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Initial Evaluation of Bone Ingrowth into a Novel Porous Titanium Coating

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RUNNING TITLE: Osseointegration of novel titanium foam coating

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ABSTRACT

Porous metals (sintered beads and meshes) have been used for many years for different orthopedic applications. Metal foams have been recently developed. These foams have the advantage of being more porous than the traditional coatings. Their high porosity provides more space for bone ingrowth and mechanical interlocking and presents more surface for implant-bone contact. The objective of this study was to evaluate *in vivo* bone ingrowth into Ti implants covered with a novel Ti foam coating. This foam contains 50% in volume of interconnected pores and a higher surface area compared to dense Ti. Both coated implants and dense Ti controls were placed transcortically in the rat tibia. The animals were sacrificed at 2 weeks after implantation, and the amount of bone in the implants was determined using backscattered electron imaging and X-ray microtomography. Already at this time interval, the pores within the Ti foam showed 97.7% bone filling and the bone-implant contact area was significantly increased compared to dense Ti controls. These initial results indicate that this novel Ti foam is biocompatible, has the capacity to sustain bone formation, and can potentially improve osseointegration.

Keywords: Biocompatibility, Bone, Osseointegration, Surface treatment, Titanium

INTRODUCTION

The geometry of an implant and its gross surface characteristics generate a biomechanical environment that significantly influences tissue repair. It is generally accepted that successful integration of an implant relies on its surface characteristics such as chemical composition, morphology and energy.¹ Surface morphology is an important factor determining the long-term implant stability, especially in poor bone quality. Accordingly, different methods such as sand-blasting,²⁻⁴ acid etching,⁵⁻⁸ machining⁹⁻¹¹ and anodization^{12, 13} have been developed to modify surface roughness, improve osteogenesis and ultimately enhance osseointegration.¹⁴⁻¹⁶ These treatments have been exploited commercially to improve the osseointegration of both orthopedic and dental implants.^{14, 17-19}

Porous metals have been exploited for several decades to increase friction force between the implant and promote the initial and long-term stability through bone ingrowth.²⁰⁻²⁵ These surfaces were proposed as a solution to problems encountered with methacrylate-based bone cement used for orthopedic implant fixation. Porous coatings offer attractive solutions since they have higher roughness than sandblasted or polished surfaces¹⁹ and they provide the initial stability without having to use bone cement. In addition, the interconnected porosity allows tissue ingrowth and integration which ensure the long term stability of the implant.

Metallic foams have been recently developed using innovative material-processing technologies and titanium foams with controlled interconnected porosity and complex surface topography have been proposed as a new type of biomaterials.^{26, 27} Their mechanical properties^{28, 29}, low density and biocompatibility make them attractive for various structural and biomedical (e.g. bone graft

substitute, orthopaedic and dental implants) applications. The fabrication process is based on a simple and flexible powder metallurgical process and can be used to produce fully porous bodies or coatings on dense implant cores. The biocompatibility of these foams has already been demonstrated with different *in vitro* models.³⁰⁻³² Noteworthy, the porous Ti foam promoted cell growth and total protein content in cultures of human alveolar bone cells.³²

While *in vitro* models provide valuable information, *in vivo* experiments in small animals are mandatory for setting up studies in larger animal models. In this context, the objective of this initial study is to report the *in vivo* evaluation of bone ingrowth into Ti foam coated rods at 2 weeks after transcortical implantation in rat tibiae, a time point at which maximal ingrowth is attained. The methodology used for evaluating the bone ingrowth as well as the biological implications of such osseointegration are presented and discussed.

MATERIALS AND METHODS

Materials

Titanium foams were produced using the process previously described.²⁶ Briefly, pure Ti powder (CpTi, -180 μm) was admixed with a polyethylene binder and a chemical foaming agent (p,p'-oxybis[benzenesulfonyl hydrazide]). The resulting powder mixture was poured around 1 mm commercially-pure Ti rods (Grade 1, Johnson Matthey, UK) into a mold and foamed at 210°C in air. The resulting material was debinded at 450°C in Ar (10^{-8} ppm O_2) and sintered at 1400°C under vacuum (10^{-5} - 10^{-6} Torr range). The cylinders obtained were machined to obtain small cylinders (approximately 2 mm diameter and 1.8 mm long) with a thin Ti porous coating (500 μm). Control Ti cylinders were cut from extruded titanium wires (99.7%, Johnson Matthey, UK). To ease implantation and optimize healing, the edges of the specimens were chamfered.

