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Composite biocompatible hydroxyapatite–silk fibroin coatings for medical implants obtained by Matrix Assisted Pulsed Laser Evaporation

F.M. Miroiu[a,*], G. Socol[a], A. Visan[a], N. Stefan[a], D. Craciun[a], V. Craciun[a], G. Dorcioman[a], I.N. Mihailescu[a], L.E. Sima[b], S.M. Petrescu[b], A. Andronie[c], I. Stamatin[c], S. Moga[d], C. Ducu[d]

[a] *National Institute for Lasers, Plasma, and Radiation Physics, 409 Atomistilor Street, RO-77125, MG-36, Magurele-Ilfov, Romania*
[b] *Institute of Biochemistry, Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, Romania*
[c] *3Nano-SAE Alternative Energy Sources-University of Bucharest, Faculty of Physics, 409 Atomistilor Street, RO-77125, Magurele-Ilfov, Romania*
[d] *University of Pitesti, Targul din Vale Str, no. 1, 110040 Pitesti, Romania*

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ABSTRACT

The aim of this study was to obtain biomimetic inorganic–organic thin films as coatings for metallic medical implants. These contain hydroxyapatite, the inorganic component of the bony tissues, and a natural biopolymer – silk fibroin – added in view to induce the surface functionalization. Hydroxyapatite (HA), silk fibroin (FIB) and composite HA–FIB films were obtained by Matrix Assisted Pulsed Laser Evaporation (MAPLE) in order to compare their physical and biological performances as coatings on metallic prostheses. We used an excimer laser source (KrF*, $\lambda = 248$ nm, $\tau = 25$ ns) operated at 10 Hz repetition rate. Coatings were deposited on quartz, Si and Ti substrates and then subjected to physical (FTIR, XRD, AFM, SEM) analyses, correlated with the results of the cytocompatibility *in vitro* tests. The hybrid films were synthesized from frozen targets of aqueous suspensions with 3:2 or 3:4 weight ratio of HA:FIB. An appropriate stoichiometric and functional transfer was obtained for 0.4–0.5 J/cm^2 laser fluence. FTIR spectra of FIB and HA–FIB films exhibited distinctive absorption maxima, in specific positions of FIB random coil form: 1540 cm^{-1} amide II, 1654 cm^{-1} amide I, 1243 cm^{-1} amide III, while the peak from 1027 cm^{-1} appeared only for HA and composite films. Osteosarcoma SaOs2 cells cultured 72 h on FIB and HA–FIB films showed increased viability, good spreading and normal cell morphology. The well-elongated, flattened cells are a sign of an appropriate interaction with the MAPLE FIB and composite HA–FIB coatings.

1. Introduction

It is already known that a composite hydroxyapatite–fibroin material should meet bioaffinity, enhanced osteoinductivity, adequate mechanical strength and flexibility for use as implant material. Hydroxyapatite (HA), with the chemical formula ($Ca_{10}(OH)_2(PO_4)_6$), constitutes the majority mineral part of natural bones and teeth, providing innate excellent biocompatibility, osteoconductivity and bioactivity [1,2]. Therefore, it is largely studied and used in different applications like bone fillers, maxillofacial reconstructions, dental applications or biomedical ceramic coatings [3–6].

On the other hand, silk fibroin (FIB), the main constituent of the natural silk, is a natural biopolymer, a protein spun in fibers by some lepidoptera larvae, as silkworms or spiders [7]. It shows three different conformations of peptide chains and a crystalline dimorphism: amorphous random coil, crystalline β-sheet and crystalline α-helix forms [8]. The silk fibroin is known and proved to be biocompatible, biodegradable, having unusual high tensile strength and elasticity. It has applications in scaffolds aimed to manipulate the osseous growth in the desired forms, sutures, artificial skin, artificial tendons, and substrate for cell culture, bone tissue engineering, coatings [9–13].

