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# Peri- and intra-implant bone response to microporous Ti coatings with surface modification

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ABSTRACT

Bone growth on and into implants exhibiting substantial surface porosity is a promising strategy in order to improve the long-term stable fixation of bone implants. However, the reliability in clinical applications remains a point of discussion. Most attention has been dedicated to the role of macroporosity, leading to the general consensus of a minimal pore size of 50–100 μm in order to allow bone ingrowth. In this in vivo study, we assessed the feasibility of early bone ingrowth into a predominantly microporous Ti coating with an average thickness of 150 μm and the hypothesis of improving the bone response through surface modification of the porous coating. Implants were placed in the cortical bone of rabbit tibiae for periods of 2 and 4 weeks and evaluated histologically and histomorphometrically using light microscopy and scanning electron microscopy. Bone with osteocytes encased in the mineralized matrix was found throughout the porous Ti coating up to the coating/substrate interface, highlighting that osseointegration of microporosities (<10 μm) was achievable. The bone trabeculae interweaved with the pore struts, establishing a large contact area which might enable an improved load transfer and stronger implant/bone interface. Furthermore, there was a clear interconnection with the surrounding cortical bone, suggesting that mechanical interlocking of the coating in the host bone in the long term is possible. When surface modifications inside the porous structure further reduced the interconnective pore size to the submicrometer level, bone ingrowth was impaired. On the other hand, application of a sol–gel-derived bioactive glass–ceramic coating without altering the pore characteristics was found to significantly improve bone regeneration around the coating, while still supporting bone ingrowth.



## 1. Introduction

Joint replacement and dental restoration have evolved enormously over the past decades, resulting in excellent clinical success rates. Ten-year survival rates up to 95.3% for hip implants and even 98.8% for dental implants have been reported [1,2]. However, due to the ever-expanding demand for implants, an increasing number of patients still suffer from implant failure and, in addition, there is a trend towards a younger and more active patient population, which raises higher expectations regarding the durability and longevity of implants [3]. Maintaining a long-term stable fixation has become a key priority in implantology. In this regard, the concept of osseointegration gave rise to the development of cementless implants relying on a close implant/bone contact for a firm retention in the host bone. Although the first generation of cementless implants was not unambiguously successful, recent studies confirm a long-term survivorship for different cementless components comparable to their cemented counterparts [4,5].

Due to the outstanding mechanical properties such as a high strength and good fatigue resistance in combination with an excellent biocompatibility, titanium and Ti alloys have become the material of choice for load-bearing implant applications [6]. The stable oxide layer at the surface enables a close bone apposition, allowing successful osseointegration under appropriate conditions (implant surface, quality of the host bone, loading condition), but in order to improve outcomes in more challenging circumstances (e.g. compromised bone), further control of the bone formation is required.

From a materials perspective, the implant surface determines the rate and extent of osseointegration. Therefore, extensive research efforts focused on modifying the bioinert Ti surface towards an improved osteoconductivity or even osteoinductivity in order to stimulate a more efficient peri-implant bone formation [7–9]. In a first approach, the surface topography was altered either on a macro- and microscale by surface roughening techniques

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(e.g. sandblasting and acid-etching), as a higher roughness promoted implant stability through an increased friction force with the bone, or on a nanoscale in order to encourage bone cell interactions [10–12]. Secondly, chemical modification of the surface (e.g. calcium phosphate (CaP) or biomolecule coatings) could mimic the natural bone interface and even stimulated bone regeneration [12,13].

Porous Ti coatings, generally applied by plasma spraying or sintering of Ti particles, present a particular surface topography combining an increased surface roughness for improved initial implant stability with the potential to achieve long-term stability through mechanical interlocking at the implant/bone interface by bone ingrowth into the pores. The eventual bone anchorage, however, strongly depends on the quality (amount and interconnectivity) of osseointegration into the porous structure. This is primarily determined by the pore characteristics of the coating. Highly porous and interconnected open-cell structures favour bone ingrowth and the optimal pore size is generally accepted to be in the 100–400 μm range as a compromise to provide sufficient space for cell migration and vascularization, while maintaining the mechanical strength of the porous material [14–16].

Modification of the Ti surface to improve the osseointegration of dense implants is well established; several recent studies have confirmed the beneficial effect of nanostructured surfaces [17] or CaP [18,19], calcium silicate and calcium titanate [20] based coatings whether or not doped with bioactive ions [21–23] on bone formation. Surface modification of the internal pore surface of porous Ti constructs has been limited mostly to macroporous Ti scaffolds, showing an improved bone ingrowth after sand blasting and/or acid etching [24], acid–alkali treatment [25], coating with CaP [26] or hydroxyapatite [27] or preparation of nanostructured calcium titanate and titanium oxide surfaces [28].

