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Biological response of human mesenchymal stromal cells to titanium grade 4 implants coated with PCL/ZrO_2 hybrid materials synthesized by sol–gel route: in vitro evaluation

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ABSTRACT

The surface modification of implantable materials in order to improve their biological proprieties, including tissue tolerance and osseointegration ability, by means of functional coating deposition is a promising strategy to provide a firm fixation of the implants. In this study, organic/inorganic hybrid materials consisting of an inorganic zirconia-based matrix, in which a biocompatible polymer, poly(ε-caprolactone) (PCL), has been incorporated at different percentages, have been synthesized via sol–gel route. Developed materials have been used to coat titanium grade 4 substrates by means of dip coating technique. Scanning electron microscopy (SEM) analysis of the obtained coatings has shown that films crack-free can be obtained for high levels of PCL. Chemical composition and interactions between organic and inorganic moieties have been studied by Attenuated Total Reflectance Fourier Transform InfraRed spectroscopy.

The bone-bonding capability of the nanocomposite films has been evaluated in vitro by examining the appearance of an apatite layer on their surface when soaked in a simulated body fluid by means of SEM equipped with EDS microanalysis.

In vitro biocompatibility assessment was performed in combination with human mesenchymal stromal cells (hMSCs). Materials were found to be non-toxic and supporting cell proliferation. Additionally, the coating material was not hampering the differentiation of hMSCs in an osteogenic medium.

1. Introduction

Commercially pure titanium (CP Ti) and its alloys are widely used in the biomedical field mainly as orthopedic and dental implants [1–3].

According to ASTM F67 standard, CP titanium is produced in 4 different degrees of purity (grade 1 to 4), upon the maximum content of interstitial impurities (the higher the content, the higher the grade). Such interstitial defects act as an obstacle to the glide of dislocations within the lattice, therefore augmenting mechanical strength and reducing ductility.

CP Ti grade 4 is most commonly used for implantology (especially in the dental field) because of its favorable combination of mechanical properties (tensile and fatigue strength), corrosion resistance, high biocompatibility and lack of inflammatory response.

From a biological perspective, titanium is classified as a biologically inert material that does not promote any adverse reaction, and is well tolerated by human tissues. However, the formation of peri-implant fibrosis may isolate the implant from the surrounding bone and can induce the mobilization of prostheses, thus reducing their performance.

Several strategies have been proposed to increase titanium bioactivity, including surface treatments which can affect the proliferation and differentiation of bone cells, improving the implant osseointegration process. In particular, surface modification by the application of coatings to biologically inert implantable materials is attracting growing attention. It offers the possibility to optimize surface properties while retaining favorable bulk properties. Titanium-based implants coated with glasses/glass ceramics and calcium phosphate, have been characterized by enhanced performances both in vitro and in vivo [4–10]. One of the most common techniques adopted to prepare glassy coating on a wide range of substrates also with complex shapes is the sol–gel dip coating method [7,11].

Sol–gel technology allows the synthesis of ceramic and glassy materials at a relatively low temperature. The transition of a sol–gel system from a colloidal solution (called ‘sol’) into a solid ‘gel’ phase occurs through hydrolysis and polycondensation of a metal alkoxide precursor [12], which proceed by a second order nucleophilic substitution [12,13]. The low processing temperature combined with the high sol homogeneity, due to mixing on the molecular scale, allows us to synthesize glass and ceramic materials doped with organic thermolabile molecules (e.g. polymers, drugs or biomolecules) by adding them, previously solubilized, to the sol during the inorganic matrix formation [14]. That

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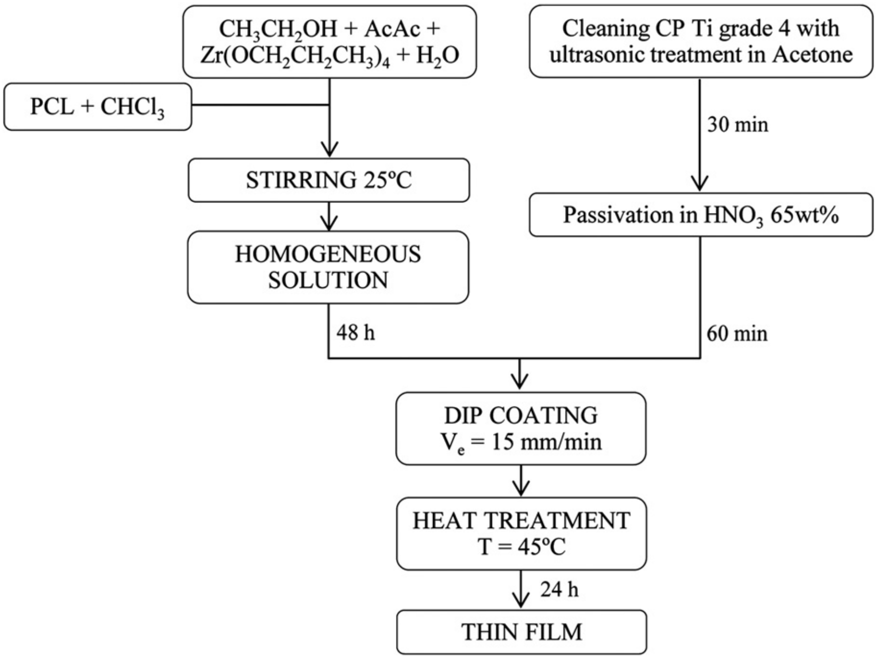

