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Title of Thesis: Evaluation of a Poly(Lactic-Co-Glycolic) Acid-Coated β-Tricalcium Phosphate Bone Substitute for Alveolar Ridge Preservation: Multicenter Randomized Control Trial

Brandon M. West, Master of Science, 2019

Thesis directed by: [Hanae Saito, DDS, MS, CRC, Assistant Professor, University of Maryland Periodontics Department]

Purpose: The aim of this study was to determine whether the efficacy of PLGA-β-TCP in Alveolar Ridge Preservation (ARP) with histological analysis of the extraction socket following four months healing was similar to FDBA + CP as the control.

Materials and Methods: 45 adult patients in this multicenter randomized controlled clinical trial were randomly assigned into treatment group A [PLGA-β-TCP] or group B [FDBA + CP] for ARP of a single extraction socket.

Results: 45 bone core samples were harvested. Vital bone was present in intimate contact with the surface of β-TCP graft particles. Histologic measurements revealed no statistically significant difference between the test and control groups in percent vital bone after four months healing.

Conclusion: This investigation demonstrated the similar efficacy of PLGA- β-TCP to FDBA as a bone substitute in ARP.

Evaluation of a Poly(Lactic-Co-Glycolic) Acid-Coated β-Tricalcium Phosphate Bone Substitute for Alveolar Ridge Preservation: Multicenter Randomized Control Trial

by
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Thesis submitted to the Faculty of the Graduate School of the
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Table of Contents

List of Tables

Table Page

List of Figures

1. INTRODUCTION

Tooth loss typically occurs when a tooth has a questionable or hopeless prognosis due to one of several factors including extensive caries, inability to restore, and severe attachment loss (Kwok & Caton, 2007). With the loss of teeth, the placement of the dental implant to replace the missing teeth has been widely accepted as one of the preferred choices. (Joda, Bragger, & Zitzmann, 2018). Before implants were available, fixed partial dentures (FPDs) and removable partial dentures (RPDs) were the primary treatment options available for missing teeth. Disadvantages of fixed partial dentures include removal of adjacent tooth structure and difficulty with daily oral hygiene under the pontic space. For removable dentures, patients were commonly less satisfied with the amount of stability and retention (Leão, Moraes, Vasconcelos, Lemos, & Pellizzer, 2018).

When tooth extraction was elected as the option of the treatment, a biological cascade starts and results in an altered morphology of the supporting alveolar ridge (Avila-Ortiz, Elangovan, Kramer, Blanchette, & Dawson, 2014; Pietrokovski, 1967; Schropp, 2003). It has been well documented that the alveolar process undergoes atrophy, and resorption can be observed more pronounced on the buccal aspect in comparison to the lingual or palatal plate (Pietrokovski, 1967). With more bone resorption on the buccal, the center of the edentulous ridge shifts lingually as well. In addition, it was noted that the amount of tissue resorption was significantly greater in edentulous molar region than in the incisor or premolar regions (Pietrokovski, 1967). In a

twelve-month observational study, it has been demonstrated that 50% of the alveolar ridge width resorbs over twelve months post-extraction. Furthermore, approximately two-thirds of this reduction occurred within the first three months (Schropp, 2003).

A. HISTOLOGY OF EXTRACTION SOCKET

Following an extraction, a sequence of biological events occurs to end with healing of the alveolar ridge. Once a tooth is removed, the socket immediately fills with blood from the surrounding vessels that were severed (Lai, 2017). This damage initiates the formation of a fibrin matrix and platelets within the blood interact with the fibrin to produce a coagulum, also known as the blood clot. It is generally accepted that the blood clot is crucial for initial healing (Lai, 2017). Once the clot is stable, it effectively blocks the severed vessels and stops the bleeding. The blood clot also contains growth factors which influence mesenchymal cells and enhance the activity of inflammatory cells. Consequently, this promotes the induction and amplification of cells migration to the site. These cells include polymorphonuclear leukocytes, macrophages, and monocytes to start the phagocytosis of necrotic tissue in the socket (Lai, 2017).

After a few days, the blood clot will begin to break down and fibrinolysis will occur. This process results in clearance of any debris as well in the socket by the macrophages, and sprouts of vascular structures begin to form from walls of the socket. Mesenchymal cells and fibroblast-like cells enter as well from adjacent bone marrow and deposit matrix components in an extracellular location. As a result, granulation tissue is formed replacing the fibrin clot (Lai, 2017). Clinically, this occurs within one week following the extraction (Araújo & Lindhe, 2005). Newly formed vessels provide oxygen

and nutrients for increasing the number of cells in new tissue. As fibroblasts continue to synthesize matrix component and angiogenesis occurs, an early form of connective tissue arises. Then, it transitions to bone when osteoprogenitor cells differentiate into osteoblasts, producing collagen fibers in a woven pattern. This is known as the osteoid and mineralization occurs within it. As osteoblasts continue to deposit bone, these cells sometimes get trapped in the matrix and become osteocytes (Lai, 2017).

In an experimental dog study performed by Araůjo and Linde, histological samples were collected at one, two, four and eight weeks of healing following extraction (Araújo & Lindhe, 2005). At one week, a provisional matrix was visible at the center of the socket, containing minimal collagen content, but a high infiltration with inflammatory cells. Small amounts of newly formed woven bone were also observed at one week around the vascular units close to the bundle bone. Osteoclasts were visualized at the crest of the walls and along the bundle bone that lines the socket walls (Araújo & Lindhe, 2005). At two weeks, there was a fibroblast rich connective tissue present and the mucosa was devoid of any inflammatory cells. Large amounts of newly formed bone occurred in the apical and lateral portions of the socket. Furthermore, no periodontal ligament could be visualized at these lateral portions of the socket. The surface of the woven bone was lined with densely packed osteoblasts and included a primitive bone marrow (Araújo & Lindhe, 2005). At four weeks, no bundle bone could be identified in the crestal region of the bone walls. In the crestal region of the buccal wall, a large portion of the lamellar bone was replaced by woven bone and the surface exhibited signs of remodeling. The most central portions still contained provisional matrix tissue. Mineralized tissue and bone marrow occupied the remaining segments of the site (Araújo & Lindhe, 2005). At

eight weeks, a wide zone of mineralized tissue extended from the buccal to the lingual bone walls. However, the crest of the buccal bone was two millimeters apical to the crest of the lingual wall (Araújo & Lindhe, 2005).

