Effect of leukocyte-platelet-rich fibrin in bone healing around dental implants placed in conventional and wide osteotomy sites: A pre-clinical study

Ernesto B. Benalcázar Jalkh[1,2] DDS, MSci; Nick Tovar[1,3] PhD, DDS; Leticia Arbex[1] MSci; Gregory Kurgansky[1] BSci; Andrea Torroni[4] MD, PhD, Luiz F. Gil[5] DDS; Brittany Wall[1] DDS; Kimia Kohanbash[1] DDS; Estevam A. Bonfante[2] DDS, MSci, PhD; Paulo G. Coelho[1,4,6] DDS, PhD; Lukasz Witek[1,7] MSci, PhD

[1] Department of Biomaterials and Biomimetics, New York University College of Dentistry, New York, NY USA

[2] Department of Prosthodontics and Periodontology, University of Sao Paulo, Bauru School of Dentistry, Bauru, Brazil

[3] Department of Oral and Maxillofacial Surgery, New York University, Langone Medical Center and Bellevue Hospital Center, New York, NY, USA

[4] Hansjörg Wyss Department of Plastic Surgery, NYU Langone Medical Center, New York, NY USA

[5] Department of Dentistry, Division of Oral and Maxillofacial Surgery, Universidade Federal de Santa Catarina, Brazil

[6] Department of Mechanical and Aerospace Engineering, New York University Tandon School of Engineering, Brooklyn, NY

[7] Department of Biomedical Engineering, New York University Tandon School of Engineering, Brooklyn, NY

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Corresponding author:

Lukasz Witek *MSci, PhD* / 433 First Avenue, Room 842 / New York, NY 10010-4086 / lukasz.witek@nyu.edu / Phone: +1-212-998-9269

Abstract

Leukocyte-platelet-rich fibrin (L-PRF) has been suggested for gap management in immediate implant placement when the distance is greater than 2 mm. However, there remains a paucity in hierarchically designed research to support this application. The present study aimed to evaluate the effect of L-PRF on the osseointegration parameters of dental implants placed after conventional osteotomy of surgically created bone defects that simulate post extraction sockets in a canine model after 3, 6, and 12 weeks *in-vivo*. Eighty dental implants (Intra-Lock, Boca Raton, FL) were placed in the radius of 13 beagle dogs. The experiment consisted of 4 groups (n=20 implants/group): 1) Regular osteotomy (Reg n/L-PRF); 2) Regular osteotomy and implant placement with L-PRF membrane (Reg L-PRF); 3) Wide osteotomy with no gap management performed, where an osteotomy/bony defect (6 mm of diameter and ~5 mm deep) was created to simulate immediate implant placement in post-extraction sockets, and the gap was left for spontaneous healing (Wide nL-PRF); and 4) Wide osteotomy with L-PRF gap management (Wide L-PRF). L-PRF membranes were obtained by blood drawn from each subject and centrifuged at 2700 rpm (408 RCF-clot) for 12 minutes. In the experimental groups where L-PRF was utilized, the membrane was inserted into the osteotomy site prior to implant placement. Six dogs had implants placed in the radius for 3 weeks; and 7 dogs had implants placed in the left radius for 6 weeks and in the right radius for 12 weeks. At the corresponding experimental time points, samples were harvested, and subjected to histological processing for qualitative and quantitative analyses, via bone-to-implant contact (BIC) and bone-area-fraction occupancy (BAFO). Qualitative analysis demonstrated increased amounts of bone formation around the implant and within the healing chambers over time for all groups. While comparable histological features were observed for both Reg groups (L-PRF and nL-PRF), the gap management performed in Wide L-PRF group resulted in effective gap filling with improved bone growth in close proximity to the implant surface. Quantitative analyses of BIC and BAFO yielded higher values for both variables at 3 weeks for Wide L-PRF (~38% and ~56% respectively) compared to Wide nL-PRF (~20% for BIC and BAFO) ($p<0.03$). No statistical differences were detected between Wide groups at 6 and 12 weeks, neither between Reg groups, independent of the association with or without the L-PRF membrane at all healing times. L-PRF placed within wide osteotomies, prior to implant placement, resulted in increased early bone formation compared to unfilled wide osteotomies (3 weeks in-vivo).

