Title: Roughness and wettability effect on histological and mechanical response of self-drilling orthodontic mini-implants.

Short title: Improvement of self-drilling orthodontic mini-implants.

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Title: Roughness and wettability effect on histological and mechanical response of self-drilling orthodontic mini-implants.

Abstract

Objectives: Self-drilling orthodontic mini-implants can be used as temporary devices for orthodontics treatments. Our main goal was to evaluate surface characteristics, roughness and wettability, of surface modified mini-implants to increase their stability during orthodontic treatment without inducing bone fracture and tissue destruction during unscrewing.

Materials and Methods: Modified mini-implants by acid etching, grit-blasting and its combination were implanted in 20 New Zealand rabbits during 10 weeks. After that, it was determined the bone-to-implant (BIC) parameter and measured the torque during unscrewing. It was also measured surface characteristics, roughness and wettability, onto modified Ti c.p. discs.

Results: Acid-etched mini-implants ($R_a \approx 1.7$ µm, CA $\approx 66^{\circ}$) significantly improved the bone-to-implant parameter, 26%, compared to as-machined mini-implants ($R_a \approx 0.3$ µm, CA $\approx 68^{\circ}$, BIC=19%) due to its roughness. Moreover, this surface treatment didn't modify torque during unscrewing due to their statistically similar wettability ($p>0.05$). Surface treatments with higher roughness and hydrophobicity ($R_a \approx 4.5$ µm, CA $\approx 74^{\circ}$) lead to a greater BIC and to a higher removal torque during unscrewing, causing bone fracture, compared to as-machined mini-implants.

Conclusions: Based on these *in vivo* findings, we conclude that acid etching surface treatment can support temporary anchoring of titanium mini-implants.

Clinical relevance: This treatment represents a step forward in the direction of reducing the time prior to mini-implant loading by increasing their stability during orthodontic treatment, without inducing bone fracture and tissue destruction during unscrewing.

KEY WORDS: Mini-implants; surface treatments; osseointegration; torque; in vivo animal studies

INTRODUCTION

Mini-implants are temporally placed miniscrews for orthodontic anchorage, with diameters ranging from 1.8 to 2.9 mm and lengths from 4.0 to 21.0 mm [1-3]. Their advantages, owing to its small size and 1-piece design, include minimal anatomic limitations, flapless surgical procedure, decrease of postsurgical discomfort and morbidity for patients, immediate loading, and lower costs [3,4]. Since mini-implants are intended for immediate load of prosthesis and provide anchorage in the orthodontic treatment of patients for specific time periods, mini-implants anchorage mostly rely on mechanical retention and do not always osseointegrate.

Different studies report short-term survival rates over 90% during the first year of implantation similar to standard width implants [1,3,5]. The reason for such success rates is principally the size of the mini-implants, increasing screw diameter and length increases the success rate but also the risk of root damage. Mini-implants of at least 1.2 mm diameter and 8 mm length have sufficient stability with minimum risk of root damage [2,6]. However, nearly no clinical studies report long-term survival more than 1 year of implantation, [3], indicating a limited scientific evidence about long-term survival. First, primary implant stability is a necessary condition to achieve immediate loading of the mini-implant. Then, after implant surgery, mechanical stability is gradually replaced by biological stability, secondary, produced by bone-to-metal interface osseointegration. The clinical appropriate stability refers to the lack of clinical mobility [7-9].

Biomechanical resistance of a rigid implant to orthodontic loads is related to both the quality and the quantity of the integrated interface [8]. Deguchi et al. [10] demonstrated that inserted mini-implants with low bone-to-implant (BIC) parameter, around 5%, successfully resisted orthodontic force. Suggesting that surface modification based in increasing bone index contact may not be decisive, but the quality of the bone, when using mini-implants as orthodontic anchors [11]. However, there is a positive linear relationship between unscrewing torque and both, BIC and bone mineral density, at the bone-implant interface [12]. Eventually, a bone density below 0.4 g/ml and 50% BIC, the removal torque can reach a minimum of 50 N·cm. Although the mechanical stability is very important for the mini-implant fixation, these stress levels could produce the bone fracture when the orthodontist removes the mini-implant. Subsequently, it is essential to find out a compromise between mini-implants stability, during orthodontic treatment, without producing bone fracture during unscrewing, to increase the use of these temporary devices by the orthodontists.

