## Sequential Bone Response to Immediately Loaded Mini-Implants, in vivo Study

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## ABSTRACT

Ti-6Al-4V alloy mini-implants were inserted in rabbit's tibiae and immediately loaded with 1 N. The healing process was analyzed by SEM in the assessment periods of 1, 4, and 12 weeks. Results showed that the tissues formed after 12 weeks were different between loaded and unloaded groups, but both of them were mechanically stable. The compression and traction areas in the loaded group did not present difference between each other. This investigation showed that the immediate load affected the healing process of the bone-implant interfacial tissue, without compromising its stability.

## **INTRODUCTION**

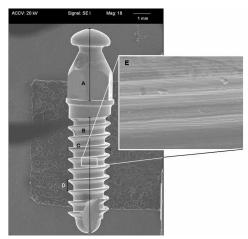
Osseointegrated titanium implants are an excellent alternative to traditional orthodontic anchorage methodologies, and they are a necessity when dental elements lack in quantity or quality, when extraoral devices are impractical, or when noncompliance during treatment is likely. In orthodontics, the mini-implants can be used to anchor different movements and are becoming widely used. They have few limitations related to the site of implantation, the insertion surgical procedure is relatively simple, and the control of direction and quantity of the force is easy. These improvements were possible due to the decrease of the implant size, but these changes can result on significant alterations at the bone-implant interface. Commercially pure titanium (c. p. Ti) is widely used as dental implant material because of its suitable mechanical properties and excellent biocompatibility [1,2]. However, c. p. Ti orthodontic mini-implants have high rate of fracture during insertion and removal procedures, because of their small size. The Ti-6Al-4V can be used to overcome this disadvantage. To simplify the mini-implant methodology they have to be loaded as fast as possible, decreasing the treatment time. The purpose of this work was to analyze the bone healing reactions of immediately loaded Ti-6Al-4V mini-implants by SEM observation, after 1, 4, and 12 weeks.

#### IN VIVO EXPERIMENTAL DETAILS

Twelve Ti-6Al-4V alloy mini-implants (Conexão Sistemas e Próteses, São Paulo, Brazil) were used. The mini-implants had a cylindrical screw design with 2.0 mm in diameter, 6.0 mm in length, and a hexagonal shaped head. The mini-implants were machined by turning, cleaned, passivated with nitric acid (HNO<sub>3</sub>), and sterilized with Co radiation. No implant surface treatment was made to change their roughness (Fig. 1). Ni-Ti closed coils spring were used to load (1N) the mini-implants.

Six-month-old male New Zealand rabbits, weighting 3.0 to 3.5 kgf, were used in the present research. The surgical procedures were common to all animals and consisted in the implantation of 4 mini-implants in the left tibial metaphyses of each animal (Fig.2). All surgeries were performed under sterile conditions in a veterinary operating room. Two mini-implants were immediately loaded using NiTi closed coil springs with 1 N. To the SEM analysis, two mini-implants of each rabbit were used, one loaded and one unloaded.

The groups were formed to investigate 3 periods of healing: 1 week, 4 weeks, and 12 weeks. In each time, one group with load and other without load was analyzed, resulting in a total of 6 groups. At the established times, the animals were euthanized by exsanguinations. The tibiae were dissected and blocks containing one mini-implant and 2.5 mm of adjacent bone were sectioned.



**Figure 1.** Cylindrical titanium alloy screw shaped. (A) Hexagonal head; (B) active area with 6.0 mm in length; (C) 2.0 mm in diameter; (D) 0.51 mm between the top of the pitches; (E) machined surface.

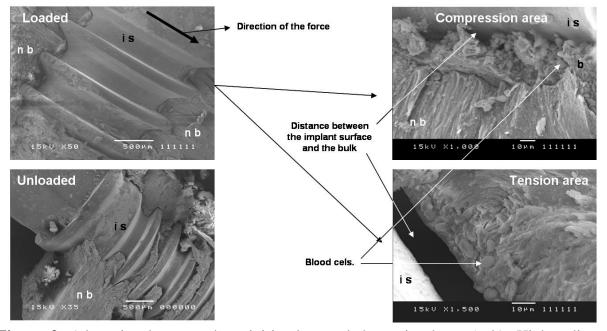


Figure 2. Four mini-implants inserted in the rabbit tibia, spaced 5 mm apart.

The blocks were immediately fixed in a solution containing 4 % formaldehyde freshly prepared in 0.1 M sodium phosphate buffer (pH 7.2) for 4 hours, dehydrated by total immersion in graded series of ethanol (50-100 %), and critical-point dried (Balzers - CPD-020). Each dried block obtained was carefully fractured with a razor blade into 2 fragments, one containing the bone and the mini-implant, and the other just with the counterpart bone. Afterwards, the samples were placed on aluminum stubs using a carbon conductive tape, coated with a thin gold layer (20 nm) (Balzers/Union FL - 9496) and examined under a scanning electron microscope (Jeol 5310), operated at 20 kV, in slow scan mode.

