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MANUFACTURING OF FUNCTIONALLY GRADED ALUMINA CERAMIC WITH DENSE CORE AND POROUS SURFACE USING CO-PRESSING METHOD

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Abstract. *Studies has shown that materials with gradients in the transition region can reduce or eliminate the problem of delamination which is common in biphasic implant design. In order to improve the mechanical performance and to enhance the cell signalling of surgical prosthesis and implants, functionally graded alumina ceramics (FGM) scaffolds with a dense core coated with a layer bearing a porosity transition in porosity from the surface towards the centre were designed. The surface of this innovative structure was covered with bioactive materials, hydroxyapatite (HA) and bioglass, under vacuum. The functionally graded structure with dense core and porous surface was prepared by co-pressing method (powder plus dry slurry) followed by sintering. Initially porous alumina ceramics with bioactive materials coating were manufactured; the material was tested in vivo on rat tibiae in order to assess the degree of adhesion of the porous surface coated with bioactive materials using push-out tests. Porous-coated and uncoated samples were compared according to the time protocol of 14 to 28 days; the coated samples presented significant difference, that is, as longer the implantation time the greater the shear stress; for the uncoated samples the correspondent values were not significant. Tukey tests were performed and p values <0.05 were considered significant. After the manufacture of the samples with functional gradient porosity, they were analyzed under scanning electron microscopy and no evidence of cracking in the dense-porous interface was observed. The results obtained demonstrated a promising in vivo adhesion of the sample porous surface convincing that the manufacturing technique used to obtain the functional gradient in alumina ceramic has a high potential to osteoimplants applications.*

Keywords: *Functional gradient material, Alumina ceramic scaffolds, Bone tissue engineering, Dental tissue engineering, Push-out tests.*

1. INTRODUCTION

Porous materials are desirable in dental and orthopaedic surgery for treatment of non-union and replacement of bone losses during trauma and tumour removal, although they have poor mechanical properties.

Research with implants in gradients with a transition region, also known as Functional Gradient Materials (FGM) provide new expectations to reduce or eliminate problems of fracture, delamination and loosening, which are common in homogeneous and / or biphasic implants [Sherwood, J.K. *et al*., 2002].

The concept of functional gradient materials has been proposed in Japan in 1984 [M. Koizumi and M. Niino, 1995]. From that date on, these materials have inspired researchers worldwide to combine properties and features not found in materials with conventional structure. Initially proposed for the aerospace engineering program, currently have arisen applications for tissue engineering and others. However, in order to design and manufacture FGMs with advanced multifunctional properties, the proper selection between components and technique that produces the transition regions is of essential importance in projects involving applications for bone tissue engineering and tooth replacement [Kawasaki and Watanabe, 2002, Watari et al. 2004].

Heterogeneous materials can be employed in several types of applications in aerospace engineering and tissue engineering. One of the biggest challenges when compared to

homogeneous materials is the manufacture of the transition regions. The selected components should be combined with a manufacturing technique suitable for obtaining implants with functional gradient.

The implant surface is the first part to come in contact with the tissue and the tissue engineering of the surfaces has received much attention because it positively modulates the host response to the implant. Among the more modified parameters, topography and surface chemistry have shown promising results [Albrektsson & Wennerberg, 2004)].

When designing porous structures or scaffolds for implants, one must plan the pore size and porosity, because they are important parameters for good tissue response. The size of pores with a minimum of 100 μm and pores larger than 200 μm are essential for the osteoconductive process. The pore size is important in cell viability and affinity, unity and spreading, intracellular signaling and transport of nutrients and metabolites [Oh, SH et al. 2007]. However, one should control the porosity so that does not compromise the mechanical properties of the implant, because the larger the porosity, the lower the mechanical strength of the implant.

However, dense materials have better mechanical strength than porous, when mechanically tested prior to deployment in biological tissue. But those often present little cellular signaling and do not result in an attachment to the host tissue as occurs with porous materials. As Anderson [2001] explains, the response at the final stage of healing biomaterials is generally fibrosis or fibrous encapsulation. Nevertheless for porous materials the encapsulation does not occur, and what happens is the regeneration which is the replacement of injured tissue by cells of the same type.

