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Surface Adjustments for Endothelialization of Decellularized Porcine Pericardium

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Abstract : The porcine pericardium is used as a material for cardiac and aortic valves substitutes. Current biological aortic heart valve prosthesis have a limited lifetime period because they undergo degeneration. In order to make them more biocompatible and prolong their lifetime it is necessary to reseed the decellularized prostheses with endothelial cells and with valve interstitial cells. The endothelialization of the prosthesis-surface may be supported by suitable chemical surface modification of the prosthesis. The aim of this study is to prepare bioactive fibrin layers which would both support endothelialization of porcine pericardium and enhance differentiation and maturation of the endothelial cells seeded. As a material for surface adjustments we used layers of fibrin with/without heparin and some of them with adsorbed or chemically bound FGF2, VEGF or their combination. Fibrin assemblies were prepared in 24-well cell culture plate and were seeded with HSVEC (Human Saphenous Vein Endothelial Cells) at a density of 20,000 cells per well in EGM-2 medium with 0.5% FS and without heparin, without FGF2 and without VEGF; medium was supplemented with aprotinin (200 U/mL). As a control, surface polystyrene (PS) was used. Fibrin was also used as homogeneous impregnation of the decellularized porcine pericardium throughout the scaffolds. Morphology, density, and viability of the seeded endothelial cells were observed from micrographs after staining the samples by LIVE/DEAD cytotoxicity/viability assay kit on the days 1, 3, and 7. Endothelial cells were immunocytochemically stained for proteins involved in cell adhesion, i.e. alphaV integrin, vinculin, and VE-cadherin, markers of endothelial cells differentiation and maturation, i.e. von Willebrand factor and CD31, and for extracellular matrix proteins typically produced by endothelial cells, i.e. type IV collagen and laminin. The staining intensities were subsequently quantified using a software. HSVEC cells grew on each of the prepared surfaces better than on control surface. They reached confluency. The highest cell densities were obtained on the surface of fibrin with heparin and both grow factors used together. Intensity of alphaV integrins staining was highest on samples with remained fibrin layer, i.e. on layers with lower cell densities, i.e. on fibrin without heparin. Vinculin staining was apparent, but was rather diffuse, on fibrin with both FGF2 and VEGF and on control PS. Endothelial cells on all samples were positively stained for von Willebrand factor and CD31. VE-cadherin receptors clusters were best developed on fibrin with heparin and growth factors. Significantly stronger staining of type IV collagen was observed on fibrin with heparin and both growth factors. Endothelial cells on all samples produced laminin-1. Decellularized pericardium was homogeneously filled with fibrin structures. These fibrin-modified pericardium samples will be further seeded with cells and cultured in a bioreactor. Fibrin layers with/without heparin and with adsorbed or chemically bound FGF2, VEGF or their combination are good surfaces for endothelialization of cardiovascular prostheses or porcine pericardium based heart valves. Supported by the Ministry of Health, grants No15-29153A and 15-32497A, and the Grant Agency of the Czech Republic, project No. P108/12/G108.

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