The surface morphology and the structure of the implants were characterized using an Hitachi S-4700 field-emission scanning electron microscope (FEG-SEM) and an X-Tek HMXST 225 x-ray microtomograph (μCT). The implant specific surface area was evaluated by gas adsorption (BET) using a Micrometrics ASAP 2010 system with krypton as adsorbate as previously described by Cheung et al.³¹

The pore size has been evaluated on the same material but on larger specimens. The evaluation is based on a qualitative evaluation of the pore size range on SEM micrographs, meaning that the majority of the pores observed fit in the 50 to 400 μm range.

Surgical Procedure

Six male Wistar rats weighting $200 \pm 10\text{g}$ (Charles Rivers Canada; St-Constant, QC, Canada) were anesthetized with an intraperitoneal injection of a mixture of Ketalean (0.05 mg/g body weight; ketamine hydrochloride; Biomeda-MTC, Cambridge, ON, Canada), Rompun (0.005 mg/g body weight; xylazine; Bayer Inc., Toronto, ON, Canada) and Acevet (0.001 mg/g body weight; acepromazine maleate; Vetoquinol Inc., Lavaltrie, QC, Canada). The antero-medial side of each hind limb was shaved and cleaned with Baxedin[®] (Chlorhexidine Gluconate; Omega Laboratories, Montreal, QC, Canada). A 1 cm incision was made through the skin with a scalpel fitted with a surgical blade (No 15C; Almedic, Montreal, QC, Canada). The skin and muscle were gently pried apart to expose the periosteum. A hole was drilled through the periosteum and across the bone cortex at 5 mm from the knee joint, using a 1.9 mm drill bit (No 49; Drill bit city, Prospect Heights, IL.). The drilling site was irrigated with physiological saline and implants were placed in both tibiae. In a randomized manner, one tibia received a dense Ti implant, while the other received the implant coated with the Ti foam. The muscle was sutured with 4-0 chromic gut sutures and the skin was closed with 4-0 sofsilk sutures (distributed by Patterson Dental Supply Inc.). The surgical site was cleaned and disinfected with Baxedin[®]. The animals received an injection of Temgesic[®] (Buprenorphine hydrochloride, Reckitt and Colman, Hull, UK) after surgery, and were fed with soft food containing Temgesic[®]. X-rays, at 10 pulses per second, were taken to verify the positioning and the stability of the implant. All experimental protocols and animal handling described above were approved by the Comité de déontologie de l'expérimentation sur les animaux of Université de Montréal.

Tissue Processing

Two weeks after the surgery, animals were anesthetized with 20% chloral hydrate solution (0.4 mg/g body weight; Fisher Scientific, Whitby, ON, Canada) and sacrificed. The tibia were dissected out and immersed in a fixative solution consisting of 4% paraformaldehyde (BDH; Toronto, ON, Canada) and 0.1% glutaraldehyde (Electron Microscopy Sciences, Washington, PA) in 0.08M sodium cacodylate (Electron Microscopy Sciences) buffer containing 0.05% calcium chloride (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada), pH 7.2, for 3 hours at 4°C. The tissues were dehydrated in graded acetone, embedded in epoxy resin (Electron Microscopy Sciences) and polymerized at 58°C for 48 hours. Tissue blocks were cut along the sagittal plane (see Figure 1), using an IsoMet[®] Low Speed saw (Buehler Canada, Markham, ON, Canada), to produce 3 sections.