Fig. 1. Flow chart of ZrO_2 + PCL gel synthesis and coating procedure.

makes sol–gel method an ideal technology for the fabrication of organic–inorganic hybrid materials by entrapping various organic polymers in a glassy matrix. Their development has the purpose of synthesizing new materials with the advantage of the best properties of each component that forms the hybrid, trying to decrease or eliminate its drawbacks.

Indeed, sol–gel process has allowed a sound control over the chemical composition of obtained materials, which have shown to be more bioactive than those prepared with different methods [12,15,16]. Enhanced bioactivity of sol–gel glasses is due to the presence of hydroxyl groups on their surface, which can promote the nucleation of calcium phosphate deposits, promoting osseointegration when those materials are implanted [15]. However, due to their poor mechanical properties, monolithic glasses cannot be used in load-bearing applications, where metallic alloys are still the materials of choice. Therefore, their use as coatings of metal implants was explored to improve biological properties at the surface level [7,17–19]. However, due to the intrinsic brittleness of inorganic sol–gel glass coatings, many works have addressed the introduction of organic components into the inorganic sol–gel to form organic/inorganic hybrid sol–gel coatings [8,20].

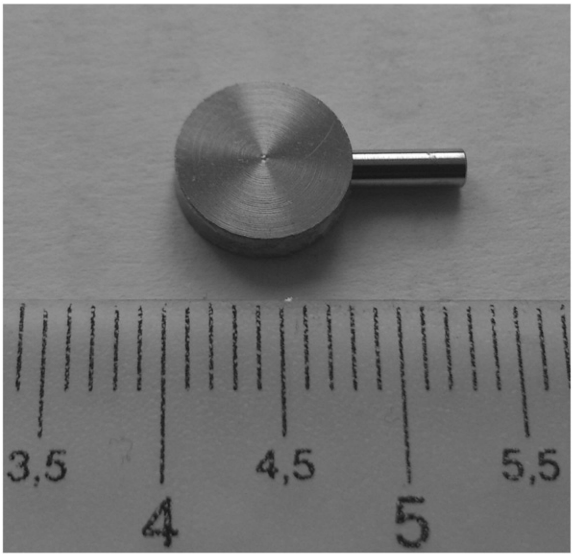

Fig. 2. ZrO_2 + PCL coating on titanium grade 4 substrate.

Sol–gel can be used to coat substrates with complex shapes and different nature by means of the dip coating technique [12,18], which requires the substrate to be dipped into the 'sol' and withdrawn at a constant speed to enable the sol drainage and its instantaneous gelation. Withdrawal speed is a key process parameter, as it influences the thickness and the morphology of the coating layer.

In this work, a sol–gel dip coating route has been optimized to coat CP Ti grade 4 substrates with films consisting of organic/inorganic hybrid materials based on zirconia (ZrO_2) and poly(ε-caprolactone) (PCL).

Several papers show the use of zirconia ceramics and glasses as biomaterials [7,21]. Also PCL (a biodegradable, synthetic, aliphatic polyester) has attracted a wide interest for its possible applications as a biomaterial: PCL-based films and three-dimensional scaffolds have been proposed for application in tissue engineering [22–24]. Moreover, composite substrates for tissue engineering have been prepared by sol–gel method, consisting of a poly(ε-caprolactone) matrix reinforced with sol–gel synthesized PCL/TiO_2 or PCL/ZrO_2 hybrid fillers [25,26] as well as several PCL-based hybrids for drug delivery, using different oxides, CaO and/or SiO_2 [27–31], TiO_2 [20,32], and ZrO_2 [33,34].

The chemical characterization of ZrO_2/PCL systems was discussed extensively elsewhere [7,34] and materials were shown to be amorphous, homogenous, and nanostructured class I hybrids: in particular, the hydrogen bond formation between Zr–OH group (H donor) in the sol–gel intermediate species and carbonyl group (H-acceptor) in the repeating units of the polymer was detected by means of FTIR and NMR analyses.

The aim of this study was to investigate, within the limits of in vitro settings, the performance of titanium grade 4 coated with organic/inorganic ZrO_2 + PCL hybrid materials synthesized via sol–gel, by analyzing proliferation and differentiation of human mesenchymal stromal cells (hMSCs).