Using Natural Extracellular Matrix Platforms Towards Engineering of Thick Cardiac-Like Tissue Constructs

Udi Sarig & Marcelle Machluf
Faculty of Biotechnology and Food Eng., Technion – Israel’s Institute of Technology
udsa@tx.technion.ac.il, machlufm@tx.technion.ac.il

Introduction: We have previously reported the successful isolation of thin porcine cardiac ECM (pcECM) slices which manifested bio-mechanical properties relevant for myocardial tissue engineering [1]. As cardiac tissue could reach a thickness of 12-15 mm, the development of thin constructs offers limited regeneration capacity. Currently, the achievement of thick myocardial-like tissue constructs is limited due to diffusion limitations (~100 µm), and the lack of proper vascular network enabling the supply of nutrients and oxygen [2]. Thus the development of a support system that would enable the cultivation of thicker constructs is required. The present work focuses on the optimization of the decellularization procedure for thicker tissue constructs and the development of a novel supportive bioreactor system.

Materials & Methods: Our previously reported decellularization procedure [1] was optimized to obtain thick pcECM (10-15 mm) was conducted by increasing trypsin activity and the introduction of sonication and/or perfusion through built-in vasculature. Histological cross-sections were evaluated using Masson trichrome and oil-red staining. Isolated thick pcECM was evaluated by SEM and two-photon microscopy for the preservation of ultrastructural morphologies. pcECM immunogenicity was evaluated using TNFα secretion by mouse bone marrow derived macrophages (BMM). Vascular network preservation was evaluated using corrosion casting and pulsate feeding of fluorescently labeled dextran in a newly developed bioreactor prototype imaged using multispectral imaging.

Results: The Increase of trypsin activity as well as the use of sonication and/or perfusion enabled a better decellularization procedure compared to control (Fig. 1). No cellular remains (Red for cytoplasm and black for nuclei) were observed with Masson trichrome staining. Oil-red showed remaining of adipocytes. SEM and multiphoton microscopy showed preserved structural characteristics, supportive of cellular growth (Fig. 2). Realtime RT-PCR analysis of the TNFα/GAPDH expression ratio in BMM, revealed low stimulation of pcECM exposed cells, compared to native cardiac tissue (Fig. 3). Vascular network functionality was preserved to the first three-four branches from the main coronary vessels (Fig. 4). A novel supportive bioreactor system is currently being developed (Fig. 5).

Conclusions: We have successfully isolated thick pcECM which is non immunogenic and preserves ultrastructural properties as well as inherent vascular network. A novel bioreactor system is currently being developed that would enable the control of pulsate flow, electrical and mechanical pre-conditioning. Future work will focus on the evaluation of different cell population seeding, cultivation under static conditions, and the optimization of dynamic cultivation parameters.

References

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Disclosures
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