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Bioglass –polymer thin coatings obtained by MAPLE for a new generation of implants♣

F. SIMA*, C. RISTOSCU, A. POPESCU, I. N. MIHAILESCU, T. KONONENKO[a], S. SIMON[b], T. RADU[b], O. PONTA[b], R. MUSTATA[c], L.E. SIMA[c], S.M. PETRESCU[c]
National Institute for Lasers, Plasma, and Radiation Physics, PO Box MG-54, RO-77125 Bucharest-Magurele, Romania
[a]*Natural Sciences Center, General Physics Institute, Moscow, Russia*
[b]*Babes-Bolyai University, Faculty of Physics & Institute for Interdisciplinary Experimental Research, Cluj-Napoca 400084, Romania*
[c]*Institute of Biochemistry, Romanian Academy, Splaiul Independentei 296, Bucharest, Romania*

We report on the transfer of novel PMMA-bioglass composites by matrix assisted pulsed laser evaporation to uniform thin layers onto chemically etched Ti. The targets were prepared by freezing in liquid nitrogen after dissolution in chloroform of mixtures containing PMMA reinforced with either 6P57 or 6P61 bioglass powders. The cryogenic targets were submitted to multipulse ablation with an UV KrF* (λ=248 nm, τ ~ 25 ns) excimer laser source. The fluence was set after optimization at 0.55 Jcm^{-2}. The morphology of synthesized coatings was investigated by scanning electron microscopy and atomic force microscopy. The deposited nanostructures evidenced rather uniform and compact morphologies and consisted of droplets in the micrometric range. The composition was monitored by energy dispersive spectroscopy, Fourier transform infrared spectrometry and X-ray photoelectron spectroscopy. The presence of both PMMA and bioglass cations was evidenced in all deposited structures. Cell proliferation after cultivation on the deposited layers was studied by fluorescence microscopy. The cells were shown in both cases to cover almost entirely the structures with which they strongly interact, as proved by the pseudopodia deeply infiltrating into the composite material. The difference in density proves that cells find a more friendly living medium on glasses with a lower silica content.

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Keywords: PMMA-bioglass composites, thin coatings, cells proliferation, MAPLE

1. Introduction

Although many materials have been used in the regeneration and substitution of injured or lost bones, no synthetic material offered till now characteristics similar to the natural tissue, in respect of both the biological and mechanical aspects. Bioglass coatings obtained by plasma spraying usually failed, due to a weak glass/metal interface or rapid dissolution in body fluids [1]. Attempts to coat metals with bioglasses by enameling techniques were also unsuccessful because of significant glass crystallization, resulting in poor adhesion to the substrate [2,3]. Composites have been recently proposed as a prospective alternative for performant coatings [4,5]. Bioactive glass particles embedded into polymer matrices could combine the bioactive performance with improved mechanical properties similar to hard tissues. In this paper, we report on the transfer by matrix assisted pulsed laser evaporation (MAPLE) of novel PMMA-bioglass composites in uniform thin layers onto chemically etched Ti substrates. As a polymer, we chose poly-methyl-methacrylate (PMMA) which is largely used as a biomedical material [6]. Bioglass particles in the system SiO_2-Na_2O-K_2O-CaO-MgO-P_2O_5 with two different compositions were embedded in the polymer matrix. The two compositions are respectively slightly lower and higher than the threshold value of 60% SiO2 corresponding to significant changes in bioactivity and degradability [7].

2. Materials and methods

Target preparation

For the preparation of targets, we used powders of commercial Poly-Methylmethacrylate (PMMA) and of two bioglasses in the system SiO_2-Na_2O-K_2O-CaO-MgO-P_2O_5 containing 57 wt% (further denoted 6P57) and 61 wt% (further denoted 6P61) silica. The bioglasses composition were 56.5% SiO_2, 11% Na_2O, 3% K_2O, 15% CaO, 8.5% MgO, 6% P_2O_5 for 6P57, and 61.1% SiO_2, 10.3% Na_2O, 2.8% K_2O, 12.6% CaO, 7.2% MgO, 6% for 6P61, respectively.

