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Structural modification of titanium surface by octacalcium phosphate via Pulsed Laser Deposition and chemical treatment

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ABSTRACT

In the present study, the Pulsed Laser Deposition (PLD) technique was applied to coat titanium for orthopaedic and dental implant applications. Calcium carbonate (CC) was used as starting coating material. The deposited CC films were transformed into octacalcium phosphate (OCP) by chemical treatments. The results of X-ray diffraction (XRD), Raman, Fourier Transform Infrared Spectroscopy (FTIR) and scanning electron microscopy (SEM) studies revealed that the final OCP thin films are formed on the titanium surface. Human myofibroblasts from peripheral vessels and the primary bone marrow mesenchymal stromal cells (BMMSs) were cultured on the investigated materials. It was shown that all the investigated samples had no short-term toxic effects on cells. The rate of division of myofibroblast cells growing on the surface and saturated BMMSs concentration for the OCP coating were about two times faster than of cells growing on the CC films.

1. Introduction

Titanium (Ti) implants are widely used for load bearing implants and inner fixation devices due to their relevant mechanical properties. Their integration with bone tissue depends on the physical-chemistry characteristics of the implant/tissue interface. Calcium phosphate coatings have been extensively applied in order to enhance the ability of the biomaterials to create bonds with the living host tissue [1]. Among calcium phosphate materials the better control of the integration process with bone tissue was provided by the hydroxyapatite (HA) coatings [2–4]. HA coatings have been extensively used in order to enhance the biological behaviour of implants and a number of deposition techniques have been developed and applied for this purpose during last two decades. They are utilized in most commercially available coated Ti implants. However, the deposition of dense, stoichiometric, and crystallised HA coating layers is frequently ineffective [5]. They have some disadvantages such as poor interfacial adhesion between coated Ti implants and host tissue and dramatic late implant failure [6].

During the last several years, growing attention has been brought to octacalcium phosphate (OCP) related to calcium phosphate materials [7]. OCP have been proposed as a possible precursor of the tooth enamel, dentine and bones in living organisms [8]. It has been reported that OCP pressed powders had high osteoconductive property when implanted in the sub-periosteal region of mouse calvaria [8,9]. Further, it has been demonstrated that OCP-based materials could stimulate osteoblastic cell differentiation *in vitro* [10,11] and had possibly osteotransductive properties, i.e. the capacity to provide new bone formation *in vivo* [7]. Therefore,

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the use of OCP material for coatings could provide a sufficient potential to enhance the biological behaviour of Ti implants.

Only recently, a number of techniques for depositing OCP coatings on implants have been proposed for implant biofunctionalization [1,12–17]. It is well known that OCP is quite difficult to deposit by means of direct physical methods because its thermal decomposition takes place at low temperature. Most of the OCP coatings were deposited from supersaturated solutions and had a very low adhesion to the substrates [13–15]. Several attempts have been performed to deposit OCP on Ti substrate by Pulsed Laser Deposition (PLD) [16,17] and Matrix Assisted Pulsed Laser Evaporation techniques [1,12]. According to the X-ray diffraction (XRD) analysis, the coatings displayed an amorphous-poorly crystalline apatite structure.

The main concept of the present study is based on PLD combined with the biomimetic approach, avoiding a direct OCP deposition. We performed first the PLD coating of Ti surface by calcium carbonate (CC), followed by the chemical treatment of the coated surface in solutions. CC has been suggested as possible precursor for biomaterials [18]. In the field of biomineralization of CC, it provides a system to allow nucleation and growth of inorganic minerals including calcium phosphates at physiological conditions. In the present manuscript the formation of OCP on CC film was invented. Structural and morphological properties of the obtained films were investigated by XRD, Raman measurements and scanning electron microscopy (SEM). In addition, adhesion and *in vitro* tests were carried out. This work is the successful attempt to produce OCP coatings for biomedical applications, applying a physical deposition method – PLD to deposit CC film with following chemical treatment to obtain OCP coating.

2. Experimental section

2.1. Target preparation

For synthesis of starting CC powder, 30 g of CaO (Cat. No: 1305–78–8, Sigma-Aldrich), 62 g of $(NH_4)_2CO_3$ (Cat. No: 506–87–6, Sigma-Aldrich), and 300 mL of distilled water were added to a vessel for grinding, carried out for 30 min at room temperature. After filtration, powders were washed and dried at 80 °C for 24 h. CC pellet-target (diameter of 1 cm and thickness of 0,5 cm) was prepared by uniaxial pressing at 100 MPa.

