA | NA | NA | NA |
| **Group 2** | NA | NA | NA | NA |
| **Group 3** | NA | NA | NA | NA |
| **Group 4** | NA | NA | NA | NA |
| **i/j Soft tissue** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | | | | |
| **Group 2** | 0.01* | | | |
| **Group 3** | 0.002* | 0.47 | | |
| **Group 4** | 0.01* | 0.94 | 0.54 | |

Table 6. Pairwise comparisons of coronal 3rd tissue distribution.

Values for new bone were omitted because analysis of variance indicated non- significant difference between groups (p=0.3) and pairwise comparisons did not take place.

*Indicates statistical significance.

| i/j Bone graft | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| **Group 1** | | | | 0.0121 |
| **Group 2** | <.0001* | | | |
| **Group 3** | 0.0002* | 0.0114* | | |
| **Group 4** | 0.0121* | 0.0006* | 0.1977 | |
| **i/j New bone** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | NA | NA | NA | NA |
| **Group 2** | NA | NA | NA | NA |
| **Group 3** | NA | NA | NA | NA |
| **Group 4** | NA | NA | NA | NA |
| **i/j Soft tissue** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | | | | |
| **Group 2** | 0.003* | | | |
| **Group 3** | 0.01* | 0.41 | | |
| **Group 4** | 0.01* | 0.54 | 0.87 | |

Table 7. Pairwise comparisons of entire core tissue distribution. *Indicates statistical significance.

| i/j Bone graft | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| **Group 1** | | | | |
| **Group 2** | <.0001* | | | |
| **Group 3** | <.0001* | 0.11 | | |
| **Group 4** | 0.002* | 0.001* | 0.05* | |
| **i/j New bone** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | | | | |
| **Group 2** | 0.02* | | | |
| **Group 3** | 0.31 | 0.16 | | |
| **Group 4** | 0.04* | 0.86 | 0.25 | |
| **i/j Soft tissue** | Group 1 | Group 2 | Group 3 | Group 4 |
| **Group 1** | | | | 0.01* |
| **Group 2** | 0.003* | | | |
| **Group 3** | 0.001* | 0.7 | | |
| **Group 4** | 0.01* | 0.56 | 0.34 | |

Table 8. Socket variables distribution among study groups.

| Socket related variables | Group 1 Mean (SD) | Group 2 Mean (SD) | Group 3 Mean (SD) | Group 4 Mean (SD) | P value |
|---|---|---|---|---|---|
| **Wound width** | | | | | |
| **Mesio-Distal** | 5.8 (1.76) | 5.8 (1.6) | 6.3 (2.2) | 6 (1.3) | 0.9 |
| **Bucco-Lingual** | 7.4 (1.5) | 7.2 (1.16) | 7.45 (1.9) | 7.4 (1.3) | 0.96 |
| **Socket width** | | | | | |
| **Mesio-distal** | 4.7 (2.1) | 5.5 (1.57) | 5.7 (1.8) | 5.2 (0.4) | 0.6 |
| **Bucco-lingual** | 5.2 (1.78) | 5.9 (1.13) | 6 (1.57) | 6.3 (1) | 0.38 |
| **Socket walls** | 3.7 (0.4) | 3.63 (0.5) | 3.9 (0.3) | 3.6 (0.5) | 0.48 |
| **Fenestration** | 1 (11%) | 1 (9%) | 1 (8.3%) | 0 (0%) | 0.74 |