Image analyses

Each section was examined using backscattered electron imaging (BEI) in a JSM-6460LV variable pressure SEM operated at 20 kV. Some samples were also imaged with an X-Tek HMXST 225 x-ray μ CT. The foam density and surface area as well as the amount of tissue ingrowth and contact surface area between mineralized bone and the implants were analyzed using a Clemex Vision 3.0 (Clemex Technologies, Longueuil, Canada) image analysis software for the SEM 2D images and a VGStudio Max 2.0 (Volume Graphics GmbH, Heidelberg, Germany) software for the μ CT images. Algorithms were developed to quantify the different foam characteristics.

Osseointegration was evaluated in 2D on 10 different images from slices taken on 4 different rats for the porous Ti and 17 pictures taken in 5 rats for the dense Ti. The image analysis procedure consisted in a sequence of operations in order to improve contrast and phase discrimination. The SEM images were obtained under normalized conditions to generate a constant grey level distribution from one sample to the other. Since the SEM images were taken right after cutting and no polishing was done (Figure 2a), horizontal grey level closing with a 2X1 structuring element was performed in the region of interest (light blue rectangle) to remove the visible cutting lines (Figure 2b). The images were then binarized, using grey level threshold, to discriminate the different phases (Figures 2c and 2d). To quantify the amount of bone in contact with the implants, the binary images of the discriminated phases were processed using the following morphological image processing algorithm. A binary opening with a circular structuring element of size 2 followed by a binary dilation of size 1 were applied to the bone phase and the resulting binary image was then intersect with the titanium phase binary image. The resultant image represents the segments of the foam perimeter in contact with bone (Figure 2e). The cumulative length of all the segments within the region of interest was assumed to be the total implant perimeter in contact with bone.

RESULTS

The structure of the foam observed by SEM is presented in Figure 3. The Ti particles used to produce the foams are visible in the coating. The pore size is significantly larger than the size of the particles used to produce the foam. The specific surface area of the implant measured by BET is relatively high ($0.05 \text{ m}^2/\text{g}$) in comparison to dense Ti ($0.00022 \text{ m}^2/\text{g}$) and comes from the porosity of the foam, the morphology of the sintered network of powder particles used to produce the foam and the presence of fine thermal etching lines produced during sintering (Figure 3c).

The reconstructed volume from an X-ray μCT image of the full implant is represented in Figure 4a. The 3D μCT image has a voxel size of $2.2 \text{ }\mu\text{m}$ and has sufficient spatial resolution to provide an evaluation of the structure of the foam. The images confirm that the particles are well sintered together and the foam is well bonded to the dense Ti substrate (Figure 4c). Figure 4b shows that the porosity is significantly larger than the size of the particles and is created by the expansion of the Ti powder suspension during foaming and not only by the porosity between the particles, as it is usually the case in sintered bead coating. The diameters of the largest pores are close to the thickness of the coating and give direct access to the surface of the dense rod. The internal structure of the foam provides space for bone ingrowth and mechanical interlocking between the implant and bone.

Image analysis done on μCT slices (2D) and volumes (3D) of the implant before the surgery indicate that the porosity is about 50% (Table 1). The foam contribution to the increase in perimeter and surface area was determined on the μCT slices in 2D (perimeter) and volume in 3D (surface area) (Table 1). Using image analysis, the measured perimeter is estimated to be 4.5

times larger than the perimeter of a dense cylinder of similar external diameter. The surface being relatively complex, the perimeter cannot simply be extrapolated geometrically into a surface area and should be evaluated directly on the 3D reconstructed images of the foam. In 3D, the surface area is approximately 7.6 times larger than that of a dense cylinder of similar dimensions. This increase is significant considering that the coating is only 500 μm thick. However, it is much lower than the surface area calculated with the BET measurements (34x). BET takes into consideration the increased surface generated by the thermal etching lines on the surface of the foam (Figure 3c) while the μCT spatial resolution is insufficient to reveal such texture.

No sign of infection at the surgical site was observed after sacrifice and all implants were stable. As shown on a representative backscattered image two weeks after the implantation (Figure 5), part of the porous implant was in contact with the cortical bone, while the other portion was in the intramedullary canal. The backscattered contrast allows seeing clearly the Ti phase (white), the mineralized matrix (grey) and the non-mineralized matrix and embedding resin (black). A closer view of the implant reveals that there is a good contact between the implant and the surrounding bone (Figure 5b). Bone was integrated in the porosity, even at locations where in 2D, the porosity appears to be closed. Indeed, bone found its way all along the complex porous network up to the dense core.

