OSSEOINTEGRATION EVALUATION OF TREATED SURFACES OF TITANIUM IMPLANTS APPLYING TENSILE PULL OUT TEST

Avaliação da osseointegração de superfícies modificadas de implantes de titânio por meio de teste mecânico de pull out

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Abstract

OBJECTIVES: The purpose of the present study was to evaluate the osseointegration of titanium implants with coin-shaped geometry and different surface roughnesses applying tensile pull out test using sheep as an animal model. **MATERIAL AND METHOD**: The tensile pull out test was performed with the application of a gradual load perpendicular to the bone-implant interface. The samples surface morphology was characterized by SEM and rugosimetry techniques. Chemical composition of the samples was obtained by EDX analyses. **RESULTS**: The tensile pull out tests showed that four weeks of healing were insufficient to achieve a satisfactory osseointegration of titanium implants. After eight weeks, the results obtained showed that the ultimate stress mean values of bone-implant interface were influenced by the different surface roughness.

Keywords: Titanium; Implants; Surface treatment; Roughness; Osseointegration.

Resumo

OBJETIVOS*: O propósito deste trabalho foi avaliar a osseointegração de implantes de discos de titânio com diferentes rugosidades de superfície por meio de ensaio mecânico do tipo pull out usando a ovelha como modelo animal.* ***MATERIAL E MÉTODO****: O teste mecânico de pull out foi realizado com a aplicação de carga perpendicular a interface osso-implante. A morfologia da superfície das amostras foi caracterizada pelas técnicas de MEV e Rugosimetria. A composição química das amostras foi obtida pela técnica de EDS.* ***RESULTADOS****: O teste de pull out mostrou que quatro semanas de cicatrização foram insuficientes para atingir uma osseointegração satisfatória dos implantes de titânio. Após oito semanas, os resultados obtidos mostraram que os valores médios de tensão de ruptura da interface osso-implante foram influenciados pelas diferentes rugosidades de superfície.*

Palavras-chave*: Implantes; Titânio; Tratamento de superfície; Rugosidade; Osseointegração.*

INTRODUCTION

Since titanium and its alloys are widely applied for endosseous implants, the constant growth of the use of titanium implants in the global landscape has required increasing efforts of industries and research centers to improve the properties of these biomaterials. Several techniques have been developed to improve the osseointegration capability of the titanium implants by enhancing biocompatibility and osseoconduction on their surfaces by morphology and chemical surface modifications, such as blasting and acid etching treatments, in order to produce roughened surfaces (1-8). According to some researchers, these methods are applied to provide an adequate surface for bone-implant integration (9-16). However, the ideal degree of roughness for an optimal clinical performance still remains unknown (1, 5, 17, 18).

Recent studies in dental and orthopedic areas have been focused on the development of new methods to evaluate the interaction between implant surfaces and bone (2-5, 19, 20). Nowadays, mechanical torque systems are the most applied mechanical tests. These tests may not provide a clearly information about bone bonding and attachment whereas the results may be strongly influenced by friction and in-growth of bone tissue on implant surface. Thus, it has proven difficult to separate biological effects from mechanical interlocking effects in this kind of tests for implants attachments (3-5, 19). On the other hand, the tensile pull out tests play an important role in this kind of evaluation, presenting several advantages towards the removal torque such as uni-axial strength application and controlled deformation rate of the bone-implant interface. Based in these advantages, the purpose of the present study was to evaluate the osseointegration of titanium implants with coin-shaped geometry and different surface roughnesses applying tensile pull out test using sheep as an animal model.

MATERIAL AND METHOD

Implant preparation

Ninety coin-shaped titanium implants ASTM grade 4 (4 mm thickness and 6 mm in external diameter) were produced by a Brazilian dental implant manufacturer, as shown on Figure 1. A 3 mm screw was adapted in the internal diameter of all implants to ensure the contact and fixation of the implants on cortical bone.

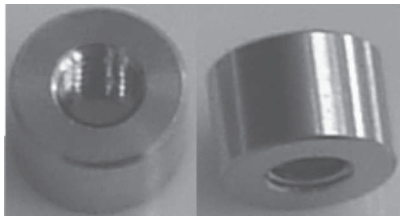

FIGURE 1 - Coin-shaped titanium implants (4 mm in thickness and 6 mm in diameter)

This implant design was developed in order to increase bone-implant contact (3-5, 19). The samples were separated in five groups, each one with 18 implants, according to surface treatment applied. Samples classification is presented on Table 1.