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The bioglasses were synthesized by mixing the due proportions of SiO_2, $CaCO_3$, $MgCO_3$, Na_2CO_3, K_2CO_3, and NaH_2PO_4, powders [8]. We dissolved 0.6 g PMMA reinforced with 0.08 g of either 6P57 or 6P61 bioglass particles in 19.3 ml chloroform. The mixture was stirred for homogenization and the solution immersed in liquid nitrogen. The obtained cryogenic targets were used in the MAPLE experiments.

MAPLE experiments

The MAPLE experiments were conducted in a stainless steel reaction chamber. An UV KrF* excimer laser source (λ=248 nm, τ=25 ns, ν=10 Hz) was used for the multipulse ablation of the cryogenic targets. The ablation flux was collected onto chemically-etched grade 2 (97 %) Ti disks of 12 mm diameter and 1 mm thickness. Prior to deposition, the Ti substrates were cleaned with alcohol in an ultrasonic bath and rinsed in deionized water.

The laser beam was incident at 45° on the target surface. The Ti substrate was placed parallel to the target, at a 3 cm separation distance. The incident laser fluence was set at 0.55 Jcm^{-2}. For deposition of every structure, we applied 12500 laser pulses. The targets were continuously rotated and translated during the multipulse laser irradiation, in order to avoid drilling and to obtain homogenous coatings. All depositions were performed on substrates heated at 30 °C, while the residual pressure inside the chamber was 2.7 Pa.

Physico-chemical characterization

The morphology of the deposited structures was examined by scanning electron microscopy (SEM) with a Jeol JSM-6460LV apparatus. The chemical composition was investigated by energy dispersive spectrometry (EDS) and Fourier Transform Infrared (FT-IR) spectrometry, with a Nicolet 380 apparatus equipped with an orbit ATR (diamond crystal). Atomic force microscopy (AFM) investigations were carried out in a semi-contact mode with AFM NTEGRA Vita equipment. X-ray Photoelectron Spectroscopy (XPS) analysis was performed with a SPECS PHOIBOS 150 MCD system with monochromatised Al Kα radiation generated by a 300 W X-ray source (hν = 1486.6 eV)

In vitro studies

Sterilization: The samples were introduced in Petri dishes, packed and sterilized by autoclaving at 121^{o}C for 30 minutes.

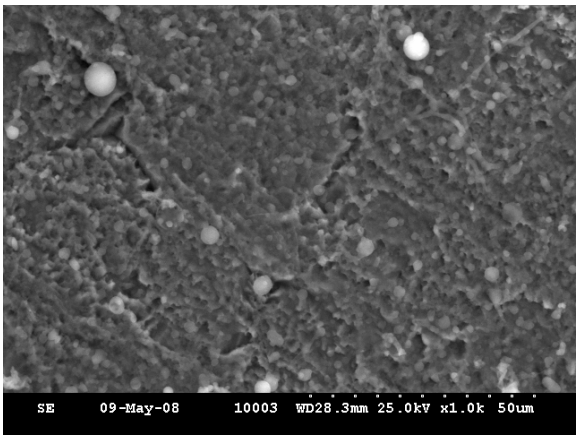
Cell culture: SaOs2 (human osteosarcoma) cells were maintained in a McCoy's culture medium supplemented with 10% inactivated bovine fetal serum, 50 U/ml penicillin, 50 mg/ml streptomycin, 1% L-glutamine. The cells were split every 2 days by a ratio of 1:3 and incubated at 37°C and 5% CO_2. All studies were done 72 hours after cell cultivation.

Cell proliferation was monitored by fluorescence microscopy. The cells were cultivated on both the MAPLE deposited samples and borosilicate glass (control). They were fixed for 20 minutes with 4% paraformaldehide (PFA). After washing and permeabilization for 3 minutes with 0.2% Triton X-100 in a phosphate buffer saline (PBS) solution, the samples were incubated for 30 minutes with an anti-Ki67 primary antibody (1:100), washed and incubated with a secondary rabbit antibody conjugated with AlexaFluor 488 (1:400, 30 min) for the visualization of the protein on the green fluorescence channel. All specimens were then washed with PBS and placed on microscope slides in a mounting medium with DAPI to label the nuclei on the blue channel. The cell visualization was performed with a Nikon Eclipse E600 microscope and the photograph were recorded with a Nikon Digital Light DS-U1.