Keywords: implants, in vivo, leukocyte- and platelet-rich fibrin, L-PRF, pre-clinical

Introduction

The development of osseointegrated implants and implant-supported restorations has transformed the available treatment modalities to restore the form, function, esthetics, and psychological wellness of fully and partially edentulous patients. [1, 2] Advances in oral implantology coupled with patients' esthetic demands have given rise to the immediate implant placement concept, which has gained popularity over the last decade.[3] The nontraumatic tooth extraction followed by immediate implant placement allows the implant to heal in a defect-like scenario, where woven bone formation is expected to bridge the gap between the implant and the socket walls. [4]

Commonly, after immediate implant placement there remains a gap between the circumferential aspect of the implant and the extraction socket wall. [5] Early literature has suggested that peri-implant defects with gaps smaller than 2 mm have the capacity to heal spontaneously in bone regeneration. The literature suggests that gaps greater than 2 mm present reduced predictability for spontaneous bone healing. [6] Therefore, several gap management treatment modalities have been introduced to prevent bone resorption and its negative effects on the soft tissues surrounding the implant. [7] These procedures include the application of bone grafting materials, different membranes, customized healing abutments, soft tissue grafting, periodontal surgery to achieve primary closure, or a combination of the aforementioned techniques. [8-13] Among them, particulate bone grafting materials have been recommended for gap management after immediate implantation. [7, 14] However, the additional cost and the potential risk for immunological reactions have been reported as disadvantages. [15]

Recently, a systematic review reported a significantly higher risk of wound healing complications in immediate implant placement compared to delayed protocols due to difficulties in achieving tension-free primary wound closure when immediate implant placement was associated with bone grafting materials. [16] Furthermore, xenografts and synthetic grafting materials present a slow degradation and turnover rate, that may affect the bone quality and composition at the implant interface with the oral cavity. [15] To the date, the optimal protocol for management of horizontal gaps greater than 2 mm has been controversial, raising concerns about the best technique to promote bone formation, preserve hard and soft tissues architecture and avoid wound healing complications in immediately placed implants. [17]

Over the years, research has shifted its focus on the exploration and development of biologics, to facilitate osseointegration and allowing for shorter turnaround time for restoring patient aesthetics and function. An increasing interest in human derived biologic compounds has emerged in implant research due to their autologous nature and ability to improve surgical outcomes. [18-21] Leukocyte-Platelet-Rich Fibrin (L-PRF) is a second-generation platelet concentrate that contains 1) platelets and their activated growth factors, 2) leukocytes and their cytokines, as well as 3) a densely polymerized fibrin matrix that has the capacity to hold cells and growth factors in place and prevent soft tissue ingrowth into the wound.[22-26] L-PRF components have been suggested to facilitate cell migration/proliferation during healing, [27] and to mediate the release of various growth factors, particularly Transforming Growth Factors (TGFβ1) and Vascular Endothelial Growth Factor (VEGF), that along with the Platelet-Derived Growth Factor AB (PDGF-AB) and different matrix proteins, play an important role in the healing process. [28]

When applied in different clinical scenarios, L-PRF has demonstrated favorable results in periodontal surgery, socket preservation following tooth extraction, and regeneration of soft and hard tissue in maxillofacial and implant procedures. [20, 21, 29-35] Along with the proven effect on accelerating wound healing, advantages of L-PRF include low cost, simple harvesting, slow release of growth factors, culminating in an excellent cost-benefit ratio. [36] While it is well known that platelet α- granules contain different growth factors such as PDGFs, TGF-β, VEGF, and epidermal growth factor (EGF), the biological properties of concentrates and their release kinetics have not been completely understood. [37] This is partly due to inconsistent centrifugation protocols and centrifuge characteristics (*i.e.*, speeds, rotor angles, tubes, etc.) among studies. [38]

Although the use of L-PRF for gap management in immediate implant placement has been previously reported in clinical studies and case reports, [39-41] the paucity of hierarchically designed investigations limits the objective evaluation of bone formation due to the inherent limitations of clinical research. Therefore, the present study aimed to evaluate the effect of L-PRF placed in regular or wide sized osteotomies prior to implant placement, on osseointegration parameters evaluated at different time points (3-, 6-, and 12-weeks) in a canine model *in vivo*. The effect of L-PRF on osseointegration parameters of dental implants placed after regular osteotomy and in surgically created bone defects (~2 mm gap) that mimics post extraction sockets was evaluated through histomorphometric analysis. The postulated

hypothesis was that the association of L-PRF to immediate implant placement would yield higher values of bone-to-implant contact and bone area fraction occupancy in regular and wide osteotomy sites.