Several studies [Chen et al., 2005; Oh S.H. et al., 2007; Hsu, Y.H.; Turner, I.G.; Miles, A.W., 2007; Singh, Berkland and Detamore, 2008; Miao X and Sun D., 2010] have investigated processes for heterogeneous materials with transition regions, such as FGMs.

Methods are proposed to fabricate FGMs, which include adhesive bonding, sintering, thermal spray, reactive infiltration, cold fusion (freeze-casting) [Macchetta et al., 2009], centrifugation of a suspension [Chen et al. 2005; Oh, Sh et al., 2007] with or without cold drying, tape casting multiple - this method manufacture parts with pore size and porosity controllable but especially poor in porous interfaces interconnectivity, which is likely to be the main cause of rolling or delamination [Werner et al., 2002].

The main advantage of the co-processing method of this work is to obtain materials with transition region as FGMs and maintaining the continuity of the different phases even after the sample sintering reducing the stress concentration. This feature provides the security of working with the material in different environments and probably in complex environments such as those required for bone tissue engineering and dental implants.

The Functionally Gradient Material under study was manufactured in cylindrical polymer moulds; alumina powder slurry with sucrose to form pores was added, followed by isostatic pressing and sintering. The presence of cracks and delamination was investigated using scanning electron microscopy. To analyze the adhesion of the implant to the bone, cylindrical samples were implanted in rat tibiae using porous alumina with pore size and porosity similar to the surface of the functionally graded alumina ceramics (FGM) scaffolds. Push-out tests were performed to evaluate the shear strength between bone and implant.

2. MATERIALS AND METHODS

The surface of bone implants should contain properties that promote osseous integration and factors such as porosity, roughness and bioactivity are crucial to the bone integration process. In order to plan the best surface of a functionally gradient material with dense core and porous surface, alumina ceramic scaffolds were covered on the surface with inert material – alumina and with bioactive materials - bioglass and hydroxyapatite (HA) and were characterized in terms of the implant to bone adhesion. The scaffolds with bioactive glass and HA on the surface were produced with the same surface of functionally graded alumina ceramics (FGM) scaffolds designed.

2.1 Sample Manufacturing

The methodology used for the sample manufacturing is shown in the flow chart in Fig. 1.

Fig. 1 – Flow chart of the methodology.

The implant samples were manufactured at the Laboratory of Tribology and Composite of the Engineering School of Sao Carlos, University of Sao Paulo – LTC-EESC-USP.

Porous samples. The manufacturing technique of porous implants samples was accomplished in previous study of Camilo et al. (2009). Briefly, follows the technique for the manufacturing of porous ceramic scaffolds. In order to compare the effect of bioactive materials coating on alumina ceramic scaffolds, two kinds of scaffolds were prepared – **coated scaffolds** and **uncoated scaffolds**, using two different ceramic slurries. The alumina ceramic scaffolds were obtained by the addition of fugitive pore forming agent method incorporating sucrose to the alumina ceramic slurry in association to isostatic pressing and sintering. The materials used for the scaffolds manufacturing were the Alumina (Alcoa & Chemicals Ltd) with particle minimum diameter of 0.6 μm and a surface area of 6 to 8 m^2/g; HAp (Sigma-Aldrich–289396) and Bioglass (Biogran–45S5®) for infiltration in the

porous alumina matrices; Polivinil-butiral (PVB) (Butvar B98) as binder and Isopropilic Alcohol (SHOVEL) as solvents, distilled water for leaching and Sucrose (SHOVEL) as the pore forming agent.