# 6 DISCUSSION

The present study indicated that inclusion of bone replacement graft suppressed new bone formation in extraction sockets during the first 8 weeks of healing. In all parts of the examined cores, new bone was consistently more in group 1, in which the socket was left to heal undisturbed without inclusion of bone graft[5, 6, 20, 21, 34, 35]. This finding is in agreement with other studies[5-7, 71, 72]. Natural healing process starts from the walls of the socket and allows for more bone formation in a given volume, if space is not occupied by graft particles[5-7]. This phenomenon was more pronounced in the apical portion suggesting that proximity between socket walls as well as less wound volume in that region might be critical factors in new bone formation. More

rapid bone regeneration in the apical portion of the socket has also been a consistent finding in previous studies[5-7, 73]. Higher percentage of soft tissue was also consistently present in group 1 compared to groups where bone graft was used. Histology of sockets selected to heal by a natural wound process demonstrated abundant woven bone formation organized in a trabecular pattern within a rich connective tissue matrix with abundant fibroblasts present. In a small number of specimens, limited number of PMNs, was also present. In the apical portion of natural healing sockets, the woven bone formation was dense and also included moderate amounts of lamellar bone. Lamellar bone was present in close proximity with woven bone forming composite bone structures. Composite bone is common in early stages of intramembranous ossification[9]. In the sockets where bone graft was used, new bone was present between and in close proximity with graft particles. New bone as well as soft tissue appeared constricted between graft particles. The formation of new bone in close proximity with graft particles is in agreement with other studies where FDBA and other composite grafts were used[8, 30, 31, 74, 75]. Becker et al[27] utilized demineralized bone allograft, autologous bone as well as a composite graft with human morphogenetic proteins. The authors reported encapsulation of bone graft particles within a soft tissue matrix without new bone formation[76]. Similar results have been reported by the same group in a previous study[27]. In the present study, such finding was not common and it only occurred in the most coronal aspect of the socket. More rapid new bone formation was noted in the apical part of the socket. This contradicts previous findings that supported that the inclusion of bone graft leads to uniform remodeling of the socket[8]. Such difference can be attributed to

differences between study design and bone graft used[8]. In the present study mineralized FDBA and β-TCP were used as a composite graft. FDBA is an osteo-conductive material that functions as a provisional scaffold to induce new bone formation[30]. Due to mineralization, the contained bone morphogenetic proteins (BMPs) are not exposed to the wound environment and cannot function to initiate bone formation from precursor osteogenic cells. Before mineralized FDBA can express osseo-inductivity, a phase of osteoclastic resorption must occur to break down the mineral content and expose the BMPs[30]. The osteo-conductive properties of FDBA have been shown to be superior to DFDBA[77], while the osteo-inductive properties of DFDBA seems to be affected by a variety of factors such as age of the donor[78]. DFDBA has not always been shown to have osteo-inductive capacity[78-82]. Despite that, FDBA has been shown to be successful as replacement graft in socket preservation scenarios in achieving space maintenance and vital bone formation[17, 30-32, 83-89]. In the present study an FDBA cancellous particulate graft was used (OraGRAFT; LifeNet Health, Inc. VA, USA). The average particle size according to the manufacturer ranged from 250-1,000 μm. The particle size has been studied as a variable that may affect the osteogenic potential of composite grafts of allogenic freeze-dried bone and marrow. A study on primates by Shappof et al[90], indicated that small particle bone graft (100-300 μm) present more favorable osteogenic response than larger size particles (1,000-2,000 μm). A synthetic ceramic material (β-TCP/GEM-21; NY, USA) was also used in groups that included bone graft in conjunction with FDBA. Tricalcium phosphate is a phosphoric acid salt that can be present with different crystalline forms (α- and β-) and has long been used in

orthopedics as a porous scaffold that is both biocompatible and biodegradable[91-94]. Biodegradation of the β- form is considered rapid due to low crystallinity compared to α- form[95]. Lindhe et al[38] used an alloplastic graft of high crystallinity (α-TCP core coated with nanocrystalline biomimetic hydroxyapatite, BPCAP- Collagen; Geistlich Pharma AG, Basel, Switzerland) to fill fresh canine premolar extraction sockets. The authors found that during 3 months α-TCP did not undergo marked resorption, although it allowed for bone fill and partially prevented ridge resorption. Another study by Hong et al[96] examined the effects of different synthetic bone fillers in canine premolar extraction sites. The authors used 4 experimental groups. In the first three groups hydroxyapatite (HA), bi-phasic calcium phosphate (BCP) and beta-tricalcium phosphate (β-TCP) were used respectively. The remaining group was used as control. According to their findings, β-TCP retarded early new bone formation compared to other biomaterials; although eventually β-TCP underwent more resorption compared to the other fillers of high crystallinity[96]. The findings were in agreement with other comparative studies, which showed that β-TCP has a significantly accelerated resorption rate compared to α-TCP[95, 97]. The inclusion of β-TCP in all bone grafting groups allowed for utilization of GEM-21 according to FDA-approved label use[98].