Methods

Eighty dental implants (Intra-Lock Osseon implants, Intra-Lock, Boca Raton, FL ; 4mm diameter and 8mm length) were divided to be placed *in vivo* in a canine model comprising four experimental groups: 1) Regular osteotomy and implant placement, used as a control group (Reg n/L-PRF); 2) Regular osteotomy and implant placement with single L-PRF membrane placed in the osteotomy site (Reg L-PRF); 3) Wide osteotomy with no gap management performed, where a bone defect of 6 mm of diameter and 5 mm deep was created to simulate immediate implant placement in post-extraction sockets, and the gap was left for spontaneous healing after implant placement (Wide nL-PRF); and 4) Wide osteotomy with L-PRF gap management, where an L-PRF membrane was placed in the osteotomy site prior to implant placement (Wide L-PRF).

The *in vivo* study comprised of thirteen adult female beagle dogs, approximately 1.5 years of age. The experimental protocol received the approval of the École Nationale Vétérinaire d'Alfort (Maisons-Alfort, Val-de-Marne, France). The beagles were acquired and remained in the facility for approximately one-week for acclimation prior to any surgical intervention. All surgical procedures were performed under general anesthesia. Intramuscular (IM) atropine sulfate (0.044 mg/kg) and xylazine chlorate (8 mg/kg) were administered for pre-anesthesia. General anesthesia was then obtained following an IM injection of ketamine chlorate (15 mg/kg). Following skin preparation, a 5 cm incision was made, and the deeper tissues were dissected to expose the diaphysis of the radius.

For the surgical models, 6 dogs had implants placed in the radius bone for 3 weeks; and 7 dogs had implants placed in the left radius for 6 weeks and in the right radius for 12 weeks (Figure 1). Each radius received one implant of each experimental group in an interpolated fashion to avoid site bias (Figure 2). Regular osteotomy sites were prepared with conventional drilling sequence with burs up to 3.8 mm of diameter. Whereas the wide bone defects were created using 6 mm diameter trephines providing a 2 mm gap and 3 mm of apical anchorage, to simulate immediate implant placement in post-extraction sockets. All osteotomies were created/prepared under constant irrigation.

L-PRF was prepared from each individual animal, by drawing venous blood into two 9 ml Red Top Blood Collection tubes (Intra-Lock, Boca Raton, FL) and then subjected to a centrifuge (CE/FDA cleared, IntraSpin, Intra-Lock, Boca Raton, FL) at 2700 rpm (408 RCF-clot) for 12 minutes. Subsequently, the XPression fabrication kit (Intra-Lock, Boca Raton, FL) was used to obtain L-PRF membranes. The osteotomy sites corresponding to L-PRF groups were filled with L-PRF membranes and the implants were then placed to the cortical plate level.

Closure was achieved with standard layered suturing techniques with VICRYL 4-0 (Ethicon Johnson, Miami, FL) for deep tissues and nylon 4-0 (Ethicon Johnson, Miami, FL) for skin. The dogs remained in the animal care facility and received antibiotics (benzyl penicillin benzathine 20.00 IU/kg) and anti-inflammatory medication (ketoprofen 1%, 1 mL/5 kg) for pain control. Euthanasia was carried out under general anesthesia (15 mg/kg of ketamine chlorate) by intravenous administration of potassium chloride (0.5 mEq/kg) at the correspondent time point evaluation. At necropsy, all radii were retrieved by blunt dissection.[42]

Histologic Preparation

Upon dissection, bone samples were initially preserved in a solution of 70% ethanol and subsequently cut into individual blocks. Bone blocks containing implants were gradually dehydrated in a series of ethanol solutions, 70-100%. Following dehydration, the samples were embedded in a methacrylate-based resin (Technovit 9100, Heraeus Kulzer GmbH, Wehrheim, Germany) according to the manufacturer's instructions. The blocks were then cut into slices (~300 μm thick) centering the implant along its long axis with a precision diamond saw (Isomet 2000, Buehler Ltd., Lake Bluff, IL) and glued to acrylic plates with an acrylate-based cement (Technovit 7210 VLC, Heraeus Kulzer GmbH, Wehrheim, Germany). After a 24-hour setting time, grinding, and polishing were performed under water irrigation with series of silicon carbide (SiC) abrasive papers (400, 600, 800, and 1200; Buehler Ltd., Lake Bluff, IL) to a final thickness of ~100 μm. The sections were then stained with Stevenel's blue and Van Gieson's picrofuchsin and samples were scanned through an automated slide scanning system and specialized computer software (Aperio Technologies, Vista, CA, USA). Qualitative and quantitative histological analyses were performed at 50x–200x magnification using image analysis software (ImageJ, NIH,