Ceramic slurries. The ceramic scaffold compositions were composed by Alumina ceramic slurry (AS) and Bioglass and HA (Hydroxyapatite) ceramic slurry for infiltration (Bg-HS). The slurry AS was obtained with raw material filled in a polyethylene jar (400ml) and milled for 6 hours, after the sucrose particles with 150 to 600 µm was added and mixed into slurries during 5 minutes, The slurry was dried in a hot air gun to obtain the sucrose-powder granules. The raw material for slurry Bg-HS composition was milled using nylon jars during 170 h in a vibratory mill with zirconia spheres milling elements.

Scaffold forming. Cylindrical test samples (Ø 2.5mm x 1.5mm) were produced for the *in vivo* tests. The ends of the implants are hemispherical, as shown in Fig. 2, according to standardized ASTM F981 - 04. The samples were obtained by uniaxial pressing at 10 MPa and compacted by isostatic pressing at 100.0 MPa during 1.0 minute.

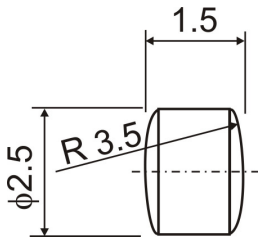

Fig. 2 - Implant samples dimensions (in mm).

Scaffold leaching. Before isostatic pressing of the samples the sucrose was removed from the samples through leaching with distilled water during 12.0 h to obtain the scaffold porosity. The remaining sucrose was burn off during the sintering.

Scaffold sintering. The green alumina samples were sintered with specific heating temperatures of 1550.0 ºC and resting in this temperature during 2.0 h. The heating rate was defined in accordance with the thermo-gravimetric analysis (TGA) of the pore forming agent (sucrose).

Alumina ceramic scaffolds coating. The alumina ceramic scaffolds samples were infiltrated with bioactive slurry Bg-HS in vacuum and after they were fired at 900.0 ºC during 1.0 h to guarantee the infiltrated adhesion. These samples are the called ***coated scaffolds*** and the ***alumina scaffolds***, without the infiltration, are called ***controls*** or ***uncoated scaffolds***. According to microstructure analysis performed in a previous study (Camilo et al., 2009) samples presented a porosity concentration near 70% and average pore size of 190 – 230 µm.

In parallel with the characterization of full porous implants scaffolds techniques for the manufacturing of the functional gradient materials were developed to obtain samples without cracks or delamination. Structures with dense core and gradual porous surfaces were designed in order to improve mechanical resistance and promote bone integration implants as well.

Functional gradient cylindrical samples. Two techniques were tested, **dipping coat** into dense cylindrical sample and **co-pressing** from both sides dense and porous cylindrical sample.

Dipping coat. Cylindrical test samples (Ø 2.5 mm x 1.5 mm) were produced to test dip coating method. Alumina ceramic slurry was dried in hot air gun to obtain the alumina powder. The samples were obtained by uniaxial pressing at 10.0 MPa and compacted by

isostatic pressing at 100.0 MPa during 1.0 minute. The green dense alumina samples were sintered with specific heating temperatures of 1550.0 ºC and followed by resting in this temperature during 2.0 h. The dense alumina cylindrical test samples were then dipped into the alumina with sucrose slurry in order to create the surface porosity onto the sample dense core. The samples were sintered and observed for the presence of detachment of porous layer.

Co-pressing. Layers of alumina slurry with sucrose were dried in the cylindrical mould using hot air gun. Then, they were compacted by isostatic pressing at 100.0 MPa during 1 minute. The samples were sintered with specific heating temperatures of 1550.0 ºC and followed by resting in this temperature during 2.0 h. The specimens obtained were observed through SEM to examine the eventual presence of cracks or delamination.

2.2 Implants Characterization

Both kind of implants, coated and uncoated, were compared using several techniques: surface roughness analysis using profilometry, distribution of bioactive materials with Energy Dispersive X-ray Spectroscopy (EDS) and *in vivo* proceedings to evaluation the bone-implant integration related to with shear strength - push-out test. Also, implants bearing functional gradient were characterized by Scanning Electron Microscope (SEM) for the presence of cracks and / or delamination.

Porous Implants

Profilometry. Surface roughness of the porous alumina specimens was quantified using a surface profilometer. Thus the surface roughness of porous alumina with and without hydroxyapatite and bioglass on the surface were compared.

