

Design of dental implants, influence on the osteogenesis and fixation

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Received: 24 November 2006 / Accepted: 29 February 2008 / Published online: 18 March 2008
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Abstract The fixation and the bone ingrowth at the interface of porous cylindrical implants (total porosity of 37% and average pores diameter of 480 μm) were compared in vivo to rough cylindrical implants ($R_a = 5.3 \mu\text{m}$), both of commercially pure titanium, made by powder metallurgy. The implants were inserted into the tibias of 20 rabbits and the animals were sacrificed 4 and 8 weeks after surgery. The percentage of bone–implant contact observed in porous implant was significantly larger than in the rough ones for all of sacrifice periods, respectively, 57% vs. 46% after 4 weeks, and 59% vs. 50% after 8 weeks. The mechanical tests showed a significant increase in the shear strength of the porous implants for the two analyzed periods, 4 and 8 weeks (14 and 20 MPa), when compared with rough ones (4 and 13 MPa). These results suggest that porous implants

improve the contact at the implant–bone interface and increase the fixation to the bone, improving the osseointegration. Thus, the porous implant might be an alternative to dental implant in less favorable conditions, and appear to be better fixed to bone, offering promising alternatives.

1 Introduction

Dental implants are usually made of commercially pure titanium or titanium alloys [1–3]. The titanium is biocompatible, has high corrosion resistance, and good mechanical properties. The microtopography of implant surfaces is considered very important for the study of osteointegration, since the events that occur at the bone–implant interface are of major importance to the osteointegration of the material [1]. The bone–implant interaction and the conditions that favor osteointegration depend on the chemical and physical properties of the implant surface [1, 4]. Several studies have demonstrated that surface roughness developed by techniques such as acid etching [1, 2], anodic oxidation [5], and sand blasting [1, 6] seems to have a direct effect on cellular proliferation and differentiation, since the osteoblast behavior is sensitive to biochemical and structural characteristics [2, 5]. It has also been demonstrated that surface treatment such as biomimetic coating, calcium phosphate deposition [7] or fluorohydroxyapatite [8], promote greater osseointegration [7, 8]. Therefore, modifications on the implant surface with the purpose of encouraging and improving bone growth, provide better fixation of the implant to the bone [9], greater bone-to-implant contact at the interface [6, 10, 11] and earlier bone formation [12–14].

Thus, the characteristics of dental implant surfaces have been changed from smooth to notched, and the porous

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surface has been used with success as an alternative to dense surface. It allows bone tissue proliferation into the pores [11–14], resulting in increased surface area for the promotion of implant immobilization in bone by enabling mechanical interlocking between the implant and tissue, leading to faster osseointegration and preventing loosening of the implant [1, 7, 9, 13, 15–17]. The pores must be interconnected to allow bone ingrowth, even into internal ones. The porous structure must also have high porosity, which provides enough space for the attachment and proliferation of the new bone tissues and facilitates the transport of the body fluids. However, the material should present the appropriate mechanical properties [18]. And characteristics such as pores diameter and porosity must still be researched. Because of great difficulties to correlate surface properties with clinical results, the ideal microtopography for commercially porous implants is not yet known [6].

However, few efficient techniques are able to manufacture these interconnected pores [18, 19], such as powder metallurgy [18, 20, 21], environmental-electro-discharging-sintering of atomized spherical titanium powders [22], multiple coating technique [23] and sintering powders techniques [6, 7, 12]. In general, the manufacture of porous-surface Ti based implants can use one of the following techniques: plasma-spraying [17, 24, 25], anodic dissolution [2], grit blasted [1, 2], or oxidation [21]; however, these techniques produce pores without interconnectivity, only cavities.

Since the most appropriate surface has not yet been identified, the techniques are continuously being developed and investigated, thus producing physicochemical and morphological modifications [4]. The aim of this study was to investigate in vivo the effects of the surface of porous and rough cylindrical implants, made by powder metallurgy technique, developed with the purpose of improving the fixation of implant to the bone, and lowering manufacture cost.

