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Effect of implant crestal position on primary stability before and after loading: an *in vitro* study

Efeito do posicionamento crestal do implante na estabilidade primária antes e após carga: um estudo *in vitro*

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Resumo

Introdução: A estabilidade primária é um dos objetivos da implantodontia moderna e, caso atingida, reduz o tempo de tratamento para a reabilitação protéticas e o número de intervenções realizadas. Diversas empresas preconizam a posição subcrestal no uso de implantes com conexão cônica interna. **Objetivo:** Este estudo *in vitro* avaliou o efeito do posicionamento de implantes de conexão conica interna sub e equicrestal sob condições estáticas e em função, considerando dois tipos de densidades ósseas. **Material e método:** um total de 200 espécimes de osso extraído do femur de suínos e padronizados por meio de radiografias e microtomografias computadorizadas foram separados em densidade alta e baixa. Implantes foram instalados no centro dos especimes e for a avaliados por meio de microCT e histomorfometria. **Resultado:** Os resultados demonstraram que a colocação de implante subcrestalmente promoveu melhor estabilidade primária e performance em todas as situações, irrespectivamente à densidade óssea. **Conclusão:** A colocação de implantes subcrestalmente melhora a estabilidade primária em todas as situações, sendo indicada quando da utilização de conexões cônicas internas.

Descritores: Implantes dentais; técnica *in vitro*; microtomografia; teste de arrancamento.

Abstract

Introduction: Primary stability is one of the goals of modern implant dentistry and if achieved, reduces treatment time for prosthetic rehabilitation and the number of interventions made in patients mouth. Several companies state as protocol for connical conection implants, a subcrestally positioning. **Objective:** This in vitro study aimed to evaluate the effect of placing a conical connection implant equicrestally and subcrestally on static and loading condition in two types of bone density. **Material and method:** A total of 200 bone cylinders were extracted from femur of pigs, standardized by means of x-rays and computerized microtomography scan (microCT) and separated in low and high density specimens. The implants were placed on the center of the bone cylinders and were evaluated before and after loading by means of microCT and histomorphometry. **Result:** The results showed that placing the evaluated implant subcrestally provided better primary stability and performance on static and loading situations on low and high density bone. **Conclusion:** Placing implant subcrestally improve primary stability outcomes under loading and static situations.

Descriptors: Dental implants; primary stability; *in vitro* techniques; X-Ray microtomography; pull out test.

INTRODUCTION

Oral rehabilitation with osseointegrated dental implants have been applied for many years since the first description of the osseointegration[1], and one of the requirements for success was a stressfree healing period of 3–6 months[1-4]. Later, immediate loading has been proposed in order to reduce the treatment time[5-7]. This is a prerequisite for immediate loading and it is influenced by many factors, such as bone density, implant geometry, cortical thickness and cortical bone density[8,9].

Implants with conical geometry and morse taper prosthetic connection have been successfully applied on oral rehabilitation with minimal marginal bone loss, usually attributed to its natural platform shift characteristics[10]. However, there is still a discussion regarding the position of the implant in relation to the bone crest[11-13]. Little is known about the influence of the subcrestal/crestal positioning on biomechanics of implant-supported rehabilitations and its stability on immediate loading situations.

In clinical settings, primary stability can be quantified within implant insertion by measuring the torque achieved with a torquimeter attached to a rachet or with the handpiece, at the end of implant insertion. It can also be measured by means of a resonance frequency analysis (RFA) made by a special apparatus[14-16]. Due to its non-invasiveness and non-destructive characteristics, RFA has been established as a quantitative measurement of implant integration by assessing changes in implant stability over time[17]. On *in vitro* settings, the pullout strength assay analyzes the resistance of the implants based on the physical and chemical properties of the screw, with the inconvenience of destroying the specimen.

Dantas et al.[18] successfully evaluated standardized porcine bone models in order to avoid the use of living subjects and reducing the use of animals to test primary stability of dental implants in static situation. This technique was capable to obtain specimens with standardized bone density, mimiquing high density and low density bone. This *in vitro* model substituted a synthetic one[19] with a considerable advantage of the ability of using non-mechanical tests such as x-rays, microtomography (microCT) and histology to evaluate periimplant bone. The use of of natural bone gives the possibility of a number of analysis including histologics slices, mimiquing the clinical setting in implant dentistry. In addition this model can also be evaluated three-dimentionally with the aid of microCT and a accurate mesurement of the effect of implant insertion in different bone densities is possible quantitavely and qualitatively. To date, no data has been published using this novel model in loading situations.

