ENDOTHELISATION OF DECELLULARIZED PERICARDIUM WITH HEPARINIZED FIBRIN COATINGS IN IN-VITRO BIOREACTOR

ROMAN MATEJKA[1,2]*, JANA STEPANOVSKA[1], JANA ZARUBOVA[2], TOMAS RIEDEL[3], ZUZANA RIEDELOVA[3], MIROSLAV KONARIK[4], JAN PIRK[4], LUCIE BACAKOVA[2]

[1] FACULTY OF BIOMEDICAL ENGINEERING, CZECH TECHNICAL UNIVERSITY IN PRAGUE, CZECH REPUBLIC
[2] INSTITUTE OF PHYSIOLOGY, CZECH ACADEMY OF SCIENCES, CZECH REPUBLIC
[3] INSTITUTE OF MACROMOLECULAR CHEMISTRY, CZECH ACADEMY OF SCIENCES, CZECH REPUBLIC
[4] INSTITUTE FOR CLINICAL AND EXPERIMENTAL MEDICINE, CZECH REPUBLIC
*E-MAIL: ROMAN.MATEJKA@FBMI.CVUT.CZ

[ENGINEERING OF BIOMATERIALS 143 (2017) 63]

Introduction
Decellularized matrices hold a great promise in advanced tissue engineering and repair of irreversibly damaged tissues in cardiovascular surgery. A cross-linked xenopericardium is commonly used as a patch in cardiac surgery but it didn't facilitate cell ingrowth and remodelling. Coating these matrices with autologous fibrin with covalently attached heparin and grow factors (FGF-1, FGF-2, VEGF) can minimize the thrombogenicity and can act as attractants to promote spontaneous endothelisation. *In-vitro* simulation of physiological conditions like those in blood vessels creates a tool for optimizing these coatings and their translation in to *in-vivo* experiments.

Materials and Methods
There are three possible ways of endothelization of patches in body: trans-anastomotic, trans-mural and blood/bone marrow-derived. For simulating these physiological conditions i*n-vitro* a special cultivation chamber with computer controlled perfusion system was created. The cultivation chamber allows fixing decellularized pericardium tissue and creates two compartments on each side of tissue. In this chamber the decellularized pericardium is coated with fibrin (with heparin and/or grow factors). Each side of pericardium can have different coating or different culture medium to creating concentration gradients. The endothelial cells (HUVEC) or stem cells (ASC) in suspension are seeded in thin strip shape on pericardium. Coating and seeding is done via sterile septum. The perfusion system creates two types of physiological stimuli simulating conditions in blood vessels. The controlled flow generates shear stress in physiological range. The pressure stimulation creates pulsatile mechanical loading.
After initial adhesion of the cells the perfusion system is activated to create dynamic physiological conditions. After defined period (7 to 21 days) the tissue removed from chamber. This tissue is histologically evaluated to get information about cell migration and proliferation and their ability to in grow into tissue based on the coatings and grow factor concentration.

Results and Discussion
Coatings of pericardium with fibrin and with covalently attached heparin and grow factors improve the cell proliferation and their migration over scaffold in contrast to only decellularized pericardium. The optimal concentration of grow factors must set based on further analysis. Also, dynamic cultivation, unlike static, provides better response of cells e.g. their orientation and morphology caused by flow and mechanical loading simulating more *in-vivo* like conditions.

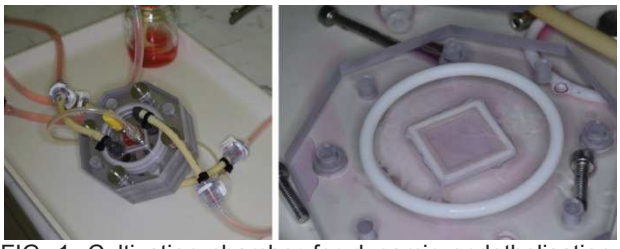

FIG. 1. Cultivation chamber for dynamic endothelisation of decellularized pericardium connected to perfusion system and sterile ports (left), fixed decellularized tissue in chamber (right).

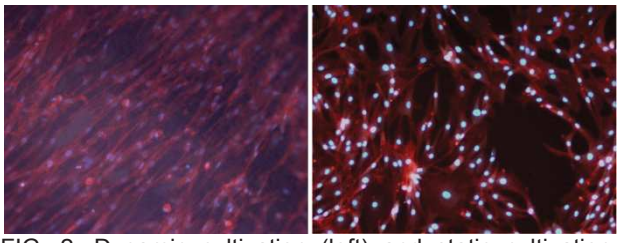

FIG. 2. Dynamic cultivation (left) and static cultivation (right).

Conclusions
Heparinized fibrin coatings with grow factors on decellularized pericardium promoted its endothelisation. Designed *in-vitro* bioreactor provides tool for optimizing these coatings and their translation to *in-vivo* experiments.

Acknowledgments
This work was supported by Ministry of Health of the Czech Republic, grant nr. 15-29153A and by 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

References
[1] Sakiyama-Elbert SE and Hubbell JA. Development of fibrin derivatives for controlled release of heparin binding growth factors. J. Control. Release 2000; 65:389-402
[2] Zisch, A.H., et al. Covalently conjugated VEGF-fibrin matrices for endothelialization J Control Release.2001; 72:101-113.
[3] Kaplan O., Zarubova J., Mikulova B., Filova E., Bartova J., Bacakova L., Brynda E. Enhanced mitogenic activity of recombinant human vascular endothelial growth factor VEGF121 expressed in E. coli Origami B (DE3) with molecular chaperones (2016) PLoS ONE, 11 (10)