Göteborg, 2020

ON TISSUE ENGINEERING OF PIG, HUMAN, AND NON-HUMAN PRIMATE TISSUES

AKADEMISK AVHANDLING

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Hjärtats Aula, Vita stråket 12 Sahlgrenska Universitetssjukhuset, fredagen den 29th Maj, klockan 13:00

av

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Avhandlingen baseras på följande delarbeten:

I. Methe K, Bäckdahl H, Johansson B R, **Nayakawde N**, Dellgren G, Sumitran-Holgersson S, 2014, An Alternative Approach to Decellularize Whole Porcine Heart. *BioResearch Open Access*, 3(6), 327-338.

II. **Nayakawde NB**, Methe K, Banerjee D, Berg M, Premaratne GU, and Olausson M, 2020, *In Vitro* Regeneration of Decellularized Pig Esophagus Using Human Amniotic Stem Cells. *BioResearch Open Access*, 9.1, 22-36.

III. **Nayakawde NB**, Methe K, Premaratne GU, Banerjee D, and Olausson M. Combined Use of Detergent and Ultrasonication for Generation of an Acellular Pig Larynx. (Submitted)

IV. **Nayakawde NB**, Sihlbom C, Thorsell A, Banerjee D, Premaratne GU, Ul Haq U, Rivas Wagner K, Berg M, and Olausson M. Investigation of Extracellular Matrix Proteins in Decellularized Pig, Human, and Baboon Esophagus by Proteomics. (in manuscript)

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR KLINISKA VETENSKAPER

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ON TISSUE ENGINEERING OF PIG, HUMAN, AND NON-HUMAN PRIMATE TISSUES

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ABSTRACT

Background: Demand for donor organs for transplantation has been increasing every year more than the actual supply of suitable donor organs. One of the major problems associated with allogeneic transplantation includes lifelong immunosuppression. Tissue engineering and regenerative medicine is a growing field that uses knowledge of stem cell biology, developmental biology, immunology, and bioengineering to replace diseased and damaged tissues or organs. Tissue engineered (TE) hollow organs and tissues derived from natural extracellular matrix (ECM) have been used in several preclinical and clinical studies. More complex three-dimensional organs such as heart, liver, lungs, and kidney have been studied extensively both *in-vitro* and *in-vivo* in preclinical settings, but clinical experience is lacking. There is an increasing demand for understanding the composition of ECM, cell-ECM interaction *in-vitro* and *in-vivo*, and how tissue engineered organs behave immunologically after implantation. The current thesis focuses on investigation of decellularization methods for heart (porcine), esophagus (porcine, baboon, and human) and larynx (porcine); and recellularization of esophagus (porcine and human). It was also investigated during various time-points of recellularization if stem cells were able to synthesize ECM proteins, tissue specific proteins and growth factors, and if stem cells were able to differentiate into tissue-specific cells. **Methods**: In Paper I, a detergent based decellularization method was developed to create acellular whole porcine hearts. The cardiac ECM was then characterized for its structural and mechanical properties. In Paper III, physical and chemical methods were developed to decellularize porcine larynx. Decellularized larynx was analyzed microscopically for its ultrastructural changes and presence of cells. In Paper II, decellularization and recellularization (with human amniotic mesenchymal stem cells and epithelial cells) of porcine esophagus was carried out. In Paper IV, decellularization of pig, baboon, and human esophagus was performed as per the method described in Paper II. Paper IV studied the cell-ECM interaction during recellularization of human esophagus with human amniotic mesenchymal stem cells by using the stable isotope labeling with amino acids in cell culture (SILAC) technique. **Results:** Decellularization of heart, larynx, and esophagus was achieved successfully, with loss of cell nuclei, preservation of major ECM proteins such as collagen and elastin, preservation of growth factors, and maintaining three-dimensional structures of the tissues and organs. Decellularized esophagus was characterized by preservation of matrisome and non-matrisome proteins in the ECM using proteomics-bioinformatics analyses. Recellularization of pig and human esophagus was evidenced by stem cell proliferation, differentiation, and tissue specific protein synthesis by seeded stem cells. SILAC assay showed synthesis of newly produced proteins in the recellularized esophagus by seeded stem cells including ECM (collagens and fibronectin), cell-ECM signaling molecules (integrins), ECM regulators, secreted factors, skeletal muscle proteins, and proteins required for contraction of striated muscle. **Conclusions:** The decellularization protocol for heart, larynx, and esophagus was effective in removing cells while preserving ECM. Recellularization of esophagus showed the potential of human amniotic-derived stem cells for different tissue engineering applications. The SILAC based proteomics method can replace use of conventional proteomics in TE field to differentiate between cell and ECM proteins

Keywords: Decellularization, esophagus, heart, larynx, proteomics, recellularization, SILAC, stem cells, tissue engineering

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