TABLE 1 - Samples groups division according to surface treatments applied on coin-shaped titanium implants. Each group contains nine titanium implants for each healing time

ROUPS	SURFACE TREATMENTS
A	Only machined (control group)
B	Fluoride acid etching
C	Al_2O_3 blasting (320*), followed by fluoride acid etching
D	Al_2O_3 blasting (100*), followed by fluoride acid etching
E	Al_2O_3 blasting (150*), followed by fluoride acid etching

* alumina particle size in Mesh scale.

Animal model

Six clinically healthy adult female sheep were used as an animal model. The animals were separated in two different groups according to healing times of four and eight weeks (45 implants each) in order to compare the bone-implant adhesion in the initial phase of bone formation and adhesion of newly formed bone. The current study protocol was approved by the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul, under registry number 06/03548.

Surgery

The surgical experiment was carried out in accordance with brazilian laws and regulations. Pre-anesthesia procedure consisted of the *IM* administration of 0.1 mg/kg acepromazine maleate (1% Acepran) and 2 mg/kg meperidine (Dolosal). After 15 minutes, 20 mg/kg cephalothin sodium was injected *IV*. Anesthesia was induced with an *IV* injection of propofol (2 – 4 mg/kg), and maintained with isoflurane in 100% oxygen. All the animals were killed with an injection of sodium thiopental. Surgical, postoperative and animals death procedures adopted are described in previous study (19).

Sample preparation and characterization

The surface roughness (R_a) of samples was measured by a Mitutoyo Surftest SJ – 201P rugosimeter apparatus. The mean value was considered based on fifteen valid measurements. Scanning electron microscopy (SEM) was carried out with a Phillips XL 30 equipment to characterize the treated surfaces of titanium implants. The micrographs were acquired in the scattering electron mode (SE) using an electron beam with 20 keV. Energy dispersive x-ray spectroscopy (EDX) was performed with a Si (Li) solid state detector to analyze the chemical composition.

After the death of the animals, the implants and surrounding tissues were removed *en bloc*, and then immersed in glutaraldehyde 2 % until the evaluation of osseointegration. Thus, the samples were embedded in acrylic resin and the 3 mm fixation screws were removed, as shown on Figure 2, followed by the attachment of ball-head pin to the center hole of all implants, allowing the sample fixation to the top jig of the tensile pull out test apparatus.