The aim of this study is to evaluate the effect of implant positioning (crestal/subcrestal) on primary stability, with or without axial or non-axial load, by means of microtomographical and mechanical analysis, in a standardized porcine model of high and low-density bone.

MATERIAL AND METHOD

Specimen Standardization Phase

A total of 200 bone cylinders from porcine origin were prepared and evaluated by digital radiography as previously described[18]. Briefly, bone was removed using an especially designed trephine burr in order to obtain specimens with 15x18mm dimension (Figure 1A and 1B). After removal, they were kept frozen and stored at -20° C.

Before implant insertion, the bone density of the cylinders was certified by two-dimensional radiographic analysis (2D analysis) using a digital X-Ray sensor (RVG Trophy, Eastman Kodak Company, Rochester, NY, EUA)) and a computer software (Image Tool for Windows, version 3.00, UTHSCSA, San Antonio TX, EUA) to determine 2D bone density, as previously described by Dantas et al.[18]. The cylinders were then grouped according to their 2D bone densities: values equal or greater than 110 were selected for High bone density cylinders (cylinders removed from femur head), and with values equal or lower than 80 were selected for Low bone density cylinders (cylinders removed from the mandibular condyle); the cylinders with intermediate values were discarded, and a total of 60 cylinders were selected (30 for low density group, 30 for high density) for the experiment.

Before insertion of the implants in bone cylinders, 3D morphometric analysis was performed using the Micro-Sky Scan 1172-160 (SkyScan, Kontich, Belgium). Micro-CT scans of each bone cylinder were taken for the evaluation of tomographic bone parameters (Figures 1C, 1D, 1E, 1F). Using the analysis software (CTan Analyser) to quantify microstructures, the volume region of interest (VOI) was determined for 3D morphometric analysis. The tomographic parameters evaluated in the bone cylinders were tridimensional bone density (BV/TV), trabecular separation (Tb.Sp); percentage of total porosity (Po.tot) and bone surface/volume ratio (BS/BV).

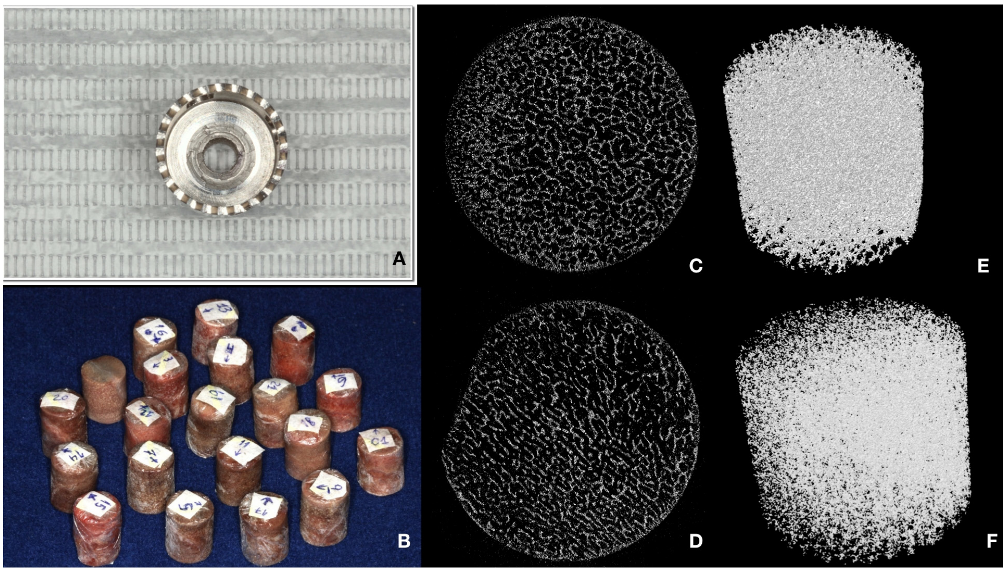

Figure 1. Custom made trephine for collection of bone specimens (A); bone cylinders prepared and identified (B); 2D and 3D analysis showing high density (C and E) and low density (D and F) specimens.

