Porous titanium for biomedical applications: An experimental study on rabbits

Luana-Marotta-Reis de Vasconcellos 1, Daniel-de Oliveira Leite 1, Fernanda-Oliveira Nascimento 1, Luis-Gustavo-Oliveira de Vasconcellos 1, Mário-Lima-de Alencastro Graça 2, Yasmin-Rodarte Carvalho 1, Carlos-Alberto-Alves Cairo 2

1 Department of Bioscience and Buccal Diagnosis, São Jose dos Campos School of Dentistry, State University of São Paulo (UNESP), São José dos Campos, Brazil
2 Department of Materials, Air and Space Institute, CTA, São José dos Campos, Brazil
3 Department of Prostodontics and Dental Materials, São Jose dos Campos School of Dentistry, State University of São Paulo (UNESP), São José dos Campos, Brazil

Abstract

Objective: The aim of this study was to carry out an in vivo assessment of bone ingrowth in two different types of porous titanium -the first being completely porous, and the second with a porous surface and dense nucleus, manufactured by powder metallurgy- and to evaluate their mechanical properties. Study design: Ten scaffolds from each group were submitted to metallographic analysis and compression tests. Next, two scaffolds of each type were inserted into 14 rabbits, which were sacrificed 8 weeks after surgery. The samples were submitted for histological examination. Results: Metallographic analysis revealed interconnected pores, and the average interconnected pore diameter was about 360 mm, with 36% total porosity. The totally porous titanium samples and the titanium samples with porous surface and dense nucleus showed an average compressive strength of 16.19 MPa and 69.27 MPa, respectively. After 8 weeks, the animals showed bone ingrowth, even into the most internal pores. Conclusions: The pore morphology was effective in permitting bone ingrowth in both groups. Titanium scaffolds with a porous surface and dense nucleus showed the best mechanical properties and most adequate interface.

Key words: Porosity, osseointegration, titanium, mechanical properties, biomedical.
Introduction
Titanium is widely used for the production of dental or orthopedic implants because direct contact occurs between bones and implant surfaces (1). Titanium is biocompatible, highly corrosion resistant and durable. Moreover, it is easily prepared in many different shapes and textures without affecting its biocompatibility (2). However, most titanium implants consist of dense components, which lead to problems such as bone resorption and implant loosening due to biomechanical mismatch of the elastic modulus (3). To overcome these problems, porous structures are being investigated extensively, since a reduction in elastic modulus can be coupled with bone integration through tissue ingrowth into pores (4). The interaction between mechanical signals and biological processes in cells and tissue is studied in mechanobiology. Mechanical load may influence cell proliferation, differentiation and metabolism and therefore have a crucial role in live tissue growth, adaptation, regeneration and bioengineering (5).

Bone ingrowth into the porous structure of the implant provides a strong implant/bone bond (6), and these pores can be interconnected three-dimensionally. The interconnected pores provide enough space for the attachment and proliferation of new bone tissues and facilitate the transport of body fluids (7). Several factors are important for promoting cell growth, such as pore shape and size, as well as their interconnectivity and spatial distribution throughout the scaffold. Scaffold architecture is crucial for allowing vascularization and the supply of nutrients to the developing tissue. Studies have shown that the optimum pore size required for scaffolds fixation remains undefined, the consensus is that in order to optimize mineralized bone ingrowth, pore sizes between 100 and 500µm are required (6-8). These porous structures have many applications ranging from spinal fixation to acetabular hip prostheses, dental implants, permanent osteosynthesis plates, and intervertebral discs (9). In general, porous-surfaced Ti-based scaffolds can be manufactured by one of the following techniques: plasma-spraying (9), anodic dissolution, and grit blasting (10), but these techniques produce only cavities and not interconnected pores. However, there are few efficient techniques for manufacturing these complex shapes with interconnected pores without the need for machining steps (11) such as powder metallurgy (PM) (6-8), the multiple coating technique (12), and powder sintering techniques (13). The powder metallurgy technique seems to be particularly advantageous because of its processing route and cost (11). In powder metallurgy, pores can originate from the particle compacting arrangement or from changes in this arrangement, when decomposition ofspacer particles causes increasing porosity, and from solid-state diffusion in the sintering step (14).

Finally, the porous structure must also present adequate mechanical strength, since large pores have a deleterious effect on the scaffold's mechanical properties. The gradient of maximum porosity must be adjusted adequately with respect to porosity and pore size, in order to ensure the scaffold's acceptable mechanical strength (11,15). The objective of the present study was to make an in vivo evaluation of bone ingrowth in two types of porous titanium manufactured by the powder metallurgy technique, and to evaluate their mechanical properties.

Materials and Methods
Scaffolds materials
Two different types of porous titanium, the first totally porous and the second with a porous surface and dense nucleus, were manufactured by powder metallurgy technique. The materials used to manufacture the scaffolds were commercially pure titanium powder with a mean particle size of about 8µm, formed by hydrogenation and dehydrogenation (HDDH) and urea with a particle size of about 200µm used as spacer material. The selection of the size of the urea particles was chosen according to previous investigations by our group, to control the amount and size of the spacer particles (6-8). These particles functioned as spacer material.