B. IMPLANT THERAPY

Dental implants provide patients with the opportunity to replace lost teeth while avoiding the need for any adjacent teeth to support a fixed restoration in edentulous sites (Joda et al., 2018). The first dental implants were placed by Dr. Per-Ingvar Brånemark in 1965 on edentulous patients (Branemark, 1983). The screwshaped dental implant was easy to insert, remained stable, and caused no undesired tissue reaction (P.-I. Branemark, U. Breine, R. Adell, B. O. Hansson, J. Lindstrom, 1969). This type became known as a fixture, consisting of a cylindrical titanium screw and perforations to allow ingrowth of bone. In theory, this would allow a secure, firm position in the jaw. From the top, there was a threaded hole for the prosthesis to be screwed into. For the original surgical protocol, teeth in the maxilla and mandible were extracted 4-6 months before insertion of the fixture. When the alveolar healing was complete, a full-thickness mucoperiosteal flap was elevated and prepared for the implant site. Special burs and screw-taps were utilized for preparation with minimal pressure, continuous irrigation, and low rotations per minute (rpm). Once the implant was successfully inserted, the flap was replaced into its original position to seal off the site from the oral cavity. After 6-8 weeks of healing, the upper surface of the implant was exposed with a small incision in the soft tissue, and a post called a strut was attached. The strut allowed the mucosa to adapt around it with final healing, and a prosthesis could be connected to it as well. Two weeks afterwards, impressions were made to manufacture prostheses in the form of bridges to anchor

directly to the struts (P.-I. Branemark, U. Breine, R. Adell, B. O. Hansson, J. Lindstrom, 1969). As time progressed, protocols suggested that implants should be placed in a healed site following three to six months after an extraction was performed (Buser, Sennerby, & De Bruyn, 2017). With the residual alveolar ridge mature, the implant could be supported entirely by native bone and achieve primary stability with placement. More contemporary protocols suggest inserting implants immediately in a fresh extraction socket or shortly after the socket has partially healed with soft tissue coverage in four to eight weeks (Buser, Chappuis, Belser, & Chen, 2017). This is known as immediate implant placement and early implant placement, respectively (Buser, Chappuis, et al., 2017). However, these protocols are limited by the residual bone volume following extraction and subsequently, a lower possibility of achieving primary stability (Buser, Chappuis, et al., 2017). Also, it has been suggested that bone grafting with implant placement is necessary if there is a gap larger than 1.5 mm from the implant and the buccal boney plate of the extraction socket (Kan et al., 2018; Wilson et al., 1998). If immediate implant placement or early implant placement are contraindicated, alveolar ridge preservation (ARP) is suggested (Buser, Chappuis, et al., 2017).

C. ALVEOLAR RIDGE PRESERVATION

When clinical evaluation of teeth indicates extraction and replacement with a dental implant, the morphology of a patient's alveolar ridge can either support or limit the implant placement. With treatment planning aimed at achieving long-term stability and prevention of failure, it is strongly recommended to surgically place implants in sites with adequate bone volume (Esposito M, Ekestubbe A, 1993; Kan et al., 2018). For this reason, hard tissue augmentation may be necessary in both the maxilla and mandible to

gain this requirement. The pre-operative dimensions of the existing alveolar ridge dictate when guided bone regeneration (GBR) should be performed. GBR is generally indicated when the alveolar ridge exhibits a horizontal and/or vertical deficiency in the future implant site. If the ridge is severely atrophic requiring a significant amount of horizontal and vertical augmentation, it may be suggested to stage GBR and the implant placement (Milinkovic & Cordaro, 2014). However, the presence of a fenestration or dehiscence with minimal deficiencies may be managed with simultaneous implant placement and GBR (Kinaia, Korkis, Masabni, & Neely, 2019; Milinkovic & Cordaro, 2014).

In contrast, it is important to acknowledge the loss of alveolar ridge volume commonly observed in a site following an extraction when planning to place an implant. The concept of alveolar ridge preservation (ARP) started initially with concern for edentulous patients and their atrophic ridges, which consequently provided less stability from the arch for the denture. To counter this deficiency, root retention was proposed as a way to prevent ridge resorption under a removable prosthesis (Osburn, 1974). However, disadvantages to this technique were the development of caries and fracture of the teeth underneath. As a better alternative to submerging retained roots, ARP was identified in the 1980's as a simple technique to add biomaterial into a fresh extraction socket and attain the same clinical result as retaining a root in the alveolus (Avila-Ortiz et al., 2014). It is well-documented that the resorption of the alveolar ridge is more severe with natural healing in comparison to the placement of a bone substitute into a fresh socket (Avila-ortiz, Chambrone, & Vignoletti, 2019; Avila-Ortiz et al., 2014; Cantín, Olate, Fuentes, & Vásquez, 2015; Frost, Mealey, Galloway, Banjar, & Huynh-Ba, 2013; Iasella et al., 2003; Ten Heggeler, Slot, & Van Der Weijden, 2011; Van Der Weijden, Dell'Acqua, & Slot,

2009; Walker et al., 2016). In response to this observation, ARP has been identified to be a successful procedure in minimizing the horizontal and vertical atrophy that is observed following extractions (Avila-ortiz et al., 2019; Avila-Ortiz et al., 2014; Ten Heggeler et al., 2011; Van Der Weijden et al., 2009). Therefore, it would be advantageous to counter these volumetric changes initially by performing ARP in preparation for future implant placement and possible avoidance of additional ridge augmentation.

ARP does not entirely prevent the alveolar ridge resorption process, but the overall changes in the ridge dimensions are significantly less compared to natural healing. In a systematic review and meta-analysis by Avila-Ortiz, it was confirmed that alveolar ridge preservation is an effective treatment in limiting the physiologic ridge reduction as compared with tooth extraction alone (Avila-Ortiz et al., 2014). The clinical magnitude of the effect was 1.89 mm in terms of buccolingual width, 2.07 mm for midbuccal height, 1.18 mm for midlingual height, 0.48 mm for mesial height and 0.24 mm for distal height changes (Avila-Ortiz et al., 2014). More specifically, the treatment protocol used by the clinician could affect the observed ridge dimensions after healing. Protocols vary with the flap elevation, barrier membrane use, type of flap closure, and the type of bone graft used (Avila-Ortiz et al., 2014). Other factors affecting ARP are the number of neighboring teeth extracted, socket morphology, periodontal biotype (bony buccal plate and soft tissue thickness), systemic (smoking, uncontrolled diabetes, bone metabolic disorders), and patient compliance (Avila-Ortiz et al., 2014).