Implant Installation

Sixty implants were selected for this study, measuring 3.5 x 10mm (Alvim Cone Morse – Neodent®, Curitiba, Brazil). According to the manufacturer, these implants have a conical body, twin screw and morse tape prosthetic platform.

The bone cylinders were fixed and immobilized in a specially designed base to avoid displacement during the drilling process (Figure 2A). The surgical site was prepared with progressive drilling sequence, at 800 rpm, with abundant saline solution irrigation, following the protocol recommended by the manufacturer (Neodent®**,** Curitiba, Brazil). After site preparation, the implants were inserted into the bone cylinders (one implant in each cylinder), according to the four experimental groups (Figures 2B to 2G):

- Experimental Group 1 (G1): implant placed in the high-density bone cylinder at crestal bone level (n = 15);
- Experimental Group 2 (G2): implant placed in the high-density bone cylinder at 2mm subcrestally (n=15);
- Experimental Group 3 (G3): implant placed in the low-density bone cylinder at crestal bone level (n =15);
- Experimental Group 4 (G4): implant placed in the low-density bone cylinder at 2 mm subcrestally (n= 15).

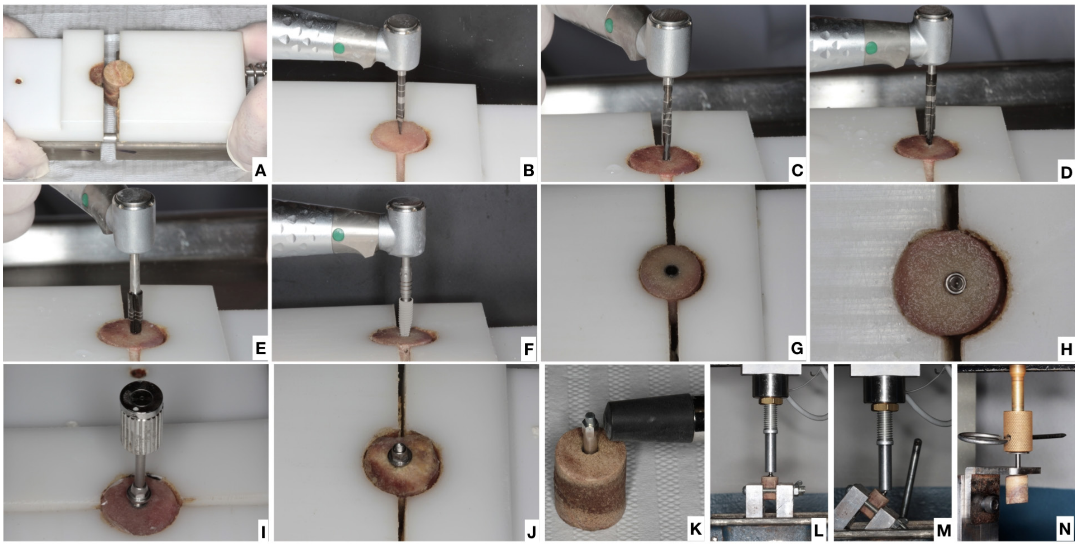

Figure 2. Device designed to hold the bone cylinder in position (A); drilling sequence (B-E); implant installation (F); implant installed on the bone cylinder (G: crestal position; H: 2mm subcrestal position); abutment connected to the implant (I and J); ISQ being measured (K); axial and non-n axial loading (L and M); and the pullout test (N).

Primary Stability Evaluation

Immediately after implant installation, insertion torque (IT) and ISQ levels were recorded. The IT measurement was performed using an micromotor and a reduction handpiece that precisely measures the handpiece torque in 0.1N.cm intervals (iChiropro, BienAir-Dental, Switzerland). The last torque value measured after implant complete insertion was taken into account for statistics as IT. Following that, Ressonance Frequency Analysis was recorded using Osstell (Osstell ISQ, Göteborg, Sweden), and the Implant Stability Quocient (ISQ) was recorded (Figure 2K). Then, another tomographic 3D analysis was performed to evaluate the tomographic bone to implant contact (Figure 3), as the intersection bone to implant surface/total implant surface (IS/TS), and all the previous described parameters, in 2 bone levels: L1- bone internal to the threads; L2 – bone immediately adjacent to the end of threads (Figure 4).