Both scaffolds were prepared from a titanium/urea powder mixture, having a ratio of 80% weight to 20% weight, respectively. The dense nucleus titanium samples were produced with pores exhibiting adequate distribution, since they were located only on the sample surface. A dense nucleus aimed to optimize the mechanical properties of the samples.

The powders were pressed uniaxially at 100MPa into a stainless steel mold and then pressed isostatically to 200MPa. The porous cylindrical samples were heat-treated at 180°C/2h in air to burn out the spacer particles. Both samples were sintered at 1200°C/1h, under vacuum (10-7 torr).

After sintering, the totally porous scaffold had an average diameter of 3.0mm and a length of 6.0mm, and the scaffold with a porous surface and dense nucleus had an average diameter of 4.5mm and a length of 6.0mm (Fig. 1). Pore size and distribution in the finished scaffold were controlled by the particle size and quantity of urea added to the titanium powder. The evaporation of the urea particles left pores in the metal microstructure, without residues.

Pore size and distribution – metallographic analysis
The scaffolds were embedded in acrylic resin and sectioned longitudinally (Labcut 1010-EXTEC). All the specimens were polished lightly using increasingly fine sandpapers. After the metallographic preparation, the specimens’ pore size, distribution and connectivity were analyzed in a scanning electron microscope (LEO 435 VPI) with 100 X magnification. The pore percen-
tage and diameter were analyzed using the Image Tool
(Windows 3.00) software program. All data were ana-
yzed by descriptive statistical analysis.

Compression test
The scaffolds thus developed were subjected to a com-
pression test to evaluate their mechanical properties. Five
specimens from each group were tested in uniaxial com-
pression, using a mechanical testing machine (MTS08
machine) at a cross-head speed of 0.5mm/min. The test
was performed at 37°C.

It was necessary to determine the area where the force
was applied, in order to determine the stress required to
break the scaffold. The average height was calculated
and used to determine the area according to the follo-
wing formula: mean area (Am) = 2πr x average height,
where r = scaffold radius. The scaffolds’ stress rupture
strength was then calculated from the Equation: σ = F/
Am, where σ = shear stress; F= peak load at failure.

All data were statistically analyzed using two-way pa-
rametric ANOVA and the Tukey test, where differences
of p ≤ 0.05 were considered statistically significant.

Surgical procedure
Fourteen New Zealand albino rabbits, five months old
and with a mean weight of 4.0kg, were used in this
study. The animals were supplied by the vivarium of
the São José dos Campos School of Dentistry and were
kept in individual cages and fed with commercial pet
food and water ad libitum. The animals received two to-
tally porous scaffolds in the left tibiae, and two titanium
scaffolds with porous surface and dense nucleus in the
right tibiae. This study was approved by the Research
Ethics Committee, Graduate School of Dentistry of São
José dos Campos UNESP (044/2002).

Prior to the surgery, the animals were weighed and an-
esthetized intramuscularly with a mixture of 13 mg/
kg of an aqueous solution of 2% hydrochloride, 2-(2,6-
xylidine)-5,6-dihydro-4H-1.3-thiazin (Rompum – Bay-
er), an analgesic, sedative and muscular relaxant, and
with 33mg/Kg of ketamine (Dopalen – Agibrands
do Brazil Ltda), a general anesthetic. A local anesthetic
composed of 3% octapressin combined with prilocaine
hydrochloride and felypressin was also used.

The procedures were performed under the usual stan-
ard sterile conditions. After trichotomy, shaving, dis-
infection, and draping, a straight skin incision of 3cm was
made in the medial portion of the tibiae. The fascia was
split, and the implantation sites were prepared slowly
and carefully using an electronic surgical drill. During
drilling, the hole was continuously cooled with saline.
Just before insertion of the scaffolds, the hole was irri-
gated with saline to remove any bone shards.

The scaffold was removed from the wrap, placed in the
perforation and pressed into the surgical cavity until it
was fixed to the cortical bone, after which the tissue was
sutured. All the animals were then given the antibiotic
penicillin. Eight weeks after implantation the rabbits
were sacrificed by applying an overdose of the anes-
thetic solution intramuscularly.

Results

Metallographic analysis
The metallographic analysis of the porous scaffolds re-
vealed different types of pores, most of them intercon-
ected (Fig. 2) and a few of them isolated. The average
interconnected pore diameter was about 360µm, with
36% total porosity.

In the metallographic analysis it was observed the pre-
sence of crack between them, suggesting appropriate
transition gradient.

