

Development of biologic scaffolds for reconstructive surgery from decellularized human skeletal muscle

Maria Martina Sfriso¹, Lucia Petrelli¹, Alex Pontini², Vincenzo Vindigni², Andrea Porzionato¹

¹ Section of Human Anatomy, Department of Molecular Medicine, University of Padova, Italy

² Clinic of Plastic Surgery, University of Padova, Italy

The development of engineered skeletal muscle tissues has recently been proposed as a potential solution for replacement of volumetric muscle losses. Experimental studies available in literature have mainly been performed in animals. The aim of the present work was to evaluate the possibility of obtaining homologous acellular scaffolds from decellularization of human skeletal muscle, also through a comparison with rats and rabbits, and evaluating the capability of muscle scaffolds to repair defects of the abdominal wall. Muscle samples of rats, rabbits and humans were treated with two different decellularization methods. Surgical tests were also performed in rabbits, implanting the decellularized scaffold in the abdominal wall of animal with surgical defects of the abdominal wall. Human skeletal muscles were sampled from body parts removed during surgery and given to the Body Donation Program of the Institute of Human Anatomy of the University of Padua. Histological stainings confirmed the effectiveness of decellularization, resulting in cell-free scaffolds with no residual cells in the matrix. The complex three-dimensional networks of collagen (azan-Mallory) and elastic fibers (Van Gieson) were maintained, in the absence of nuclear material (DAPI staining and DNA extraction/quantification). Ultrastructural analyses with transmission and scanning electron microscopy also showed the preservation of collagen and elastic fibres, together with proteoglycan components. The vascular structures in the tissue were also still visible, with preservation of collagen and elastic wall components and loss of endothelial (anti-CD31 and -CD34 immunohistochemistry) and smooth muscle (anti-alpha smooth muscle actin) cells. Scaffold implantations in rabbits gave good results in terms of integration with surrounding structures (in vivo US analyses and post mortem macro/microscopic analyses), although recellularization by surrounding muscle cells was not satisfactory. In conclusion, not only animal (rat and rabbit) skeletal muscles but also human samples may be decellularized to obtain complex 3D scaffolds preserving tissue architecture potentially suitable for recellularization. In rabbits, these scaffolds have maintained some biomechanical properties once implanted, although spontaneous recellularization was not achieved. Further analyses will be necessary to verify the possibility of *in vitro* recolonization of scaffolds by homologous myoblasts and autologous satellite cells, before *in vivo* re-implantation.

Keywords

Skeletal muscle, scaffold, decellularization, regenerative medicine.