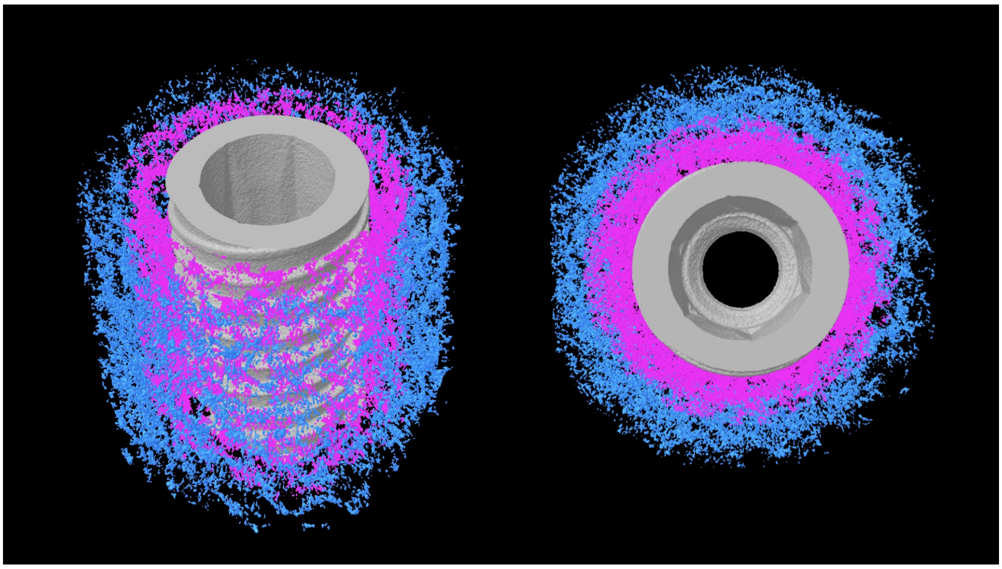

Figure 3. 3D reconstruction of the implant and the two areas of microCT analysis (in pink L1 and in blue L2).

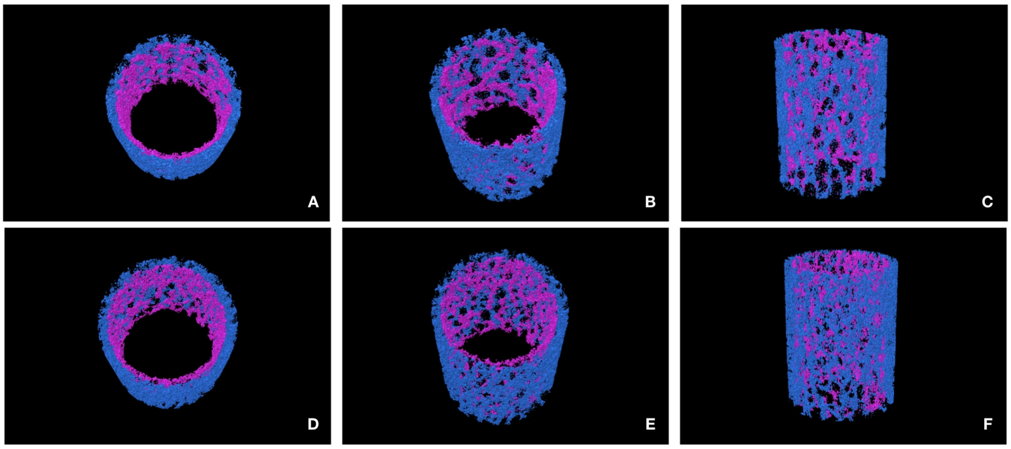

Figure 4. 3D reconstruction of L1 (pink) and L2 (blue) for high bone density (A, B and C) and low bone density (D, E and F) cylinders.

Loading Phase

Five implants from each group were submitted to axial (Figure 2L) and non- axial (Figure 2M) (non-axial load, in 45º degree angulation) loads, while the other 5 did not receive loading forces. Loading was performed as previously described[20]. Briefly, the implants were placed in a Universal Testing Machine (EMIC®, DL-10000N- São José dos Pinhais, Brazil) and loading was progressive applied until it reaches 150N/cm with 1mm/min speed. After applications of axial and non-axial forces in the selected bone cylinders, new Micro-CT scans and micro-tomographic reconstructions were performed, evaluating the same tomographic parameters previously described. A new resonance frequency analysis was performed and, after that, pull out test was accomplished.