2 Materials and methods

2.1 Implants

The porous and rough cylindrical implants were manufactured by using the powder metallurgy techniques. The materials used to manufacture the implants were commercially pure titanium powder with mean particles size of around 80 μm , and urea particles around 250 a 350 μm in size as spacer material.

Titanium/urea powder mixture, in the ratio of 80% weight to 20% weight, respectively, was used to manufacture the porous cylindrical implants (PI) and only pure

titanium powders was used to manufacture the rough cylindrical implants (RI). The powders were uniaxially pressed at 100 MPa into a stainless steel mold and isostatically pressed at 200 MPa. The porous cylindrical samples were heat treated at 180°C/2 h in air to burn out the spacer particles. Both samples were sintered at 1,200°C/1 h, under vacuum (10^{-7} torr).

The sintered samples presented average diameter of 3.0 and 6.0 mm in length. The size and the pores distribution, in the finished implant, are controlled by size and quantity of urea particles added to the powder titanium. The evaporation of the urea particles leaves pores in the metal microstructure, without residues.

2.2 Metallographic analysis and profilometer equipment

The metallographic analysis of the porous and rough cylindrical implants evaluated size, distribution and pores interconnection. The samples were embedded in acrylic resin and cut in the radial direction with a cutting machine for hard tissue (Labcut 1010-EXTEC). Then these specimens were ground in a polishing machine (Labpol 8-12, EXTEC) using an increasing sequence of sandpapers (800 and 1,200). After metallographic preparation, the specimens were observed in scanning electron microscopy (SEM) with original magnification of 100 \times , to characterize the microtopography, morphology and porous interconnection. The image program used for the metallographic analysis of the porous percentage and diameter was *Image Tool* (Windows 3.00). The obtained data were submitted to Mann–Whitney statistic test, $p \leq 0.05$ were considered significant.

The average surface roughness (R_a) of rough cylindrical implants was evaluated by a profilometer equipment S8P *Perthen* (Mahr), with a diamond tip of 5 μm in diameter. The test was performed in five rough implants samples on five different areas each. R_a is the arithmetical mean deviation of the profile and is calculated as the arithmetical mean of the absolute values of the profile deviations from the mean line. The porous cylindrical implants were not submitted to the profilometer equipment, since the mechanical feeler that surveys the roughness would be stopped into pores, and would not make the reading of the surface topography.

2.3 Surgical procedure

Twenty New Zealand albino rabbits, aged between 6 and 8 months and with mean weight of 4.5 kg, were used in this study. The animals were provided by the Vivarium of the Sao Jose dos Campos School of Dentistry and were kept in individual cages and fed with commercial pet food

(Coelhil R—Socil) and water ad libitum. The animals received three porous cylindrical implants in the left tibiae and three rough ones in the right tibiae. This study was approved by the Research Ethic Committee, Graduate School of Dentistry of Sao Jose dos Campos—UNESP (044/2002).

Prior to the surgery, the animals were weighed and anesthetized intramuscularly with a mixture of 13 mg/kg of aqueous solution of 2% hydrochloride of 2-(2,6-xylylidine)-5,6-dihydro-4H-1,3-thiazin (Rompum—Bayer), an analgesic, sedative and muscular relaxant substance, and with 33 mg/kg of ketamine (Dopalen—Agibrands do Brazil Ltda.), a general anesthetic. A local anesthetic composed of 3% octapressin combined with prilocaine hydrochloride and felypressin (3% Citanest—Dentsply®) was also used.

The procedures were performed under standard usual sterile conditions. After trichotomy, shaving, disinfection, and draping, a straight 3 cm skin incision was made over the medial portion tibiae. The fascia was split, and the implantation sites were prepared slowly and carefully using a surgical electronic drill. Three 3 mm diameter perforations were made bilaterally, the distance among the perforations was 0.5 cm. During drilling, the hole was continuously cooled with saline. Just before insertion of the implants, the hole was irrigated with saline to remove any shards of bone.

