Influence of Interimplant Distance on Bone Microstructure: A Histomorphometric Study in Dogs

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ABSTRACT

The microstructure of the crestal alveolar bone is important for both the maintenance of osseointegration and the location of the gingival soft tissues. The aim of this study was to evaluate and compare the bone microstructure of the alveolar bone and of the interimplant bone in implants inserted at different interimplant distances. The mandibular bilateral premolars of six dogs were extracted, and after 12 weeks, each dog received eight implants, for a total of 48 implants. Two pairs of implants, one for each hemiarch, were separated by 2 mm (group 1) and by 3 mm (group 2). After 12 weeks, the implants received temporary acrylic prostheses. After four more weeks, metallic crowns substituted the temporary prostheses. After an additional 8 weeks the animals were sacrificed and the hemimandibles were removed, dissected, and processed. The longitudinal collagen fiber orientation was 43.2% for the alveolar bone; it was 30.3% for the 2-mm group and 43.9% for the 3-mm group. There was a statistically significant difference between the 2-mm and 3-mm groups ($p < .05$). The orientation of transverse collagen fibers was 47.8% for the alveolar bone; it was 37.3% for the 2-mm group and 56.3% for the 3-mm group. There was a statistically significant difference between the 2-mm and 3-mm groups ($p < .05$). The marrow spaces were 34.87% for the alveolar bone, 52.3% for the 2-mm group, and 59.9% for the 3-mm group. There was a statistically significant difference between the alveolar bone and the 3-mm group ($p < .05$). The low mineral density index was 36.29 for the alveolar bone, 46.76 for the 2-mm group, and 17.91 for the 3-mm group. There was a statistically significant difference between the 2-mm and 3-mm groups ($p < .05$). The high mineral density was 87.57 for the alveolar bone, 72.58 for the 2-mm group, and 84.91 for the 3-mm group. There was a statistically significant difference between the alveolar bone and the 2-mm group ($p < .05$). The collagen fiber orientation resulted in statistically significant differences in both the 2-mm and 3-mm groups compared with the alveolar bone. The marrow spaces appeared significantly increased in the 3-mm group compared with the alveolar bone. The low mineral density index was significantly higher in the 2-mm group, while the high mineral density index was significantly higher in the alveolar bone. In conclusion, the interimplant distance should not be less than 3 mm.

KEY WORDS: bone, bone formation, bone structure, dental implants, implant esthetics, interimplant distance

INTRODUCTION

The success of oral rehabilitation with implants depends on the integration of the components with soft and mineralized tissues. Soft-tissue stability around dental implants is crucial for the predictable and routine restoration of single-tooth and partially edentulous patients. In turn, the soft tissues are supported by the underlying alveolar crest. An early crestal bone loss of approximately 1.5 mm is frequently observed during the first year after the implant loading, followed by a bone loss of 0.2 mm in the following years. Crestal bone loss

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produces changes in the soft-tissue arrangement and vice versa. Possible etiological factors associated with this initial bone loss are surgical trauma, overload, peri-implantitis, presence of microgap, implant design, and the formation of biological distances.[1] The biological width around the teeth was reported by Gargiulo and colleagues,[2] who studied the dimensions of the physiological attachment apparatus. Likewise, a biological width has also been reported around implants, after the implant placement.[3] The composition of the gingiva and mucosa around dental implants was compared by Berglundh and colleagues,[4] who found no differences between clinically healthy soft tissues surrounding both the teeth and the implants. Both were covered by a keratinized epithelium continuous to the junctional epithelium and had a 2-mm extension. The epithelium was separated from the alveolar bone by a connective-tissue area larger than 1 mm. Characteristics similar to the implant/mucosa union (ie, junctional epithelium and connective tissue) were reported in studies that used one- and two-stage implants.[5] Cochran and colleagues[6] evaluated the biological width around implants, comparing unloaded and loaded nonsubmerged implants, and concluded that the dimensions and relations of the mucosa/implant junction are similar to those found in dento-gingival tissues.

A possible explanation for the bone loss around implants is that after implant exposure and abutment placement, an implant/abutment interface is established and bone resorption of 1.5 to 2.0 mm may occur in an apical direction.[7] The biological reason for this phenomenon is that if a chronic irritant, such as bacteria, reaches the implant/abutment interface, or if the abutment is removed after initial healing, bone resorption will occur, creating a distance from the irritated area. Tarnow and colleagues[8] previously reported a similar bone response in subgingival prosthetic crown preparations that violate the attachment apparatus on natural teeth.

