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The formation of calcium phosphate coatings by pulse laser deposition on the surface of polymeric ferroelectric

E.N. Bolbasov[a], I.N. Lapin[b], V.A. Svetlichnyi[b], Y.D. Lenivtseva[a], A. Malashicheva[c,d,e], Y. Malashichev[d], A.S. Golovkin[c], Y.G. Anissimov[f], S.I. Tverdokhlebov[a,*]

[a] Tomsk Polytechnic University, 30 Lenin Avenue, Tomsk 634050, Russian Federation
[b] Tomsk State University, 36 Lenin Avenue, Tomsk 634050, Russian Federation
[c] Federal Almazov Medical Research Centre, 2 Akkuratova St., St. Petersburg 197341, Russian Federation
[d] St. Petersburg State University, 7/9 Universitetskaya nab., St. Petersburg 199034, Russian Federation
[e] ITMO University, Institute of translational Medicine, St. Petersburg, Russian Federation
[f] Griffith University, School of Natural Sciences, Engineering Dr., Southport, QLD 4222, Australia

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ABSTRACT

This work analyses the properties of calcium phosphate coatings obtained by pulsed laser deposition on the surface of the ferroelectric polymer material. Atomic force and scanning electron microscopy studies demonstrate that, regardless of the type of sputtering target, the calcium phosphate coatings have a multiscale rough surface that is potentially capable of promoting the attachment and proliferation of osteoblasts. This developed surface of the coatings is due to its formation mainly from a liquid phase. The chemical and crystalline composition of the coatings depends on the type of sputtering target used. It was shown that, regardless of the type of sputtering target, the crystalline structure of the ferroelectric polymer material does not change. Cell viability and adhesion studies of mesenchymal stromal cells on the coatings were conducted using flow cytometry and fluorescent microscopy. These studies indicated that the produced coatings are non-toxic.

1. Introduction

Implants made from fluoropolymers are ubiquitous in modern medicine [1]. Such implants are used in ophthalmology [2,3] vascular surgery [4], surgery [5], orthopedics [6], traumatology [7] and tissue engineering [8,9]. The widespread use of fluoropolymers in medicine is due to their beneficial properties, such as: high chemical resistance, good mechanical properties, heat resistance, thermal stability and biological inertness [10,11]. Some fluoropolymers, such as polyvinylidene fluoride (PVDF), vinylidene fluoride with tetrafluoroethylene copolymer (VDF-TeFE) and vinylidene fluoride copolymer with trifluoroethylene (VDF-TrFE) have piezoelectric and ferroelectric properties [12].

Piezoelectric and ferroelectric properties of these polymer materials arise from strong dipole moment of perpendicular polymer chain occurring at the specific conformation of the macromolecule. A significant dipole moment arises due to the higher electronegativity of fluorine atoms in comparison with hydrogen and carbon ones [13]. There are three main crystalline modifications of the polymers (α, β, γ) that are differing in chain conformation. The α – modification is characterized by a monoclinic lattice in which the chain conformation (*TGTG-*) has opposite dipole moments, so in general it is non-polar. The β –modification has an orthorhombic lattice with a polar cell in which chains have a planar zigzag conformation. The γ –modification is comprised of a polar cell with the chain conformation $T_3GT_3G^-$. In addition the β –modification is the most electrically active phase as it has the most significant dipole moment ($\approx 8 \times 10^{-30}$ C m) [14].

The ferroelectric property of PVDF or its copolymers gives the opportunity to actively influence the pool of biological cells placed on its surface. It has been demonstrated that an increase in the ferroelectric β phase stimulates the differentiation of mesenchymal stem cells (MSC) in osteoblasts [15,16] and accelerates the regeneration of bone tissue [17,18] and stimulates attachment of neurons [19,20]. Devices made using polymeric ferroelectric materials were previously used for mechanical stimulation of osteoblasts [21,22].

It is known that the processing conditions of PVDF and copolymers (the rate of crystallization from the melt or solution,

* Corresponding author. Tel.: +7 382260 64 74.
E-mail address: tverd@tpu.ru (S.I. Tverdokhlebov).

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orientation drawing, annealing, hardening, *etc.*) significantly affect its crystal structure and hence the physical characteristics of the formed product [13].

The main disadvantage of implants made of PVDF and its copolymers is a limited capacity for integration within tissues due to its low surface free energy. To overcome this shortcoming a series of studies on the surface modification of fluoropolymers was conducted in different research centers to improve the integration of the implants into the surrounding tissues [23–25]. For applications associated with the regeneration of bone tissue on the surface of fluoropolymers calcium phosphate coatings (CaP) are applied, usually by RF magnetron sputtering [26,27]. It is known that CaP coatings have a high biological compatibility and promote the attachment and proliferation of osteoblasts on their surface. It is desirable for bio-compatibility that the surface of coatings is multiscale rough, and the coating structure has considerable number of macro- and micro-pores [28]. However, the RF magnetron sputtering CaP coatings usually do not have the multiscale rough surface [29,30], which has the potential to reduce the degree of integration with the surrounding tissues. During the coating formation by RF magnetron sputtering there is a substantial heating of the coated sample. For the case of coating PVDF and its copolymers this heating is capable of causing an undesirable change in the polymer's crystal structure [31].

Thus, to provide the possibility of additional osteoblasts stimulation, the ferroelectric polymer materials should have a rough biocompatible calcium phosphate coating. At the same time the process of calcium phosphate coating formation should not affect the polymer conformational structure to avoid the transformation of β phase to α phase in order to preserve the crystal structure of the polymer material with ferroelectric properties.