The major goal of this study was to evaluate and control the effect of surface characteristics of modified self-drilling orthodontic mini-implants, by grit-blasting and acid-etching procedures, to find out a compromise between an adequate osseointegration and acceptable unscrewing, avoiding bone fracture and tissue destruction. All the results were supported by *in vivo* studies with New Zealand rabbits of the mini-implant osseointegration.

EXPERIMENTAL METHODS

Surface modification of titanium surfaces and mini-implants

8 mm diameter discs, for surface characterisation, and screw-shaped mini-implants of c.p. titanium (HDC^R 2 mm diameter, 9 mm length), for animal experiments, were used (Fig. 1D). Modified surfaces and mini-implants, and their control, were codified as follows:

- as-machined (Ctr);
- acid-etched (AEtch) in 0.35 M HF for 15 s at 25 °C;
- grit-blasted (GBlast) with alumina particles (600 µm size) with 0.25 MPa blasting-pressure until roughness saturation;
- grit-blasted with acid-etched treatment (GBlast+AEtch)

After surface treatments were performed, all titanium c.p. dics and mini-implants underwent a cleaning protocol consisting of 15 min with acetone, 15 min with bidistilled MilliQ water and drying with nitrogen gas.

Surface characterization

Surface roughness was evaluated by means of a white light interferometer microscopy (Wyko NT1100 Optical profiler, Veeco Instruments, USA) on Ti c.p. discs. A Gaussian filter was used to separate waviness and form from the roughness of the discs surface. The following cut-off values were applied according to ISO 16610-21:2011 standard: λ_c = 2.5 mm, for micro-rough AEtch, GBlast, and GBlast+AEtch surfaces and λ_c = 0.25 mm for control discs surfaces. Data analysis was performed with Veeco Vision 4.10 software (Veeco Instruments, USA). Amplitude, R_a-arithmetic deviation profile, and spacing, P_c-peak density, roughness parameters were determined.

The measurements of static contact angle (CA) on Ti c.p. discs were performed using a contact angle video based system (OCA 15 plus, Dataphysics, Germany) through the sessile drop method and analysed with the SCA20 software (Dataphysics, Germany). The measurements were obtained with ultra-pure distilled water at 25 °C under saturated humidity.

Roughness and wettability parameters were measured by triplicate for each disc and for each surface treatment.

In vivo animal experiments

Ti c.p. mini-implants were sterilized with ethylene oxide at 37 °C during 5 h at 760 mbar, 18 h forced aeration and 24 h natural aeration prior to *in vivo* studies. A total of 20 mini-implants divided into four groups according to referred surface finish were implanted, two per animal, during 10 weeks. Ten female adult New Zealand White rabbits were operated under general anesthesia performed by intramuscular injections following a protocol approved for the study by the Faculty of Veterinary Sciences of the University of Córdoba (Spain). The self-drilling mini-implants were inserted bone centred at the lateral condyle with a 10 N·cm torque, after lateral bilateral knee arthrotomy. The New Zealand White rabbits were euthanized under general anesthesia after 10 weeks implantation. Torque necessary to unscrew was evaluated with a torque scale.

Femoral condyles were harvested and peripheral soft tissue was removed. Specimens were fixed for 7 days in 4 % formaldehyde neutral solution, rinsed in water, dehydrated in graded series of ethanol (from 70 to 100 %) and then embedded in polymethyl methacrylate (Technovit 7200 VLC, Kulzer-Heraus, Germany). Finally, each implant was sectioned along the longitudinal axis with a diamond circular saw (Leica SP1600, Wetzlar, Germany). Block sections were observed by FESEM (Supra 40, Carl Zeiss AG, Germany) using the backscattered electrons (BSE) mode that allows differentiating between Ti implant, soft tissue and new mineralized bone based on their gray levels. Global histomorphometry was carried out using a semi-automatic image processing system (Quantimet 500MC, Leica, Cambridge, UK) Osseointegration of the implants was assessed by calculating the percent of direct bone-to-implant contact (BIC) parameter, between mineralized bone and titanium mini-implant along the total length covered by the pictures.