D. DIFFERENT ARP PROTOCOLS: 1. Bone Substitutes

When performing ridge augmentation, there are several bone replacement graft options for the clinician to choose from to include autogenous, allograft, xenograft, and alloplastic materials. Autogenous bone is acquired from the same individual and harvested from either an extraoral or intraoral site (Hsu & Wang, 2013). Allografts are obtained from different individuals of the same species, and xenografts are obtained from a different species than the recipient (Hsu & Wang, 2013). Without the need for a donor, alloplastic materials are synthetically derived (Hsu & Wang, 2013). Autogenous bone is considered the gold standard (Demetter, Calahan, & Mealey, 2017; Hsu & Wang, 2013). This is due to its osteogenesis potential and no risk of disease transmissibility (Hsu & Wang, 2013). However, there are disadvantages to autogenous bone use, which include the need for an additional surgical site for harvesting followed by morbidity with the donor site and the need to adapt the graft properly (Bizenjima, Takeuchi, Seshima, & Saito, 2016).

To avoid these considerations, many clinicians prefer to use allografts instead. Commonly used allografts are Freeze-Dried Bone Allograft (FDBA), demineralized FDBA (DFDBA), or a mixture of both FDBA and DFDBA (Hsu & Wang, 2013). It has been demonstrated that allografts perform similarly to autogenous bone (Demetter et al., 2017). FDBA has also been shown to out-perform alloplastic materials in regards to dimensional stability and new bone formation (Avila-Ortiz et al., 2014; Ten Heggeler et al., 2011). In favor of regeneration, allografts (DFDBA and FDBA) are capable of being resorbed and replaced by new bone formation in the site. An early histological study confirmed the success of periodontal regeneration with the use of DFDBA in submerged

defects (Gerald Bowers, Brian Chadroff, Robert Carnevale, James Mellonig, Russel Corio, Jane Emerson, Mark Stevens, 1982). However, it has been documented that many defects still contain residual bone particles with adjacent new bone formation at six months (Bowers, Gerald M, Reynolds, 1996). In contrast, xenografts such as bovine bone substitutes have a slower resorption rate (Hsu & Wang, 2013). This characteristic is beneficial for providing space maintenance within the defect while new bone formation occurs. Histologically, this was demonstrated in a randomized ARP study (Ramaglia, Luca, Matarese, Giovanni, Williams, 2018). At sixteen weeks healing, biopsies demonstrated bovine bone particles embedded and partially encapsulated in connective tissue with coarse collagen fibers. In addition, there was no apposition of newly formed bone and no inflammatory reaction. At thirty-two weeks, mature lamellar bone was observed with integrated xenograft particles by following ARP (Ramaglia, Luca, Matarese, Giovanni, Williams, 2018).

Without the acquisition of tissue from a donor, alloplastic materials such as β-Tricalcium Phosphate (β-TCP) provide a bone replacement graft with no risk of disease transmission. It has also been documented to have a slow resorption rate similar to xenograft (Hsu & Wang, 2013). When comparing the effect on midbuccal alveolar bone height preservation, the use of alloplastic materials has been identified to be less beneficial in comparison to a xenograft or an allograft (Ortiz 2014). However, the alloplastic materials could provide better handling for the clinician when the properties of the graft are altered and combined with additional materials. For example, β-TCP can be coated by poly (lactic-co-glycolic acid) (PLGA) and become more sticky when activated

by an additional liquid, N-methyl-2-pyrrolidone (M. Leventis et al., 2018). This will be discussed further in the study protocol.

E. DIFFERENT ARP PROTOCOLS: 2. Use of Barrier Membrane

ARP involves the use of at least one biomaterial, but it is typical to use a combination of materials to include barriers to contain the bone graft and exclude epithelial migration into the defect. Barriers can be resorbable or non-resorbable. Non-resorbable barriers are more rigid, thus optimizing the potential for vertical bone augmentation. However, non-resorbable barriers require the need to be removed and have an increased risk of post-operative exposure or infection. Resorbable barriers are commonly less rigid and have fewer complications when exposed. Overall, the use of barriers is aimed at the concept of providing epithelial exclusion and promotes periodontal regeneration (Rakhmatia, Ayukawa, Furuhashi, & Koyano, 2013).

When using barriers for ARP, the use of barriers alone reported more vertical bone change than the use of grafts alone (Vignoletti et al., 2011). This was further supported in another systematic review and meta-analysis that the use of a membrane had a strong beneficial effect on the preservation of midbuccal and midlingual alveolar bone height among the experimental sites (Avila-Ortiz et al., 2014). However, there is no evidence to support the use of a barrier either internally or externally to address small fenestrations or dehiscences (Frost et al., 2013). Furthermore, with the use of a poly(lactic-co-glycolic) acid-coated β-Tricalcium Phosphate (β-TCP) bone substitute, an in-situ hardening can occur and stabilize the graft within the extraction socket

(Schmidlin, Nicholls, Kruse, Zwahlen, & Weber, 2011). This biomaterial reduces the need for a barrier to contain the graft and allows epithelial migration without the primary wound closure (Kakar, Rao, Hegde, Deshpande, & Lindner, 2017; M. Leventis et al., 2018).

F. DIFFERENT ARP PROTOCOLS: 3. Flapless/Flap Elevation

It has been suggested that elevating a flap with extraction leads to further bone loss (Fickl, Zuhr, Wachtel, Bolz, & Tissue, 2008; Frost et al., 2013). For this reason, atraumatic extractions have been suggested to minimize bone loss in future implant sites. In a beagle dog study, sites with flap elevation were observed to have 0.7 mm greater volumetric hard and soft tissue loss opposed to flapless sites (Fickl et al., 2008). Contrary to these findings, it has been reported that similar bone remodeling occurs when performing extractions with or without flap reflection after six months healing (Araujo, 2009). In more recent systematic reviews and meta-analyses, it was reported that that ARP with flap elevation resulted in less horizontal ridge resorption (Avila-Ortiz et al., 2014; Vignoletti et al., 2011). It was also reported that flap elevation could have a beneficial effect on preservation of the midbuccal and midlingual alveolar bone height (Avila-Ortiz et al., 2014). These findings could be attributed to the possibility that many clinicians attempt primary closure when replacing the flap and provide a more optimal result for augmentation (Avila-Ortiz et al., 2014; Frost et al., 2013; Vignoletti et al., 2011).

