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PlasmaNanoSmart

Plasma- and electron beam-assisted nanofabrication of two-dimensional (2D) substrates and three-dimensional (3D)

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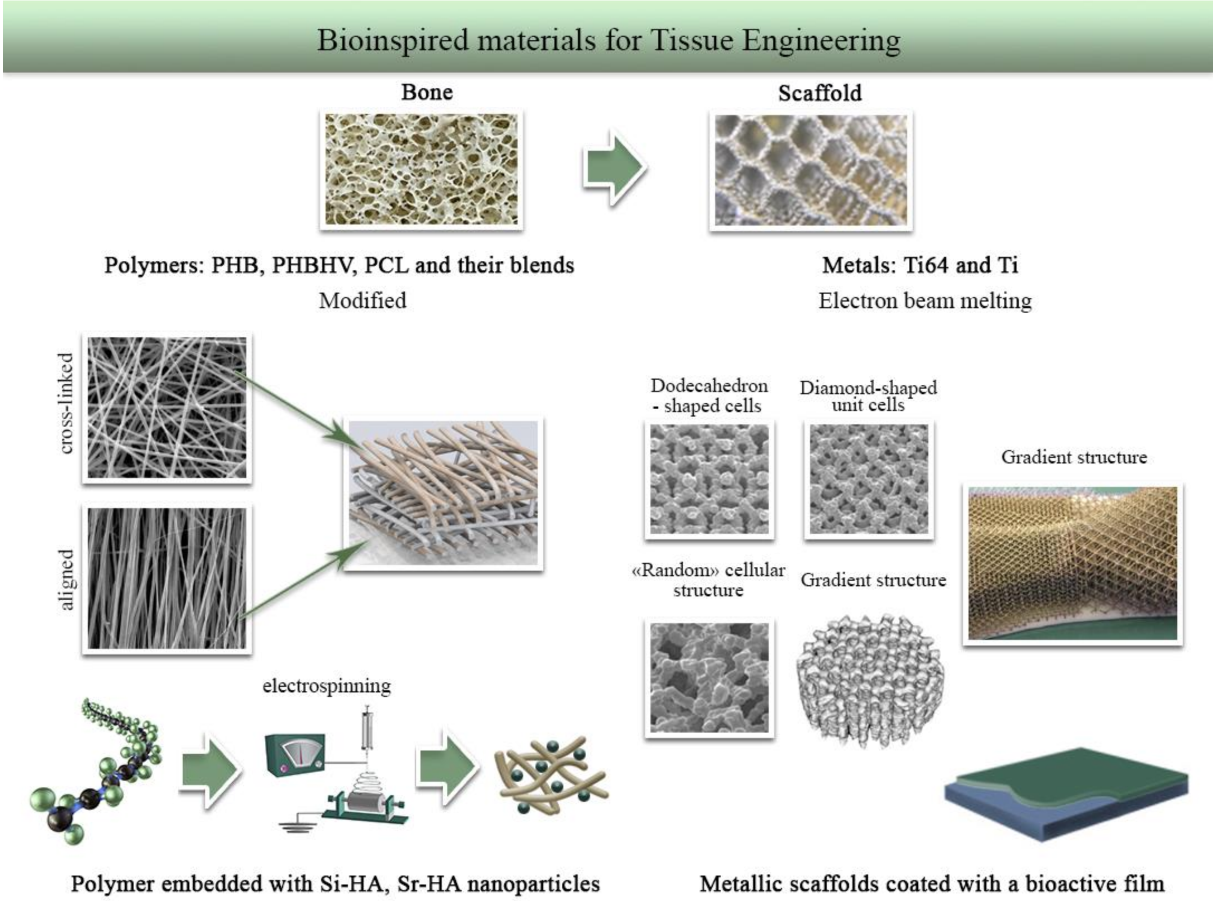
An overall scheme of the proposal can be presented as follows. Novel biomaterials fabrication, their surface modification and functionalization followed by *in vitro* and *in vivo* studies analyses of the tissue regeneration processes were done in the frames of the proposal.



DURING THE IMPLEMENTATION OF THE PROPOSAL THE FOLLOWING MOST IMPORTANT RESULTS WERE ACHIEVED:

1. Novel ways of the fabrication of 2D or 3D scaffolds based on poly-3-hydroxybutyrate, polycaprolactone, poly-3-hydroxybutyrate-co-hydroxyvalerate (PHB, PCL, PHB-HV) and their blends were elaborated and their surface nano/micro structuring was done. The surfaces of metallic alloys (Ti-, Mg- or Fe-based) were modified using RF-magnetron sputtering which allowed us to prepare a thin nanocrystalline hydroxyapatite (HA) coating. The results obtained revealed that low crystalline HA-based coatings with the different structure (columnar and equiaxed), morphology, stoichiometry and thickness can be prepared. These characteristics of the coatings can be controlled by variation of the deposition control parameters. The coatings have nanostructured features with the crystallite size between 45 and 70 nm. These differences in grain size were corroborated by imaging with SEM. HA films exhibited (002) preferred orientation. The preferred orientation becomes weaker when the negative bias on the substrate was applied during thin film deposition. *In vitro* cell studies represented a perfect adhesion of cells on the surface of the coating. Within 7 days cell vitality and number exceeded that found in case of the uncoated substrate.

