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Research Article

What Happens at Orthodontic Mini-Implants Apex?

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Abstract

Objectives: This study aimed to describe bone healing around self-drilling orthodontic mini-implants apex, with or without immediate orthodontic load.

Materials and Methods: One hundred forty-four self-drilling mini-implants (*MIs*-TOMAS®, Dentaurum, Germany) were inserted into 18 white rabbit's tibiae, elevating full thickness flaps. An immediate load (50 cN) was applied to 50% of the MIs. Two rabbits were sacrificed soon after the surgery and served as control group. Four rabbits each, were sacrificed at day 15, 21, 30, and 60 after the surgeries. Digital radiographs were obtained to measure the cortical bone thickness (CBT) below the MIs apex (MIA) and MIs apex-internal border distance (AIB). The sections were stained for histologic and histomorphometric analysis. Bone quantity (BQ), bone to implant contact (BIC), CBT and AIB were statistically evaluated using Kruskal-Wallis test.

Results: At day 0, fractures were visible at the cortical surface around the MIA. At days 15 and 21, intense bone proliferation was visible as woven bone, followed by lamellar bone. After 30 days, primary bone was visible with less proliferation activity. At day 60, primary bone was visible in the remodeling process for the secondary bone. CBT and AIB was increased throughout the healing period ($p = 0.016$ and $p < 0.001$, respectively), being higher values in the loaded group. BIC showed statically difference only between 21 and 60 days. BQ did not show differences.

Conclusions: Unintentional contact between MIA and the cortical bone didn't generate any type of damage, but there was a gain in the amount of bone-implant contact.

Keywords: *Orthodontics; Orthodontic Anchorage Procedures; Implants; Radiography; Histology; Bone*

Introduction

Anchorage control is primordial for orthodontic treatment success in most of cases. Among the anchoring accessories available, the most effective are orthodontic mini-implants [1-4].

Primary stability, obtained after the mini-implant insertion, is the key for success [5-10]. Already, secondary stability is obtained by osseointegration, that is originally defined as newly formed bone and implant microscopic contact [11,12].

Some studies have suggested that *MIs* stability is produced by mechanical imbrication between bone and implant, allowing immediate force application [10,13-15]. *MIs* primary stability is affected by: implant design, bone quality, implantation site preparation, and angle of insertion [10,16-18].

Bone remodeling process at bone-implant interface has been the subject of experimental studies carried out on animals [2,19-22]. Histomorphometric and histological analysis revealed during the early stages of healing bone deposition in direct contact with self-tapping implants metallic surface [19-25].

Self-drilling *MIs* shows an extremely sharp and thin tips, and, in majority of the cases, additional procedures for bone perforation is not needed [8,15]. Even though the bone healing around grade-V orthodontic mini-implants has been described by many studies in the literature, none was described about what happens around *MIs* apex.

This study aimed to histologically, histomorphometrically and radiographically describe, characterize, and quantify the bone healing process around mini-implants apex during different periods of time (days: 0, 15, 21, 30, and 60) in *MIs* previously sterilized in-office by the researchers, submitted or not to orthodontic load.

Materials and Methods

In this study were used eighteen 6-month-old New Zealand white male rabbits weighing 3.5 to 4.0 kg. In the right and left tibiae of each animal were inserted four orthodontic *MIs*. A total of 144 self-drilling orthodontic *MIs* (TOMAS - Temporary Orthodontic Micro Anchorage System, Dentaurum, Inspringen, Germany) made of grade V titanium alloy (Ti6Al4V), 6 mm X 1.6 were inserted (Figure 1). Implants were cleaned and sterilized by the researchers [26]. All surgical procedures were previously described [23].

