Abstract (ANNIC, Barcelona 2016)
http://premc.org/annic2016/
9th November - 13:30 - Poster Session - Gallery - Poster presentation - Abstract ID: 110

Preparation and testing of collagen-based nanocomposite scaffolds for tissue engineering and bone implantology.

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Introduction

Nanotechnologies represent a perspective approach which enable regeneration or substitution of an impaired connective tissues. In our study we focused on preparation and testing of biocompatible nanocomposite scaffolds which can imitate a bone matrix and could be potentially applied in bone surgery and implantology.

Methods

Our scaffolds are based on natural collagen matrix isolated from fish skin supplemented with sodium hyaluronate and natural calcium phosphate nano-particles (bioapatite) isolated from bovine bone and reinforced by poly(DL-lactide) electrospun nanofibers.

Structure, degradation, and chemical properties of the scaffolds were characterized using infrared spectrometry (FTIR), scanning electron microscopy (SEM), by means of the determination of mass loss, swelling ratio and pH.

We tested three different cross-linking agents to improve the mechanical properties and stability of the scaffolds: N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide in an ethanol solution (EDC/NHS/EtOH), EDC/NHS in a phosphate buffer saline solution (EDC/NHS/PBS) and genipin. To find out the most suitable scaffold for cell adhesion and tissue engineering application we monitored the effect of these cross-linkers and preparation conditions on the pore size, structure and mechanical properties of the scaffolds. The swelling ratio and also the pH of the scaffolds were assessed using their immersion in a cell culture medium. Moreover, we measured the metabolic activity of human mesenchymal stem cells (hMSCs) cultivated in scaffold infusions for 2 and 7 days as well as cell adhesion, proliferation and cell penetration into our scaffolds using confocal microscopy visualization.

Results and Discussion

Based on these tests we found out that EDC/NHS/PBS and genipin formed the most effectively cross-linked and stable biomaterials. The scaffolds cross-linked with EDC/NHS/PBS embodied a low degradation together with a low swelling ratio. The genipin cross-linked scaffold has shown the best conditions for hMSC cultivation. No cytotoxicity was proved in infusions from all the tested scaffolds. The results of our experiments suggest that our collagen-based scaffolds cross-linked by both genipin and EDC/NHS/PBS are perspective biomaterials for further *in vivo* testing and bone surgery applications.

Acknowledgments: This study was supported by a grant project provided by the Ministry of Health of the Czech Republic (NV 15-25813A).