This effect is most prominent in case of the coating prepared at the grounded substrate holder. Investigations of the HA-coated AZ91 and Fe-TCP (60-40, % in wt.%) alloys revealed that a low crystalline HA coating was prepared. The results of the chemical, molecular and phase composition of the biocomposites resulted in significantly higher cells activity for the CaP coated surfaces. It can be readily observed that the level of DNA and ALP is higher in the case of CaP coating. The cells were well-spread on the surface coated with CaP, however, no filipodia were observed in case of uncoated metallic substrates.

2. The polarization tests in a 3.5 wt.% NaCl solution were performed to examine the corrosion behaviour of the HA-coated AZ31 magnesium alloy. Two HA coating thicknesses of 700 nm and 1500 nm were studied. The coatings homogeneously covered the entire surface of the substrates. In the case of a greater coating thickness, larger crystallites were formed. The potentiodynamic polarization test demonstrated that a 1500-nm thick nanocrystalline HA coating significantly improved the corrosion resistance of the bare alloy. In the case of a magnesium–calcium (Mg–Ca) alloy a thin film of hydroxyapatite (HA) was coated on a magnesium–calcium (Mg–Ca) alloy using radio frequency (RF) magnetron sputtering. The thickness of the HA coating was in the range of 550–750 nm. In vitro degradation behaviour of the HA coated alloy was evaluated in simulated body fluid (SBF) using electrochemical methods. The ultrathin coating has significantly improved the degradation resistant of the alloy. The polarization resistance (Rp) of the coated alloy was more than two-order of magnitude higher and the corrosion current density (i_{corr}) reduced by ~98% as compared to the base alloy.

3. A multifunctional biocomposite based on a HA coating and silver nanoparticles (AgNPs) was prepared. AgNPs synthesized by a wet chemical reduction method were deposited on Ti substrates using a dripping/drying method followed by deposition of CaP coating via radio-frequency (RF) magnetron sputter-deposition. The negatively charged silver nanoparticles (zeta potential −21 mV) have a spherical shape with a metallic core diameter of 50 ± 20 nm. The HA coating was deposited as a dense nanocrystalline film over a surface of AgNPs. The RF-magnetron sputter deposition of HA films on the AgNPs layer did not affect the initial content of AgNPs on the substrate surface as well as NPs size and shape. SEM cross-sectional images taken using the backscattering mode revealed a homogeneous layer of AgNPs under the CaP layer. The diffraction patterns from the coatings revealed reflexes of crystalline HA and silver. The concentration of Ag ions released from the biocomposites after 7 days of immersion in phosphate and acetate buffers was estimated. The obtained results revealed that the amount of silver in the solutions was 0.27 ± 0.02 μg mL^{-1} and 0.54 ± 0.02 μg mL^{-1} for the phosphate and acetate buffers, respectively, which corresponded well with the minimum inhibitory concentration range known for silver ions in literature. Thus, new routes to prepare a biocompatible layer using embedded AgNPs to achieve a local antibacterial effect were obtained.

4. The effect of the low temperature plasma treatment on the properties of biodegradable polymer scaffolds was studied. It was revealed that significant changes in the surface wettability occur after plasma modification. Moreover, a hydrophilic or hydrophobic state was achieved under particular set of the treatment parameters such as RF power, working gas atmospheres and treatment time. Significant changes were achieved in respect with the surface energy. Its polar part increase was responsible for the

observed improvements of the surface wettability. The surface properties of medical implants which stimulate cell adhesion, proliferation and differentiation were revealed.

5. The structure, porosity and nanoparticles distribution of the polymer scaffolds were investigated. The ultrastructure of the scaffolds enabled us to reveal the significance of the material composition on its biodegradation and corrosion resistance behaviour. The visualization of the internal structure of the scaffolds was performed. The surface and bulk porosities distribution of the pure polymer and hybrid polymer-HA nanoparticles was obtained using X-rays. The nanoparticles distribution in the scaffolds was studied as well as HA nanoparticles size and size distribution were obtained.

6. Polymer-inorganic composites containing HA-based nanoparticles were studied using X-rays. It was revealed that polymers enrichment with inorganic particles improved their mechanical properties, mineralizability and biological properties, e.g. bioactivity and adhesion, proliferation and differentiation of bone-forming cells. X-ray radiation, which proved to be a powerful analytical tool to determine size and spatial distribution of HA nanoparticles with high resolution, was used to study the nanoparticles distribution in the polymer scaffolds. The homogeneous HA-based nanoparticles distribution within the polymer scaffolds was achieved.

IMPACT:

We expect that the newly developed implants will be successfully integrated in the orthopaedic practice and market. During the last years, it was reported that the number of the accidents in European Union and across the world has increased, and also the average age of the population is increasing. This led to the continuous development of the orthopedic implants in order to improve the patients' life quality. The commercialization of the developed technologies can allow not only to treat bone tissue failures, but improve the rate of post-implantation patients recovery and decrease operational costs. It enables an overall resource economy in EU and Russia. Moreover, the costs of the personalized implants fabrication are expected to reduce after project implementation. Existing international and national contacts will also be used for further dissemination of the results including invited lectures at MSc and PhD courses, topic workshops and popular-science presentations.

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