Use of growth factors in conjunction with bone replacement grafts has been gaining momentum over the past 20 years[57, 64, 67]. In a cornerstone manuscript[66], Marx et al outlined the effect of combining PRP with bone grafts. Platelets are megakaryocyte fragments that circulate for about 7-10 days in the peripheral blood and play a major role in the initial stages of wound healing, specifically clot

formation. PRP is a concentrated autologous clot that contains 4 to 7 times the usual concentration of platelets found in the peripheral blood (200,00 platelets/μL) resulting in an approximate concentration of $1x10^6$ platelets per μL[65]. Platelets contain a variety of growth factors such as three isomers of PDGF (PDGFaa, PDGFbb and PDGFab), vascular endothelial growth factor (VEGF), and two isomers of transforming growth factor (TGFa and TGFb) as well as epithelial growth factor (EGF)[66, 99]. These factors are packaged in inactive forms in cytoplasmic alpha granules. During blood clot formation, alpha granules fuse with the outer membrane and become active in the tissue after the addition of carbohydrate chains and histones. Each growth factor has different functions in the early stages of wound healing. PDGF causes proliferation and mitosis of different cells through membrane receptors[66]. Osteoprogenitor cells are differentiated to osteoblasts and secrete osteoid while endothelial cells produce basal lamina for neoangiogenesis and fibroblasts secrete collagen matrix. TGFs promote differentiation for bone formation while VEGF promote formation of new blood vessels. These factors are secreted within the first minutes of clot formation and play a vital role in the early stages of wound healing in osseous defects. Molecules such as vitronectin, fibrin and fibronectin create a protective matrix around the grafted area that provides wound stability and enhances cell migration and vascularization[66]. As mentioned previously, the life of a platelet does not exceed 10 days and the beneficial effect of PRP has been established to perform within that timeframe[64, 66, 100]. Marx et al[66], studying mandibular continuity restoration procedures, demonstrated that the use of PRP in conjunction with bone grafting resulted in rapid mineralization and maturation of

bone grafts compared to control sites. In test sites after 4 months healing, the authors were able to demonstrate 80% bone density with mature lamellar architecture featuring well-formed Haversian systems[66]. Under this light, the combination of PRP with bone replacement grafts in sockets is thought to promote maturation and mineralization processes[58].

Another growth factor that has shown promising results is human recombinant PDGFbb. GEM 21 is a synthetic bone grafting system that contains about 1,000 times more PDGFbb than found in available PRP harvesting preparations. As mentioned previously, PDGF is a potent molecule that induces cell proliferation and recruitment towards bone formation. Two large multicenter studies have demonstrated the effectiveness of PDGF in periodontal regeneration[67, 101]. Nevins and Reynolds[57] have also shown beneficial effects of off-label use of PDGFbb in conjunction with FDBA and DFDBA in ridge preservation, lateral ridge augmentation and sinus lifts procedures. Potential beneficial effects of GEM-21 composite grafts in ridge preservation would lead to acceleration of implant site development and allow the clinician to utilize a completely synthetic, off-the-shelf product to achieve superior results. In the present study, tissue distribution between groups where bone graft was used revealed a trend of less residual bone particles in groups 3 and 4 where growth factor enhancement was used. This phenomenon was more pronounced in the apical and middle thirds as well as in the entire core. This finding implies that rh-PDGFbb and PRP preparations in this study may induce more rapid bone graft remodeling. The cores were harvested at 8 weeks with the aim being to examine the effect of growth factors in the early stages of wound healing. A

further comparison between groups 3 and 4 reveals that group 4 had less residual bone graft particles, although the two groups did not differ significantly in new bone or soft tissue formation.