Statistical analysis

All data are represented as mean values ± standard deviations (SD). Statistical analysis of the obtained results was performed with ANOVA tables using Fisher's test to determine statistically significant differences between groups ($p\text{-value} < 0.05$) and confidence intervals (95%). Statistical analysis was performed using Minitab software (Minitab Inc, United States).

RESULTS AND DISCUSSION

The clinical success of implant dentistry depends on fast and predictable osseointegration by controlling surface characteristics [8]. Among different surface characteristic surface topography, energetic and physico-chemical properties are crucial for the exit or the failure of dental implants [13-17].

In our work, different surface treatments, based in increasing real surface area, were applied to orthodontic mini-implants in order to increase device stability, during orthodontic treatment, without producing bone fracture, during unscrewing, and evaluated by *in vivo* studies on New Zealand White rabbits, throughout 10 weeks implantation. Previously, reproduced mini-implants surface modifications on c.p. Ti discs were characterised by means of surface roughness and wettability.

The values of the roughness and wettability parameters for the different surfaces studied are shown in Table 1. From the results, the applied physical and chemical surface treatments on c.p. Ti discs increased the mean-roughness and decreased peak density parameters. GBlast and GBlast+AEtch surfaces were significantly rougher than AEtch and Ctr surfaces, and AEtch surfaces were significantly rougher than Ctr in accordance with findings by others authors [8, 16, 18]. The influence of grit-blasted and acid-etched rough Ti surfaces on wettability measurements indicated that GBlast and GBlast+AEtch surfaces were significantly hydrophobic than AEtch and Ctr surfaces. Moreover, AEtch surfaces did not change significantly the wettability of Ctr surfaces.

Fig. 2 shows representative histology images of Ctr, AEtch, GBlast, and GBlast+AEtch tested mini-implants, respectively, after 10 weeks of insertion in New Zealand White rabbits. Relevant differences of osseointegration are observed depending on the applied surface treatments. Ctr surfaces are the ones with a lower bone tissue in contact with surface implant (Fig. 2A). The observed soft tissue surrounding the mini-implant is a keratinized tissue. Mini-implants osseointegration was higher for AEtch series (Fig. 2B) and considerably higher for GBlast and GBlast+AEtch (Fig. 2C and D). These results are summarized in Table 2 where the BIC parameter quantifies the osseointegration of the implants and determines the torque necessary to unscrew the mini-implants. A 1-week healing period in rabbits is equivalent to a 3-week period in humans. Ctr implants had lower percentages of BIC and torque values, 19% and 18 N·cm, respectively, lower than AEtch mini-implants (26%, 22 N·cm) and with statistically significant differences compared to GBlast (75%, 52 N·cm) and GBlast+AEtch (79%, 57 N·cm).

Differences in roughness are due to the grit-blasting procedure where the big abrasive particles used induce a sharp and considerable increase of roughness compared to acid etching procedures where rounded and smoother irregularities were found. Moreover, a consequence of treating metallic surfaces with a blasting method is that some particles remain embedded in the surface, [15], increasing the R_a value. Afterwards, when these surfaces are acid etched, GBlast+AEtch, the particles are removed and that's the reason why this series has a lower R_a value compared to GBlast. Finally, GBlast and GBlast+AEtch surfaces did not have significantly different values of P_c roughness showing that the number of pairs peak-valley by unit length was nearly the same. This indicates that the sizes of these nano-topographic features are smaller than the lateral resolution of the interferometry technique used to measure roughness. Concerning to the wettability results some cautions must be considered since rough surfaces affect to contact angles/wettability because for the same nominal area; the total real area is higher for rougher surfaces [9]. The increase of contact angle is directly related to the increase of R_a

