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Titanium alloy mini-implants for orthodontic anchorage: Immediate loading and metal ion release ☆

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Abstract

Removable osseointegrated titanium mini-implants were successfully used as anchorage devices in orthodontics. The early load is necessary to simplify the mini-implant methodology, but can lead to failure during osseointegration. The Ti–6Al–4V alloy was used instead of commercially pure Ti due to its superior strength. However, the corrosion resistance is low, allowing for metal ion release. The purpose of this work was to analyze the immediately loaded mini-implant fixation and to gauge the vanadium ion release during the healing process. Titanium alloy mini-implants were inserted in the tibiae of rabbits. After 1, 4 and 12 weeks, they were submitted to removal torque testing. There was no increase in the removal torque value between 1 and 4 weeks of healing, regardless of the load. Nevertheless, after 12 weeks, a significant improvement was observed in both groups, with the highest removal torque value for the unloaded group. The kidney, liver and lung were also extracted and analyzed by atomic absorption spectrometry. In comparison with the control values, the content of vanadium increased slightly after 1 week, significantly increased after 4 weeks and decreased slightly after 12 weeks, without reaching the 1 week values. A stress analysis was carried out which enables both the prediction of the torque at which commercially pure (CP) Ti and Ti–6Al–4V deform plastically and the shear strength of the interface. This analysis reveals that the removal torques for CP Ti dangerously approach the yield stress. The results of this rabbit model study indicate that titanium alloy mini-implants can be loaded immediately with no compromise in their stability. The detected concentration of vanadium did not reach toxic levels in the animal model.

Keywords: Titanium alloy; Mini-implant; Orthodontic anchorage; Ion release

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1. Introduction

Anchorage has long been a challenge since the introduction of fixed appliances in orthodontics [1]. Typically, orthodontic movement of a tooth is anchored by a large group of teeth so as to minimize undesired displacements of anchoring teeth. Adequate anchorage becomes difficult when posterior teeth are missing. Intra- and extra-oral auxiliary devices can be used to assist movement, but the effectiveness of these measures is dependent upon the level of patient cooperation [1].

Conventional titanium implants have emerged as an excellent alternative to traditional orthodontic anchorage methodologies, mainly when anchorage dental elements are insufficient in quantity or quality [2]. Unfortunately, conventional dental implants can only be placed in limited sites, such as the retromolar and edentulous areas [2,3]. In

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addition, conventional dental implants are troublesome for patients because of the severity of the surgery, the discomfort of the initial healing and the difficulty of maintaining oral hygiene [4].

Due to these disadvantages, Kanomi [5] proposed titanium mini-implants (1.2 mm in diameter and 6.0 mm in length) for orthodontic anchorage. They are widely used since they have few implantation site limitations, a simple insertion procedure and easy mechanical force control [6]. The methodology for implementation of mini-implants has been continuously improved. Some complications persist, and the sources of failure include the inflammation of the soft tissue around the mini-implant and fracture of the mini-implant [6].

A period of healing is usually necessary before applying load to conventional dental implants. This period varies from 4 to 6 months in humans [7,8]. When the load is placed prematurely, histological analyses have suggested that there is no uniform intimate bone-implant contact due to interplayed fibrous tissue [9,10]. This phenomenon could be favorable for implants for orthodontic anchorage purposes, since it facilitates the surgical removal of the implant at the end of the orthodontic treatment. On the other hand, the excess of interplayed fibrous tissue could lead to implant failure.

Commercially pure titanium (CP Ti) is widely used as implant material because of its suitable mechanical properties and excellent biocompatibility [11,12]. However, CP Ti has lower fatigue strength than titanium alloys. Ti–6Al–4V can be used to overcome this disadvantage [12,13]. However, the corrosion resistance of the mini-implant decreases when the alloy is used, favoring metal ion release, which has been associated with clinical implant failure, osteolysis, cutaneous allergic reactions, remote site accumulation [14], kidney lesion [15], cytotoxicity, hypersensitivity and carcinogenesis [16].

The purpose of this work was to measure the bone anchorage of immediately loaded Ti–6Al–4V mini-implants by removal torque test, and the amount of vanadium ion release in remote tissues by atomic absorption spectrometry.

2. Experimental

2.1. Materials

Seventy-two Ti–6Al–4V alloy mini-implants (Conexão Sistemas de Próteses, SP, Brazil) were used. The mini-implants had a cylindrical screw design, were 2.0 mm in diameter and 6.0 mm in length, and had a hexagonal-shaped head that was 3.4 mm in length. The mini-implants were machined by turning, cleaned, passivated with nitric acid (HNO_3) and sterilized. No surface treatment was applied to alter the roughness (Fig. 1). Ni–Ti closed coil springs were used as loading devices for half of the mini-implants.

