Localized Controlled Drug Delivery from Mesoporous Implants

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ABSTRACT

Mesoporous materials possess both a well-defined topography on the nanometer scale, and they may serve as hosts for drugs. In this work, titanium implants coated with mesoporous TiO_2 thin films have been evaluated both *in vitro* and *in vivo*. Material characterization showed that, long-range ordered mesoporous TiO_2, with a pore-size of 6 nm, and a narrow pore-size distribution were obtained. An *in vivo* study demonstrated that the films were robust enough to withstand the implantation procedure. The *in vitro* apatite formation experiments showed that formation of apatite was higher on the mesoporous surface compared to its nonporous counterpart. In a separate *in vivo* study, two osteoporosis drugs, alendronate (ALN) and raloxifene (RLX), were immobilised into the nanoporous oxide films. The *in vitro* drug release tests carried out showed a sustained release of both drugs. The osteogenic response after 28 days of implantation of the drug-loaded implants showed a significantly improved bone fixation.

Keywords: osseointegration, implant coating, mesoporous titania, nanotopography, drug delivery

1 INTRODUCTION

Since the pioneering introduction of osseointegrated implants, impressive clinical and commercial outcomes have emerged. There is still, however, a need to improve the materials technologies, especially those related to bone regeneration and infection. An important issue in this context is specific patient groups with reduced bone healing capacity, for instance patients suffering from osteoporosis. Properties that have been suggested to affect the osseointegration are surface topography, surface chemistry and physical properties such as the surface charge and energy [1]. Topographical modifications on different length scales; macro, micro and nanometer have all been identified to be of importance in order to obtain good osseointegration of implants [2]. Whether the nanotopography alone promotes osseointegration has recently been discussed, however, the challenge still remains in order to conclude its actual effects [3]. Moreover, the formation of apatite, which is the major building block of bone tissue, is another property being influenced by the nanostructure of the surface [4]. A more recent approach in implantology is to deliver appropriate drugs at the time of implantation, and the most common delivery routes are oral and systemic administrations. However, there is for several reasons a need to develop a more suitable drug administration at the sites of implantation. Systemic side effects could then be avoided, and lower and safer drug quantities would suffice with more localized efficacy and efficiency. A local administration of drugs from the implant surfaces provides a particularly interesting alternative to traditional medicine, and may broaden the number of useful drug candidates. As a first step, the design of implants with optimal drug-loading and release profiles is needed.

Mesoporous structures have the ability to serve for drug-delivery purposes. One property that can be adjusted to control the drug administration is the pore size, which can affect the release rate to a large extent [5]. Moreover, the presence of meso-sized pores will highly increase the drug loading capacity of the implant [6]. Local delivery from mesoporous implants would meet the abovementioned challenges such as prolonged bioavailability and efficiency, lowered systemic toxicity, controlled release rate over longer periods of time and lower clinical costs [7].

In this work, titanium implants coated with a thin layer of mesoporous TiO_2 has been evaluated. TiO_2 was the material of choice since it has been established to be a suitable material for hard tissue applications. The mesoporous TiO_2 thin films were formed using the evaporation-induced self-assembly (EISA) method, which results in meso-sized pores with a narrow size distribution. Using EISA, without the presence of a template, results in thin films without pores, but with identical chemistry, hence the method can be used in order to evaluate the real contribution from the nano-sized pores. How the mesoporous structure promotes osseointegration was investigated both *in vitro* and *in vivo* [8]. The *in vitro* study was performed using the well-established simulated body fluid (SBF) method, which is commonly used to evaluate how well a material can promote the formation of apatite [9]. The ion adsorption and apatite formation was monitored using quartz crystal microbalance with dissipation monitoring (QCM-D), which gives detailed information at an early time point. An *in vivo* study using a rabbit model was performed and the removal torque and

histomorphometry analysis were implemented to evaluate the osseointegration. Both mesoporous implants (test) and nonporous implants (control) were evaluated. Two healing times, 3 and 12 weeks were used in order to study both the early bone response and the osseointegration after complete healing. In a separate study, two well-known osteoporosis drugs with different effector mechanisms, alendronate (ALN) and raloxifene (RLX), were absorbed into the 200 nm thick mesoporous TiO_2 coatings on titanium screws [10]. The outcomes in rat tibia were compared to implants without drugs with respect to implant fixation and *de novo* bone growth. The rationale for the current drug selection was based on the fact that two different mechanisms of osteoclast suppression is underlying their efficacy. Bisphosphonates selectively suppress osteoclast metabolism while RLX, a specific oestrogen receptor modulator (SERM), decreases the access of RANKLs to osteoclasts, and thereby decrease the bone degradation rate.

