Title of the project: Muscle remodelling on the basis of extracellular matrix seeded with functionally characterized cells

Grant Agency: Czech Republic ***Project Number:*** 15-09161S

Principal Investigator: S. Filip

Co-investigators: J. Mokrý, G. Dayanithi, O. Forostyak, H. Hrebíková, R. Pisal, J. Chvátalová, D. Čížková

Starting date: 1.1.2015 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 5794

Summary of 2015 results

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Authors: S. Filip (1), J. Mokrý (2), G. Dayanithi (2), O. Forostyak (2), H. Hrebíková (2), R. Pisal (2), J. Chvátalová (2), D. Čížková (2).

Department of Oncology and Radiotherapy (1); Department of Histology and Embryology (2).

In the proposed project, we suggest a novel approach to muscle organ reconstruction based on colonization of decellularized extracellular matrix (ECM) isolated from the skeletal muscle with muscle-derived cells. Optimalized cell types for recellularization are selected on the basis of their phenotypical and electrophysiological properties. Analysis of bioscaffolds alone and recellularized scaffolds implanted to animals performed at defined survival intervals includes a spectrum of morphological and molecular biological methods aimed at assessment of changes in ECM remodelling inside the graft, examination of cell activity and evaluation of function of reconstructed muscles. The results could be exploited for skeletal muscle reconstruction as well as repair of other organs.

Preliminary results show that muscle derived cells respond to caffeine, ryanodine, CPA, high K+ and glutamate but the responses to ATP were highly pronounced, observed in the absence of external Ca2+, inhibited by P2Y2 inhibitor suggesting the important involvement of P2Y signalling. Mitochondrial and ER Ca2+ uptake seem to play a major role in Ca2+ homeostasis. Our results demonstrate that Ca2+ entry induced by high K+ can be activated by specific L-type Ca2+ voltage-operated calcium channels. Histological examination of decellularized muscle scaffolds confirmed absent nuclei and sarcoplasmic components, presence of glycosaminoglycans and preservation of collagen fibres. Hydroxyproline assay proved preservation of collagen. Preservation of basal lamina was determined with immunostaining of collagen IV. DNA content was significantly reduced under 50 ng of DNA per mg of tissue. Adhesion and viability of the scaffold was confirmed by re-seeding with C2C12 murine myoblasts. Histological examination of scaffolds implanted into the murine tibial anterior muscle confirmed its good integration with the muscle and rich colonization by recipient cells.

We succeeded in producing decellularized skeletal muscle tissue with well-preserved major components (basal lamina, collagen fibres and glycosaminoglycans) that fulfilled requirement for successful decellularization (elimination of cell and nuclear components, reduction of DNA content and preservation of 3D architecture of ECM). Cytocompatibility of scaffold was proved with a successful in vitro recellularization technique and implantation of decelularized scaffold into the mouse muscle, which resulted in rich scaffold recellularization.

Address for correspondence: S. Filip, Dept. of Oncology and Radiotherapy, Charles University in Prague. Faculty of Medicine and Faculty Hospital in Hradec Králové, Šimkova 870, 50038 Hradec Králové, Czech Republic, fillip@fnhk.cz