Influence of Periodontal Status on Bone Formation in Extraction Sockets After Alveolar Ridge Preservation: A Clinical Histological Pilot Study

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LISTS OF ABBREVIATIONS

- PD: Periodontal disease
- Pg: Porphyromonas gingivalis
- OCN: Osteocalcin
- Micro-CT: Microcomputed tomography
- NB: New bone
- RBG: Residual bone graft particles

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ABSTRACT

Background: Periodontal disease (PD) is common and is a leading cause for tooth loss. PD is associated with dysbiosis and chronic inflammatory destruction of the alveolar bone. ARP is a procedure aims at reducing dimensional ridge changes after tooth extraction and the need for additional bone augmentation at the time of implant placement. Periodontal inflammation and active PD may affect the healing outcomes of extraction socket that underwent ARP.

Aim: To compare new bone formation and residual bone graft particles in extraction socket that underwent ARP between periodontally healthy (control group) and PD patients (experimental group).

Methods: Bone core biopsies were harvested from healed extraction sites that underwent ARP. Micro-CT imaging, Masson's trichrome, immunohistochemistry for osteocalcin were performed on the harvested biopsies. In addition, correlation analyses were performed between micro-CT and histological analyses (Masson's trichrome and OCN immunohistochemistry) results for NB and RBG.

Results: No statistically significant difference between control and experimental groups. The correlation between micro-CT and Masson's Trichrome showed a statistically strong association, while the other correlation analyses showed a weak to a very association that was not statistically significant.

Conclusion: No difference was found between periodontal healthy and stable periodontitis patients with regards to new bone formation and bone graft remodeling in extraction sockets that underwent ARP. Adequate sample size calculation and standardization of the healing time between ARP and implant placement are critical to obtain robust results from which clinically relevant conclusions can be drawn.

I. INTRODUCTION

1.1 Periodontal Disease and Inflammatory Bone loss

Periodontal disease (PD) is one of the most common oral diseases affecting nearly half of the United States adult population (Eke et al., 2015). Globally, the most severe form of PD has a prevalence of 11.2% (Kassebaum et al., 2014). PD is a collective term that encompasses a wide array of diseases and conditions affecting the tooth supporting structure, the periodontium consisting of the gingiva, periodontal ligament, cementum and alveolar bone. Those conditions and diseases range from gingival diseases and conditions (biofilm induced gingivitis or non-biofilm induced gingival conditions), periodontitis (necrotizing periodontal diseases, periodontitis or periodontitis as a manifestation of systemic diseases) or other conditions affecting the periodontium (systemic diseases or conditions affecting the periodontal supporting tissue, periodontal abscesses and endodontic periodontal lesions, mucogingival deformities and conditions, traumatic occlusal forces and tooth and prosthesis related factors) (Caton et al., 2018). The common thread between those conditions is a microbial challenge in the periodontium that provokes a destructive host-inflammatory response that is reversible in its early stages (gingivitis) then becomes irreversible at its later stages (periodontitis). Histologically, Page and Schroeder described the progression of periodontal lesion from initial, early and established lesions limited to the gingival sulcular and junctional epithelial and the underlying connective tissue to an advanced lesion characterized by apical and lateral extension of the inflammatory lesion, apical migration of the junctional epithelium, pocketing and alveolar bone loss (Page & Schroeder, 1976). The histological findings of the progression of the periodontal lesion described by Page and Schroeder were later revisited by Hajishengallis and Korostoff who outlined the involvement of a

wide array of innate and adaptive immune cells and the interaction between immune mechanisms and bone cells in the progression of periodontal lesions (Hajishengallis & Korostoff, 2017).

The dental plaque-biofilm has been well-established as the major triggering factors underlying PD (Loe, Theilade, & Jensen, 1965). At its early stages, the plaque-biofilm is mostly composed of commensal (non-disease causing) microbiota. Accumulation of the plaque biofilm at the gingival margin increases the commensal microbial biomass eliciting a breakdown of host-microbe homeostasis. This triggers an inflammatory response that is initially reversible and localized to the gingiva called gingivitis (Murakami, Mealey, Mariotti, & Chapple, 2018). If the plaque biofilm is left undisturbed for an extended duration, the inflammatory response becomes chronic and advances deeper subgingivally. This results in an environment that favors the emergence of keystone pathogens (species that remodel the microbial community to induce disease), and pathobionts (commensal species that can cause disease when host microbe homeostasis is broken down). Both keystone pathogens and pathobionts feed on the nutrients present in the inflammatory exudates in the inflamed periodontium and the lower redox potential characteristic of an aged plaque (Lamont, Koo, & Hajishengallis, 2018; Loesche & Grossman, 2001). Those shifts in the periodontal environment promote microbial diversity in the periodontal environment with more gram-negative disease-inducing species dominating the periodontal microbiota (Marsh, 1994). This shifts in the periodontal microbial community is termed dysbiosis (Hajishengallis, 2015). Dysbiosis promotes immune dysregulation and homeostasis breakdown resulting in a non-resolving and destructive chronic inflammatory response that is responsible for hard and soft tissue damage within the periodontium (Kumar, 2021).

Progressive chronic inflammatory bone loss is a hallmark of PD. The underlying chronic inflammatory response includes components of the innate and adaptive immune systems. In this respect, a wide network of cytokines and chemokines orchestrate the local host response that is mainly aimed at mediating defense mechanisms against invading periodontal pathogens (Graves, Li, & Cochran, 2011). The invasion of periodontal pathogens into the connective tissue of the gingiva and periodontal ligament induces the secretion of proinflammatory cytokines (e.g. IL-6, and TNF-a), chemokines (e.g. CXCL8) and proteases (e.g. matrix metalloproteinases) and the expansion and chemotaxis of phagocytic leukocytes (neutrophils and macrophages) to limit pathogens invasion. However, those pro- inflammatory mediators and immune cells induce bone marrow stromal cells, osteoblasts and periodontal ligament fibroblasts to secrete the receptor activator of nuclear factor kappa-B ligand (RANKL) that binds to its receptor RANK on osteoclasts precursors resulting in the differentiation and maturation of bone resorbing osteoclasts (Graves et al., 2011). The imbalance in the local concentration of RANKL and its decoy receptor osteoprotegrin (OPG) within the periodontium has been proposed the major driving mechanism underlying uncoupled bone remodeling and inflammatory bone loss during the course of PD (Belibasakis & Bostanci, 2012).

Porphyromonas gingivalis (Pg) is one of the most studied keystone pathogens driving immunopathology and destructive host response during the course of PD (Hajishengallis, Darveau, & Curtis, 2012). Several mechanistic studies have been proposed to explain the effect of Pg in driving uncoupled bone remodeling and inflammatory bone loss in PD. In this regard, the Pg lipopolysaccharide (LPS) was found to induce the secretion of RANKL and increase osteoclasts numbers in vitro and in vivo in mice in a toll-like receptor 2 (TLR-2) dependent manner (Kassem et al., 2015). In bone marrow stromal cells, Pg derived LPS and proteases (gingipains) resulted in

increased expression of RANKL and decreased expression of OPG at both the protein and gene levels (Reddi et al., 2008). Infection of murine primary osteoblast cultures with viable Pg resulted up-regulated mRNA expression of RANKL and IL-6 (Okahashi et al., 2004). In murine primary calvarial osteoblasts cultures, Pg derived lipids inhibited osteoblasts differentiation via downregulation of the osteoblast markers RUNX2, ALP, OC and DMP1, along with upregulation of RANKL, TNF-a and MMP3 (Y. H. Wang et al., 2010). All the aforementioned studies underline the negative impact of Pg, as one of the major pathogenic factors in PD, on bone remodeling favoring uncoupled remodeling via upregulation of osteoclastic bone loss and downregulation of osteoblastic bone formation.

1.2. Periodontal Disease and Tooth Loss

As one of the most common causes oral diseases and owing to its association with alveolar bone loss, PD has been implicated as one of the major causes of tooth loss (Ong, 1998). As opposed to tooth loss due to dental caries, tooth loss due to PD was found to be associated with anterior more than posterior teeth (Jaafar, Razak, & Nor, 1989). According to the 2017 World Workshop (2017 WW) Classification of Periodontal and Peri-implant conditions, tooth loss related to periodontitis was incorporated in the disease staging as one of the major indicators of disease severity and the need for complex rehabilitation for patients with advanced disease (Stage IV periodontitis (Tonetti, Greenwell, & Kornman, 2018). Along the same lines, patients with severe (Stage IV) and rapidly progressing PD (Grade C) according to the 2017 WW classification were at higher risk of periodontitis related tooth loss during both the active and supportive periodontal therapy phases (Siow, Goh, Ong, & Preshaw, 2023; Takedachi et al., 2022). Finally, several

studies pointed out a correlation between periodontitis related tooth loss and all-cause mortality, colorectal cancer and dementia (Asher, Stephen, Mäntylä, Suominen, & Solomon, 2022; Momen-Heravi et al., 2017; Wu et al., 2021).

