

JIACD Continuing Education

Osteocyte Density in Woven and Lamellar Peri-Implant Bone

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Abstract

Background: Considering the possible association between osteocytes and bone remodeling, this study evaluated the osteocyte density in woven and lamellar bone around loaded implants.

Method: Bilateral mandibular premolars of 6 dogs were extracted, and after 12 weeks each dog received 8 Morse-cone connection implants immediately loaded. Four experimental groups were constituted: subcrestal with interimplant distance of 2mm and 3mm; and equicrestal with interimplant distance of 2 mm and 3 mm. After eight weeks, the animals were euthanized, the retrieved specimens were

prepared and histomorphometry was used to evaluate the osteocyte index in the groups.

Results: The differences in osteocyte density between woven and lamellar bone were statistically significant in all 4 groups, but not between them.

Conclusion: Woven bone presented significant higher number of osteocytes when compared to lamellar bone and this might be explained by different bone remodeling rates. However, different implant placement depths and interimplant distances did not influence the osteocyte number.

KEY WORDS: dental implants; lamellar bone; osteocyte density; osteocytes; woven bone

Learning Objectives

After reading this article, the reader should be able to:

1. Discuss aspects of early peri-implant bone remodeling following dental implant placement.
2. Discuss the significance of osteocyte density in peri-implant bone crestal bone loss.

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INTRODUCTION

As a dynamic environment, functional adaptation is required for bone survival.^{1,2} The mechanical integrity of bone is a result of the replacement of old or damaged bone by newly formed bone over time.^{2,3} Concerning implant dentistry, loading seems to play a decisive role in bone formation and bone mineral density.⁴

The function of the osteocyte in skeletal health and disease has been a focus of recent study.⁵ Osteocytes are not only the most abundant cells in mature bone, but also the longest-lived and the best-connected in the mineralized matrix.⁶ They seem to have a putative role in mechanotransduction, actively participating in the modulation of bone remodeling and turnover.^{1,2,7} There is a theory that osteocytes may sense external mechanical loads, which could be understood as a vital function for the maintenance of bone mass and architecture.⁸ The osteocyte cell bodies are positioned in lacunae, and they are in contact with neighboring osteocytes via long slender cell processes located in canaliculi, which are filled with interstitial fluid.^{9,10} When bones are loaded, the resulting strain acts as a driving force that causes a flow of interstitial fluid through the lacuno-canalicular network.¹¹ This fluid flow is sensed by osteocytes, which respond by producing signaling molecules that stimulate osteoblast recruitment while inhibiting osteoclast recruitment and activity, resulting in a gain of bone mass.^{12,13} However, this process remains partially understood.

Woven bone is produced in response to a need of quick bone formation, thus it lacks regular orientation and organization of the collagenous matrix.¹⁴⁻¹⁶ This type of structure is thought to result from accelerated matrix production by the osteoblasts.¹⁵ Moreover, woven bone tends

to undergo remodelling more rapidly than lamellar bone,¹⁶ and considering the possible existence of specific mechanisms by which osteocytes influence bone resorption and remodelling,¹⁷ osteocyte density may play an important role in determining the biological properties of woven bone.¹⁷ Hernandez et al,¹⁷ for example, reported that lacunar density between woven and lamellar bone can differ by as much as 40-100%.

Based on this, the aim of the present study was to evaluate osteocyte density in woven and lamellar peri-implant bone around loaded implants in a dog model, investigating the influence of different interimplant distances and depth placement on osteocyte cellularity.

MATERIALS AND METHODS

This research was conducted in parallel with another study that evaluated the influence of different interimplant distances and different placement depths of adjacent implants in papillae formation and crestal bone resorption through clinical/radiographic analysis¹⁸ and histomorphometric evaluation¹⁹ in a dog model. In a previous study²⁰ it was reported that osteocytes surrounding implants are important in the regulation of bone remodeling. In the present study the objective was to evaluate if loaded implants placed in different positioning conditions will influence osteocyte presence and morphology.

The study protocol was approved by the Ethical Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, SP, Brazil (Process 07.1.122.53.8) and involved two surgical interventions that were performed in six young adult male mongrel dogs, weighing approximately 20 kg.

The animals presented intact maxillas, no general occlusal trauma, and no oral viral or fun-

gal lesions. They were in good general health, with no systemic involvement as determined during clinical examination by a veterinarian.

Two weeks before the surgery, the dogs received antiparasitic treatment, multivitamins and vaccines. Ultrasound prophylaxis was administered to remove supragingival calculus and a solution of chlorhexidine gluconate 0.12% was applied on the teeth with gauze.