Histological analysis
All the animals presented satisfactory postoperative
results, with no evidence of inflammation or infection.
in the surgical sites. New bone was observed at the scaffold-bone interface, regardless of the type of scaffold, leading to osseointegration (Fig. 3). Moreover, both types of scaffolds also showed new bone in their pores. This new bone was constituted of mature bone trabeculae with a lamellar arrangement and different sized medullar spaces. There was a distinct edge between newly formed and preexisting bone, emphasizing the biocompatibility of the material and the adequate surface for new bone proliferation.

All the animals showed bone ingrowth, even into the most internal pores. New bone was also observed above and below the scaffolds, and the pores of these areas also presented new bone. No fibrous tissue was observed at the bone-scaffold interface.

Compression test

The totally porous titanium samples and the titanium samples with porous surface and dense nucleus showed an average compressive strength of 16.19MPa and 69.27MPa, respectively.

Discussion

In the present study, two types of titanium scaffolds were manufactured by powder metallurgy techniques, one totally porous or and the other with porous surface and dense nucleus. The pores of both scaffolds allowed for bone ingrowth, even into the innermost pores, evidencing the three-dimensional growth. However, the scaffolds with porous surface and dense nucleus displayed greater compressive strength. Porous materials can provide early mechanical stabilization, preventing early mobility and micromotion due to their increased surface (6-8). Their capacity for osseointegration also offers the advantage of better long-term fixation, good biofunctionality and biocompatibility (6). Titanium foam presenting porosity not only at the scaffold surface but throughout the entire structure may allow for a higher degree of bone infiltration, while also reducing the stiffness of the foam when compared with that of solid titanium, thereby addressing the stress shielding problem (4). In the present study, the totally porous scaffold showed bone ingrowth inside its pores, but mechanically, this porous metallic structure was relatively weak. An alternative to totally porous Ti were the samples with porous surface and dense nucleus, which also showed bone ingrowth within pores and adequate elastic modulus. From a histological standpoint, the two samples of porous titanium showed a similar amount and quality of bone ingrowth.

The porous surface acts as a gradient with respect to material hardness, where the bone shows greater flexibility and reduced hardness, followed by the porous surface filled with bone and finally the dense titanium, which presents the lowest elasticity and greatest hardness. The porous structure filled with bone is a composite, an important area of transition between the titanium core and original bone. Metals like titanium are
much stiffer than bone and an implant of solid Ti can carry a disproportionate amount of the biological load. The surrounding bone is then stress shielded, which can lead to bone resorption (16). The porous surface titanium offers certain advantages over solid Ti, including enhancing bone-implant interface strength by promoting bone ingrowth and reducing the bone-implant modulus mismatch, which can lead to stress shielding. Therefore, biomaterials must exhibit hardness similar to bone (16), without producing a systemic or localized immune response (17). In addition, the material to be implanted must present mechanical properties adequate for the propose, while also presenting elasticity values similar to bone (17).

The mechanical properties of titanium products made of PM depend on the material’s composition, and on the density and final microstructure of the compact. Therefore, specially prepared metal powders of round shapes and sizes of 10-30µm are required (3). In this study, both scaffolds were produced with titanium powders whose particles were spherical and had a size of 8µm, and the final microstructure of the compact was adequate. Thus, the higher compressive strength was due to the presence of the dense nucleus. Characteristics such as pore diameter and porosity still require investigation. Because of the great difficulties to correlate surface properties with clinical results, the ideal microtopography for commercial porous scaffolds is not yet known (13). The diameter of interconnecting pores seems to dictate the quality of tissue growing into the porous spaces. Although there are alternative views, the consensus seems to be that the optimal pore size for mineralized bone ingrowth is 100-500µm (6-8). The pores of both porous titanium samples had an average diameter of 360µm, which sufficed for the consistent ingrowth of new bone within the porous spaces. A suitable porous network is required to promote extensive vascularization for bone ingrowth, rapid bone regeneration and good scaffold integration. Bone ingrowth rates depend to a large extent on pore morphology, the degree of pore connectivity, and pore volume (15), and it is known that pore connectivity is necessary to enable access of blood and nutrients for bone mineralization. On the other hand, larger pore sizes could weaken the porous scaffolds as a result of an associated reduction in density (18).

Dental and orthopedic implants can make use of osteoconductive surfaces such as hydroxyapatite to promote increased implant fixation to bone. There may be failure between this coating and the metal substrate. However, porous implants have been studied to promote implant fixation through bone growth into the porous structure and to provide an alternative to osteoconductive surfaces (19). The porous surface and dense nucleus implants developed in this study were produced in a single step; therefore, there was no failure between porous coating and the metal substrate, as sometimes reported for other surfaces. The porous surface might be an alternative to dental implant in less favorable conditions (7), such as upper maxilla—posterior region (20). The porous surface seems to be better fixed to bone, offering promising alternatives (7).

The scaffolds with a porous surface and dense nucleus, manufactured by powder metallurgy, combined important aspects such as greater compressive strength, efficient space for bone proliferation and improvement of the elastic modulus of titanium.

References