The implant was removed from the wrap, placed in the perforation and pressed into the surgical cavity until it was fixed to the cortical bone. The muscular tissue was sutured with absorbable thread and the skin with mononylon 4–0 surgical thread. After that, all animals received the antibiotic penicillin. The rabbits were sacrificed, using an overdose of the anesthetic solution intramuscularly, 4 and 8 weeks after implantation, 10 animals for each period, providing 30 implants per experimental condition.

2.4 Histological and histomorphometric examination

Seven implant sites for each period were removed and prepared for histology. The specimens were fixed in 10% formalin for 48 h, and were dehydrated in increasing sequence of alcohol (50%, 75%, 90% and 100%) for 24 h each. The specimens were then embedded in polyester resin and sectioned longitudinally with a cutting machine for hard tissues (Labcut 1010-EXTEC) in serial sections of about 80 μm each, and ground to a thickness of 30–40 μm in a polishing machine (Labpol 8-12, EXTEC) using an increasing sequence of sandpapers (400, 600 and 1,200). A microscopic analysis was performed using an optical microscope (OM) and SEM combined with a Sony digital camera (DSC-S85, Cyber-shot).

The percentage of new bone at the bone–implant interface was evaluated in three sections of each implant. Two

fields of each section were digitized (100 \times), representing the medial and distal interface of the implant. Therefore, 126 sections were analyzed for each type of implant, and for each sacrifice period. New bone rate and bone ingrowth to the interior of the pores were calculated using the Image J software (NIH).

2.5 Mechanical testing

After each sacrifice period, the bone fragment, of three rabbits, containing the implant were preserved in distilled water in a freezer at -20°C until the mechanical testing, which was performed at room temperature. For the push-out test, each specimen was mounted on a special platform with a central circular opening. This jig was designed to keep the pushing load parallel to the long axis of the implant. The pushing load was applied to the implant end using universal testing equipment Instron 2301, at cross-head speed of 0.5 mm/min until the peak load was obtained.

It was necessary to determine the area where the force was applied, in order to determine the tension needed for the displacement of the implant. Therefore, the cortical thickness of each specimen was measured at three locations for each push-out sample. The average thickness was calculated and used to determine the contact area according to this formula: mean area (A_m) = $2\pi r \times$ average cortical thickness, where r = implant radius. Following that, the shear stress was calculated using the Equation: $\sigma = F/A_m$, where σ = shear stress; F = peak load at failure.

2.6 Statistical analysis

All data were expressed as mean \pm standard deviation (SD) and statistically analyzed using the two-way parametric ANOVA, the kind of implant was considered as repeat factor. Tukey's test was also used, and differences with $p \leq 0.05$ were considered statistically significant.

The descriptive statistical analysis and Tukey's test were used for all values obtained in the mechanical test, in order to identify the implant with better fixation to the bone.

3 Results

3.1 Metallographic analysis and surface roughness

The photomicrography of the porous cylindrical implants showed different types of pores, interconnected pores and few isolated pores. The average interconnected pore diameter was about 480 μm (210 μm), and 37% (2.0%) total porosity (Fig. 1). The photomicrography of the rough cylindrical implants showed only isolated smaller pores, with average pore diameter of about 180 μm (80 μm) and