Silk fibroin–hydroxyapatite is a quite new composite particularly studied as 3D scaffolds, nanocomposites or thick coatings [14–18]. In our studies, silk fibroin is added to the hydroxyapatite in view to induce the surface functionalization of the coating and to improve the mechanical properties (elasticity). The HA–polymer composites mimic the natural bone, formed by the inorganic phase (nanometric biological HA) and organic compounds (mainly collagen). The intimate synergy between inorganic and organic phase provide the hard tissues with the providential features as high fracture toughness, flexibility and strength. The nanometric dimension of the inorganic element, of high specific surface, similar to the one in the bony apatite is important from the point of view of the mechanical and biological properties [19,20].

The aim of our work was to obtain biomimetic ceramic–polymer composite coatings for metallic medical implants. We have cho-

* Corresponding author. Tel.: +40 21 4574491; fax: +40 21 4574243.
E-mail address: marimona.miroiu@inflpr.ro (F.M. Miroiu).

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Table 1
Selected samples of hydroxyapatite, fibroin and composite thin films deposited by MAPLE.

Code	Sample type
FIB5	Fibroin (5 wt% in suspension)
HA5	Hydroxyapatite (5 wt%)
FIBNaOH	Fibroin (5% suspension) with NaOH added
FIBNaCl	Fibroin (5% suspension) with NaCl added
HA3–FIB2	Hydroxyapatite (3 wt%)–fibroin (2 wt%)
HA3–FIB4	Hydroxyapatite (3 wt%)–fibroin (4 wt%)
C	Control–borosilicate glass

sen to synthesize the hybrid hydroxyapatite–polymer coatings and simple hydroxyapatite and simple polymer coatings, respectively on titanium substrates (as basis of the orthopedical implants) by a novel fabrication method for this composite, Matrix Assisted Pulsed Laser Deposition (MAPLE), as being the most appropriate for synthesis of large organic molecule thin films [21].

2. Experimental procedure: methods, materials and analyses

2.1. Matrix Assisted Pulsed Laser Evaporation (MAPLE) method

MAPLE is an advanced laser technique, based on a cryogenic approach, which has been developed since 1998, to produce a "protected" accurate transfer of organic and polymeric materials in form of thin films [22,23].

The material subjected to the laser irradiation – which is called "target" – is a frozen composite. It is obtained by freezing following the dissolution of the "active" biomaterial (up to 5 wt%) in an appropriate volatile solvent, highly absorbing the laser wavelength, but not reacting under laser exposure. The laser pulses intensities are adjusted to avoid the biomolecules damage. The structural and functional fidelity is preserved inducing a non-direct laser–material interaction in a vacuum chamber. Due to the low concentration of biomolecules in the frozen target, the laser photons preponderantly interact with the matrix (the solvent), which is vaporized. The complex biomolecules are released undamaged and, by means of the collisions with the other molecules, moved toward the substrate, where they form a uniform thin film. In the same time, the volatile solvent is pumped away by the vacuum system. During the deposition process the target is kept at low temperature by a cooler.

As its precursor, Pulsed Laser Deposition, MAPLE is a layer-by-layer growing method, which can deposit thin films with dopants and may also be applied with masks, in order to induce a controlled distribution of the local structure parameters by the nature and type of the coating.

2.2. Materials, set-up and MAPLE experiments

We used in our experiments commercial 2 μm granulation polymer powder of degummed (i.e. sericin-free) *Bombyx mori* fibroin (Wuxi Allied Technologies, Inc., China), and commercial Merck hydroxyapatite powder.

For MAPLE experiments we prepared different fibroin and hybrid solutions or suspensions. According to literature, the water-insoluble fibroin can be solubilised in organic substances (N-methyl morpholine N-oxide, MMNO N-dimethyl acetamide, copper-ethylendiamine, $Ca(NO_3)_2$/MeOH) or in saturated solutions of calcium chloride or lithium bromide, simple or with ethylic alcohol [24–26]. Thus, a number of films were grown from fibroin subjected firstly to a solubilisation process, and then to one of separation.