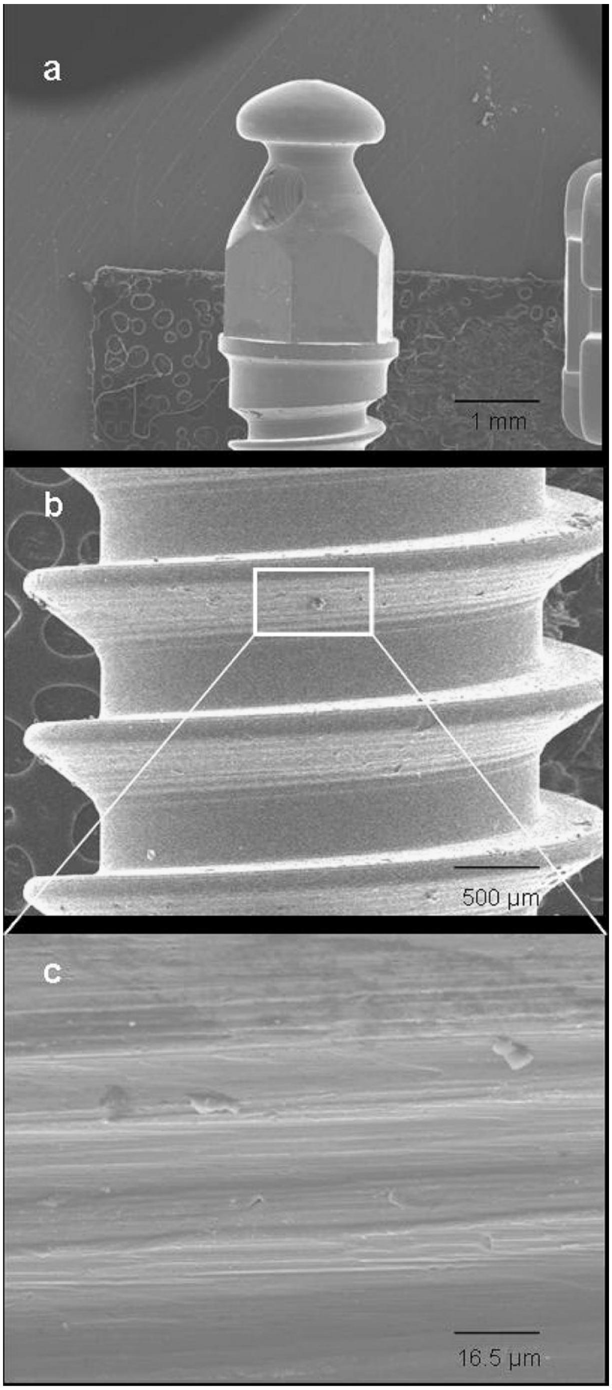

Fig. 1. Titanium alloy mini-implant: (a) hexagonally shaped head; (b) cylindrical screw design; (c) as-machined surface.

2.2. Animals

Twenty-three 6-month-old male New Zealand white rabbits, weighing between 3.0 and 3.5 kg, were used in the research. The surgical procedures exercised were common to the 18 experimental animals and consisted of the implantation of four mini-implants into the left tibial metaphyses of each animal. All surgeries were performed under sterile conditions in a veterinary operating room.

Five rabbits were used as controls for the metal ion release study.

The protocol for the animal study was approved by the standing ethics committee on animal research of Oswaldo Cruz Institute and all procedures were done based on Canadian Council of Animal Care guidelines. In the pre-surgical period, the rabbits were conditioned in a vivarium, where they remained during an observation period. For the duration of the study, they had free access to pellets and water, and were maintained at temperature from 22 to 26 °C with daily illumination.

2.3. Experimental design

Each animal had four mini-implants inserted, two of which were immediately loaded with a force of 1 N. Thirty mini-implants were used in the removal torque test and the other 42 mini-implants were used in other analysis. The groups were formed to investigate three periods of healing: 1 week, 4 weeks and 12 weeks. In each assessment period, one group was loaded and another was unloaded, giving a total of six groups. The removal torque test was carried out to analyze the bone fixation of the mini-implants during the healing process.

