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Evaluation of standardized porcine bone models to test primary stability of dental implants, using biomechanical tests and Micro-CT. An *in vitro* pilot study

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ABSTRACT

Aim This study evaluated a new porcine bone model to test the primary stability of different implants, analyzing Micro-CT, insertion torque, and pull-out strength.

Materials and methods Bone cylinders were prepared from porcine bone and separated into 2 groups: 10 high density bone cylinders (HDB), and 10 low density bone cylinders (LDB). Then, 3D pre-implant analyses were performed, evaluating tridimensional bone density (ratio of trabecular bone volume and total tomographic volume, BV/TV), trabecular separation; percentage of closed pores; percentage of open pores; percentage of total porosity, in 3 bone levels (L1 bone volume corresponding to the internal part of the threads; L2 corresponding to the area between 0 to 0.5 mm from the end of threads; L3 corresponding to the area between 0.5 to 1.5 mm from the end of threads). Twenty implants of two different macrostructures were inserted in the bone cylinders, and divided into 4 groups (5 implants each): Group 1, e-Fix HE implant placed in HDB cylinder; Group 2, e-Fix HE implant in LDB cylinder; Group 3, e-Fix HE Silver implant placed in HDB cylinder; Group 4, e-Fix HE Silver implant in LDB cylinder. The insertion torque was recorded and bone cylinders were re-evaluated by Micro-CT (post-implant analysis). Then a pull-out strength test was performed.

Results 3D analysis showed that pre- and post-implants intra-groups evaluation had statistically significant differences in Group 3 and 4, for all tomographic parameters assessed. Group 3 showed the best values for biomechanical tests (Friedman Test, $p<0.05$).

Conclusion This methodology can produce standardized bone cylinders of high and low bone density, in which different implant designs are able to promote different effects, evidenced by biomechanical and image analysis.

KEYWORDS Bone density; Computerized tomography; Implant primary stability; Insertion torque; Pull-out strength.

INTRODUCTION

Primary stability is achieved when an implant is positioned into the host bone and there is a direct mechanical connection between its surface and the surrounding bone (1). The success of this adaptation, however, depends on several factors, including the implant geometry (length, diameter and shape), the quality and quantity of the host bone, and the surgical site preparation technique (2, 3).

Tapered implants have been used to improve esthetics and facilitate implant placement between adjacent natural teeth (3), and were initially designed for immediate placement after tooth extraction. Tapered implants were devised to provide a degree of compression of the cortical bone in an implant site with poor bone quality (4, 5), thereby creating a more uniform compaction of bone when compared to parallel-walled implants.

The design of threads is also crucial for the success of the implant placement in the host bone (6). Indeed, threads maximize the bone-implant contact, improve primary stability, increase the surface area of the dental implant (7) and promote a more favorable dissipation of masticatory tensions (8). Clinical studies have shown that implants with square threads possess certain beneficial qualities promoting bone condensation; furthermore, incorporating more threads per surface area can make the implants more stable (6, 9).

The quality and quantity of bone in the receptor site is considered one of the most important factors for achieving and maintaining the success of oral implants. Lekholm and Zarb (10) classified the bone quality into four different types: homogenous cortical bone (type I), a thick layer of cortical bone that surrounds a central part of dense trabecular bone (type II), a thin layer of cortical bone that surrounds a dense trabecular bone of favorable strength (type III), and a thin layer of cortical bone that surrounds a low density trabecular bone (type IV) (10). Placing implants in type I to type III bones leads to good clinical outcomes, whereas type IV is linked to a lower success rate related to the lack of adequate implant primary stability (11).

Computerized Tomography (CT) introduced three-dimensional evaluation of bone structures, thus expanding the analysis obtained with the two-dimensional radiographic images (12, 13). Recently, Micro-computed tomography (Micro-CT) scanners were developed, allowing to study structures of a few micrometers such as bone trabeculae (14).

Implant stability can be measured by noninvasive (such as insertion torque, Osstell) or invasive (Pull-out and Removal Torque) (15) test methods. In the insertion torque (IT) method, the main purpose is to quantify the torque (in Ncm units) required to seat the implant into the socket during surgery and thereby assess bone support and density (16).

It is unknown how much torque is necessary to achieve sufficient primary stability for individual implant systems (17), but some evidences suggest that a minimum of 35 Ncm should be achieved with immediate implant loading (18). There is a correlation between IT and bone mineral density, which can be determined using CT (19). There are also biometric tests related to the design of implants widely used in research, such as the axial pull-out strength (PS), an invasive method well established in orthopedic medical studies (20, 21). A previous study demonstrated the correlation between IT and axial PS, showing that screw retention in bone tissue can be predicted through IT (22).

Several manufacturers have introduced implants specially designed for areas of low bone quality, stating better results in these clinical situations. However, according to our knowledge, there are no comparative studies regarding these implants, aiming to tridimensionallly assess bone variability, as well as IT and axial PS. Therefore, the purpose of the present study was to evaluate a new standardized bone model, with high and low density bone, comparing with biomechanical tests and Micro-CT the primary stability of two different implant designs.

MATERIALS AND METHODS

Preparation of bone cylinders

Fresh porcine bone was used in this study. Using an especially designed trephine bur, osteotomy was conducted and 80 bone cylinders with 15 mm in diameter and 18 mm in length were prepared from porcine bone, removed from the mandibular condyle (40 cylinders, low-density bone LDB) or from the femur head (40 cylinders, high-density bone, HDB). The samples were kept frozen, and stored at -20° C until the experiments (Fig. 1).