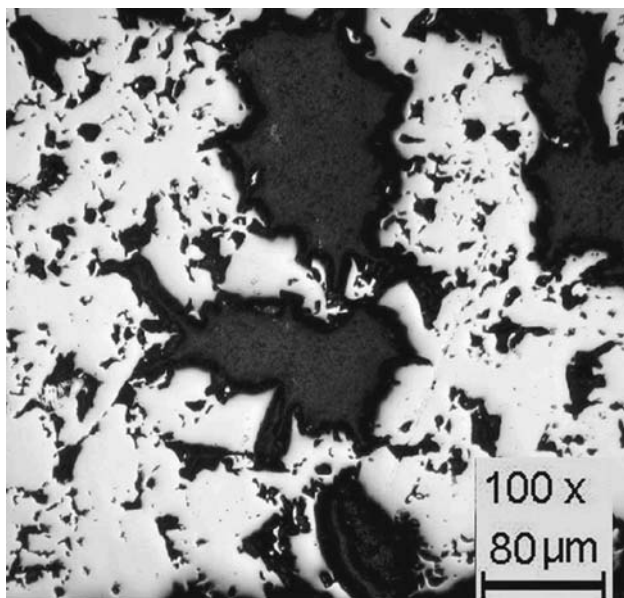


Fig. 1 Photomicrography of metallographic analysis obtained for SEM of porous implants—pores with various diameters and shapes, interconnectivity pores

3% (0.8%) total porosity. The rough cylindrical implants presented surface roughness of R_a 5.30 μm .

3.2 Histological examination

All animals presented satisfactory postoperative results, without any evidence of inflammation or infection in the surgical site. No adverse reaction was observed during the procedure. During the clinical evaluation, the implants were not loose manually.

At 4 and 8 weeks, new bone was observed at the implant–bone interface, regardless of the type of implant, leading to an osseointegration (Figs. 2 and 3), and in the porous implants new bone was also noticed into the pores. This new bone was similar in the two periods of sacrifice; it was constituted of mature bone trabeculae that presented lamellar arrangement and of different size medullar spaces. There was, especially in the rabbits sacrificed in the 4 weeks period, a distinct border between newly formed bone and preexisting bone (Fig. 4), emphasizing the biocompatibility of the material and the adequate surface to new bone proliferation.

Bone ingrowth was observed in all animals that received porous implants. Regardless of the sacrifice period, bone ingrowth into the pores was observed, even into more internal pores. In general, for both periods the small pores were totally filled with bone, whereas in the 4-week period bigger pores presented partial filling (Fig. 4), and in the 8-week period bigger pores were total filling (Fig. 5). New bone was also observed above the implants and in the

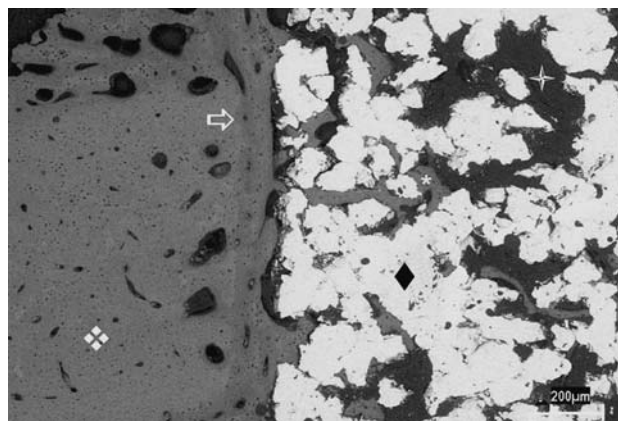


Fig. 2 Photomicrography obtained for SEM of porous implants (\blacklozenge), 4 weeks after surgery of inserting of implant in the rabbit tibia (\blacklozenge): distinct border between new bone and preexisting bone (\rightarrow), bone ingrowth (*), and resin (\times)