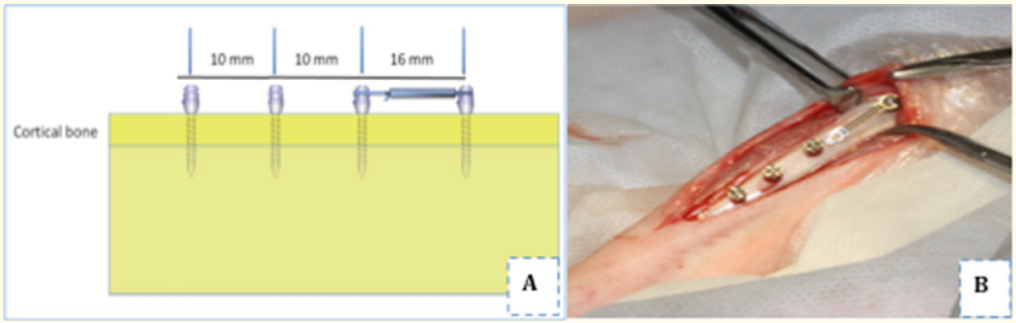

Figure 1: *MIs insertion.*

At the end of the surgical procedure, there were four *MIs* in each tibia, two loaded and two unloaded, for a total of eight *MIs* per animal. The animals were sacrificed, two on day 0, immediately after the insertion of the *MIs*, being the control group and four at each of the four time periods under assessment.

Both tibiae of each rabbit were dissected and after the soft tissue removal, digital radiographs were taken of each metaphysis containing the *MIs* (XDENT D70, Xdent Equipamentos Odontológicos Ltda, Ribeirão Preto, Brazil; Dr. Suni Plus Intraoral Sensors, Suni Medical Imaging, Inc, Calif., USA).

The distance from MI apex to cortical bone internal border (AIB) and the cortical bone thickness (CBT) was measured on the radiographic images, in millimeters, using Image J® software (Java version 1.1.4. for Windows, Microsoft, Redmond, Wash.). The thickness of the cortical bone was measured as the distance between outer and inner surface, exactly below the MIs apex (Figure 2).

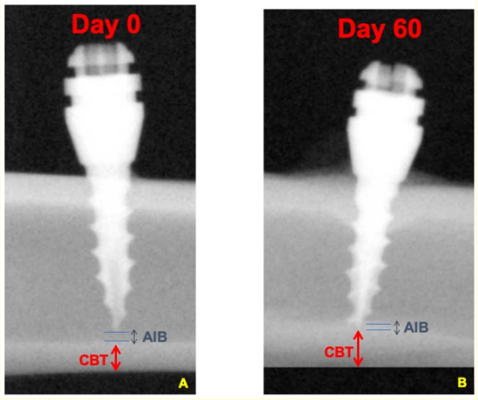

Figure 2: *Tibia's radiographs.*

The *MIs* were removed with at least 4 mm of surrounding bone and fixed in 4% formaldehyde. The blocks were then sectioned on the long axis of the mini-implant using a microtome (Exakt®**,** Kulzer, Norderstedt, Germany). The sections, of approximately 30 µm, were examined under an AxioCam® HRc digital camera (Zeiss, Jena Germany) and an AxioScope® microscope (Zeiss, Jena, Germany) to obtain polarized digital images with 10x magnification. After polarized imagens, the sections were stained with Stevenel's blue, counterstained with Alizarin red S [27] and new images were obtained with same equipment and magnification. Histomorphometric measurements (BQ and BIC), were obtained using Image J® software.

Polarized light images were used to view apposition and resorption bone lines, showing newly formed bone areas. Digital images, obtained under bright-field microscopy, were analyzed to determine the cellular and morphological characteristics of each specimen. Data from the different groups were compared to determine the similarities and differences between the tissue characteristics after different periods of healing and orthodontic load.

Data from both groups (WL + WOL) for cortical bone thickness and the MI apex-internal border of cortical bone distance at all study periods, were compared using Kruskal-Wallis test with Tukey multiple comparisons when it was significant. To compare the amounts of bone and BIC for the different time periods under assessment, Kruskal-Wallis test was applied. In results significant, multiple comparisons of Turkey were performed (5% of significance).