## DISCUSSION

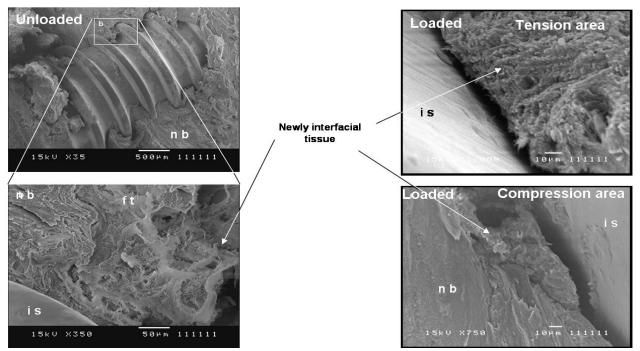
Unloaded-1-week and loaded-1-week groups demonstrated great adaptation with the native bone (Fig. 3). In the loaded group, just the first thread of the mini-implant inserted inside the cortical bone was considered compression and tension area. The injuries caused by drilling and by the mini-implant insertion produced a bulk of blood clot, necrotic bone, proteins, and macromolecules [3]. This bulk was clearly identified in both groups. In the loaded-1-week group, the bulk seemed to be closer to the titanium surface in the compression side than in the tension side. The elastic feature of the bone and the little displacement described in orthodontically loaded implants could explain the reduction of the interfacial distance observed [4,5,6]. The morphological characteristics of the interfacial tissue did not demonstrate changes due to the loading until one week of healing.



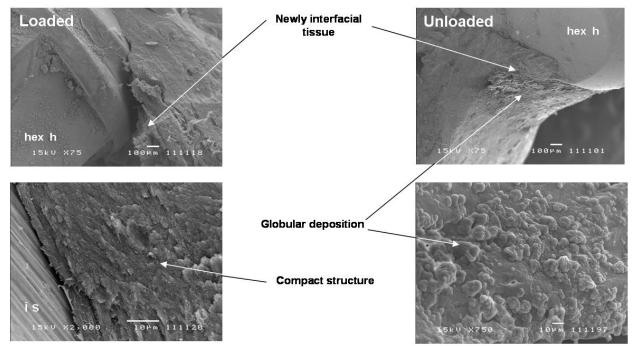
**Figure 3.** Adaptation between the mini-implant and the native bone (n b); Higher distance between implant surface (i s) and the interfacial bulk (b) in the tension area than in the compression area.

Following the healing process, a rich fiber tissue was observed in both 4-weeks groups (Fig 4). The unloaded group presented a more fibrous interfacial tissue than the loaded group and the difference between native bone and healing tissue was less clear in this time. Collagen fibers oriented perpendicularly to the implant surface has been described [4] in this phase. However, we did not observe a defined fiber orientation in both 4-weeks groups. Differences between the compression and tension area were not observed anymore in the loaded group.

Findings such as the bone-like tissue formation on the hexagonal mini-implant's head in both groups after 12 weeks of healing indicated a rigid union relation (Fig.5). Nevertheless, the unloaded-12-weeks group demonstrated a globular mineral deposition typical of the primary bone, while the loaded group presented a more compact bone with a lamellar architecture. The compression and tension areas of the loaded-12-weeks group kept the same tendency observed in the 4-weeks groups, without differences between each other. Roberts *et al.*[7] described that forces ranging between 1-3 N did not affect the implants stability and Büchter *et al.*[4] concluded that tip forces higher than 9 N can result in osseointegration failure of the implant. Isidor [8] defined that high forces trend to damage the interface integration. This overloading limit is influenced by the implant design. The first tread of implants with screw design has stress concentrations after lateral or oblique load, causing marginal bone loss [5,9]. The present study demonstrated that forces of 1 N modified the interfacial tissue formed, but did not cause failure on mini-implant integration or supra crestal bone loss, in rabbit tibia.



**Figure 4.** Newly more fibrous interfacial tissue in the unloaded group. (f t - fibrous tissue) (n b - native bone) (I s - implant surface).



**Figure 5.** Tissue-like-bone formation on the mini-implant hexagonal head (hex h) and different features of new tissue formed after 12 weeks of healing with or without load. (i s - implant surface)

The success of the rigid orthodontic anchorage by mini-implants is related to a sequence of factors [10]. First, the used material needs to be nontoxic and biocompatible [10,11]. In addition, the design of the mini-implant has to provide primary stability and to support immediate load, transmitting the forces with no overloading or damage to the host bone [4,5,9]. These features depend on the relation between the host bone quality and quantity at the insertion site and the implant form. This relation is fundamental to the maximal force that the system will sustain [4,10,12,13]. During the healing process, the micromotion and the peri-implant tissue inflammation are associated with the implant failure and must to be avoided [13,14].

# CONCLUSION

- 1. After 1 week of healing, the single difference between loaded and unloaded group was the decrease of the distance between the interfacial tissue and the mini-implant surface in the compression area.
- **2.** After 4 weeks of healing, the loaded group presented less fibrous tissue than the unloaded group.
- **3.** After 12 weeks of healing, both groups presented a tissue-like-bone formation on the miniimplant hexagonal head. The quality of the interfacial tissue was lamellar in the loaded group, but the unloaded group presented an unorganized globular tissue.

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