The pullout test was conducted at the Laboratory of Bioengineering from the Faculty of Medicine of Ribeirão Preto. The bone cylinder containing the implant was positioned in the Universal Testing Machine (EMIC®, DL-10000N- São José dos Pinhais, Brazil) and connected to a mobile base by a device specially designed and screwed to the implant. After that, a load cell of 200kg was adjusted, and, after a pre-load of 10N for 30 seconds, an axial tensile strength with constant speed of 2mm/min was applied (Figure 2N). The force that broke up bone to implant interface was then measure in Newtons and recorded as the Pullout strength force for that specimen.

Statistical Analysis

All variables were tested for normality of data; according to the result, parametric (t Test) test was used for intra-groups analysis, and nonparametric test (Mann-Whitney Test Whitney Test) for inter-group evaluation. For all analysis, a significance level of 5% was considered.

RESULT

Trabecular Separation (Tb.Sp)

The intra-group analysis showed significant differences for all the groups, pre and post implant placement, indicating bone compacting within the implant insertion. There was statistical significance between G2 *versus* G4 regarding the difference between before and after loading. After non-axial load, there were significant differences between G2 *versus* G4 and between G3 *versus* G4 for the adjacent area. The trabecular separation after loading significantly decreased for all groups, showing that there was a significant difference of bone compaction after loading, with higher values associated to the low density bone cylinders (Table 1).

Table 1. Mean Values ± SD of Trabecular Separation (Tb.Sp) between groups

	Axial		**Non-Axial**	
	Intra- Thread	Adjacent	Intra- Thread	Adjacent
G1(w)	0.07 ± 0.06	0.02 ± 0.20	0.08± 0.10	0.05 ± 0.11
G2(x)	0.03 ± 0.05	-0.02 ± 0.11	0.04 ± 0.03	0.01 ± 0.11
G3(y)	0.10 ± 0.09	1.59 ± 0.06	0.11 ± 0.01	-0.04 ± 0.06
G4(z)	0.14 ± 0.01	-0.09± 0.23	0.13 ± 0.03	-0.15 ± 0.03
	Difference between groups (p)			
w/x	ns	ns	ns	ns
y/z	ns	ns	ns	$p<0.05$
w/y	ns	ns	ns	ns
x/z	$p<0.05$	ns	$p<0.05$	$p<0.05$

Percentage of Total Porosity (Po.tot)

There was a significant reduction of Po.tot for all groups after implant placement. When the differences in Po.tot (ΔPo.tot) were considered, there was a significant difference between all groups after axial load, when compared to the pre-load situation. The non-axial load showed an increase in Po.tot values for all the groups, with significant differences between G2 *versus* G1 and between G2 *versus* G4. In G2, it was observed a reduction of the Po.tot after both loading situations (Table 2).

Table 2. Mean Values ± SD of Percentage of total porosity (PO TOT) between groups

	Axial		**Non-Axial**	
	Intra- Thread	Adjacent	Intra- Thread	Adjacent
G1(w)	18.74 ± 3.16	-1.19 ± 4.11	16.00 ± 1.35	-6.59 ± 1.01
G2(x)	-15.80 ± 2.24	-0.14 ± 1.44	4.41 ± 1.77	-0.20 ± 1.02
G3(y)	14.06 ± 8.40	-4.39 ± 4.48	16.60 ± 3.72	-6.76 ± 2.51
G4(z)	17.00 ± 1.19	0.12 ± 4.90	15.70± 6.58	0.58± 3.14
	Difference between groups (p)			
w/x	$p<0.05$	ns	$p<0.05$	$p<0.05$
y/z	ns	$p<0.05$	ns	$p<0.05$
w/y	ns	$p<0.05$	ns	$p<0.05$
x/z	$p<0.05$	ns	$p<0.05$	ns

Bone Surface / Volume Ratio (BS/BV)

When the bone density was high (G1 and G2), no differences were found after loading. In low-density bone, there was a significant difference between G3 *versus* G4 for the ratios obtained before and after implant installation. The axial load numerically increased the BS/BV in all groups, and there were significant differences between G3 *versus* G4, G1 *versus* G3, and G2 *versus* G4. When non-axial load was applied, there were significant differences between G2 *versus* G4, and G1 *versus* G3 (Table 3).

