HIGHLY MACROPOROUS CHITOSAN/AGAROSE/HA BONE SCAFFOLD PRODUCED BY COMBINATION OF FREEZE-DRYING WITH GAS-FOAMING AGENT

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Introduction

Typical bone scaffolds have a three-dimensional (3D) porous structure, which provides mechanical support and space for migration and proliferation of osteoblasts as well as mesenchymal stem cells. Importantly, open and interconnected porosity have the most significant biomedical importance since they facilitate bone tissue ingrowth and new blood vessel formation [1]. The aim of this study was to simultaneously apply freeze-drying method with a foaming agent to produce biocompatible and highly macroporous chitosan/agarose/nanoHA biomaterial for bone tissue engineering applications.

Materials and Methods

Biomaterial fabrication. Bone scaffold made of chitosan/agarose matrix reinforced with hydroxyapatite nanopowder (nanoHA) was produced using sodium bicarbonate as a source of CO_2 and freeze-drying method (Polish patent application no. P.426788). The resultant sample, marked as chit/aga/nanoHA, was made of 2% chitosan, 5% agarose, and 40% nanoHA.

Porosity determination. The porosity was evaluated by microcomputed tomography (µCT). CTAnalyser software (Bruker microCT) was used to determine pores diameter as well as total, open and closed porosity.

Biodegradation test. *In vitro* biodegradation test was performed in an enzymatic solution [2] and in citric acid (pH=3) and Tris-HCl (pH=7.4) buffer according to the international procedure for ceramic materials described in ISO 10993-14. Degradation of polysaccharides was determined by detection of reducing sugars, whereas degradation of ceramic component by assessment of the concentrations of Ca^{2+} and HPO_4^{2-} ions.

Biocompatibility tests. The study was conducted using mouse calvarial preosteoblasts (MC3T3-E1 Subclone 4) and mesenchymal stem cells. The cytotoxicity of the biomaterial was assessed according to ISO 10993-5:2009 by agar diffusion indirect test. Osteoblast growth on the biomaterial was visualized by fluorescent staining of cell nuclei (DAPI) and cytoskeleton (AlexaFluor635phalloidin). Osteogenic differentiation on the scaffold surface was evaluated using mesenchymal stem cells by ELISAs and immunofluorescent staining.

Bioactivity test. *In vitro* apatite-forming ability assay was conducted in accordance with ISO 23317 procedure (2014). Briefly, bone scaffolds were immersed in the simulated body fluid (SBF) for 28 days and then the samples were analysed using SEM equipped with EDS detector to calculate the Ca/P atomic ratio (confirmation the presence of apatite crystals).

Results and Discussion

Produced scaffold exhibited highly macroporous structure (total porosity 63%) with a high share of macropores with diameter in the range 100-410 µm (FIG. 1, TABLE 1). According to available literature, macroporous structure of biomaterial provides good osseointegration, vascularization, and oxygenation of the implant *in vivo* [3,4]. Biomaterial was prone to enzymatic degradation, degradation in acidified microenvironment (e.g. osteoclast-mediated), and slow degradation under physiological pH of 7.4. Biocompatibility tests showed that novel scaffold was non-toxic, favoured osteoblasts growth (FIG. 2), promoted osteogenic differentiation, and induced apatite formation, indicating its high bioactivity which is essential for good implant osseointegration.

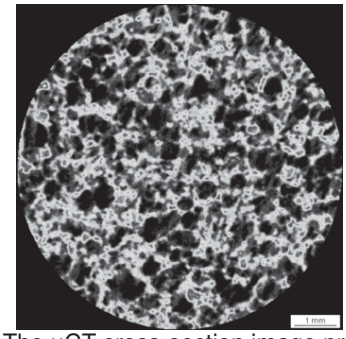

FIG. 1. The µCT cross-section image presenting microstructure of the chit/aga/nanoHA scaffold.

TABLE 1. Porosity of the scaffold assessed using µCT.

Type of porosity		
closed [%]	**open [%]**	**total [%]**
25.80	37.24	60.05

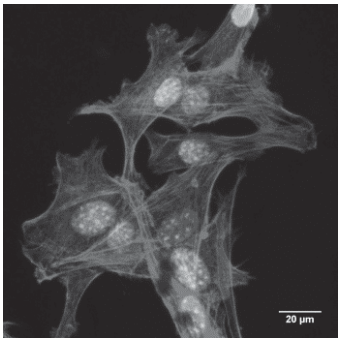

FIG. 2. Confocal microscope image of MC3T3-E1 cells cultured on the biomaterial.

Conclusions

Produced by novel method macroporous biomaterial has great potential to be used in regenerative medicine for acceleration of bone healing process.

Acknowledgments

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References

[1] M. Mour et al., Materials. 3 (2010) 2947–2974.
[2] S. Gorgieva, V. Kokol, J. Biomed. Mater. Res. - Part A. (2012). doi:10.1002/jbm.a.34106.
[3] N. Sachot, E. Engel, O. Castano, Curr. Org. Chem. 18 (2014) 2299–2314.
[4] A. Przekora, Mater. Sci. Eng. C. 97 (2019) 1036–1051. doi:10.1016/J.MSEC.2019.01.061.