Profilometry tests were performed using a Veeco, Wyko NT 1100 device in order to obtain the *Ra* (roughness average) and *Rt* (total roughness) parameters. For the analysis, statistical significance with paired *t* test. Differences were considered significant if $p < 0.05$.

Evaluation of coated scaffold for Energy Dispersive x-ray Detector (EDS or EDX). Energy Dispersive x-ray detector (EDX) was used to characterize the chemical composition and the bioactive materials (bio-glass and HA) depth of the coated scaffolds. EDX experiment was performed on fractured surfaces and the presence of Ca and P elements was investigated on the porous alumina (Al_2O_3) matrix.

In vivo proceedings. The proceedings realized *in vivo* related to the animal experiments were approved by the Ethic Committee of the *Clinics Hospital* of the *Faculty of Medicine - University of Sao Paulo.* Twenty rats were randomized by the stratified weight method into two groups with five subjects each: Control (engrafted with uncoated scaffolds) and Coated scaffolds (engrafted with bioactive coating scaffolds). The animals were operated on under anaesthesia (ketamine – 40mg/kg; xilazine – 5mg/kg body weight). Cortical bone defects measuring ø 2.5 mm × 1.6 mm were created on the medial aspect of the rat tibia. For the *in vivo* study, scaffolds were fixed in perforations in the rat tibias. Implant length allow them to reside in the cortex and the medulla without excessive protrusion beyond the periosteum. After 14 and 28 days, the animals were submitted to euthanasia in a carbon dioxide chamber. The implant pushing was performed in a shear strength push-out test.

Push-out test. To perform the shear tests, rat tibiae were dissected free of soft tissue, contra lateral to the cortical bone defect was removed and the pushing was done on the surface of the implant. The shear strength required to detach the implant from bone was measured by push-out testing.

Retention tests or shear were performed in the Mechanical Testing Laboratory of the Materials, Aeronautical and Automotive Department of the School of Engineering of Sao

Carlos - University of Sao Paulo. A mechanical test machine EMIC was used, with a load cell of 500.0 kgf (5 kN), temperature of 23 º C, full scale of 450.0 kgf and test speed of 0.5 mm / min (Hing et al, 2004). The tests were stopped after reaching maximum strength. The sample test which presented misalignments was discarded. The calculation of the shear stress was based on the shear surface and the implantation site and the actuator radius (*r*) Eq. (1) was used for the calculations:

$$Sc=F/S \qquad \text{Eq. (1)}$$

where *Sc* is the shear stress (MPa), *F* is the applied maximum force and *S* is the shear surface area. The lateral shear surface area of the cylindrical sample is $2.\pi.r.h$, where *r* is the radius of the actuator and h is the height of the implant.

Functional Gradient Implants

Scanning electron microscopy. Functional gradient implants with dense core and porous surface were embedded into epoxy resin, sliced transversely and polished with diamond paste. To obtain the SEM images the epoxy resin was removed and the presence of crack or delamination between the porous and dense region was checked.

Statistical analysis. Statistical analysis was performed with the software Graph Pad InStat 3.0. Tests were performed and differences were considered significant if $p < 0.05$. For the push-out tests, Tukey test method was used and for the superficial roughness Paired *t* test was performed.

3. RESULTS AND DISCUSSION

Porous Implants

Profilometry. The arithmetic average of the absolute values of the roughness (*Ra*) and total roughness (*Rt*) were obtained as well the average and standard deviation as shown in Table 1.

Table 1: Superficial Roughness values

Roughness (µm)*	*Coated*	*Uncoated*	*Statistical difference***
Ra	74,18 ± 5,15	60,43 ± 5,00	P=0,0049 (very significant)
Rt	429,05 ± 27,13	491,25 ± 2,02	P=0,2395 (not significant)

* Average and standard deviation - *Ra* – Roughness Arithmetic Average, *Rt* – Roughness total. **Paired *t* test - $p < 0,05$ considered significant.