All treatment modalities in the present study achieved a significant amount of new vital bone that ranged from 28-37% at 8 weeks post extraction. Lian et al[102] showed that an average of 58-60% of bone-to-implant contact is present around successful dental implants. However, this author is not aware of any evidence that directly correlates the amount of vital bone at the time of implantation with implant success. However, implant success has been associated with primary stability,[103] and primary stability has been associated with bone mineral density[104]. The use of bone replacement graft allows for successful implantation with satisfactory primary stability. The latter may not be possible in a socket that is left to heal undisturbed without bone graft.

The present study did not identify any association between tissue distribution and race, gender, age or smoking. The effect of age on osteoinductivity of DFBDA has been identified in a study by Schwatz et al[78]. In the same study, gender of the donor did not affect osteoinductivity of the allograft. Unlike the study by Schwatz et al[78] that accounted for young adult donors, the mean age in our study was 52 years with subjects not deviating significantly from the mean. This did not allow for conclusions to be drawn on the effect of age on early socket healing. Smoking has been consistently shown to be a leading risk factor for negative outcomes in periodontal regeneration[105-107], guided bone regeneration[108], sinus augmentation[109-112] and long term implant survival[113]. 29% of patients who participated in our study

were smokers and were evenly distributed among the treatment groups. Smoking did not affect tissue distribution significantly and no adverse events were reported throughout the 8 weeks of post-extraction healing. Subjects were instructed prior to and throughout the study, to refrain from smoking. This may have averted some of the immediate negative effects of tobacco during the study.

Socket and wound-related variables such as bucco-lingual and mesio-distal dimensions, as well as presence of fenestrations were evenly distributed among groups. No association between these variables and tissue distribution was identified. Socket volume has been identified as a factor that affects rate of healing and tissue formation[114]. Sockets of larger volume present delayed tissue proliferation and maturation[114]. However, the sockets in this study did not differ significantly in dimensions since the majority of extracted teeth were premolars. The results of the present study may not be reproducible in different populations. The majority of the subjects were North American Caucasian females with a mean age of 52 years old.

Taking under consideration that the core was harvested form the center of the socket, the study design did not allow for evaluating changes that take place in the walls of the socket at 8 weeks post-extraction. According to previous studies[5-7], the walls of the socket, as well as remnants of the periodontal ligament, collapse towards the center of the socket, where they are degraded by osteoclasts and macrophages during the normal remodeling process. We were unable to detect parts of the sloughed socket walls in all groups, including the undisturbed healing sockets group. One explanation for that might be that in 8 weeks there are no visible remnants of

necrotic bone because the initial osteoclastic process in the center of the wound is complete[11].

Significant differences have been noted in platelets, white cells and growth factor concentration between different harvesting and separation methods[115]. Contradictory results have emerged on the efficacy of the one versus two-step procedure of platelet separation[115, 116]. Variability has also been noted between repeated blood draws from the same subject with the same method[115]. In the present study, the Cascade Fibrinet System (MTF, NJ,USA) was used. This system has been successfully used in orthopedic surgery for treatment of knee osteoarthritis[117]. Published comparison studies reported that the Cascade system is dependable in providing high concentrations of platelets and growth factors such as PDGF and VEGF as well as low concentrations of white blood cells[117-119]. Low concentrations are thought to reduce the inflammatory response after PRP injection in the wound site[117]. In the present study, the PRP harvesting method was identical for every subject and the harvesting system was operated by the same experienced operator.