2.2. Pulsed Laser Deposition

Thin films were deposited onto titanium substrates by PLD technique, using a Nd:YAG laser source (Handy YAG-Quanta System, $\lambda = 532$ nm, $\tau = 7$ ns, 10 Hz). The laser beam, oriented with an inclination of 45° with respect to the target surface, is focused by a lens system. The pure Ti substrates (1×1 cm^2 squares of 3 mm of thickness) were sandblasted with a 60-grid SiC abrasive powder. Before depositions, the substrates were boiled in aqua regia for 30 min, in order to remove any contaminant from the surface. During depositions, the Ti substrates were kept at room temperature. The CC targets were supported onto a rotating holder, in order to minimize laser induced craterization effect. The target-substrate distance was kept at 2 cm, for a deposition time of 5 h. The laser fluence was fixed at 30 J/cm^2.

2.3. Chemical treatment: preparation of octacalcium phosphate coatings

In order to transform the CC coatings into OCP, the procedure described elsewhere has been followed [7]. Briefly, an aqueous solution was prepared by dissolving of 115 g of $NH_4H_2PO_4$ (Cat. No: 7722–76–1, Sigma-Aldrich) in 500 mL of distilled water at room temperature, pH 4.1 ± 0.1. The CC coated Ti substrates were placed into the solution and were shaken in a sealed glass vessel for 168 h at 40 °C. After that, the coated Ti substrates were thoroughly washed in distilled water at least 5 times and dried overnight at 37 °C. Then, the obtained samples were placed in a second solution. The second solution was prepared by dissolving 95.2 g of CH_3COONa (Cat. No: 127–09–3, Sigma-Aldrich) in 700 mL of distilled water at 40 °C and pH 8.2 ± 0.2. The so-obtained samples were again shaken in a sealed glass vessel for 168 h at 40 °C. Finally, they were thoroughly washed in distilled water at least 5 times and dried overnight at 37 °C.

2.4. Characterization of octacalcium phosphate coating

Phase composition was analyzed by conventional XRD technique (X'Pert Pro MPD diffractometer, PANalytical, Netherlands). The XRD pattern has been obtained using the CuKα radiation ($\lambda = 1.54184$ Å) and performing the scan in 2 theta(θ) mode, keeping the incidence angle at 1.5°. A 0.03125° divergence slit on the incident beam path has been used and the 2θ scan step size was 0.02°. The phase analysis of the obtained pattern has been performed using the PANalytical High Score Plus software package. Micro-Raman measurements were carried out in backscattering configuration by a HORIBA LabRam 800 HR apparatus (HORIBA Scientific, Japan) equipped with an edge filter, two gratings (600 lines/mm and 1800 lines/mm). Excitation was performed with 632.8 nm radiation form a He-Ne laser source. The laser spot size impinging on the samples surface was about 5 μm in diameter when the 100x microscope objective was used. The spectrometer is connected to a Peltier cooled CCD detector. A spectral resolution of about 4 cm^{-1} was obtained by the holographic grating with 600 lines/mm. Fourier Transform Infrared Spectroscopy (FTIR) investigation was carried out using an IR microscope (Nicolet Avatar 330 FTIR spectrometer, England) in transmission mode. The microscope provides a control stage movement, aperture setting and focusing directly from the PC screen. Before FTIR analyses coatings was removed from titanium surface and mixed 1 mg of sample with 300 mg of KBr powder, followed by compacting those into a thin pellet in a stainless steel die of 1 cm inner diameter. FTIR data were recorded over the range of 4000 to 400 cm^{-1} with 128 scans. The morphology of samples was investigated using a Carl Zeiss NVision 40 high resolution Scanning Electron Microscope (SEM), equipped with an Oxford Instruments X-Max energy dispersive detector (80 mm^2) (Germany). The images were obtained at 1 kV acceleration voltage (SE2, magnifications up to ×100 k). The samples were analyzed by SEM without deposition of a conductive surface layer. Finally, the adhesive bonding strength of the obtained coatings was determined according to the ASTM C633-79 standard. A universal testing system, Instron 4204 (UK), was used for the adhesive strength measurement with a tensile speed of 1 mm/min. Five samples were tested to get an average value.

2.5. Cell culture

Prior to *in vitro* test, the samples were sterilized by heating at 120 °C for 2 h [7]. Afterwards, they were placed in the wells of 12 well plates (Greiner, Germany), one sample in the hole. After that, the wells were filled with culture medium Dulbecco''s modified Eagle's medium (DMEM) (Sigma-Aldrich, USA) plus 10% fetal calf serum (Gibco, USA). Gentamicin sulphate, up to a final concentration of 0.7 mM (Mosagrogen, Russia), was introduced into the culture medium, in order to assure the coefficient of molar absorption of $\varepsilon_{256} = 530$ l/mol. Then, the cells seeding on the material sample surface was performed at a concentration of 5×10^3 cells/