3. Results and discussion

Physical-chemical characterization

We observed by SEM (Fig. 1) that the structures obtained from cryogenic targets of 6P57-PMMA are uniform and compact. They consist of droplets of variable size, characteristic of MAPLE or PLD depositions [9-11]. The droplets are in the micrometric range, and are randomly spread across the surface. These features are considered favorable [12,13] for enhancing the growth and proliferation of cells which can easily and deeper infiltrate their pseudopodia into surface imperfections. The observations were supported by AFM evidence in 2D, 3D and phase mode respectively (Fig. 2 a, b, c).Thus, the irregular shape of the droplets is visible from Figs. 2 a, b, while their uniform outer surface is evidenced from Fig. 2 c.



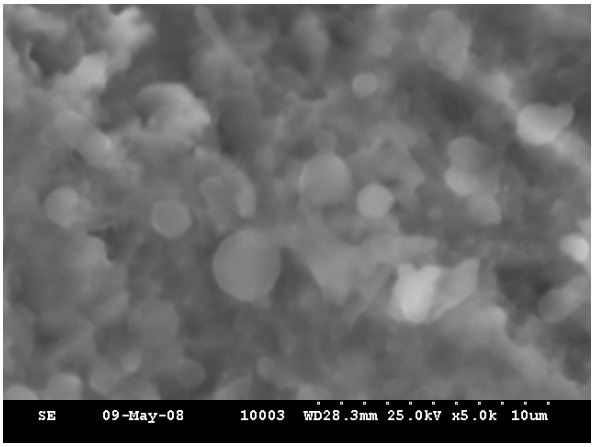
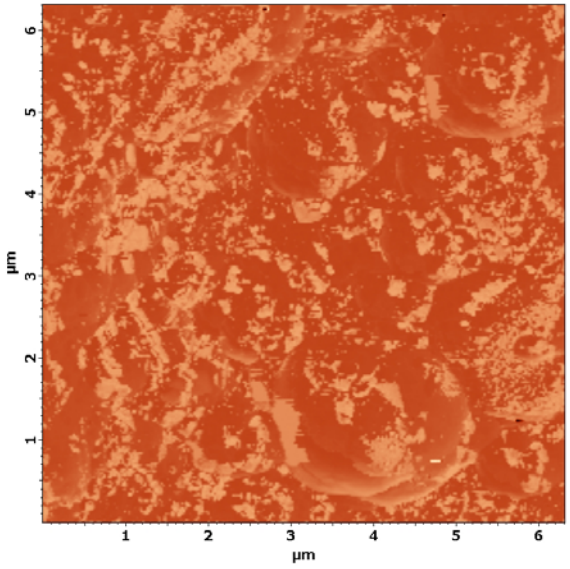
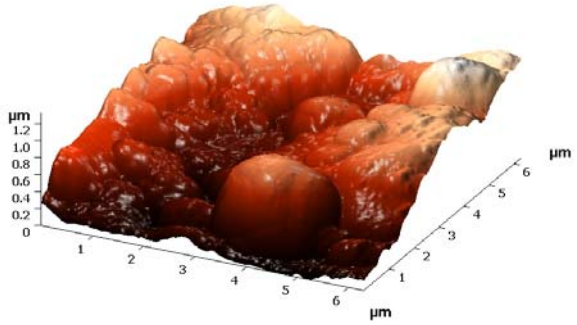


Fig. 1. SEM micrographs showing a typical surface morphology of a 6P57-PMMA coating: (a) general view; (b) detail from (a)





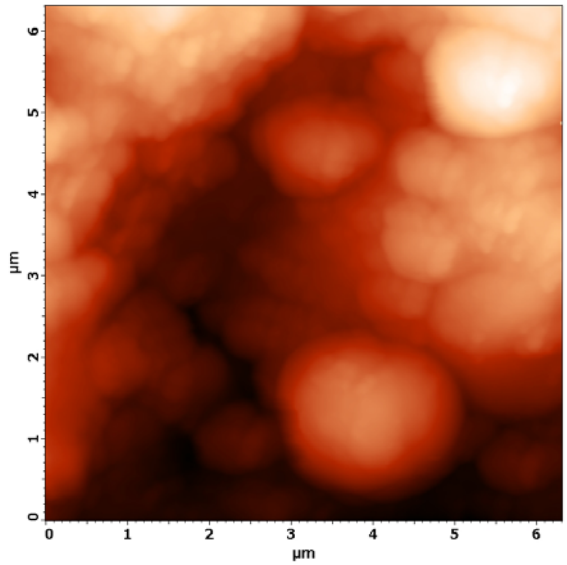


Fig. 2: 3D (a), 2D (b) and phase (c) typical AFM images recorded in case of a 6P57-PMMA coating.