One of the strengths of the present study was the utilization of the Nikon Elements software. This software has been used in various applications for the detection of cells to evaluate photoactivation and photobleaching in living cells[120, 121]. The software provides a potent pixel classifier that utilizes different filters such as intensity values, RGB values (colors are specified in terms of the three primary colors: red, green, and blue), HIS values (hue, intensity and saturation) or RGB values ignoring intensity. It is worth mentioning that different pixel classifier settings were chosen for each studied specimen to accommodate different staining results of

different tissues between specimens. To accomplish that, extended training of the classifier took place on every specimen by manually identifying different tissues. The operator (AN) was blinded and unaware of the group that each specimen belonged to. Once the pixel classifier was trained, the settings were saved and could be loaded at any point of time. The use of pixel classifier allowed for accurate quantitative analysis and imaging of the different tissues in the histologic sample[121].

The present study demonstrated a moderate effect of PRP as well as rh-PDGFbb in bone graft turnover in human extraction sockets. Future fields of study on the use of growth factors in ridge preservation should include the effect of growth factors on soft tissue healing and epithelial migration. Possible beneficial effects would mean accelerated time of re-entry for implant placement as well as minimizing prevalence of early cover screw exposures after implant placement[122-124]. Also, the effect of growth factors should be evaluated in relation to the dimensional changes that take place after tooth extraction, and whether or not they offer an additional advantage to the preservation of socket wall dimensions and socket volume[58].

The ridge preservation technique utilized in this study calls for flapless extraction of teeth without the use of barrier membrane and no effort for primary closure. Several studies have reported less remodeling with flapless extraction procedure[125-127]. A meta-analysis of nine studies by Vignoletti et al[17] indicated that flap elevation is crucial for the ridge remodeling after tooth extraction. Interestingly, flap elevation appeared to cause less horizontal remodeling. According to the authors, that was associated with achieving primary closure[17]. An advantage of not achieving primary closure is that the mucogingival junction does not shift coronally, which preserves

the architecture and position of keratinized tissue. The effect of barrier membranes remain unclear[17]. The inclusion of a membrane appears to inhibit horizontal changes, while it causes more changes in vertical height compared to bone graft alone[17]. In the present study, the inclusion of PRP and rh-PDGFbb failed to induce significantly more new bone formation overall. Although, a moderate effect on accelerating bone graft turnover was noted. As mentioned previously, the effect of vital bone concentration at the time of implantation remains unknown. The use of growth factors that accelerate turnover of graft materials may prove efficacious.

# 7 SUMMARY AND CONCLUSION

The present study is a prospective randomized clinical trial with multiple arms. This manuscript presents the outcomes of histologic and histomorphometric analysis of samples harvested 8 weeks post extraction from 41 sockets randomized in 4 different groups. Participants were stratified for smoking status and randomized to one of four extraction groups; three experimental groups utilizing three different bone grafting applications and a control group where extraction sockets were allowed to heal naturally. Each participant contributed one random socket in the analysis. The study evaluated the healing response of grafted sockets when PRP and rhPDGFbb were combined with the graft. The following conclusions can be drawn from this study:

a) Inclusion of bone replacement graft suppressed new bone formation in extraction sockets during the first 8 weeks of healing.

b) Less residual bone graft particles were noted in PRP and rh-PDGFbb enhanced groups, indicating more rapid turnover of bone graft in human extraction sockets.

c) The inclusion of PRP and rh-PDGFbb failed to induce significantly more new bone formation overall.

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# APPENDIX A

# IRB APPROVAL FORM

# Project Revision/Amendment Form

Form version: June 26, 2012

*In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.*

- Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for Investigators for additional information.
- Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator's Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.



| 1. Today's Date | November 6, 2012 |
|---|---|

## 2. Principal Investigator (PI)

| | | | |
|---|---|---|---|
| Name (with degree) | Michael S. Reddy, DMD, DMSc | Blazer ID | mreddy |
| Department | Periodontology | Division (if applicable) | |
| Office Address | 406 SDB | Office Phone | 4-4720 |
| E-mail | mreddy@uab.edu | Fax Number | 5-6544 |