2 MATERIALS AND METHODS

2.1 Material Preperation

Mesoporous TiO_2 films were prepared by evaporation-induced self-assembly (EISA) [11]. The inorganic precursor, titanium(IV) ethoxide (TEOT) was dissolved in HCl. Pluronic P123 was used as template, it was dissolved in ethanol. The solutions were vigorously stirred and mixed together. Spin-coating was used to prepare thin sol-gel films on glass slides, titanium discs, QCM-D sensors and screw shaped titanium implants. These were stored at room temperature (RT) overnight, allowing self-assembly to occur. Simultaneously, evaporation of HCl and ethanol took place. Calcination was thereafter performed by heating up to 350°C, 1°C per min, followed by a constant held temperature during 4 h followed by spontaneous cooling to room temperature.

2.2 Drug Loading

Alendronate and raloxifene were absorbed into hydrophilic and hydrophobic mesoporous TiO_2 films, respectively. Their respective water solubilities are 10 mg/ml and 0.25 mg/mL. Since the RLX polarity is lower than that of ALN, thin films were methylated with dichlorodimethylsilane (DCDMS, 1% methanol, 30 min). RLX (0.8 mg/ml) was absorbed and dissolved in methanol and then loaded into the hydrophobic pores (RT, 1h). ALN was dissolved in and absorbed from milli-Q water (0.8 mg/ml, RT, 1h).

2.3 Material Characterization

Transmission electron microscopy (TEM) was used for detailed microstructural analyses, such as visualization of long-range order and pore widths. It was performed with a FEI Titan 80-300 TEM/STEM (Hillsboro, USA) FEG equipped microscope operated at 300 kV. Before imaging, the thin TiO_2 films were scraped off from glass slides, and dispersed in ethanol. 2 µl of the dispersion was added onto a TEM grid and dried prior to analysis.

Scanning electron microscopy (SEM) was used to visualize the pore directions, pore widths and thicknesses of mesoporous TiO_2 thin films. A Leo Ultra55 (Zeiss, Oberkochen, Germany) with a field emission electron gun (FEG) operated at 5 kV was used for the visualization with SEM.

2.4 *In Vitro* Apatite formation

An established method to evaluate apatite formation *in vitro* is to use simulated body fluids (SBF) [12]. SBF is a solution which contains nearly the same ion concentration as the human blood plasma, giving a good simulation to the real clinical situation as an *in vitro* analysis for apatite formation [13]. *In situ* monitoring of ion adsorption and apatite formation was performed using quartz crystal microbalance with dissipation monitoring (QCM-D).

2.5 *In Vitro* Drug Adsorption and Release

ALN and RLX *in situ* absorption and release rates were investigated with QCM-D. The measurements were performed with a Q-Sense E4 instrument (Q-Sense, Sweden). Mesoporous and nonporous TiO_2 thin films were prepared on QCM-D sensors (QSX 310, Q-Sense, Sweden). In ALN experiments a flow of milli-Q H_2O was first passed through sample chamber where both the test and control samples were inserted. Upon a stable baseline, ALN (0.8 mg/ml) was added and absorption followed by changes in frequency (Df, Hz) and energy dissipation (D). The frequency shift (Df) corresponds to the amount of absorbed/released substance and was converted to mass (ng/cm^2) by the Sauerbrey equation [14]. After 15 min of absorption, rinsing was applied with milli-Q water and ALN desorption monitored by following f(t) and D(t). In RLX experiments, DCDMS hydrophobised mesoporous and nonporous sensors were inserted into the flow modules. A flow of methanol gave a stable baseline. The flow was then switched to a flow containing 0.8 mg/ml RLX.

