Sequential bone healing of immediately loaded mini-implants

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Introduction

The goals of the orthodontic implants are to obtain primary stability, to support immediate loading with continuous low unidirectional forces during all treatment and to be easily removed after. The required implant size decrease results in loss on mechanical properties and, sometimes, in fractures of mini-implants. In addition, the early load has an important influence on the characteristic of the new bone formed. Hence, the aim of this study was (1) to biomechanically compare the sequential bone response to titanium alloy mini-implants (Ti6Al4V) submitted to immediate loading by removal torque test and (2) to analyze the interfacial tissue evolution during bone healing by SEM.

Materials and Methods

Eighteen adult male New Zealand white rabbits, weighting 3.0 to 3.5 kilograms, were used in the present research. The surgical procedures were common to all animals and consisted in the implantation of 4 mini-implants (Fig.1) in the left tibial metaphyses of each animal. The mini-implants were threaded at the first cortical of the tibia, and the two central mini-implants were loaded with NiTi closed coil spring with 1 N (Fig. 2). After the surgical procedures, each animal had 4 mini-implants, being 2 loaded and 2 unloaded, in all 72 mini-implants. 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. The removal torque test (RTT) was done using 5 samples from different animals and the SEM analysis was accomplished in 2 samples in each group (Table 1). The removal torque value and the stiffness were compared by 1-way analysis of variance (ANOVA) and when significant differences were found; the post hoc Tukey test was used (p < 0.05).

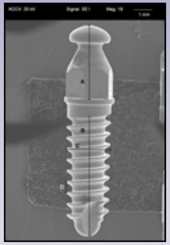

Fig. 1: Experimental implant: cylindrical screw of titanium alloy. (A) Hexagonal head with 3.4 mm height; (B) Active area with 6.0 mm in length; (C) 2.0 in diameter; (D) 0.51 mm between the top of the pitches. A total of 9 screw pitches and in the last 1.5 mm a flange to improve primary stability.

Fig. 2: Surgical procedure.

New Zealand Rabbits	18	
Mini-implants	72	2.0 mm x 6.0 mm
Load	Immediate	1 N
Time healing	1, 4, and 12 weeks	
Groups	6	
1 week unloaded (Un1w)	RTT (n=5)	SEM (n=2)
1 week loaded (Lo1w)	RTT (n=5)	SEM (n=2)
4 week unloaded (Un4w)	RTT (n=5)	SEM (n=2)
4 week loaded (Lo4w)	RTT (n=5)	SEM (n=2)
12 week unloaded (Un12w)	RTT (n=5)	SEM (n=2)
12 week loaded (Lo12w)	RTT (n=5)	SEM (n=2)

Table 1: Experimental design.

Removal torque test

The test was performed by applying a counter-clockwise rotation to the implant at a rate of 0.1º/s. The test was done with the mechanical traction on the vertical axis of the device resulting on removal torque force (Fig 3). In each removal test, the curve was recorded and the maximum value (N.mm) was considered as the removal torque value (RTV). The test was stopped when the implant had done a 90º rotation. The interfacial stiffness (STF) was defined as the slope of torque removal curve (Table 2).

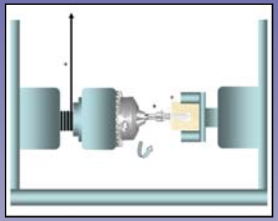

Fig. 3: Schematic of removal torque device. a, Vertical axis of universal test machine. Its vertical activation results in counter clockwise rotation. b, Insertion-removal key attached in the left grip. c, mini-implant inside the bone and the block attached in the right grip. The system maintains just a horizontal axis where the tests were realized.

Results

All mini-implants were removed without deformation in the head or in the long axis. The biomechanical performance of the mini-implants is demonstrated in a graph of RTV vs healing time (Fig. 4). There was no increasing in the bone/implant interface resistance between 1 and 4 weeks groups, but after 12 weeks both groups had higher values. The interfacial stiffness increased with the time healing as expected (Fig 5). Nevertheless, the statistical significance was just observed between 1 and 12 weeks groups, regardless the load.