In a study of interdental sites, if the distance was 5 mm or less, the interproximal papilla was present in 98% of the cases.[9] Although other variables, such as degree of inflammation, probing depth, fibrous or edematous tissue, position of teeth (anterior or posterior), surgical or nonsurgical history, and proximal restorations, can also contribute to the presence or absence of papilla, Tarnow and colleagues[9] evaluated 288 sites and concluded that the distance between the base of the interproximal contact point and the bone crest is a determining factor for the presence of the gingival papilla. Similar to the importance of the contact point between teeth, Garber and colleagues[10] reported that when the implant is in position, the surrounding tissues of the restorative gingival interface needs to be maintained. According to the authors just mentioned, the esthetically crucial dimension of soft tissues, which extends from the free gingival margin coronally to the osseous crest apically, for a distance of approximately 3 mm, has to be supported entirely by the tooth or the implant-supported restoration, emphasizing that this tissue is not supported by the osseous structure. It was also verified that this tissue requires gentle lateral pressure to support the height of the papilla. In another study, radiographs from 36 patients with two adjacent implants were evaluated.[11] The lateral bone loss was measured from the alveolar crest of the bone to the implant surface, and the data were divided into two groups based on the distance between the implants.

The results showed that the crestal bone loss for implants with a 3-mm or larger distance was smaller than that for implants with a distance of less than 3 mm. These data show an influence not only of vertical components but also of lateral components on the bone loss around dental implants. The clinical significance of this finding is that the increase in the crestal bone loss results in an increase in the distance between the base of the interproximal contact of the crowns and the bone crest and that this determines if papilla will be present or absent between implants. The authors suggested the use of implants with smaller diameters to improve aesthetics in multiple implants, especially in the anterior region.[11] Novaes and colleagues[12] analyzed, clinically and radiographically, in dogs, the amount of crestal resorption and absence/presence of the papilla between contiguous implants with 2- or 3-mm interimplant distances and a contact point to bone crest distance of 5 mm, and Papalexiou and colleagues[13] evaluated the same parameters histomorphometrically. It was concluded that there was not a complete fill of the interimplant spaces by the papilla in any of the groups and that both groups showed similar papilla formation and crestal bone resorption. Scarano and colleagues,[14] in a histologic study in dogs, measured crestal resorption in relation to interimplant distances of 2 to 5 mm. The histomorphometry revealed statistically significant differences for the 4-mm and 5-mm groups but not for the 2-mm and

3 mm groups. Scarano and colleagues[14] concluded that the crestal resorption increases the distance from the contact point to the crestal bone, and that this can determine that absence or presence of the papilla between adjacent implants. The bone microstructure, mainly in the interimplant area could be a key factor for the long-lasting maintenance of osseointegration and stability of the biological width between adjacent implants. The bone is a two-phase porous composite material composed primarily of collagen and mineral, which together provide its mechanical properties.[15] The presence of collagen in the bone matrix is crucial in the determination of the quantity of energy required to produce matrix failure.[16] Skedros and colleagues[17] reported that collagen fiber orientation reflects the load suffered by the bone. The peri-implant bone adjusts its architecture in relation to its functional load bearing. Wang and colleagues,[16,18] studied the effect of collagen integrity on the mechanical properties of bone. They concluded that collagen plays a substantial role in the toughness of bone; that mineral content plays a substantial role in bone strength and stiffness; and that collagen reorganization accounted for the maintenance of mechanical properties of bone. In previous studies, we focused on the relationship between collagen fiber orientation and loading of osseointegrated dental implants.[19–22] Even if Novaes and colleagues and Papalexiou and colleagues[12,13] did not find differences regarding papilla formation or crestal bone resorption, differences in the bone microstructure of the interimplant bone could exist. The orientation of collagen fibers in the bone could provide information about the relationship between implant design, interimplant distance, distribution of stress applied to the bone, and bone formation and resorption. Furthermore, collagen fibers have been implicated in the initiation of matrix calcification with many other matrix proteins, including chondrocalcin, proteoglycans, osteonectin, and osteocalcin.[23] The elasticity of bone, and thus its resistance to fracture, is related to the collagen fiber orientation, to the degree of mineralization, and finally to the water content. Because an increase in stiffness and brittleness of bone tissue follows the replacement of its water content by mineral, the relationship between the mineral content and the strength of bone has been studied extensively.[24,25] Increasing mineralization density increases the ability of bone to absorb impact energy, although this relationship is not linear, and, in fact, a very high mineralized bone becomes liable to fracture, because microfractures can more readily propagate through it.[26] The optimal mineralization density value for bone strength has not yet been determined.[27] Collagen fiber orientation, mineral density, and extension of the marrow spaces are all factors able to influence the bone adaptation to loading.