FIG. 1 Bone cylinders prepared and identified.

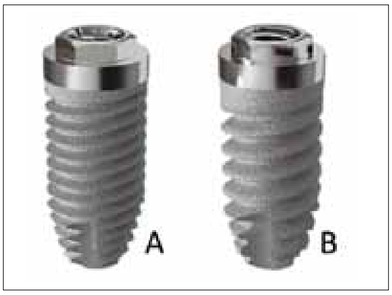

FIG. 2 A) e-Fix HE implants (3.75x10 mm); B) e-Fix Silver implants (3.75x10 mm).

Implant selection

Twenty implants of two different types, both 3.75 mm wide and 10 mm long, were selected for this study: e-Fix HE (10 implants) and e-Fix HE Silver (10 implants) (TitaniumFix, São José dos Campos, SP, Brazil) (Fig. 2). According to the manufacturer, these tapered implants have self-drilling threads with double entry and conical rounded apex with four cutting chambers. Furthermore, the e-Fix HE Silver implants have large external threads and more space between them in order to increase the bone-implant contact surface and the compression to the bone, providing higher primary stability, particularly in poor bone quality, as stated by the manufacturing company.

Standardization of bone cylinders, two-dimensional radiographic analysis

Bone cylinders were prepared from porcine bone and separated into 2 groups: Group A, cylinders removed from the femur head (HDB); and Group B, cylinders removed from the mandibular condyle (LDB). Digital radiographs were taken from each cylinder, using a digital sensor (RVG Trophy, Eastman Kodak Company, Rochester, NY, EUA), and the images obtained had the gray levels calibrated with the use of a specific software. Then, the radiographic density values were obtained by the mean gray value of the pixels using the histogram command. Three standardized regions of interest (ROIs), which corresponded to 16 square pixels, were defined for each cylinder, all of them situated in the central portion of the radiographic image: one coronal, one central and one apical. The radiographic density of each ROI was calculated, and the average of the three ROIs was assumed as the 2D radiographic density of the bone cylinder. The cylinders were then grouped according to their 2D bone densities values: cylinders with values equal or greater than 110 were selected for Group A (HDB), and with values equal or lower than 80 were selected for Group B (LDB). The cylinders with intermediate values were discharged, and at the end of this process there were 20 bone cylinders, 10 from group A and 10 from group B.

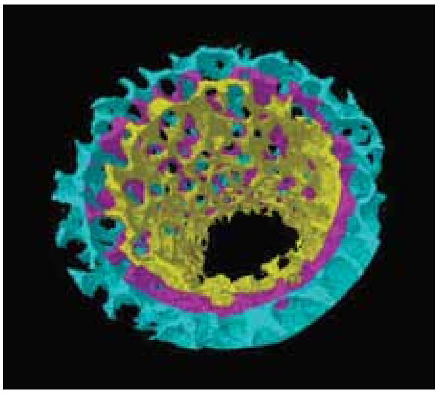

FIG. 3 Pre-implant tomographic image. Yellow: Level 1 pre-implant;. Purple: Level 2 pre-implant;. Blue: Level 3 pre-implant.

Standardization of bone cylinders, three-dimensional tomographic analysis

Micro-CT scans of each bone cylinder were made for the evaluation of tomographic bone parameters. Three-dimensional analysis was performed using the Micro-Sky Scan 1172-160 (SkyScan, Antwerp, Belgium). After image reconstruction, from the center of the bone cylinder, a tomographic cylinder measuring 7 mm in diameter and 5 mm in height, starting 2 mm below the top of the bone cylinder was defined, and three-dimensional bone density (ratio of trabecular bone volume and total tomographic volume, BV/TV) was measured.

Tomography prior to implant placement

Before insertion of the implants in bone cylinders, another 3D morphometric analysis was performed. For that, three volumetric evaluation levels were determined for the bone cylinders: Level 1 (L1) pre-implant: morphometric analysis in the first area, corresponding to the bone volume that will be internal to the threads after the insertion of the implant; Level 2 (L2) pre-implant: morphometric analysis in the second area (0 to 0.5 mm from the end of threads), corresponding to the bone volume that will be, after insertion of the implant, immediately adjacent to the end of the threads up to 0.5 mm; Level 3 (L3) pre-implant: morphometric analysis in the third area (0.5 to 1.5 mm from the end of threads), corresponding to the bone volume that will be, after insertion of the implant, 0.5 mm to 1.5 mm distant from the end of the threads (Fig. 3).
For all the evaluation levels, the following tomographic parameters were analyzed: three-dimensional bone density (BV/TV); trabecular separation (TbSp) (maximum separation between trabeculae in volume assessed); percentage of closed pores (POcl); percentage of open pores (POop); percentage of total porosity (POtot).