The decision to extract a tooth due to periodontal disease or decay is governed by several tooth prognostic systems. One of the earliest prognostic systems is the one developed by Becker and colleagues who conducted a retrospective study on patients treated for PD but did not comply with the maintenance program (Becker, Becker, & Berg, 1984). Becker and colleagues identified a list of problems that were found associated with a tooth before it was given a hopeless prognosis. They posited that at least two of those problems must be present in order for it to be considered "hopeless". This list included the following: loss of > 75% of the supporting bone, probing depth greater than 8 mm, class III furcation involvement, class III mobility, poor crown-root ratio, root proximity with minimal interproximal bone and evidence of horizontal bone loss, and history of repeated periodontal abscess formation. Another prognostic system was developed by McGuire and Nunn based on a retrospective evaluation of treated PD patients who were enrolled in a maintenance program for 5 years and were followed-up for up to 8 years (McGuire & Nunn, 1996). This prognostic system considers several clinical (such as percentage bone loss, probing depth, mobility, furcation involvement, whether the tooth serves as an abutment, etc...) and patient related factors (Age, hygiene, maintenance interval and parafunctional habits, etc...). McGuire and Nunn suggested that some clinical factors (poor crown/root ratio, tooth malposition, teeth used as fixed abutments and smoking) are more significant than other factors that are indicative of disease progression (increased probing depth, severe furcation involvement and increased mobility). According to this prognostic system, the tooth prognosis categories vary from "good" to "hopeless". A good prognosis is assigned when the control of etiologic factors and adequate

periodontal maintenance would be relatively easy. On the other hand, a hopeless prognosis is assigned when there is inadequate periodontal attachment to maintain the tooth. The previous 2 systems were based on tooth mortality as an endpoint, and therefore could have limited predictability or usefulness in patient management. For this reason Kwok and Caton developed a therapeutic prognostic system that considers the likelihood of achieving periodontal stability as indicated by the probability of controlling patient-related/systemic (such as compliance to periodontal maintenance, cigarette smoking and diabetes mellitus) and local (deep probing depth and attachment loss, anatomic factors such as furcation involvement and tooth position, tooth mobility and trauma from occlusion and parafunctional habits) factors after therapy (Kwok & Caton, 2007). According to Kwok and Caton's system, a tooth is considered hopeless and requiring extraction if periodontal stability cannot be achieved with periodontal treatment and maintenance. The usefulness of Kwok and Caton prognosis system lies in the ability of the clinician to assign an individual tooth and overall prognosis during initial treatment planning and completion of comprehensive treatment, at which point the prognosis can be revised based on the treatment outcomes and future treatment needs can be discussed.

1.3. Spontaneous Healing of the Tooth Extraction Socket

Animal and humans studies examining the spontaneous healing of the tooth extraction socket showed that this process follows a well-ordered sequence of events (Amler, Johnson, & Salman, 1960; G. Cardaropoli, Araújo, & Lindhe, 2003; Evian, Rosenberg, Coslet, & Corn, 1982; Scala et al., 2014). Bone formation starts from the apical and lateral walls of the socket and gradually progresses towards the central and coronal regions, while coronal soft tissue closure

proceeds at a relatively faster pace by inwards migration of epithelial cells at the socket entrance. This complex healing process can be affected by a number of local (tooth position, surgical technique and buccal plate thickness) and systemic factors (smoking and diabetes) that could alter the healing outcomes (Udeabor et al., 2023).

A myriad of clinical studies reported that dimensional changes in alveolar ridge take place during the immediate and delayed post-extraction healing period. In areas where extraction site is bound by neighboring teeth, most of the dimensional changes occur in the horizontal rather than the vertical dimension most likely due to the maintenance of the mesial and distal bony peaks by the adjacent teeth (Favero et al., 2013). A landmark study by Schropp and colleagues reported a 50% reduction of ridge width in premolar and molar sites evaluated at 12 months post-extraction (Schropp, Wenzel, Kostopoulos, & Karring, 2003). In this study, minimal to no change in the vertical dimension was found. Similarly, Covani and co-workers studied the pattern of alveolar ridge remodeling at premolar and molar sites 6-24 months after extraction (Covani et al., 2011). This study demonstrated that the buccal alveolar crest tends to shift lingually from its original position, denoting more bone resorption on the buccal, rather than the lingual aspect. In the esthetic zone, Famer and Darby examined the early dimensional changes following single tooth extraction (Farmer & Darby, 2014). In this respect, bone sounding measurements revealed a 15% reduction in the total ridge width after 6-8 weeks of healing, with buccal plate only present in 25% of subjects.

The studies above indicate that the buccal plate is prone to more resorption following tooth extraction than its palatal/lingual counterpart. This notion is supported by the fact that the buccal plate is generally thin averaging at 0.8 mm in anterior teeth and 1.1 mm in premolars (Huynh-Ba et al., 2010). Additionally, a study in beagle dogs showed that the buccal bone is composed mostly

of bundle bone that is maintained by the presence of adjacent tooth structure, while it undergoes resorption during the post-extraction remodeling (Araújo & Lindhe, 2005). Along the same lines, a prospective case series study using pre- and post-extraction CBCTs showed that a thin buccal plate of < 1mm was associated with significantly greater horizontal and vertical bone loss than a thick buccal plate of > 1 mm in the esthetic zone (Chappuis et al., 2013). Thus, preservation of the buccal bone plate can be considered as a prophylactic measure precluding horizontal and vertical dimensional changes of the alveolar ridge following tooth extraction.

1.4.   Assisted Healing of the Tooth Extraction Socket

Several strategies have been employed to hamper the loss of buccal plate and subsequent changes in alveolar ridge dimensions post-extraction. The most common strategy is alveolar ridge preservation (Depaolo, Lathan, Rollins, & Karpus) via socket grafting. This procedure aims at decreasing post-extraction alveolar ridge atrophy by filling the space previously occupied by the extracted tooth with a biomaterial that would emulate a root retention effect that favors bone preservation and minimize the need for additional grafting procedures at the time of endosseous implant placement (Avila-Ortiz, Elangovan, Kramer, Blanchette, & Dawson, 2014). From a biological standpoint, the complete or partial presence of the buccal plate following extraction is expected to enhance the success rate of ARP procedures due the presence of more bony walls that allow for graft containment. In guided tissue regeneration procedures, the presence of 3 remaining osseous walls (rather than 1 or 2 osseous walls) at sites of infra-bony defects was designated as a predictor of successful regeneration (Weinberg & Eskow, 2000). This concept can be applied in the context ARP procedures where the presence of more bony walls, including the buccal/lingual

plate and interdental bony peaks, would be favor successful outcomes in terms of dimensional ridge stability following healing. Similarly, the preservation of buccal plate would allow for the creation and maintenance of the space needed and undisturbed bone ingrowth within the extraction socket after ARP. Along with primary wound, closure angiogenesis and stability of the wound, space creation/maintenance was suggested to enhance the predictability of guided bone regeneration procedures (H. L. Wang & Boyapati, 2006).

With regards to the effect of buccal plate thickness on the outcomes of ARP procedure, one study indicated that patients that underwent ARP procedure exhibited less reduction in alveolar ridge dimensions compared to the in the spontaneous healing group, regardless of the baseline buccal plate thickness (D. Cardaropoli, Tamagnone, Roffredo, & Gaveglio, 2014). Similarly, a recent systematic review concluded that ARP could be beneficial in reducing alveolar ridge atrophy in post-extraction sockets with absent buccal wall (García-González et al., 2020). Another strategy to preserve the facial bone plate is to employ minimally invasive surgical procedure in tooth extraction. More recently, a retrospective cohort study showed that every 1 mm increase of baseline facial bone thickness reduced the need for simultaneous bone augmentation at the time of implant placement by almost 8 times (Couso-Queiruga et al., 2022). This suggests that thicker facial bone may provide a better prognosis for socket preservation and may help to reduce the need for additional bone augmentation procedures.

In addition to the influence of the buccal plate, several bone grafting materials have been investigated for their efficacy in ARP procedures. Notably, early ARP techniques were originally proposed in the 1970's and involved the decoronation of root canal treated teeth followed by their submergence beneath the oral mucosa (Lam, 1972; Simon & Kimura, 1974; Von Wowern & Winther, 1976). This concept has originally evolved from the higher susceptibility of overdenture

abutments to dental caries and periodontal disease, but has been later adopted as an approach to preserve ridge volume and esthetics at pontic sites as part of tooth or implant supported fixed partial dentures (Salama, Ishikawa, Salama, Funato, & Garber, 2007). One limitation of this technique is the high incidence of root exposure and its unsuitability for sites planned for later endosseous implant placement (Du Toit, Salama, Gluckman, & Nagy, 2021).

With the widespread adoption of dental implants in daily practice and the significant advancements in material sciences and clinical research, different biomaterials have been developed for socket grafting and guided bone regeneration applications. It has been proposed that the selection of socket grafting material should be guided by the treatment plan for the grafted site. Based on the resorption rate of the socket grafting materials, Bartee classified ARP procedures into long-term, transitional or short-term ridge preservation (Bartee, 2001). For long-term ridge preservation, particulate dense hydroxyapatite (HA), porous coraline HA and bioactive glass have been used. HA and Bioglass-based materials exhibit varying degrees of osteoconductivity and their particles size is proportional to the rate at which they resorb (Fischer, Layrolle, Van Blitterswijk, & De Bruijn, 2003; Schepers, Ducheyne, Barbier, & Schepers, 1993; Stanley et al., 1997). Transitional ridge preservation is indicated for patient who may consider implant placement on the intermediate term rather at the time of tooth extraction. Anorganic bovine bone matrix (ABM), beta tri-calcium phosphate (B-TCP) and microporous resorbable HA are the materials recommended for transitional ridge preservation. These materials are osteoconductive in nature and undergo resorption within 3-5 years period to be replaced by host bone. As for short-term ridge preservation, the goal is to maintain bone mass at the extraction site in preparation for implant placement within a 3–6-month period. This can be accomplished by using a mixture of grafting materials that will result in socket fill with bone of adequate density

within the intended time frame. To that end, demineralized freeze-dried bone allograft (DFDBA)

or autogenous bone combined with ABM, low density HA or B-TCP at a ratio of 50:50 or 75:25.

Those combinations exhibit both osteogenic and osteoconductive properties, and have been

reported to be effective in ARP procedures (Borg & Mealey, 2015; Serrano, Castellanos, &

Botticelli, 2018). The choice of socket grafting material for ARP should be preceded by a careful

treatment plan that is tailored to the patient needs. A systematic review by Majzoub and colleagues

revealed minimal differences between the different bone grafting materials used for ARP, where

allografts, xenografts and alloplasts resulted in significantly less reduction in ridge resorption

following extraction compared to unassisted healing (Majzoub, Ravida, Starch-Jensen, Tattan, &

Suárez-López Del Amo, 2019).

