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Biomaterials 26 (2005) 2381–2389
Biomaterials
www.elsevier.com/locate/biomaterials

Human osteoblast response to pulsed laser deposited calcium phosphate coatings

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Received 18 May 2004; accepted 23 July 2004
Available online 11 September 2004

Abstract

Octacalcium phosphate (OCP) and Mn^{2+}-doped carbonate hydroxyapatite (Mn-CHA) thin films were deposited on pure, highly polished and chemically etched Ti substrates with pulsed laser deposition. The coatings exhibit different composition, crystallinity and morphology that might affect their osteoconductivity. Human osteoblasts were cultured on the surfaces of OCP and Mn-CHA thin films, and the cell attachment, proliferation and differentiation were evaluated up to 21 days. The cells showed a normal morphology and a very good rate of proliferation and viability in every experimental time. Alkaline phosphatase activity was always higher than the control and Ti groups. From days 7 to 21 collagen type I production was higher in comparison with control and Ti groups. The level of transforming growth factor beta 1 (TGF-β1) was lower at 3 and 7 days, but reached the highest values during following experimental times (14 and 21 days). Our data demonstrate that both calcium phosphate coatings favour osteoblasts proliferation, activation of their metabolism and differentiation.

Keywords: Calcium phosphate coating; Octacalcium phosphate; Mn^{2+}-doped carbonated hydroxyapatite; Laser ablation; Human osteoblasts; Differentiation

1. Introduction

One of the most important and successful biomedical applications of calcium phosphates is in coatings of endosseous implants [1]. Titanium and its alloys are the most widely used materials for the production of metal implants, due to their excellent mechanical and biomedical properties [2]. The deposition of calcium phosphate coatings on these materials is aimed to enhance the bioactivity of the surface, which improves fixation between hard tissue and metal implant, and stimulates bone apposition [1,3].

Pulsed laser deposition (PLD) technique was recently extended to deposit calcium phosphate coatings on Ti substrates [4–7]. PLD utilizes a short, generally UV pulsed laser beam, which is focused onto a rotating target inside a vacuum chamber, where a controlled atmosphere can be introduced. The species that are expulsed by each subsequent laser pulse conform the coating as they reach the substrate, which can also be heated to a fixed temperature. The stoichiometry and the crystallinity of the deposited material can be selected by a proper choice of the deposition parameters. A further advantage of PLD with respect to other physical

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doi:10.1016/j.biomaterials.2004.07.057

techniques is that the pollution of the deposited material is greatly reduced due to the use of the laser light [8–10]. By optimisation of the deposition conditions, we were able to obtain thin films of nanocrystalline octacalcium phosphate (OCP) [11], and thin films of Mn^{2+}-doped carbonate hydroxyapatite (Mn-CHA) [12] on pure Ti substrates. The choice of these two phosphates was dictated by biomimetic strategies. OCP is a basic calcium phosphate with a close resemblance to the more stable hydroxyapatite. From a structural point of view, OCP is described as a "hydrated HA". It easily hydrolyses to HA, and it is considered as the most likely precursor of biological apatites [13–15]. Accordingly, OCP coatings and even more nanocrystalline OCP coatings should dissolve relatively quickly, and stimulate fast bone new growth. At variance, Mn-CHA should dissolve more slowly, but it has the advantage of a composition more similar to that of bone carbonated apatite. In addition, the presence of Mn^{2+} ions increases the ligand-binding affinity of integrins through conformational changes. The integrins are a quite large family of receptors, which mediate cellular interactions with extra-cellular matrix, and activate cell adhesion [16].

One essential step to the understanding of the biological events occurring at the bone tissue/material interface is the biological investigation by in vitro tests. Cell lines are commonly used for biocompatibility tests (cytotoxicity), and are very efficient because of their reproducibility and culture facility. Even if the use of sarcoma and transformed osteoblasts in in vitro biocompatibility is widely accepted [17–19], we note that with respect to osteoconductive and inductive properties of biomaterials, the use of freshly obtained osteoblasts is mandatory. Primary cultures of osteoblasts are able to expand in vitro, maintain their capacity to differentiate and to synthesize an extracellular matrix. Primary cultures of osteoblasts were previously used for biological investigation of pulsed laser-deposited HA, α- and β-tricalcium phosphate, and amorphous calcium phosphate [20–22]. In this paper, we report the results of a study on the response of primary cultures of human osteoblast cells to OCP and Mn-CHA coatings deposited by PLD on Ti substrates.

2. Materials and methods

2.1. Films deposition

The PLD were performed using a UV KrF* excimer laser source generating pulses of $\tau_{FWHM} \leqslant 30$ ns at $\lambda = 248$ nm. The frequency repetition rate was of 2 Hz. The experiments were carried out in a stainless steel enclosure. It was evacuated down to a residual pressure of 10^{-4} Pa prior to every deposition. To avoid drilling during the multipulse irradiation, the targets were rotated with a frequency of 0.4 Hz. The disk-shaped ($\varnothing$ 15 mm) Ti substrates were mechanically polished with a roughness of less than 2 μm and subsequently submitted to acid etching to get an extended active surface [23]. They were placed parallel to the target at a separation distance of 4 cm and heated during the thin-film deposition at a fixed temperature. The targets were prepared from OCP and Mn-CHA (HA containing 0.55% Mn^{2+} and 5% carbonate) powders, synthesized in aqueous medium as reported previously [24,25]. OCP thin-film deposition was carried out in the presence of water vapours, in the range of 35–80 Pa, on Ti substrates heated at 150 °C [11]. Mn-CHA deposition was performed under dynamic oxygen pressure of 10 Pa on Ti substrates heated at 400 °C [12]. The number of applied laser pulses in all cases was 15,000. To promote the crystallization further, each structure was heat post-treated for 6 h in a flux of hot water vapours at the same temperature as applied during deposition.The thickness of the coatings was about 800 nm for Mn-CHA, and about 1 μm for OCP.

2.2. Physico-chemical characterization

Thin-film XRD measurements were performed on the coatings employing an X'Pert Philips Diffractometer using CuKα radiation and a grazing angle of 0.3–1.0°. The 2θ angles ranged from 3° to 60° with a 0.005°/s scanning speed.

Morphological investigations of synthesized products were performed using a Philips XL-20 Scanning Electron Microscope. The samples were sputter coated with gold before examination. EDS analysis was performed on specimens coated with carbon.

2.3. Isolation of osteoblasts

Human primary osteoblasts (hOBs) were prepared using a described protocol approved by Ethics Committee (University Hospital, Strasbourg). An informed consent was obtained from patients after the nature of the study had been fully explained. Human osteoblasts were prepared from bone explant obtained during implant placement. The bone explant was collected in a complete Dulbecco's modified Eagles medium (DMEM) containing 3.7 mg/ml $NaHCO_3$ (Life Technologies, Grand Island, NY, USA) supplemented with antibiotics (penicillin, 100 Units/ml; streptomycin, 100 μg/ml) and washed twice with phosphate buffer saline (PBS) supplemented with both antibiotics. The bone explant was treated for 2 h at 37 °C with 50 μg/ml of collagenase I (Sigma, Saint-Louis, MO, USA) in 1 ml of PBS, washed twice with PBS and placed for two weeks on a plain polystyrene Petri dish containing 0.5 ml of DMEM medium supplemented with 10% foetal calf