Implant placement

The bone cylinders were fixed in a basis specially designed to immobilize them, and the site was prepared with progressive drilling sequence, at 800 rpm, with abundant saline solution irrigation, following the protocol recommended by the manufacturer of the implants for poor bone quality (Fig. 4). After site preparation, the implants were inserted into

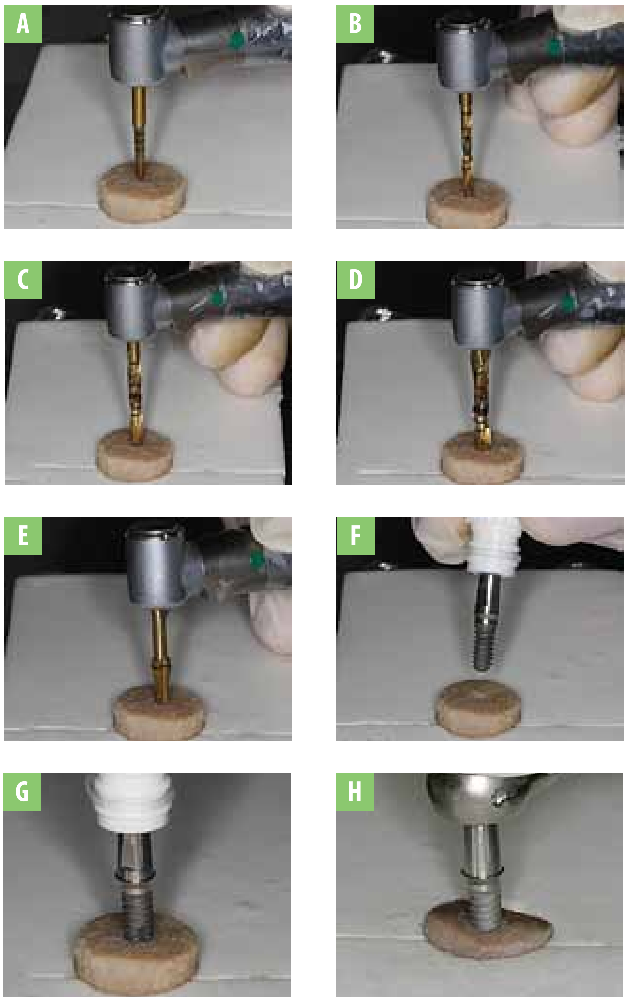

FIG. 4 A) Start of the perforation; B) 2.0 mm in diameter drill; C) 2.5 mm in diameter drill, D) 3.0 mm in diameter drill; E) counter sink drill, F, G, H) e-Fix HE Silver implant of 3.75 x 10 mm installation; I) implant in final position.

the bone cylinders (one implant in each cylinder) in the following manner: Group 1: e-Fix HE implant placed in HDB cylinders (n=5); Group 2: e-Fix HE implant placed in LDB cylinders (n=5); Group 3: e-Fix HE Silver implant placed in HDB cylinders (n=5); Group 4: e-Fix HE Silver implant placed in LDB cylinders (n=5).
The insertion of implants on cylinders was performed using the manual surgical ratchet of the kit, which has a torque control. The insertion was always initiated with the torque of 10 Ncm; when this torque was reached, a gradual increase in the ratchet torque was made, in multiples of 5 Ncm, until the complete insertion of the implant. The rotation of the ratchet was interrupted when the implant was fully inserted and positioned at the bone level, and the last value recorded in the ratchet was considered the value of IT for each implant.

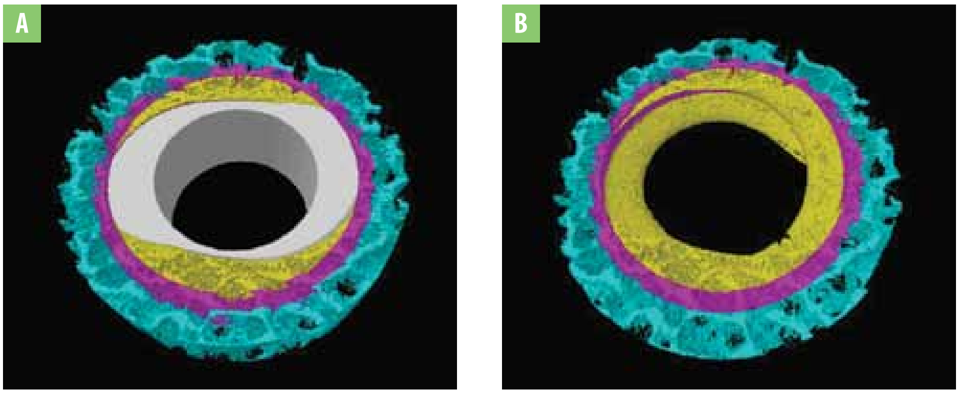

FIG. 5 (A, B) Post-implant tomographic image.
Yellow: Level 1 post-implant.
Purple: Level 2 post-implant.
Blue: Level 3 post-implant.

Tomography after implant placement

After installation of the implants new Micro-CT scans and micro-tomographic reconstructions of the cylinders were performed.

Using the analysis software (CTan Analyser; Skyscan, Antwerp, Belgium) to quantify microstructures, the volume region of interest (ROI) was determined for 3D morphometric analysis. For that, three new volumetric evaluation levels were determined in the bone cylinders: Level 1 (L1) post-implant: morphometric analysis in the first area, corresponding to the bone volume internal to the threads; Level 2 (L2) post-implant: morphometric analysis in the second area (0 to 0.5 mm from the end of threads), corresponding to the bone immediately adjacent to the end of the threads up to 0.5 mm; Level 3 (L3) post-implant: morphometric analysis in the third area (0.5 to 1.5 mm from the end of threads), corresponding to the bone volume 0.5 mm to 1.5 mm distant to the end of the threads (Fig. 5). For all the evaluation levels, the same tomographic parameters previously described were analyzed.