Groups	RTV (mean ± sd)		Stiffness (mean ± sd)
Un1w (n =5)	15.21 ± 4.2 N.mm		0.55 ± 0.4 Nmm/ degree
Lo1w (n = 4)	12.76 ± 5.1 N.mm		0.34 ± 0.1 Nmm/ degree
Un4w (n = 5)	13.10 ± 5.7 N.mm		1.10 ± 0.7 Nmm/ degree
Lo4w (n = 4)	11.11 ± 5.4 N.mm		1.42 ± 0.3 Nmm/ degree
Un12w (n = 4)	54.38 ± 12.8 N.mm		1.88 ± 0.6 Nmm/ degree
Lo12w (n = 5)	32.90 ± 12.8 N.mm		2.19 ± 0.6 Nmm/ degree
Analysis of Variance	**p = 0.000001**		**p = 0.00011**
Post hoc Tukey test significant differences	**Un12w *vs* Un1w**: p= 0.0001	**Lo12w *vs* Un1w**: p= 0.0364	**Un12w *vs* Un1w** p = 0.0106
	Un12w *vs* Lo1w p= 0.0001	**Lo12w *vs* Lo1w** p= 0.0217	**Un12w *vs* Lo1w** p = 0.0046
	Un12w *vs* Un4w p= 0.0001	**Lo12w *vs* Un4w** p= 0.0156	**Lo12w *vs* Un1w** p = 0.0008
	Un12w *vs* Lo4w p= 0.0001	**Lo12w *vs* Lo4w** p= 0.0114	**Lo12w *vs* Lo1w** p = 0.0004
	Un12w *vs* Lo12w p= 0.0129	**Lo12w *vs* Un12w** p= 0.0129	**Lo12w *vs* Un4w** p = 0.0328

Table 2: Removal torque (RTV), Stiffness (STF) means and standard deviations of load and unload groups at the 3 time of analysis (1, 4, and 12 weeks). Comparisons statistically significant were showed (p<0.05).

RTV

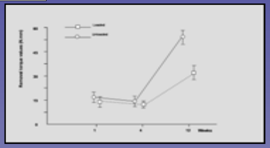

Fig. 4: RTV sequential graphs of load and unload groups after 1, 4, and 12 weeks of healing.

STF

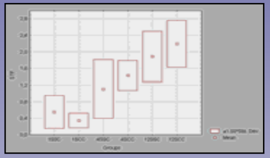

Fig. 5: Implant/bone interfacial stiffness values (N.mm/degree), elastic slope per angular degree of the load and unload groups in the healing periods.

SEM analysis

The blocks were dehydrated in graded series of ethanol (50-100%), and the critical point dried. Then, each block was divided into 2 halves, one of them containing the bone and the implant and the other just with the bone. The beginning of the division was done with a blade and then, the bone was cleaved by insertion of a wedge into the created area. Thus, the features of the bone/implant interface were preserved (Fig. 6). Afterwards, the samples were sputter coated with gold and examined in a SEM microscopy by secondary electrons incidence.

Fig. 6: Division of bone/titanium implant block.

Results

1 Week

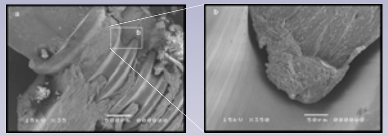

Fig. 7: (a) Unload bone/mini-implant interface after 1 week of healing. (b) Native bone present in the screw thread associated with wound tissue.

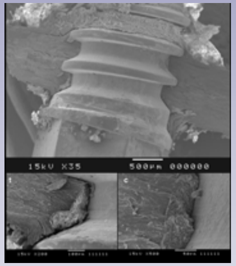

Fig. 8: (a) Loaded group after 1 week of healing. The distance between wound tissue and implant surface in tension side (t) is higher than in compression side (t) with 1 N application.

4 Weeks

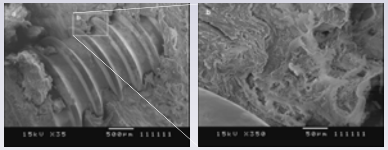

Fig.8: (a) The Un4w group maintained the association between the native bone and the wound healing tissue. (b) This tissue presented high amount of collagen fibers adjacent to the implant surface and the difference between the native bone and the new formed tissue was clearly observed.

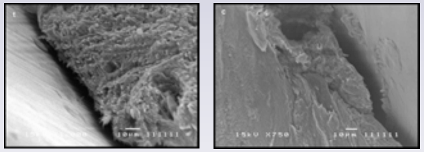

Fig.9: The Lo4w group presented less clear difference between the new bone formed and the host bone, with a new tissue less fibrous. The compression (c) and tension (t) did not demonstrated different interfacial tissue.

12 Weeks

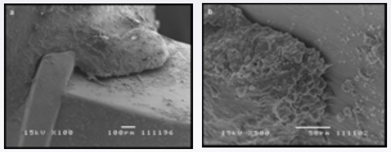

Fig.10: Bone formation after 12 weeks of healing without load. (a) Bone growing in the mini-implant head. (b) Globular feature of interfacial bone formation.

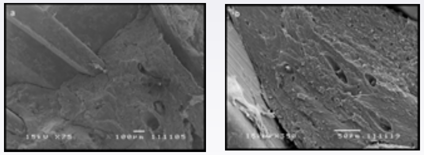

Fig.11: (a) Lo12w group demonstrated bone formation in the six-sided implant head. (b) Lamellar organization was observed in the new bone formed.

Conclusion

The sequential analysis of the interfacial tissue formed under immediate loading protocol indicated that:

(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, but the removal torque values were not statistically different.

(3) After 12 weeks of healing, the loaded group presented interfacial tissue more lamellar and lower removal torque value than the unloaded group, but suitable for orthodontic anchor purposes.