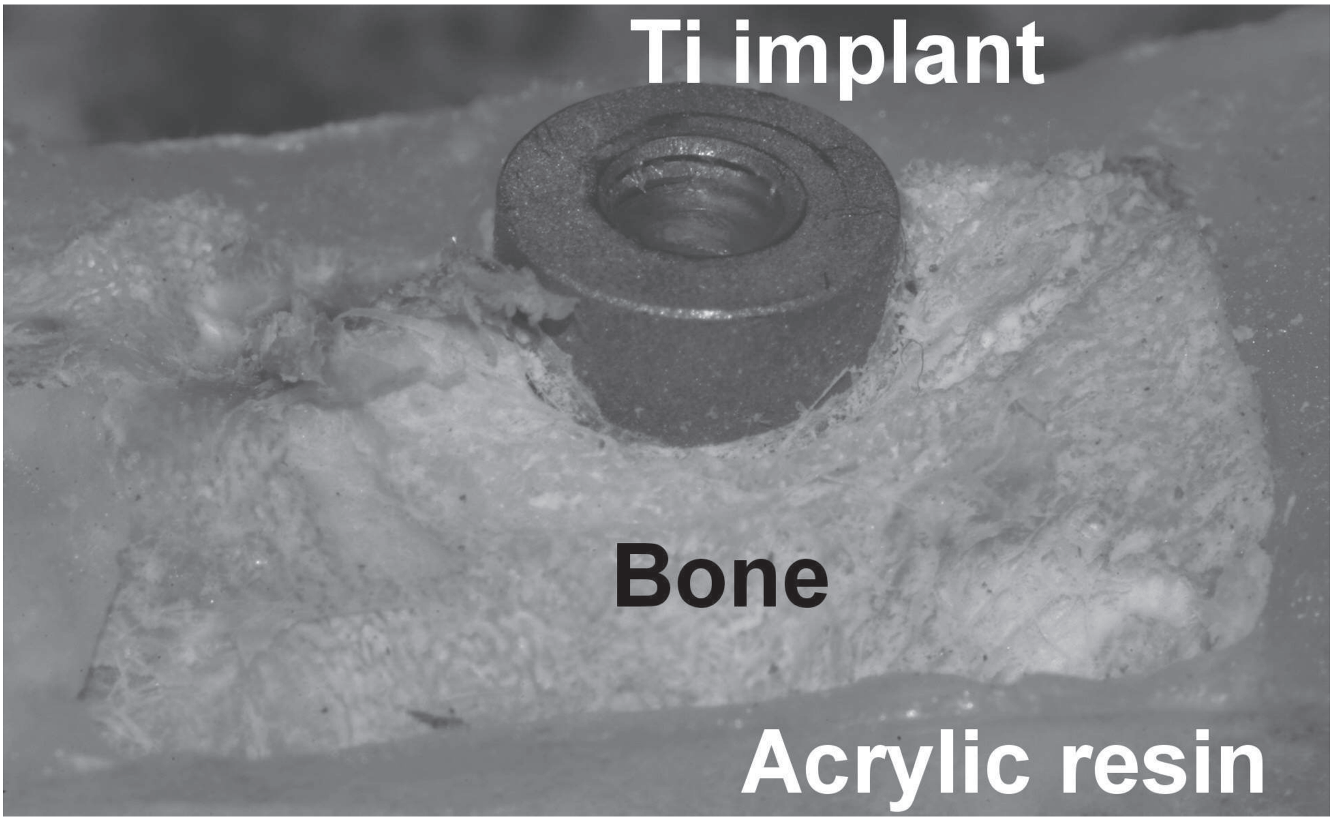

FIGURE 2 - Bone-implant *en bloc* embedded in acrylic resin ready to perform the osseointegration evaluation. The titanium treated surfaces are in contact with the sheep cortical bone

Pull out tests were performed using an EMIC DL 2000 testing machine. The measurements were made using a load-cell of 500 N and a constant deformation rate of 1 mm/min, according to an adaptation of ASTM C 633. The load was applied until the complete sample detachment of the bone. A schematic illustration of the pull out apparatus and the experimental analysis are showed on Figure 3.