G. DIFFERENT ARP PROTOCOLS: 4. Primary or Secondary Intention Healing

Periodontal wound healing has been determined to be affected by the type of flap closure and subsequent healing pattern (Lai, 2017). With primary closure, the flap margins are replaced together with sutures and healing by first intention is observed (Lai, 2017). In regards to ARP, primary closure has been identified by a meta-analysis to have a slight tendency towards less horizontal bone loss (Vignoletti et al., 2011). However, many providers do not attempt to achieve primary closure for simple ridge preservation procedures and allow secondary intention healing with the grafts exposed (Frost et al., 2013). Without elevating a flap to achieve primary closure, it would be beneficial to minimize surgical trauma and possible post-operative complications (Frost et al., 2013). Furthermore, another study comparing the use and absence of primary closure observed similar vital bone and residual bone graft when performing ARP (Kim, Angelis, & Camelo, 2013). A recent randomized control trial by Hong et al. observed more hard and soft tissue thickness in the alveolar ridge following ARP with the use of a cross-linked collagen barrier over allograft and intentional exposure in comparison to primary closure with allograft and non-crosslinked collagen barrier (Hong, Chen, Kim, & Machtei, 2018). This study also found with CBCT analysis that the crestal width had a significant reduction with primary closure compared to an exposed barrier, which provides contradictory results to findings of the meta-analysis by Vignoletti et. al (Hong et al., 2018; Vignoletti et al., 2011).

In regards to secondary intention healing with ARP, a poly(lactic-co-glycolic) acid (PLGA)-coated β-Tricalcium Phosphate (β -TCP) bone substitute (Easy-graft®

CLASSIC) hardens within minutes when exposed to blood within the extraction socket. This alloplastic material forms a stable porous scaffold for bone regeneration without the need for a membrane. For this reason, better handling can be expected by the clinician (Saito, Shiau, Prasad, & Reynolds, 2017). By following an atraumatic protocol and intentional exposure of a PLGA- β -TCP bone graft, it could be hypothesized that a maximum of keratinized tissue can be achieved with attenuated loss of alveolar bone reduction.

H. STUDY AIM

This multicenter randomized control trial study evaluated the performance of PLGA-β-TCP bone graft in ARP in comparison to the control of FDBA with containment by collagen plug. A previous pilot study determined that the effectiveness of PLGA-β-TCP bone graft was not inferior to those of other leading bone graft substitutes even when PLGA-β-TCP was used without a membrane or a plug covering the defect site (Saito et al., 2017). These findings support the reduction of necessary treatment time and cost of a membrane or a plug to both dentists and patients. Therefore, the aim of this study was to determine whether the efficacy of PLGA-β-TCP in ARP with histological analysis of the extraction socket following four months healing was similar to FDBA + collagen plug as the control.

2. MATERIALS AND METHODS

A. PARTICIPANT ENROLLMENT

In order to answer the hypothesis, the study was designed as a randomized control prospective study with two study centers. Forty-four patients equally distributed among University of Maryland School of Dentistry, Baltimore, MD and University of Iowa College of Dentistry, Iowa City, IA were included in this investigation. Each patient was treatment planned for single tooth extraction, requiring alveolar ridge preservation prior to dental implant placement in a posterior site, including premolars and molars except for third molars. Patients were consecutively enrolled from November 2016 to June 2017. Radiographic verification of ridge integrity was completed using available cone-beam computed tomographic (CBCT) images prior to extraction. The study was approved by the Institutional Review Board of the University of Maryland (IRB #HP-00068397) and registered at Clinicaltrials.gov (NCT02702609). Informed consent to enroll in study was obtained from each patient with verbal and written review of the procedures, scheduled appointments, purpose of investigation, and rights as a patient.

B. STUDY POPULATION

Fourty-four adult patients, (16 males and 29 females) ranging from 23 to 82 years of age (mean 58), were invited to participate according to the inclusion and exclusion criteria. One subject was withdrawn in early phase of study (4 weeks) and additional patient was enrolled as a result. Later in the study, only one patient was lost due to early

withdrawal and did not receive an implant, but a core sample was harvested to be included in the final results.

Subjects and respective tooth sites were included in the study if all of the following inclusion criteria were met: 1) Provision of informed consent; 2) At least 18 years old; 3) In need of one posterior tooth, excluding third molar molars, planned for extraction and replacement with a dental implant (If the subject requires two adjacent socket preservation, they can still be enrolled in the study but only one site will be used for the study); 4) Intact ridge as verified with cone-beam CT scan; 5) At least one natural tooth adjacent to the study site present.

Any of the following criteria was regarded as reason for exclusion from the study: 1) Insufficient interocclusal space to allow for implant supported prosthesis; 2) Dehiscence or fenestration identified at the time of reviewing CBCT; 3) No previous interventions performed involving soft and/or bone grafting; 4) Non-treated caries or uncontrolled periodontal disease present affecting the teeth in adjacent to study site; 5) Any of natural teeth adjacent to the study site presents active periapical endodontic lesion ("active periapical endodontic lesion" will be determined per consultation by endodontics specialist); 6) Adjacent tooth (mesial and distal) to study site was extracted within last 6 months; 7) Smoker using more than 10 cigarettes or equivalent per day; 8) Smokeless tobacco use or e-cigarette use; 9) Current alcohol or drug abuser; 10) Systemic or local disease or condition that would compromise post-operative healing and/or osseointegration e.g. uncontrolled diabetes; 11) Need for systemic corticosteroids or any other medication that would influence post-operative healing and/or osseointegration; 12) Pregnancy, as indicated by positive urine human chorionic gonadotropin (HCG) test

result; 13) Unable or unwilling to return for follow-up visits for a period of 5 months; 14) Unlikely to be able to comply with study procedures according to Investigators judgement; 15) Subject in other clinical trials.

C. STUDY DESIGN

This multicenter randomized controlled clinical trial assigned patients for ridge preservation of a single extraction socket site (premolar or molar excluding third molars) randomly into treatment group A (GUIDOR® Easy-Graft CLASSIC - Poly{Lactic-Co-Glycolic} Acid-Coated β-Tricalcium Phosphate Bone Substitute [PLGA-β-TCP]) or group B (Freeze-dried bone allograft {FDBA} with collagen plug {cp}). Each center enrolled 22 evaluable subjects and 11 were assigned to each treatment group.

D. STUDY MATERIAL CHARACTERISTICS

The test material, GUIDOR® easy-graft CLASSIC is a bone graft substitute consisting of pure beta-triphosphate calcium (β-TCP) coated by poly (lactic-co-glycolic acid) (PLGA) and BioLinker™. BioLinker™ is comprised of N-methyl-2-pyrrolidone (NMP). The particle size is 500– 1000 μm and is sterilized by gamma sterilization process. The mixture of grafting material and BioLinker get hardening into a stable, porous immediately after the mixture contacts blood or body fluid in a defect.

The control material Mineralized cortical freeze-dried particulate bone allograft (FDBA) has a particle size of 250-1000 μm (Lifenet). It is sterilized via Allowash XG® technology. Collagen plug (Zimmer Dental Inc.), is made from a bovine type I collagen.