Table 2 presents different values calculated by image analysis on the backscattered images obtained from sections of the implant two weeks after the surgery. The porosity measured on the porous coating is 46.9%. This value compares well with the values evaluated on 2D and 3D images obtained by μCT on the implants before implantation (Table 1). Appropriate image processing enabled obtaining reliable images corresponding to the mineralized matrix observed

on the original SEM micrograph (compare Figures 2a and 2d). The amount of mineralized bone in the porosity is 46% of the initial porosity surface. In order to calculate the fraction of bone ingrowth, the amount of bone in the porosity was compared with the density of bone surrounding the implant (i.e. 48.9%) as measured on the field of similar size and adjacent to the coating (see Figure 2f). Taking into consideration these numbers, the pores appear to be completely filled with bone (97.7%). This confirms that after 2 weeks, bone filled the porosity in the foam coating and the density of bone in the porosity was similar to that of the bone surrounding the implant. The amount of mineralized bone into the pores was also evaluated at later time points (4 and 6 weeks, data not shown) but there was no significant difference in the total quantity of bone that filled the pores. These data suggest that maximal bone ingrowth is achieved at 2 weeks, that the implant is well osseointegrated and that this newly formed bone is stable.

The contact region obtained after image processing corresponds relatively well with the bone-implant contact perimeter observed on the original image when Figures 2a and 2e are visually compared. The amount of bone in contact with the implant evaluated by image analysis is 65% that of the perimeter of the foam (Table 3). On the dense implant, the amount of bone in contact with the dense implant is estimated at 36%. In absolute value, the contact surface between bone and the porous coated implant is 11 times larger than with the dense Ti.

DISCUSSION

The structure of the foam characterized in this study is unique and different from that of standard materials used in orthopedics and dentistry. The pores in the foam are not produced by the space between the particles but by the expansion of the foaming agent. Contrary to sintered beads, the metal foam has pore size significantly larger than the size of the particles used. The particles are much finer than those generally used to produce sintered beads. Since the driving force for sintering is the reduction of surface energy, small particles have much more energy stored in their surfaces and are therefore much easier to sinter than large beads. The high surface area ($0.05 \text{ m}^2/\text{g}$) of the foam is provided by the porosity, the particulate nature of the starting material and the thermal etching lines produced during sintering. The thermal etching lines, first observed by Evans³³, come from the evaporation and condensation of Ti during sintering. These etching lines are very fine (as small as 15 nm thick) and contribute significantly to the surface area of the material, as measured by BET.

The results from the present study confirmed *in vivo* the biocompatibility of a new Ti foam intended for orthopedic and dental applications. No adverse effects were observed and all animals recovered normally from the surgery. After 2 weeks of implantation, the implants were well integrated in the surrounding bone and new bone filled 97.7% of the porosity, relative to the bone density adjacent to the implant. To assess the bone growth kinetics, other tests with larger implants (thicker foam coatings or fully porous implants) and more time points prior to maximal ingrowth are, however, required.

The various approaches used for the evaluation of bone ingrowth into porous structure are either qualitative,^{25, 34} or quantitative by calculating the surface of the pores filled with mineralized matrix³⁵ or the contact surface with bone.³⁶ In orthopedics, the amount of bone in the pores and bone penetration length are generally used to quantify bone integration.³⁵⁻³⁷ In dentistry, since rough surfaces rather than porous materials are employed, osseointegration is generally analyzed in terms of bone contact surface area.³⁸⁻⁴¹ Traditionally, 2D image analyses have been used to quantify the amount of bone in the pores and to evaluate the bone-implant contact by measuring the contact lines between the two phases (i.e. Ti and bone) by optical, radiographical techniques or SEM. Although useful information can be drawn from these 2D images, 3D-based analyses provide distinct advantages on the assessment of the relationship between complex 3D structures and bone ingrowth: For example, the structure of the foam and surface area are more accurately evaluated in μ CT which enables better measurements of bone-implant contact.