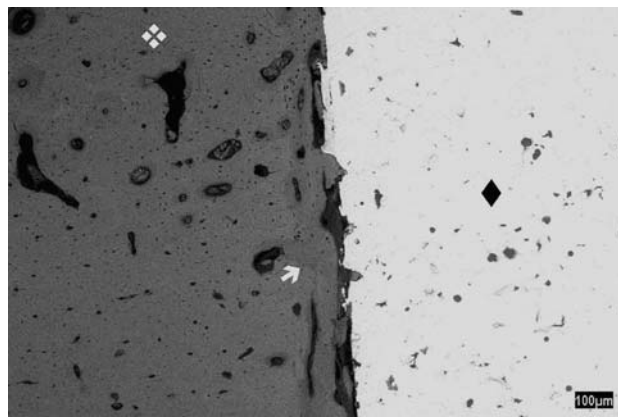


Fig. 3 Photomicrography obtained for SEM of rough implant (\blacklozenge), 4 weeks after surgery of inserting of implant in the rabbit tibia (\blacklozenge): distinct border between new bone and preexisting bone (\rightarrow)

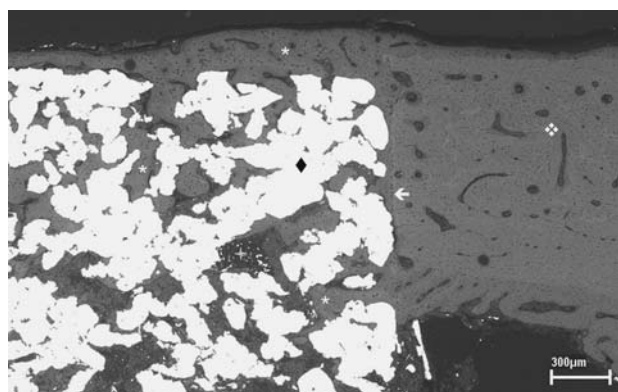


Fig. 4 Photomicrography obtained for SEM of porous implants (\blacklozenge), 4 weeks after surgery of inserting of implant in the rabbit tibia (\blacklozenge): bone ingrowth (*), growth new bone above of the implants (*), resin (\times), and distinct border between new bone and preexisting bone (\rightarrow)

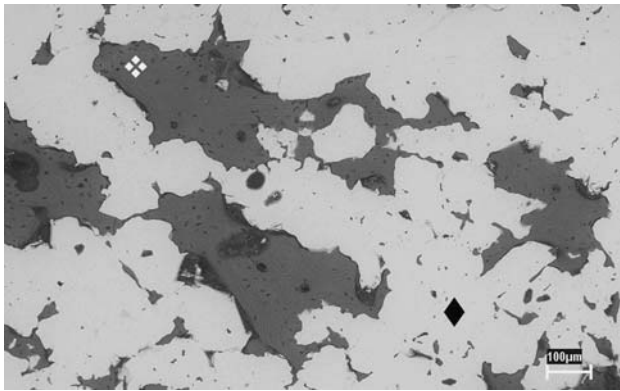


Fig. 5 Photomicrography obtained for SEM of porous implants (♦) 8 weeks after surgery: pore filled with bone (◆)

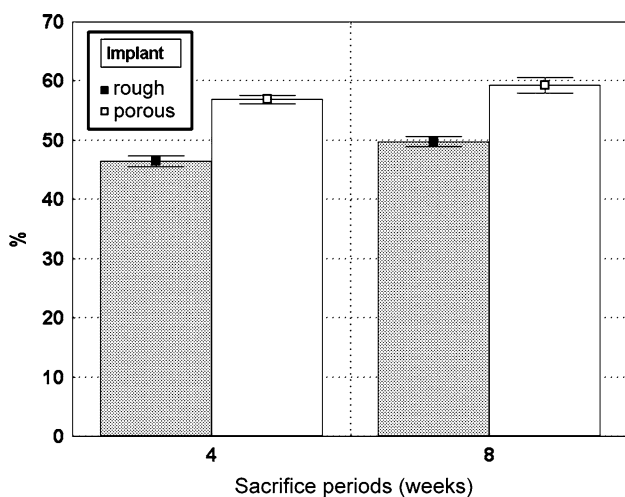


Fig. 6 Mean of values obtained of percentage of implant–bone contact (%)—in different types of implants and period of sacrifice

inferior region of the implants and the pores of these areas also presented new bone (Fig. 4).

