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Osteoblast responses to different oxide coatings produced by the sol–gel process on titanium substrates ☆

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Abstract

In order to improve the osseointegration of endosseous implants made from titanium, the structure and composition of the surface were modified. Mirror-polished commercially pure (cp) titanium substrates were coated by the sol–gel process with different oxides: TiO_2, SiO_2, Nb_2O_5 and SiO_2–TiO_2. The coatings were physically and biologically characterized. Infrared spectroscopy confirmed the absence of organic residues. Ellipsometry determined the thickness of layers to be approximately 100 nm. High resolution scanning electron microscopy (SEM) and atomice force microscopy revealed a nanoporous structure in the TiO_2 and Nb_2O_5 layers, whereas the SiO_2 and SiO_2–TiO_2 layers appeared almost smooth. The R_a values, as determined by white-light interferometry, ranged from 20 to 50 nm. The surface energy determined by the sessile-drop contact angle method revealed the highest polar component for SiO_2 (30.7 mJ m^{-2}) and the lowest for cp-Ti and 316L stainless steel (6.7 mJ m^{-2}). Cytocompatibility of the oxide layers was investigated with MC3T3-E1 osteoblasts in vitro (proliferation, vitality, morphology and cytochemical/immunolabelling of actin and vinculin). Higher cell proliferation rates were found in SiO_2–TiO_2 and TiO_2, and lower in Nb_2O_5 and SiO_2; whereas the vitality rates increased for cp-Ti and Nb_2O_5. Cytochemical assays showed that all substrates induced a normal cytoskeleton and well-developed focal adhesion contacts. SEM revealed good cell attachment for all coating layers. In conclusion, the sol–gel-derived oxide layers were thin, pure and nanostructured; consequent different osteoblast responses to those coatings are explained by the mutual action and coadjustment of different interrelated surface parameters.

Keywords: Titanium; Sol–gel coating; Osteoblast; Titanium oxide; Niobium oxide

1. Introduction

Titanium has been used in medical applications for a long time, because of its superior bulk and surface properties as compared to other metallic biomaterials. It has been extensively used for biomedical devices in dental, orthopaedic and cardiovascular fields, e.g. for dental implants, hip-joint prostheses and coronary stents. In fact, it is not titanium itself but a dense oxidized layer (TiO_2) that forms naturally on its surface (2–6 nm thick) which interacts with the living environment. This oxide layer is thermodynamically stable and chemically inert, and has very low solubility in body fluids [1]. This is the reason why titanium materials have excellent corrosion resistance [2] and biocompatibility [3]. Nevertheless, this TiO_2 layer is very thin and irregular. The application of titanium-based materials for implants may sometimes cause problems because of their relatively poor surface hardness and wear resistance [4], causing metal release in the tissue [2]. The most important requirement for the long-term success of implants is the stable interface between the biomaterial and surrounding

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tissue. By means of oxide coating modification of titanium or its alloys, it is hoped to improve the tissue response to the implant surface. Such surface modification can be achieved by changing the surface composition and/or the surface structure, including oxide layer thickening. In comparison with a titanium surface with only a naturally grown oxide layer, it has already been shown that corrosion resistance can be enhanced by a thicker (100 nm) oxide layer coating, independent of the oxidation procedure [5]. In addition to TiO_2, as the main candidate for oxide coatings on titanium implants, other stable oxides, such as niobium oxide (Nb_5O_2), silicon oxide (SiO_2), etc., may also play a promising role in the tissue–material interaction.

Besides the surface chemistry, the topography of biomaterials is commonly accepted as an important factor to improve the cell or tissue response [6]. Improving the implant–tissue interface by forming a particular surface topography and microstructure has attracted much attention. The possible influence of nanosized structures on the cellular response has been a much-discussed subject. It is now increasingly acknowledged that the surface structure of biomaterials with nanoscale topographies can influence cellular interactions such as osteoblast adhesion and further biomineralization [7,8].

