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TRANSPLANTATION OF NORMAL AND DECELLULARIZED SYNGENEIC, ALLOGENEIC AND XENOGENEIC CARDIAC TISSUE IN MICE AND NON-HUMAN PRIMATES

AKADEMISK AVHANDLING

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i M106 K Isaksson hörsal, Medicinargatan 16, fredagen den 27 Mars, klockan 9.00

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.; An Alternative Approach to Decellularize Whole Porcine Heart, *BioResearch Open Access*. 2014 December; 3(327-336).

II. **Methe K**., Wagner K.R., Nayakawde N.B., Banerjee D., Patil P., Antony D., Travnikova G., Premaratne G.U., Olausson M.; Immune response to normal and decellularised scaffolds in mouse, pig and non-human primates (Submitted)

III. **Methe K**., Nayakawde N., Ghosh S., Sihlbom C., Wagner K.R., Patil P., Premaratne G.U., Olausson M.; Comparative proteomics of decellularized cardiac scaffolds from mouse, pig and non-human primates (Submitted)

IV. **Methe K**., Nayakawde N., Banerjee D., Travnikova G., Olausson M.; Cross-talk Between Different Immune Regulatory Pathways Determines the Fate of Decellularized Grafts After Implantation (Submitted)

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Introduction

Because of the restricted inherent capacity of regeneration and healing, transplantation is the only treatment for end-stage heart failure but it has limitations of donor shortage and graft rejection. Cardiac tissue engineering strategies might help to alleviate this problem. Organ specific, biocompatible and biodegradable extracellular matrices (ECM) could be the preferred option for a suitable scaffolding material. However, clinical trials with these matrices is still not routine practice. Experimental and clinical data suggest variable outcomes after implanting decellularized (DC) scaffolds. Hence, the immunological properties of ECM and future perspectives need to be addressed. In this report we chose to study cardiac ECM to better understand its immune potential.

Methods

A decellularizing protocol using whole porcine heart using Triton X-100 and SDC was developed. The resultant acellular cardiac matrix was analyzed for its structural, functional and mechanical strength. The same protocol was adapted to DC mouse, pig and baboon cardiac hearts. The DC ECM was further tested for its immunological potential by implantation into mouse and baboon recipients, followed by histological and immune histological examinations. Further evaluation of the immunological properties was carried out by proteomics-bioinformatics studies using Mass Spectrometry analysis at the University Core facility. Finally, DC ECM from mouse and pig were implanted into mouse recipients to better understand responses to ECM in syngeneic, allogeneic and xenogeneic settings.

Results

Serial perfusion of Triton X-100 and SDC was effective in removing all cellular and nuclear materials from whole porcine hearts. It thoroughly decellularized ECM scaffolds from cardiac tissue with cytoskeletal elements of cardio-myocytes remaining largely intact. We observed differences in the immune response for the same ECM scaffolds in mouse and baboon recipients, respectively. In mouse, the responses were more donor specific, and allogeneic scaffolds had a higher immune potential than syngeneic scaffolds. Furthermore, proteomic-bioinformatic analyses revealed the presence of protein S-100, α-laminin and annexin A1 in the mouse DC ECM. In pig scaffolds, we identified, coagulation factor V (fV), fibrilline, spondin 1, and hyaluronan, whereas insulin growth factor (IGF) and periostin were observed in DC ECM of baboon scaffolds. These proteins are known to be immunomodulatory and their immune potential in regard to DC ECM scaffolds should be further tested. We found a distinct immune response for syngeneic, allogeneic and xenogeneic scaffolds after implantation. The allogeneic and xenogeneic immune responses were both T-cell driven, however, the development of these responses were different for DC ECM of allogeneic and xenogeneic scaffolds, respectively.

Conclusion

DC ECM has favorable properties as a scaffolding material. The evaluation protocol of DC processes described in this thesis requires further development of the immunological potential. The host immune responses cannot be generalized as they are donor species specific. The ECM proteins themselves seem to be immunogenic which might explain differences in the distinct immune responses shown by the recipients.

Keywords: Decellularization, Cardiac tissue, Syngeneic, Allogeneic, Xenogeneic, Non-human primate

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