The purpose of this study was to evaluate and compare the effect that a 2- or 3-mm interimplant distance may have on the collagen fiber distribution within the bone, on the proportion of marrow spaces to mineralized tissues, and on the mineral density of the interimplant bone.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Animal Care Committee of the University of São Paulo, Brazil. Six young-adult male mongrel dogs weighing approximately 10 kg were used in this study. They had intact maxillae and mandibles and no general occlusal trauma. The animals were in good general health and had no viral or fungal oral lesions. The animals were kept fasting from the night before surgery. They received an intramuscular injection of a preanesthetic (2% Rompun, 20 mg/kg, 0.5 mL/10 kg) and were then anesthetized intravenously with thiopental (1 mL/kg; 20 mg/kg thiopental diluted in 50 mL saline). A total flap was raised in the region of the four mandibular premolars, and the teeth were sectioned in the buccal-lingual direction and extracted with forceps. The flaps were repositioned and sutured with absorbable 4-0 sutures. After a healing period of 3 months, the animals received 20,000 IU penicillin and streptomycin (1.0 g/10 kg) the night before surgery. This dose provided antibiotic coverage for 4 days, and thus another dose was given 4 days later to provide coverage for a total of 8 days. This large-spectrum antibiotic is commonly used to treat infections in small animals.[28] After the same sedation and anesthesia as in phase 1 were repeated, a horizontal crestal incision was made in keratinized gingiva from the distal region of the canine to the mesial region of the first molar, at the top of the alveolar crest, and implants were placed according to manufacturer instructions. Four 4.5 × 10 mm Frialit (Dentsply® Friadent, Mannheim, Germany) implants, with a blasted and acid-etched surface, were placed on each side of the mandible of each animal, for a total of 48 implants. The implants were placed, as reported previously,[12,13] so that two adjacent implants were 2 or 3 mm distant from each

other. For this, a stainless steel device was made to standardize both the angle and the distance between implants. Contralaterally, the distances between implants were repeated but with variation in the position of the implants, following the random cross-location method. The flaps were repositioned and sutured with absorbable sutures, so that the implants were totally submerged. Sutures were removed after 10 days. During the healing period, the animals received monthly hygiene prophylaxis with ultrasonic scalers. After 12 weeks, prosthetic restoration was started. The same method of sedation and anesthesia previously described was followed; the implants were exposed and loaded with provisional acrylic prostheses. These temporary restorations were made so that the distance between the contact point and the bone crest was 5 mm. After 4 weeks, metallic crowns substituted the provisional prostheses, maintaining the distance of 5 mm from the contact point to the bone crest. Hygiene prophylaxis continued on a weekly basis.

Eight weeks after the prosthetic restoration, the animals were sedated and then sacrificed with an overdose of thiopental. The hemimandibles were removed, dissected, and fixed in 4% formalin, pH 7.0, for 10 days, and then transferred to a solution of 70% ethanol until processing. The specimens were dehydrated in increasing concentrations of alcohol up to 100%, infiltrated and embedded in LR White (London Resin Company, Berkshire, UK) resin, and sectioned using the technique described by Donath and Breuner.[29] After the preparation of the histological slides, the specimens were evaluated for the following.

Birefringence Analysis for Collagen Fiber Orientation

The specimens were analyzed for collagen fiber orientation in an Axiolab light microscope (Zeiss Oberchen, Germany) equipped with two linear polarizers and two quarter-wave plates arranged to have a transmitted circularly polarized light. Collagen fibers that aligned transverse to the direction of the light propagation (parallel to the plane of the section) appeared "bright." The microscope was connected to a high-resolution digital camera FinePix S2 Pro (Fuji Photo Film Co. Ltd., Minato-Ku, Japan). The measurements were performed on digital images at ×100 after they were converted into 8-bit color. For each pixel in the grid, a value between 2 and 256 colors was assigned. A threshold of gray-value levels (170–250), related to the transverse collagen fibers areas, was made before quantitative analysis using a software Image J 1.32j (Wayne Rasband National Institute of Health, USA). To ensure accuracy, the software was calibrated for each experimental image. The analysis considered the areas occupied by transverse collagen fibers (measured in pixels) because they were related to the bone areas subjected more to compressive than to tensile stress as are longitudinal collagen fibers. The mean value of all measurements for each group was considered for statistical analysis.