The resorption period is 10-14 days. Lifenet Health® mineralized cortical freeze-dried particulate bone allograft (FDBA) has a particle size of 250-1000 μm. It is sterilized via Allowash XG® technology, consisting of rigorous screening and assessment of donor tissues; cleaning with hypotonic solutions and antimicrobial reagents; decontamination, disinfection, and cleaning regimens with hydrogen peroxide and isopropanol alcohol solutions; and terminal sterilization with gamma irradiation. The particulate material comes packaged in a sterile vial in a blister pack.

Collagen Plug (CP) is a biocompatible, sterile resorbable highly porous collagen wound dressing for bleeding control, stabilizing the blood clot, and protecting the wound bed. Its dimensions are 10 mm X 20 mm. CP was used as an alternative to a resorbable collagen barrier for graft containment in order to provide a similar clinical scenario when directly comparing FDBA to PLGA-β-TCP. It has been reported that use of a barrier in ARP was strongly associated with more preservation of midbuccal ridge height (Avila-Ortiz et al., 2014). If a barrier was used with FDBA in this protocol, an additional ridge volume may have been preserved in comparison to PLGA-β-TCP alone. Thus, the data could inherently support superiority of FDBA and barrier use.

E. STUDY VISITS

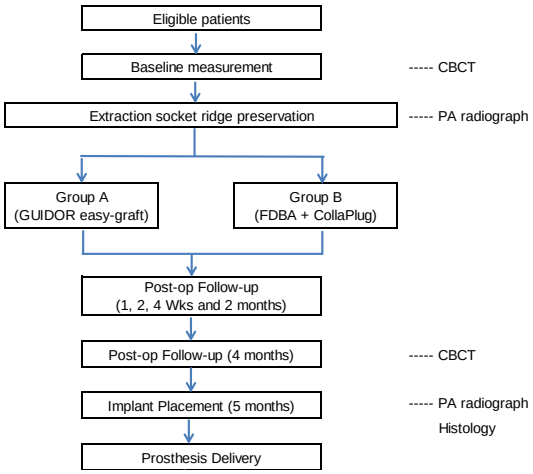
Subjects in need of ARP for a single tooth extraction socket in premolars or molars prior to dental implant surgery were deemed eligible for treatment in this study after meeting inclusion and exclusion criteria. Third molars were excluded as a study site. The screening process included evaluation of general dental and systemic health. It also included a clinical and radiographic assessment of the potential study site and the

adjacent teeth. Pre-existing intraoral (periapical) radiographs not older than 6 months were accepted.

Individuals meeting all the inclusion and none of the exclusion criteria were enrolled in the study. Patients were further evaluated only after completing all appropriate consent forms. Each subject was informed orally and in writing about the study prior to signing the appropriate consent forms. HIPAA authorization was included in addition to the consent. Subjects meeting inclusion and exclusion criteria were randomly allocated to Group A or B.

Patients were observed for five months after ARP completion up to re-entry for implant placement. The treatment period included ridge preservation followed by implant placement at 5 months from ARP. Follow-up visits following the ARP were completed at one week, two weeks, 4 weeks, 2 months, 4 months (CBCT) and 5 months (re-entry and implant placement) after screening/enrollment as presented in Figure 1.

FIGURE 1. Study Appointments



F. SURGICAL PROTOCOL: ALVEOLAR RIDGE PRESERVATION

After completed day of treatment consent and delivery of local anesthesia deemed appropriate by the clinician, pre-surgical measurements of the alveolar ridge were recorded with the use of a pointy caliper intraoperatively. The bucco-lingual width was measured one millimeter apical to the level of the alveolar crest. Study teeth were extracted as atraumatically as possible without raising a mucoperiosteal flap. Next, extraction sockets were thoroughly debrided with bone currettes and the integrity of the buccal plate was verified with a UNC periodontal probe. If a fenestration and/or dehiscence was identified through the sulcus around the tooth or fracture of the buccal bone was noted upon extraction, the subject was excluded from the study and treated as a normal patient of the clinic (Incorrect enrollment). If exclusion criteria was not identified, the site was randomly selected for Group A (Easy-graft® CLASSIC) or B (FDBA) with computer randomization.

After thorough debridement of granulated tissue and rinsing with sterile saline, the prepared Easy-graft® CLASSIC (Group A) or FDBA (Group B) was delivered to the extraction socket and adapted to the internal morphology. Both graft materials were condensed to achieve a fill of the socket to level of crestal bone. Easy-graft® CLASSIC (Group A) was prepared according to manufacturer instructions by mixing PLGA β-TCP granules with N-methyl-2-pyrrolidone (NMP) liquid activator to form a permeable, moldable material. The graft hardened over several minutes. FDBA (Group B) was rehydrated by sterile saline before grafting followed by CP adapted on top of the graft and secured with sutures. Immediately following surgery, a post-operative standardized periapical radiograph was obtained to provide assessment of graft material in relation to

residual alveolar ridge. This positioning device was fabricated with bite registration material and disinfected after the procedure for future use.

For both groups, no attempt was made to adapt the wound margins or to achieve primary wound closure. Post-operative medications to include antibiotics, analgesics, and oral antiseptic rinses were prescribed at the discretion of the clinician.

G. POST-OPERATIVE AND FOLLOW-UP ASSESSMENT

At one week, two weeks, four weeks, two months, and four months following extraction with ARP, post-operative appointments were conducted to evaluate wound healing and documented with intra-oral photographs. At four months healing, an additional standardized radiograph was exposed while utilizing the previous radiographic positioning device fabricated on the day of ARP surgery to document graft maturation. The patient also had a second CBCT exposed to provide radiographic analysis of the site and plan future implant placement to be performed at five months post-operative.

Small segmental cone beam computed tomography (CBCT) imaging was obtained for all patients 4 months following the ridge preservation surgical procedure. Images were used baseline data of horizontal and vertical bone locations. CBCT scans were obtained using i-CAT Next Generation (Imaging Sciences International Inc., Hatfield, PA, USA) or equivalent system. Only the arch that contained the site of interest was scanned to minimize the radiation exposure. Based on previous investigations, the field of view was approximately 6 cm and the machine settings were fixed at 120 kVp

and 18.66 mAs for all scans. Subjects with an intact ridge, verified by the CBCT scan, were eligible to continue enrollment to the study.

