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Pulsed laser deposited bioactive RKKP-Mn glass-ceramic coatings on titanium☆

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ABSTRACT

The study is aimed at the design of materials, *i.e.* glass-ceramics, containing trace elements with a specific role in the biological process of regenerating osteoporotic bones. Among these, manganese has a key function in bone osteogenesis and in the maintenance of bone mass. In this work, a 2 wt% of manganese containing RKKP glass-ceramic material has been prepared by sol-gel synthesis. Pulsed laser deposition technique has been applied to deposit films on titanium from the prepared glass-ceramic target. The films have been deposited at two different substrate temperatures (room (RT) and 500 °C) and investigated by X-ray diffraction, Raman spectroscopy, and SEM-EDX techniques. Both the films are characterized by a compact, crack-free and rough surface. The films deposited at 500 °C are composed of crystalline wollastonite and hydroxyapatite phases. For the assessment of films bioactivity *in vitro*, the standard Kokubo protocol for simulated body fluid (SBF) preparation has been followed. The films deposited at RT and 500 °C have been soaked in SBF for 3, 7, 14 and 28 days. For the film deposited at RT, apatite phase is registered after 7 days of soaking, whereas for the film deposited at 500 °C the apatite appeared only after 14 days. Moreover, the film deposited at RT induces a faster crystallization of the apatite layer.

1. Introduction

With an aging population, the incidence of fractures caused by osteoporosis is increasing [1]. As defined by the World Health Organization "*Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture*" [1,2]. This pathology prevails in postmenopausal women, but several studies demonstrated that men can suffer as well [3,4].

One of the most common fractures caused by osteoporosis is the hip fracture, which requires the substitution with a prosthesis. Certainly, this type of surgery in patients with osteoporosis can cause more complications that in healthy patients: the lack of calcium in the surrounding bone and its abnormal metabolism can slow down the healing process. Therefore, it is considered useful to include, in materials designed for regeneration of osteoporotic bone, trace elements with a specific role in the biological process upon implantation. Among these elements, calcium plays definitely the most important role in bone metabolism; nevertheless, the key function of manganese in the maintenance of bone mass has been demonstrated [5,6].

Mn is an important cofactor of several enzymes involved in extracellular matrix (ECM) remodelling and synthesis of proteoglycans, important constituents of skeletal and cartilage structural matrices [7,8]; therefore, its deficit retards osteogenesis. Low content of Mn in the body is connected with the increasing of the extracellular concentration of calcium and phosphates [4]. The research group of Landete-Castillejos individuated a strong connection between the dietary lack of Mn and the bone fragility. They studied the genesis of the brittleness of deer antlers (an excellent model for studying bone biology) after a very hard winter. They discovered that cause was Mn deficit, because frozen plants are usually characterized by an increasing in Si content, and as a consequence, a Mn content reduction [9]. After several studies, they indicated the loss of calcium as a consequence and not the cause of the osteoporosis [10]. The incorporation of Mn ions could be a powerful way to improve bone healing in terms of bioactivity, cell differentiation and mineralization rate [11]. Furthermore, Mn increases the activity of the integrins, a family of receptor that mediate the cellular interaction with the extracellular matrix [12], thus Mn incorporated in bioceramics can improve the cell adhesion.

☆ The authors declare no competing interests.

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Given the roles of manganese in the bone osteogenesis and osteoporosis, in this work a small percentage (2%) of manganese oxide has been added to the RKKP glass-ceramic composition. RKKP (stands for Ravaglioli, Krajewski, Kirsch and Piancastelli), developed at ISTEC_CNR (Faenza, Italy) [13], has been widely studied and in the last two decades is drawing the attention of many researchers [14–19]. In the literature [13,20,21], the RKKP is reported as a great potential glass-ceramics; its good performances of biological interaction, including high bioactivity and osteoconduction, have been largely proved; furthermore, *in vivo* studies revealed also its capability to bond both healthy and osteopenic bone [22]. The adding of manganese in such successful material should improve the capacity of RKKP to induce the bone regeneration and implant integration.