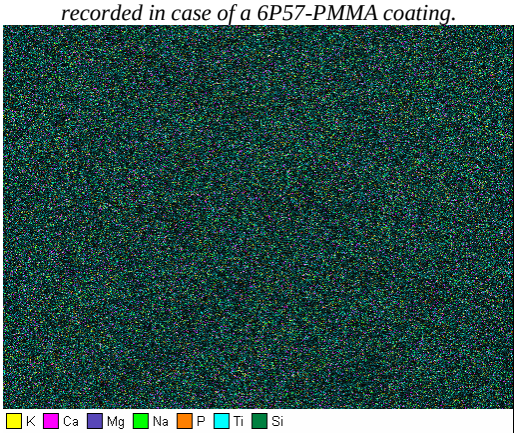


Fig. 3. EDS cartography of the 6P57-PMMA coating

From EDS cartography of the 6P57-PMMA coating (Fig.3) we observe that all bioglass elements have been preserved in deposited nanostructures and were homogeneously distributed over the whole investigated area.

The corresponding XPS spectra of the same structures pointed to the clear presence of bioglass cations (as marked in the Fig. 4).

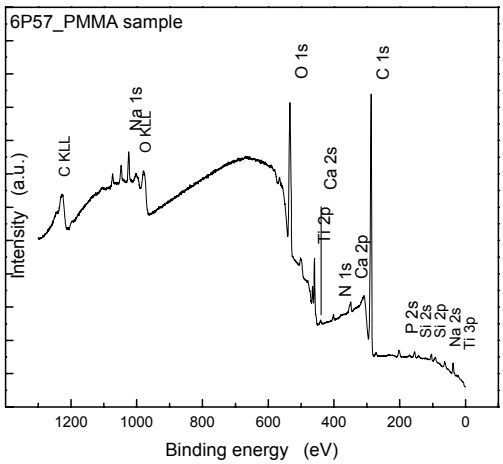


Fig. 4. XPS survey spectrum of the 6P57-PMMA coating

We give in Fig. 5 the FT-IR spectra recorded in the case of a PMMA powder and of a 6P57-PMMA coating respectively.

We note that FT-IR analysis proved that PMMA is stoichiometrically transferred from powder to film. Indeed, all peaks characteristic of PMMA visible in case of the powder are preserved for the film. Nevertheless, the intensities are slightly higher in the case of films, while the corresponding peaks became narrower. This could prove a reinforcement of the PMMA film by bioglass inclusion [14]. On the other hand, the peaks of the 6P57 bioglass are very hardly visible, probably due to the small quantity of material and the reduced thickness of the coatings.

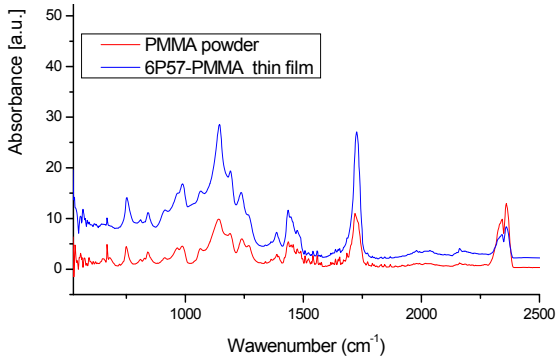


Fig. 5: The main vibration bands present in the FT-IR spectra of PMMA powders and of a 6P57-PMMA coating obtained by MAPLE.

All peculiarities evidenced by the physical-chemical investigation of PMMA-6P57 coatings (as visible from Figs. 1-5) were similar in the case of the coatings PMMA-6P61. This sustains the conclusion that the two kinds of coating are identical from the points of view of their physical-chemical parameters.