Recently, we developed a new processing route for porous Ti coatings with predominantly micropore sizes [29]. Few studies have considered the possible effect of microporosity (0.5–10 μm) on osseointegration and only recently research on CaP has shown that micropores in scaffold struts can be employed as additional space for bone ingrowth [30–32]. Therefore, the first objective of the present study was to assess the early peri-implant tissue response to porous pure Ti coatings with significant microporosity. Secondly, it was hypothesized that biofunctionalization of the internal pore surface could improve the bone regeneration in the vicinity of the coatings. However, when envisaging the modification of the internal surface of a porous coating, direct line-of-sight techniques, such as plasma spraying, are not qualified. Wet chemical techniques based on a solution penetrating the entire porous structure are more suitable [9]. We proposed three wet chemical techniques for the application of an additional surface layer in the porous structure. Anatase $TiO_2$, which is known to enhance the bioactivity of Ti [33], was applied by a hydrothermal treatment (HT) [34]. Furthermore, micro-arc oxidation (MAO) was used to produce a $TiO_2$ surface layer containing $Ca^{2+}$ and $PO_4^{3-}$ ions [35]. Previous studies have shown that MAO coatings enhance the bone response for flat Ti substrates [36], especially when $Ca^{2+}$ and $PO_4^{3-}$ ions are incorporated in the surface layer [37,38]. Alternatively, $Ca^{2+}$ and $PO_4^{3-}$ ions can also be introduced in the form of dissolution products released from a bioactive glass (BAG) matrix [39]. Both melt and sol–gel-derived BAGs have proven to support bone bonding in vivo, but especially sol–gel BAG is associated with osteogenesis due to an increased release of ionic species from its intrinsically higher surface area [40,41]. Moreover, as sol–gel synthesis is more compatible with the coating of porous structures, this was the preferred processing route in this study [42]. Coated Ti implants were inserted in the rabbit tibia, applying a bone cavity model featuring a regeneration compartment [43]. Histological and histomorphometrical analysis of the bone response after 2 and 4 weeks was done using transmission light microscopy (LM) and scanning electron microscopy (SEM).

## 2. Materials and methods

### 2.1. Materials and characterization

As substrate material, commercially pure Ti (thickness 1 mm, grade 2, Goodfellow) was laser cut into discs 15.5 mm in diameter for the surface characterization and discs 4 mm in diameter as implant material for the in vivo experiment. Next, the discs were decontaminated by ultrasonically cleaning in acetone (Acros Organics) and rinsing in distilled water followed by acid etching in a 4 vol.% HF (40%, Riedel-de Haën) and 20 vol.% $HNO_3$ (60%, Chemlab) solution for 60 s. After excessive rinsing in distilled water, the samples were autoclave sterilized.

As the unmodified reference coating, a porous pure titanium coating was applied using electrophoretic deposition (EPD) of titanium hydride ($TiH_2$) powder suspensions followed by dehydrogenation and sintering in vacuum, as described elsewhere [29]. The coating will be referred to as EPD Ti. Subsequently, this coating was functionalized using three different additional treatments. First, a hydrothermal treatment (Jožef Stefan Institute, Slovenia) was applied creating a thin microanatase $TiO_2$ layer on the outer and inner surfaces of the porous Ti coating [34]. Alternatively, MAO (University of Bayreuth, Germany) was used to produce a pore filling $TiO_2$ layer containing $Ca^{2+}$ and $PO_4^{3-}$ ions [35]. Finally, a micrometer-thin bioactive glass coating was applied on the internal surface of the Ti coating using an all-alkoxide sol–gel synthesis (KU Leuven, Belgium) [42]. The samples are denominated EPD Ti + HT, EPD Ti + MAO and EPD Ti + BAG, respectively. Prior to implantation, the samples were sterilized either using an autoclave (EPD Ti and EPD Ti + HT) or by heating to 200 °C in a vacuum furnace (EPD Ti + MAO and EPD Ti + BAG) in order to avoid dissolution of the functionalized coatings.

White light interferometry (WLI, Wyko NT 3300 Optical Profiler, Veeco Metrology Inc.) was used to obtain three-dimensional (3-D) roughness measurements. Ten spots divided over three different samples were measured per experimental surface. A quantitative analysis of the roughness data was performed using MountainsMapH Premium software (Digital Surf). Further qualitative analysis of the surface roughness was done by SEM (XL30-FEG, FEI).

The main pore structure characteristics (porosity, pore size, interconnecting pore channel (IPC) size) was done by mercury intrusion porosimetry (MIP, AutoPore IV 9500, Micromeritics) in combination with image analysis (PPM2OOF software, NIST) on SEM images of representative metallographic cross-sections.

### 2.2. Surgical procedure and tissue processing

The animal handling and experimental protocol used in this study was approved by the Animal Ethics Committee of KU Leuven and was performed according to the Belgian national legislation concerning the protection and wellbeing of animals. (Approval ID: P122/2008)

Six mature New Zealand white rabbits (average weight 3.17 ± 0.18 kg) underwent surgery as described elsewhere [43]. In short, following anaesthesia, four double-stepped cavities were drilled exclusively in the cortical bone of the diaphysis at the medial side of the proximal tibia. The outer step diameter was 4 mm in size for a press-fit mounting of the coated implants; the smaller inner cavity was 2 mm in diameter with a depth of 0.5 mm (Fig. 1a). To ensure a standardized blood supply to the cavity during healing, a perforation (0.5 mm diameter) to the bone marrow was made into the base of the cavity centre. Next, the sterilized implants