Coronal seal on top of the bone grafting material within the extraction socket is also a

crucial factor for the success of ARP procedures. Clinical closure of socket entrance by firm

epithelialized soft tissue and evidence of radiographic bone fill mark the latest stage of the socket

healing process. Both parameters exhibit a wide range of variability among individuals with

regards to the timing of completion. A period of 10-20 weeks post-extraction has been reported

in literature for completion of socket entrance closure by soft tissue in humans (Johnson, 1969).

In a mongrel dog model of socket healing, Cardaropoli and colleagues observed the formation of

a woven bone bridge separating the socket from oral mucosa by day 60 post-extraction (G.

Cardaropoli et al., 2003).   On the other hand, a period of 3-6 months was required for detecting

an evidence of radiographic bone fill following extraction in humans (Schropp et al., 2003). The

aforementioned studies indicate that natural coronal seal of the extraction socket takes at least 8-

10 weeks to be completed. This is a relatively long period of time that may allow for soft tissue

ingress into the healing socket and inadequate bone fill especially in its most coronal aspect. For

this reason, achieving a coronal seal becomes especially important when alveolar ridge preservation (Depaolo et al.) procedures are attempted to minimize post-extraction dimensional change in height and width. Several materials/techniques have been employed to achieve coronal seal following socket grafting. One technique involves the use of resorbable and non-resorbable barrier membranes to secure intra-socket grafting materials. Regarding resorbable membranes, a number of randomized controlled trials evaluated the use of collagen membrane, collagen matrix or acellular dermal matrix in combination with xenogenic or allogenic grafts for socket preservation (D. Cardaropoli, Tamagnone, Roffredo, Gaveglio, & Cardaropoli, 2012; Iasella et al., 2003; Natto et al., 2017) . The results from those studies showed superiority of using resorbable membranes in combination with grafting materials in terms of reduction of ridge resorption, as opposed to spontaneous healing or using a resorbable membrane/matrix alone. Similar positive outcomes in terms dimensional stability were achieved with employing non-resorbable barrier membranes such as expanded or high-density polytetrafluoroethylene (ePTFE or dPTFE) membranes in conjunction with bone allografts or xenografts (Avila-Ortiz et al., 2020; Lai, Michalek, Liu, & Mealey, 2020; Sun, Lim, & Lee, 2019). These membranes were originally introduced as inert barrier membranes for guided tissue regeneration (Bartee, 1995; Dahlin, Gottlow, Linde, & Nyman, 1990). They were later modified, and their use was later expanded for other applications such as ARP and guided bone regeneration (Fontana et al., 2008).

Regardless of whether a resorbable or a non-resorbable membrane was used, achieving primary closure does not seem to provide additional benefits in terms of bone preservation or graft remodeling (Aladmawy et al., 2019; D. M. Kim et al., 2013). Primary closure by flap advancement has been reported to result in repositioning of the mucogingival junction with crestal displacement of the keratinized mucosa, along with increased incidence of postoperative discomfort and edema

(Engler-Hamm, Cheung, Yen, Stark, & Griffin, 2011) . To circumvent those drawbacks, primary soft tissue closure using autogenous soft tissue grafts without flap advancement has been suggested. This can be achieved either via harvesting a free gingival graft (FGG) from the palate or using a rotated palatal flap (in case of anterior maxilla) (Bitter, 2010; Landsberg, 2008). When used alone as a socket sealer, FGG has been reported to minimize the reduction in alveolar ridge height in the anterior maxilla compared to spontaneous healing (Karaca, Er, Gülşahı, & Köseoğlu, 2015). The technique involving the use of a free gingival graft to cover the socket orifice to secure an intra-socket bone grafting material was referred to as "the socket seal surgery" (SSS) (Landsberg, 2008). The original rotated palatal flap is also combined with socket grafting to preserve ridge dimensions post-extraction (Bitter, 2010). Both autogenous grafting techniques have been reported to preserve soft tissue contours especially in the anterior maxilla.

An alternative strategy to autogenous soft tissue graft for socket sealing is the "BioCol" technique. This technique involves the use of a collagen plug to seal the socket filled with bovine bone mineral. The collagen plug is secured in place with a horizontal mattress suture along with the application of cyanoacrylate to harden the plug and reduce its permeability (Sclar, 1999). Following the introduction of the original BioCol technique, several modifications have been proposed. One modification involves the use of a collagen plug trimmed to 1/5 of its original size, placed over the intra-socket graft and secured by provisional fixed or removable prosthesis that will help molding an esthetic soft tissue profile (Fowler & Whicker, 2004). Another modification of the BioCol technique entails the substitution of xenogenic graft with a mineralized human allograft (FDBA) that filled the extraction socket up to 1-2 mm below the crest and secured by a collagen plug stabilized by a cross-mattress suture (H. L. Wang & Tsao, 2007). This technique was coined by the authors as the "mineralized bone allograft-plug" and was shown to result in

satisfactory clinical and histological outcomes. Based on the above, it can be concluded that achieving a stable coronal seal on top of socket grafts is critical factors for the success of ARP procedures. When selecting a specific technique/material, clinicians should consider the post-extraction socket anatomy, especially with regards to the buccal wall integrity. In this regard, Steigmann and colleagues proposed a decision tree based on the buccal bone morphology after extraction (Steigmann, Di Gianfilippo, Steigmann, & Wang, 2022). This decision tree reflects the need to tailor the ARP technique based on individual clinical scenarios.

1.5. Effect of Periodontal Health on Socket Healing Outcomes

Several human, animal and in-vitro studies have shown that chronic inflammation of bone tissue results in downregulation of osteoblastic activity and upregulation of osteoclastic activity causing uncoupled bone remodeling and net bone loss (Baum & Gravallese, 2014; Maruyama et al., 2020; Terkawi et al., 2022). This concept can be extended to extraction socket healing where a previous chronic inflammatory process at the extraction site or at adjacent site can compromise bone intra-socket bone formation and negatively impact ARP outcomes. In this regard, long-standing chronic periodontal or endodontic infections can result in the formation of a reactive granulomatous tissue within the periodontal space of the offending tooth termed intra-socket reactive tissue (ISRT) (Shehabeldin et al., 2023). ISRT is a form of infected chronic inflammatory tissue that is often found on the internal socket walls following the extraction of periodontally or endodontically involved teeth. A comparative histopathological analysis of ISRT dissected from extraction sockets of periodontally or endodontically involved teeth showed that this tissue showed that this tissue harbored of large bacterial aggregates surrounded by polymorphonuclear (PMN) cells, along with areas of discrete osteoclasts and total absence of osteoblasts or pre-osteoblasts.

(Canullo et al., 2020). Two mechanisms can be described to explain the possible negative effects of ISRT on extraction socket healing. First, the reactive granulomatous tissue serves as a niche for a non-resolving chronic inflammatory response, which could detrimentally affect the intra-socket bone formation, leading to compromised bone fill. Second, the reactive granulomatous tissue harbors unwanted epithelial and connective tissue cells that could occupy the space needed during bone formation. Both mechanisms can negatively impact socket healing and compromise ARP success. This highlights the importance of debriding ISRT to eliminate the presence of these unwanted cells, which can negatively impact socket healing and the success of ARP procedures.

The detrimental biological effect of residual ISRT is illustrated in two studies investigating spontaneous socket healing. The first study showed that sockets of periodontally involved teeth took twice as long to form the same proportion of new bone compared to those of periodontally healthy teeth when no debridement was done at the time of extraction (Ahn & Shin, 2008). The second study is a retrospective radiographic analysis that revealed a 5% rate of erratic socket healing among teeth extracted due to various reasons, with periodontal involvement being the most common factor associated with erratic healing (J. H. Kim et al., 2014). Despite the expected negative effects of periodontal inflammation on extraction socket healing, several clinical trials demonstrated the efficacy of ARP in preserving alveolar ridge dimensions at extraction sites with severe periodontitis (Wei, Xu, Zhao, Hu, & Chung, 2022; Zhang et al., 2022; Zhao, Xu, Hu, & Chung, 2018). Along the same lines, a recent systematic review revealed that periodontal involvement does not seem to compromise the outcomes of ARP with regards to minimizing dimensional ridge changes and reducing the need for ancillary bone augmentation at the time of implant placement (Atieh, Alnaqbi, Abdunabi, Lin, & Alsabeeha, 2022). Notably, most studies investigating ARP following extraction of periodontally compromised teeth focus on assessing the

dimensional ridge changes as the primary outcome but does not report on the quality of newly formed bone within extraction sockets that underwent ARP. In this regard, the ratio of newly formed bone and residual bone graft particles as a functional readout of adequate bone remodeling within grafted extraction sockets remain unexplored. It has been suggested that implant primary stability is generally lower in regenerated versus native bone (Vallecillo-Rivas et al., 2021). Accordingly, a higher percentage of residual bone graft particles is expected to result in an even lower primary stability at the time of implant placement due to lower bone quality. In this regard, one systematic review showed that lower bone quality was associated with a higher implant failure rate (Chrcanovic, Albrektsson, & Wennerberg, 2017). Thus, studying the influence of periodontal status on bone quality within grafted extraction sockets could provide valuable insights into the association between periodontal inflammation and dental implant therapy success.

2.   Statement of the Problem

Most of studies on ARP outcomes focus on assessing the efficacy of a certain technique or a new material on the dimensional ridge changes or new bone formation within extraction sockets. In those studies, teeth with periodontal involvement or lack of buccal plate at the time of extraction are often excluded because those factors are difficult to standardize among patients and can confound the results regarding the efficacy of a new material or technique in improving ARP outcomes. However, PD is one of the major causes of tooth loss and periodontal inflammation could negatively impact on new bone formation and graft remodeling in sockets that underwent ARP. While clinical studies have shown that the efficacy of ARP on dimensional ridge changes in patients with severe periodontitis, there is still a gap in knowledge with regards to our understanding of the effect of periodontal status on the quality of newly formed bone within grafted extraction sockets, which may affect implant stability and long-term success.