Table 3. Mean Values ± SD of Bone Surface/ volume ratio (BS/BV) between groups

	Axial		Non-Axial	
	Intra- Thread	Adjacent	Intra- Thread	Adjacent
G1(w)	3.01 ± 4.50	-5.99 ± 5.72	1.97 ± 4.45	-7.04 ± 1.02
G2(x)	1.58 ± 2.94	-6.00 ± 7.42	-3.18 ± 5.69	-8.00± 1.01
G3(y)	-12.86 ± 11.08	-9.69± 5.20	-7.21 ± 6.38	-10.76 ± 9.04
G4(z)	5.20 ± 3.59	30.32 ± 9.20	17.59 ± 1.29	-13.68 ± 1.20
	Difference between groups (p)			
w/x	ns	ns	ns	ns
y/z	p<0.05	p<0.05	ns	ns
w/y	p<0.05	ns	ns	p<0.05
x/z	p<0.05	p<0.05	p<0.05	p<0.05

Tridimensional Bone to Implant Contact (IS/TS)

Overall results showed no signifcant differences on Tridimensional Bone to Implant contact before and after loading on high density bone. On low density bone, there was a significant decrease on IS/TS after oclusal and axial loading.

After axial load it was observed a significant difference between G2 *versus* G4 and between G1 *versus* G2. After non-axial load, there were significant differences between G1 *versus* G3, and G2 *versus* G4 (Table 4).

Table 4. Mean Values ± SD of Bone Implant Contact (BIC) between groups

	Post Load	
	Axial	Non-Axial
G1(w)	-13.67 ± 8.48	-5.58 ± 6.93
G2(x)	-7.08 ± 1.88	-5.30 ± 1.90
G3(y)	-13.90 ± 8.21	-16.70 ± 4.52
G4(z)	-15.02 ± 1.18	-17.13 ± 6.42
	Difference between groups (p)	
w/x	p<0.05	ns
y/z	ns	ns
w/y	ns	p<0.05
x/z	p<0.05	p<0.05

Resonance Frequency Analysis

There were no significant differences between G1 *versus* G3 and between G2 *versus* G4 at implants placement and after loading. Implants placed subcrestally (G2 *versus* G4) exhibited higher numeric ISQ values at both densities in static and loading situations, although without significant differences.

Biomechanical Tests

The mean and standard deviation insertion torque values were: 40.70 ± 6.74 N.cm, 45.46 ±7.99 N.cm, 26.86 ± 7.42 N.cm and 30.44 ± 8.18 N.cm for G1, G2, G3 and G4, respectively. The differences were statistically significant between G1 *versus* G3 and between G2 *versus* G4 ($p<0.05$).

In the Pullout strength test, the mean and standard deviation values of implants that received axial and non axial forces, were respectively: Group 1: 472.10 ± 232.30 and 511.50 ± 184.60; Group 2: 506.40 ± 94.01 and 395.30 ± 63.15; Group 3: 190.10 ± 106.80 and 647.20 ± 172.80 and Group 4: 268.30 ± 98.13 e 392.70 ± 186.50. There were significant differences between G1 *versus* G3, and G1 *versus* G4 at static situation. After axial load there were significant differences between G1 *versus* G3, and between G2 *versus* G4. When the non- axial load was applied to the low bone density, the values were higher than pre-load, and a significant difference could be detected between G3 *versus* G4.

DISCUSSION

This is a novel *in vitro* study, evaluating the effects of the position of the implant after insertion in different bone densities on the biomechanics and adjacent bone, in non-loaded and loaded situations. Overall results showed that inserting a morse taper implant 2 mm subcrestally represents better biomechanical settings than inserting it at crestal level.

To date, few studies had used standardized *in vitro* models to evaluate tridimensional changes caused by implant placement in different bone densities. This model resembles with a good reproducibility high-density and low-density bone, comparable to types 1 and 2, and 3 and 4, respectively. This is the first study to mimic axial and non-axial loading to analyze its effect on implant primary stability and bone compacting in this bone model.