Cells proliferation

The cell proliferation on MAPLE structures was very close to that of the control (borosilicate glass) (Fig. 6). The number of cells seeded on surfaces was rather low, to ensure a good survival over 72 hours.

We recorded 5 images for each sample with 10X magnification, and counted all cells labeled with DAPI or Ki67. The data were calculated as the ratio Ki67/DAPI x 100 and presented in Fig. 6.

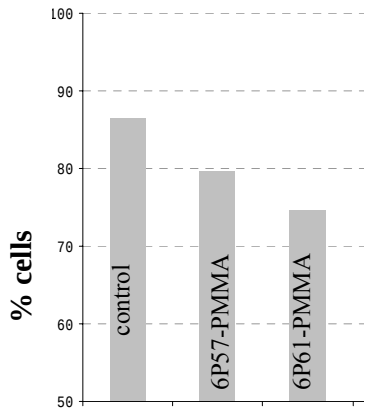
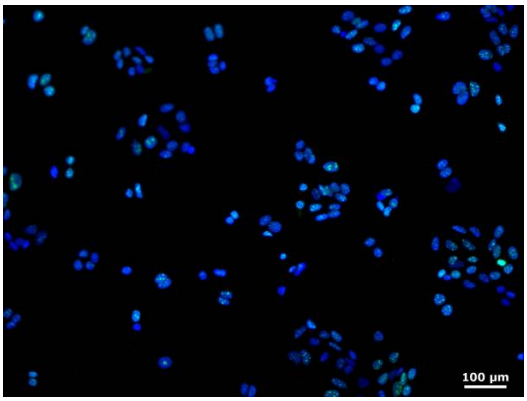


Fig. 6: Proliferation 72 hours after cell cultivation on MAPLE deposited structures and a control, respectively.

We noticed that cells proliferated very well on both structures, but slightly less well than onto the control sample. However, a difference in the cell proliferation was evident between the structures containing the 6P57 or 6P61 bioglasses. Cells multiplied in a larger number, in the case of the 6P57-PMMA structure. The observed difference between the proliferating cells percentage (80% for 6P57 vs 75% for 6P61) was indicative that cells found a more friendly living medium on the bioglass with a lower silica content. This statement is supported by results recently reported [15].

The cell density on the surface was monitored by fluorescence microscopy. Cells were labeled with fluorescent markers (Ki67 and DAPI) for nuclei visualization (Fig. 7). One can see from Fig. 7 that the cells covered uniformly deposited surfaces with a larger density for the 6P57-PMMA structure.



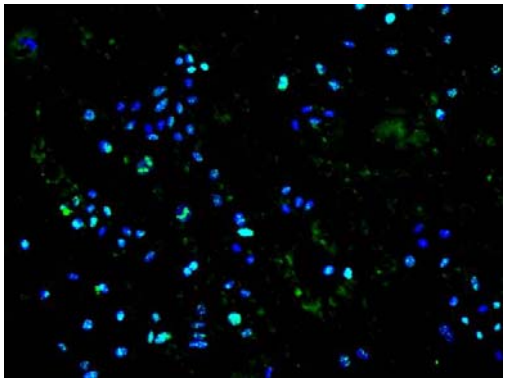


Fig. 7. Fluorescence microscopy of cells cultured on control (a) and MAPLE deposited structures 6P57 – PMMA (b) and 6P61 – PMMA (c) respectively. 100×.

4. Conclusions

Thin layers of PMMA-bioglass composites were synthesized by matrix assisted pulsed laser evaporation onto chemically etched Ti disks. The thin structures had a uniform morphology, and were composed of droplets with different sizes. The EDS and XPS spectra of these structures confirmed the presence of bioglass particles inside MAPLE synthesized thin structures. FT-IR investigations of PMMA powders and of 6P57-PMMA coatings showed that PMMA was stoichiometrically transferred by MAPLE. SaOs2 cells were seeded on the surfaces of the synthesized structures. They multiplied in larger numbers in the case of structures containing bioglass particles with a lower silica content (6P57). The difference between the proliferating cell percentages indicated that cells found a more friendly living medium in the bioglasses with lower silica contents.

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*Corresponding author: felix.sima@inflpr.ro