EVALUATION OF CELLULOSE/HYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE ENGINEERING: STUDIES *IN VITRO* AND *IN VIVO*

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Introduction

Nowadays, development of three-dimensional (3D) scaffolds for bone regeneration is one of current challenges in the tissue engineering. A variety of materials are proposed for the fabrication of 3D scaffolds. The main requirements for such scaffolds are as follow: (i) the structure similar to that of a natural bone; (ii) interconnected pores suitable for fast nutrition and metabolites diffusion; (iii) biocompatibility; (iv) osteoconductive and osteoinductive properties [1].

The aim of this work was to evaluate novel cellulose-based scaffolds with immobilized microhydroxyapatite (cellulose/μHA) and nanohydroxyapatite (cellulose/nHA) for the bone tissue engineering. Previously it was shown that the morphology of the scaffolds, i.e. porosity, pore size, framework thickness, corresponds to that of natural bone [2]. In this work results of *in vitro* and *in vivo* studies are presented.

Materials and Methods

Biocompatibility and potential toxicity of the cellulose/HA scaffolds were tested by determination of cell viability, cell membrane integrity and the response to insulin. Hepatocytes and the extensor digitorum longus muscle tissue isolated from rats were used for the studies. Membrane integrity was tested by evaluating of the lactate dehydrogenase (LDH) and aldolase release from the cells after their incubation with the scaffolds. Metabolic effects of cellulose/HA composites were studied by evaluating liver and muscle cells sensitivity to insulin after 90 min incubation with the composite samples. To analyze the insulin-induced glucose uptake by the cells the 2-D-[^{3}H] glucose was used.

In vivo studies were performed using the mouse as well as the rabbit model. The scaffolds were implanted subcutaneously in the back of mice and harvested after 2 weeks, 1 and 3 months of the implantation for the histological examination. Using rabbit model the scaffolds were implanted into a calvaria bone and harvested after 2, 4 and 12 weeks after the implantation. Then X-ray spectroscopy, microcomputed tomography and histology were used for the examination of the samples.

Results and Discussion

In vitro studies with hepatocytes and the extensor digitorum longus muscle tissue have confirmed that the cellulose/μHA scaffold is not cytotoxic and can be used in contact with biological systems. However, the cellulose scaffolds containing nanoparticles have decreased liver cell viability and increased the release of lactate dehydrogenase and aldolase from hepatocytes and extensor digitorum longus muscle myocytes, respectively (FIG. 1).

Moreover, the cellulose/nHA scaffold significantly reduced the insulin stimulated glycogen synthesis in the liver cells and glucose uptake by myocytes.

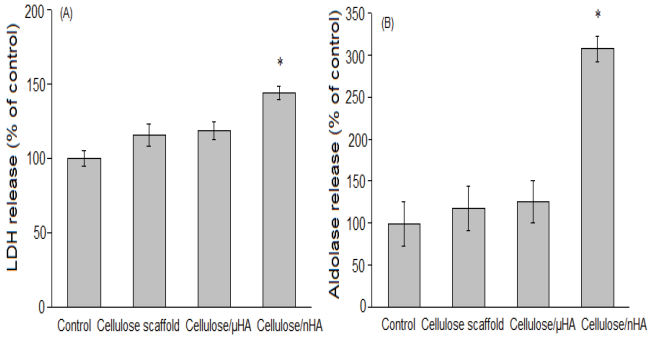

FIG. 1. Effect of the scaffolds on LDH (A) and aldolase (B) release in the incubation medium of cells.

In vivo studies with mice did not show significant differences between cellulose/μHA and cellulose/nHA. In both cases no wound complications were observed. After 2 weeks of implantation, the histological analysis showed that there was the inflammatory response of the surrounding tissue. The scaffold was surrounded with a fibrous and collagenous tissue capsule. The histological analysis of specimens harvested after 1 and 3 months revealed the biocompatibility of the scaffolds. The connective tissue proliferated within the scaffolds, angiogenesis was also expressed.

In vivo studies with rabbits revealed osteoconductive properties of the scaffolds. The scaffolds induced a fast new bone formation. After 12 weeks of implantation appropx. 20 % of a newly formed bone within the defect was observed (FIG. 2).

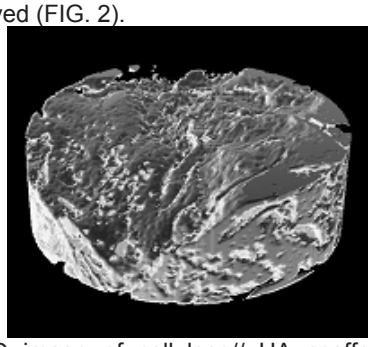

FIG. 2. 3D image of cellulose//μHA scaffold after 12 weeks of implantation in the rabbit calvaria.

The results were similar to those obtained with commercial allogenic and xenogenic bone blocks implanted for comparison.

Conclusions

The *in vitro* and *in vivo* studies showed that the cellulose//μHA scaffold is osteoconductive and non-cytotoxic. Thus, it has a high potential to be used as an implantable material in bone defects. However, cellulose scaffolds with nanosized HA particles have demonstrated slight cytotoxicity in the studies *in vitro*.

Acknowledgments

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