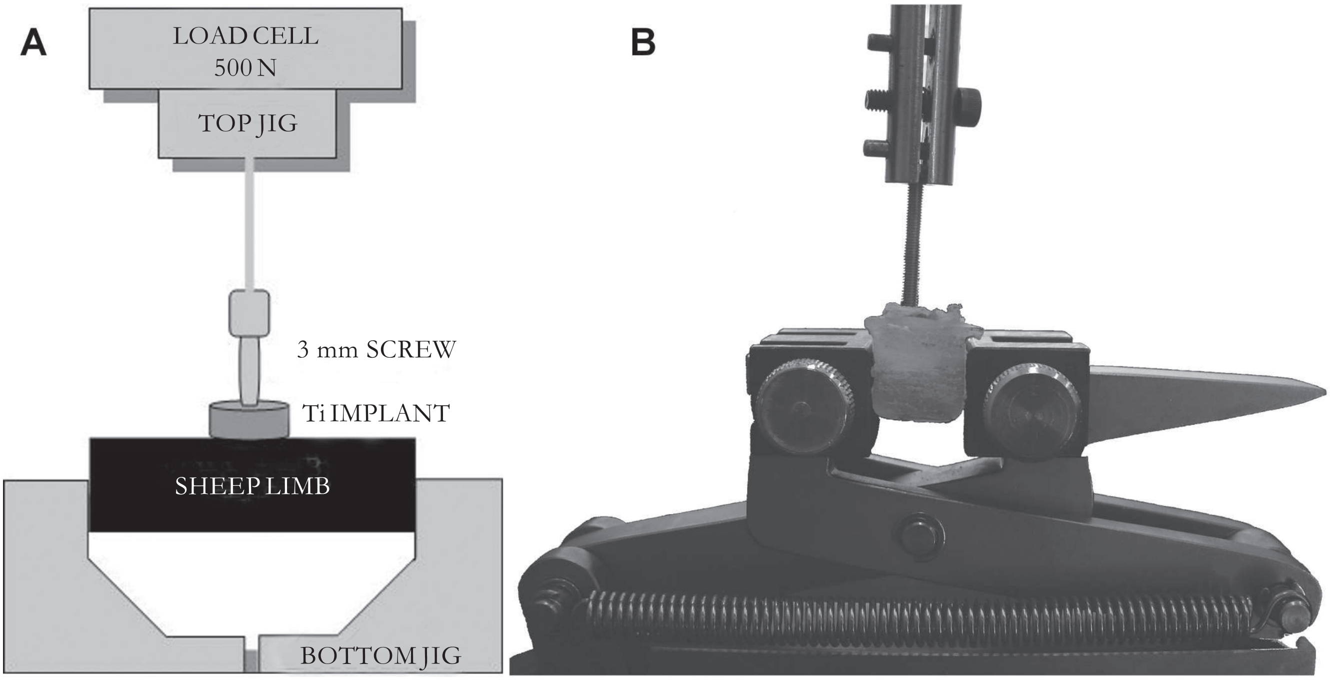

FIGURE 3 - In (A) schematic illustration of the pull out test apparatus. The samples embedded in acrylic resin are fixed in a bottom jig and the top jig is fixed in the ball-head pin located in the through hole of all implants. In (B) the implants are pulled out until the completely rupture of the bone-implant interface

Statistical analysis

Statistical analyses for determination of differences among the groups and confidence intervals were accomplished using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Titanium implants characterization

The SEM images of the implant surfaces showed similar morphology between groups B and E, and between groups C and D, Figure 4. The sample micrographs of groups C and D shown deformations generated by the alumina blasting treatment, which could be from the impact of particles on the implant surfaces. However, no appreciable differences could be observed among the textures achieved for each surface treatment at higher magnification, which indicates relative homogeneity among these structures. Comparing to the control group (A) all the treated surfaces showed a clearly morphology modifications.