Clinically, implants are placed subcrestally due to esthetic demands, to improve initial stability, especially in case of immediate implants, or in cases presenting insufficient inter-occlusal height for proper restoration[21,22]. Primary stability is influenced by various factors, including bone quality and quantity, implant geometry, and cortical bone thickness[8,23,24]. It has been reported that the primary stability is affected by cortical bone thickness and trabecular bone density[9]. In the present study, the highest mean values for insertion torque were obtained in high density bone (G1 and G2), with significant difference between implants with the same position and different bone densities (G1 *versus* G3 and G2 *versus* G4). Those results are in agreement with a previous study in a different *in vitro* model, that showed a significant difference in micromovimentation of implants in soft bone (low density), including in implants with insertion torque considered clinically acceptable to immediate loading[25].

When the primary stability was measured by means of RFA, no difference could be detected between groups. However, the mean values of ISQ were higher in the high density bone groups for subcrestal implant positioning (G1=74.4 *versus* G2=76.7; G3=72.7 *versus* G4=75.3;), suggesting that subcrestal placement of implants could improve the initial stability in low density bone such as in posterior maxilla[26,27]. After loading, an increase in ISQ values was observed in the groups that received subcrestal implants placement (G2: 83.3 for axial load and 86.3 for non - axial load; G4: 84.7 axial load and 85.3 for non-axial load), while a reduction was observed for the implants placed at crestal level (G1: 71.50 for axial load and 73.45 for non-axial load e G3: 73.06 for axial load, 70.20 for non-axial load), suggesting a higher bone compression for the subcrestal implants. The lowest values were found in low-density bone groups and it is in agreement with a previous clinical study, that reported mean values of 68.51 and 72.84 in maxilla and mandible, respectively.

The pullout test has been previously used to evaluate primary stability *in vitro* (66-69). In the present study, the highest values were observed in the high-density groups G1: 564.66 and G2: 443.45, while the low-density groups (G3 and G4) showed 226.38 and 298.74, respectively. Similar results were reported in studies using high-density substrates such as pine wood and a polyurethane 40PCF bone simulator[28].

The use of microCT to analyze bone density is considered an objective and reliable method[29]. It was first described in 1987[30]. In the past years it has been used in studies to evaluate bone density and implant stability[31,32].

Trabecular separation showed statistically significant difference between all the groups for the pre- implant *versus* post- implant comparisons ($p<0.05$). Therefore, it was observed an increasing in bone compression during insertion, more evident in low bone densities groups. The variation values showed statistically significant differences between G2 *versus* G4; the higher values were observed after non- axial loading, and confirms the deleterious effects of lateral forces on dental implants. The inappropriate immediate loading can cause failure in oral implants, as previously showed in literature[33-37].

The total porosity showed significant differences between pre and post-implant at all groups ($p<0.05$). Those differences are expected due to a bone compaction after implant placement, as well after loading. Delta values showed significant differences between all the groups, and subcrestal implant placement promoted reduced values of porosity showing an additional bone compacting in comparison to crestal placement.

The three-dimensional BS/BV analysis showed significant differences for same bone level and different bone densities (between G1 *versus* G3 and G2 *versus* G4) indicating that the BS/BV is affected by bone density. BV/TV analysis showed significant differences for all the groups between pre-loading and post-loading. However, the lower variations were observed for G2, with higher stability after the loading challenge.

The intersection bone to implant surface/total implant surface (IS/TS) values showed significant reduction after the non-axial load in low bone densities groups, indicating trabecular disorganization after loading. Osseous density shows an important role on primary stability of implants, and has been focus of research in recently[38]. The IS/TS measured in the present study refers to primary stability and cannot be compared with *in vivo* studies reporting histological data. The deleterious effect of non-axial loading to IS/TS values is an important data regarding the protection of non-physiological loading, especially on initial healing phase in order to avoid disruption of the osseointegration biological process.

This is an *in vitro* study and the influence of secondary stability (osseointegration) is not being evaluated. The tridimensional bone to implant contact values after load represents an *in* vivo/clinical situation immediately after the loading and cannot be extrapolated to a live tissue setting. On the other hand, to date, it would be impossible to achieve an optimal standardization of bone quality and density considering an *in vivo* setting in order to perform biomechanical studies.

Within the limitations of this study, the subcrestally positioned dental implant had shown advantages for the primary stability of implants placed in low-density and high-density bone. Further *in vivo* studies might be necessary to confirm this statement and evaluate the effect of this primary stability on the osseointegration and secondary stability of dental implants.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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