2.6 *Ex Vivo* Characterization

Removal Torque (RTQ) vs. rotation angle were followed with 0.1 degree/s and real time monitoring. The RTQ-values were recorded at the plastic deformation point following the rotational friction and maximum force point where the bone-implant integration was broken (ultimate strength).

Quantitative histomorphometry was done measuring the Bone Implant Contact (BIC) and Bone Area (BA) within the threads, considering threads available in the cortex, endosteal bone and bone marrow compartments. All

specimens were evaluated with a light microscope (Nikon Eclipse E600, Japan).

Ultrastructural interface analysis was conducted using an *in situ* lift-out TEM technique [15]. Focused ion beam (FIB) was used to prepare the TEM sample to maintain the integrity at the interface between the mesoporous TiO_2 thin film on the implant and the surrounding tissue. It was performed with a FEI Strata 235 DualBeam (FIB/SEM, Hillsboro, USA), equipped with an ominiprobe. For TEM imaging, a FEI Tecnai T20 LaB6 ST (TEM, Hillsboro, USA) operating at 200kV was used. Elemental analysis over the interface area was evaluated using energy-dispersive X-ray spectroscopy (EDS) using a nano-probe in STEM mode.

3 RESULTS AND DISCUSSION

3.1 Material Characterization

A TEM micrograph showing the mesoporous TiO_2 is presented in Figure 1a. The pore size distribution is narrow, possessing an average pore diameter of 6 nm. A SEM micrograph of the thin mesoporous TiO_2 film is presented in Figure 1b and the image shows that the material possesed a high degree of porosity and that the pores were accessible from the surface. The film thickness based on the SEM analysis was estimated to be around 200 nm.

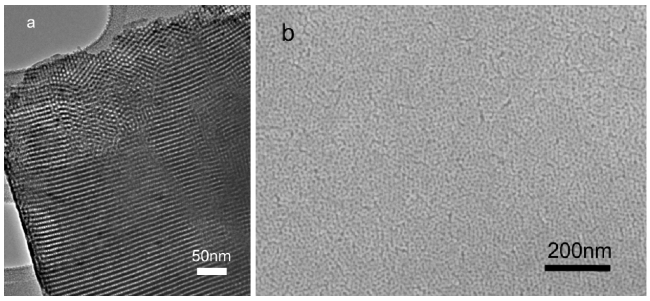

Figure 1: (a) A TEM image and (b) a SEM image of the mesoporous titania matrix.

3.2 *In Vitro* Apatite Formation

The apatite formation on mesoporous and nonporous TiO_2 surfaces was evaluated with QCM-D using SBF. The monitored frequency (f) and dissipation (D) as a function of time is presented in Figure 2. The adsorbed mass onto the materials can be calculated from the frequency shift using the Saurbrey equation [14]. A 3.8 times higher ion adsorption was obtained on the mesoporous surface compared to its nonporous counterpart. Moreover, the dissipation shift (D) is about the same for both surfaces which indicate that the ions could access the mesopores. The formation of apatite began after 22 h, which is shown in Figure 2b. The apatite formation was approximately 1.5 higher on the mesoporous surface compared to the nonporous.

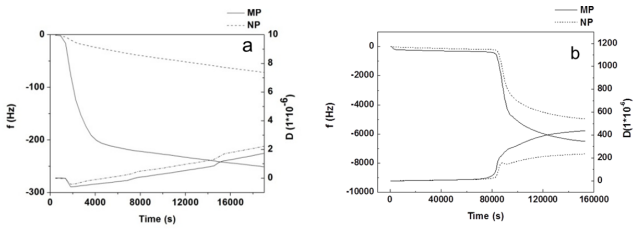

Figure 2: (a) Ion adsorption and (b) apatite formation from SBF on mesoporous (MP) and nonporous (NP) titania surfaces monitored by QCM-D.

3.3 Evaluation of Biomechanical Stability

To evaluate the biomechanical interlocking of the implant to the surrounding tissue, removal torque measurements were performed as *ex vivo* study. The mean value (SD) for the removal torque after 3 weeks of healing was 6.56 Ncm (4.56) and 4.67 Ncm (1.66) for the test and control, respectively. After 12 weeks of healing the mean value was 14.13 Ncm (5.59) and 13.00 Ncm (6.32) for test (mesoporous) and control (nonporous), respectively. From the results after 3 weeks healing, there is a tendency that the mesoporous samples enhanced the osseointegration, however, no significance between the test and control could be determined. The histology images showed that the bone formation was successfully integrated to the implant. No significant difference was observed of the mean bone area of new bone formed in the implant threads between the test and control groups.

