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ORIGINAL ARTICLE

Lateral static overload on immediately restored implants decreases the osteocyte index in peri-implant bone: a secondary analysis of a pre-clinical study in dogs

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Abstract

Objectives This animal study was conducted to evaluate the osteocyte index in the peri-implant bone around immediately restored implants under static lateral overload.

Material and methods Seven mongrel dogs received three implants on each side of the mandible. Forty-two implants were distributed into three groups (14 implants per group); each animal received two implants connected to a 4.5-mm opened expansion device (experimental group); in the other mandible side, two implants were connected into an expansion device without activation (control group); one implant each side of the mandible was left submerged (unload group). After 4 months under daily mechanical and chemical plaque control, the animals were euthanized; dental implants and surrounding bone were removed and processed to obtain thin ground sections. Histomorphometry was used to evaluate the osteocyte index in the peri-implant bone contact to implant.

Results A higher, statistically significant mean number of osteocytes × 10^{-5} μm^2 (54.74 ± 23.91) was found in the control group compared with the test group (22.57 ± 22.55) ($p = 0.0221$). The correlation between percentage of bone-implant contact and osteocyte index for submerged implants was not statistically significant ($p = 0.2667$), whereas the value for immediately loaded implants was statistically significant ($p = 0.0480$).

Conclusion The lower number of osteocytes in the peri-implant bone around overloaded implants could be related to the need for functional adaptation of the bone tissue to overloading and to the hypothesized involvement of the osteocytes in the maintenance of the bone matrix in the control group.

Clinical relevance Osteocytes play a pivotal role in bone adaptation to mechanical loading, and the osteocyte network has been regarded as being the main mechanosensory mechanism.

Keywords Dental implants · Osteocytes · Bone matrix · Bone remodeling

Introduction

Primary or mechanical stability of dental implants is a prerequisite concept in initiating the process of osseointegration. Several factors, such as metabolism, genetics, and nutrition, may affect the bone tissue structure that is characterized by a constant turnover process, in response to mechanical stimuli such as occlusal loading. Osteocyte cells play an essential role in the regulation of bone mass and structure, together with the coordinated actions of osteoblasts and osteoclasts that lead to the mechanical adaptation of bone, which are orchestrated by the osteocytes that have been considered the main mechanosensitive cells in the bone [1, 2].

Balanced mechanical loading is essential for the maintenance of skeletal homeostasis and bone tissue formation [1, 2]. Therefore, the transformation of mechanisms of mechanotransduction, i.e., the conversion of a physical stimulus into a cellular response or damage, is an actively updated

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topic under research [3, 4]. Under a slight load, the mechanosensation of osteocytes inhibits the regulatory signal of osteoblasts for bone formation, whereas excessive loading and bone microdamage have been associated with higher osteocyte apoptosis followed by an increased RANKL in bone, leading to osteoclastogenesis and bone resorption [3–7].

Self-regulating stress on bone tissue produces strain; the load-induced fluid flow regulates the bone matrix at tissue level due to the shear stress created [6, 7]. Previous studies [1, 2] have reported that loaded dental implants showed more total bone tissue volume and more newly formed bone trabeculae than unloaded dental implants. The functional role of osteocyte cells is being extensively researched at present, and their important role in the regulation of skeleton remodeling has been suggested. Replacement of bone tissue with fatigue microdamage occurs through local osteocyte apoptosis, probably related to the attenuation of the inhibitory signals released by the osteocytes [3]. In fact, the effects of prosthetic component misfit or static lateral loading on immediate loading protocols could also influence this process. Modifications in the osteocyte environment release growth factors and cytokines that affect both osteoblast and osteoclast activities [6, 7]; additionally, osteocyte apoptosis determines the remodulation of bone matrix [8]. A previous study has shown the association between osteocyte loss and bone microdamage [8, 9]. Nevertheless, the literature is not clear about the level of loading necessary for homeostasis without bone damage [10]. The recent consensus report of periodontal and peri-implant diseases and conditions [11] has classified "occlusal overloading" as a force that surpasses/exceeds the adaptative capacity of tissues. Moreover, a recent systematic review [10] has indicated that up to now, the literature still lacks reports about the effect of traumatic forces on peri-implant tissues. Thus, the aim of this animal study was to evaluate the effect that static lateral overload on immediately restored implants has on the osteocyte index.

