Effects of various decellularization processes on the ECM of skeletal muscles: impact on tissue engineering applications

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Abstract

Decellularization of organs and tissues has been widely applied to produce bioactive ECM (extracellular matrix) scaffolds for tissue engineering applications. The natural protein content and tridimensional organization of these scaffolds represent important advantages over synthetic ones. Several decellularization protocols have been described. However, finding an optimal procedure that provides a cell-free matrix whereas preserves the composition, bioactivity and tridimensional structure of the ECM is crucial for future in vivo and in vitro applications. In this study we analyzed the influence of freezing temperatures and distinct detergents on the maintenance of 3D architecture of decellularized skeletal muscles of rats. Biceps femoris, tibialis anterior, medial gastrocnemius and rectus abdominis muscles were harvested from adult male Sprague Dawley rats. Samples having 5mm of thickness were washed in ultra-pure water, frozen at -20°C and -150°C and compared to samples that were kept at 4°C for 24 hours. This was followed by static immersion in either 1% SDS or 1% SDS/10mM Tris solution for 48 hours. Samples were then subjected to incubation in 1% Triton X-100 at 4°C for 2 days and again washed in ultra-pure water. Decellularized muscles were fixed in 4% paraformaldehyde and processed for histological and scanning electron microscopic (SEM) analyses. Preliminary results indicated that freezing muscle samples at -20°C prior to incubation with detergents may enhance and favor decellularization over storage at 4°C or at -150°C. Lower temperature (-150°C) may lead to a more disorganized ECM structure. Immersion of samples in SDS/Tris solution for at least 48 hours favors muscle decellularization over incubation in SDS, at the same period of time. Optimal decellularization process is tissue-dependent. Freezing skeletal muscles prior to decellularization procedures optimize the effects of detergents. Studies have been conducted in order to analyze and compare the bioactivity of these scaffolds prepared by distinct protocols.