ARTICLE IN PRESS

Ceramics International (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Ceramics International

journal homepage: www.elsevier.com/locate/ceramint

Vitroceramic coatings deposited by laser ablation on Ti-Zr substrates for implantable medical applications with improved biocompatibility

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ARTICLE INFO

Keywords:
Vitroceramics
Biocompatible coatings
Pulsed laser deposition
Implants

ABSTRACT

The influence of vitroceramic coatings deposited onto Ti-Zr alloy plates on the biological properties of the final biomaterials was investigated. In this regard, two vitroceramic masses, different from compositional point of view, were synthesized by a sol-gel route, being subsequently converted into ceramic targets, suitable for ablation experiments. The film depositions were conducted in oxidative atmosphere, on substrates heated at 300 or 400 °C. The coated samples were characterized by X-ray diffraction, scanning electron microscopy, transmission electron microscopy coupled with selected area diffraction and energy-dispersive X-ray spectroscopy, contact angle measurements and *in vitro* biological analyses (MTT cell proliferation assay, GSH oxidative stress assay, optical and fluorescence microscopy). All results sustained the applicative potential of Ti-Zr alloy substrates covered with a thin vitroceramic layer for medical implant applications.

1. Introduction

Concerns for the restoration of damaged or lost parts of living bodies have always existed, the first attempt consisting in the reconstruction of lost teeth using gold dental prostheses [1]. Since then, the biomaterials science has continuously evolved to higher performances, newer techniques and a better understanding of the interactions that take place between the implantable materials and surrounding tissues [2–5]. Thus, the biocompatibility is the main requirement that a synthetic material must meet before being subjected to perform a specific function in a physiological media [6,7]. However, in recent years, the bioactivity property has gained ground due to the fact that the corresponding materials favour or even induce tissue regeneration and obviously accelerate the healing process [8,9].

Over time, metals such as Fe, Cr, Co, Ni, Ti, Ta, Nb, Mo and W were well and rapidly tolerated by the patients as inert materials for bone substitution, especially in dentistry and orthopaedics [10]. Furthermore, they exhibit desirable mechanical properties (excellent moldability, increased hardness, ductility and tenacity, great wear resistance etc.) [11,12], fact that makes them the most common materials for orthopaedic implants. Still, there are some problems regarding the maximum accepted concentration of some metallic elements, difference in stiffness against the living tissues or adverse reactions occurrence [13–15].

The metallic biomaterials are classified by their nature in three main groups: stainless steel, Co alloys and Ti with its alloys [16]. Ti stands out by having a high corrosion resistance due to the formation of a titanium oxide layer on its surface, which speeds up the process of bone-to-implant adhesion without unwanted side effects [17]. The disadvantages of Ti (low wear resistance, difficult manufacturing process etc.) have been solved through its alloys, that have found applications as dental pivots, orthopaedic screws, skull plates etc. [11,18,19]. In the particular case of Ti-Zr material, all studies have shown good osseointegration and high implant success rate [20,21].

The biocompatibility of metallic surfaces poses a major problem because of their possible corrosion in hostile environment, with undesirable effects, like material loss, strength weakening, damaging surrounding tissue, overall a toxic character. In order to overcome these impediments, the metallic implants can be coated with a bioactive layer [22–25], by different techniques: magnetron sputtering, laser ablation, electrophoretic deposition or chemical methods (impregnation, vapour deposition etc.) [22,26–30]. Thus, to avoid clinical problems (weak interface, corrosion, allergy, inflammation, infection, rejection etc.) and also to make the metallic implants more biocompatible, researchers and producers have coated pure Ti and its alloys with a bioactive layer, which can have different natures: glasses [31,32], ceramics [9,11,19,23,26,27,30] or glass-ceramics [22,33]. Moreover, apart from the surface layer composition, an important issue is

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http://dx.doi.org/10.1016/j.ceramint.2017.01.070
Received 21 November 2016; Received in revised form 2 January 2017; Accepted 13 January 2017
0272-8842/

represented by the morphological properties of the coating, which have a strong influence on the osseointegration process [19,24].

Concerning the film deposition route, the physical vapour deposition methods are simple, precise and versatile due to their potential to generate *2D* structures with controlled thickness and texture [22,32–34]. Indeed, the necessity of a material source requires either the purchasing of a commercial target or the fabrication of a stoechiometric and compact disc of the desired material; the last choice is more appropriate in the case of complex or exotic systems. Further, the variation of the processing parameters during the pulsed laser deposition experiment enables the adjusting of the morphological characteristics (average grain size, porosity, roughness etc).

In this paper, vitroceramic coatings were grown on Ti-Zr substrates in order to improve their biological behaviour and demonstrate the suitability of the coated structures for medical applications. The sol-gel derived targets were subjected to laser ablation in different experimental conditions, the resulting samples being investigated in terms of structure, morphology and biological effect. As far as we know, our work represents the first attempt to deposit a vitroceramic surface layer on Ti-Zr alloy with a with the aim of providing a milder joint between the implantable device and the neighbouring living cells.

2. Experimental

The targets were prepared in our laboratory, following a previously reported procedure [22]. Briefly, starting from tetraethyl orthosilicate (TEOS, $Si(OC_4H_9)_4$, 98%, Sigma-Aldrich), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, 99.8%, Sigma-Aldrich), triethyl phosphate (TEP, $PO(OC_2H_5)_3$, 99.8%, Sigma-Aldrich) and calcium fluoride (CaF_2, 99.9%, Sigma-Aldrich), two vitroceramic masses were synthesized by the sol-gel method, in SiO_2–CaO–P_2O_5–(CaF_2) systems (without CaF_2 – called A and with CaF_2 – called B). The calcined powders were pressed as large thin discs and sintered in order to achieve dense targets. Afterwards, the vitroceramic films were grown by pulsed laser deposition (PLD) on Ti-Zr alloy substrates, a material that appears to have superior strength, corrosion resistance and biocompatibility to commercially pure Ti [12,18,20,21].

