Colloids and Surfaces B: Biointerfaces 135 (2015) 386–393

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Nano-hydroxyapatite-coated metal-ceramic composite of iron-tricalcium phosphate: Improving the surface wettability, adhesion and proliferation of mesenchymal stem cells in vitro

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 19 July 2015
Accepted 21 July 2015
Available online 26 July 2015

Keywords:
Bioceramic composite
Bioresorbable alloy
Hydroxyapatite coating
RF magnetron sputtering
Cell adhesion

ABSTRACT

Thin radio-frequency magnetron sputter deposited nano-hydroxyapatite (HA) films were prepared on the surface of a Fe-tricalcium phosphate (Fe-TCP) bioceramic composite, which was obtained using a conventional powder injection moulding technique. The obtained nano-hydroxyapatite coated Fe-TCP biocomposites (nano-HA-Fe-TCP) were studied with respect to their chemical and phase composition, surface morphology, water contact angle, surface free energy and hysteresis. The deposition process resulted in a homogeneous, single-phase HA coating. The ability of the surface to support adhesion and the proliferation of human mesenchymal stem cells (hMSCs) was studied using biological short-term tests in vitro. The surface of the uncoated Fe-TCP bioceramic composite showed an initial cell attachment after 24 h of seeding, but adhesion, proliferation and growth did not persist during 14 days of culture. However, the HA-Fe-TCP surfaces allowed cell adhesion, and proliferation during 14 days. The deposition of the nano-HA films on the Fe-TCP surface resulted in higher surface energy, improved hydrophilicity and biocompatibility compared with the surface of the uncoated Fe-TCP. Furthermore, it is suggested that an increase in the polar component of the surface energy was responsible for the enhanced cell adhesion and proliferation in the case of the nano-HA-Fe-TCP biocomposites.

1. Introduction

A principal requirement for the biodegradable material used as bone implant material in trauma surgery and orthopaedics includes a degradation rate that matches new bone growth rates. The implants also must be mechanically stable during the entire healing process [1]. In addition to magnesium, iron is a metal used for implant materials, and it is degradable in physiological fluids. Furthermore, iron is an essential element for metabolic processes such as oxygen transport and has advantageous mechanical properties [2].

An approach to create a degradable bone substitute material for load-bearing areas combining pure iron and a fast-degrading but brittle β-tricalcium phosphate (TCP) was proposed [2]. It was reported that the addition of pure iron to β-TCP is a promising approach because of its increased strength. The powder injection moulding process is cost-effective and enables the production of complex structures. It also allows for selective control of the material properties such as density and porosity, which are crucial when developing materials with high mechanical strength. Previous research found that iron samples with 40% β-TCP lost <1% (NaCl) and 9% (PBS) of their compressive yield strength following immersion, and the addition of β-TCP led to an increase in

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http://dx.doi.org/10.1016/j.colsurfb.2015.07.057
0927-7765/

degradation; however, the bioactivity of this composite remains a challenge for biomedical applications [2]. Therefore, the objective of this study was to prepare a biocompatible HA coating to enhance the bioactivity of bare Fe-TCP bioceramic composites.

With the development of modification technologies for biomaterials surfaces, the field of the bioinert or bioactive coating fabrication has been broadened, from wet-chemical to physical deposition [1,3–5]. To date, HA coatings have been widely studied and have attracted a broad range of scientists. Different physical and chemical deposition techniques have been developed [5–7]. Recent progress in RF magnetron sputtering of biocompatible HA films was discussed in a previous study [5], and the significance of calcium-phosphate (CaP) coatings on the enhancement of new bone osteogenesis was also examined [1]. Our previous results showed that the deposition of the 700 nm-thick RF magnetron sputter deposited HA film on a Ti substrate enhanced the mechanical properties of the biocomposite [6]. A thin CaP-based coating enabled a significant reduction in the release of toxic nickel from the NiTi substrates [8]. Low-crystallinity HA coating with different thicknesses stimulated cells to attach, proliferate and form mineralized nodules on the surface better than uncoated titanium substrates [9]. Therefore, it was hypothesized that thin HA films fabricated by RF magnetron sputtering, while covering the surface of the composites and maintaining open pores and rough microstructure, could also enhance the biological properties of the Fe-TCP composite.

It is known that the surface characteristics, including the morphology, surface chemistry, functional groups and wettability of biomaterials, affect cell attachment and adhesion [1]. The surface adhesion of cells is a complex process involving initial cell attachment and spreading, focal adhesion formation and cytoskeleton organization. Cells may encounter surfaces with different physical and chemical properties that profoundly influence their behaviour. The investigation of the surface-cell interaction is a key step for the further improvement of biomaterials and their applications.

The deposition of the HA films on Fe-TCP bioceramic composites has not yet been reported in the literature. This study examined the influence of the chemical and physical properties of the novel HA-coated Fe-TCP composite on the short-term cell adhesion, proliferation and growth. The biological effects of the coated surfaces are highlighted.