The animals began fasting the night before surgery. Anesthesia consisted of an intramuscular injection of a preanesthetic (acepromazine 0, 2% - 0, 05 mg/kg) followed by intravenous administration of thiopental (1 ml/kg; 20 mg/kg thiopental diluted in 50 ml saline). The animals were then moved to the operating room and maintained on gas anesthesia (1-2 % isoflurane/ O₂ titrated to effect).

In the first phase of the study, full-thickness flaps were elevated bilaterally and the four mandibular premolars of both hemi-arches of each animal were extracted. The teeth were sectioned in the buccolingual direction at the bifurcation, and the roots were individually extracted using a periosteal elevator in order to not damage the bony walls. The flaps were repositioned and sutured with non-absorbable 4-0 sutures. The animals received analgesic and anti-inflammatory injections and multivitamins.

After a healing period of eight weeks, the dogs received 20,000 IU penicillin and streptomycin (1,0 g/10 kg) the night before second surgery. This dose provided antibiotic coverage for 4 days, thus another dose was given 4 days later to provide coverage for a total of 8 days.

After repeating the same sedation and anesthesia protocol previously described, horizontal crestal incisions were bilaterally made from the distal region of the canine to the mesial

region of the first molar. Four implants (Neodent, Curitiba, Brazil) 4.5 X 9.0mm with internal Morse cone connections and sandblasted and acid-etched surfaces were placed 1.5mm subcrestally on one side of the mandible and the other four implants were placed crestally on the contralateral side. A total of 48 implants were placed in the study. Distances between two adjacent implants were alternatively 2 or 3mm in both side of the mandible. Thus four groups were constituted: (1)subcrestally with 2mm of interimplant distance (2 SCL); (2)subcrestally with 3mm of interimplant distance (3 SCL); (3)equicrestally with 2mm of interimplant distance (2 ECL); (4) equicrestally with 3mm of interimplant distance (3 ECL). The distances between the implants as well as the positions of implant placement were arbitrary determined through a coin toss.

After implant placement, metallic crowns were immediately installed with 3mm of distance between the contact point and the bone crest. The soft tissues were sutured around the crowns for non-submerged healing. The animals were maintained on a soft diet for 14 days until the sutures were removed. Healing was evaluated weekly and plaque control was maintained by flushing the oral cavity with chlorhexidine gluconate. The remaining teeth were cleaned monthly with ultrasonic points.

Eight weeks after restoration, the animals were anesthetized and then euthanized with an overdose of thiopental. The implants and the surrounding tissues were retrieved and immediately stored in 10% buffered formalin. They were then processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).²¹ Firstly, the specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200,

VLC, Kulzer, Wehrheim, Germany). After polymerization, the blocks were sectioned longitudinally along the major axis of the implants with a high-precision diamond disc at about 150 μm and ground down to about 30 μm . Three slides were obtained from the central part. The slides were stained with acid fuchsin and toluidine blue.

Histomorphometry was used to evaluate the osteocyte index (O_i) that was calculated using the equation $O_i = N.Ot / B.Ar$, where $N.Ot$ is the number of osteocytes observed at 200X magnification on the section plane for an infinitely thin section, and $B.Ar$ is the total area of the evaluated bone expressed in μm^2 (or in square pixels). The specimens were analyzed under a transmitted light microscope (Laborlux S, Leitz) that was connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVCs, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intels, Santa Clara, CA, USA). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and controlled by a software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc, Milano, Italy). Woven and lamellar bone areas and cell number were analyzed using image managing software (Adobe Photoshop CS, version 8.0.1, Adobe Systems, Beaverton, OR) and image analysis software (Image J1.32j, Wayne Rasband, National Institutes of Health, Bethesda, MD)(figure 1). Digital maps of histologic images at 200X magnification of the bone around loaded dental implants were reconstructed and evaluated. The acid fuchsin and toluidine blue staining facilitated the separation of woven and lamellar bone. Intensely red stained areas belonged to low mineral den-