H. IMPLANT PLACEMENT AND CORE HARVESTING

Implant placement was completed per standard procedure of the institution's clinic. Selection of implant system was left to the judgment of study sites; however the same system was used in all the cases treated at the site. Implant site preparation and placement was performed according to the implant system manufacturer recommendations. For patients in each treatment group, a trephine drill was used to harvest a core of bone for descriptive histology. A trephine drill was used to harvest a core of bone corresponding with the planned implant length. A trephine drill of 2.8 mm in diameter was used as the first choice. If another diameter of a trephine was used, it was recorded. The length of the trephine did not exceed 8 mm. Following harvesting, the trephined bone core was immediately transferred to a solution of 10% neutral buffered formalin (NBF). These samples were used for histomorphometric assessment. Bone Density Classification) was evaluated and recorded (Misch CE, Hoar J, Beck G, 1998). When one-stage approach was chosen, a healing abutment was placed with hand tightening. Depending on the buccal soft tissue thickness, the crestal tissue was either be removed or deepithelialized and inverted to the buccal to increase the soft tissue thickness. When two-stage procedure was chosen, a cover screw was placed and remained uncovered to promote undisturbed healing. Once healing period passed, a

second surgery was performed to attach an abutment for preparation of crown fabrication. Selection of approach was at the dentist's discretion.

An intraoral periapical radiograph was taken to assess the depth and angulation of dental implant placement. Buccal and occlusal photographs of the surgical site were obtained for both extraction and implant surgeries. A final intraoral periapical radiograph was obtained for final implant placement.

I. HISTOLOGIC PREPARATION

Each bone specimen was processed and polished to a thickness of 45 µm according to Donath's protocol followed by staining with Stevenel's Blue and van Gieson's picrofuchsin. Photomicrographs were obtained with a digital microscope camera under constant magnification and used for histologic comparisons.

3. RESULTS

A. PATIENT DEMOGRAPHICS

One subject was withdrawn in early phase of study (4 weeks) and additional subject was enrolled as a result. Therefore, within forty-four participants enrolled, twenty-three patients were assigned to Easy-graft® CLASSIC (Group A) and twenty-one patients were assigned to FDBA (Group B). In Group A, there were nine males and fifteen females, average age of 61. In comparison, seven males and fourteen females, average age of 53, were included in Group B. The total number of premolars included in the study were thirteen in the Group A and thirteen in Group B. As for molars, eleven were included in Group A and eight in Group B (**Table 1**).

Table 1. Patient Gender, Age and Tooth Sites.

GROUP	Males	Females	Total Group	Average Age	Premolars	Molars
BTCP (A)	9	14	23	61	13	10
FDBA (B)	7	14	21	53	13	8

B. CLINICAL AND HISTOLOGICAL OUTCOMES

Healing was uneventful at each extraction site following alveolar ridge preservation. Surgical reentry was completed after five months healing in forty-four healed sites. Forty-four bone core samples were harvested, but one site was not adequate for implant placement due to perforation of the Schneiderian membrane during osteotomy near the maxillary sinus. All other sites exhibited adequate alveolar ridge

preservation to support implant placement as these sites did not require any additional bone grafting.

Microscopic examination of the specimens revealed evidence of vital bone formation at each graft site (**Figure 2 and Figure 3**). Vital bone was present in intimate contact with the surface of β-TCP graft particles. Residual graft particles, exhibiting varying degrees of resorption, were evident in all specimens. Soft tissue matrix exhibited no evidence of inflammation or foreign body reaction adjacent to residual graft particles.

Figure 2. Histologic Analysis of β-TCP Core Sample

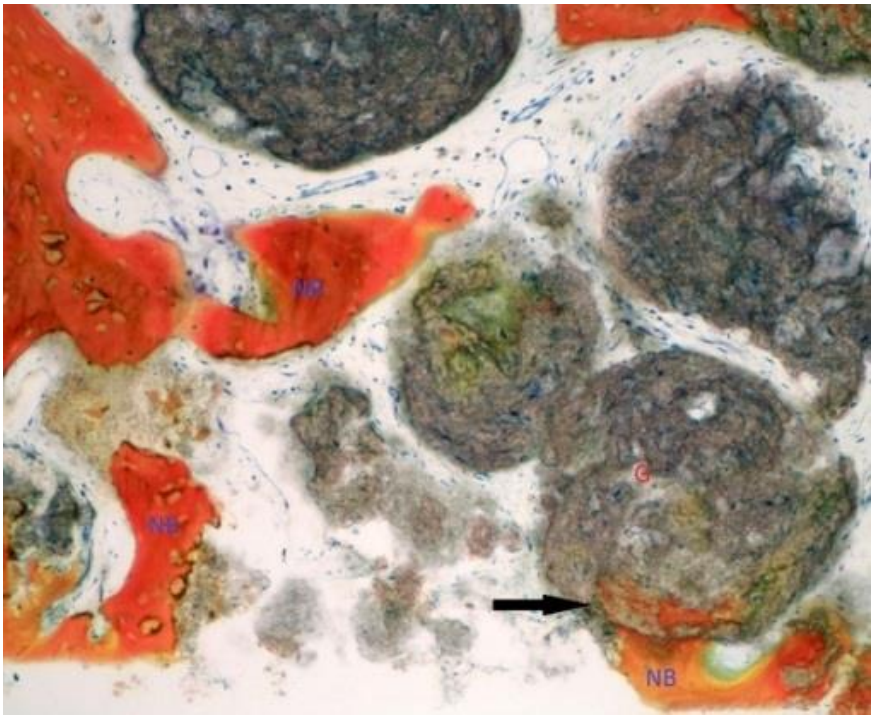
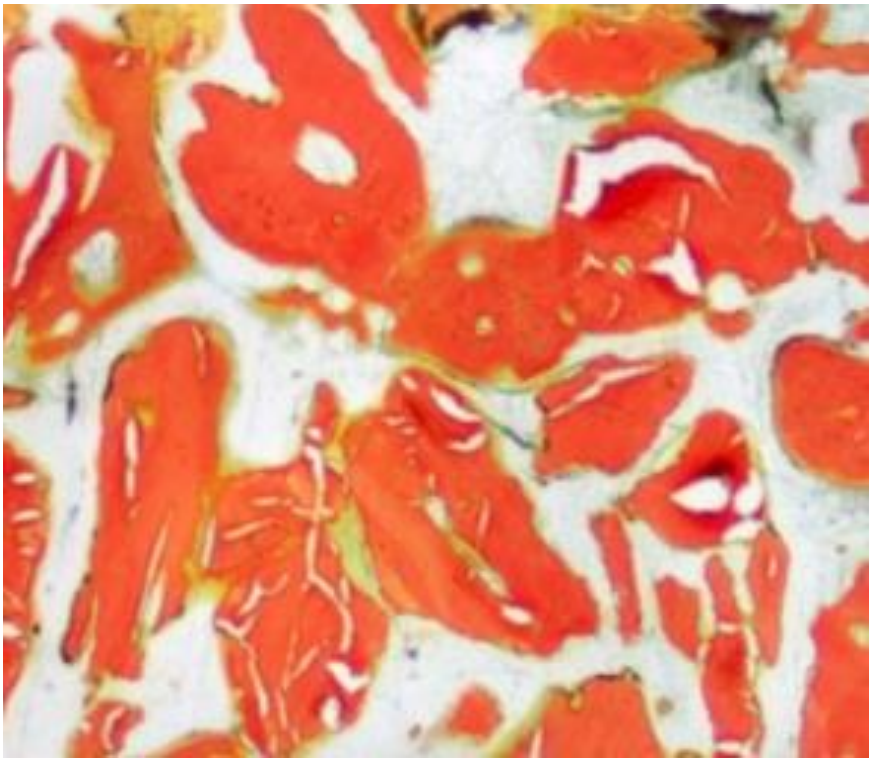