3.   Objectives

The primary aim of this study was to compare the quality of newly formed bone within extraction sockets of single rooted teeth that underwent ARP in periodontitis and periodontal healthy patients. In this regard, the amount of newly formed bone and residual bone graft particles was quantified both radiographically using micro-computed tomography measurements and histologically using different enzymatic and immune-histochemical stains.

The secondary objective was to assess whether there is a correlation between radiographic and histological results in terms of assessing newly formed bone and residual bone graft particles. Furthermore, we investigated whether this correlation is affected by the periodontal status of the patient.

4.   Hypothesis

Null Hypothesis (H0): There is no significant difference between the periodontally health and periodontitis patients in terms of newly formed bone and residual bone graft particles within extraction sockets of single rooted teeth that underwent ARP.

Alternative Hypothesis (H1): There is a significant difference between the periodontally health and periodontitis patients in terms of newly formed bone and residual bone graft particles within extraction sockets of single rooted teeth that underwent ARP.

II.   MATERIAL AND METHODS

1.   Study Design and Participants

This study was conducted in adherence to a protocol (HUM00203875/Ame00147900) approved by the Institutional Review Board at the University of Michigan. The subjects pool consisted of a total of 7 patients (3 Females and 4 males) who were referred to the Department of Periodontics at the University of Michigan School of Dentistry for extraction of a hopeless tooth and replacement with dental implant. Inclusion criteria included patients who were at least 18 years old and had a single anterior or premolar edentulous site where ARP was performed at the time of tooth extraction and presented for delayed implant placement 4.5-7 months after extraction and ARP were done. Exclusion criteria included pregnancy, current smokers, patients with diabetes mellitus or any other systemic conditions that may affect extraction socket healing (osteoporosis, osteopenia and patients on antiresorptive medications) and a healing period between tooth extraction/ARP that is less than 4.5 months or more than 7 months.

2.   Surgical Procedures

After confirming the hopeless prognosis of the tooth in question, minimally traumatic extraction was performed by the graduate resident provider. Following tooth extraction, the extraction socket was debrided of any ISRT using a serrated bone curette and under magnification. The integrity of the buccal plate was evaluated using a periodontal probe to confirm that at least two thirds of the buccal bone plate of the extraction socket is intact. Next, ARP was performed by packing the socket with mineralized cancellous particulate bone allograft (Puros ®) filled to the level of the alveolar bone crest. The bone allograft was then covered with a Collagen Plug (Zimmer ®) secured in place with cross-mattress and single interrupted sutures. For the initial

follow-up, patients were seen 2-3 weeks following tooth extraction/ARP to remove the sutures and assess initial healing. Patient were brought back again at 4 months following tooth extraction/ARP to assess soft tissue and perform digital impression and cone beam computed tomography scanning needed to devise the digital plan and design the surgical guide that will be used for implant placement.

Implant placement was performed 4.5-7 months after extraction/ARP. On the day of implant surgery, a 2 mm x 22 mm trephine drill (Salvin ®) was used to perform initial drilling at the future implant site guide by the digitally designed surgical guide without flap reflection. A 2 mm x 5 mm bone core biopsy with crestal soft tissue was harvested from the implant site. Next, the drilling protocol was followed to enlarge the osteotomy to appropriate diameter and length for implant placement.   The decision to reflect or not to reflect a full thickness mucoperiosteal flap was left for the clinical judgement of the operator.

3.   Micro-computed Tomorgraphy (micro-CT) scanning and Analysis

The harvested bone core biopsies were fixed overnight in 10% buffered formalin.   The next day, the bone core biopsied were rinsed with double distilled water and stored in 70% Ethyl alcohol until they are ready for micro-CT scanning. For micro-CT scanning, specimens were placed in a 14 mm diameter specimen holder and scanned using a micro-CT system (µCT100 Scanco Medical, Bassersdorf, Switzerland). Scanning settings were as follows: voxel size 10 µm, 70 kVp, 114 µA, 0.5 mm AL filter, 1000 projections around 180° and integration time 500 ms. DICOM files for each specimen were exported and were used for u-CT analysis.

Micro-CT analysis was performed using Dragonfly software which allows visualization and artificial intelligence-based segmentation and quantification of 2D and 3D imaging studies. A protocol tailored for the quantification of newly formed bone and residual bone graft particles

within the scanned bone core biopsies was developed. In brief, the DICOM files for each specimen were imported into the software. Next, using the 2D views, the radio-opaque metal particles within observed within the specimen were masked by matching their grayscale with the background's grayscale. The step that followed was to create a median filtered image on the 2D views to reduce background noise. A region of interest (ROI) was then defined by creating a cylinder around the specimen in the 2D views. Next, automatic image thresholding was performed using Otsu's method, and applied to the cylinder ROI to separate pixels into 2 classes, foreground for bone volume (BV) and background for total volume (TV). To define the newly formed bone NB and residual bone graft RBG within the BV pixels, a second Otsu operation was performed to separate the pixels for NB (background with lower grayscale) and RBG (foreground with higher grayscale). Finally, the relative ratio of the NB and RBG voxels was computed and generated using the bone mineral density tool.

4. Histological Processing

Before histological processing, the formalin fixed bone core biopsy samples were first demineralized for 48 hours in EDTA solution, then embedded in paraffin. Next, 5 μm thick apico-coronal sections were obtained. A minimum of 10 tissue sections were acquired from each specimen. Those tissue sections were used for hematoxylin and eosin (H and E), masson's trichrome and immunohistochemical staining for osteocalcin. H and E staining was performed for only one slide for representative purposes.

5. Masson's Trichrome Staining and Analysis

Masson's Trichrome staining was performed on three equidistant tissue sections from each specimen. The stained tissue sections have two main colors: a red color, denoting mineralized

tissue indicative of residual bone graft particles, and a blue color that denotes newly formed bone or osteoid. The stained sections were imaged using a Nikon microscope under bright field and the images were exported for quantitative analysis. Quantitative analysis was performed using Fiji software, which is an open-source biological image analysis software. In brief, individual RBG images were imported into Fiji software, then a split channel operation was performed to split the image components into red, blue and green channels. Next, an identical ROI outlining the specimen border in the red and blue channels was created and the threshold was adjusted until a clear no overlap can be observed within the components of the red and blue channels. Finally, the percent surface area of the visible tissue within the ROI in the red and blue channels was quantified. The red channel was used to denote RBG particles, and the blue channel was used to denote newly formed bone. The area fractions of NB and RBG from each of the 3 slides within each specimen were averaged. Paraffin embedded demineralized tissue section from stock bone graft particles were used as a negative control.

6. Immunohistochemistry (IHC)

To assess the osteoblastic activity within the bone core biopsies, three equidistant tissue sections were stained with osteocalcin (OCN) antibody, where osteocalcin was used as a late marker for osteoblasts differentiation (Tsao et al., 2017). In brief, three equidistant tissue sections from each specimen were first baked into a slide oven at 60 °C for 1 hour. Next, the slides were deparaffinized then antigen retrieval was performed by incubating the slides for 10 minutes in TEN buffer with 1:400 Proteinase K (Ambion). The slides were then blocked with 5% normal goat serum for 1 hour at room temperature then incubated overnight with 1:400 osteocalcin rabbit polyclonal antibody (Abcam) in 3% BSA at 4 °C. The next day, the slides were washed in TBS solution then incubated with 1:400 goat anti-rabbit HRP-conjugated secondary antibody (Thermo

Fisher) diluted in 3% BSA in the dark at room temperature for 45 minutes. Finally, the slides were incubated with the DAB substrate for 5 minutes then counterstained with hematoxylin and cover slipped using aqueous mounting medium compatible with IHC (Ab64230). A slide that went through all the previously described steps except for the incubation with the primary antibody was used as a negative control.

The osteocalcin-IHC stained slides were then imaged using a Nikon microscope under a bright field. The images were then exported for analysis using Fiji software. In brief, the images were first imported into the software and converted to grayscale 8-bit images. Next, a ROI was manually drawn to outline the specimen then the image threshold was adjusted until a clear distinction between the positively and negatively stained areas is observed. Finally, the fraction of the positively stained area was quantified. The final value was presented as the average area fraction from the 3 equidistant stained slides from each specimen.

7. Statistical Analysis

Statistical analysis was performed in GraphPad Prism version 10. Unpaired t-test was used to compare between periodontally healthy and periodontitis patients followed by Tukey post-hoc test. Results were presented as mean ±SD and statistical significance was considered at p-value < 0.05. For correlation analysis, a very strong association was considered at R^2 values greater than 0.8, whereas the R^2 was 0.6-0.79 for strong association, 0.4-0.59 for moderate association, 0.2-0.39 for weak association and 0-0.19 for very weak association (Bewick, Cheek, & Ball, 2003).

III.   RESULTS

1.   Study population

A total of 7 patients were recruited in the study (3 females and 4 males). The patients' age ranged from 54-78 years old with an average age of 64 years old.   4 patients were periodontally healthy (control), and 3 patients had periodontitis classified as stage III grade B periodontitis according to the 2017 WW classification. The patients in the periodontitis were compliant with periodic periodontal maintenance recalls for at least 1 year before ARP was performed.   In all patients, ARP site belonged to a single root tooth (incisor or premolar). The ARP site and healing time after ARP in each patient are shown in table 1 below.

Table 1. ARP site and healing time after ARP in control (no periodontitis) and experimental (periodontitis) groups.

No Periodontitis (Control) ARP site – Healing time after ARP	Periodontitis (Experimental) ARP site – Healing time after ARP
Patient #1: Maxillary central incisor – 4.5 months	Patient #1: Maxillary central incisor – 6 months
Patient #2: Maxillary lateral incisor – 5 months	Patient #2: Maxillary lateral incisor – 4.5 months
Patient #3: Maxillary central incisor – 6.5 months	Patient #3: Maxillary second premolar – 7 months
Patient #4: Maxillary second premolar – 5.5 months	

2.   Surgical procedures Outcomes

Healing was uneventful after ARP procedure in all patients. Implant placement with adequate primary stability ($\geq$ 35 N/cm^2) was achieved in all patients. There was no need for additional bone augmentation at the time of implant placement.