The ablation experiments were performed at National Institute for Laser, Plasma and Radiation Physics, Magurele, Romania. A Nd:YAG laser which operates in pulses (5 ns pulse duration) and emits a radiation of 355 nm wavelength (UV domain) was employed. The laser parameters were adjusted to acquire energies around 80–82 mJ/pulse and fluences of the order of 1 J/cm^2. The substrates were placed at a distance of 40 mm from the target and heated during the deposition at 300 °C (samples called A1 and B1) or 400 °C (samples called A2 and B2) in order to increase the mobility of the constituents so that to restore the chemical bonds and rebuilt the crystal lattice of the original compound. The working medium was oxygen atmosphere, provided by an oxygen flux that was continuously purged in the deposition chamber to a pressure of 100 mTorr.

A Shimadzu XRD 6000 diffractometer with Ni filtered Cu Kα radiation (λ=1.5406 Å), 2θ was ranging between 10 and 80°, was used for the compositional and structural analyses by X-ray diffraction (XRD). The surface topography was visualized with a FEI Quanta Inspect F scanning electron microscope (SEM), while the detailed morphological investigation was performed with a Tecnai™ G2 F30 S-TWIN transmission electron microscope (TEM), equipped with selected area electron diffraction (SAED) and energy-dispersive X-ray spectroscopy (EDS). The biocompatibility was analysed on amniotic fluid-derived mesenchymal stem cell cultures, in accordance with the law in force and following standard procedures. The viability of the cells in the presence of the obtained supports was assessed by fluorescent microscopy, using Red CMTPX fluorophore; the images were taken with a Carl Zeiss digital camera. Furthermore, in order to evaluate the cell proliferation and cytotoxicity of the investigated samples, MTT biochemical assay (Vybrant® MTT Cell Proliferation Assay, Molecular Probes) was employed, for which the absorbance was read at 570 nm with a Tecan spectrophotometer. The cellular response to oxidative stress was estimated on the basis of GSH assay (GSH-Glo™ Glutathione Assay, Promega), the luminescence being recorded with a Titertek-Berthold luminometer.

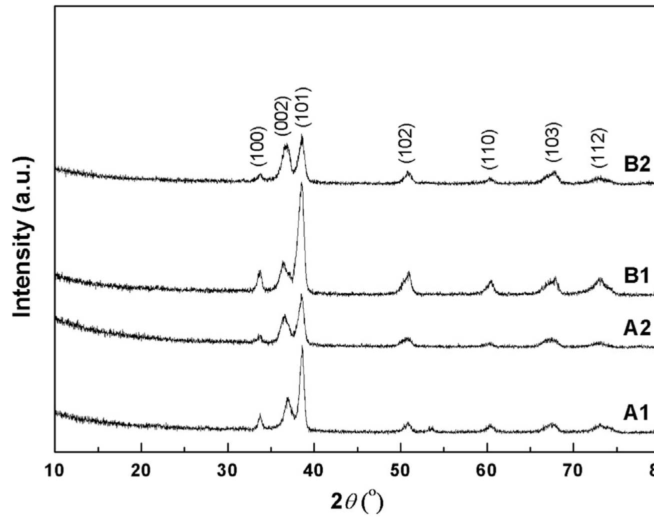

Fig. 1. XRD patterns of the vitroceramic coatings deposited by PLD from A and B targets, on Ti-Zr substrates.

3. Results and discussion

3.1. XRD investigation

In the XRD patterns of the vitroceramic layers deposited on Ti-Zr alloy plates (Fig. 1), specific maxima of $Ti_{0.5}Zr_{0.5}$ alloy (ICDD 04-003-1466) with hexagonal symmetry (*P*63/*mmc* space group), originated from the metallic substrate, occurred. It was expected to detect the presence of Ti_xO_y and Zr_xO_y compounds, formed due to oxidizing processes suffered by the substrate during the PLD deposition in oxygen atmosphere. Furthermore, no other crystalline phases were identified, which means that the modification of the laser parameters compared to our previous paper (the fluence was decrease from 3.7, 2.7 and 2.2 J/cm^2 to 1 J/cm^2) [22], had important repercussions on the material transfer from the target to the substrate. In the mentioned case, the composition of the target, consisting of calcium silicate and silicon dioxide crystallites embedded in a vitreous matrix, was partially maintained in the vitroceramic layers as wollastonite crystalline phase distributed in the parent glassy matrix. Also, the replacement of Ti plates with Ti-Zr alloy plates could have a certain impact on the thermodynamics of plasma condensation, as well as domains nucleation and growth. Analysing the intensity of the highest diffraction peak, it can be stated that the substrate signal is stronger in the case of the depositions made at lower temperature (300 °C), which means that these films shield to a lesser extent the information coming from the underlying support, probably due to a greater degree of disorder. However, it is possible to have the following situation: in the coating volume, one or more crystalline phases are distributed as nanometric regions and their concentration is placed below the detection limit of the equipment.

3.2. SEM investigation

Fig. 2 presents the SEM images of the neat metallic substrate and also of the four samples obtained after the ablation experiments. Thus, the alloy plates have a plain surface, with low roughness; the only noticeable defects consist in longitudinal hollows appearing as a consequence of the fabrication process (Fig. 2a). Considering the