Even though the Ti foam coating is thin, it allows a significant increase in the area of contact between the bone and the implant. Evaluated by image analysis on 2D images, the contact surface is augmented by about 11 times. While some authors have reported that nanotexture may affect the biocompatibility of materials *in vitro*,⁴² very few have studied the effect of nanotexture on the *in vivo* biocompatibility of Ti, including features such as the nanoscale thermal etching lines observed in this study. While nanotextures may have an impact on the early mechanisms responsible for osseointegration,⁴³ they do not generate more space for cells to grow on. On the other hand, the porosity in the foam is sufficient to provide space for cells to grow in and may significantly affect the bone-implant contact surface area. It is likely that combination of nano- and microscale surface topography will generate synergistic influences that will provide optimal implant integration.⁴⁴

Although surface area and contact surface are important parameters for implant stability, the direction of loading with respect to the implant surface (surface orientation) must also be taken into consideration. Loading direction significantly affect the mechanisms of rupture. In most implants, the load direction can be relatively complex and is most likely not parallel to the surface of the implant. In standard implants, the surface and loading orientation is affected by the design and rely mostly on the anatomy. When there is little or no mechanical interlocking between the implant surface and bone, excessive loading causes rupture at the bone-implant interface. Increasing the surface area and complexity of the surface improve the mechanical interlocking, and can increase the implant stability. In foams, bone forms into pores that are larger than the channels interconnecting them. This provides a mechanical interlock, a mechanism that is not observed on flat or rough surfaces. This mechanical interlock should enhance the strength on the interface between the implant and the bone since the force needed to extrude the bone through the porosities can be much higher than the bone mechanical strength. In these cases, the rupture will take place in the bone rather than at the bone-implant interface. Additional *in vivo* biomechanical studies are, however, required to better assess the effect of bone ingrowth and interlocking on the stability of implants produced with foams, such as the one presented in this study.

The porous coating approach is now widely used for hip and knee “cementless” procedures. Metallic foams have recently been proposed as an alternative to other porous materials traditionally used in orthopedic (sintered beads, meshes). These materials allow not only improving treatments but also developing novel approaches such as bone augmentation and graft free fusion devices that were not conceivable with previous generations of materials. However, foams are generally produced using complex processes which have an important impact on

production costs. In addition, the materials cannot always be easily integrated with other materials when assembling is required. The present process is based on a simple and flexible metallurgy process adapted for large scale manufacturing of devices that could be used in various applications.

Metallic foams have not yet been used in dentistry. Indeed, few technologies allow the deposition of foams on the surface of small implants such as dental implants. Most of the treatments referenced in the literature for dental implants are based on press-fit implants with sintered beads.⁴⁵⁻⁴⁸ While such implants are easy to place and may provide good fixation with time, they do not, however, have the initial stability provided by threaded implants. With the growing tendency towards faster loading and the need for shorter implants for cases where the amount of good quality bone is not sufficient, there is an interest in developing technologies to further increase the initial and long term stability of screwed implants. Better interlocking between the implants and surrounding bone can help reducing the implant length and avoiding bone graft procedures when bone height is insufficient. Ti foams offer an interesting and promising alternative to address these problems.

CONCLUSION

A novel porous Ti foam coating has been evaluated *in vivo*. Small foam implants (i.e. 2 mm diameter) were implanted transcortically in rat tibiae. Two weeks after implantation bone had integrated into the porous structure of the foam and bone filled the porous network. While the thin foam coating used significantly augmented the contact surface area between the implant and surrounding bone, even larger contact surfaces could be achieved by increasing the thickness of

the coating. The present results pave the way for additional studies in animal models aimed at assessing the bone ingrowth kinetics and the strength of the mechanical interlocking between the implant and the surrounding bone.

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FIGURE LEGENDS

Figure 1: X-ray (inverse mode) showing the surgical placement of a dense Ti implant within a rat tibia. The red lines and the drawing illustrate the positions where sections were cut (A, B, C).