value, this effect was statistically significant for GBlast and GBlast+AEtch surfaces since higher roughness average values were obtained compared to Ctr. Finally, GBlast and GBlast+AEtch were the treatments that most decreased surface wettability, besides roughness, this increase of the CA could also be due to the metastable character of the deposited water drops produced by the surface roughness features. Demonstrating that highly rough ($R_a \approx 4.5$ μm) and hydrophobic surfaces improves bone integration compared to as-machined smooth and less hydrophobic surfaces ($R_a \approx 0.3$ μm). Moreover AEtch series ($R_a \approx 1.7$ μm) significantly increase the BIC value, 26%, compared to Ctrl mini-implants placed during 10 weeks on lateral condyle of New Zealand White rabbits due to its roughness since the wettability was statistically equal ($p>0.05$).

Regarding chirurgical procedures and torque levels of the in vivo studies on rabbits, the applied torque of 10 N·cm for orthodontic mini-implants placement on lateral condyle of New Zealand White rabbits, did not produce the nucleation of micro-cracks in bone. Then, this level of stress might be regarded as optimal for our conditions. But it should be considered, that longer implants and thicker cortical bone layers might need and increase of insertion torque moment. After insertion, there was observed absence of tissue inflammation and clinically detectable mobility in all modified mini-implants. None of the 20 mini-implants placed in New Zealand White rabbits failed during the studies.

All mini-implants placed presented unscrewing torque values higher 15 N·cm. When comparing the levels of osseointegration through the BIC parameter with unscrewing torque values, it was observed a direct correlation between both parameters. Then, GBlast and GBlast+AEtch series presented a higher level of osseointegration and consequently, higher values of torque during unscrewing when compared with Ctr and AEtch treated mini-implants. Moreover, after GBlast and GBlast+AEtch mini-implants removal, it was observed cracks and broken bone tissue in a greater proportion than on Ctr and AEtch mini-implants. The clinical experience indicated that GBlast and GBlast+AEtch surfaces could be advantageous in areas of poor bone quality [11]. Contrary, AEtch series showed a statistical equal torque value during unscrewing compared to Ctr mini-implants and nearly no bone fracture was observed. Indicating that wettability, and not roughness, is the surface parameter that controls the torque removal force of mini-implants.

CONCLUSIONS

The present study demonstrates the effectiveness of surface modification on orthodontic mini-implants to enhance osseointegration and then, stability of the dispositive. Moreover, it was found a compromise between implant osseointegration/stability and removal torque without causing bone fracture correlated with surface characteristics. It was found that, wettability was the main parameter to control the torque necessary to unscrew a mini-implant. Specifically acid-etched mini-implants, with a R_a value of 1.69 μm and CA of around 69°, 26 % of BIC and a removal torque of 22 N·cm are vey interesting candidates for temporary mini-implants. This surface modification could be used to enhance a rapid stability of self-drilling orthodontic mini-implants through adequate osseointegration levels and acceptable by the surrounding tissues to prevent major destruction or fatigue during unscrewing.

COMPLIANCE WITH ETHICAL STANDARDS

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

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Ethical approval: All procedures in this study were performed in accordance with the ethical standards of the Ethics Committee of Faculty of Veterinary Sciences of the University of Córdoba (Spain).

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

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FIGURES LEGENDS

Fig. 1 A) Lateral view of c.p. Ti mini-implants inserted in human maxilla in an orthodontic treatment; B) c.p. Titanium mini-implant (Ctr)

Fig. 2 Histology images of A) Ctr: as machined, B) AEtch: acid-etched with hydrofluoric acid, C) GBlast: grit-blasted with alumina particles, and D) GBlast+AEtch: grit-blasted and acid-etched treated mini-implants after 10 weeks of insertion in New Zealand White rabbits. Relevant differences of osseointegration are observed depending on the applied surface treatments. Ctr surfaces are the ones with a lower bone tissue in contact with surface implant. Conversely, mini-implants osseointegration was higher for AEtch series and considerably higher for GBlast and GBlast+AEtch

Table 1. Surface roughness and wettability of the different treated mini-implants. Statistical differences vs. smooth surfaces for each column are indicated by asterisks-symbol ($p<0.05$).