The atomic absorption spectrometry analysis was performed on the kidney, liver and lung in order to analyze whether vanadium ion release occurs and if these metal ions accumulate in remote tissues. The three tissues were extracted from the 18 experimental rabbits at the times previously established (1, 4 and 12 weeks) and from the five control rabbits, in which no treatment was administered, totaling up to 12 groups (Table 1).

2.4. Surgical procedure

The animals were anesthetized with an intra-muscular injection of Tiletamine (5 mg/kg) and Zolazepan (5 mg/kg), followed by continuous delivery of 2% Halothane and Isofluthane throughout the surgery. The hair on the medial surface of the upper portion of the left leg was removed and the skin was cleansed with iodinate surgical soap. A 70% alcohol solution was used for the local prophylaxis. A 50 mm-long incision was made parallel to the longitudinal axis of the tibia and the periosteum was stripped, denuding the bone. The implantation holes were drilled under profuse saline irrigation, employing a drill with a bit diameter of 1.6 mm operating at low rotatory speed. Four perforations were made at 5 mm intervals. The mini-implants were threaded at the first cortex of the tibia, using a holder key. In each animal, the two central mini-implants were loaded with Ni–Ti coil springs with 1 N force (Fig. 2). Afterwards, the soft tissues were closed in layers with absorbable sutures.

Table 1

Experimental design	
New Zealand rabbits	23
Mini-implants	Ti–6Al–4V
Removal torque test	
Groups	6
Time assessment	1, 4 and 12 weeks
Atomic absorption analysis	
Groups	12
Time assessment	Control, 1, 4 and 12 weeks
Tissue analyzed	Kidney, liver and lung

2.5. Removal torque test

Following the healing time, the animals were euthanized by exsanguination. The tibiae were dissected, and blocks containing one mini-implant and at least 2 mm of surrounding bone were sectioned (Fig. 3). The blocks containing the mini-implant and the adjacent tissues were refrigerated and hydrated until testing. The tests were

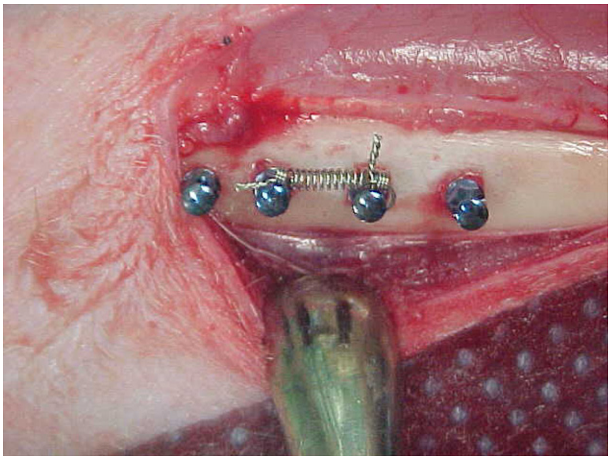

Fig. 2. Immediate loading (1 N) of the two central mini-implants with Ni–Ti closed coil springs.

Fig. 3. Experimental block with mini-implant and about 2 mm of bone in each side.

performed at room temperature. A device containing a rotational grip aligned with a fixing grip was used to achieve alignment during the test and to avoid bending forces. This device was used in association with a universal mechanical testing machine in which the vertical traction resulted in torsion of the rotational grip and the removal torque test. To set up the mechanism, a holder key was attached to the rotational grip of the device, the head of the mini-implant was attached to the holder key and the bone block was attached to the fixation grip. The tests were performed with the mechanical traction on the vertical axis of the device resulting in a counter-clockwise rotation to the implant at a rate of $0.1° \, s^{-1}$ and an increasing removal torque force. For each removal test, the curve was recorded and the maximum value in N mm was taken as the removal torque value (RTV); the test was stopped when the implant had undergone a 90° rotation.

2.6. Atomic absorption spectrometry

The selected organs (kidney, liver, and lung) were removed from each rabbit, weighed, washed with deionized water, and stored in plastic vessels at −30 °C for 24 h. The samples were initially dehydrated at 60 °C for 48 h, chopped up and kept in the stove at 60 °C for 7 days, until total dehydration. Two samples of 0.5 g of each tissue were weighed in glass beakers and calcinated at 400 °C for 5 days to remove the tissues organic portion. The resulting powders were poured in glass tubes and mixed with 2 ml of 65% nitric acid (HNO_3). The tubes were sealed for 24 h and then placed in a bath at 60 °C for 4 h for sample digestion. Empty beakers were used as blanks and subjected to all steps of analysis [17].