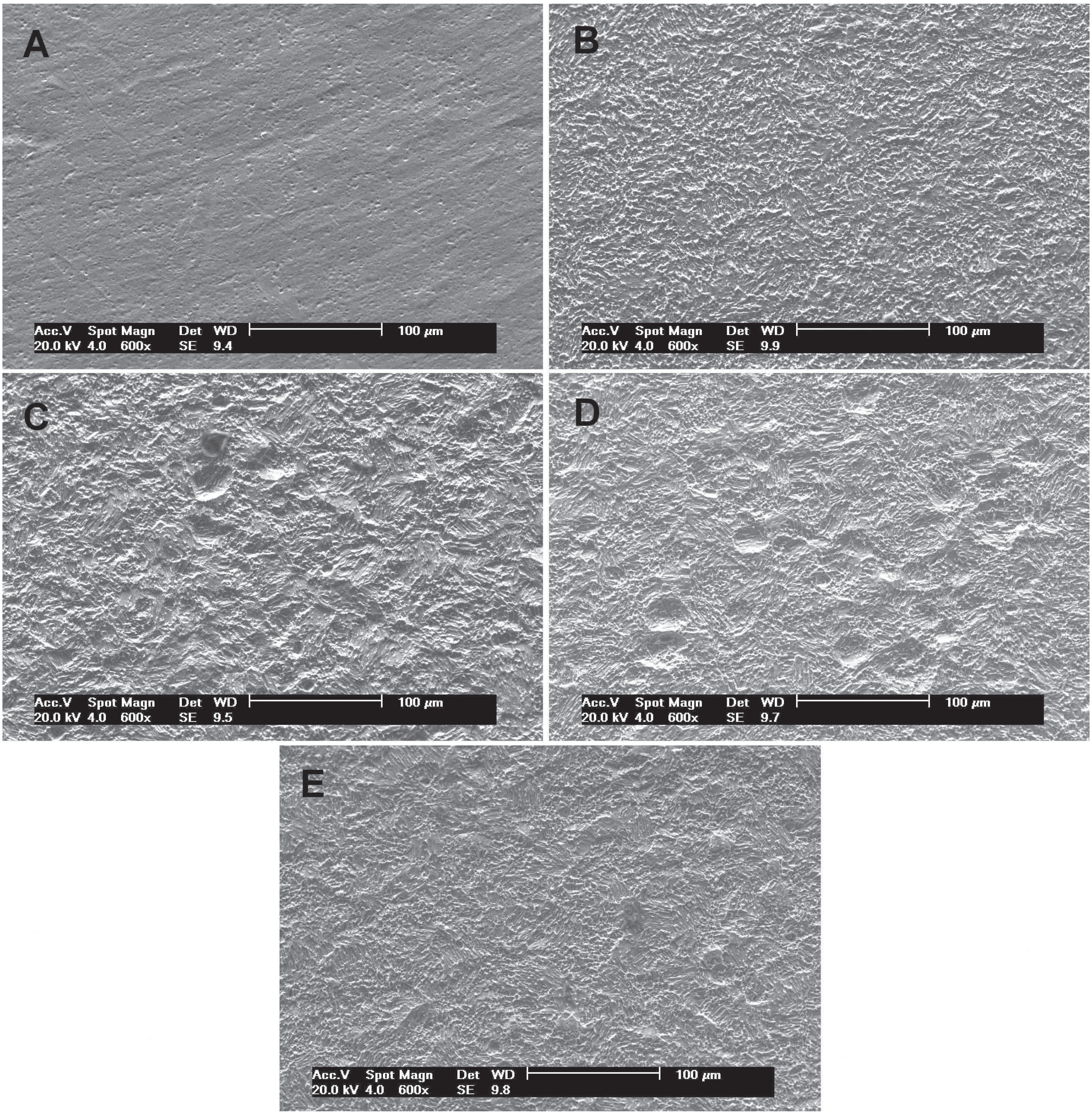

FIGURE 4 - Scanning electron micrographs of Titanium implants after the surface treatments. In (A) Control Group; (B) Group B; (C) Group C; (D) Group D; (E) Group E

The roughness values are shown on the Table 2. The control group exhibited the lowest average roughness, whereas the samples of the group D showed the highest values. The ANOVA statistical analysis indicated no significant difference among the R_a mean values of the groups B, C, D and E.

TABLE 2 - Roughness values (R_a) of titanium implant surfaces (n=15)

GROUPS	TITANIUM SURFACES ROUGHNESS VALUES (μm)		
	Minimum	Maximum	(Mean ± SD)
A	0,12	0,23	(0,14 ± 0,03)
B	0,43	0,63	(0,49 ± 0,06)
C	0,44	0,69	(0,53 ± 0,08)
D	0,48	0,86	(0,66 ± 0,11)
E	0,41	0,55	(0,49 ± 0,04)

The EDS analyses of all groups showed the x-rays peaks of K_a, K_b and L_a titanium transitions. The spectra do not show any oxide particles from blasting or other contaminants in all sample surfaces.

Osseointegration evaluation

Fifteen samples were lost for 4 weeks of healing, while for 8 weeks only one sample was lost. A significant difference was found between the ultimate stress values obtained for 4 weeks and 8 weeks. After 4 weeks, any numerical and statistical significant differences were found among the groups. The results of roughness and pull out tests for the 8 weeks indicated a correlation between the increase of surface roughness and the ultimate stress mean values, until reach surface roughness of group C. A further increase in roughness did not result in additional improvement in ultimate stress values, as can be seen on Figure 5.

