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Osseointegration properties of titanium dental implants modified with a nanostructured coating based on ordered porous silica and bioactive glass nanoparticles

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ABSTRACT

The fabrication of a nanoporous silica coating loaded with bioactive glass nanoparticles (nBG/NSC) on titanium dental implant surface and its *in vitro* and *in vivo* evaluation is presented. The coating was produced by a combined sol–gel and evaporation induced self-assembly process. *In vitro* bioactivity was assessed in simulated body fluid (SBF) and investigating the osteogenic differentiation of human bone marrow mesenchymal stem cells (hBMSCs). A rat tibial model was employed to analyze the bone response to nBG/NSC-modified titanium implant surface *in vivo*.

The nBG/NSC coating was confirmed at nano level to be constituted by a highly ordered nanoporous silica structure. The coating nanotopography in conjunction with the bioactivity of the BG particles accelerate the *in vitro* apatite formation and promote the osteogenic differentiation of hBMSCs in absence of osteogenic supplements. These properties accelerate the formation of bone tissue in the periphery of the implant after 3 weeks of implantation. Backscattered scanning electron microscopy images revealed the presence of gaps and soft tissue in the unmodified implant after 6 weeks, whereas the nBG/NSC-modified implant showed mature bone in intimate contact with the implant surface. The nBG/NSC coating appears promising for accelerating the osseointegration of dental implants.

1. Introduction

Dental implants have become a widely accepted and predictable treatment to replace single or multiple missing teeth [1,2]. Although they present an elevated clinical success rate [3], efforts are made in order to obtain faster and more stable osseointegration, which has stimulated greatly the developments on implant design [4,5]. Different strategies have been established including the treatment of the surface of the implant to create physical and chemical changes [6].

Nowadays, most of the commercially available dental implants have surface modifications in order to increase their surface roughness [7]. Several studies have observed a positive effect on modifying the surface topography of implants compared to smooth surfaces [8]. However, majority of current commercially available implant present surface morphologies that are controlled only to the micron level, though the events involved in osseointegration occur in a nanoscale setting [7,9]. Therefore, it is possible that understanding and controlling tissue responses at the nano level, by providing nanoscale topography to dental implants, could help to eliminate rejection and improve osseointegration processes [9,10].

Different techniques have been developed to produce surface modifications at the nano level on implants [10], including optical lithography [11], crystal deposition [12], and chemical treatment [13], among others. However, the use of these methods makes difficult to control the distribution and homogeneity of nanostructures on the implant surface. The use of novel techniques derived from soft matter physical chemistry and inorganic or hybrid sol–gel chemistry allows a high control of material nanostructure. By using the evaporation-induced self-assembly (EISA) technique, in which colloids self-organize into ordered layers during evaporation of the solvent [14], together with sol–gel processing is feasible to achieve

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regularly-patterned nanoporous surfaces [15,16]. This method is simple, inexpensive, scalable, and has a high reproducibility. In a previous study, we demonstrated that titanium surfaces modified with silica coatings with highly ordered sub-10 nm porosity produced by EISA/sol–gel technique improves the osteoblast adhesive responses and stimulates the osteogenic differentiation of stem cells, probably through a mechanotransduction mechanism [17]. On the other hand, the loading of the implant surface with bioceramic particles also constitutes a common approach to accelerate the osseointegration process. The most commonly tested are calcium phosphate (hydroxyapatite and tricalcium phosphate) and bioactive glasses, which when in contact with fluids tend to form a carbonated apatite layer, similar to the mineral phase of osseous tissue [9]. Several studies have reported a higher rate and extent of bone formation adjacent to implants coated with microsized bioceramics than in uncoated implants [18,19]. Bioceramic nanoparticles are expected to have improved bioactive properties, due to the higher aspect ratio exhibited by the nanodimensional materials. In a previous study, we compared the ability of hydroxyapatite and bioactive glass nanoparticles to induce the formation of apatite, finding that bioactive glass nanoparticles produce a faster formation of apatite in physiological medium [20]. It is expected that the incorporation of bioactive glass nanoparticles into the patterned nanoporous silica coating could generate a novel nanocomposite coating material combining the osteogenic properties of the porous nanotopography with the chemical bioactivity of the ceramic nanoparticles.

The aim of this work is to prepare a highly ordered nanoporous silica coating loaded with bioactive glass nanoparticles (nBG/NSC) on titanium implant surfaces and assess the osseointegration properties of the modified surface. It is hypothesized that this nanostructured coating stimulate the bone-like apatite mineralization and the osteogenic differentiation of stem cells, thus accelerating the formation of bone in intimal contact with the implant surface. The *in vitro* bioactive response of the modified titanium surfaces was evaluated on apatite formation in simulated body fluid (SBF) and on osteogenic differentiation of stem cells. In addition, the *in vivo* osseointegration process of titanium implants modified with the novel nanostructured coating was assessed by using rat tibial model.