Pull-out strength test

After the Micro-CT analysis, the biomechanical PS test was performed in each implant placed in the bone cylinders, evaluating the resistance force of the bone-implant interface. The test was conducted at the Laboratory of Bioengineering of the Faculty of Medicine of Ribeirão Preto, according to the technical standard ASTM F543.

The 20 implants placed in individual bone cylinders (five from each group, Group 1, 2, 3 and 4) were used. The cylinder with the implant was positioned in the Universal Testing Machine and connected to a mobile base by a device specially designed and screwed to the implant. After that, a load cell of 200 kg was adjusted, and, after a pre-load of 10 N for 30 seconds, an axial tensile strength with constant speed of 2 mm/min was applied. The curve of load versus deformation was evaluated using the software Tesc 1.13: the higher value in this curve was considered the pull-out force.

Statistical analysis

All variables were tested for normality of data; according to the result, parametric or nonparametric tests were chosen. The Friedman test was used for intra and between-groups comparisons for the averages of insertion torque, pull-out test and intra group analysis for the three-dimensional analysis. The Kruskal-Wallis test was used for comparisons between groups for the three-dimensional analysis. For the 2D classification of bone cylinders the Mann-Whitney test was used. To evaluate the occurrence of correlation between 2D and 3D analysis in the bone cylinders classification process, Spearman Rank Correlation Coefficient was used. For all analysis, a significance level of 5% was considered.

RESULTS

Classification of bone cylinders

In 2D analysis, the mean $\pm$ standard deviation (SD) for Group A was 113 $\pm$ 2.58; and for Group B it was 72.70 $\pm$ 11.46, a statistically significant difference was found between groups (Student t test; $p<0.0001$). The three-dimensional bone density (BV/TV), in 3D analysis (mean $\pm$ SD) was 25.73 $\pm$ 2.83 for Group A and 17.92 $\pm$ 5.70 for Group B (Student t test; $p=0.0002$). In addition, statistically significant correlation was observed between the 2D (Bone Density) and 3D (BV/TV) analysis (Spearman correlation test; correlation coefficient of 73%; $p<0.0003$) (Fig. 6).

Three-dimensional tomographic analysis

Three-dimensional bone density: The intra groups evaluation of BV/TV in L1 pre- and post-implant showed statistically significant differences in Group 3 (L1 pre-implant: 8.01 $\pm$ 0.45%; L1 post-implant: 10.75 $\pm$ 0.75%) and Group 4 (L1 pre-implant: 5.00 $\pm$ 2.63%; L1 post-implant: 7.07 $\pm$ 2.21%). In the intra group results in L2 pre and post implant, statistically significant differences were found among all groups. In L3, statistically significant differences in Group 1 (L3 pre-implant: 8.34 $\pm$ 1.00%; L3 post-implant: 6.97 $\pm$ 0.57%), Group 3 (L3 pre-implant: 9.46 $\pm$ 0.54%; L3 post-implant: 8.21 $\pm$ 0.97%) and Group 4 (L3 pre-implant: 6.05 $\pm$ 2.89%; L3 post-implant: 4.93 $\pm$ 2.26%) (Friedman test, $p<0.05$) were observed (Table 1).

The analysis among experimental groups (Group 1, 2, 3

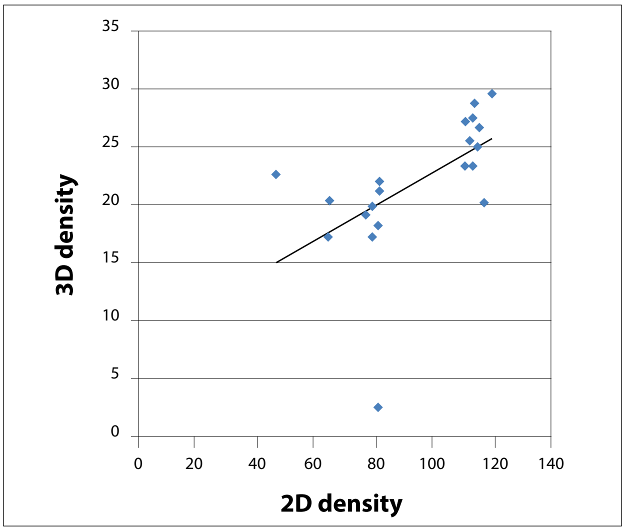

FIG. 6 Statistically significant correlation between the 2D (Bone Density) and 3D (BV/TV) analysis (Spearman correlation test; p<0.0003).

and 4) showed significant differences between Group 2 and Group 3, in L1 and L3 pre-implant. In L1 post-implant, statistical difference between Group 2 and Group 3 (Kruskal Wallis test, p<0.05) was also observed (Table 1).

Trabecular separation: The intra group analysis of this parameter has shown in Levels 1, 2 and 3, a statistically significant reduction of the values for all groups from pre- to post-implant situations (Friedman test, p<0.05), but no difference was found among groups (Kruskal Wallis test, p>0.05) (Table 2).