Bethesda, MD). Bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO) evaluations were performed by a single, blinded user to evaluate the osseointegration parameters around the implant's surface and within the thread area, respectively.

Statistical Analysis

All data was assessed for normality using the Shapiro-Wilk. Statistical Analysis was conducted utilizing a general linear mixed model (GLMM) ANOVA (at a 95% confidence interval, α=0.05). The independent values considered (individually or in combination) were the size of osteotomy site and presence of L-PRF. The dependent variables evaluated were BIC and BAFO (IBM SPSS v25, IBM Corp., Armonk, NY, USA).

Results

During the surgical procedures primary stability was achieved in all the implants. At follow-ups after respective healing times, no complications, signs of infection, or other clinical concerns were observed.

Qualitative analysis of histological micrographs after 3- (Figure 3), 6- (Figure 4), and 12- (Figure 5) week revealed a significant increase in quality and maturation of bone with respect to time, with an increase in osteocyte and lacunae formation in samples evaluated at 6 and to 12 weeks in comparison to those at 3 weeks evaluation. This trend was observed independent of osteotomy size and the presence or absence of L-PRF membranes. When evaluating implants placed in regular osteotomy sites with and without L-PRF, analogous histological features were observed for Reg L-PRF and Reg nL-PRF. However, implants placed in wide osteotomy sites without L-PRF gap management yielded increased levels of soft tissue infiltration at the cervical portion of the implants, and more soft tissue islands within the newly forming bone than implants associated with L-PRF. This was evident during the analysis at three weeks in the Wide L-PRF and Wide nL-PRF groups, where gap management with L-PRF membranes evidenced effective filling of the gap between the implant surface and the defect walls, with improved bone growth in close proximity to the implant surface.

The BIC results as a function of time *in vivo* and presence of L-PRF presented no statistical difference (p>0.05) between implants placed at regular osteotomy sites with and without L-PRF at 3, 6 and 12 weeks (Figure 6a). For implants placed in wide osteotomy sites, higher values of BIC (p=0.03) were

observed after 3 weeks when gap management was performed with L-PRF membranes in comparison with spontaneous healing. No significant differences were observed for Wide L-PRF and nL-PRF groups at 6 and 12 weeks.

BAFO results as a function of time *in vivo* and presence of L-PRF yielded no statistical difference ($p > 0.05$) between implants placed at regular osteotomy sites with and without L-PRF at 3, 6, and 12 weeks (Figure 6b). Similar to BIC analysis, a statistical difference ($p=0.002$) was observed for BAFO at the 3 weeks between Wide L-PRF and Wide nL-PRF groups. Furthermore, statistically significant differences in BAFO were not observed between implants placed in wide osteotomy sites with and without L-PRF at 6 and 12 weeks ($p>0.05$).

Discussion

The present study evaluated through histomorphometric analyses the effect of L-PRF in the gap management of implants placed in wide bone defects, as well as the potential effect of L-PRF to reduce osseointegration times in regular implant placement. The results demonstrated that gap management with L-PRF is efficient in promoting faster bone formation to bridge the gap between the implant surface and the defect walls in wide osteotomy sites. However, no additional benefits were observed in the association of L-PRF and implant placement in regular osteotomy sites. Therefore, the postulated hypothesis of the present study was partially accepted.