However, many authors have focused primarily on either the chemical or the topographical aspects of the materials' surface structure. For this reason, the correlation between these properties and the cell response remains unsatisfactory in most cases.

In order to determine what kind of surface chemistry and topography may improve osteoblast response, different sol–gel-derived oxide layers with a thickness of 100–130 nm were produced on titanium surfaces. Unlike many other studies, and some of our previous work [7,9], this study paid closer attention to the physical and the chemical structure of these substrates and, furthermore, not only the topography and the roughness of the surface but also the morphology of the whole film (e.g. porosity) was taken into account, especially by studying cut cross-sections with appropriate methods. To investigate the mechanism of cell–substrate interactions, the cellular and molecular activity of osteoblastic cells grown on these different novel oxide layers were compared with untreated, mirror-polished titanium and stainless steel (316L). Since the proteins present in the extracellular matrix (ECM), cytoskeleton and membrane are generally involved in cell–substrate interactions and consequently influence cell adhesion, growth and differentiation, qualitative analyses of key proteins such as actin and vinculin were also assessed via osteoblast cell lines.

2. Materials and methods

2.1. Preparation of titanium substrates

The substrates used for the coating procedure are commercially pure titanium (cp-Ti, grade 2, ISO5832) discs of 15 × 2 mm (diameter × thickness). All disc samples have been mirror polished before coating. After a primary abrasion with silicon carbide (SiC) emery paper, the samples were mechanically polished with a monocrystalline diamond suspension, and finally with a colloidal suspension of silica particles (0.05 μm in size).

2.2. Oxide layer processing by the sol–gel method

For starting this process, a sol was prepared consisting of one or several precursors/metal alcoxides (depending on the type of the desired oxide coating) and of various solvents (butanol and acetylacetone) (Table 1). The acetylacetone serves to form a complex with the precursor to prevent an uncontrollably fast hydrolysis of the precursor with the water present as a trace impurity in the solvent. Nevertheless, a controllable hydrolysis could also be a favourable chemical reaction in the sol–gel process in order to generate a homogeneous layer without any micro-fissures [9]. The reaction with water in the air, however, needs to occur after the sol has been deposited on the substrate.

The sol was deposited on the mirror-polished titanium discs by spin-coating at 6000 rpm for 15 s. The evaporation time for the solvents was 10 min. As the result of two simultaneous and rapid processes, i.e. hydrolysis and condensation, a gel consisting of metal oxide particles, alcohol and water was obtained after a 30 min period of drying at 150 °C [9]. The water and the alcohol evaporation densified the layers, which still remained amorphous. Furthermore, the coatings were annealed by heating from room temperature up to 450 °C and maintaining this temperature for 1 h. This process definitely eliminated all residual water and organic content in the layers. A thin and compact coating layer was realized. In addition, during the annealing, the oxides could crystallize.

2.3. Physicochemical characterization of the oxide coatings

2.3.1. Infrared spectroscopy

The purity of the final oxide layers, especially the possible presence of organic residues from the sol–gel processing, was examined by infrared (IR) spectroscopy (Biorad FTS 3000 Excalibur FTIR spectrometer with a Harrick Seagull® reflection unit). Attenuated total reflection

Table 1
Concentrations of precursors and acetylacetone for the production of different oxide coatings

Coating type	Precursor	Precursor concentration (mol kg^{-1})	Acetylacetone concentration (mol kg^{-1})
TiO_2	Orthobutyltitanate $[Ti(OC_4H_9)_4]$	1	1
Nb_2O_5	Niobium ethoxide $[C_{10}H_{25}NbO_5]$	0.75	1.125
SiO_2	Tetraethylorthosilicate named TEOS $[Si(OC_2H_5)_4]$	1	1
SiO_2–TiO_2	Orthobutyltitanate + TEOS	0.5/0.5	1

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