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ScienceDirect
Acta Biomaterialia 4 (2008) 1885–1893
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Strontium-substituted hydroxyapatite coatings synthesized by pulsed-laser deposition: In vitro osteoblast and osteoclast response

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Received 14 December 2007; received in revised form 30 April 2008; accepted 9 May 2008
Available online 23 May 2008

Abstract

The increasing interest in strontium incorporation into biomaterials for hard tissue repair is justified by the growing evidence of its beneficial effect on bone. We successfully synthesized hydroxyapatite (HA) thin films with different extents of strontium substitution for calcium (0, 1, 3 or 7 at.%) by pulsed-laser deposition. The coatings displayed a granular surface and a good degree of crystallinity, which slightly diminished as strontium content increased. Osteoblast-like MG63 cells and human osteoclasts were cultured on the thin films up to 21 days. MG63 cells grown on the strontium-doped HA coatings displayed normal morphology, good proliferation and increased values of the differentiation parameters, whereas the number of osteoclasts was negatively influenced by the presence of strontium. The positive effect of the ion on bone cells was particularly evident in the case of coatings deposited from HA at relatively high strontium contents (3–7%), where significantly increased values of alkaline phosphatase activity, osteocalcin, type I collagen and osteoprotegerin/TNF-related activation-induced cytokine receptor ratio, and considerably reduced values of osteoclast proliferation, were observed.

Keywords: Hydroxyapatite coating; Strontium; Laser ablation; Osteoblast; Osteoclast

1. Introduction

Synthetic hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA) is widely applied as a biomaterial, thanks to its similarity to the inorganic phase of bone. The surgical treatment of bone defects and coating of metallic implants are among its chief biomedical applications. Metallic implants, such as those necessary in total hip-joint replacements and artificial tooth sockets, meet mechanical stability requirements, but do not form mechanically stable bonds to bone tissue. The high incidence of bone implant failures is mainly blamed on incomplete osteointegration and stress shielding due to significant differences in mechanical properties between the implant and surrounding bone [1,2]. Improved implant fixation to hard tissues can be achieved by coating the metallic surface with a thin film of calcium phosphates [3–5]. The different methods that have been employed to modify metallic surfaces include plasma-spraying, magnetron sputtering, ion-beam coating, electrophoretic deposition, anode oxidation, anodic spark deposition, pulsed-laser deposition (PLD) and biomimetic deposition from supersaturated solutions [5–10]. PLD has proved to be a successful technique for growing thin calcium phosphate structures on metallic substrates. PLD uses short, mostly UV, pulsed-laser beams to expel the species and then focus them onto a target that rotates under a controlled atmosphere in a reaction chamber. The stoichiometry and crystallinity of the deposited material that forms the coating can be selected by a proper choice of the ablation and deposition parameters [11–14]. The development of the drug strontium rane-

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doi:10.1016/j.actbio.2008.05.005

late, which has recently been shown to reduce the incidence of fractures in osteoporotic patients [15,16], is the main reason for the growing interest in strontium-enriched calcium phosphate bioceramics and bone cements [17,18]. Strontium is present in the mineral phase of bone, especially in the regions of high metabolic turnover [19]. In vitro, it increases the number of osteoblasts and reduces the number and activity of osteoclasts [20,21]; in vivo, it inhibits bone resorption and improves bone formation [22–24]. Strontium can replace calcium in the HA structure for the whole range of compositions, inducing a linear variation in the lattice constants [25,26]. The aim of this study was to deposit thin films of strontium-substituted HA on titanium substrates and investigate the influence of strontium ions on the biocompatibility and bioactivity of the coating. To this end, we used PLD to coat the metallic substrates with thin films of HA synthesized in the presence of different strontium concentrations up to 10 at.%. Osteoblast-like cells MG63 and human osteoclasts were cultured on the deposits.