The vanadium content was measured by graphite furnace atomic absorption spectrometry (AAS) [14,17,18] with background correction by a transverse microprocessor-modulated bipolar Zeeman magnetic field, using an AAS ZEEnit® 60 with an MPE 60 z autosampler (CGS Analitical Instrumentation Ltda, São Paulo, Brazil).

The statistical analysis for reporting the mean and standard deviation of data from RTV and AAS analysis were performed for all the groups. For significance of differences the data were evaluated by a one-way analysis of variance (ANOVA) test followed by the post hoc Tukey test. The significance limit was predetermined in the confidence interval of 5%.

3. Results and discussion

3.1. Sequential removal torque test

All 30 mini-implants were inserted and removed without fracture or deformation. The rabbits did not exhibit any complication, such as infection or leg fractures, during the healing process. Three mini-implants were excluded from the test due to the high mobility in the bone site during the sample preparation. These samples were considered lost implants. The 10% of failure observed was similar to other in vivo studies [19,20], where it was considered a good result. Since the bone healing around the mini-implant depends on various factors that are difficult to control in vivo (i.e., the micromotion), and since it occurred in both the loaded and unloaded groups, we believe that the three failures found are not related to the immediate load.

The fixation torques of the mini-implants did not demonstrate improvement after 1 or 4 weeks of healing (Tables 2 and 3), with any statistical difference ($p < 0.05$) between the loaded and unloaded groups. After 12 weeks, both groups demonstrated increased removal torque values, with the highest values for the 12-week-unloaded group. In this manner, until 4 weeks of healing, the immediately applied load did not result in any improvement or worsening of the fixation of the mini-implant. When comparing the 1-week and 4-week groups with the 12-week groups, the measurements were statistically different. The comparison between the 12-week groups indicated significantly higher removal torques values to the unloaded group (Tables 2 and 3, Fig. 4).

The required reduction of the size of the mini-implant could result in fracture during the insertion and removal procedures, mainly when they reach osseointegration [6].

Table 2
Descriptive statistic of removal torque values (RTV) in Nm

Groups	Mean	SD	*N*
1-week-unloaded	15.21	4.2	5
1-week-loaded	12.76	5.1	4
4-weeks-unloaded	13.10	5.7	5
4-weeks-loaded	11.11	5.4	4
12-weeks-unloaded	54.38	12.8	4
12-weeks-loaded	32.90	12.8	5

SD: standard deviation; *N*: number of samples.

Table 3
Two-way ANOVA and post hoc Tukey test for comparison of means of removal torque values

Term	Probabilities for post hoc tests interaction: 1 × 2					
Load	Load × Unload → 0.0174					
Time	1w × 4w → 0.8869		1w × 12w → 0.0001		4w × 12w → 0.0001	
Load and time	{1} 15.21	{2} 13.10	{3} 54.38	{4} 12.76	{5} 11.11	{6} 32.90
Un 1w {1}	–	0.9986	0.0001	0.9984	0.9823	0.0364
Un 4w {2}	0.9986	–	0.0001	1.000	0.9994	0.0156
Un 12w {3}	0.0001	0.0001	–	0.0001	0.0001	0.0198
Lo 1w {4}	0.9984	10.000	0.0001	–	0.9997	0.0320
Lo 4w {5}	0.9823	0.9994	0.0001	0.9997	–	0.0176
Lo 12w {6}	0.0364	0.0156	0.0198	0.0320	0.0176	–

Un: unloaded; Lo: loaded; w: weeks.

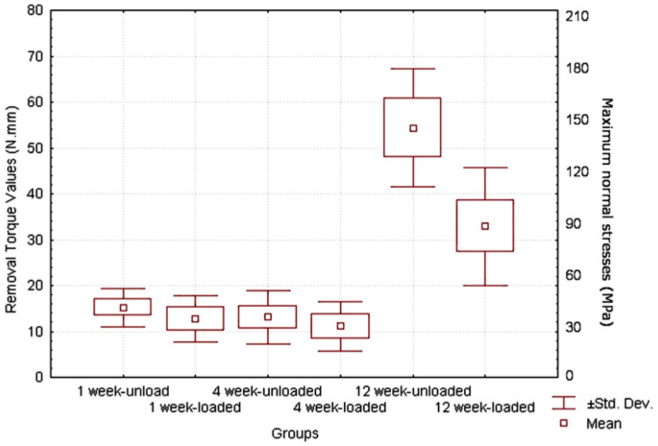

Fig. 4. Box plot of the removal torque test; the left hand side shows removal torques; the right hand side shows normal stress on mini-implant.

