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Mesenchymal Stem Cells on Fibrin Assemblies with Growth Factors

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Abstract : Decellularized vessels have been evaluated as small-diameter vascular prostheses. Reseeding autologous cells onto decellularized tissue prior implantation should prolong prostheses function and make them living tissues. Suitable cell types for reseeding are both endothelial cells and bone marrow-derived stem cells, with a capacity for differentiation into smooth muscle cells upon mechanical loading. Endothelial cells assure antithrombogenicity of the vessels and MSCs produce growth factors and, after their differentiation into smooth muscle cells, they are contractile and produce extracellular matrix proteins as well. Fibrin is a natural scaffold, which allows direct cell adhesion based on integrin receptors. It can be prepared autologous. Fibrin can be modified with bound growth factors, such as basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF). These modifications in turn make the scaffold more attractive for cells ingrowth into the biological scaffold. The aim of the study was to prepare thin surface-attached fibrin assemblies with bound FGF-2 and VEGF, and to evaluate growth and differentiation of bone marrow-derived mesenchymal stem cells on the fibrin (Fb) assemblies. Following thin surface-attached fibrin assemblies were prepared: Fb, Fb+VEGF, Fb+FGF2, Fb+heparin, Fb+heparin+VEGF, Fb+heparin+FGF2, Fb+heparin+FGF2+VEGF. Cell culture poly-styrene and glass coverslips were used as controls. Human MSCs (passage 3) were seeded at the density of 8800 cells/1.5 mL alpha-MEM medium with 2.5% FS and 200 U/mL aprotinin per well of a 24-well cell culture. The cells have been cultured on the samples for 6 days. Cell densities on day 1, 3, and 6 were analyzed after staining with LIVE/DEAD cytotoxicity/viability assay kit. The differentiation of MSCs is being analyzed using qPCR. On day 1, the highest density of MSCs was observed on Fb+VEGF and Fb+FGF2. On days 3 and 6, there were similar densities on all samples. On day 1, cell morphology was polygonal and spread on all sample. On day 3 and 6, MSCs growing on Fb assemblies with FGF2 became apparently elongated. The evaluation of expression of genes for von Willebrand factor and CD31 (endothelial cells), for alpha-actin (smooth muscle cells), and for alkaline phosphatase (osteoblasts) is in progress. We prepared fibrin assemblies with bound VEGF and FGF-2 that supported attachment and growth of mesenchymal stem cells. The layers are promising for improving the ingrowth of MSCs into the biological scaffold. Supported by the Technology Agency of the Czech Republic TA04011345, and Ministry of Health NT11270-4/2010, and BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University" project (CZ.1.05/1.1.00/02.0109), funded by the European Regional Development Fund for their financial supports.

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