Materials and methods

In this animal study, a secondary analysis of a clinical and histological study is presented, which was designed and powered to compare the effects of static lateral loading on immediately restored implants. The primary outcome variable was the difference between the mean value of the bone-to-implant (BIC) contact. The materials and methods were previously detailed [12]. The main aspects of this study design have been summarized below.

Experimental design

Forty-two implants were distributed into three groups (14 implants per group); each animal received two implants connected to an expansion device that was opened to 4.5 mm (experimental group). On the other side of the mandible, in the control group, two implants were connected to an expansion device without activation; one implant on each side of the mandible was left submerged (unload group).

Animals and surgeries

After obtaining approval from the Institutional Animal Care of University of Guarulhos, seven male dogs (average age of 2 years and weight of 18 kg) received three implants bilaterally as previously described [12] (total of 42 implants). All surgical and clinical procedures, as well as implant-supported restoration procedures, were performed under general anesthesia at the Veterinary School of University of Guarulhos. The number of animals was based on previous preclinical studies [13–17]. Animal selection, management, and surgical protocol were conducted in compliance with routines approved for this study by the Institutional Animal Care of University of Guarulhos. Briefly, all mandibular first molar and premolars were extracted creating an edentulous ridge. In addition, maxillary premolars were extracted to prevent negative impacts of trauma from interfering during the healing period and avoid possible bias to the study model involving immediately restored implants [12]. Bacterial biofilm control was performed with 0.12% chlorhexidine daily and scaling and root planing once a month for 2 months.

After a healing period of 2 months, a periodontist (J.A.S) performs all surgeries and prosthetic procedures. A total of 42 external hexagon dental implants (Ø3.75 × 10 mm) with sandblasted acid-etched surface topography were randomly allocated among the dogs as follows: control group (static loading) (n = seven pairs/restorations)—crowns connected to an orthodontic expansion screw without activation; test group—static overloading ($n = 7$ pairs/restorations)—crowns connected to an orthodontic expansion screw opened to 4.5 mm; unloaded group ($n = 14$ single implants)—implants that were left submerged. The implants of the unloaded group were evaluated and the mean value found for each animal was subjected to statistical analysis. An external surgeon not related with the surgical procedures (G.I.) performed the randomization. Tossing a coin was used to randomize which side (left or right) was assigned as control or test load protocol. Dental implants from unloaded group were placed in both mandibular ridges.

Immediate restoration and static lateral load

Immediately after dental implant placement procedures, impression posts were tightened directly into the implants and connected to each other with a self-curing composite resin. Laboratory implant analogs were attached to the impression posts and a master cast was constructed. Prosthetic titanium abutments were welded to an orthodontic expansion screw. All orthodontic expansion screws (control and test groups) were placed at 10 mm between dental implants (margin-to-margin) and fitted to the implants. All the experimental prosthetic devices were delivered within 2 h. For the test group (static lateral overloading), the expansion screw was opened to 4.5 mm, resulting in a force of 20 kg. Figure 1 shows a schematic diagram of the expansion device. The control group received only the restoration without any expansion device; abutment screws were tightened in accordance with the manufacturer's instructions. The screw access of the restoration was then covered with light-cured temporary resin.

At the time of delivery of the implant-supported restoration, periapical radiographs were taken to check the implant position and fit of the prosthetic components. Once a month, all restorations were checked to replace the loosened abutment screws. If the implant restoration showed mobility or abutment screw loosening, the orthodontic device was closed, then repositioned, and re-tightened onto the implants. The abutment screws were checked once a month, and then, the expansion screws were opened again (in order to test the groups).

Histological procedures

The animals were euthanazied after 4 months when the healing period ended. Dental implants and surrounding tissues were dissected and immediately fixed at 10% buffered formalin immersion and processed [12].

The ground mesiodistal sections were evaluated under normal transmitted light microscope connected to a high-resolution video camera (Leica DFC 320) and interfaced with a monitor and a computer. The optical system was associated with a software package with image-capturing capabilities (Leica QWin Plus V 3.5.0, Leica Microsystems, Heerbrugg, Switzerland). Bone-to-implant contact (BIC%), i.e., the amount of mineralized bone in direct contact with the entire extent of the dental implant, was measured.

