Growth Factor Delivery Systems that Mimic Natural Extracellular Matrix and Supply Biological Molecules in Bone Tissue Engineering

W. Swieszkowski, E. Kijeńska, K. Zalewska-Wierzbicka, K.J. Kurzydlowski
Faculty of Materials Science and Engineering, Warsaw University of Technology, Woloska 141, Warsaw 02-507, Poland.

Statement of Purpose: Every year more than million patients suffer from the consequences of large critical-sized bone defects caused by injury or resection. A treatment of such defects remains a challenge for orthopaedic surgeons. Bone grafts or permanent implants, which are most common way of treatment these days do not guarantee recovery of the bone tissue or full mobility of the organ. This stimulates the search for other strategies for bone repair. On of the most promising approach is a tissue engineering (TE) which is based on delivery cells to the site of implantation with proper scaffolds, which must be biocompatible, biodegradable and mimic the multidimensional hierarchical structure of native bone. Since in vivo bone healing and repair are driven by a complex cascade involving a number of growth factors and cytokines, a delivering scaffold and cells alone may not be sufficient for tissue regeneration. Thus, it is crucial to address the issue of controlled delivery of growth factors in strategies for bone tissue engineering. An ideal scaffold should be able to delivery bioactive molecules e.g., BMPs, TGFs, VEGF etc., to accelerate extracellular matrix production and tissue integration. However, a delivering appropriate combinations of factors, in proper concentration, timing, kinetics and sequence in which they are introduced are still a challenge [1],[2].
The aim of this project is to develop a novel hybrid growth factors delivery system that would mimic extracellular matrix of the native tissue and allows for localized and controlled delivery of biological molecules in bone tissue engineering.

Methods: Synthetic polymer P(LLA-CL) (Poly-L-lactic acid -co-poly-ε-caprolactone) [3] and model proteins as well as nanobioceramics were used in this study. Electrospinning method was utilized to produce nanofibrous meshes from polymer-protein blend, while coaxial electrospinning was used to obtain core-shell protein-polymer nanofibers. By using this core-shell technology the release kinetics of the bioactive molecules separately or together can be manipulated.
The setup for fabrication of core-shell nanofiberes was developed based on coaxial electrospinning method. In a coaxial electrospinning process, two polymer solutions were concomitantly eletrospuned in an electrostatic field through two coaxial capillaries and result in a core-shell structured nanofiber. The shell thickness of nano-objects was controlled in the process to have different "membranes" controlling release profile. The method of fabrication of polymeric nanofibers containing nanobioceramic was also elaborated and tested.
The obtained delivery systems based on nanofibers were then investigated in order to characterize in detail the original 3D structure in terms of pore size, struts thickness and degree of pore interconnection using HRSEM, TEM and AFM. Moreover, the nano-microCT analysis allowed studying the morphological properties of composite nanofibers. The degradation of the scaffold structure and properties was also evaluated. FTIR spectroscopy was used to assess the presence of the proteins in the nanofibers.
Results: The core-shell biocomposite nanofibers with incorporated a model protein was fabricated using developed setup (Figure 1). Nanofibrous systems with encapsulated model protein with a diameter from 300 to 400 nm and porosity from 70 to 80 % were successfully fabricated and comparative studies on the release of the model protein have been carried out along with morphology, topography and mechanical properties evaluation. The FTIR investigation shown the model proteins survived the electrospinning process.

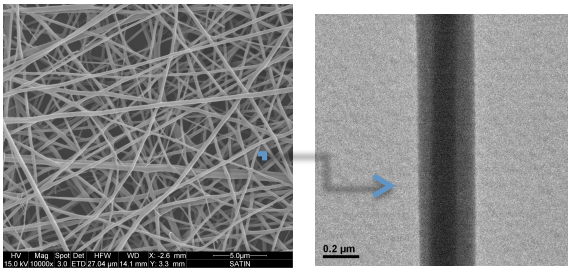

Figure 1. SEM and TEM images of core-shell electropsun fibres

Conclusions: Our studies confirmed that application of different method of fabrication of the nanofibers allows for obtaining bio-active agent delivery systems with different kinetics of release of growing factors, based on different mechanism of releasing. Blended systems reveal initial burst release of the proteins. However, release from the core-shell nanofibrous systems presented sustained manner, which is related to incorporation of the protein within the different nanofibers.

References: [1] T.P. Richardson, M.C. Peters, A.B. Ennett and D.J. Mooney, Polymeric system for dual growth factor delivery. Nat. Biotechnol. 19 (2001), pp. 1029–1034. [2] R. Vasita, D.S. Katti: Nanofibers and their applications in tissue engineering Internat. JNanomedicine 2006:1 (1) 15-30. [3] E. Kijeńska, M.P. Prabhakaran, W. Swieszkowski, K.J. Kurydlowski, S. Ramakrishna: Electrospun bio-composite P(LLA-CL)/collagen I/collagen III scaffolds for nerve tissue engineering. J. Biomed. Mat. Research Part B: Applied Biomaterials 2012,100(4):1093-102
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