Percentage of closed pores: Concerning this parameter, for all groups, an increased trend in the number of closed pores from pre-implant to post-implant situation was observed. The pre versus post-implant intra-groups analysis showed statistically significant differences in L1 for Group 1 (L1 pre-implant: 0.24 ± 0.08%; L1 post-implant: 1.69 ± 0.69%), and Group 3 (L1 pre-implant: 0.38 ± 0.09%; L1 post-implant: 1.31 ± 0.41%); in L2, for all groups; and in L3, for Group 2 (L3 pre-implant: 0.27 ±

	Three-dimensional Bone Density (BV /TV)														
	Leve l Pre-Implant			Level Post-Implant			Intra Groups Difference (p)*								
	Level l (a)	Level 2 (b)	Level 3 (c)	Level l (d)	Level 2 (e)	Level 3 (f)	a/b	a/c	b/c	a/d	b/e	c/f	d/e	d/f	e/f
Gl (w)	7.16±0.95	5.21±0.61	8.34±1.00	7.57±1.06	6.79±0.65	6.97±0.57	ns	ns	<0.05	ns	<0.05	<0.05	ns	ns	ns
G2 (x)	5.47±0.62	4.14±0.59	6.53±0.87	6.06±0.65	6.57±1.07	6.06±1.05	ns	ns	<0.05	ns	<0.05	ns	ns	ns	ns
G3 (y)	8.01±0.45	6.04±0.42	9.46±0.54	10.75±0.75	7.02±0.97	7.20±0.97	ns	ns	<0.05	<0.05	<0.05	<0.05	ns	ns	ns
G4 (z)	5.00±2.63	3.90±2.06	6.05±2.89	7.07±2.21	4.92±1.80	4.93±2.26	ns	ns	ns	<0.05	<0.05	<0.05	ns	ns	ns
Between Groups Difference** w/x	ns	ns	ns	ns	ns	ns									
w/y	ns	ns	ns	ns	ns	ns									
w/z	ns	ns	ns	ns	ns	ns									
x/y	<0.05	ns	<0.05	<0.05	ns	ns									
x/z	ns	ns	ns	ns	ns	ns									
y/z	ns	ns	ns	ns	ns	ns									

*: intra -group analysis- Friedman Test. **: between group analysis- Kruskal Wallis Test. ns: no significant difference

TABLE 1 Mean values ± SD (mm) of Three-dimensional Bone Density (BV/TV) intra and between groups (pre- and post-implant).

	Trabecular Separation (Tb.Sp)														
	Leve l Pre-Implant			Level Post-Implant			Intra Groups Difference (p)*								
	Level l (a)	Level 2 (b)	Level 3 (c)	Level l (d)	Level 2 (e)	Level 3 (f)	a/b	a/c	b/c	a/d	b/e	c/f	d/e	d/f	e/f
Gl (w)	2.21±0.02	2.74±0.02	2.69±0.02	0.80±0.01	0.83±0.003	0.78±0.004	ns	ns	ns	<0.05	<0.05	<0.05	ns	ns	<0.05
G2 (x)	2.21±0.03	2.75±0.01	2.67±0.03	0.78±0.01	0.83±0.007	0.78±0.01	ns	ns	ns	<0.05	<0.05	<0.05	ns	ns	ns
G3 (y)	2.20±0.01	2.75±0.02	2.69±0.02	0.75±0.01	0.83±0.03	0.78±0.003	ns	ns	ns	<0.05	<0.05	<0.05	ns	<0.05	ns
G4 (z)	2.34±0.24	2.88±0.22	2.78	±0.18	0.73±0.02	0.82±0.01	0.79±0.01	ns	ns	ns	<0.05	<0.05	<0.05	<0.05	ns
Between Groups Difference** w/x	ns	ns	ns	ns	ns	ns									
w/y	ns	ns	ns	ns	ns	ns									
w/z	ns	ns	ns	ns	ns	ns									
x/y	ns	ns	ns	ns	ns	ns									
x/z	ns	ns	ns	ns	ns	ns									
y/z	ns	ns	ns	ns	ns	ns									

*: intra -group analysis- Friedman Test. **: between group analysis- Kruskal Wallis Test. ns: no significant difference

TABLE 2 Mean values ± SD (mm) of Trabecular Separation (Tb.Sp) intra and between groups (pre- and post-implant).

0.09%, L3 post-implant: 0.33 ± 0.11%), Group 3 (L3 pre-implant: 0.41 ± 0.10%, L3 post-implant: 0.55 ± 0.18%) and Group 4 (L3 pre-implant: 0.23 ± 0.10%, L3 post-implant: 0.42 ± 0.30%) (Friedman test; p<0.05). There were no statistically significant differences among groups (Kruskal Wallis test, p>0.05) (Table 3).

Percentage of open pores: For all groups, from pre to post-implant evaluation, a trend of reduction in POop, in L1 and L2, and of increase in L3 was observed. In L1, statistically significant differences were found in Group 2 (L1 pre-implant: 94.52 ± 92.29%; L1 post-implant: 93.89 ± 0.66%), Group 3 (L1 pre-implant: 91.96 ± 0.46%; L1 post-implant: 89.11 ± 0.76%) and Group 4 (L1 pre-implant: 94.98 ± 2.63%; L1 post-implant: 92.87 ± 2.24%); in L2, in all groups; and in L3, in Group 1 (L3 pre-implant: 91.63 ± 1.01 %; L3 post-implant: 93.00 ± 0.58%) and Group 3 (L3 pre-implant: 90.50 ± 0.55%; L3 post-implant: 91.74 ± 0.99%) (Friedman test, p<0.05) (Table 4).