A single masked and calibrated examiner (C.D.C) performed the histometric parameters. A total of 12 ground sections (4 of each group) were used for the calibration exercise. The sections were analyzed twice with a 1-week interval between measurements. Paired *t* test statistics showed no significant differences ($P > 0.05$) in intra-examiner reproducibility. The standard errors of the mean differences of histometric analysis were 5.5%. The osteocyte index was calculated by the ratio of the number of osteocytes (counted in bone tissue [for each slide] at a magnification of × 200) to the bone-area (μm^2) with the above-mentioned software package. The measurements were performed in bone tissue (in the thread length, mean 0.45 ± 0.05 mm) along the entire perimeter of the dental implants. The measurements were performed in the coronal (the first 1/3 of the length of the implant with bone-to-implant contact region of interested—ROI). Threads were used to guide ratio calculation (Fig. 2).

Statistical analysis

Osteocyte index and BIC% were calculated for each implant, then for each group (control, test, and unloaded). Differences among the groups were compared by nonparametric mixed models to evaluate the data clustered, within the dog. The Spearman rank correlation was used to evaluate the correlations between percentage of bone-implant contact values and osteocyte index. The significance test was two-tailed and conducted at a 0.05 level of significance.

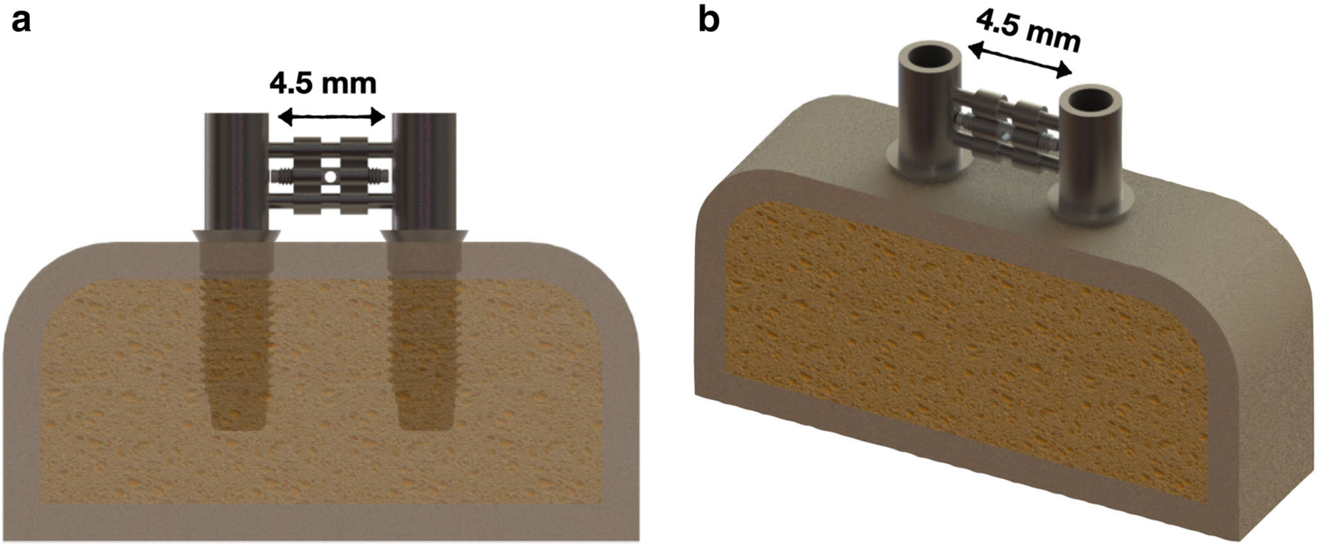

Fig. 1 **a** Frontal view and schematic figure represents abutments connected though expansion device which was opened to 4.5 mm to create static lateral overload. **b** Lateral view