The implants coated with bioactive materials showed higher average roughness and significance when compared to the uncoated implants.

Some features on the surface of the implant, as the roughness and the presence or absence of bioactive materials, may interfere with the bone integration of the implant and the bone response changes during the shear tests. According to Kangasnieni, et al. (1994) interfacial shear strength and the friction between the bone and implant, are generally difficult to make, because depends on the roughness and contact area of the surface.

The obtained average roughness of the coated implants was higher than the uncoated. Also, it can be noted that the coated implants bear two characteristics that theoretically provide a greater integration with bone tissues, which are the presence of bioactive materials and the increased surface roughness.

Evaluation of coated scaffold for Energy Dispersive x-ray Detector-EDS or EDX. EDX spectra observation of the bioactive surface of the fractured scaffold showed a perceptual value of the oxygen (*O*) of 50.65%, aluminium (*Al*) 34.20%, phosphorus (*P*) 5.18% and calcium (*Ca*) 9.97%. The EDX mapping shows the presence of the HA and bioglass elements as phosphorus (*P*) and calcium (*Ca*), in a depth nearly 100.0 µm.

In vivo proceedings

Mechanical push-out testing. Push-out test is emerging as an important experimental tool for the characterizing of bone-implant interface behaviour. Fig. 3 (A) shows a rat tibia during mechanical push-out testing. The mechanical tests performed showed the bone-implant interface integrity; in other words, there was no shear between the bone and implant when the implant was pushed by its core from the bone as shown in Fig. 3 (C). This confirms the strong adhesion of the bone to the implant or the bone-implant interface integrity. On the other hand, the implants that remained during 28 days in situ fractured into three parts during the tests as shown in Fig. 3 (B), like was observed by Hing K. A., *et al*. (2004).

Table 2 shows values of shear stress on the average and the standard deviation.

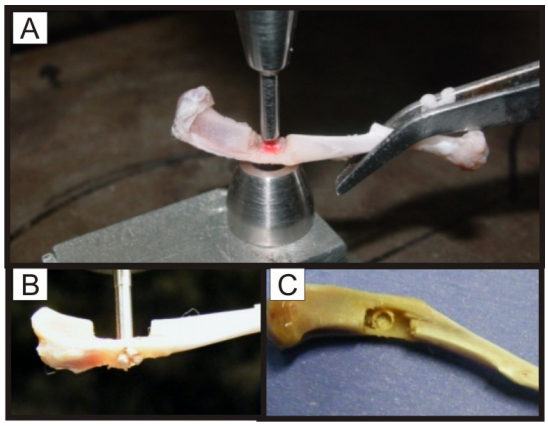

Fig. 3 – Mechanical push-out testing. (A) Rat tibia under mechanical push-out testing. (B) Implant fractured into three parts. (C) Implant with the centre pushed and bone-implant interface entirety.

The push-out test data (Fig. 4 and Tab. 2) indicate that the longer the deployment the more is the shear stress. The values of shear stress to the implant cover were higher in both seasons, but the difference was not significant between implants covered and not covered. Tukey test - $p < 0, 05$ considered significant.

Considering the results of Table 2 and Fig. 4, it can be observed that the **samples covered with uncoated during the 14 days** after the implantation surgery presented a the difference in shear stress which was not significant for this period, that is, the two-tailed P value is 0.6989 was considered not significant.

Table 2: Values of shear stress

Stress strength (MPa)	***Coated 14 d***	***Coated 28d***	***Uncoated 14d***	***Uncoated 28d***
Average	2.38	3.656	2.35	2.92
standard deviation	0.31	0.78	0.17	1.33

Also (Tab. 2 and Fig. 4), the samples **covered with uncoated during the 28 days** after the implantation surgery and the difference in shear stress, as observed, was not significant for this period and the two-tailed P value is 0.4082 was considered not significant. The p value for the shear stress between **14 days and 28 days for the coated implants** after the implantation surgery and the difference in shear stress was significant for this period in the same implant. The two-tailed P value is 0.0315 was considered significant. The p value between **14 days and 28 days for the uncoated during the 28 days** after the implantation surgery and the difference in shear stress was not significant for this period in the same implant. The two-tailed P value is 0.3320 was considered not significant.

