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Modulation of the nanometer pore size improves magnesium adsorption into mesoporous titania coatings and promotes bone morphogenic protein 4 expression in adhering osteoblasts

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ABSTRACT

Objective. Mesoporous (MP) titania films used as implant coatings have recently been considered as release systems for controlled administration of magnesium to enhance initial osteoblast proliferation *in vitro*. Tuning of the pore size in such titania films is aimed at increasing the osteogenic potential through effects on the total loading capacity and the release profile of magnesium.

Methods. In this study, evaporation-induced self-assembly (EISA) was used with different structure-directing agents to form three mesoporous films with average pore sizes of 2 nm (MP1), 6 nm (MP2) and 7 nm (MP3). Mg adsorption and release was monitored using quartz crystal microbalance with dissipation (QCM-D). The film surfaces were characterized with atomic force microscopy (AFM), scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS). The effect of different Mg release on osteogenesis was investigated in human fetal osteoblasts (hFOB) using pre-designed osteogenesis arrays and real-time polymerase chain reaction (RT-PCR).

Results. Results showed a sustained release from all the films investigated, with higher magnesium adsorption into MP1 and MP3 films. No significant differences were observed in the surface nanotopography of the films, either with or without the presence of magnesium. MP3 films (7 nm pore size) had the greatest effect on osteogenesis, up-regulating 15 bone-related genes after 1 week of hFOB growth and significantly promoting bone morphogenic protein (BMP4) expression after 3 weeks of growth.

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Significance. The findings indicate that the increase in pore width on the nano scale significantly enhanced the bioactivity of the mesoporous coating, thus accelerating osteogenesis without creating differences in surface roughness.

1. Introduction

One of the main goals in placing a dental implant is to achieve a rapid integration with the bone to attain a firm anchorage. Both surface topography and surface chemistry have proven to be crucial for bone apposition and formation around the implant [1–6] and numerous modifications have been undertaken to contribute to the improvement of so-called osseointegration.

The application of mesoporous titania films to the surface has been proposed as a method to improve the bioactivity of an implant [7]. The improvement is due to the ability to incorporate bioactive substances into the films, facilitating their release at the peri-implant region in a controlled and sustained manner. One approach is to load the films with different drugs, *e.g.* osteoporosis medications, in order to obtain stronger biomechanical interlocking of the implant into the host bone [8–10]. Alendronate and raloxifene have been used as candidates to assess the capability of mesoporous films to act as carrier frameworks at the bone-implant site *in vivo* [11,12]. These studies showed an increased local bone density around implants loaded with the drugs. In order to adjust the drug content that can be released from the implant, mesoporous films with different pore sizes have been investigated. It was demonstrated that the pore diameter can be modulated using different structure directing agents and that the amount of drugs and their release profiles is affected by the pore-size [13,14].

Despite the positive results using osteoporosis medications to improve osseointegration, clinical trials are needed prior to the launch of such drug-releasing medical devices to ensure that there are no negative clinical effects. For example, the intravenous application of bisphosphonates has shown negative side-effects when used in high concentrations, which can result in necrosis of the jaw (BRONJ), an impaired bone turnover which leads to the exposure of necrotic bone in the oral cavity [15,16].

An alternative strategy is to bio-functionalize the implant surface with bioactive elements that participate in the osseointegrative process, such as calcium, phosphate, fluoride and magnesium [17,18]. Sul et al. have reported that Ca- and Mg-incorporation into titanium implants significantly increased the strength and speed of osseointegration compared to non-ion incorporated commercially pure titanium implants [19]. Mg- and Ca-incorporated titanium surfaces may electrostatically bond with polyanionic proteins, such as proteoglycans, collagen, fibronectin, vitronectin, osteoadherin, osteopontin and bone sialoprotein. This process triggers the recruitment of osteoprogenitor cells and osteoblasts, which possibly explains the rapid and strong bone formation seen with Mg- and Ca-incorporated titanium surfaces. Magnesium ions also play a critical role in the DNA polymerase binding site, catalysing the DNA polymerase mechanisms for repair and synthesis [20].

The concept of drug delivery from smooth mesoporous surfaces is of significant interest since excessively rough surfaces, designed to stimulate bone growth, have been proved to provoke negative biological consequences. This is most likely bcause they can act as a substrate for colonization by oral bacteria, which can induce progressive peri-implant bone loss [21–23]. In addition, even moderately rough surfaces have been proved a tendency for bacterial biofilm accumulation *in vitro* compared to smooth ones [24]. Recent reports have suggested that implant surfaces with smoother microtopographies function as well as rougher surfaces *in vivo* and clinical studies [25,26].

Thus bone integration could be enhanced by the controlled release of osteogenic substances from mesoporous smooth surfaces. We previously investigated the use of mesoporous thin films as a carrier for magnesium that was gradually released when in contact with bone cells and bone tissues. Both *in vitro* and *in vivo* studies demonstrated that the magnesium release positively influenced differentiation of progenitor cells into cells with an osteoblast phenotype and activity as well as improving the osteogenic environment and bone anchorage in a rabbit model [27–30]. These mesoporous films possessed a smooth surface (S_a lower that 0.5 μm) in accordance to the Wennerberg and Albrektsson guidelines [31], an average pore size of 6.0 nm and successful loading of Mg onto the coating. Although positive biological responses were observed from these studies, we speculated that there were still possibilities to increase the Mg content and, therefore, its osteogenic potential in biological systems.

Thus, in the present study, the effect of pore size of mesoporous thin films as well as magnesium release from them was investigated *in vitro*. Three mesoporous titania films with 2 nm, 6 nm, and 7 nm average pore diameters, comprehensively characterized in terms of material properties and drug delivery by Karlsson et al. in a recent investigation [14], were used in this study. Mg adsorption and release, and its osteogenic effect were investigated using human fetal osteoblasts. It was hypothesized that larger pores would show increased magnesium adsorption, giving a stronger effect on the expression of bone-related proteins in the osteoblast population.

2. Materials and methods

2.1. Surface preparation

Commercially pure titanium (CpTi, grade 4) discs with a diameter of 12 mm and a thickness of 1 mm were used in this study. Samples were coated with mesoporous titania thin