No fibrous tissue was observed on the interface regardless of the implant type or sacrifice period.

3.3 Histomorphometric examination

The bone ingrowth rates are presented in graph above (Fig. 6). The averages values obtained for the percentage of implant–bone contact in the porous cylindrical implants versus the rough ones were, respectively, 57% (0.7%) vs. 46% (0.9%) after 4 weeks, and 59% (1.3%) vs. 50% (0.8%) after 8 weeks.

3.4 Mechanical testing

The shear strengths of porous and rough implants 4 weeks after surgery were 14 MPa (1.1 MPa), and 4 MPa

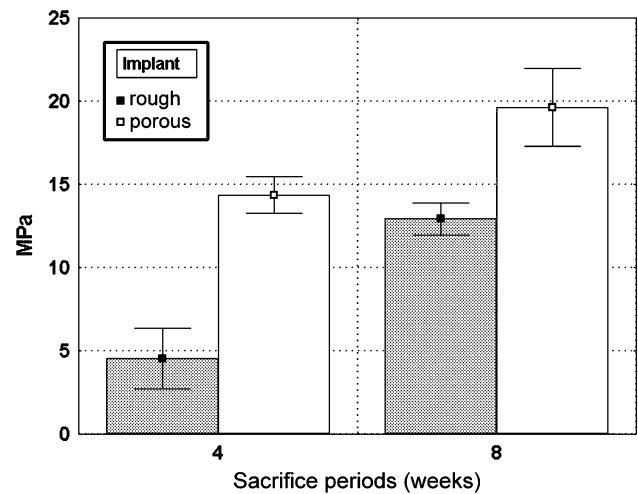


Fig. 7 Mean of values obtained of mechanical test (MPa)—in different types of implants and period of sacrifice

(1.8 MPa), respectively. At 8 weeks its shear strength was higher, 20 MPa (2.3 MPa) for porous implants and 13 MPa (0.95 MPa) for rough implants. It can also be observed that, regardless of the implant type, the shear strength increased with the sacrifice period as shown in the graph above (Fig. 7). The porous implants of the animals sacrificed at 8 weeks of bone repair exhibited the highest shear strength level.

4 Discussion

The variation of the substrate morphology on surgical implants was initially based on the observation that there was greater bone–implant contact when rougher surfaces were used [1, 6, 24, 25]. Actually, various types of morphologies have been employed to improve the new bone proliferation, and the porous surface have successfully supported bone ingrowth [2, 5, 6, 9, 12–15, 24–28]. In this porous surface, besides chemical interaction of material with the bone, greater mechanical contact between them is favored by bone ingrowth [9, 17].

In this study the quantity and quality of new bone in the porous and rough cylindrical implant was evaluated and compared by means of histological and histomorphometrical analysis and mechanical test. The porous cylindrical implants, developed by powder metallurgy technique, showed a complex structure of interconnected pores exhibiting 480 μm average diameter and 37% total porosity, whereas the rough cylindrical implant exhibited a dense structure of 3% closed pores. In the porous implants, new bone was observed on the surface and into the pores, even into the more internal ones, due to the intercommunication between them. Whilst, in the rough implants, new

bone was observed only on the surface, since the pores were isolated and small. These findings resulted in better fixation for the porous implant than for the rough implant.

Both porous and rough cylindrical implants can become osseointegrated under appropriated conditions. But although osseointegration occurs through mechanical interlock of bone with the implant in both cases, the extent and nature of this mechanical interlock is very different for the two designs. The porous implants become 3-dimensionally interlocked with bone as a result of bone ingrowth into the three-dimensional, open-pored structure that characterizes the surface region of this design [26]. The interconnected pores are important to its interlocking with bone, obtaining the maximum shear strength in shorter time [26, 27], since the rough and smooth implants present only juxtaposition of bone at their surfaces [28]. The pore interconnection increased the implant–bone contact, and bone proliferation increased with time. Therefore, the development of porous implants in this study provided better stabilized and efficient fixation of titanium implants, as demonstrated in previous studies [9, 14, 16, 28].