Immediate implant placement after tooth extraction offers several advantages when compared to delayed protocols.[4] However, a recently published systematic review reported a higher risk of early failures (~3.5%) and complications (~21.1%) for immediate implant placement when compared to implants placed into a healed socket. [16] Such differences have been associated with difficulties in achieving primary stability. [3, 16] During implantation in fresh extraction sockets, a reduced initial bone-to-implant-contact along with the presence of gaps are expected due to the size difference between the implanted device and the tooth diameter.[43] While small gaps have been found to fill spontaneously, grafting procedures have been suggested for the management of larger gaps (2 mm or larger).[5] Although several materials and techniques have been reported in the literature, the optimal protocol for the management of large horizontal gaps remains controversial. [17]

Particulate bone grafting materials have been commonly used in gap management to stimulate bone formation and maintain the architecture of the alveolar ridge after immediate implantation.[7] The use of bone grafting materials has been also combined with several approaches, such as soft tissue grafting,[8, 9] customized healing abutments,[10, 11] periodontal surgery,[12, 13] and the use of L-PRF membranes[40], aiming to synergistically enhance healing and facilitate the achievement of predictable outcomes in implant therapy. However, xenografts and synthetic grafting materials present a slow degradation and turnover rate, that may affect the quality and composition of the newly formed bone at the interface with the implant surface and the oral cavity. [15] Furthermore, the use of grafting materials has been associated with increased risk of complications and additional economic costs to the treatment. [3, 16] Therefore, autogenous and fully resorbable materials, that promote predictable bone formation and do not require the creation of additional surgical sites are highly desirable.

In the present study, L-PRF showed promise of increasing the evaluated osseointegration parameters at the earlier time point evaluation which may eventually shorten the time to achieve secondary stability for implants placed in wide osteotomy sites. The higher values of BIC and BAFO at 3 weeks when L-PRF was utilized for gap management corroborated the qualitative observations presented in the histological analyses, where higher woven bone formation was observed around the implant surface and within the implant's threads in the Wide L-PRF group. Gap management with L-PRF not only produced a significant increment in BIC (from 20 to 40%) and BAFO (from 30 to 55%) at 3 weeks, but also reduced the soft tissue infiltration in the cervical portion of the implant, as reported in the qualitative analyses of the histological micrographs. These findings are in agreement with a previous study performed in a canine model that reported increased values of BAFO when L-PRF was associated to immediate implant placement in fresh extraction sockets. [44] The substantial increase in BIC and BAFO may be accounted by the improved osteogenic cell migration through the L-PRF fibrin matrix present between implant and the socket walls. Furthermore, the continued release of growth factors associated with L-PRF components, along with its densely polymerized structure, may have had a crucial role in hastening bone formation around the implant.[22, 23]

The use of L-PRF for gap management in immediate implant placement has been presented in several case reports [45-49] and clinical studies[39-41, 50-52], frequently in association with bone grafting materials. [53] However, only a handful of pre-clinical studies evaluating histologically the effect of L-PRF

in this particular scenario have been reported in previous literature. [44, 54, 55] Considering the number of variables surrounding immediate implant therapy (implants macro/micro designs, adjunctive biomaterials, surgical procedures, and the combination of different clinical approaches), it is evident that further pre-clinical investigations are required not only to better elucidate the effects of L-PRF in tissue formation, but also to provide scientific support for its clinical application in different settings. Additionally, pre-clinical studies including different gap management protocols are warranted to further elucidate the optimal treatment to expedite bone formation around immediate dental implants. The pre-clinical model used in the present study allowed for the creation of defects to simulate post-extraction sockets for immediate placement of regular sized dental implants. The radius bone of the beagles provided a dense cortical bone that has been widely used in pre-clinical research[56-58] due to the similarities of cortical bone composition (ash weight, hydroxyproline, extractable proteins and IGF1 content) between dogs and humans. [59]

It the present study, L-PRF was also tested in regular osteotomy sites in an attempt to promote faster bone apposition to the implant surface. The rationale behind this application relied in the interplay between implant macrogeometry and the surgical instrumentation for implant placement. It has been reported that screw-type implants with large thread pitch and outer to inner thread diameter differences may interplay with the osteotomy dimensions to form healing chambers between threads, implant inner diameter, and bone instrumented walls.[60, 61] After implant placement, the healing chambers are expected to be filled with a blood clot that may evolve toward an osteogenic connective tissue configuration that will ossify through an intramembranous-like pathway.[62] By including the L-PRF membrane within the osteotomy site prior to implant placement, it was expected that the healing chamber would be filled with the L-PRF components and facilitate osteogenic cell migration and bone formation in intimate contact with the implant surface. However, no significant differences in the qualitative and quantitative analyses were observed at 3-, 6-, or 12-weeks, regardless of the presence or absence of L-PRF.