The entire experimental procedure was performed with the approval of the Animal Ethics Committee of the Institute of Biomedical Sciences (n° 059/87/02) and of the School of Veterinary Medicine and Animal Science of the University of X (n° 2016/2010).

Results

All *MIs* inserted showed success. Out of the 144 *MIs* inserted, 71 (49.3%) had contact with cortical bone (CB) opposite to that of its insertion and 73 (50.7%) showed their apexes located in medullar bone (MB). At day 0, 50% of the screws tips was at cortical bone and 50% at MB. At day 15, 12 *MIs* (37.5%) were CB, being 50% from WOL and 50% WL groups. At day 21, 19 *MIs* (59.4%) had contact with

the CB, 10 from WL and 9 WOL group. After 30 days, 16 *MIs* (50%) had CB contact (10 WL and 6 WOL group). At day 60, 16 *MIs* (50%) had CB contact, however, 9 were WL and 7 WOL group.

The results described below refer to *MIs* whose apexes were in contact with the cortical bone opposite to their insertion site. Due to reduced numbers of samples in WL and WOL groups, it was not possible to apply statistic tests between these groups. Therefore, load was not considered in statistic tests to compare CBT, AIB, BQ and BIC over the study time intervals.

Histological analysis

Peri-implant bone remodeling was continuous throughout healing periods in both groups. At day 0 small fracture of the edge bordering on the *MIs* apex contact on the internal cortical bone were noticed; fragments were displaced to medullar bone areas (Figure 3 - FC).

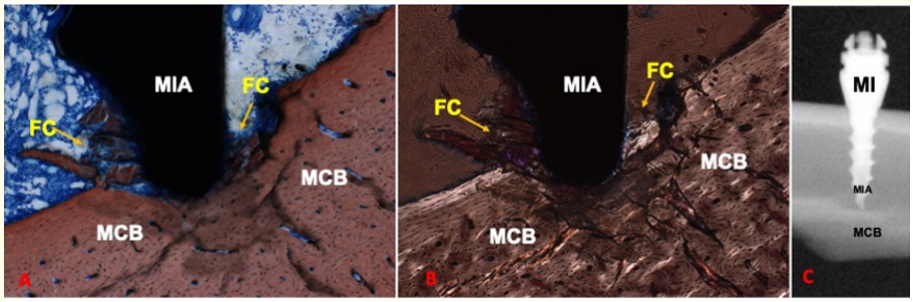

Figure 3: *MIA day0 (10x). A–(BFM)bright-field microscopy; B-(PM)polarized microscopy; C- RX; MCB – mature cortical bone; FC – location of fractures in the coronal region.*

After 15 days, CB fractures in the contact of MI apex were still evident, as well as areas filled with differentiating tissues. Osteoblasts harvesting the osteoid matrix at bone-implant interface. Cortical bone surface presented extensions of immature reticular bone towards the mini-screw and lamellar bone coated with osteoblasts and osteoid matrix (Figure 4).

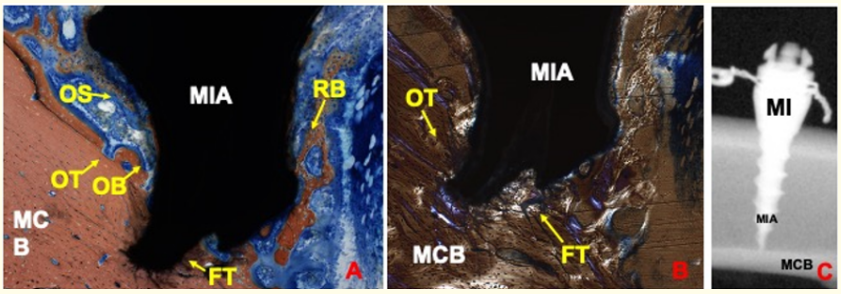

Figure 4: *Day 15; OB – osteoblasts; OT – osteocytes; RB – immature reticular bone; OS – osteoid matrix; FT –MIA's thread fracture.*

At 21-days, there was active cell proliferation, with amounts of osteoblasts forming the osteoid matrix between the fractured pieces and towards the screw interface. As healing process progress, new bone formation originated by the cortical bone, exhibiting one or more layers of osteoblasts and immature reticular bone were evident (Figure 5A/B).