Miyawaki et al. [19] described the relation between the variation of the diameter of the mini-implant and failure. They obtained a rate of 100% of failure in the use of mini-implants 1.0 mm in diameter in humans. Büchter et al. [21] reported fractures during insertion and removal of mini-implants in a pig study. Huang et al. [22] described how the use of titanium alloys could overcome this disadvantage. In fact, in the present study, no fractures were observed during the insertion or removal of Ti–6Al–4V alloy mini-implants.

The success of the early loading of the implants is related to the primary stability just after implantation [23–26]. The primary stability depends mainly on the implant design and the quality of the host bone [6,26–28], but the relation between the diameter of the drill and the implant diameter is also an important factor, especially when the insertion is into cancellous bone [27]. In the present study, all 30 mini-implants were clinically immobile after insertion and immediate loading was applied. The RTV after 1 week of healing indicated that the primary stability was not affected by the immediate loading. Paik et al. [25] used mini-implants of 1.6 mm diameter for intermaxillary fixation of surgical patients. A total of six self-drilling mini-implants were inserted under immediately loaded conditions. All were fixed and were easily removed after 2 weeks. In the present study, the immediate loading of the mini-implants also did not compromise its efficiency, despite the analysis period being longer (12 weeks) than that of Paiks's work.

Deguchi et al. [29] described the reduction of the bone-implant contact 3–6 weeks after the mini-implant insertion as a normal occurrence in the course of healing events. This is consistent with our results, which did not show any statistically significant improvement in the removal torque values after 4 weeks of healing, with or without load. Histological analysis is required to determine the quality of new tissue formed after 4 weeks of healing, but the immediate load did not compromise the fixation of the mini-implants in this phase of the study.

After 12 weeks of healing, both 12-week groups were significantly higher than the 1-week and 4-week groups. This indicates that the mini-implants reached fixation values sufficient for orthodontic purposes, with or without load. Although the 12-week-unloaded group presented the highest RTV, this can be interpreted as a positive result, as the mini-implants have to be removed after the orthodontic treatment. Comparison between human and rabbit bone physiology indicates that the relative bone metabolic rate is three times faster in the rabbit [23]. In this manner, a longer term study would be interesting.

Some researchers have tested CP Ti subjected to early loading [25,30–32]. Freudenthaler et al. [31] have studied mesial molar movement in anchored mini-implants loaded immediately with 1.5 N. One mini-implant was lost in a total of 15 tested after 3 weeks. Using Ti–6Al–4V alloy we lost three implants out of a total of 30 tested with 1 N immediate loading.

The forces in the orthodontic treatment vary greatly depending upon the type of movement required. Orthodontic forces of low value, ranging from 0.2 to 2 N, are used to move few teeth. Orthodontic forces from low to medium values are used to move a high number of teeth. Orthopedic forces are also required in orthodontic treatment to move maxillary and mandibular bone. This force has high values, ranging from 4 to 15 N [2]. A force of 1 N was chosen to be used in this study due to the fact that it is the most used force in orthodontic treatment and has been used in many animal studies [21,23,32,33]. Szmukler-Moncler et al. [27] concluded that a force less than the overload limit does not cause the loss of stability, and Bütcher et al. [21] showed that tip forces higher than 9 N could compromise the fixation of the mini-implant. Oyonarte et al. [33,34] added that the overloading limit depends on the implant design. In screw design implants, the susceptibility of overload is highest along the first treads used to place the implant into the bone, and approaching the limit induces marginal bone loss. Roberts et al. [23] reported that forces ranging between 1 and 3 N applied after 6–8 weeks of healing do not compromise the stability of the implants. Infact, the 1 N immediate load force applied in the Ti–6Al–4V mini-implant screw design affected the fixation of the mini-implants, but not so substantially as to disrupt adequate orthodontic anchorage.

3.2. Stress analysis

From the torque values it is possible to obtain both the maximum torsional stresses in the mini-implant and the interfacial shear stresses.

3.2.1. Maximum stresses in implants

The maximum torsional stress $\tau_{\max}$ in a cylindrical body with diameter D is related to the torsional moment T [35]:

$$\tau_{\max} = \frac{16T}{\pi D^3}. \qquad (1)$$

The corresponding normal stress is equal to [36]:

$$\sigma_{\max} = \frac{\tau_{\max}}{\sqrt{3}} = \frac{16T}{\sqrt{3}\pi D^3}. \tag{2}$$

Eq. (2) enables the calculation of the maximum stress acting on the mini-implants. At the highest torque observed of 5.38×10^{-2} Nm, $\sigma_{\max} = 148$ MPa. The yield stresses for CP Ti and Ti–6Al–4V are:

Grade 2 CP titanium: $\sigma_y = 250$ MPa;
Grade 5 Ti–6Al–4V: $\sigma_y = 880$ MPa.
If one computes safety factors:
(SF) CP Ti = 1.7;
(SF) Ti–6Al–4V = 6.0.