Figure 3. Histologic Analysis of FDBA Core Sample



In **Table 2**, histologic measurements revealed statistically significant difference between the test and control groups in percent vital bone, Group A (27.01%) and Group B (38.16%), p=0.05*. In addition, Group A had more percent marrow or fibrous tissue than Group B with 52.52% and 46.10%, respectively (p=0.004*).

Table 2. Histologic Analysis of All Core Samples

	Percent Vital Bone (%)	Percent Non-vital Bone (%)	Percent Non-bone Particles (%)	Percent Marrow or Fibrous Tissue (%)
BTCP (A)	27.01	0	20.35	52.52
FDBA (B)	38.16	28.81	0	46.10
P-Value	0.05*	0.004*	0.190	0.004*

In **Table 3 and Table 4**, subgroup analysis for tooth type (premolar and molar) are presented. In **Table 3**, the histologic measurements revealed statistically significant difference between the test and control groups when evaluating only first and second premolar sites with the percent vital bone, Group A (22.23%) and Group B (37.97%) . However, analysis of only molar sites (**Table 4**) demonstrated no statistically significant difference ($p = 0.58$) among the percent vital bone present in Group A (33.22%) and Group B (38.47%). Furthermore, the differences of percent marrow or fibrous tissue in both groups were not statistically significant in both premolar and molar sites.

Table 3. Histologic Analysis of Premolar Core Samples

	Percent Vital Bone (%)	Percent Non-vital Bone (%)	Percent Non-bone Particles (%)	Percent Marrow or Fibrous Tissue (%)
BTCP (A)	22.23	0	25.08	52.77
FDBA (B)	37.97	31.23	0	44.08
P-Value	0.03*	0.158	0.055	0.421

Table 4. Histologic Analysis of Molar Core Samples

	Percent Vital Bone (%)	Percent Non-vital Bone (%)	Percent Non-bone Particles (%)	Percent Marrow or Fibrous Tissue (%)
BTCP (A)	33.22	0	14.20	52.20
FDBA (B)	38.47	24.88	0.0	49.38
P-Value	0.58	0.001*	0.034*	0.369

4. DISCUSSION

This investigation compared the use of Poly{Lactic-Co-Glycolic} Acid-Coated β-Tricalcium Phosphate Bone Substitute [PLGA-β-TCP]) to freeze-dried bone allograft {FDBA} with collagen plug {cp} for ARP. The study was designed as a multicenter, randomized controlled clinical trial with the aims of evaluating PLGA-β-TCP use without a membrane or a plug covering at the ARP site. This goal was completed by analyzing the histology of the socket from a bone core harvested at time of implant placement after at least 4 to 5 months healing and directly compared to the histology obtained from the FDBA control group. As a result, this investigation would demonstrate that the effectiveness of PLGA-β-TCP bone graft was not inferior to those of other leading bone graft substitutes and support the reduction of necessary treatment time and cost of a membrane or a plug to both dentists and patients.

Histological analysis revealed that there was a statistically significant difference in vital bone formation between the groups treated with β-TCP and FDBA. In comparison to other studies, Demetter et al. found on average 24.54 to 28.81% and Wood et al. observed 24.63% new vital bone formation in approximately 5 months following ARP (Demetter et al., 2017; Wood & Mealey, 2012). In our study, FDBA demonstrated 38.16% new vital bone formation after 5 months healing. Kakar et al. found 21.3% new vital bone formation after 5 months healing (Kakar, Rao, Hegde, Deshpande, & Lindner, 2017). In comparison to this investigation, 27.01% new vital bone was identified in the BTCP group. In both groups, the results seem to be consistent with previous reports. The statistically significant difference between both groups in vital bone formation remained the same when performing a subgroup analysis for premolars, but not the molars.

Although the subgroup analysis of tooth type was presented, the number of premolar and molar sites was not stratified at the time of enrollment.

Although autogenous bone is considered the gold standard (Demetter, Calahan, & Mealey, 2017; Hsu & Wang, 2013), there are disadvantages its use, which include the need for an additional surgical site for harvesting followed by morbidity with the donor site (Bizenjima, Takeuchi, Seshima, & Saito, 2016). For this reason, other bone substitutes that eliminate the need for a donor site are more attractive to use by clinicians. It has been demonstrated that allografts perform similarly to autogenous bone (Demetter et al., 2017). FDBA has also been shown to out-perform alloplastic materials in regards to dimensional stability and new bone formation (Avila-Ortiz et al., 2014; Ten Heggeler et al., 2011). In addition, allografts such as DFDBA and FDBA are capable of being resorbed and replaced by new bone formation in the site (Demetter et al., 2017; Hsu & Wang, 2013). These histological observations provide the basis for selecting FDBA as the control group in this investigation. In contrast, PLGA-β-TCP has been demonstrated to provide an alloplastic option capable of limiting resorption of the alveolar ridge following extraction and promote new bone regeneration within the alveolar defect (Kakar, Rao, Hegde, Deshpande, Lindner, et al., 2017; M. Leventis et al., 2018; M. D. Leventis et al., 2016; Naenni, Bienz, Jung, Hämmerle, & Thoma, 2019; Saito et al., 2017).