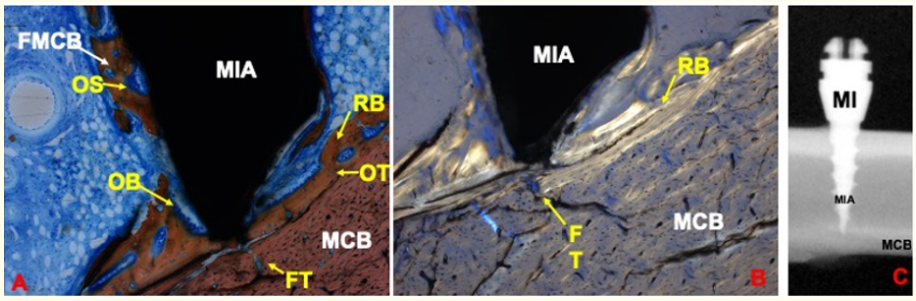

Figure 5: *21 days WOL: FMCB – MCB fragments.*

At 30 days, in both groups, there were immature bone areas surrounded by osteoblasts and exhibited osteoid deposition. All the MI apex showed the deposition of immature lamellar bone at the interface. In most samples, it was possible to observe newly bone deposition towards the MI body, not only at the MI tip that contacted CB. However, the regions not filled yet, showed osteoblasts and active deposition of osteoid. Just beneath the fractured fragments of cortical bone surface promoted by MI apex it was possible to observe deposition of the lamellar bone (Figure 6).

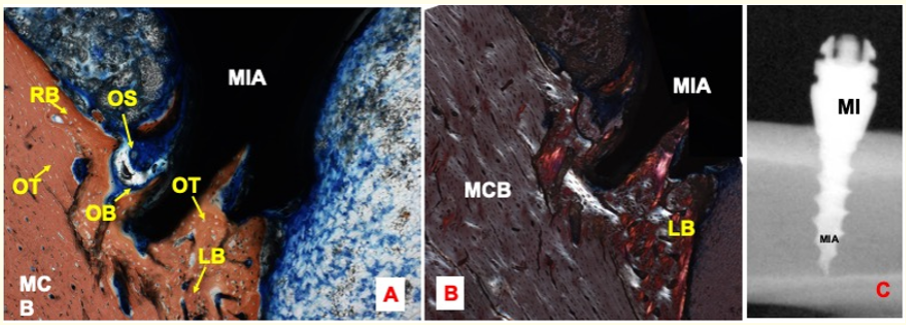

Figure 6: *30 days WL: MB–mature bone; LB – immature lamellar bone.*

After 60 days, better organized, well-formed, maturing primary bone, was seen at the interface with the implant. The distinction between the young bone and pre-existing bone was evident. There was maturating bone with less amounts of osteoblasts and osteoid than at 30 days. Bone proliferation towards mini-implant body was evident (Figure 7).

Histological analysis showed no significant differences between the WL and WOL groups in the processes of bone healing.

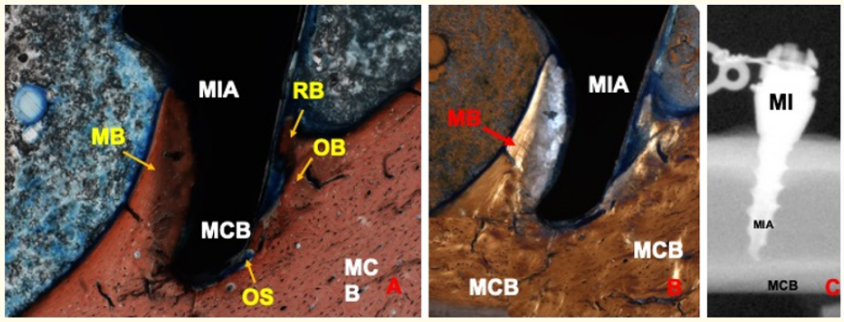

Figure 7: *60 days WL.*

Some interesting images of the mini-implants apexes were found at medullar bone, in which, even without direct cortical bone contact, there was bone proliferation in the screw body (Figure 8 to 11). Such bone growth proved to be compatible with the time of the study, when compared with the images of the mini-implants that were in contact with the cortical bone.