3.   Evaluation of NB and RBG using micro-CT

To assess new formation and bone graft remodeling within the extraction socket following ARP, we performed volumetric analysis of NB and RBG within bone core biopsies harvested from ARP sites at the time of implant placement. This analysis was performed using Dragonfly software that uses artificial intelligence-based segmentation operations to distinguish between different radiographic entities based on their relative voxel radiodensity. Histograms for the relative Hounsfield units (Hu) generated from the analyzed bone core biopsies revealed 3 distinct peaks representing the background, NB and RBG (Figure 1). The peak with the lowest Hu was considered representative of the background, whereas the peak with the light Hu was considered representative of the RBG. Accordingly, the intermediate peak was considered representative of the NB.
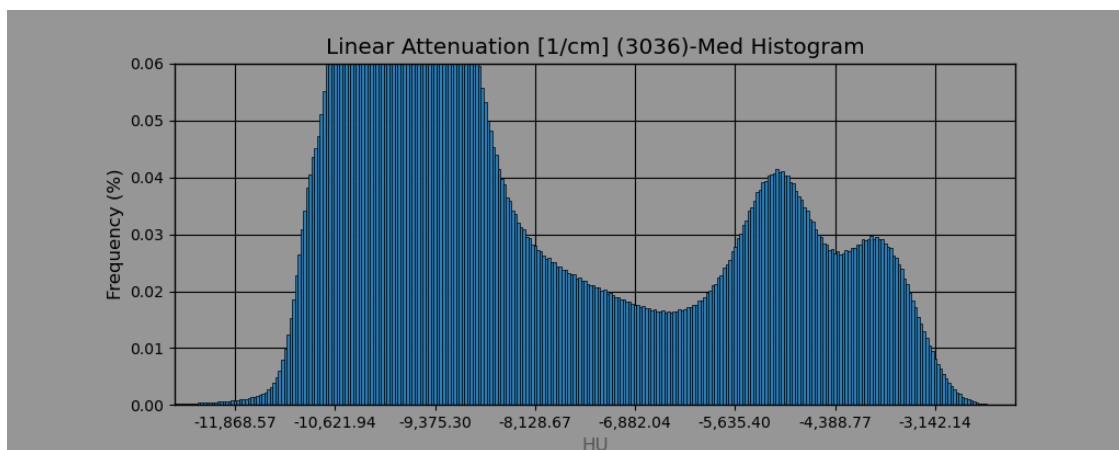
Figure 1. **Representative histogram showing the 3 different radiographic entities (background, NB and RBG) based on the relative Hu.**

Using Dragonfly software segmentation capability, we quantified the percentage of NB and RBG within the bone core biopsies in both the control and the periodontitis groups (Table 2). Our quantification showed that the percentage of RBG volume was consistently higher in all bone core biopsies from both groups except for one bone core biopsy in the control group where the percentages of NB and RBG were relatively close. Using Dragonfly, the segmented NB and RBG areas were labeled with different colors in the apico-coronal 2-dimensional view of the bone core biopsies (Figure 2).

Table 2. **Percentages of NB and RBG in bone core biopsies from the control and experimental groups as determined by micro-CT analysis.**

No Periodontitis (Control)			Periodontitis (Experimental)		
Patient	**NB%**	**RBG%**	**Patient**	**NB%**	**RBG%**
#1	15.5%	32.8%	**#1**	18.7%	45.8%
#2	17.4 %	29.8%	**#2**	7.05%	13.1%
#3	21.1%	34.8%	**#3**	13%	24.6%
#4	15.2%	16.7%			
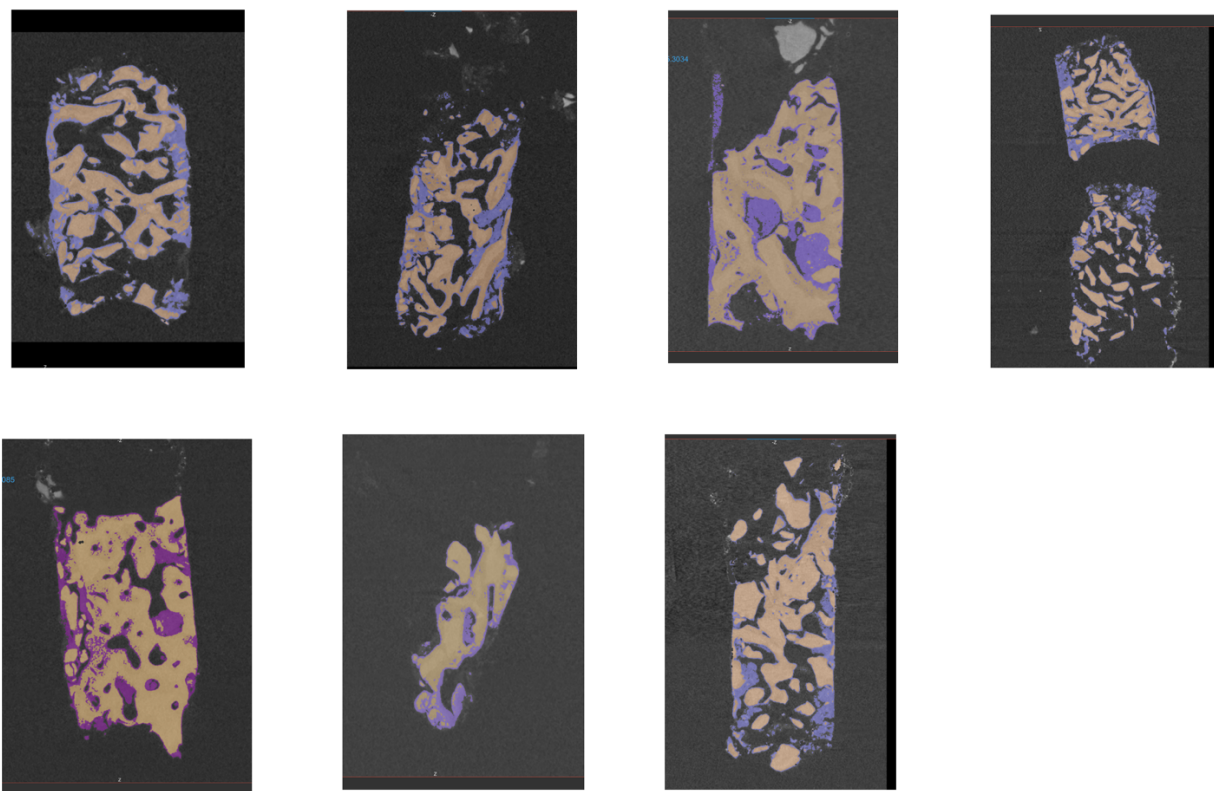
Figure 2. Representative 2D views of the segmented NB (purple) and RBG (Brownish) voxels of the bone core biopsies. Top row includes the biopsies from the control group and bottom row shows biopsies form the experimental group.

Statistical analysis was performed to compare the differences in NB, BG and NG/RBG ratio between the control and experimental groups. The results of these analyses showed no statistically significant difference between both groups (Figure 3).
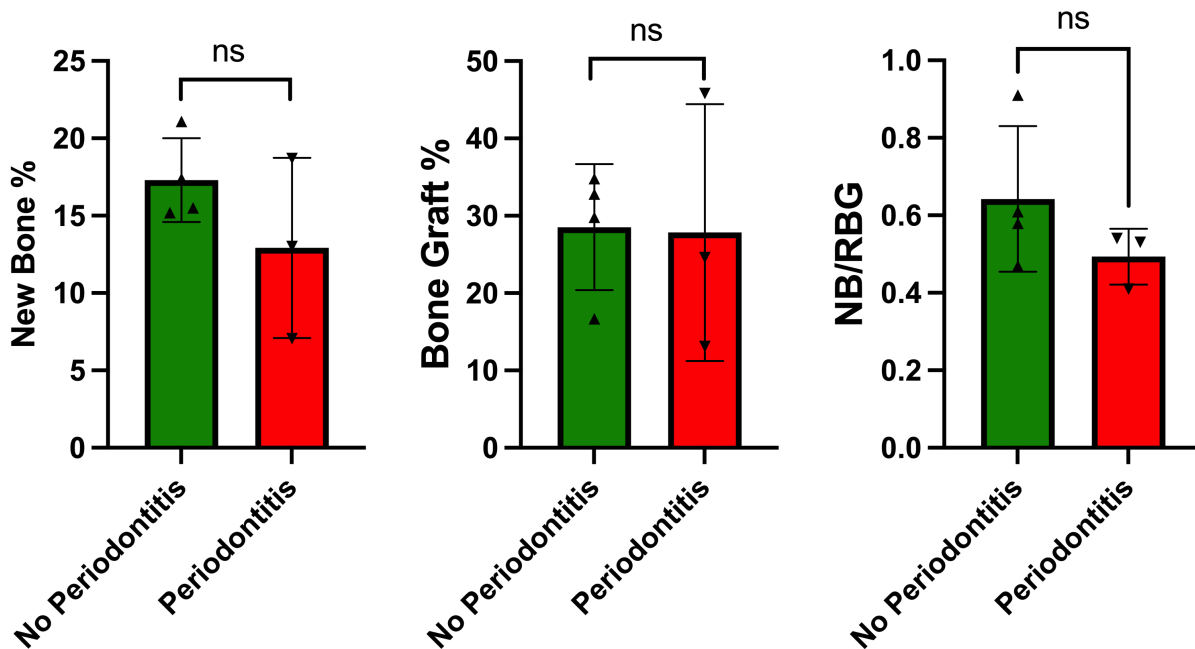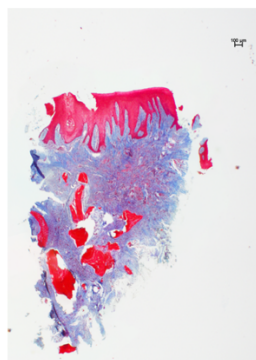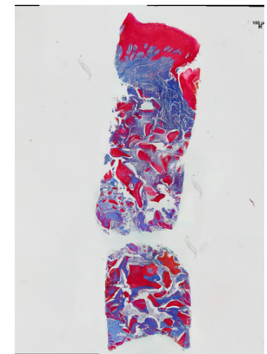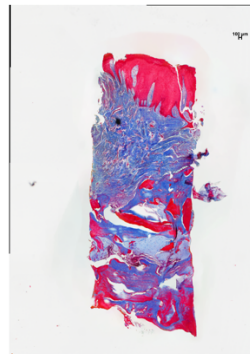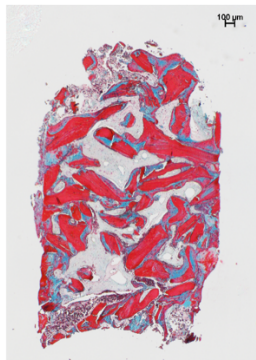
Figure 3. Bar graphs comparing the percentage of NB, RBG and the NB/RBG ratio in both the control (no periodontitis) and the experimental (periodontitis) groups using microCT. Unpaired t-test was performed with post-hoc Tukey test to determine statistical significance (P < 0.05).