Back Scattering Electrons (BSE) Analysis

The thin sections were polished with 0.5-μm alumina to an optical finish and lightly sputter-coated with gold in an Emitech K 550 (Emitech Ltd., Ashford, Kent, UK). The specimens were placed on the storage of a scanning electron microscopy (SEM) LEO 435 Vp (LEO Electron Microscopy Ltd., Cambridge, UK), equipped with tetra solid-state BSE detector. SEM operating conditions included 30-kV accelerating voltage; 15-mm working distance; 1,2 nA probe current. The images (MxN 1,024 × 768 grid of pixels) were captured in the BSE mode with nine scans using a frame average technique. SEM operating conditions were stored in the computer's memory and restored prior to the capture of every image. A total of three images were taken for each implant section, at ×66 magnification, and these were then used for a map reconstruction using an image managing software (Adobe Photoshop CS version 8.0.1, Adobe Systems Inc., San Jose, CA, USA).

Calibration of the Gray Levels

The BSE signal (gray scale) was calibrated using the "atomic number (Z) contrast" of reference materials. Using this method, two different reference materials of known Z were imaged under defined conditions. The BSE images were calibrated using both carbon ($Z = 6$) (gray levels 25 ± 1) and aluminum ($Z = 13$) (gray levels 255 ± 1) as reported by Roschger and colleagues.[30] Under the same brightness and contrast conditions, the BSE gray level was calibrated for bone mineral density values as follows: the BSE gray levels ranging from 6 to 110 were assumed as standard for the bone with low mineral density, while the BSE gray levels ranging from 111 to 230 was used as standard for bone with high mineral density.

Analysis of the SEM Digital Images

All images were calibrated using the software Image J 1.32j applying the Pythagorean Theorem for distance

calibration, which reports the number of pixels between two selected points: the scale bar was overimposed on each image by SEM. The linear remapping of the pixel values was used to calibrate the intensity of the images. For each field measured, the following were recorded:

- the total area (in pixels) in the field that might have contained bone;
- the area (in pixels) in the gray-level range defined by low (50–125) or high (137–254) mineral density;
- the mean of the gray values of the area defined by low (94.7) or high (178.4) mineral density.

Special Procedure

The mineralization index was evaluated for each image based on different gray levels forming the region of interest. Low mineral density index (LMDI) and high mineral density index (HMDI) were evaluated using the following equation:

$$_{L-H}MDI = \sum_{i=i}^{k} \frac{A_i \times \overline{X}GL}{A_t}$$

where A_i was the bone area of interest with gray levels comprised among i to k, XGL was the mean of the gray level range considered in the area of interest, while A_t was the total area of the image occupied by bone tissue without marrow spaces and osteocytes lacunae being considered.

Statistical Analysis

Statistical analysis was performed using a software package (Sigma Stat 3.0, SPSS Inc., Ekrath, Germany). All the data were checked using the Kruskall–Wallis one-way ANOVA on ranks to tests the null hypothesis (H0) between all groups and the Tukey test for multiple comparison pairwise to determine which groups were different. For all the tests performed, the confidence level alpha chosen was 5%. Because during the healing period, one implant in the 2-mm group and three implants in the 3-mm group were lost, the missing values were substituted by the mean of the other five in order not to diminish the sample number. Among the methods utilized for the analysis of lost data, the mean substitution method, which replaces a variable's mean values, computed from available cases to fill in the missing data by the remaining cases, was chosen.[31,32]

RESULTS

Collagen Fiber Orientation

The mean area of longitudinal collagen fiber orientation for the alveolar bone was 2,871,171 ± 24.979 pixels (mean ± SD) or 43.2%, while for the 2-mm group it was 1,903,225 ± 44.024 pixels (mean ± SD) or 30.3%, and for the 3-mm group it was 2,323,163 ± 69.324 pixels (mean ± SD) or 43.9%, of the total interimplant bone area (Figure 1, A1,B1,C1). A statistically significant difference was present between the 2-mm and the 3-mm groups ($p < .05$; Figure 2). The mean area of transverse collagen fiber orientation for the alveolar bone was 3,174,311 ± 10.975 pixels (mean ± SD) or 47.8%, while for the 2-mm group it was 2,339,249 ± 66.895 pixels (mean ± SD) or 37.3%, and for the 3-mm group it was 2,980,623 ± 72.188 pixels (mean ± SD) or 56.3% (Figure 1, A2,B2,C2). A statistically significant difference was found between the 2-mm and 3-mm groups ($p < .05$; Figure 3).