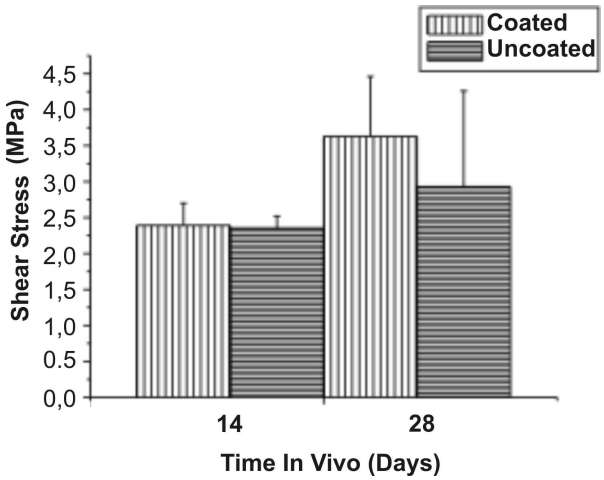

Fig. 4 – Values of mean shear stress obtained from push-out testing of porous alumina coated and uncoated samples implanted for 14 and 28 days.

Functional Gradient Implants

Dipping coat. The sample implants that were submitted to the dipping coat showed delamination after sintering. This technique has not been approved for the manufacture of FGMs. The technique of co-pressing was then performed.

Co-pressing. Fig. 5 indicates the delamination of the porous surface in one of the first remanufactured parts, when the co-pressing technique was still being refined. Co-pressing improved the continuity of the dense porous phase without the presence of detachment in the region of interface according to Fig 6 (B, C and D).

Scanning electron microscopy. During the development of the co-pressing technique, the samples obtained initially showed delamination as shown Fig. 5.

The delamination probably occurred because there was a failure during the drying of the slurry into the mold, that is, the coated layer was not so dry before to fill with dried powder.

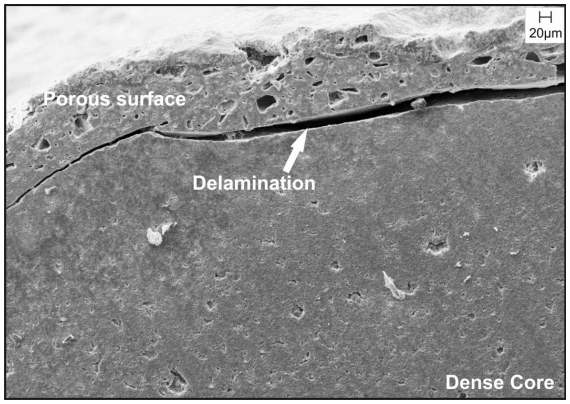

Fig. 5 – Scanning electron microscopy. The arrow indicates the delamination of the porous surface in one of the first remanufactured parts, when the co-pressing technique was still being refined.

With co-pressing technique the thickness of the porous region expressed as mean and standard deviation of the implants after sintering were as follows: 296 µm ± 37; 917 µm ± 14; 1292 µm ± 153.

The subsequent manufactured samples showed no delamination as shown in Figure 6.