Among the metallic materials used in biomedical field, titanium (Ti) is one of the most widely employed in orthopaedics and dentistry due to its biocompatibility. In addition, for its excellent mechanical properties, Ti is widely used for hard tissue implants, in particular, for load-bearing applications. Moreover, in order to avoid the fibrous encapsulation of the implant, due to the bioinertness of the metal, it is possible to coat the surface with bioactive materials. For this purpose, a wide range of coating techniques has been exploited, such as plasma spraying [23], magnetron sputtering [24], electrophoretic deposition [25], and pulsed laser deposition (PLD) [26,27]. Among these techniques, PLD allows the congruent transfer of target composition to deposited film, high adhesive strength of film to substrate, the control of film's thickness (from tens of nm to tens of μm) [28], crystallinity and surface morphology by variation of the experimental parameters, such as laser pulse length and wavelength, deposition time and temperature [26,27].

In this work, the RKKP-Mn glass-ceramic material, obtained by sol-gel synthesis, was used to coat Ti substrates by PLD, in order to obtain an improved bioceramic coating for bone implant, adequate for the replacement of osteoporotic bone. In this preliminary work, structure and composition of RKKP-Mn bulk material have been characterized, then the physical-chemical properties and the *in vitro* bioactivity of the deposited films have been investigated.

2. Materials and methods

RKKP-Mn glass-ceramic material has been synthesized by sol-gel procedure. This synthesis has been performed according to the details given in [16]. The final glass-ceramics, obtained by the sol-gel, had the following composition [wt%] SiO_2 [43.29], P_2O_5 [11.00], CaO [31.02], Na_2O [4.49], MgO [2.76], CaF_2 [4.88], K_2O [0.19], La_2O_3 [0.50], Ta_2O_5 [0.99], MnO [0.89]. An aqueous solution of TEOS (tetraethyl orthosilicate), $(P(OEt)_3$, $P(C_2H5O)_3$, $(Ca(NO_3)_2{\cdot}4H_2O$, $NaNO_3$, $Mg(NO_3)_2{\cdot}6H_2O$, KNO_3, NH_4F, $La(NO_3)_3{\cdot}6H_2O$, $Ta(OC_2H_5)_5$ and $Mn(NO_3)_2{\cdot}4H_2O$ balanced in stoichiometric amounts, underwent hydrolysis and polycondensation to obtain the desired composition. To catalyse the TEOS and $P(OEt)_3$ hydrolysis, HNO_3 (0.1 M) has been utilised. All products were Sigma-Aldrich, > 99.9% purity and have been used as received. The synthesis has been carried out in a Teflon bottle. Reactants have been added to the mixture one by one, under vigorous stirring, every 30 min. The addition followed the order: TEOS, triethyl phosphite, calcium nitrate tetrahydrate, sodium nitrate, magnesium nitrate hexahydrate, ammonium fluoride together with manganese nitrate tetrahydrate, potassium nitrate, lanthanum nitrate hexahydrate and tantalum ethoxide. The sol has been left at room temperature for 10 days, and then placed in an oven a 70 °C for 72 h to obtain a gel. This gel has been dried at 120 °C for 48 h and then stabilized at 700 °C (heating rate 5 °C/min, cooling rate 20 °C/min) to obtain sol-gel granules. In order to obtain a compact pellet, the granules have been pressed at 400 MPa and sintered in air at a peak temperature of 1100 °C (heating rate 5 °C/min, cooling rate 20 °C/min).