Marrow Spaces. The percentages of the marrow spaces measured for the alveolar bone was 120,740 ± 11.294 or 34.87%, while for the 2-mm group it was 221,144 ± 27.884 or 52.3%, and for the 3-mm group it was 360,505 ± 16.514 or 59.9% (Figure 4, A1,B1,C1). A significant difference was found between the alveolar bone and the 3-mm group ($p < .05$; Figure 5).

Mineral Density. The LMDI, calculated with the equation reported in the methodology section, was 36.29 for the alveolar bone, 46.76 for the 2-mm group, and 17.91 for the 3-mm group (Figure 4, A2,B2,C2). A statistically significant difference was found between the 2-mm and 3-mm groups ($p < .05$) (Figure 6). The HMDI, calculated with the equation reported in the methodology section, was 87.57 for the alveolar bone, 72.58 for the 2-mm group, and 84.91 for the 3-mm group (Figure 4, A3,B3,C3). A statistically significant difference was found between the alveolar bone and the 2-mm group ($p < .05$; Figure 6).

DISCUSSION

The crestal bone loss around dental implants following restoration has been a topic of discussion and has been used as a reference for evaluating implant success.[33] Changes in crestal bone height have been reported during both the healing time and the first year of

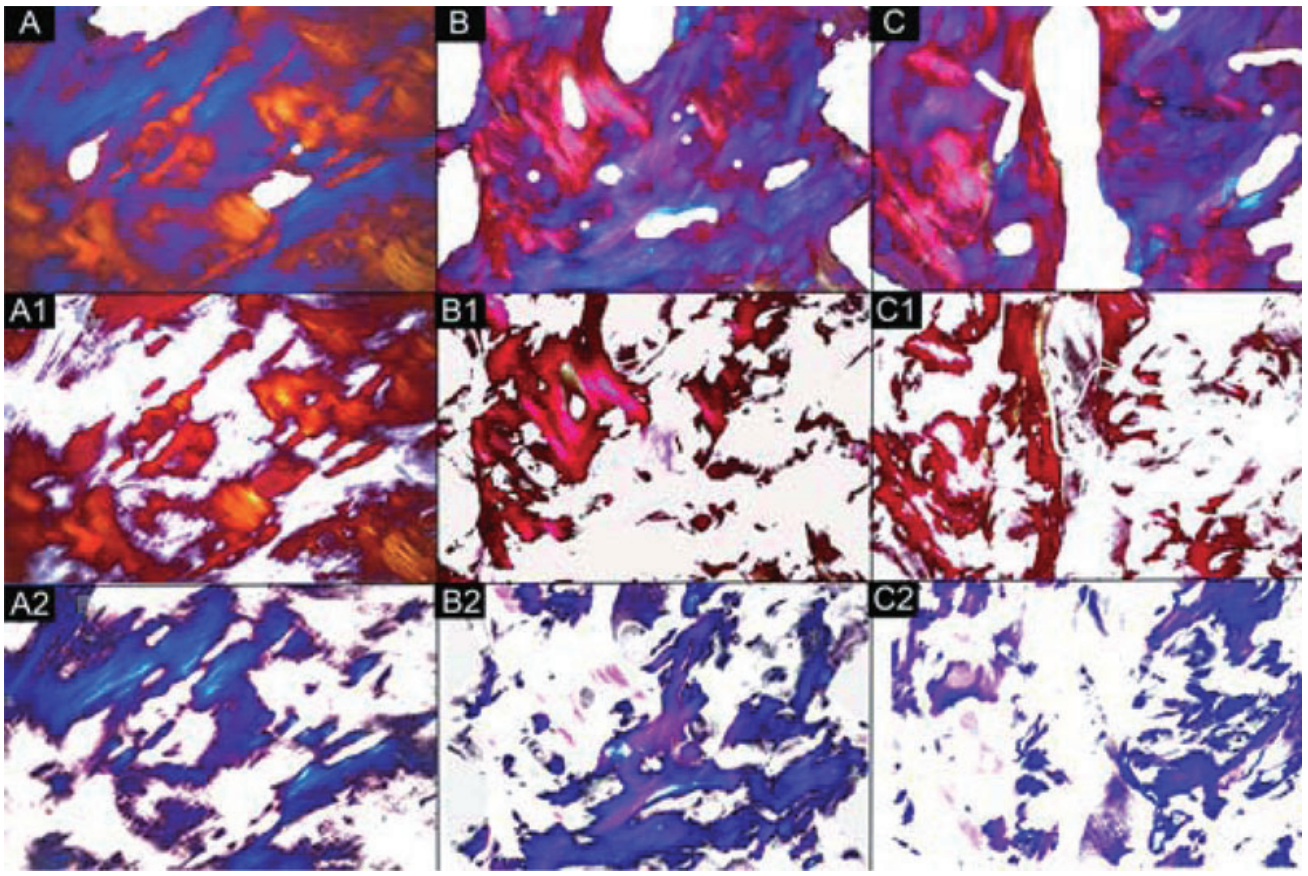