Implant surfaces	Roughness		Wettability
	R_a (μm)	P_c (cm^{-1})	CA (º)
Ctr	0.33 ± 0.1	150.9 ± 69	66.29 ± 4.62
AEtch	1.69 ± 0.1 *	198.3 ± 34	68.84 ± 4.97
GBlast	4.74 ± 0.2 **	82.1 ± 10 *	76.93 ± 2.94 *
GBlast+AEtch	4.23 ± 0.2 ***	92.1 ± 13 *	72.11 ± 5.15 *

Table 2. Bone implant contact, BIC, and mini-implant unscrewing torque of the different mini-implants inserted for 10 weeks on New Zealand White rabbits. Statistical differences vs. smooth surfaces for each column are indicated by asterisks-symbol ($p<0.05$). Values are given as mean ± standard deviation (SD) and 95% confidence interval (95% CI)

	BIC (%)			**Torque (N·cm)**		
		95% CI			95% CI	
Implant surfaces	Mean ± SD	Lower	Upper	Mean ± SD	Lower	Upper
Ctr	19 ± 7	12,9	25,1	18 ± 3	15,4	20,6
AEtch	26 ± 6	20,7	31,3	22 ± 4	18,5	25,5
GBlast	75 ± 15 *	61,8	88,1	52 ± 10 *	43,2	60,8
GBlast+AEtch	79 ± 12 *	68,5	89,5	57 ± 8 *	49,9	64,0

Fig.1

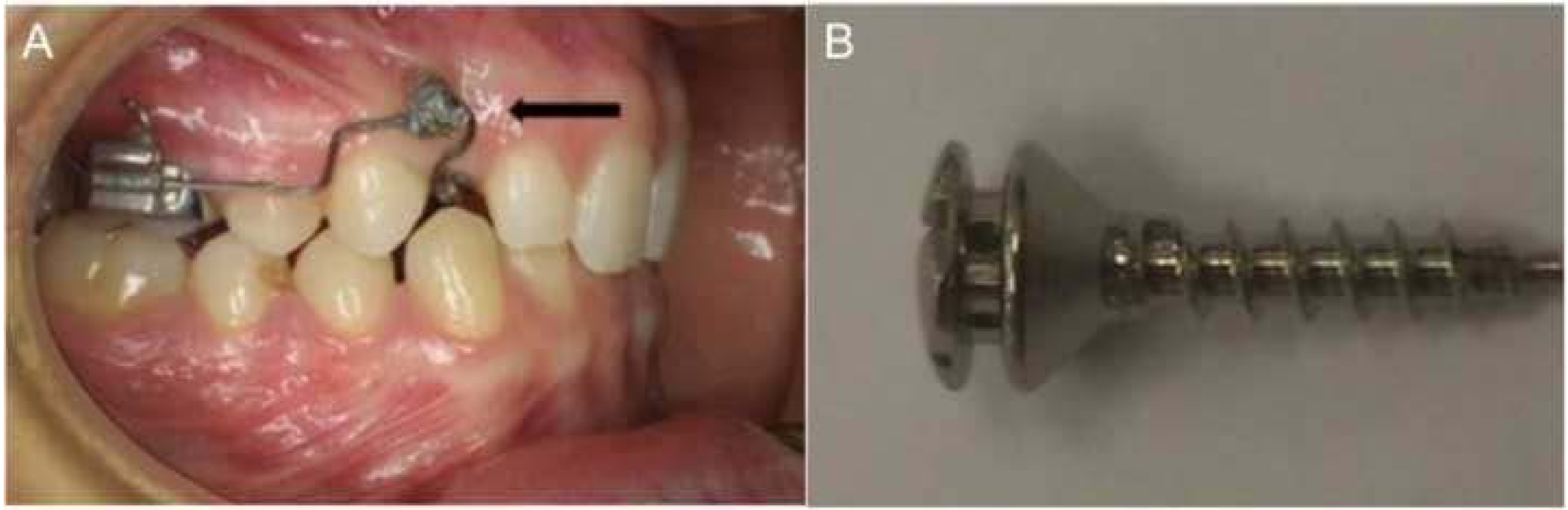

Fig. 2