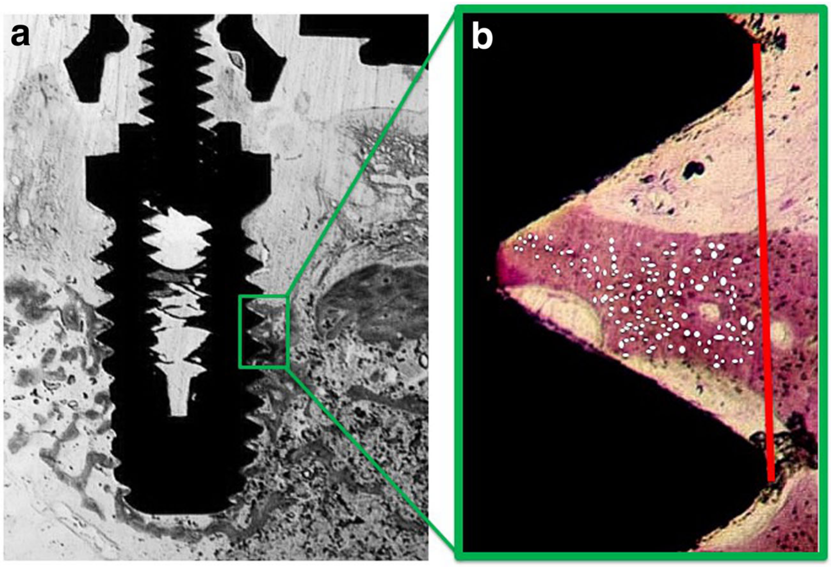

Fig. 2 (**a**) Implant threads were used to guide osteocyte ratio calculation (× 16 magnification) (**b**) at higher magnification (× 40 magnification)

Results

Osteocyte index and bone-to-implant contact (BIC%) are shown in Figs. 3 and 4. Implants of the test group showed a sharp decrease in the osteocyte index values when compared with the implants of the control and unloaded groups.

The major portion of peri-implant bone was trabecular, and there were osteocytes in their lacunae, while the newly formed bone exhibited different stages of maturation and remodeling, mainly in the loaded groups. Evident differences were observed among the groups, mainly in the dental implants of the test group that exhibited extensive peri-implant bone loss. A higher, statistically significant osteocyte index × 10^{-5} μm^2 (54.74 ± 23.91) was found in the control group compared with the test group (22.57 ± 22.55) (p = 0.0221). The unloaded group had a mean osteocyte index of 45.59 ± 4.33. The correlation between the percentage of bone-implant contact and osteocyte index for submerged implants was not statistically significant (p = 0.2667) whereas for the control group, it was statistically significant (p = 0.0480).

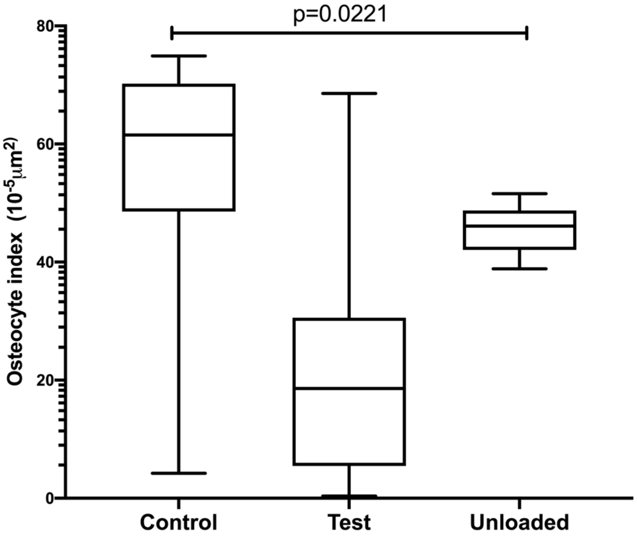

Fig. 3 Box-plot (min.max) of osteocyte index (μm^2) around implants of control, test, and unloaded groups—animals (n = 7 dogs). Kruskal-Wallis test (p = 0.0162)–Dunn post hoc test $p < 0.05$ (control > unloaded > test)

Discussion

The results of the present study showed a lower osteocyte index around overloaded implants; this result could be attributed to the lack of capacity for adaption of peri-implant bone tissues under static lateral overloading. Nevertheless, the role of osteocytes in maintaining the bone matrix in this group could be hypothesized.

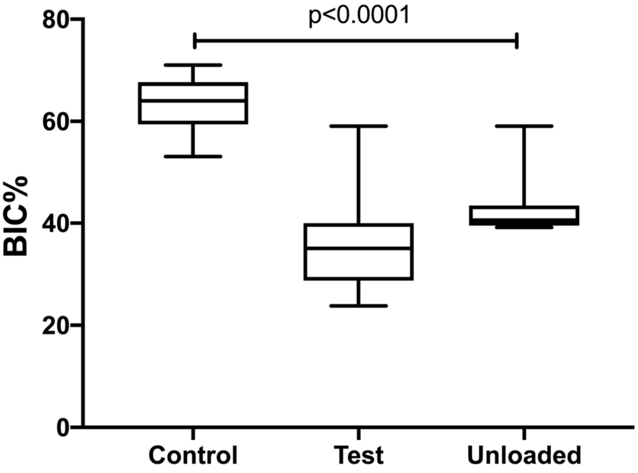

Fig. 4 Box-plot (min.max) of bone-to-implant contact (BIC%) around implants of control, test, and unloaded groups—animals (n = 7 dogs). Kruskal-Wallis test ($p < 0.0001$)–Dunn post hoc test $p < 0.05$ (control = unloaded > test)