Figure 1 Representative histological images of collagen fibers orientation as appeared under circularly polarized light microscopy (×100 original magnification). *A* was for the alveolar bone group; *B* was for the 2-mm interimplant distance group, and finally *C* was for the 3-mm interimplant distance group. The images *A1*, *B1*, and *C1* show the amount of longitudinal collagen fiber orientation, while the images *A2*, *B2*, and *C2* show the amount of transverse collagen fibers orientation.

function without affecting the long-term implant success.[34] In case of two or more adjacent implants, however, the crestal bone resorption will affect the position of the soft-tissue margins, which in turn will have a significant impact on the aesthetic outcome of the implant therapy. Cardaropoli and colleagues[35] reported that a reduced horizontal interimplant distance produced an increased loss in the height of interproximal

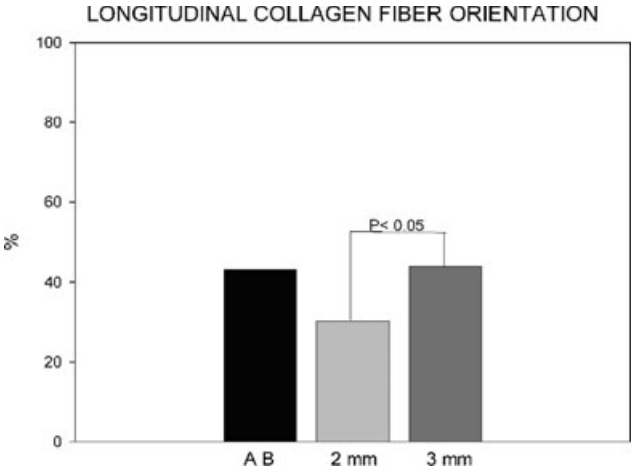

Figure 2 Graph showing the results for longitudinal collagen fiber orientation among the alveolar bone (AB), the 2-mm, and the 3-mm interimplant distance groups. Significance level of 5%.

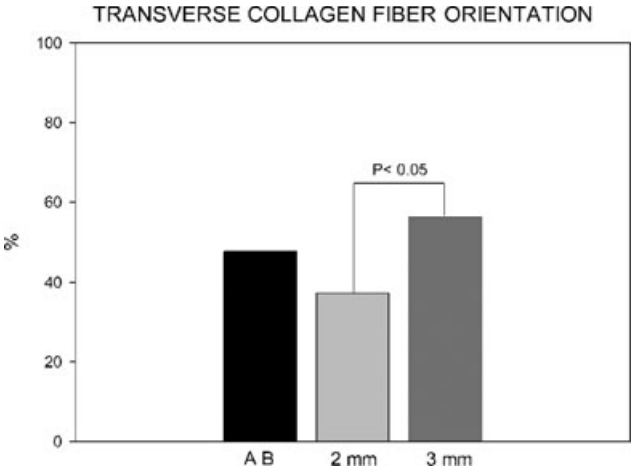

Figure 3 Graph showing the results for transverse collagen fiber orientation among the alveolar bone (AB), the 2-mm, and the 3-mm interimplant distance groups. Significance level of 5%.