In general, the manufacture of Ti based porous-surface implants involves one of the following techniques: plasma-spraying [17, 24, 25], anodic dissolution [2], grit blasted [1, 2], or oxidation [21], which produce only cavities or craters denominate pores. The powder metallurgy technique represents an ideal approach for manufacturing complex shaped components without the need for machining steps [29], enabling the manufacture of implants with interconnected pores, such as a three-dimensional net [17, 19], a characteristic observed in this study. This manufacture process has an advantage over other conventional metallurgy techniques, such as economy of raw materials, reduction of manufacture costs, and reduction of the number of complementary stages in the samples [26]. Additionally, this technique provides the control of chemical composition and improves the material mechanical properties, by means of powder control and sintering process [15]. The pore size and the pore shape can also be changed by choosing the particle spacers [18, 20].

With regard to porosity and pore diameter, studies showed that pore diameters ranging from 100 to 500 μm are required to improve tissue ingrowth [6, 10, 14, 18, 21, 26]. Other investigations reported that the adequate percentage of pores for titanium sample is about 25% and 66% [10, 16, 20, 21, 30], and Takemoto et al. [17] suggested that porous titanium with 40% porosity might be an alternative for clinical application. However, samples with 5% and 80% porosity also presented bone proliferation [18, 25]. The increased porosity allowed tissue ingrowth and subsequent mineralization [16, 27], but it is necessary to maintain the appropriated mechanical properties of the implant [18].

In this study, the mechanical tests showed that porous implants had significantly higher push-out strength in all sacrifice periods. The porous implants achieved greater bond strength and bone ingrowth at shorter periods than the rough ones (14 and 4 MPa, respectively). The porous implants of animals sacrificed at 8 weeks showed bigger fixation to the bone tissue than the other conditions (20 and 13 MPa, respectively). Therefore, bone ingrowth into pores provides a more effective fixation of porous implant to bone, due to the development of resistant areas to shear strength. These resistant areas were directly related to the quantity of open pores in the surface. Thus, in order to occur the dislodging of porous implant, the fracture of bone that proliferated into pores was necessary. These results corroborates with previous studies of Svehla et al. [6] and Nischiguchi et al. [10] who obtained shear strengths of about 18 and 13 MPa, respectively, for porous implants at sacrifice period of the 4 weeks. While Svehla et al. [6] observed shear strength of about 6 MPa for rough implants. Furthermore, other authors observed increased shear strength with time [6, 10], which was also demonstrated in this research.

An important prospective clinical use for porous implants, as the ones developed in this study, will be the manufacture of short implants for clinical situations such as cases of limited available bone height, poor quality bone [14, 26], or orthodontic loading [31].

The small segment of porous implant allowed an effective osseointegration, due to increased contact area provided by its surface configuration. The porous implants of this study were manufactured with dimensions that could be used in these dental clinical situations, since they presented small diameter and height, and exhibited three-dimensional bone ingrowth and mechanical interlocking.

5 Conclusion

Porous cylindrical implants with 37% porosity, average pore diameter of 483 μm and shear strength of 19 MPa have been successfully manufactured by powder metallurgy. The results confirmed that the porous implant provided an optimal surface for bone ingrowth and interlocking, as expected, whereas the surface of rough cylindrical implants exhibited lower new bone proliferation. Furthermore, the powder metallurgy proved to be a simple and low cost technique for the manufacture of this type of implant.

The longer sacrifice time contributed to proliferation of new bone and of bone into pores, it allowed better osseointegration and better mechanical interlocking of both implants.

These results suggest that the porous implants might be an alternative to dental implant in less favorable

conditions, and seems to be better fixed to bone, offering promising alternatives.

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