Figure 2: Scanning electron images showing the different steps for digital image processing. (A) Original image. (B) Filtration to remove cutting lines. (C) Binarization of Ti phase (blue). (D) Binarization of Ti foam (blue) and mineralized matrix (green). (E) Contact perimeter (red) between bone and the specimen. F) Bone (cyan) at the proximity of the implant.

Figure 3: Scanning electron micrographs of the surface of a Ti foam coated implant. (A) Low magnification image of the Ti foam. (B) Thermal etching lines on the surface of the foam. (C) High magnification image revealing the fine features provided by thermal etching on the Ti foam surface.

Figure 4: X-ray microtomography images of a Ti foam coated implant. (A) 3D reconstruction, (B) perpendicular cross-section, (C) close-up on the region, boxed area delimited in 3B, between the Ti rod and the coating showing the bonding between the individual particles and the dense rod.

Figure 5: Scanning electron micrographs in backscattered mode showing the dense Ti implant (A, B) and porous coated (C, D) specimen implanted transcortically in the rat tibia. (A, C) General view and (B, D) Closer view. The Ti implant appears white, while the mineralized and non-mineralized matrices are grey and black, respectively.

Table 1: Porosity, perimeter and surface area of implants coated with 500 μm thick Ti foam coating, as measured on X-ray microtomography (μCT) images. Relative increase of the perimeter and surface area were compared with the surface of a dense Ti cylinder of same dimensions.

2D		
Foam porosity	Implant perimeter increase	
<i>Image analysis</i>	<i>Image analysis</i>	
% area		
50.2	4.5X	
3D		
Foam porosity	Implant surface area increase	Implant surface area increase
<i>μCT Image analysis</i>	<i>μCT Image analysis</i>	<i>BET^a</i>
%Volume		
50.4	7.6X	34X

^a BET, gas adsorption

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Table 2: Foam porosity, bone density and bone ingrowth, measured by image analysis on backscattered images, in the pores after two weeks of implantation in the rat tibia.

	Porosity Foam	Density Bone	% Bone (in porosity)	Bone Filling
	% area	% area	% area	% area
Average	46.9	48.9	46.4	97.7
Stdev	3.3	13.5	10.0	16.6

Table 3: Bone-implant contact perimeter and surface, measured by image analysis on backscattered images, after two weeks of implantation in rat tibia. The contact surface were extrapolated from the perimeter measured in 2D and the perimer/surface ratio measured on the X-ray microtomography images (1.7X).

	Relative contact Bone-implant	Relative contact Bone-implant	Contact surface increase (absolute) Bone-implant
	Foam	Dense	Foam Micro
	%	%	X
Average	65.0	36.3	10.5
Stdev	8.8	15.7	1.7

Figure 1: X-ray (inverse mode) showing the surgical placement of a dense Ti implant within a rat tibia. The red lines and the drawing illustrate the positions where sections were cut (A, B, C).
80x32mm (300 x 300 DPI)

Figure 2: Scanning electron images showing the different steps for digital image processing. (A) Original image. (B) Filtration to remove cutting lines. (C) Binarization of Ti phase (blue). (D) Binarization of Ti foam (blue) and mineralized matrix (green). (E) Contact perimeter (red) between bone and the specimen. (F) Bone (cyan) at the proximity of the implant. 130x138mm (300 x 300 DPI)

Figure 3: Scanning electron micrographs of the surface of a Ti foam coated implant. (A) Low magnification image of the Ti foam. (B) Thermal etching lines on the surface of the foam. (C) High magnification image revealing the fine features provided by thermal etching on the Ti foam surface.

Figure 4: X-ray microtomography images of a Ti foam coated implant. (A) 3D reconstruction, (B) perpendicular cross-section, (C) close-up on the region, boxed area delimited in 3B, between the Ti rod and the coating showing the bonding between the individual particles and the dense rod. 170x51mm (300 x 300 DPI)

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Figure 5: Scanning electron micrographs in backscattered mode showing the dense Ti implant (A, B) and porous coated (C, D) specimen implanted transcortically in the rat tibia. (A, C) General view and (B, D) Closer view. The Ti implant appears white, while the mineralized and non-mineralized matrices are grey and black, respectively.
118x84mm (300 x 300 DPI)