4. Quantification of NB and RBG using Masson's Trichrome stain

To evaluate the new bone formation and bone graft remodeling within the bone core biopsies at the histological level, we stained equidistant tissue sections from the bone core biopsies using Masson's Trichrome stain. This stain yields two colors: a blue color that stains collagen fibers representative of the osteoid matrix of the NB and a red color representative of the mineralized RBG particles (Figure 4). Fiji software was used to measure the NB and RBG areas within a defined ROI outlining the specimen on imaged histological slides stained with Masson's Trichrome.

Table 3. **Percentages of NB and RBG in bone core biopsies from the control and experimental groups on Masson's Trichrome stained histological slides.**

No Periodontitis (Control)			Periodontitis (Experimental)		
Patient	**NB%**	**RBG%**	**Patient**	**NB%**	**RBG%**
#1	11.2 %	47.6%	**#1**	1.5%	56.9%
#2	13.7%	20.2%	**#2**	10.1%	13.8%
#3	47.3%	35.3%	**#3**	15.3%	40.2%
#4	20%	27.9%			

Figure 4. Representative images of the Masson's Trichrome staining of bone core biopsies from the control (top row) and experimental (bottom row) groups. NB is stained as blue and RBG particles are stained as red.

Statistical analysis was performed to compare the differences in NB, BG and NG/RBG ratio on Masson's Trichrome stained slides from the control and experimental groups and showed no statistically significant difference (Figure 5).
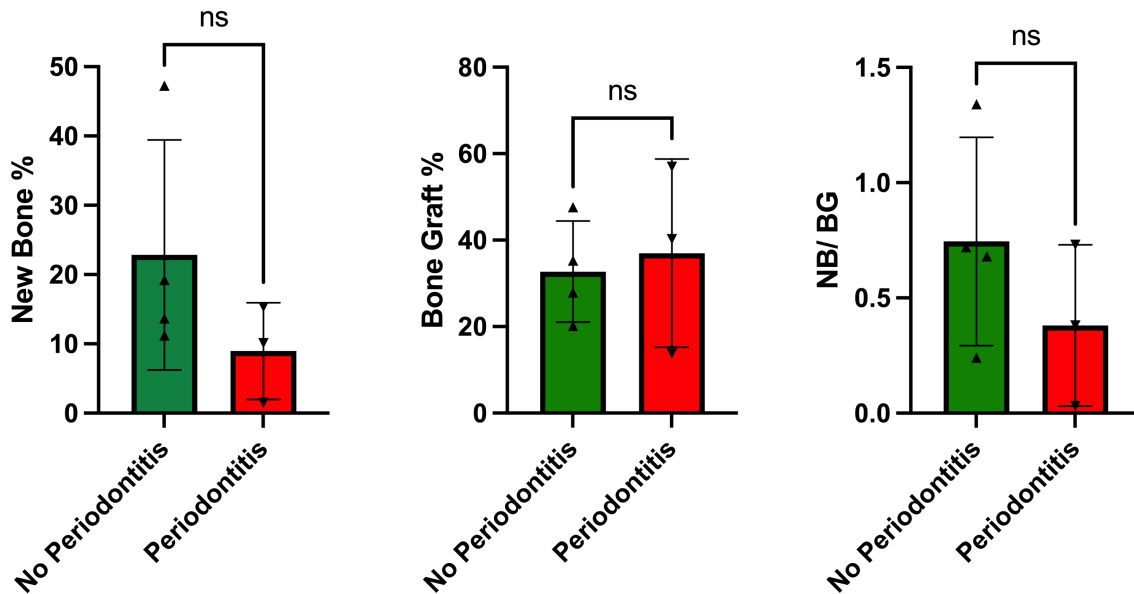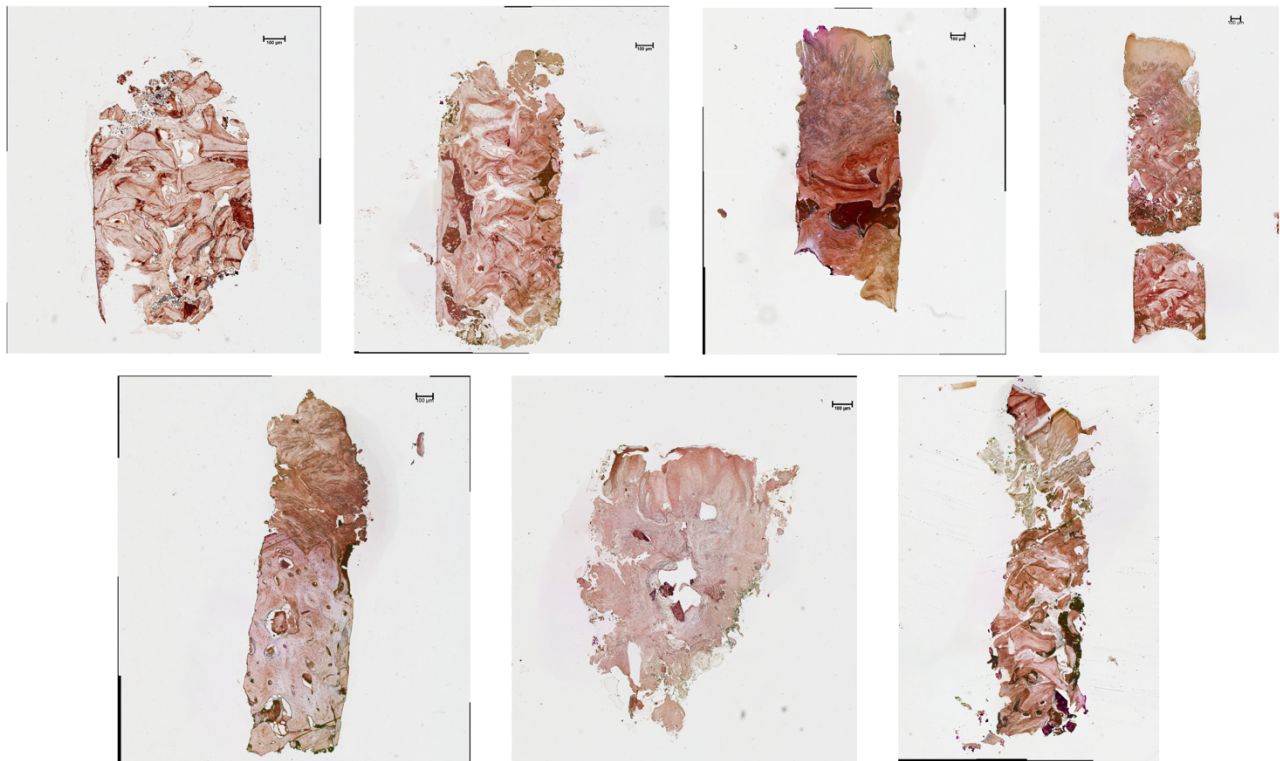

Figure 5. Bar graphs comparing the percentage of NB, RBG and the NB/RBG ratio in both the control (no periodontitis) and the experimental (periodontitis) groups using microCT. Unpaired t-test was performed with post-hoc Tukey test to determine statistical significance (P < 0.05).

5.   Assessment of OCN expression using IHC

To further assess and quantify new bone formation within the bone core biopsies, we performed immunohistochemical staining and analysis of OCN expression. OCN is a late marker for osteoblasts differentiation and was used as a functional readout for newly formed bone within the bone core biopsies. OCN expression was measured by quantifying the areas with a positive stain (dark brown color) using Fiji software on equidistant slides from each bone core biopsy (Figure 6).

Figure 6. Representative images of the OCN immunohistochemical staining of bone core biopsies from the control (top row) and experimental (bottom row) groups. A positive stain is shown as brown stained areas and were used to represent NB.

Statistical analysis comparing the OCN IHC staining results between the control and experimental group showed no statistical significance between both groups (Figure 7).
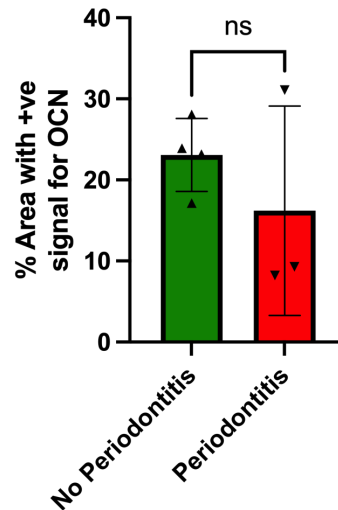
Figure 7. A bar graph comparing the percentage area with positive OCN staining in both the control (no periodontitis) and the experimental (periodontitis) groups using IHC. Unpaired t-test was performed with post-hoc Tukey test to determine statistical significance (P < 0.05).