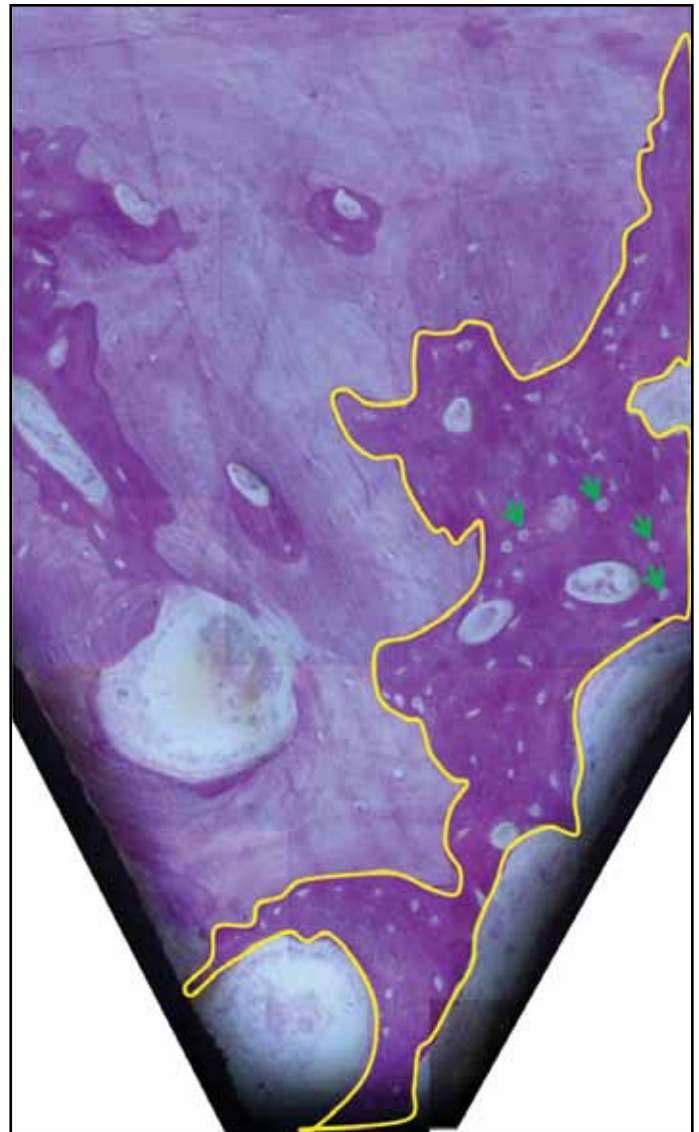


Figure 1: In order to determine the osteocyte density, the bone area was delineated (yellow line) and the osteocytes were counted (green arrows) at 200x magnification of the slides stained with acid fuchsin and toluidine blue.

sity bone (woven bone), while unstained or pale stained areas belonged to highly mineralized bone (lamellar bone) (figure 2). All images were calibrated using the Pythagorean Theorem for distance calibration, which reported the number of pixels between two selected points.



Figure 2: Digital map reconstruction of histologic images (magnification x200) of the bone around loaded dental implants. The section was stained with toluidine blue and acid fuchsin. The white arrows indicate areas intensely red stained belonging to low mineral density bone (woven bone) while the black arrows referring to unstained or pale stained areas belonging to highly mineralized bone (lamellar bone). Asterisks (*) indicate marrow spaces. Double asterisks (**) indicate implant.

Figure 3: (right) Differences between osteocyte lacunae observed in woven bone (yellow arrows) and in lamellar bone (green arrows).