In view to homogenise them, the calcium chloride and lithium bromide solutions were heated at 95 °C [27] and 60 °C [28] and kept at these temperatures for 8 and 4 h, respectively. Subsequently, to separate the fibroin from salt, the solutions were subjected to a chemical dialysis process in bidistilled water, for 4 days. In the end,

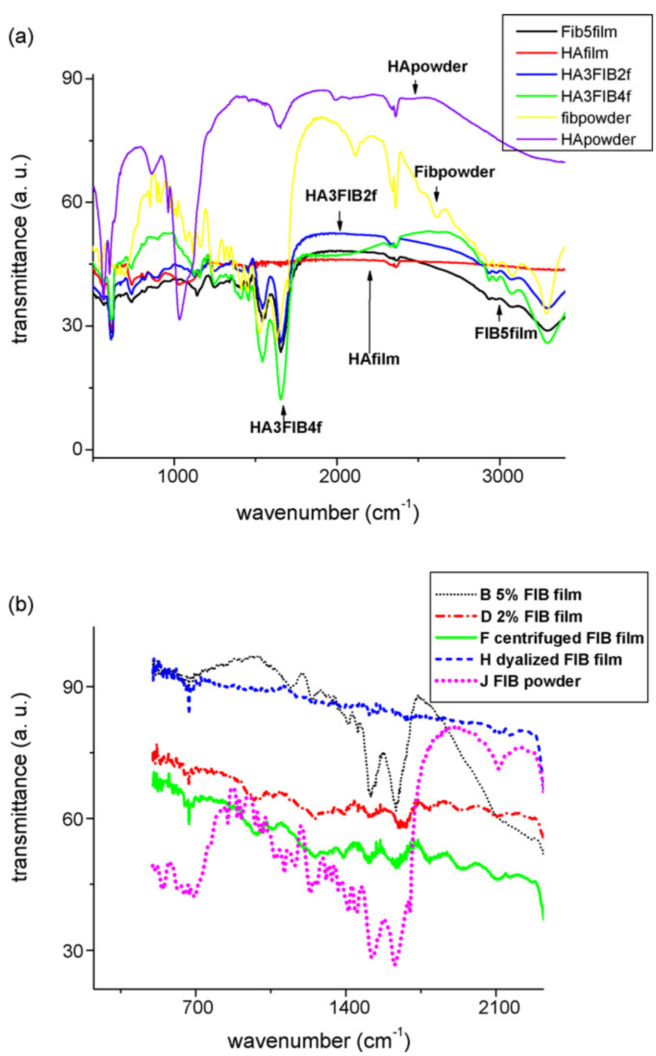

Fig. 1. FTIR spectra of (a) HA, FIB and hybrid HA–FIB films on silicon, compared to those of fibroin and HA powders; (b) fibroin thin films (from different fibroin solutions) on silicon compared with the original powder.

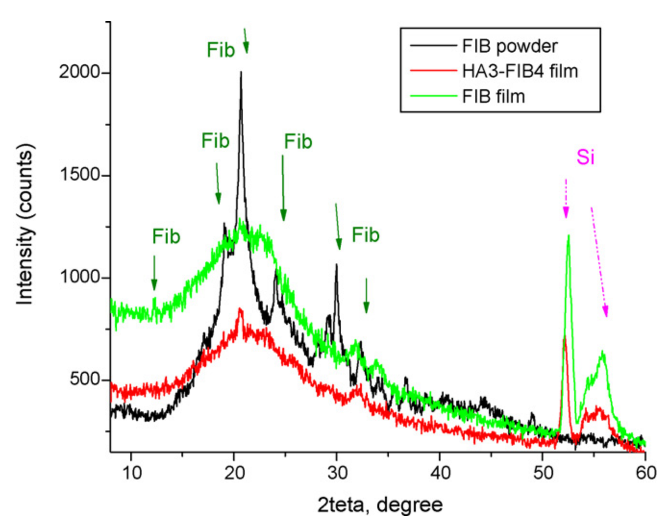

Fig. 2. XRD spectra of the fibroin and HA–fibroin thin films on silicon substrates deposited by MAPLE.