6. Correlation Analyses between micro-CT and histological staining results

To assess whether the quantification results of NB and RBG can be correlated across micro-CT, Masson's Trichrome and OCN IHC, we performed a Pearson's correlation analysis for NB and RBG between micro-CT and Masson's trichrome results for NB and RBG, and between micro-CT and OCN IHC results only for NB. With regards to NB, the results of the correlation analyses between micro-CT and Masson's Trichrome staining (Figure 8A) and between micro-CT and OCN

IHC (Figure 8C), showed very weak to weak correlation, respectively. On the other, we found a strong correlation between micro-CT and Masson's Trichrome staining for RBG, and this correlation was statistically significant (Figure 8B).
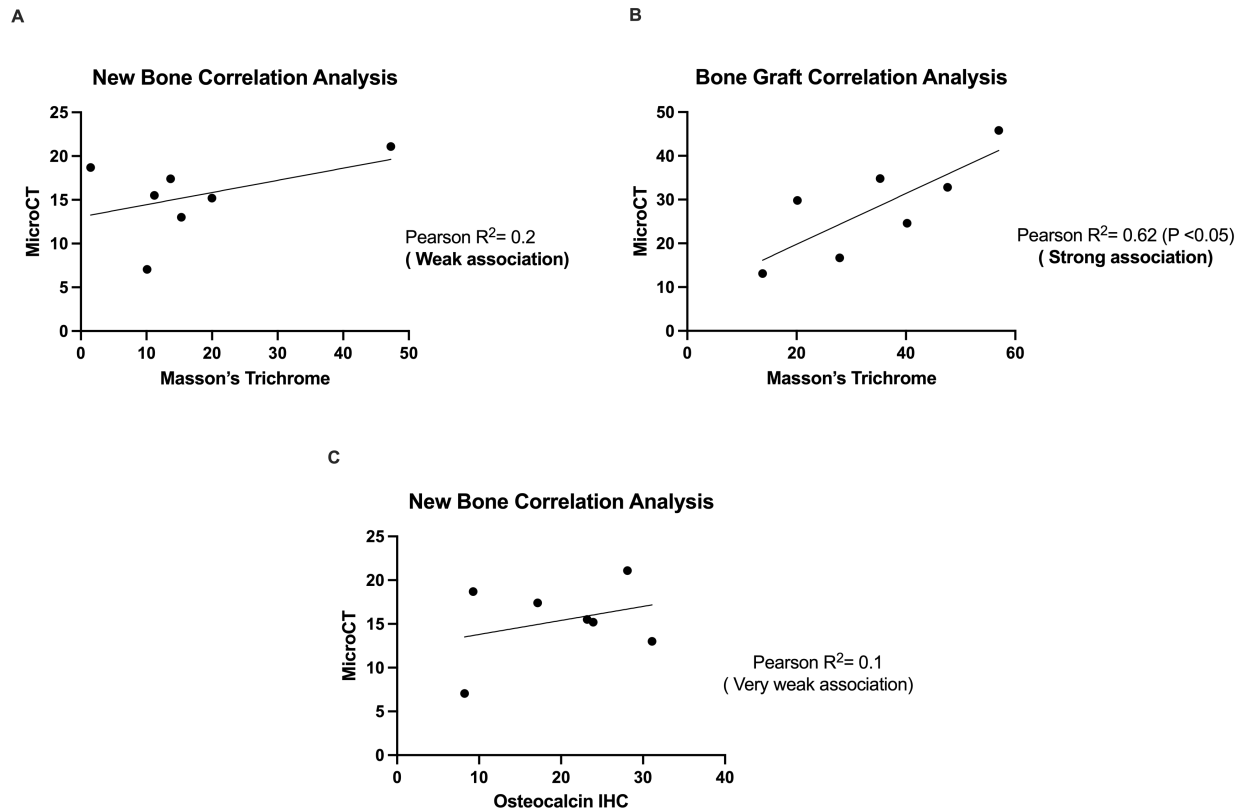
Figure 8. Correlation analyses between microCT and Masson's Trichrome (A and B) and between micro-CT and OCN IHC (C). Pearson's correlation coefficient and the degree of association are shown. Statistical significance was considered at P < 0.05.

To validate the correlation analysis results between micro-CT and Masson's trichrome, we repeated this analysis using select micro-CT slices that match the location and morphological appearance of the Masson's trichrome and Osteocalcin IHC stained slides. The results of this

analysis were consistent with the previous correlation analysis that correlated between the mico-CT results of the whole specimen and those from Masson's trichrome and the Osteocalcin IHC stained slides (Figure 9).
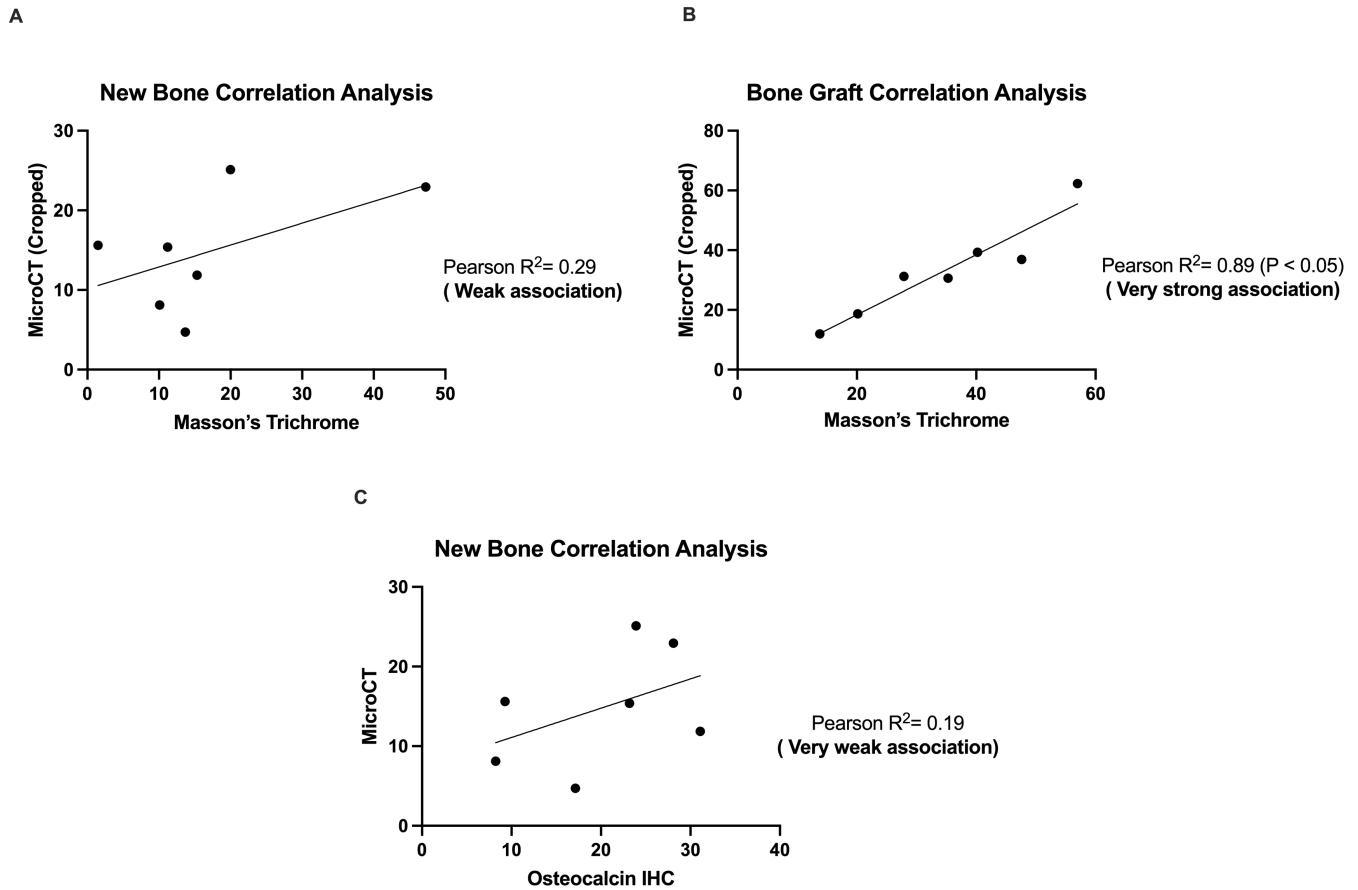
A

New Bone Correlation Analysis

Pearson R^2= 0.29
(Weak association)

B

Bone Graft Correlation Analysis

Pearson R^2= 0.89 (P < 0.05)
(Very strong association)

C

New Bone Correlation Analysis

Pearson R^2= 0.19
(Very weak association)

Figure 9.   Correlation analyses between select-micro-CT slices matching Masson's Trichrome (A and B) and OCN IHC stained slides (C). Pearson's correlation coefficient and the degree of association are shown. Statistical significance was considered at P < 0.05.