Previous clinical and pre-clinical studies have reported the use of different platelet concentrates to enhance implant stability and accelerate osseointegration in regular osteotomy sites. Despite the differences in protocols and composition, the majority of the results reported suggest faster bone formation and increased implant stability with the use of platelet/grow factors concentrates.[63-67] Among them, L-

PRF present additional desirable features for clinical application, such as low cost, simple harvesting, fast processing, and remarkable cost-benefit ratio. [68]

Although L-PRF have been extensively used in dentistry since first described by Choukroun and Dohan,[25] the exact biological mechanism of platelets derivates remains unclear in bone regeneration. Furthermore, it has been reported that different protocols and equipment may affect dramatically the biological signature and bone regeneration potential of platelet concentrates.[69, 70] Such variability along with the high heterogeneity of clinical studies and the limitations of *in vitro* to *in vivo* extrapolations, warrants further well-designed pre-clinical and clinical investigations to further assess the biological effect of L-PRF to promote bone regeneration and enhance the outcomes of implant therapy.

Conclusion

L-PRF placed within wide osteotomies, prior to implant placement, resulted in increased early bone formation compared to unfilled wide osteotomies at early implantation times (3 weeks in vivo) relative to empty controls. Over extended periods of time (6 and 12 weeks in vivo), the effect of L-PRF placed within osteotomies was not significant.

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Conflict of Interest

All listed authors have viewed the manuscript and given their permis- sion for submission.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures and Figure legends:

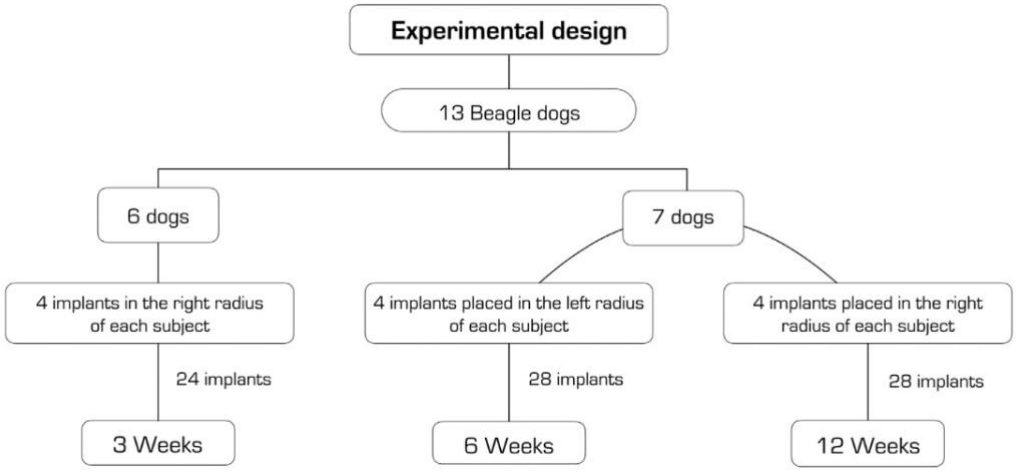

Figure 1: Experimental study design of the thirteen beagle dogs - six dogs were randomly assigned into the three weeks evaluation time point where each subject received 4 implants (one of each experimental group) in the right radius yielding a total of 24 implants across 6 dogs. The remaining seven dogs received 4 implants in the right radius and 6 weeks later, a follow up surgery was performed on the left radius adding an additional 4 implants (a total of 28 implants per time point; 12- and 6-weeks) and were evaluated after 12 weeks post first surgical intervention (right radius – 12 weeks and left radius – 6 weeks).

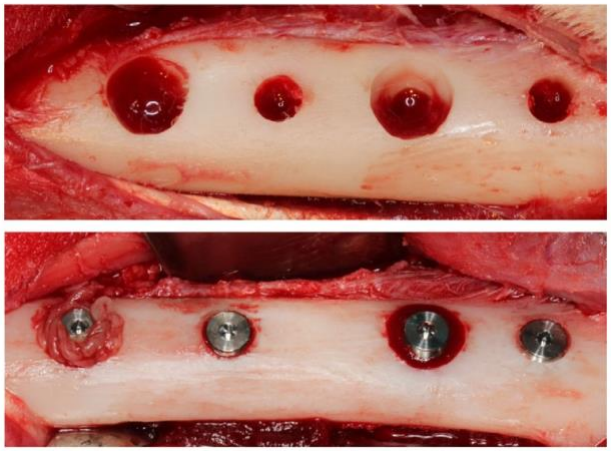

Figure 2: Surgical image of the beagle's radius to demonstrate (A) Osteotomy sites distributed in an interpolated fashion to avoid site bias, and (B) Implants with respective L-PRF membranes according to the group distribution.