When evaluating β-TCP histologically, it is known to have a slow resorption time in comparison to FDBA (Hsu & Wang, 2013). With this observed resorption rate, it would also be expected to observe a residual bone graft in addition to newly formed bone and well vascularized, uninflamed connective tissue in a sample following ARP (Kakar,

Rao, Hegde, Deshpande, Lindner, et al., 2017; M. Leventis et al., 2018; M. D. Leventis et al., 2016). A prospective study by Kakar et al. obtained histological samples from sites of previous ARP with PLGA-β-TCP after an average of five months healing before implant placement and performed histomorphometric evaluation (Kakar, Rao, Hegde, Deshpande, Lindner, et al., 2017). In their results, the grafted sites were composed of an average 21.34 ± 9.14% new bone, 26.19 ± 9.38% residual graft material, and 53 ± 7.51% connective tissue (Kakar, Rao, Hegde, Deshpande, Lindner, et al., 2017). Another multi-center case series with a similar study design and re-entry at 4 months for implant placement revealed that sites grafted with PLGA-β-TCP had 24.4 ± 7.9% new bone, 12.9 ± 7.7% residual graft material, and 60.5 ± 7.4% connective tissue/bone marrow (M. D. Leventis et al., 2016). In this investigation, the histological samples harvested at the time of implant placement after 5 months healing contained 27.01% new bone, 20.35% residual graft material, and 52.52% connective tissue/bone marrow. Therefore, the findings of this study seem to be similar to previous investigations.

For this study, an attempt at primary closure was not performed in either Group A or B, and the PLGA-β-TCP was not covered by a barrier. In order to provide a similar clinical scenario within the control group to the test group, the FDBA group had a rapidly-absorbable collagen plug placed over the site. In a previous systematic review, it was indicated that the use of a barrier membrane for ARP alone resulted in more vertical bone change than the use of grafts alone (Vignoletti et al., 2011). Furthermore, the use of membranes obtained better results than grafts (either alone or the combination of membrane and graft) in terms of horizontal bone changes as well (Vignoletti et al., 2011). With these reported findings, it was supported to use a rapidly-absorbable collagen plug

instead of a barrier membrane in the control group. In a more recent systematic review, it was determined that alloplastic materials left exposed rendered a more favorable result than particulate allograft covered with a rapidly-absorbable collagen sponge for preserving vertical mid-buccal dimension (Avila-ortiz et al., 2019). However, for this investigation, Group A and Group B did not have a statistically significant difference in the alveolar ridge dimensional changes (data not shown).

With comparison to the PLGA-β-TCP group, the percent of non-vital bone was higher in the FDBA group. This finding could be explained by comparison to other studies that observed the histological fate of allografts in periodontal regeneration and augmentation procedures. An early study by Reynolds and Bowers noted that 75% of the grafted intrabony defects with demineralized FDBA had remaining bone particles present after six months healing (Bowers, Gerald M, Reynolds, 1996). A more recent randomized control clinical trial found that sites grafted with 100% cortical FDBA had significantly greater residual graft material after eighteen to twenty weeks healing in ARP than sites grafted with cancellous or cortico-cancellous bone (Demetter, Calahan, & Mealey, 2017). This same study also observed that the harvested bony cores exhibited 24.54% to 28.81% mean vital bone percentages and 18.82% to 28.14% of residual bone percent depending on the allograft used (Demetter et al., 2017). Therefore, the results of this study seem to be similar to previous studies with 28.81% non-vital bone in Group B.

Implant related outcome measures from other studies reported that the sites that received PLGA-β-TCP for ARP provided adequate primary stability with implant placement (Kakar, Rao, Hegde, Deshpande, Lindner, et al., 2017; M. D. Leventis et al., 2016). In a multi-center case series, the final average seating torque of the implants was

43.6 ± 8.3 N/cm and the mean ISQ measurement of the implants were 69.0 ± 11.1 (M. D. Leventis et al., 2016). In another clinical trial, the mean ISQ measurements at time of implant placement were 70.3 ± 9.6 (Kakar, Rao, Hegde, Deshpande, Lindner, et al., 2017). In our investigation, ISQ measurements were not recorded, but the bone density (Misch CE, Hoar J, Beck G, 1998) was noted at time of implant placement. A recent prospective study concluded that the tactile sense of a surgeon can approximate the histologic properties of the bone (Rokn, Labibzadeh, Ghohroudi, Shamshiri, & Solhjoo, 2018). It was found that the correlation between the tactile sense and the mean superficial cortical bone thickness, haversian canal number, and trabecular distance was statistically significant, and the bone volume was higher for Type II and III bone quality (Misch CE, Hoar J, Beck G, 1998; Rokn et al., 2018). Although majority of the sites in this study were classified as Type III or IV bone density from the surgeons day of implant placement (Misch CE, Hoar J, Beck G, 1998), implants were successfully placed in all sites except one, which had a complication with sinus floor perforation. In a study analyzing β-TCP use in maxillary sinus augmentation, it was determined that β-TCP presented with different amounts of resorption from very little to complete disappearance after 6 months (Zijderveld, Zerbo, van den Bergh, Schulten, & ten Bruggenkate, 2005). These studies support the fact that it is not necessary for β-TCP to entirely resorb to promote implant success and osseointegration. β-TCP may have a slow resorption rate, but it is a viable bone substitute to facilitate future implant placement.

There are some limitations in this study. First, the bone cores were harvested at the time of the implant placement during the osteotomy procedure. This allowed only the "center" part of the bone available for sampling of the bone, which could be considered

as the last part of the grafted socket to be matured. However, the sub-analysis of tooth type (premolars compared to molars) did not show statistical significance with the assumption that molar sites would require more time to completely mature due to increased socket volume. In this study design, it was only possible to make it single blinded with the treatment as the surgeons recognized the bone graft materials at the time of ARP. However, the histologist was not aware of the group assignment for each sample. Although this was blinded, the histology of β-TCP was not difficult to differentiate from FDBA samples due to the increased non-vital bone particles noted. Furthermore, many previous randomized control trials ended the observation period at the time of implant placement (Avila-ortiz et al., 2019). Few have an additional observation period of twelve months following the implant restoration delivery (Avila-ortiz et al., 2019). Therefore, there is a need for more randomized controlled clinical studies with extended follow-up period after the implant being in function to evaluate implant success rate over time including esthetic parameters such as soft tissue stability.

5. CONCLUSION

The results of this multicenter randomized control clinical trial demonstrated the similar efficacy of PLGA- β-TCP to FDBA as a bone substitute in ARP. Histologic measurements revealed no statistically significant difference between the test and control groups in percent vital bone after four months healing. Although the FDBA group had more percent bone than PLGA- β-TCP, FDBA had more non-vital bone than PLGA- β-TCP. Both groups provided adequate stability for implant placement. Therefore, clinicians can expect similar treatment outcomes with the use of PLGA- β-TCP or FDBA for alveolar ridge preservation.

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