INNOVATIVE MACROPOROUS CHITOSAN/AGAROSE MATRIX-BASED BIOMATERIAL FOR BONE TISSUE ENGINEERING APPLICATIONS

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Introduction

Scaffolds for bone tissue engineering applications should possess good biological and mechanical properties as well as high porosity, especially open and interconnected one. The porosity enhances adhesion, proliferation, and differentiation of osteoprogenitor cells/osteoblasts promoting faster rate of bone ingrowth [1]. The aim of this study was to develop novel macroporous chitosan/agarose matrix-based biomaterial for bone tissue engineering applications and to evaluate its basic structural, mechanical, and biological properties.

Materials and Methods

Porous biomaterial made of chitosan/agarose matrix and hydroxyapatite nanopowder (nanoHA) was fabricated using sodium bicarbonate as a gas-foaming agent and freeze-drying method. The mix of chitosan solution, agarose suspension, gas foaming agent and nanoHA was subjected to a high temperature and subsequently to freezing. Then, the sample was lyophilized to obtain highly porous structure of the biomaterial. The resultant scaffold, marked as chit/aga/nanoHA, was composed of 2% chitosan, 5% agarose, and 40% nanoHA.

Microstructure visualization. The porosity and surface of the biomaterial were determined by microcomputed tomography (μCT) and scanning electron microscopy (SEM), respectively.

Compression test. The Young's modulus value and compressive strength of cylinder-shaped samples were evaluated by Zwick Roell Z2.5 testing machine.

Cell culture experiments. The study was conducted using mouse calvarial preosteoblasts (MC3T3-E1 Subclone 4) obtained from ATCC. The cytotoxicity of the biomaterial was assessed according to ISO 10993-5:2009 by indirect test using fluid extract of the chit/aga/nanoHA scaffold. Cell viability upon exposure to the extract was determined by MTT assay. Cell spreading and morphology on the biomaterial was evaluated by fluorescent staining of nuclei with DAPI and F-actin filaments with AlexaFluor635phalloidin. Stained cells were analysed under confocal microscope.

Statistical analysis. The unpaired t-test was carried out to assess statistical differences between control cells and the cells cultured in the presence of the extract. Statistical significance was considered at a probability with $p < 0.05$ (GraphPad Prism 5, Version 5.03 Software).

Results and Discussion

Microstructure analysis showed that developed biomaterial is characterized by rough surface, highly macroporous structure (total porosity approx. 50%, pore diameter > 100 μm), and relatively high interconnected and open porosity (FIG. 1). According to available literature, interconnected and macroporous structure of biomaterial provides good osseointegration, vascularization, and oxygenation of the implant *in vivo* [2]. However, the chit/aga/nanoHA material revealed low compressive strength and Young's modulus values, which were equal to 1.4 MPa and 13.98 MPa, respectively (TABLE 1). It is worth to emphasize that it is common phenomenon that introduction of high porosity into the biomaterials inferiors their mechanical parameters [3]. MTT assay revealed that chit/aga/nanoHA biomaterial is non-toxic to the cells because osteoblast viability was near 100% compared to the control cells. Moreover, confocal microscope observation confirmed non-toxic character of chit/aga/nanoHA since cells cultured on the biomaterial were well attached and spread (FIG. 2).

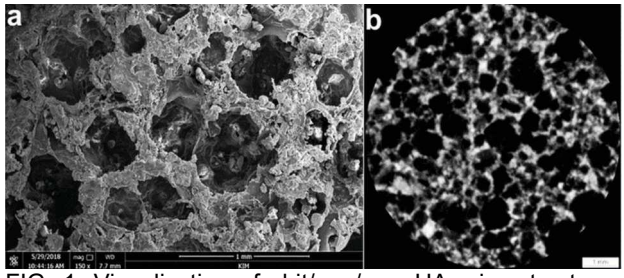

FIG. 1. Visualization of chit/aga/nanoHA microstructure: (a) SEM image of the surface, magn. 150x; (b) μCT cross-section presenting porosity of chit/aga/nanoHA.

TABLE 1. Mechanical properties of chit/aga/nanoHA.

chit/aga/nanoHA scaffold	
Compressive strength [MPa] ± SD	1.4 ± 0.18
Young's modulus [MPa] ± SD	13.98 ± 1.8

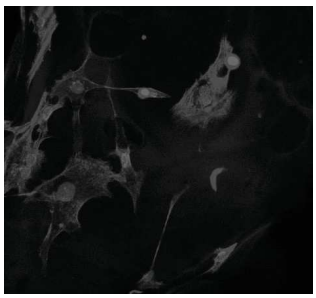

FIG. 2. Confocal microscope image of MC3T3-E1 cells cultured on the biomaterial, magn. 200x.

Conclusions

Obtained results demonstrated that simultaneous application of foaming agent and freeze-drying technique for biomaterial fabrication allows to gain highly macroporous, non-toxic, and supportive to osteoblasts growth biomaterial. Despite relatively poor mechanical properties of fabricated biomaterial limiting its application to non-load bearing implantation areas, the scaffold may be potentially used to generate living grafts under *in vitro* conditions.

Acknowledgments

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