Thus, we can conclude that CP Ti is indeed stressed on average close to its yield value, which is an undesirable situation, since it is certain some mini-implants will have a release torque above the mean value used above. On the other hand, the Ti–6Al–4V alloy is stressed significantly below its yield stress and is indeed a much safer choice.

3.2.2. *Interface stresses*

The shear stress acting on the interface, τ_i can also be calculated from the torsional moment T:

$$\tau_i = \frac{2T}{DA_T}. \tag{3}$$

The area A_T is the total resisting area that has to be computed from the dimensions shown in Fig. 5. Approximately three threads penetrate through the cortical bone and it is assumed that the trabecular bone does not contribute significantly to interfacial bonding.

The area A_T is computed from:

$$A_T = 3(A_1 + A_2 + A_3) = 3\left[\pi(1.48/2)^2 \times 0.225 + \pi(1.75/2)^2 \times 0.275 + \pi(1.75/2)^2 \times 0.26\right] = 3 \times 1.675 \approx 5\ \text{mm}^2. \tag{4}$$

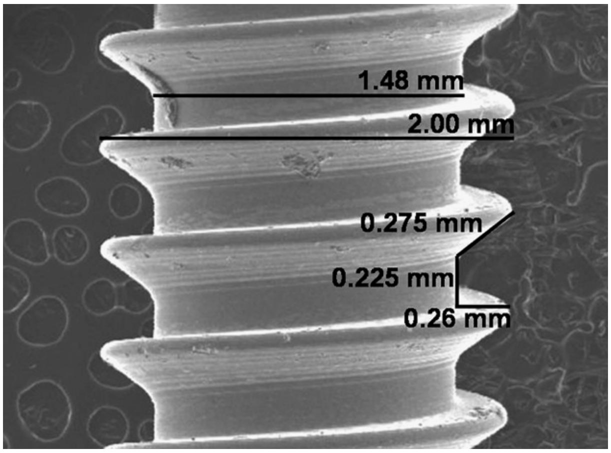

Fig. 5. Dimensions of the mini-implant.

Taking a mean diameter of 1.75 mm, one obtains, from Eqs. (3) and (4):

$$\tau_i = \frac{2T}{1.75 \times 5 \times 10^{-9}}.$$

The maximum removal torque was 54.38×10^{-3} Nm. This corresponds to:

$$\tau_i = 12.34\ \text{MPa}.$$

It is instructive to compare this with the mechanical resistance of bone. The strength of canine cortical bone is approximately 190 MPa [37]; the corresponding shear strength is equal to 85 MPa. This value is significantly higher than the interfacial strength. On the other hand, the strength of canine trabecular bone is of the order of 13 MPa [37], giving a shear strength of 6.5 MPa. It can be concluded that the interface shear strength is slightly higher than the strength of trabecular bone but significantly lower than cortical bone.

3.3. *Vanadium remote site accumulation*

Any metal or alloy which is implanted in the body is a potential source of toxicity [38]. Titanium has been the material most commonly used for dental implants. It is considered chemically inert, biocompatible with human tissue and resistant to corrosion by human body fluids. However, due to the small size required by orthodontic mini-implants, CP Ti can fail during insertion or removal procedures, as demonstrated by the calculations in Section 3.2. To overcome this disadvantage, the material of choice for orthodontic mini-implants is Ti–6Al–4V alloy. The small percentages of vanadium and aluminum atoms contained in the alloy are potentially toxic [12] because of the corrosion fatigue which implants suffer in body fluids [40]. The most harmful components in alloys were determined to be cobalt from the Co–Cr alloy, nickel from stainless steel and vanadium from the Ti–6Al–4V alloy [13].