3.4 *In Vitro* Drug Adsorption and Release

The drug adsorption and desorption data (Figure 3) showed an uptake of approximately 550 ng/cm^2 maximum ALN into the hydrophilic porous oxide and 900 ng/cm^2 RLX into the hydrophobic pores. A 30 times higher ALN absorption was observed into porous TiO_2 films compared to nonporous films. Three times more RLX absorbed into methylated porous TiO_2 compared to the methylated nonporous TiO_2 control. Both drugs showed a sustained release pattern. Furthermore, no difference in the QCM-D energy dissipation was observed between porous and nonporous surfaces, indicating that both drugs absorbed into the mesopores.

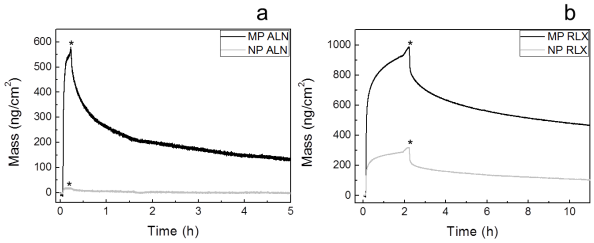

Figure 3: Adsorption and release patterns of (a) ALN and (b) RLX as monitored by QCM-D. First a drug flow was introduced and adsorption monitored on mesoporous (MP) and non-porous (NP) samples. This was then followed by rinsing flow (*).

3.5 *In Vivo* Performance with Drugs

The biomechanical bone-implant anchorage was tested by removal torque (RTQ) analysis. The drug-loaded mesoporous implants displayed a significantly higher bone-to-implant attachment than their references [10].

A quantitative *ex vivo* analysis of Bone Areas (BA) and Bone Implant Contacts (BIC) The BA-value of ALN-samples was significantly higher than for all other implant types. Moreover, from the observed BIC data ALN indicated the lowest BIC value and RLX indicated the highest. However, no significant differences in BICs were observed between the differently prepared surfaces.

In order to try to understand the high RTQ value of the RLX coated implant, an ultrastructural interface analysis was performed. A captured TEM image and an elemental analysis line scan over the mesoporous implant and surrounding bone is presented in Figure 4. An intimate contact was observed between the mineralized bone tissue and the RLX surface. Interestingly, the elemental analysis showed the presence of Calcium (Ca) and Phosphorus (P) inside the porous film. The interpretation is that RLX loaded surfaces induced precipitation of calcium phosphate mineral (CaP) inside the mesopores.

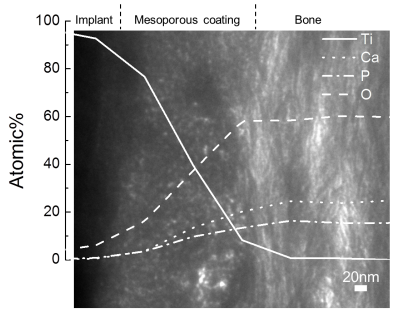

Figure 4: A cross-sectional TEM image of the RLX-releasing surface after 28 days *in vivo*. The EDS line scan was performed across the implant, the mesoporous TiO_2 coating and the surrounding bon. At.% of Ti, Ca, P and O is presented on the y-axis.

4 CONCLUSIONS

Ordered mesoporous TiO_2 thin films was successfully formed using the evaporation induced self-assembly (EISA) process. The pore-width was 6 nm, with a narrow size distribution, and the pores were accessible from the surface. An SBF *in vitro* study showed that formation of apatite could occur inside the mesopores, which means that the accessible surface area for apatite formation is much higher for mesoporous coatings. It was demonstrated *in vivo* that the mesoporous thin films was biomechanical stable towards the shearing forces during implantation into bone. In another study two osteoporosis drugs, alendronate and raloxifene, both showed a sustained local release *in vitro*. The *ex vivo* evaluation demonstrated improved bone implant fixation by both drug releasing implants.

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