**Contact person who should receive copies of IRB correspondence (Optional)**

| | | | |
|---|---|---|---|
| Name | Sandra Haigh | E-Mail | shaigh@uab.edu |
| Phone | 4-7513 | Fax Number | 4-7901 |
| Office Address (if different from PI) | 412 SDB | | |

## 3. UAB IRB Protocol Identification

| | |
|---|---|
| 3.a. Protocol Number | F071006001 |
| 3.b. Protocol Title | A Prospective Study of Bone Augmentation Techniques in Extraction Sockets and Implant Surface Textures |

3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable

- ☐ Study has not yet begun — No participants, data, or specimens have been entered.
- ☐ In progress, open to accrual — Number of participants, data, or specimens entered:
- ☐ Enrollment temporarily suspended by sponsor
- ☒ Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)
  - Date closed: 04/15/10
  - Number of participants receiving interventions: 69 ✓
  - Number of participants in long-term follow-up only:
- ☐ Closed to accrual, and only data analysis continues
  - Date closed:
  - Total number of participants entered:

## 4. Types of Change

**Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.**

- ☐ **Protocol revision (change in the IRB-approved protocol)**
  In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.
- ☐ **Protocol amendment (addition to the IRB-approved protocol)**
  In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.
- ☐ **Add or remove personnel**
  In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See "Change in Principal Investigator" in the IRB Guidebook if the principal investigator is being changed.
- ☒ **Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication**
  In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP). ✓
- ☐ **Change in source of funding; change or add funding**
  In Item 5.c., describe the change or addition in detail, include the applicable OSP proposal number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.

☐ **Add or remove performance sites**
In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.

☐ **Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)**
To assist you in revising or preparing your submission, please see the IRB Guidebook for Investigators or call the IRB office at 934-3789.

☐ **Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)**
In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.

☐ **Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)**
In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.

☐ **Revise or amend consent, assent form(s)**
Complete Item 5.d.

☐ **Addendum (new) consent form**
Complete Item 5.d.

☐ **Add or revise recruitment materials**
Complete Item 5.d.

☐ **Other (e.g., investigator brochure)**
Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable.
Include a copy of all affected documents, with revisions highlighted as applicable.

▶

## 5. Description and Rationale

**In Item 5.a. and 5.b, check** Yes **or** No **and see instructions for** Yes **responses.**
**In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.**

☐ Yes ☒ No **5.a. Are any of the participants enrolled as normal, healthy controls?**
If yes, describe in detail in Item 5.c. how this change will affect those participants.

☐ Yes ☒ No **5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?**
If yes, FAP-designated units complete a FAP submission and send to fap@uab.edu. Identify the FAP-designated unit in Item 5.c.
For more details on the UAB FAP, see www.uab.edu/cto.

**5.c. Protocol Changes: In the space below, briefly describe—and explain the reason for—all change(s) to the protocol.**

▶ Thanos Ntounis, DDS, department of Periodontology, will perform an analysis of data collected under this IRB-approved HSP and will use the results to develop his Master's degree thesis. The working title: "A Prospective Study of Bone Augmentation with Growth Factors in Extraction Sockets."

Dr. Ntounis will assess and compare quantitative and qualitative bone-fill after eight weeks post-extractions across the four treatment groups; his analysis falls within the original objectives of the study. Dr. Ntounis has no conflicts of interest to disclose. ✓

**5.d. Consent and Recruitment Changes: In the space below,**
**(a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them;**
**(b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and**
**(c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary (not applicable for recruitment materials).**

**Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:**
- **a copy of the currently approved document (showing the IRB approval stamp, if applicable)**
- **a revised copy highlighting all proposed changes with "tracked" changes**
- **a revised copy for the IRB approval stamp.**

▶

Signature of Principal Investigator _____ Date 1-6-12

**FOR IRB USE ONLY**

☐ Received & Noted ☑ Approved Expedited* ☐ To Convened IRB

f Clubhole MD — Nov 13, 2012

Signature (Chair, Vice-Chair, Designee) Date

DOLA 3-7-12

Change to Expedited Category **Y / N / NA**

*No change to IRB's previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111