The comparisons among groups showed statistically significant differences between Group 2 and Group 3 in L1, L2 and L3 pre-implant, and between the same groups in L1 post-implant (Kruskal Wallis test, p<0.05) (Table 4).

Percentage of total porosity(PoTot): This parameter showed a reduction trend from pre to post-implant evaluations for all levels. In L1, statistically significant differences in Group 3 (L1 pre-implant: 91.99 ± 0.45%; L1 post-implant: 89.25 ± 0.75%) and in Group 4 (L1 pre-implant: 95.00 ± 2.63%; L1 post-implant: 92.92 ± 2.21%) were observed; in L2, all groups showed statistically significant differences; and in L3 there were significant differences for Group 1 (L3 pre-implant: 94.78 ± 0.61%, L3 post-implant: 93.03 ± 0.57%), Group 3 (L3 pre-implant: 93.96 ± 0.42%, L3 post-implant: 91.78 ± 0.97%) and Group 4 (L3 pre-implant: 96.10 ± 2.06%, L3 post-implant: 95.07 ± 2.26%) (Friedman test; p<0.05) (Table 5). The analysis among groups showed differences between Group 2 and Group 3 in L1 and L3 pre-implant. In L1 post-implant, it was also observed a statistically significant difference between Group 2 and Group 3 (Kruskal Wallis test, p<0.05) (Table 5).

		Percentage of closed pores (POcl)														
		Leve l Pre-Implant			Level Post-Implant			Intra Groups Difference (p)*								
		Level I (a)	Level 2 (b)	Level 3 (c)	Level I (d)	Level 2 (e)	Level 3 (f)	a/b	a/c	b/c	a/d	b/e	c/f	d/e	d/f	e/f
	G1 (w)	0.24±0.08	0.24±0.09	0.27±0.10	1.69±0.69	0.57±0.04	0.34±0.04	ns	ns	ns	<0.05	<0.05	ns	ns	ns	<0.05
	G2 (x)	0.24±0.10	0.23±0.07	0.27±0.09	0.89±0.19	0.51±0.12	0.33±0.11	ns	ns	ns	ns	<0.05	<0.05	ns	ns	ns
	G3 (y)	0.38±0.09	0.04±0.10	0.41±0.10	1.31±0.26	0.80±0.29	0.55±0.18	ns	ns	ns	<0.05	<0.05	<0.05	ns	<0.05	ns
	G4 (z)	0.23±0.05	0.21±0.09	0.23±0.10	0.73±0.41	0.50±0.33	0.42±0.30	ns	ns	ns	ns	<0.05	<0.05	<0.05	<0.05	ns
Between Groups Difference**	w/x	ns	ns	ns	ns	ns	ns									
	w/y	ns	ns	ns	ns	ns	ns									
	w/z	ns	ns	ns	ns	ns	ns									
	x/y	ns	ns	ns	ns	ns	ns									
	x/z	ns	ns	ns	ns	ns	ns									
	y/z	ns	ns	ns	ns	ns	ns									

*: intra -group analysis- Friedman Test. **: between group analysis- Kruskal Wallis Test. ns: no significant difference

TABLE 3 Mean values ± SD (mm) of Percentage of closed pores (POcl) intra and between groups (pre- and post-implant).

		Percentage of open pores (PO.op)														
		Leve l Pre-Implant			Level Post-Implant			Intra Groups Difference (p)*								
		Level I (a)	Level 2 (b)	Level 3 (c)	Level I (d)	Level 2 (e)	Level 3 (f)	a/b	a/c	b/c	a/d	b/e	c/f	d/e	d/f	e/f
	G1 (w)	92.82±0.95	94.77±0.61	91.63±1.01	92.29±1.12	93.17±0.65	93.00±0.58	ns	ns	<0.05	ns	<0.05	<0.05	ns	<0.05	<0.05
	G2 (x)	94.52±0.62	95.85±0.59	93.35±0.87	93.89±0.66	93.38±1.07	93.92±1.05	ns	ns	<0.05	<0.05	<0.05	ns	ns	<0.05	<0.05
	G3 (y)	91.96±0.46	93.94±0.43	90.50±0.55	89.11±0.76	92.74±1.00	91.74±0.99	ns	ns	<0.05	<0.05	<0.05	<0.05	ns	<0.05	<0.05
	G4 (z)	94.98±2.63	96.09±2.06	93.94±2.90	92.87±2.24	95.04±1.82	95.04±2.28	ns	ns	ns	<0.05	<0.05	ns	ns	<0.05	<0.05
Between Groups Difference**	w/x	ns	ns	ns	ns	ns	ns									
	w/y	ns	ns	ns	ns	ns	ns									
	w/z	ns	ns	ns	ns	ns	ns									
	x/y	<0.05	<0.05	<0.05	<0.05	ns	ns									
	x/z	ns	ns	ns	ns	ns	ns									
	y/z	ns	ns	ns	ns	ns	ns									

*: intra -group analysis- Friedman Test. **: between group analysis- Kruskal Wallis Test. ns: no significant difference

TABLE 4 Mean values ± SD (mm) of Percentage of open pores (PO.op) intra and between groups (pre- and post-implant).