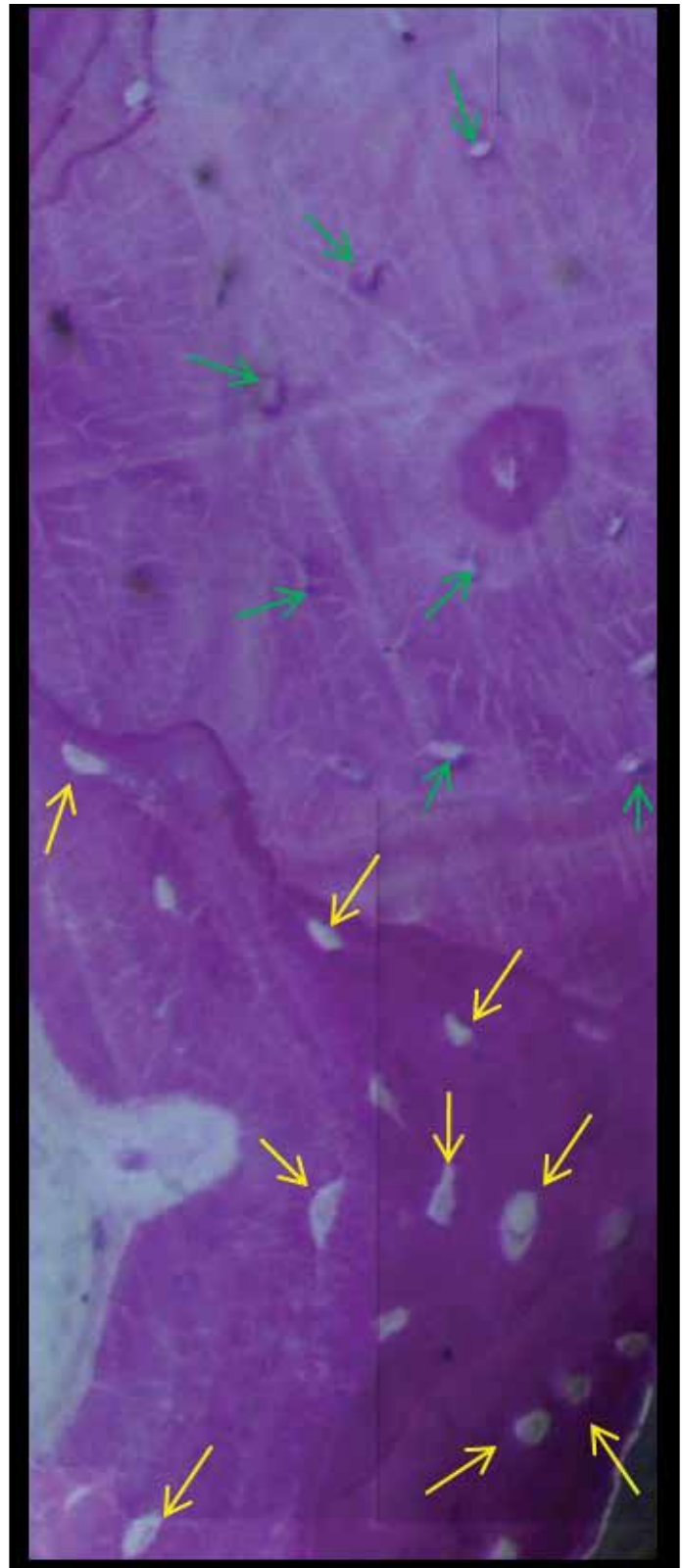


Table 1: Analysis of Osteocyte Density in Woven and Lamellar Bone for all Experimental Groups

Groups	Number of Osteocytes		Intra-group Analysis
	Woven Bone	Lamellar Bone	
2ECL	0.015 ± 0.006	0.007 ± 0.005	p=0.0027*
3ECL	0.014 ± 0.004	0.006 ± 0.002	p=0.0032*
2SCL	0.016 ± 0.005	0.009 ± 0.003	p=0.0059*
3SCL	0.018 ± 0.006	0.011 ± 0.006	p=0.0250*
mean ± standard deviation	p=0.276 Inter-group Analysis	p=0.032	*statistically significant

Statistical analysis

Comparisons of the differences in osteocyte density in woven and lamellar bone within the four groups separately were carried out using the non parametric Mann-Whitney U-test, for independent samples. However, comparisons between the four groups were carried out using the non-parametric Kruskal-Wallis Test. Results were presented as means ± standard deviation (SD), and the confidence level of 95% was assumed for analysis.

RESULTS

Qualitative differences in osteocyte lacunae shape and canalicular organization were observed among the different tissue types (figure 3). Lacunae and osteocytes in woven bone appeared to be larger than in pre-existing cortical bone. Osteocytes in lamellar bone varied from well organized lines when constituting an osteon unit to apparently random distributions in other specimens. However in woven bone they always appeared spontaneously.

The differences in osteocyte density between

woven and lamellar bone were statistically significant in each group individually, but not between the experimental groups (table 1).

DISCUSSION

Woven bone forms rapidly during tissue growth, following injury, and in response to certain anabolic stimuli, thus it is not found in the adult skeleton in normal conditions.²² It has an important function helping to fill, quickly, bone defects. It provides initial bone continuity that guarantees some strength to a bone weakened by injury,²² although it can be considered weaker when compared to the pre-existent cortical bone in terms of bone density.

Several features of woven bone may cause functional differences when compared to lamellar bone. For example, the extracellular matrix of woven bone is loosely organized and there is considerable evidence that the composition of the extracellular matrix in woven bone is different from that of lamellar bone.²³ In the present study, the shape and distribution of the lacu-

nae appeared different in woven and lamellar bone. While in the first, the osteocyte lacunae appeared larger and randomly distributed; in the second they were smaller and appeared organized in their location, accompanying the concentric lines that usually determine an osteon unit. These qualitative differences could be the result of the rapid rate of matrix synthesis in woven bone.¹⁷ Lastly, the osteocyte population in woven bone has been estimated to be larger than that found in lamellar bone,²⁴ however, data supporting this fact have been only rarely reported in the literature. The size of the osteocyte population may be especially important in determining the biological properties of woven bone, as cell number certainly has an important role in the growth and size of many organs.^{25,26} Moreover, recent studies of lamellar bone have implicated osteocytes as regulators of remodeling activity, formation rate and tissue volume.²⁷⁻²⁹ Whether similar relationships exist in woven bone is unknown.

