A Biologic Scaffold Composed of Skeletal Muscle Extracellular Matrix

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Statement of Purpose: Traumatic skeletal muscle injuries are a common occurrence in both the civilian and military population. Though skeletal muscle possesses a relatively high capacity for regeneration, large volumetric defects or chronic repetitive injuries will result in the formation of non-functional scar tissue and significant morbidity. Clinically, there are no treatment options available for the restoration of functional muscle tissue following large volumetric loss. Biologic scaffolds composed of extracellular matrix (ECM) scaffolds have been shown to promote constructive tissue remodeling when implanted in a site of injury including musculoskeletal injuries. Since ECM is produced by the resident cells of each tissue, it possesses unique, tissue specific characteristics. Therefore, ECM scaffolds prepared from different tissues may provide different signals following in vivo implantation. This tissue specific ECM hypothesis implies that a muscle derived ECM scaffold would provide the optimal environment for muscle regeneration. The goal of the present study was to identify an effective method for skeletal muscle decellularization and to characterize the composition and cell compatibility of the resultant ECM scaffold both *in vitro* and *in vivo*. The findings were compared to a well characterized non-muscle ECM scaffold, specifically, small intestinal submucosa (SIS).

Methods: Muscle ECM (M-ECM) scaffolds were prepared by chemically decellularizing canine skeletal muscle tissue. Cadaveric quadriceps and hamstring muscles were cut into thin cross-sectional sheets and subjected to lipid extraction using chloroform/methanol, partial enzymatic digestion with trypsin, and removal of cellular content with sodium deoxycholate followed by extensive rinsing. Complete decellularization was confirmed using basic histology, PicoGreen quantification of residual DNA content, and gel electrophoresis of remnant DNA base pair size. Surface topology was imaged via scanning electron microscopy (SEM), and structural basement membrane proteins were examined by using immunolabeling techniques. The biochemical composition of M-ECM was determined for sulfated glycosaminoglycan (GAG) content via the Blyscan assay and total soluble protein using the bicinchoninic acid (BCA) assay. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) content were determined with ELISAs. Cell compatibility was assessed with C2C12 myoblasts and perivascular stem cells, which were cultured on M-ECM and examined histologically. Host compatibility *in vivo* was assessed by implanting M-ECM scaffolds in a rat abdominal wall partial thickness defect model. After 14 and 35 days, scaffolds were evaluated using histomorphometric methods.

Results: Skeletal muscle was decellularized by the described methods. Decellularization was confirmed by lack of visible cellular and nuclear material on histologic examination, negligible DNA content of 7.4 ± 1.6 ng DNA/dry weight M-ECM scaffold compared to 1748 ng/mg for intact skeletal muscle, and no visible DNA fragments after gel electrophoresis. SEM of the surface of M-ECM showed long compact collagen bundles arranged in a net like pattern and no evidence of cellular remnants. M-ECM showed positive immunolabeling for laminin, collagen IV (Figure 1B), and fibronectin, all of which were localized to the basement membrane in intact muscle ECM (Figure 1A). Total extractable protein from M-ECM was 2.10 ± 0.51 mg protein/g, which contained 10.15 ± 2.34 ng /g of bFGF (Figure 1C) and undetectable amounts of VEGF. M-ECM retained 0.61 ± 0.01 µg/mg sulfated GAGs after decellularization. C2C12 myoblast and perivascular stem cell lines were all cultured to confluent monolayers on the surface of M-ECM scaffolds for 7 days.

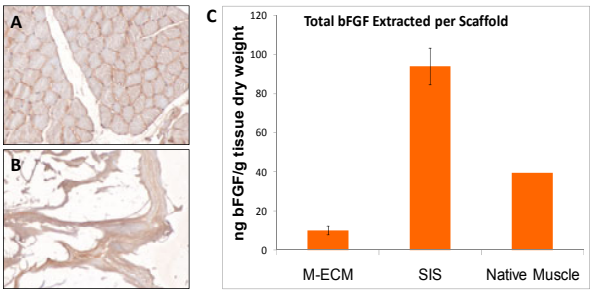

Figure 1. Collagen IV immunostaining of native muscle (A) tissue and M-ECM scaffolds (B), and total extractable bFGF from M-ECM, SIS, and native muscle (C) (error bars represent ± sem).

In vivo, M-ECM was extensively infiltrated by host mononuclear cells and substantial angiogenesis was observed after 14 days of implantation in the rat. After 35 days, the host cell response had greatly subsided and most of the scaffold had degraded and remodeled. There was no evidence of scar tissue formation at the site of scaffold remodeling.

Conclusions: Skeletal muscle can be completely decellularized by enzymatic and chemical treatments resulting in an acellular M-ECM scaffold sheet. The M-ECM scaffolds retained a number of bioactive factors found in native ECM including basement membrane proteins, bFGF, and sulfated GAGs. M-ECM was cell compatible *in vitro* for different cell types including C2C12 myoblasts and perivascular stem cells, supporting their growth and differentiation. M-ECM scaffolds show a favorable host tissue response *in vivo*, with angiogenesis and extensive scaffold degradation and remodeling after 35 days. The retention of bioinductive factors, in vitro cell compatibility, and host remodeling indicates that M-ECM suggests a therapeutic application for the repair and constructive remodeling of skeletal muscle tissue following injury.

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