		Percentage of total porosity (PO.tot)														
		Leve l Pre-Implant			Level Post-Implant			Intra Groups Difference (p)*								
		Level l (a)	Level 2 (b)	Level 3 (c)	Level l (d)	Level 2 (e)	Level 3 (f)	a/b	a/c	b/c	a/d	b/e	c/f	d/e	d/f	e/f
	Gl (w)	92.84±0.95	94.78±0.61	94.78±0.61	92.43±1.06	93.21±0.65	93.03±057	ns	ns	<0.05	ns	<0.05	<0.05	ns	<0.05	<0.05
	G2 (x)	92.53±0.62	95.86±0.59	95.86±0.59	93.94±0.65	93.42±1.07	93.94±1.05	ns	ns	<0.05	ns	<0.05	ns	ns	<0.05	<0.05
	G3 (y)	91.99±0.45	93.96±0.42	93.96±0.42	89.25±0.75	92.81±0.97	91.78±0.97	ns	ns	<0.05	<0.05	<0.05	<0.05	ns	<0.05	<0.05
	G4 (z)	95.00±2.63	96.10±2.06	96.10±2.06	92.92±2.21	95.08±1.80	95.07±2.26	ns	ns	ns	<0.05	<0.05	<0.05	ns	<0.05	<0.05
Between Groups Difference**	w/x	ns	ns	ns	ns	ns	ns									
	w/y	ns	ns	ns	ns	ns	ns									
	w/z	ns	ns	ns	ns	ns	ns									
	x/y	<0.05	ns	<0.05	<0.05	ns	ns									
	x/z	ns	ns	ns	ns	ns	<0.05									
	y/z	ns	ns	ns	ns	ns	ns									

*: intra -group analysis- Friedman Test. **: between group analysis- Kruskal Wallis Test. ns: no significant difference

TABLE 5 Mean values ± SD (mm) of Percentage of total porosity (PO.tot) intra and between groups (pre- and post-implant).

Biomechanical tests

The mean IT values were 35 ± 3.54 Ncm, 19 ± 5.48 Ncm, 44 ± 2.24 Ncm and 22 ± 8.37 Ncm for groups Group 1, 2, 3 and 4, respectively. The difference was statistically significant between Group 3 and Group 2 and between Group 3 and Group 4 (Friedman test; $p < 0.05$); not statistically significant differences between Group 1 and Group 3, and Group 2 and Group 4 (Fig. 7) were observed.

In the PS test, the mean values were 430.29 ± 29.81 N, 157.48 ± 11.78 N, 507.20 ± 54.11 N and 178.09 ± 93.98 N for Group 1, 2, 3 and 4, respectively. The difference was statistically significant between Group 3 and Group 2, and between Group 3 and Group 4 (Friedman test; $p < 0.05$) (Fig. 8).

DISCUSSION

Primary implant stability depends on bone quality and quantity, implant geometry and the site preparation technique. Primary implant stability can remarkably decrease in 'poor bone quality' and thereby jeopardize the osseointegration process (23). Although various designs of implants have shown favorable clinical outcomes, there are not to our knowledge comparative studies controlling the bone variability factors (trabecular density, trabecular separation, porosity). In the present study the standardization of bone cylinders comprised 2D and 3D analysis, and statistically significant correlations were observed between (Spearman correlation test; correlation coefficient of

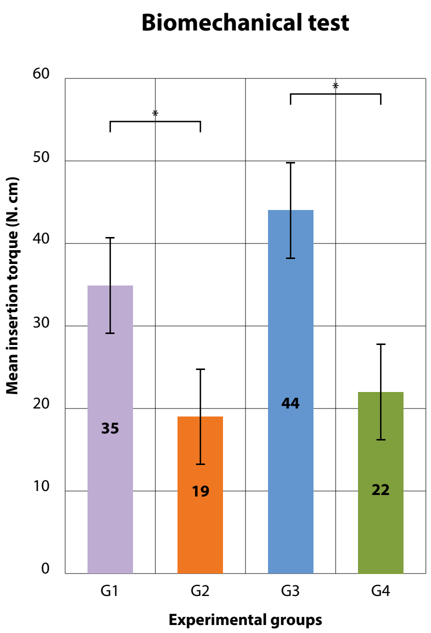

FIG. 7 Implant insertion torque - mean values ± SD for G1, G2, G3 and G4. The signal * denotes statistically significant difference between groups (Friedman test, p<0.05).

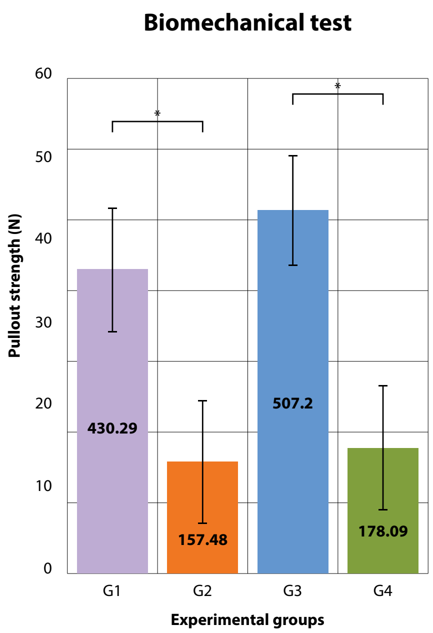

FIG. 8 Implant pullout strength test - mean values ± SD for G1, G2, G3 and G4. The signal * denotes statistically significant difference between groups (Friedman test, p<0.05).