2. Materials and methods

2.1. Synthesis and characterization of HA and SrHA nanocrystals

HA nanocrystals were grown as previously reported [26], in N_2 atmosphere by dropwise addition of $(NH_4)_2HPO_4$ to $Ca(NO_3)_2 \cdot 4H_2O$ solution of pH adjusted to 10 with NH_4OH. Sr-substituted HAs were synthesized by using the appropriate amounts of $Ca(NO_3)_2 \cdot 4H_2O$ and $Sr(NO_3)_2$ to prepare the nitrate solution. Different compounds were prepared from solutions containing 0, 1, 5 and 10 at.% Sr ($[Sr/(Ca + Sr)] \cdot 100$) and were labelled HA, Sr1, Sr5 and Sr10, respectively.

Powder X-ray diffraction (XRD) patterns were recorded using an X'Pert Philips diffractometer at a scanning speed of $0.5° \text{ min}^{-1}$. Cu Kα radiation ($\lambda = 0.154$ nm, 40 mA, 40 kV) was used. The powder XRD pattern of the solid products confirmed that the compounds grown in the presence of different strontium concentrations were constituted of HA as the sole crystalline phase [26].

Calcium and strontium contents were determined using a GBC 901 atomic absorption spectrophotometer ($\lambda(Ca) = 422.7$ nm; $\lambda(Sr) = 460.7$ nm). The results confirmed that Sr incorporation into HA nanocrystals increased on increasing ion concentration in the solution [26]. The data indicated that the Sr content was 0.5, 3.0 and 7.0 at.% for Sr1, Sr5 and Sr10, respectively, as reported in Table 1.

Disk-shaped targets (diameter = 13 mm, thickness ⩾ 1 mm) were made for PLD by pressing HA, Sr1, Sr5 and Sr10 powders at 3 MPa and sintering at 380 °C for 6 h.

2.2. Synthesis and characterization of HA and SrHA coatings

Disk-shaped (diameter = 15 mm, thickness = 0.5 mm) Ti substrates were mechanically polished and subsequently submitted to acid etching to obtain an extended active surface [27]. The films were pulsed-laser deposited on etched Ti substrates using an UV KrF^* excimer laser source ($\lambda = 248$ nm, $\tau \sim 7.4$ ns). Prior to each deposition, the reaction chamber was evacuated down to a residual pressure of 10^{-4} Pa. Films were deposited in 50 Pa water vapor flux on substrates heated to 400 °C. The substrates were placed parallel to the targets, 4 cm away from them. Fluence was set at 2.4 J cm^{-2}, and 25,000 subsequent laser pulses were applied at a frequency repetition rate of 2 Hz for the deposition of each film. The as-deposited samples were submitted to annealing treatments in water vapor and ambient pressure for 6 h at the same temperatures as those applied during deposition. The average thickness of the obtained structures, as measured by profilometry, was ~1 μm.

Table 1
Strontium contents of solid crystalline powders synthesized on varying strontium concentration in solution

Sr at.% in solution	Sr at.% in the solid product	Label of the solid product	Label of the coating
0	0	HA	TiHA
1	0.5	Sr1	TiSr1
5	3.0	Sr5	TiSr5
10	7.0	Sr10	TiSr10

The labels used for both the crystalline powder and the related coating are reported.

Grazing-incidence XRD measurements were performed on the coatings with an X'Pert Philips diffractometer using Cu Kα radiation and a grazing angle of 0.3–1.0°. The 2θ angles ranged from 10° to 40° with a scanning speed of $0.005° \text{ s}^{-1}$.

The morphology of the synthesized products was investigated by scanning electron microscopy (SEM) using a Philips XL-20 microscope. The samples were sputter coated with gold before examination. Energy dispersive X-ray spectrometry (EDS) analyses were also performed on uncoated specimens.

Cell experiments were carried out on coatings deposited on Ti substrates and sterilized by γ-rays (^{60}Co) at a dose of 25 kGy.

2.3. Osteoblast culture

MG63 human osteoblast-like cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma, UK), supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U ml^{-1} penicillin, 100 mg ml^{-1} streptomycin). The cells were detached from culture flasks by trypsinization, and centrifuged. Their number and viability were determined by the trypan blue dye exclusion test.

MG63 osteoblast-like cells were plated at a density of 2×10^4 cells ml^{-1} in 24-well plates containing sterile samples of HA-coated titanium (TiHA), as reference, and samples of HA-coated Ti that had been prepared with different strontium concentrations (TiSr1, TiSr5, TiSr10). The same