Vanadium is an ultratrace element occurring in most mammalian cells [18]. The main source of vanadium intake is food [15]. Although vanadium has been assumed as being an essential trace element [18,38], no biological function has been identified [15]. The pharmacological and physiological actions of vanadium have been investigated. Short-term clinical trials with diabetic patients suggest that vanadium may have a potential role in the adjunctive therapy of these patients due to its insulin-like properties [18]. The acute and chronic toxic effects of this element when absorbed in greater amounts are well documented [18]. Vanadium can be cytotoxic for macrophages and fibroblasts [38,39], can be bound by various iron proteins (ferritin and transferrin), which affects the distribution and accumulation of vanadium in the body [18,38], can incite local and systemic reactions, and can inhibit cellular proliferation [16]. Kidney lesions have also been observed in animal studies [15]. Vanadium exhibits appreciable tissue binding and can accumulate in tissues such as liver, kidneys, bone and spleen [18]. Studies on mice show that within the first day after intra-venous injection of vanadium the highest levels are found in the kidney [38]. Urinary excretion is the main elimination pathway for injected vanadium in humans [18].

The statistics of the vanadium content in the kidney are shown in Tables 4 and 5. The control group had the lowest vanadium content at 0.423 ppb. After 1 week, the content increased slightly to 0.488 ppb. At 4 weeks, the highest values were measured to be 0.758 ppb. After 12 weeks, the vanadium content decreased somewhat (0.558 ppb), but it did not reach the values observed after 1 week of healing. All the means were statistically different from each other ($p < 0.05$).

The means and standard deviations of the vanadium content in the liver are shown in Tables 6 and 7. The lowest vanadium content was seen in the control group (0.434 ppb). This value increased after 1 week (0.508 ppb), even more at 4 weeks (0.785 ppb), and had a slightly decrease after 12 weeks (0.572 ppb). All the means were statistically different between each other ($p < 0.05$).

For the lung, the means and standard deviations of the vanadium content can be seen in Tables 8 and 9. For this tissue, the lowest vanadium content was seen in the control group (0.428 ppb), followed by the 1-week group (0.461 ppb). After 4 weeks the value was higher (0.812 ppb), with a decrease at 12 weeks (0.553 ppb). There was no statistical difference between the control and the 1-week groups. For all other comparisons the means were statistically different ($p < 0.05$).

This pattern of vanadium release may be associated to the electrochemical and mechanical behavior of the surface oxide film. The surface film on Ti–6Al–4V is TiO_2, containing a small amount of Al_2O_3, hydroxyl groups and bound water. Vanadium is not responsible for the formation of the surface film of Ti–6Al–4V [13,40]. When Ti–6Al–4V alloys are implanted, changes in the alloy's protective surface oxide occur and these changes may influence the release of alloy corrosion products. These changes occur because the concentration of chloride ion in serum and interstitial fluid makes these seriously corrosive environments for metallic materials; body fluid contains various amino acids and proteins that influence metallic corrosion; the concentration of dissolved oxygen in body fluid is one-quarter of that in air, delaying the regeneration of surface oxide film; the pH of the hard tissue into which material is implanted decreases to approximately 5.2 then recovers to 7.4 within 2 weeks, and cells also behave as charging bodies which may influence the corrosion of metallic materials [13].

Therefore, surface oxides and their properties play a significant role in the corrosion process since the mechanical

Table 4
Descriptive statistic of vanadium content in the kidney in ppb (ng mg^{-1})

Groups	Mean	SD	*N*
Control	0.423	0.053	10
1-week	0.488	0.021	12
4-weeks	0.758	0.042	12
12-weeks	0.558	0.049	12

SD: standard deviation; *N*: number of samples.

Table 5
Post hoc Tukey test for comparison of means of vanadium content in the kidney

Groups	Probabilities for post hoc tests main effect: groups			
	{1} 0.4230	{2} 0.488	{3} 0.758	{4} 0.558
Control {1}	–	0.0054	0.0002	0.0002
1-week {2}	0.0054	–	0.0002	0.0012
4-weeks {3}	0.0002	0.0002	–	0.0002
12-weeks {4}	0.0002	0.0012	0.0002	–

Table 6
Descriptive statistic of vanadium content in the liver in ppb (ng mg^{-1})

Groups	Mean	SD	*N*
Control	0.434	0.033	10
1-week	0.508	0.047	12
4-week	0.785	0.046	12
12-weeks	0.572	0.056	12

SD: standard deviation; *N*: number of samples.

Table 7
Post hoc Tukey test for comparison of means of vanadium content in the liver

Groups	Probabilities for post hoc tests main effect: groups			
	{1} 0.434	{2} 0.508	{3} 0.785	{4} 0.572
Control {1}	–	0.0035	0.0002	0.0002
1-week {2}	0.0035	–	0.0002	0.0104
4-weeks {3}	0.0002	0.0002	–	0.0002
12-weeks {4}	0.0002	0.0104	0.0002	–

Table 8
Descriptive statistic of vanadium content in the lung in ppb (ng mg^{-1})

Groups	Mean	SD	*N*
Control	0.428	0.027	10
1-week	0.461	0.040	12
4-week	0.812	0.054	12
12-weeks	0.553	0.043	12

SD: standard deviation; *N*: number of samples.