73%; p<0.0003) and intra-groups (2D analysis - Group A: 113 ±2.58; Group B: 72.7 ±11.46; p<0.0001 and 3D analysis – Group A 25.73 ± 2.83; Group B 17.92 ± 5.70; p=0.0002).

After determining the two bone groups according to bone density, the implants were inserted in the bone cylinders. Both implants were tapered in design, but the e-Fix HE Silver implants have a greater thread pitch to increase the bone-implant contact surface and the compression of the bone. The tapered implant and thread design provide higher primary stability, particularly in poor bone quality and, thus, it should increase IT and PS (20, 24).

In the evaluation of the biomechanical performance of screws placed into bone of different densities, methods such as IT and axial PS tests are more often used in orthopedics and in oral and maxillofacial surgery (21, 25-24). Several studies have examined the correlation between IT and axial PS to determine whether IT can predict screw retention in bone tissue (22, 25-28). According to many Authors, there is a correlation between IT and axial PS (22, 28), although other investigations are not of the same opinion (26, 27). The present study agrees with the majority of the literature demonstrating a correlation between IT and axial PS: the highest values of both tests were observed in Group 3 (IT: 44 ± 2.24 Ncm; PS: 507.20 ± 54.11 N) and the lowest in Group 2 (IT: 19 ± 5.48 Ncm; PS: 157.48 ± 11.78 N); statistical differences were found between Group 2 and Group 3, and between Group 3 and Group 4 (IT: 22 ± 8.37 Ncm; PS: 178.09 ± 93.98 N); the lowest values were correlated to low bone density and the highest to high bone density.

Computerized tomography (CT) is an established method for acquiring bone images before oral implant surgery (29) and it is also used for objective quantification of trabecular and cancellous bone mineral densities, and direct density measurements (30). Clinical studies using CT before oral implant surgery observed correlation between IT and bone density in designated implant sites, and also concluded that the CT could be a tool to predict the primary stability (31, 32). The results of the present study agree with this observation: the groups with higher 3D bone density also showed the highest values of IT and PS.

The analysis of BV/TV on bone inside the threads (L1 pre and post implant) showed that the e-Fix Silver implants placement lead to the highest condensation in different bone densities (Group 3 L1 pre-implant 8.01 ± 0.45%, L1 post-implant 10.75 ± 0.75%; Group 4 L1 pre-implant 5.00 ± 2.63%, L1 post-implant 7.07 ± 2.21%). This probably occurred due to greater thread pitch of the implant, increasing compression during insertion (33, 34).

These results were also observed for TbSp (trabecular separation) (Group 3 L1 pre-implant 2.20 ± 0.01 mm, L1 post-implant 0.75 ± 0.01 mm; Group 4 L1 pre-implant 2.34 ± 0.24 mm, L1 post-implant 0.73 ± 0.02 mm), in the POop (Group 3 L1 pre-implant 91.96 ± 0.46%; L1 post-implant 89.11 ± 0.76%; Group 4 L1 pre-implant 94.98 ± 2.63%; L1 post-implant 92.87 ± 2.24%), and POtot (Group 3 L1 pre-implant 91.99 ± 0.45%, L1 post-implant 89.25 ± 0.75%; Group 4 L1 pre-implant 95.00 ± 2.63%, L1 post-implant 92.92 ± 2.21%).

The bone area immediately adjacent to the end of the threads (L2) also showed greater post-implant values for BV/TV, TbSp, POop and POtot, mainly in Groups 3 and 4, demonstrating an appropriate bone condensation made by the implant during insertion and resulting in an increased bone density in the adjacent area, that contributes to the enhancement of primary stability. However, the analysis of pre-and post-implant tomographic parameters in Level 3 (0.5 mm to 1.5 mm distant to the end of the threads), revealed a decrease in BV/TV and POop in different bone densities, numerically more evident in Group 4 (BV/TV, L3 pre-implant 6.05 ± 2.89%, L3 post-implant 4.93 ± 2.26%; POop, L3 pre-implant 93.94 ± 2.90%, L3 post-implant 95.04 ± 2.28%). Probably the implant insertion caused a trabecular structural disorganization in this surrounding area, and this effect was greater in Group 3 and Group 4 that showed a higher bone compression during insertion.

The development of in vitro models with standardized bone characteristics to evaluate mechanical aspects of bone fixation devices is an important area of interest both in medical and in dentistry field. Particularly in dentistry, artificial bone has been used to study treatment of facial fractures, and, although these models allow a good reproducibility, they do not enable image evaluation. The present study describes a standardized bone model of high and low density that could be useful in future studies to evaluate different aspects of dental implants macro-structure and primary stability, providing crucial information on immediate implant loading protocols.

CONCLUSION

The results of this study allow to conclude that:
- this methodology can produce standardized bone cylinders of high and low bone density;
- there is correlation among insertion torque and pull-out strength values;
- different implant designs are able to promote different effects, in presence of high and low density bone.

Further studies should be focused on the behavior of bone structure against different threads, sizes, diameters, and shapes of implants.

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