One of the promising ways to create CaP coating on the surface of ferroelectric polymer materials satisfying the above requirements is a method of pulse laser deposition (PLD) [32]. PLD method is based on a laser ablation of target due to powerful laser pulses in a vacuum or in the presence of a background gas. As a result of a rapid local heating of the target material a plasma plume is formed, which spreads in a vacuum or a rarefied gas and is deposited on the surface of the substrate, forming a thin film. The properties of the film depend on the parameters of radiation, the material of the target and the coated substrate [33]. The selection of the experimental conditions aims to minimize the impact of the process on the properties of the substrate material. PLD method using hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) target is widely used for the creation of biocompatible CaP coatings on surfaces of metal, ceramic and polymeric materials [34,35]. Another promising target compound for the formation of CaP coatings is Dicalcium Phosphate Anhydrous ($CaHPO_4$, DCPA) [36,37]. Hitherto there is no literature data on obtaining CaP coatings by PLD using DCPA targets. The influence of the formation of coatings by PLD on the crystal structure of ferroelectric polymer substrates is poorly understood, which restricts further development of the technology.

Thus, the main objectives of our work were: to investigate the possibility of formation of CaP coatings by PLD on the surface of the ferroelectric polymer material and to study the properties of the coatings and their dependence on the sputtering target material. Impact of the formation of coatings on the crystal structure of the ferroelectric polymer substrate materials was also investigated.

2. Materials and methods

Formation of the polymer film on plates was performed as described in [38]. In this work two types of plates were used: stainless steel (321 stainless steel, dimensions of 20 × 20 mm) and glass (microscope slide, dimensions of 20 × 40 mm). The CaP coatings were formed using PLD method on the surface of the ferroelectric polymer films. The fundamental harmonic of a repetitively pulsed solid-state Nd:YAG laser (Lotis TII, Belarus) was used with wavelength of 1064 nm, pulse energy 170 mJ, pulse duration 7 ns and pulse repetition frequency of 15 Hz. The radiation was focused onto the target surface by a spherical lens with a focal length of 250 mm and spot diameter of 2 mm. Maximum pulse power and energy density on the target surface were 0.75 GW/cm^2 and 5 J/cm^2, respectively. Distance from an ablation point on the target to the substrate was 3 mm. The working vacuum chamber, where the target and substrate were placed, had the diameter of 70 mm and the height of 40 mm (volume $V \sim 150$ cm^3). The vacuum in the chamber was created by a rotary vane pump HP-2 and the residual pressure was less than 1 Pa. The target, consisting of the pressed powders of analytical grade HAP or DCPA, had a cylindrical shape with the diameter of 16 mm and the height of 5 mm. The compression of the powder was performed in a hydraulic press T-40 (SPECAC, UK) with a nominal pressure of 20 tons.

Investigation of the surface morphology of the resulting coatings was performed using scanning electron microscopy (SEM, ESEM Quanta 400, FEG). A thin layer of gold was applied to the surface of the investigated sample for 60 s using a magnetron sputtering system SC7640 (Quorum Technologies Ltd).

Morphological characteristics of the coatings (*e.g.* particle size), were measured using the software Image J 1.38 (National Institutes of Health, USA).

Surface properties were also studied using the high resolution atomic force microscopy (AFM) using Solver-HV (NT-MDT). The topology and surface potential of the coatings were determined by the method described previously [39].

The study of the elemental composition of the samples was performed by X-ray fluorescence analysis (XRF) using X-Ray Fluorescence Spectrometer (Shimadzu XRF 1800) with the accelerating voltage of 40 kV, current 95 mA, scanning speed 8°/min and the scanning step 0.1°.

Examination of the chemical structure of the samples was performed for the films obtained on the glass substrate. For this purpose, the film was mechanically separated from the plate. The result was a sample on one side of which was the CaP coating. Both sides of the sample were investigated using the method of Attenuated Total Reflectance (ATR) Fourier Transform Infrared spectroscopy (FTIR). Spectrometer Tensort 27 (Bruker) with ATR attachment (PIKE MIRacle, with the angle of incidence 45°) on the crystal ZnSe was used. Investigations were carried out in the spectral range of 500–2000 cm^{-1} with a resolution of 4 cm^{-1}.

The crystal structure of the samples was investigated using X-ray diffraction analysis with Shimadzu XRD 6000 diffractometer. The samples were exposed to a monochromatic Cu K-alpha (1.54056 Å) radiation. The accelerating voltage and the beam current were set to 40 kV and 30 mA respectively. The scanning angle range, scanning step size and signal collection time were 6–55°, 0.0200° and 1.5 s respectively. As references we used the following entries from the PDF – 4 database: Hydroxyapatite (#00-055-0592) and Dicalcium Phosphate Anhydrous (#01-071-1759). The average size of the crystals (l_c) was evaluated using the Debye–Scherrer equation:

$$l_c = \frac{k\lambda}{\cos\theta\sqrt{\beta^2 - \beta_r^2}}, \quad (1)$$

where λ is the wavelength of the incident radiation, β the width of the reflection at half height, β_r is the broadening reflex of the apparatus, θ is the angle of diffraction and $k = 0.9$.

Evaluations of the effectiveness of cell adhesion to the modified surface were conducted by fluorescent microscopy using universal