Finally, we performed a correlation analysis between the healing time after ARP and the percentage of NB and RBG as calculated by micro-CT and Masson's trichrome analyses. This

analysis revealed a very weak association between the healing time and NB or RBG as quantified

using micro-CT or Masson's trichrome staining (Figure 10).
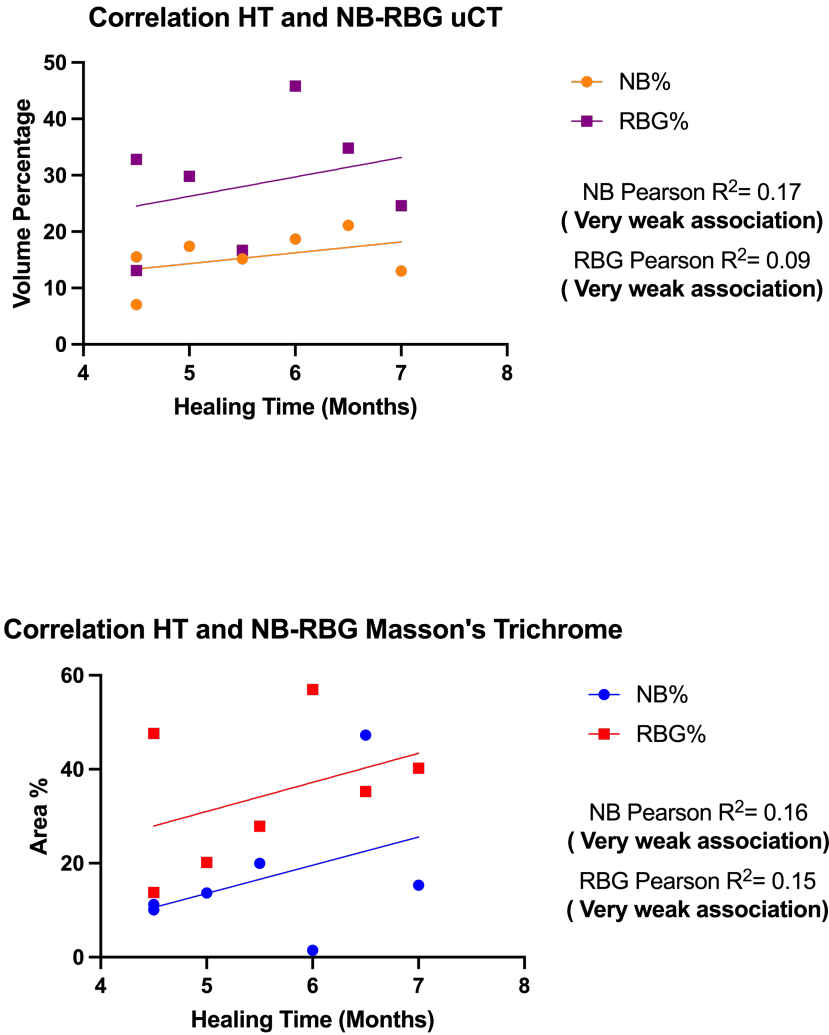
Correlation HT and NB-RBG uCT

NB Pearson R^2= 0.17
(Very weak association)

RBG Pearson R^2= 0.09
(Very weak association)

Correlation HT and NB-RBG Masson's Trichrome

NB Pearson R^2= 0.16
(Very weak association)

RBG Pearson R^2= 0.15
(Very weak association)

Figure 10. Correlation analyses between healing time after ARP and micro-CT or Masson's Trichrome in terms of NB or RBG percentages. Pearson's correlation coefficient and the degree of association are shown. Statistical significance was considered at P < 0.05.

IV. DISCUSSION

A myriad of studies investigated the efficacy of ARP at sites of periodontally involved teeth in the preservation of alveolar ridge contours and minimizing the need for ancillary bone augmentation at the time of delayed implant placement. The results from those studies showed the effectiveness of ARP at sites where teeth with severe periodontitis were extracted (Atieh et al., 2022; Fok, Pelekos, & Jin, 2024; Lee et al., 2021; Wei et al., 2022; Zhang et al., 2022; Zhao et al., 2018). Those studies focused mainly on assessing the dimensional alveolar ridge changes of the ARP site, the need for additional bone augmentation at the time of implant placement, and histomorphometry evaluation of new bone formation and residual bone graft particles within the healed sockets. While the results from those studies provide clinically valuable information, they do not address whether the nature and quality of bone tissue formed within extraction sockets is influenced by a history of PD or the presence of periodontal inflammation at the ARP sites or around adjacent teeth. To the best of our knowledge, there are no studies to date that investigated the influence of periodontal status on the healing outcomes of ARP in terms of newly bone formation and bone graft remodeling in a side-by-side comparison between periodontitis and periodontally healthy patients.

In this study, we sought to assess whether the periodontal health status would affect tooth extraction socket healing following ARP. We hypothesized that extraction sockets that underwent ARP in periodontitis patients would exhibit different radiographic and histological healing outcomes compared to those in periodontally healthy patients. The two main parameters that we quantified were new bone formation (NB) and the residual bone graft particles (RBG). To that

end, we recruited periodontitis and periodontally healthy subjects who underwent ARP and were presenting for implant placement at 4.5-7 months after. On the day of implant placement, bone core biopsies from the ARP site were harvested and processed for micro-CT and histological analysis.

Our micro-CT analysis revealed no statistically significant difference between periodontitis and periodontally healthy patients in terms of NB or RBG volume within bone core biopsies harvested at the time of implant placement. Similarly, Masson's trichrome analysis did not show a significant effect for periodontal status on NB or RBG surface areas using histological sections representative of the bone core biopsies. Finally, OCN expression was not statistically different between both patient cohorts as determined by immunohistochemistry staining of representative histological sections. One possible explanation for our inability to observe an effect for periodontal health on socket healing is the wide variation in the healing time after ARP across samples. A recently published randomized controlled trial showed that a longer healing time following ARP with a mixture of demineralized and mineralized bone allografts correlated with greater vital bone formation and fewer residual bone graft particles as analyzed on histological sections, as opposed to a shorter healing time (Zellner, Allen, Kotsakis, & Mealey, 2023). Thus, the variation in healing time between ARP and bone core biopsies harvesting could have masked any effect the periodontal status may have had on NB formation or bone graft remodeling within the extraction sockets. Along the same lines, we performed a correlation analysis between the healing time and the percentages of NB and RBG as analyzed by micro-CT and Masson's trichrome and found a very weak correlation between these parameters that was not statistically significant. This correlation, if present, could have also been diluted by the small sample size we had in this study.

The subjects in the experimental group in our study were classified as stage III Grade B periodontitis patients according to the 2017 WW on periodontal classification. Those patients had already completed the active periodontal therapy phase and had been compliant with maintenance recalls for periodontal supportive therapy for at least one year prior to the tooth extraction and ARP procedure. In addition, the two most updated periodontal charting at the last periodontal maintenance recall prior to the ARP procedure in those patients revealed stable probing depth and clinical attachment levels, as well as low bleeding on probing (BOP) scores. BOP has been widely used as a clinical indicator for periodontal inflammation, and a lower BOP incidence at repeated maintenance recalls has been considered as an indicator of periodontal stability with a lower chance of periodontal breakdown and PD progression (Lang, Joss, Orsanic, Gusberti, & Siegrist, 1986). This indicates that the patients in the periodontitis group were compliant to periodontal maintenance, and as a result, were periodontally stable and had minimal periodontal inflammation at the time of ARP and throughout the healing period that followed. In the same regard, several studies have shown that patients who are compliant to periodontal maintenance had lower total bacterial counts of periodontal pathogens (such as T. denticola and A. actinomycetemcomitans) and had better periodontal clinical parameters compared to irregular compliers (Cortelli et al., 2020; Costa et al., 2018). Thus, even though the patients in the experimental group have had a periodontitis diagnosis at the time ARP, it was unlikely that their periodontal condition would cause a significant change in the local microbial load or host response that would be detrimental to extraction socket healing process. It would be interesting to retrospectively evaluate the outcomes of ARP in patient who were periodontal unstable or non-compliant to supportive periodontal care at the time of tooth extraction. Furthermore, a recent split-mouth experimental

gingivitis study showed that localized plaque-induced inflammatory response in the periodontium could affect distant healthy sites that may become more susceptible to inflammatory damage (Kerns et al., 2023) . Thus, in patient with localized periodontitis, the potential detrimental effect of periodontal instability and inflammation on extraction socket healing could extend to sites that are not directly affected by PD or in close proximity to periodontally diseased teeth.

Among all the analyses we performed in this study, the correlation analysis between micro-CT and Masson's trichrome staining showed a strong and statistically significant association only for the RBG particles. On the other hand, this association was weak and non-statistically significant for NB. The micro-CT analysis was done using Dragonfly software that leverages artificial intelligence (AI) and machine learning (ML) to enable the segmentation and quantification of 2D and 3D imaging studies. Repeated use of this tool to analyze data for larger and well-designed studies could set the basis for streamlined, time-efficient and reproducible AI and ML based protocols for implant site evaluation and planning on the same day of implant surgery. AI and ML based diagnostic and surgical planning tools are being currently developed and integrated in daily medical and dental practice to enhance patient care and treatment outcomes (Kerns et al., 2023; Zhu et al., 2023).

LIMITATIONS

The limitations of this study are summarized below:

- Small sample size that likely resulted in the lack of statistical significance in the analyses performed in this study.

- Lack of standardization in the healing time following ARP across all subjects recruited in this study.

- The fact that patients in the experimental group were periodontally stable with no evidence of disease activity or progression, which could have masked any hypothesized effect of periodontal inflammation may have had on extraction socket healing after ARP.

V.   CONCLUSIONS

Within the limitations of this study, our data showed that there is no difference between periodontal healthy and stable periodontitis patients with regards to new bone formation and bone graft remodeling in extraction sockets that underwent ARP. Adequate sample size calculation and standardization of the healing time between ARP and implant placement are critical to obtain robust results from which clinically relevant conclusions can be drawn to guide patient care and clinical practice guidelines.

FUTURE DIRECTIONS

Larger and well-designed future studies are needed to assess the effect of periodontal inflammation on extraction socket healing. An interesting avenue of research would be to retrospectively assess whether an active untreated PD or treated PD in erratic compliance patients would negatively impact extraction socket healing and ARP outcomes. Another avenue would be to leverage the widespread use of animal models of PD to conduct mechanistic studies aimed at evaluating the pathways by which periodontal inflammation and dysbiotic oral microbiota can impact oral hard and soft tissue healing.

Conflict of interest

The authors have states explicitly that there are no conflicts of interest in connection with this article.

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