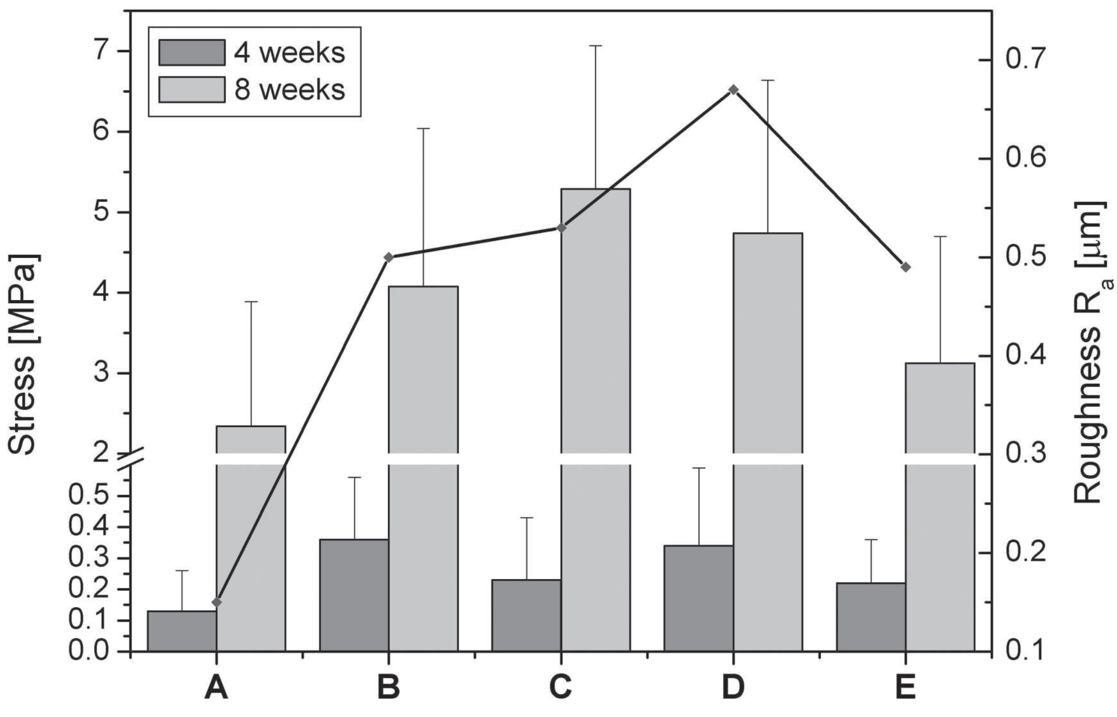

FIGURE 5 - Comparison between the ultimate stress mean values and the roughness (R_a) mean values for both healing times

Even the statistical results indicating no significant differences among the surface roughness of the treated groups, the ultimate stress values were influenced by the small roughness variance.

The osseointegration evaluation obtained was similar to those described in previously studies (Table 3). The comparison among different studies is limited because of the diverse mechanical test applied. However, no influence of mechanical interlocking on periimplant bone was detectable in this work. Thus, comparison is valid in a quantitative way considering the ultimate tensile stress and implant contact area.

TABLE 3 - Comparison among ultimate stress mean values obtained in this study and in similar investigations. Some studies showed here, were carried out with different implanted materials and tests to evaluate the osseointegration process

AUTHORS	BIOMATERIAL/ ANIMAL MODEL	SURFACE ROUGHNESS (μm)	HEALING TIME (Weeks)	ULTIMATE STRESS (MPa)
Li et al (1997)/[20]*	Ti/rabbit	Ra = 1,5	6	0,5
Vercaigne et al (1998)/[20]*	Ti-6Al-4V/goat	Ra = 4,7	12	2,9
Rønold et al (2002)/[3]	Ti grade 2/rabbit	Sa = 1,43	8	0,11
		Sa = 0,63		0,33
		0,89		0,36
		1,25		0,68
Rønold et al	Ti grade 2/rabbit	1,30	10	0,78
		1,80		0,94
		2,02		1,40
		3,62		1,79
		5,52		1,53
		Sa = 3,9		0,54
Rønold et al	Ti grade 2/rabbit	5,07	8	0,35
		11,03		0,09
		Ra = 0,14		0,13
		0,49		0,13
This Study	Ti grade 4/sheep	0,53	4	0,36
		0,66		0,23
		0,49		0,22
		Ra = 0,14		2,33
		0,49		4,07
		0,53	8	5,28
		0,66		4,73
		0,49		3,12

* Mechanical test applied: push out.

As can be seen, the ultimate stress mean values obtained are higher than similar studies considering the healing time and metabolism of the animal model used. Compared to the values obtained in the study using goat (20), the osseointegration results for 8 weeks are similar, but take into account the healing time, our outcomes showed that it was possible to achieve greater osseointegration in a shorter time. Considering the values of roughness and ultimate stress achieved in another studies, this study showed that there is an improved relationship between these parameters, because a lower value of roughness towards those showed on Table 3, reached up a higher values of ultimate stress.

CONCLUSIONS

The tensile pull out tests showed that four weeks of healing was insufficient to achieve a satisfactory osseointegration of titanium implants. However, the outcomes of groups B, C e D showed the best results and are comparable to those obtained by other researchers.

The surface treatments applied increased the surface roughness of titanium implants without produce any chemical modification or contamination of the surfaces. The surfaces treatments applied presented an improvement in bone-implant attachment towards the control group (only machined surfaces), increasing the osseointegration of titanium surfaces. According to our roughness results, the mean value of 0.53 μm provided the best conditions for the formation of a stable bone tissue–implant interface. Compared to other surface treatments, the Al_2O_3 blasting (grain size of 320 mesh) followed by fluoride acid etching shows a significantly better performance than others treatments. The results also showed that the tensile pull out test is a valid technique to evaluate quantitatively the osseointegration process.

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