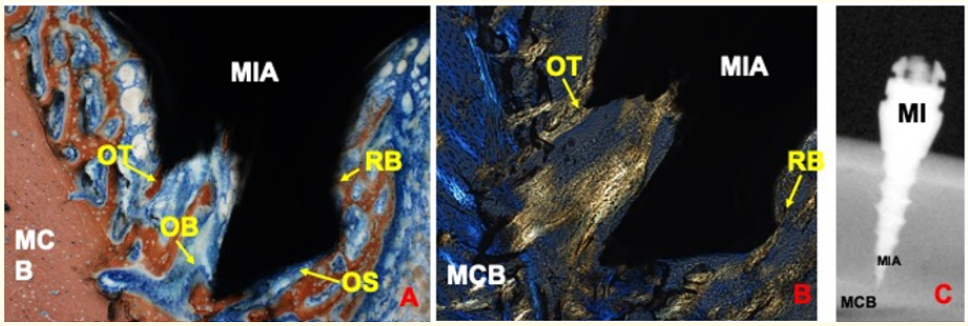

Figure 8: *MIA at medullar bone 15 days WOL.*

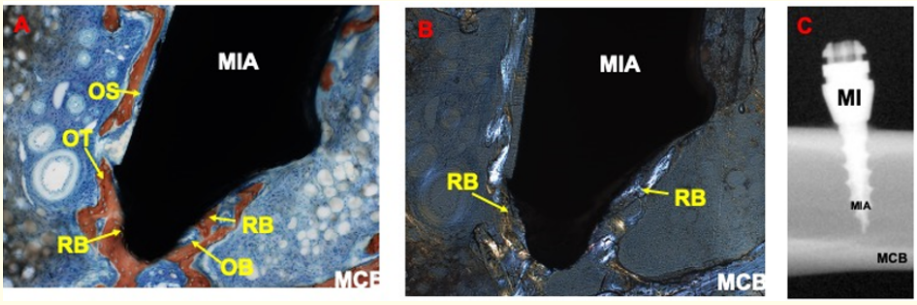

Figure 9: *At medullar bone 21 days WOL.*

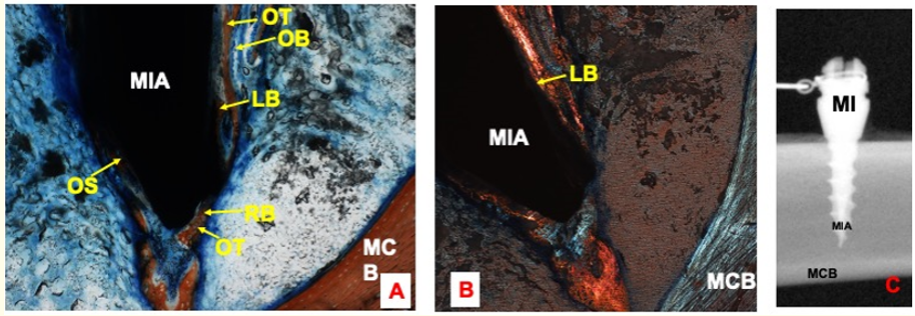

Figure 10: *MIA at medullar bone 30 days WL.*

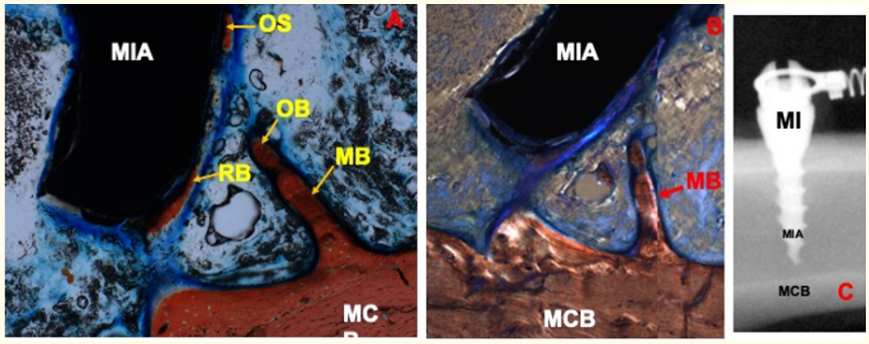

Figure 11: *MIA at medullar bone 60 days WL.*

Cortical bone thickness

The average minimum and maximum bone thickness ranged from 1.16 ± 0.40 mm (day 0) to 1.85 ± 0.56 mm (60 days). It was possible to observe slightly higher values for the group with load in all study periods. Cortical bone thickness means values increased over the healing periods of this study, with statistically significant differences (p = 0.016) (Table 1).

	Time						Results	
	0	**15**	**21**	**30**	**60**	**Kruskal-Wallis (p) test**		
AIB	Mean	0,37	-0,78	-0,31	-0,43	-0,42		(15 x 21 x 30 x 60), p=0,082	
	SD	0,34	0,46	0,39	0,66	0,20	< 0,001*	(0 x 15) p < 0,001* ; (0 x 21) p=0,011*	0 > 15 = 21 = 30 = 60
	N	8	11	14	16	16		(0 x 30) p = 0,001* ; (0 x 60) p=0,001*	
CBT	Mean	1,16	1,85	1,75	1,73	1,85		(15 x 21 x 30 x 60), p=0,975	15 = 21 = 30 = 60
	SD	0,40	0,53	0,47	0,51	0,56	0,016*	(0 x 15) p = 0,035* ; (0 x 21) p = 0,076	0 = 21 = 30
	n	8	11	14	16	16		(0 x 30) p = 0,082 ; (0 x 60) p = 0,020*	0 < 15 = 60

Table 1: *Mean and standard deviation of cortical bone thickness (CBT) and MIs apex-internal border distance (AIB) at the time periods under assessment. Kruskal-Wallis test was used with a 5% level of significance. *Statistically significant.*