The crystalline phases have been investigated by the X-ray diffraction (XRD) technique using a X-Perth-Pro Philips X-ray diffractometer in the following conditions: CuKα radiation (wavelength of 1.5406 Å)

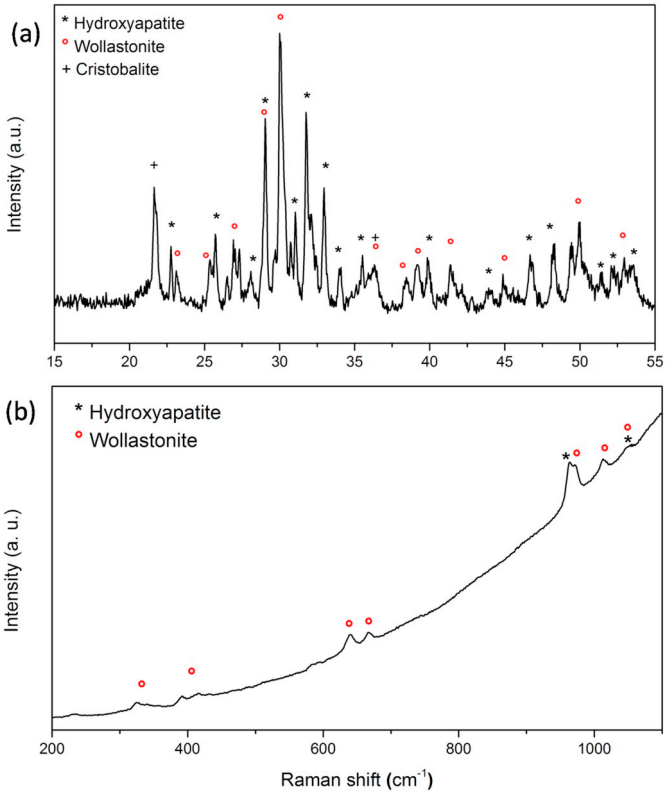

Fig. 1. XRD pattern (a) and Raman spectrum (b) of RKKP-Mn target material.

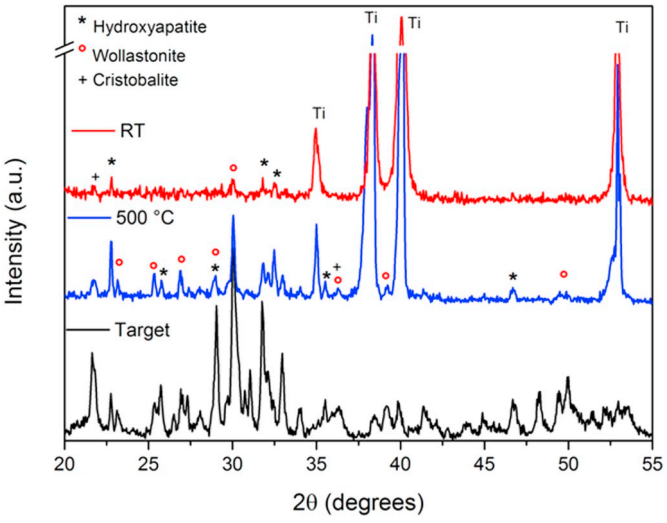

Fig. 2. XRD patterns of RKKP-Mn films deposited on Ti at RT and 500 °C, compared to the target one.

in a θ-2θ configuration, step size 0.020 θ, time per step 4 s. The material has been analysed by Micro-Raman spectroscopy, performed by the means of a Jobin-Yvon Horiba LabRam apparatus equipped with edge filter, which excludes Raman shift below 150 cm^{-1} from detection, a He–Ne laser (λ: 632.8 nm) and an Olympus microscope with 10×/50×/100× objectives. A spectral resolution of about 4 cm^{-1} has been obtained by a holographic grating with 600 lines/mm. Measurement times have been integrated in the range of 50–150 s.

For the PLD experiments, a doubled Nd:YAG laser source (λ = 532 nm, τ = 10 ns, repetition rate = 10 Hz) has been used. The depositions have been conducted at 12 J cm^{-2} fluence, optimized in our previous work, for the pure RKKP target [16]. The films deposition time has been of 4 h; substrate has been positioned at 2 cm from the