The present study evaluated the osteocyte density in woven and lamellar bone around loaded implants, taking into consideration different interimplant distances and crestal or subcrestal implant placements. It was thought that quantifying their cellularity might be a way to better understand the regulation of their functions.

Mechanosensors in bone are able to sense a load-induced strain and to translate this information to cells at the bone surface⁵ and this is one theory to explain how osteocytes could be directly involved in bone remodeling. Vashishth et al³⁰ showed an age-related decline in osteocyte density that was correlated with microcrack accumulation in human femoral mid-diaphyses. Qiu et al³¹ have confirmed that osteocyte defi-

cit is related to increased microdamage, but not necessarily to animal age. Mann et al⁵ also found that the presence of fewer osteocytes in aging specimens has been related to an impairment of the ability to remove injured bone, which determines a reduced level of remodeling activity. This remodeling activity, coordinated between osteocytes, osteoblasts and osteoclasts, provides a basis for the adaptation of bone to external stimuli.¹ Osteocytes have a probable supervision of the biomechanical regulation of bone mass and architecture, modulating the activity of osteoblasts and osteoclasts by the production of chemical signals.⁸

The results of the present study showed a highly significant statistical difference in the osteocyte density of woven and lamellar peri-implant bone. In some cases the values differed by more than 100%. Similar results have been reported by Hernandez et al.¹⁷ The significance of the higher osteocyte density in woven peri-implant bone supports the hypothesis that this bone needs to be most rapidly remodeled, and the concentration of relevant signaling factors seemed to be increased in tissues with high cell numbers.¹⁷ Furthermore it was suggested that a large cell density would encourage the removal of tissue and possible replacement by tissue with a more typical cell density,¹⁷ enhancing the bone strength with superior levels of bone mineral density.

The loading conditions of the implants could also have an impact on the osteocyte density. In a study done in our laboratory,²⁰ on retrieved human samples, it was found that the osteocyte density was higher in immediately loaded implants when compared to submerged, unloaded implants. This study results

demonstrated that loading influenced positively the osteocyte density, however it was also observed that the different conditions in terms of interimplant distances and crestal or subcrestal implant placement did not affect the number of osteocytes in woven or lamellar bone.

CONCLUSION

The woven peri-implant bone presented significant higher number of osteocytes when compared to the lamellar bone, probably because of their different bone remodeling rates. However the differences of crestal or subcrestal implant placement and interimplant distances did not influence the number of osteocytes. ●

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Disclosure

This work was partly supported by the Coordination for the Development of Personnel in Higher Education (CAPES), Brazil, and by the National Research Council (C.N.R.) and the Ministry of Education, University, Research (M.I.U.R.), Rome, Italy.

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Continuing Education JACD Quiz #6

1. **True or False: Loading seems to play a decisive role in bone formation and bone mineral density.**
 - a. True
 - b. False

2. **The most abundant cells in mature bone are:**
 - a. Fibroblasts
 - b. Osteocytes
 - c. Epithelial cells
 - d. Multinucleated giant cells

3. **Bone lacunae house which of the following?**
 - a. Osteocyte cell bodies
 - b. Polymorphonucleocytes
 - c. Mast cells
 - d. Red blood cells

4. **True or False: Strain on bone results in osteoblast recruitment and osteoclast inhibition.**
 - a. True
 - b. False

5. **In response to a need of quick bone formation, which bone is formed?**
 - a. Lamellar bone
 - b. Woven bone
 - c. Cortical bone
 - d. Demineralized bone

6. **Which bone undergoes more rapid remodeling?**
 - a. Lamellar bone
 - b. Woven bone
 - c. Both remodel at the same speed
 - d. Neither bone is remodeled

7. **Lacunar density between woven and lamellar bone can differ by as much as:**
 - a. 1-2%
 - b. 5-10%
 - c. 10-25%
 - d. 40-100%

8. **True or False: Woven bone is typically found in the adult skeleton under normal conditions.**
 - a. True
 - b. False

9. **With advancing age, osteocyte density and numbers have been shown to:**
 - a. Increase
 - b. Decrease
 - c. Remain the same
 - d. Oscillate

10. **Osteocyte density in woven peri-implant bone is:**
 - a. Higher
 - b. Lower
 - c. The same as lamellar bone
 - d. No osteocytes exist in peri-implant bone