PHYSICOCHEMICAL AND *IN VITRO* BIOLOGICAL PROPERTIES OF POROUS PLLA/HAp-Zn COMPOSITES MODIFIED WITH SODIUM ALENDRONATE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 61]

Introduction

Thanks to the appropriate microstructure, porous composite implants, are gaining more and more interest in regenerative medicine for filling bone defects e.g. in osteoporosis. A well-known first-line drug in the treatment of osteoporosis is sodium alendronate from the bisphosphonates (BP) group. The mechanism of action of this drug is based on binding to hydroxyapatites in the bones and inhibiting the activity of osteoclasts, which cause bone tissue resorption [1,2]. However, it does not inhibit the bone formation process and thus causes a gradual increase in bone mass. Unfortunately, BPs show extremely low bioavailability (<1%), poor gastrointestinal absorption and a number of adverse effects when administered orally [3,4]. Hence, there are examples of developing new methods of local delivering of bisphosphonates to the body, e.g. in the form of BP-loaded hydrogels, or drug-eluting implants [5,6].

The aim of this study was to obtain non-cytotoxic, mechanically reinforced, biodegradable, porous composites with a combined osteogenic effect of apatite fillers and sodium alendronate (ALN). Alendronate was introduced into the structure of the porous composite by surface adsorption, and the apatite particles were additionally enriched with bactericidal Zn^{2+} ions.

Materials and Methods

Synthesis of hydroxyapatite whiskers with Zn^{2+} ions (HAp-Zn) was conducted by a homogenous precipitation method with urea with using of aqueous solutions containing sodium dihydrogen phosphate, calcium nitrate tetrahydrate and zinc nitrate hexahydrate (Chempur). The whiskers were characterized by XRD and SEM.

Porous composites were prepared in a thermally induced phase separation process of frozen suspensions of hydroxyapatite whiskers doped by Zn^{2+} ions in 5% solution of polylactide (5%PLA) (PLA-Resomer® LR706, Sigma-Aldrich) in 1,4-dioksane (Avantor). The whiskers content in composites was 25 wt.% (5%PLA25Hap-Zn). The obtained composites were diped for 24 h in sodium alendronate solution (1mg/ml) and dried by lyophilization (5%PLA25Hap-Zn/ALN). The morphology and average pore size of composites was evaluated by SEM. Mechanical properties of the composites were evaluated by compressive test on the Zwick Roell 5kN ProLine test machine for more than 5 specimens at a cross-head speed of 0,6 mm/min.

Cytotoxicity of the obtained composites was determined using standard direct contact cytotoxicity assay with L929 mouse skin fibroblasts, according to ISO10993-5:2009. Analogous tests were performed using human osteoblasts hFOB1.19, a model more relevant to bone implantation. The cytotoxicity assays were complemented using monocyte/macrophage-mediated inflammation sensing system to exclude the activation of human THP1-Blue™ cells in the presence of investigated biomaterials

Results and Discussion

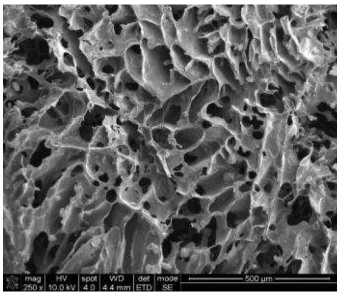

FIG. 1. SEM image of porous composite with the addition of HAp-Zn whiskers.

SEM observations of the obtained composites show that HAp whiskers are embedded in thin walls of the porous scaffolds. Mechanical analysis showed that inclusion of whiskers reinforced the composites. Their compressive strength reaches the level of 0.271±0.057 MPa and 0.373±0.043 MPa for 10% and 20% of strain respectively. The pore size is within the wide range up to 360 μm and can be suitable for the growth of new bone tissue.

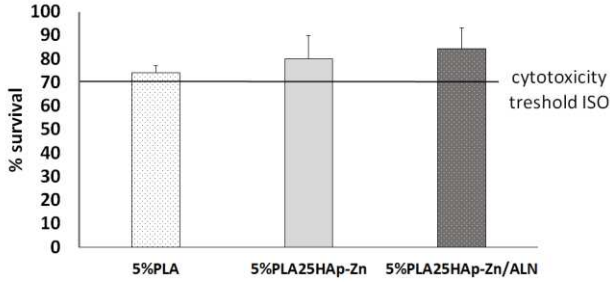

FIG. 2. The results of cytotoxicity assessment according to ISO10993-5:2009.

The effect of direct contact of composites on L929 mouse skin fibroblasts was evaluated in an MTT reduction assay. Percent of survival is shown, in relation to cells grown in the absence of any composite treated as 100%. Percent of survival above 70% is not indicative of a potential cytotoxic effect.

Conclusions

The addition of HAp-Zn whiskers affects the mechanical strength and morphology of porous composites. The surface modification of composite with sodium alendronate allows for a higher % survival of L929 cells.

Acknowledgments

The „Multifunctional composites biologically active for applications in regenerative medicine of bone system" project (POIR.04.04.00-00-16D7/18) is carried out within the TEAM - NET programme of the Foundation for Polish Science financed by the European Union under the European Regional Development Fund.

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