Table 9
Post hoc Tukey test for comparison of means of vanadium content in the lung

Groups	Probabilities for post hoc tests main effect: groups			
	{1} 0.428	{2} 0.461	{3} 0.812	{4} 0.553
Control {1}	–	0.2785	0.0002	0.0002
1-week {2}	0.2785	–	0.0002	0.0002
4-weeks {3}	0.0002	0.0002	–	0.0002
12-weeks {4}	0.0002	0.0002	0.0002	–

properties and electrochemical behavior of the oxides affect the fracture and repassivation processes. After fracture of the Ti–6Al–4V surface oxide, it takes a long time to reform, and metal ions are released during repassivation [13,41]. The initial ion release is dominated by ion exchange events at the solid–liquid interface, which occur before thermodynamic equilibrium of the surface protein adsorption and desorption events has been reached. Once the latter is achieved, further ion exchange events may be inhibited [42]. We concluded that at 1 week of implantation there was not sufficient interaction at the solid–liquid interface. At 4 weeks, large numbers of ion exchange events were taking place at the interface. The thermodynamic equilibrium seems not to be reached at 12 weeks. More refined experimental protocols are needed to identify when this event occurs.

Dietary intake of several elements has been surveyed by various investigators [15,43]. The daily intake of a specific element may vary considerably according to different eating and drinking habits and geographical location, while large discrepancies may occur between the various compilations with regard to intake of a specific element [43]. The amounts of vanadium released in rabbits from the presently surveyed orthodontic Ti–6Al–4V alloy mini-implants were far below the daily intake of this element through food and drink ($1.8\ \mathrm{mg\ day}^{-1}$) [15,43]. Gioka et al. [44] measured in vitro traces of vanadium (2 ppm) released from Ti–6Al–4V orthodontic brackets and it was considered that vanadium release was minimal. The authors pointed out that long-term release may be higher than that occurring within the first weeks. However, in contrast to the long-term biomedical applications of Ti alloys in orthopedics, the orthodontic use of Ti alloy mini-implants has a limited service life [44]. Thus, the minute levels of vanadium release may not constitute an alarming situation, since they did not reach toxic levels in the animal model.

The use of rabbits in implant studies is widely diffused [3,17,23] due to the correlation between rabbit and human physiology. The bone turnover in humans is 18 weeks, whereas it is about 6 weeks in rabbits, suggesting that a factor of 3 is a good rule for extrapolating rabbit data to the clinical situation [23]. However, care should be taken in extrapolating the clinical behavior from animal tests since the dimensions of the tested material in relation to the biological system of the rabbits could greatly influence the results [44].

The presence of elements with potential biologically hazardous action, especially vanadium, has increased the interest in adopting other alternatives, such as the development of new titanium alloys employing Nb as a beta stabilizer (Ti–6Al–7Nb) [44], and CP titanium with nanoscale grains, which has greater strength than the conventional Ti–6Al–4V alloy. These implant materials are corrosion resistant and biocompatible with human body organs and fluids, so they can remain in the body for years [12].

4. Conclusions

The results of this rabbit model study indicate that immediately loaded and unloaded titanium alloy mini-implants reached fixation values appropriate for the purposes of orthodontic anchorage. The unloaded group had a higher RTV than the loaded group. Since the mini-implants have to be removed at the end of the treatment, immediate loading could be a favorable option in treatment.

The absence of fractures during insertion and removal of the mini-implants indicated that the Ti–6Al–4V alloy has adequate mechanical properties for this application. Despite the tendency of greater ion release when using the titanium alloy, the amount of vanadium detected did not reach toxic levels in the animal model, even at 4 weeks, when the maximum concentrations were measured.

The normal stresses acting on the mini-implants and the shear stresses acting at the interface were calculated from the removal torques. The maximum normal stress values (148 MPa) approach the yield stress of CP Ti ($\sim$250 MPa) but are much lower than the yield strength of Ti–6Al–4V. This analysis suggests that the 2 mm-diameter mini-implants of CP Ti are unsafe and proves that Ti–6Al–4V is a safe choice. The shear strength of the interface was calculated and its value was found to be between the shear strengths of cortical and trabecular bone.

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