The mean of the AIB measurement varied from 0.37 ± 0.34 mm (Day 0) to - 0.78 ± 0.46 mm (15 days). This increase in the number of screws inserted in the cortical bone, was statistically significant ($p < 0.001$) (Table 1).

Histomorphometric analysis

Minimum and maximum values for the amount of bone varied between 11.448 ± 5.2 (15 days) and 17.755 ± 6.7 (21 days). BIC minimum and maximum values varied between 64.208 ± 24.06 (60 days) and 127.26 ± 84.1 (21 days).

The comparison of BQ and BIC over healing periods of this study showed statistically significant differences only to BIC with higher percentage at 21 days when compared to 60 days ($p = 0.014$) (Table 2).

15		Time			Kruskal-Wallis			Results
		21	30	60	Test (p)			
BQ	Mean	11,448	17,755	16,476	13,672			
	SD	5,162	6,706	9,376	5,533	0,074	----------	15 = 21 = 30 = 60
	n	12	19	16	16			
% BIC	Mean	104,339	127,261	96,012	64,208		(15 x 21 x 30), p = 0,471	15 = 21 = 30
	SD	51,284	84,185	53,823	24,066	0,013*	(15 x 30 x 60), p = 0,253	15 = 30 = 60
	n	12	19	16	16		(21 x 60) p = 0,014*	21 > 60

Table 2: *Comparison between BQ and BIC values for MIs at time periods under assessment. Kruskal-Wallis test was used with a 5% level of significance. *Statistically significant.*

Discussion

The present study obtained one hundred percent of success rate, so in-office sterilization allows for using non-sterilized *MIs*, that have small costs than sterilized ones, with no biosafety reduction.