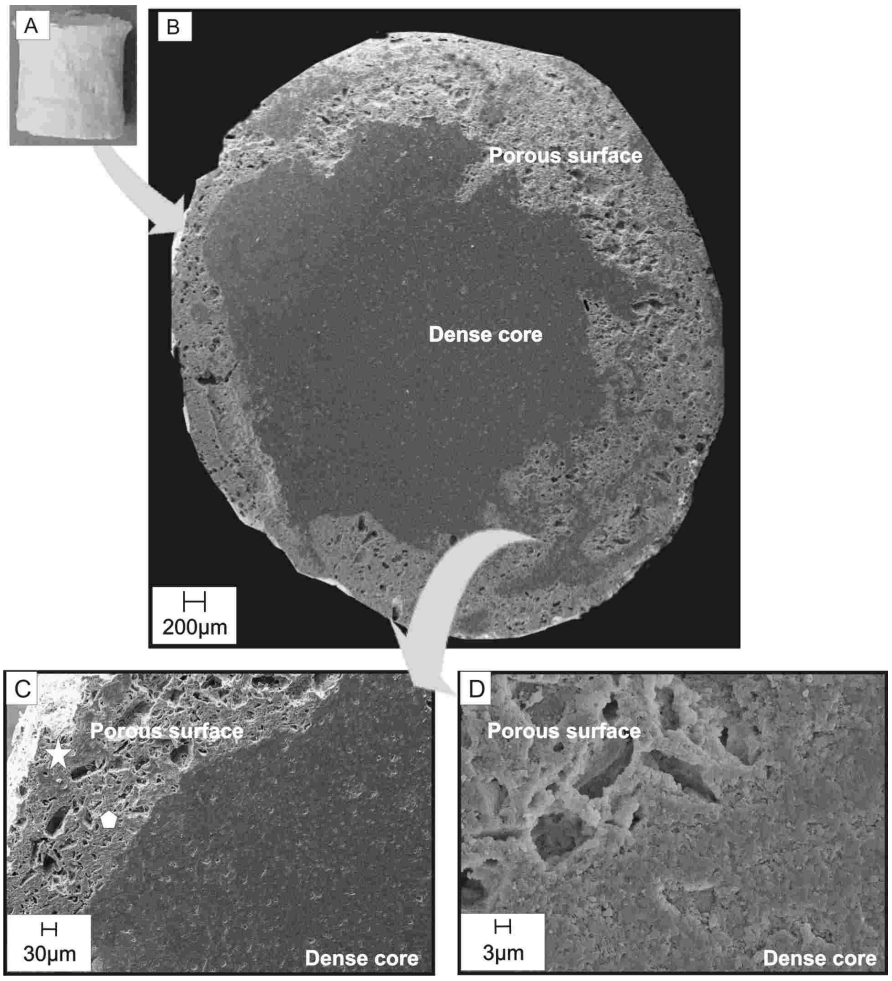

Fig. 6 – Images of scanning electron microscopy of the FGM implants. Note that no delamination showed up after the technique of co-pressing improvement. In C it can be observed a surface region with larger pores (☆) and a region with smaller pores (⬭), as well. In D it can be observed the continuous dense-porous interface.

In Figure 6 (C) a higher proportion of larger pores on the surface and the lower house with the denser core can be observed. In Figure 6 (D) there is continuity in the dense-porous interface, which highlights the lack of cracking or delamination.

4. CONCLUSIONS

The samples were coated with bioactive materials showed that the arithmetic average roughness (Ra) is significantly higher than samples not covered; this result shows that the coating promotes the potential for osteointegration of implants.

Coated implants bear two characteristics that theoretically provide a greater integration with bone tissues, which are the presence of bioactive materials and the increased surface roughness.

Furthermore, the failure mechanisms during push-out testing varied as a function of time *in vivo* for both samples. However, for coated implants the difference was significant between the two periods.

The application of co-pressing to manufacture FGMs implants showed to be promise with respect to the obtaining of parts without delamination. However, the FGM technique for manufacturing is still in a continuous improvement process by the research group of the LTC-EESC-USP. The present method provides obtaining of parts without cracking or delamination at the interface dense to porous, but the implants provided by the current technique does not present yet a homogeneous porous thickness. In some samples the current technique provided spread porosity and pores around the porous region, in other words, the pores should be larger and in greater quantities to the surface, defining the various porous interfaces. Devices for perfecting the technique have been designed by the LTC team, but are in process of manufacture for future searches.

However, the results obtained demonstrated a promising *in vivo* adhesion of the sample porous surface convincing that the manufacturing technique used to obtain the functional gradient in alumina ceramic has a high potential to osteoimplants applications.

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