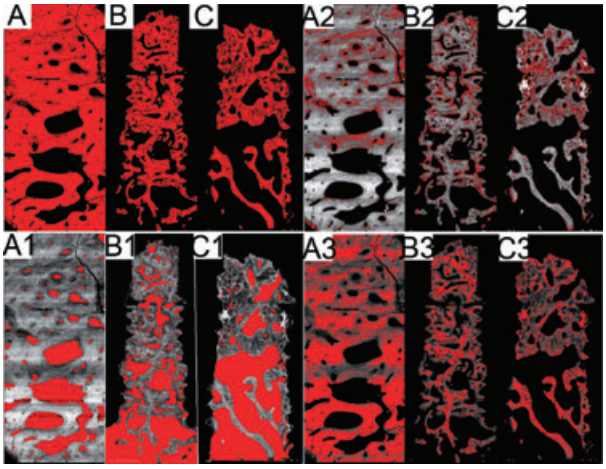

Figure 4 Map reconstruction of SEM images (×66 original magnification) showing the alveolar bone in *A*, the 2-mm group in *B*, and finally the 3-mm group in *C*. The extension of the medullary spaces was in red in images *A1*, *B1*, and *C1* for the respective groups. The low mineral density index areas are shown in red in *A2*, *B2*, and *C2*, while the high mineral density index areas are shown in red in *A3*, *B3*, and *C3*.

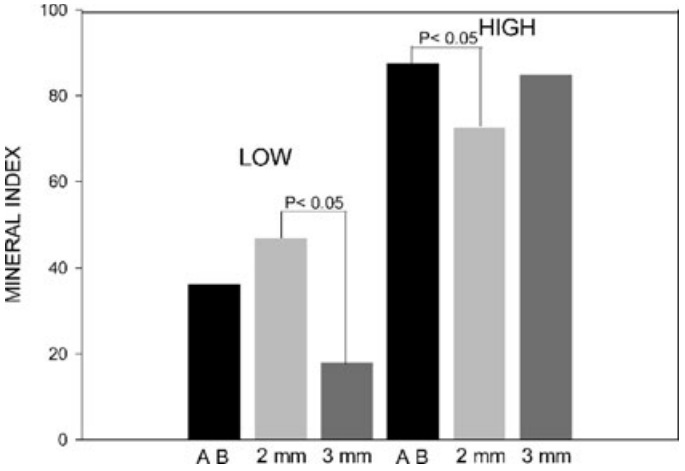

Figure 6 Graph showing the results for high and low mineral density index among the alveolar bone (AB), the 2-mm, and the 3-mm interimplant distance groups. Significance level of 5%.

bone crest. As reported in previous studies,[12,13] papilla formation or crestal bone resorption surrounding contiguous dental implants separated by 2 or 3 mm showed no significant differences. However, differences in the interimplant bone microstructure may be possible, and this was the objective of this study. Determination of the orientation of collagen fibers, the extension of the marrow spaces, and the mineral density indexes in the bone tissue around adjacent dental implants is indispensable when studying the relationship between interimplant distances and the bone microstructure. From the viewpoint of biomechanics, such a determination will be useful when estimating the mechanical stress acting on the interimplant bone tissue and the physiological response. The results of the present investigation on the collagen fibers orientation demonstrate the absence of a predominant orientation in the alveolar bone, and, in fact, longitudinal and transverse collagen fibers presented almost the same values (43.2% vs 43.9%). When the interimplant distances of 2 and 3 mm were analyzed and compared with the alveolar bone, a statistically significant difference only between the 2-mm and 3-mm groups for both longitudinal and transverse directions was noted. The longitudinally oriented collagen fibers in the 2 mm group were significantly less represented when compared to both 3 mm and AB groups. Transverse collagen fibers were a little more represented in the 3-mm group than in the alveolar bone group but significantly different ($p < .05$) when compared with the 2-mm group (37.3% vs 56.3%). In general, the 2-mm group showed a lower amount of collagen fiber organization.

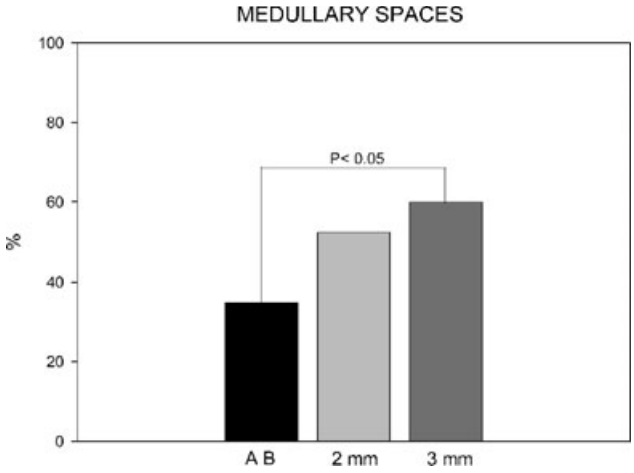

Figure 5 Graph showing the results for medullary spaces among the alveolar bone, the 2-mm, and the 3-mm interimplant distance groups. Significance level of 5%.