All mini-implants had satisfactory primary stability, being 49.3% contacted with opposite cortical bone. The researchers had no intention to obtain bicortical anchoring; this probably occurred due to rabbit's tibia anatomical variation. In all the apexes that touched the internal surface of the opposite cortical bone, bone microfractures were found, with small displacements of bone fragments, which over the study time span, were repaired by the new bone formation that was completed at 30 days of healing, in both, with and without load groups. Such fracture areas were the result of contact with the extremely thin and sharp tip of self-drilling mini-implant apex, which generated crush points in the internal cortical superficial bone reached.

Histological and polarized light analysis of all MI apex that reached opposite cortical bone, showed continuous bone remodeling process around the apex of the mini-implant. Bearing in mind that such description does not exist in the literature, it was possible to compare it with data from histological studies of screw's cervical region. Cellular events and bone neo-formation sequences that occurred in mini-implant apex were the same as described in the literature over the time intervals evaluated in the present study [11,19,23,28,29].

As in some studies carried out with self-tapping or self-drilling mini-implants, the histological characteristics showed continuous bone remodeling. Although there is some variation in the animal model (New Zealand rabbits or Beagle dogs) and in time intervals evaluated in previous literature studies, all are in agreement with the present study, regarding the healing sequence [20,21,23,25].

Radiographic images showed an increased bone volume surrounding MIs apexes of touched cortical bone, over the time intervals of the study. However, no comparing information about that aspect was found in the examined literature.

Histological and radiographic images of the areas surrounding the apexes of the mini-implants showed a significant increase in bone volume, with projection towards the screw threads, located in the medullary bone. This also reinforced the view that bone tissue integrates grade V titanium alloy mini-implants without showing foreign body reaction, even after in-office sterilizing process.

Histomorphometric analysis showed direct bone-implant contact at all times of the study. Unfortunately, it was not possible to analyze statistically significant difference between with and without load groups due to small sample. However, when load was not considered, higher BIC and BQ values over the time intervals of the study were statistically significant. Due to all previous studies being performed at cervical bone level, we had no comparison parameters with other literature reports.

In this study, MIs apex position had not been standardized in terms of insertion and or the amount of contact with the opposite cortical bone was merely casual, so it was not possible to compare BIC data with similar studies. However, it was possible to confirm that the amount of BIC is time-dependent, as already well described in the literature of cervical MIs studies [12,19,23].

The presence of immediate, light and continuous load (50cN) did not negatively affect healing patterns. When compared to previous studies, carried out in the cervical region of mini-implants in animals and humans [24,25,30], in relation to this amount of load, Catharino., *et al.* (2014) found that load induced greater initial reparative inflammatory response at 15 days, showing greater amount of bone for the WL group.

This study aimed to evaluate the cortical bone in contact with the MIs apex, however, some apexes did not reach the opposite cortical bone remaining in medullar bone showing some bone growth surrounding the implant tip. Histological analyses showed characteristics of bone remodeling processes identical to those found in the cortical bone at the respective study times. Perhaps, a hypothesis for explaining growth in medullar bone, is the displacement of bone chips resulting from mini-implant insertion procedure. Further studies are needed to better understand this histological finding, but the good biocompatibility of the grade V titanium alloy is a fact.

Bone remodeling cycle, in humans, lasts approximately 18 weeks, while in rabbits 6 weeks long [21,31]. Thereby, it's possible to use a ratio of 1:3 to interpret the rabbit studies data for human [12]. Results of this animal model (rabbit tibia) restricts the applicability to bones with thicker and denser cortical layers such as the mandible [32].

This study monitored the healing and remodeling process over a period of 60 days. Additional *in vivo* animal studies using μCT are needed to analyze in 3D the evolution of bone growth at the interface with titanium alloys over time.

Conclusions

- Unintentional contact between MI apex and the cortical bone does not generate any type of damage, on the contrary, there is a gain in the amount of bone-implant contact.

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