2. Material and methods

2.1. Synthesis of nBG/NSC-modified titanium surfaces

Nanoparticles of bioactive glass (nBG) were synthesized by the sol–gel method using the following molar composition: $58SiO_2$:$40CaO$:$5P_2O_5$ reported in a previous work [20]. nBG/NSC modified titanium surfaces were prepared on sheets of Ti_6A1_4V titanium alloy (Zimmer Dental) using the EISA sol–gel technique. Titanium sheets ($15 \times 15 \times 1$ mm) were sanded with silicon carbide paper (800 grit) and cleaned ultrasonically with acetone and ethanol before use. The coating sol solutions were prepared using the amphiphilic triblock copolymer Pluronic P123 (P123; $EO_{20}PO_{70}EO_{20}$, Mw 1/4 5800; Aldrich) as pore structure-directing agent (SDAs). Briefly, 3.7 g of tetraethyl orthosilicate (98%; Aldrich) were prehydrolyzed in a solution containing 10 mL of ethanol (95%) acidified with 0.5 mL of HCl 0.5 N (pH 2.0) under vigorous stirring at room temperature for 20 min. Appropriate amount of nBG were added into 10 mL ethanol to produce a 10 wt.% suspension. Both solutions were added to a solution containing 2 g of P123 dissolved in 20 mL of ethanol. The resulting solution was then submitted to an aging period at room temperature for 24 h with stirring, and films were prepared by slip coating on the titanium sheets.

For the slip-coating procedure the titanium sheet was suspended in an inverted position with a pair of tweezers attached to a clamp fixed loosely enough to a stand to allow rotation of the tweezers. The polished side was brought in contact with the silica sol. The titanium sheet was kept in this half-immersed position for 20 s, slipped away horizontally by rotating the tweezers, and then dried in a vertical position for 40 s. The silica coatings were kept for 24 h at 35 °C, and then calcined by heating at a rate of 0.5 °C/min to 400 °C, holding that temperature for 4 h to remove the SDA.

In the case of *in vivo* animal study, orthodontic titanium mini-implants (Biomaterials Korea®, Ti_6A1_4V) with 1.5 mm in diameter and 7 mm in length were used. The implants were coated using a dip-coating procedure. For this purpose, the implants were suspended in an inverted position with a device that allows vertical movement. The implants were kept in the sol during 1 min and then vertically removed at a 0.3 mm/s speed using a precision micro-geared motor. The coated implants were kept for 24 h at 35 °C, and then calcined, by heating at a rate of 0.5 °C/min and holding it for 4 h at 400 °C. After that, implants were sterilized for 1 h with UV light and then kept in sterile containers.

2.2. Materials characterization

The unmodified and nBG/NSC-modified titanium sheet and implant surfaces were examined by scanning electron microscopy (SEM; Zeiss, DMS 940) after coating the surfaces with gold. The structural order of the porous silica coatings was analyzed by low-angle X-ray diffraction (XRD) within a 2θ range of 0.5–5°. XRD patterns were collected on a Siemens D 5000 diffractometer using Cu Kα radiation at a scanning speed of 0.2°/min. The porous nanostructure was examined by high-resolution transmission electron microscopy (HR-TEM) on a FEI-Tecnai G2 F20 S-Twin high-resolution transmission electron microscope equipped with a field emission gun operating at an accelerating voltage of 120 kV. The specific apparent surface areas (Sg) of coating material was measured by N_2 adsorption at 77 K in a Micromeritics ASAP 2010 sorptometer and calculated using the Brunauer–Emmett–Teller (BET) equation.

2.3. In vitro *bioactivity assays*

The ability of the nBG/NSC modified titanium surfaces to induce the formation of apatite was assessed in acellular SBF, which has inorganic ion concentrations similar to those of human extracellular fluid. The SBF solution was prepared according to the procedure described elsewhere [21] using the standard ion composition (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 mM). The fluid was buffered at physiological pH 7.4 at 37 °C with tri-(hydroxymethyl) aminomethane and hydrochloric acid. The unmodified and nBG/NSC modified titanium sheets (1.5 cm^2) were individually soaked in 20 mL of SBF in polyethylene containers at 36.5 °C using a thermostatic bath. After incubation for 3 days, the samples were removed from the SBF, rinsed with distilled water, and dried at 60 °C. Apatite mineralization on the surfaces was analyzed by SEM with a Jeol JSM 5410 microscope equipped with energy-dispersive X-ray spectroscopy (EDX). The structural order of the surfaces was analyzed by low-angle XRD, collected on a Siemens D 5000 diffractometer using Cu Kα radiation at a scanning speed of 0.2°/min.

2.4. Cell culture

Human bone marrow stem cells (hBMSCs) were used to evaluate the osteogenic differentiation capacity of the nBG/NSC-modified titanium surfaces. Approximately $1 \times 10^3/cm^2$ cells were seeded on sterilized unmodified and nBG/NSC-modified titanium sheets (10 mm × 10 mm) and maintained at 37 °C in a humidified air atmosphere containing 5% CO_2, in Dulbecco's modified Eagle medium