In a previous study in humans,[19] an increase of transverse collagen fibers near immediately loaded dental implants compared with the alveolar bone (32.96% vs 26.99%) was reported, while almost the same values were reported for the transverse and longitudinal collagen fibers in the alveolar bone (26.99% vs 22.25%). In another study in humans,[20] collagen fiber orientation in peri-implant bone of immediately loaded and unloaded dental implants was compared; an increase of the transverse collagen fibers under the lower

flank of the loaded implant threads and higher values for the longitudinal collagen fibers near the unloaded implants were observed. The present results confirm the trend reported previously for a progressive increase of transverse collagen fibers near immediately loaded dental implants, and also in the case of progressive bone loading like in the present study. In fact, after an unloaded healing phase of 3 months, acrylic restorations were applied for a month, and then metal restorations were placed. Moreover, the present results for the collagen fiber orientation in the alveolar bone group in dogs confirm our previous data in humans. In this study, the marrow spaces appeared significantly more expressed in the 3-mm group than in the alveolar bone, while in the 2-mm group the extension of marrow spaces was intermediate. An increase for marrow spaces should be considered positive because osteoblasts are thought to differentiate from bone marrow stromal stem cells[36]; moreover, bone vascularization plays an important role in bone remodeling, with a functional link between vessels, osteoblasts, and osteoclasts. Osteoblasts and osteoclasts are metabolically demanding cells with high levels of energy consumption and therefore require an adequate blood supply. In an animal study, Barou and colleagues[37] reported a relationship between bone formation and vascularization in the trabecular bone. Following this, the bone in the 3-mm group appear to be more able to adapt to the physiological needs induced by loading. The LMDI was significantly more represented in the 2-mm group than in the 3-mm group, while the alveolar group showed an intermediate value. The HMDI appeared significantly more expressed in the alveolar bone than in the 2-mm group, while the 3-mm group showed an intermediate value. It has become clear over the last several years that the osteocyte syncytium is the mechanosensory system of the bone tissue, and the lacunocanalicular porosity is the structure that mediates mechanosensing.[38] It has been shown that mechanical load induces fluid flow in the canalicular network.[39] This fluid flow has been suggested as a physical mediator of mechanosensing by osteocytes in vivo.[40] The osteocytes respond to mechanical stimuli with the production of signaling molecules which modulate the activities of osteoblasts and osteoclasts, thus converting mechanical stimuli into cellular signals.[41] It is our opinion that the insertion of titanium screw implants produces a structural change inside the hierarchical microstructure of the dog mandibular bone, which is mainly osteonal with a few marrow spaces, and this results in an increase of marrow space extension comparing to the alveolar bone. The presence of two loaded adjacent dental implants placed at a distance of 2 or 3 mm produces a difference in bone microstructure. A prevalence of bone with LMDI in the 2-mm group and an HMDI in the 3-mm group produces a change in stiffness, with a number of implications for how tissues perceive the load at the interface, and the type of functional response in bone density and assembly of trabeculae (architecture or connectivity). The variation of bone stiffness alters the mechanical signals perceived by the osteocytes in the adjacent 1-mm of peri-implant bone.[42] Osteocytes play a key sensory role; they are highly sensitive to the fluid shear forces as electrolytic tissue fluids pass through the canaliculi upon application of tissue stresses[43,44] or through direct connections to other cells (especially the cambium layer of the periosteum) and the extracellular matrix.[45,46] The majority of the microstructural variations described in the present study can be explained by Frost's Mechanostat Theory of mechanically induced bone adaptation.[47,48] According to this theory, as illustrated by Frost's example of an idealized portion of a limb bone loaded in bending, the mechanical stimulus for modeling/remodeling activities is strongly influenced by strain magnitude thresholds.[49–51] Skedros and colleagues[52,53] reported that the lower mineral content, higher porosity, and different osteon microstructural features of the bone were primarily the consequence of locally increased activation of basic multicellular units.

CONCLUSIONS

Within the limitation of the present study, the following conclusions could be made:

- The bone microstructure between two implants placed at 2 mm of distance showed a lower amount of collagen fiber orientation, fewer marrow spaces, and a significantly higher LMDI.
- The bone microstructure between two implants placed at 3 mm of distance showed a significant increase of collagen fiber orientation, a relevant extension of marrow spaces, and a higher level of mineralization.

Considering the bone microstructure characteristics evaluated in the present study, the interimplant distance should not be less than 3 mm.

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