Bonewald and Johnson [18] previously demonstrated that osteocytes were mandatory for the maintenance of bone tissue mass in response to "regular" loading; their presence was probably associated with the ability of bone tissue to efficiently remodel itself to maintain the normal levels of mineralization and to repair microdamage [18, 19]. It could be speculated that the excessive loading jeopardized this process, increasing microdamage and reducing the ability of the bone tissue adaptative capacity to maintain its level. A minimum number of osteocytes seem to be crucial for their operational network [7, 20]; however, the scientific evidence about the cut-off point of cells to maintain the homeostasis process is still lacking.

The osteocyte canaliculi, which must enter into the narrow bone spaces and recruit osteoclast precursor cells or induce mesenchymal stem cell differentiation [18], may have been interrupted. Mechanical loading of bone tissue stimulates the interstitial fluid flow through the pericellular space surrounding osteocytes and their mechanisms. Consequently, this flow activates signaling molecules in the cells, which are able to regulate the activity of osteoblasts and osteoclasts [21, 22] as was indirectly observed in the control group.

In addition, a previous study [23] has suggested that cytoplasmic processes were more mechanosensitive than cell bodies. The cytoplasmic processes probably have a cell body provided with a more robust structure which might explain how osteocytes transduce a mechanical signal into a chemical response, mainly under excessive loading. These aforementioned factors could partly explain how difficult it is to compare studies about overloading tissues in both implant-supported restorations [23–25] and restored teeth.

The dissipation of the static lateral force onto the dental implants might show distinct effects: increase in the bone osteocyte index when there are controlled forces or peri-implant bone loss as result of mechanical disruption of osteocytes. However, the unclear definition or even the measurement of occlusal overloading must be emphasized. Several studies have discussed the mechanical behavior, stress, strain, and viscoelasticity of peri-implant bone under different loading conditions [9, 26, 27] in an endeavor to clarify these questions.

The present study was limited to evaluating different levels of loading force to determine threshold: control, test (overloaded), and unloaded. The five dental implants lost from the experimental group during the study clearly showed that static lateral overloading resulted in the excessive transmission of force and consequently jeopardized the bone healing around peri-implant bone tissue. The mechanical performance of peri-implant bone that governs alveolar function and the mechanism of osteocyte are complex. The properties of bone tissue are closely related to load conditions, especially during growth and development [8, 26], with the latter being the target in the present study. The dental implants were immediately restored, and excessive lateral forces were applied in experimental group while a lower or incipient force was applied on the control group and no force on the unloaded group. Loading in the early phases of peri-implant wound healing has an osteogenic effect on bone healing and could be also enhanced by implant surface topography. In addition, long-term survival of the implant-supported restorations is more dependent on the ability of bone tissue to adapt effectively under the occlusal loads than merely on the amount of the peri-implant tissue and the mechanical properties of the bone [12, 28].

The expansion screws of experimental groups were almost totally opened, creating an excessive lateral force throughout the entire study period. An adaptive remodeling process of the peri-implant bone was observed in the control group, although no statistical significance was found [29–31]. Restorations were checked once a month and re-tightened and this clinical situation might also have increased the peri-implant bone loss as previously demonstrated [32, 33]. Finally, the use of external hexagon dental implants showed a detrimental effect on loading transfer as has previously been found [32, 34]

Therefore, within the limitations of this animal model, it could be concluded that static lateral overloading negatively affected the osteocyte index around the peri-implant bone of immediately restored implants. Further studies are needed to gain better understanding of the impact of load transmission on peri-implant bone behavior.

Conclusion

Within the limitation of this preclinical study, it could be concluded that immediately restored dental implants under static lateral overload had a lower number of osteocytes in the peri-implant bone.

Authors' contributions C. D. C, D. B, A. P, G. I, A.N., and J.A.S wrote the draft, conducted the experiment, collected data, and wrote the manuscript. J.A.S was the leader responsible for the conception and quality standards of the manuscript. All authors have approved the final version of this document.

Compliance with ethics standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international guidelines for the care and use of animals were complied with according to the Institutional Animal Care Guidelines of the University of Guarulhos and Veterinary School of University of Guarulhos, Guarulhos, SP, Brazil.

Informed consent For this type of study, formal consent is not required.

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