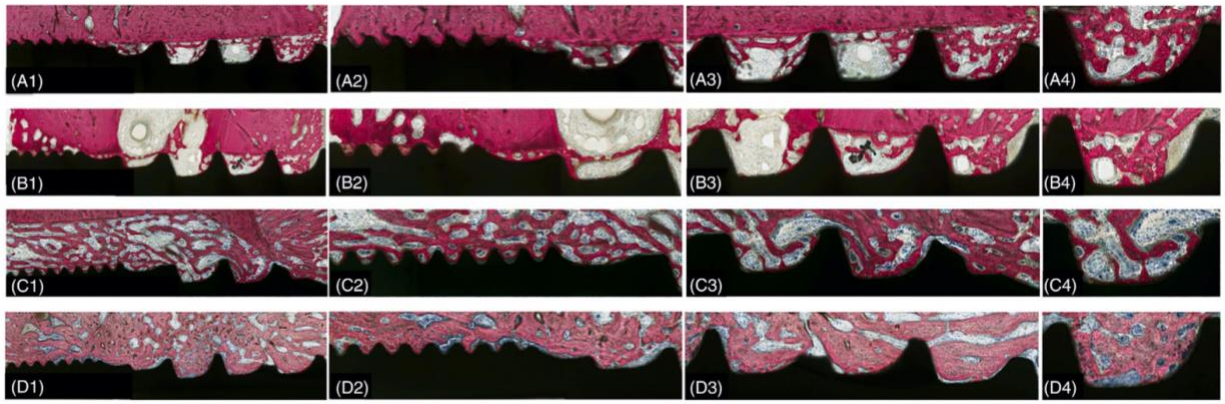

Figure 3: Histological micrographs of representative samples from the evaluated groups at 3 weeks. a) Reg n/L-PRF, b) Reg L-PRF, c) Wide nL-PRF and d) Wide L-PRF. Numbers represent 1) Overview, 2) Cervical, 3) Middle/apical threads, and 4) High magnification of a healing chamber.

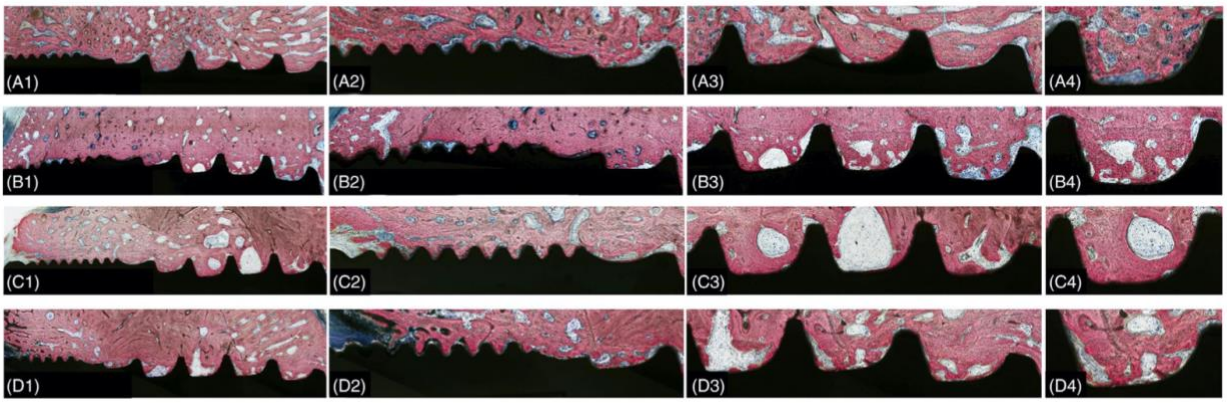

Figure 4: Histological micrographs of representative samples from the evaluated groups at 6 weeks. a) Reg n/L-PRF, b) Reg L-PRF, c) Wide nL-PRF and d) Wide L-PRF. Numbers represent 1) Overview, 2) Cervical and 3) Middle/apical threads, and 4) High magnification of a healing chamber.