2. Materials and methods

2.1. Injection moulding process to fabricate Fe-TCP composites

For the injection moulding process, iron (Diafe2000, $d(50) = 1.29\,\mu m$, $\rho = 7.87\,g\,cm^{-3}$, Dr. Fritsch, Germany) and β-TCP (318S, $d(50) = 5.69\,\mu m$, $\rho = 3.07\,g\,cm^{-3}$, Plasma Biotal, UK) powders were mixed with a polymer-wax-based binder system to obtain a mouldable feedstock. The final step in the injection moulding process was to sinter the unbound samples under an argon atmosphere (Ar) in a MIM3001 furnace (Elnik Systems, USA) at temperatures of up to 1270 °C. Details of the powder injection moulding and the Fe-TCP bioceramic composites fabrication process are reported elsewhere [2].

2.2. Coatings fabrication and characterization

A pure HA target was prepared according to the procedures previously described [10,11]. A commercially available apparatus with an RF (13.56 MHz, COMDEL) magnetron source was used to deposit the HA coatings. The target-to-substrate distance was 40 mm. The coating was fabricated at an RF power level of 500 W in an argon atmosphere (0.4 Pa) for 8 h onto a substrate mounted on a grounded substrate holder, which resulted in the coating thickness 650 ± 50 nm. The surface morphology and composition of the deposited coatings were investigated using an environmental scanning electron microscope (ESEM Quanta 400 FEG from FEI) equipped with energy-dispersive X-ray analysis (EDX, Genesis 4000, S-UTW-Si(Li) detector). Before examination by SEM, the samples with the nonconductive CaP film were sputter-coated with a gold/palladium layer. The coating composition was determined with the Genesis 4000 software, and then the Ca/P atomic ratio was measured. The EDX-spectra were collected under high vacuum for 60 s with a dead time of 30% and electron energy of 15 keV. To determine the phase composition of the studied thin films, a Panalytical Empyrean instrument with Cu Kα radiation (1.54 Å; 40 kV and 40 mA) was used. The coated samples were investigated using grazing incidence X-ray diffraction (GIXD) with an incident beam angle of $\Phi = 1.0°$ (with respect to the sample surface), at 2θ varying over a range from 5 to 90° with a step size of 0.05°. To calculate the average crystallite size and the lattice parameters, Rietveld refinement (using the Le Bail method) with the TOPAS 4.2 program package from Bruker was performed. For each Rietveld refinement, the instrumental correction as determined with a LaB6 standard powder sample from the National Institute of Standards and Technology (NIST) as the standard reference material (SRM 660b). The ICDD database: #9-0432 was used for the HA reference pattern. The phases assigned to the substrate were indexed as α-Fe (ICDD card no. 98-000-0064) and β-TCP (ICDD card no. 9-169). Optical ellipsometry (Ellipse 1891-S AG, Institute of Semiconductor Physics, RAS, Siberian Branch) was used to determine the thickness of the deposited coatings. Ellipsometric measurements were performed at an incidence angle of 70° and over the wavelength region of 250–1000 nm with a spectral resolution of 2 nm. The coating thickness was derived from the changes in ellipsometric parameters between the bare and coated substrates with a three-phase model (substrate-layer-air). Single-crystal (1 0 0) silicon wafers were used as substrates for the thickness measurements. The molecular composition, including the presence of O—H and P—O groups, was analyzed using FTIR-spectroscopy (Bruker Vertex 70 FTIR spectrometer) at the Analytical Centre of National Research Tomsk Polytechnic University. The coatings were deposited onto IR-transparent KBr single crystals, and the measurements were performed over the wavelength range from 400 to 4000 cm^{-1}. Contact angle analyses were performed with an optical contact angle apparatus (OCA 15 Plus Data Physics Instruments GmbH, Germany) using the SCA20 software (Data Physics Instruments GmbH, Germany). The contact angle (CA) of water in air was measured using a sessile drop method. A minimum of 10 droplets (2 μl, 5 $\mu l\,s^{-1}$) of water and three droplets of diiodmethane or ethylene glycol were examined for each sample, and the resulting mean CA values were then used for the calculations. The surface free energy σ was calculated using the Owens–Wendt–Rabel–Kaelble (ORWK) method. Three different media (water, diiodmethane and ethylene glycol) were used for these calculations.

2.3. Sterilization

For the biological analyses, samples were placed in 6-well tissue culture plates for gamma sterilization (25 kGy).

2.4. Isolation, culture and characterization of human mesenchymal stem cells (hMSCs)

Bone marrow aspirates from healthy donors (two 24 and 25 years old males and one 45 years old female) were purchased from Lonza (Walkersville, USA), and the hMSCs were isolated by density centrifugation and plastic adherence according to [12]. Cell culture was conducted in a mesenchymal stem cell medium (MSCGM™