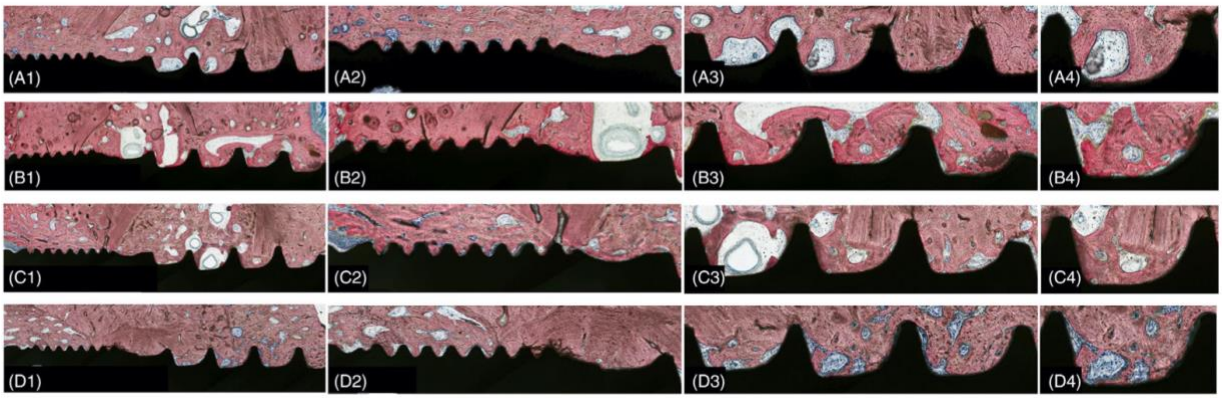

Figure 5: Histological micrographs of representative samples from the evaluated groups at 12 weeks. a) Reg n/L-PRF, b) Reg L-PRF, c) Wide nL-PRF and d) Wide L-PRF. Numbers represent 1) Overview, 2) Cervical and 3) Middle/apical threads, and 4) High magnification of a healing chamber.

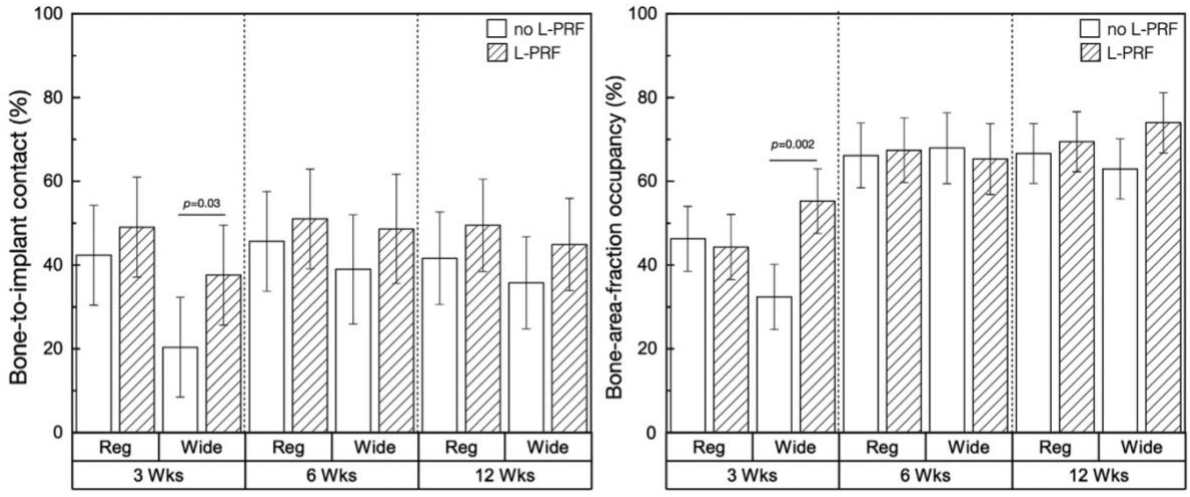

Figure 6: Statistical summary of (A) BIC and (B) BAFO results in function of osteotomy size, presence, or absence of L-PRF and experimental time-points.