Engineered Skeletal Muscle Units for Repair of Volumetric Muscle Loss in the Tibialis Anterior Muscle of a Rat

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Disclosures:

Introduction: Multiple pathological conditions including muscle trauma, congenital defects, post-operative damage, and degenerative myopathies can lead to volumetric loss of skeletal muscle tissue. Volumetric muscle loss (VML) is the loss of skeletal muscle resulting in impaired muscle function and physical deformity. To date, clinical treatments for VML - the reflected muscle flap or transferred muscle graft - are inadequate due to limited tissue availability and donor site morbidity. To address the need for greater flexibility in skeletal muscle repair, our laboratory has developed scaffoldless tissue engineered skeletal muscle units (SMUs), multiphasic tissue constructs composed of skeletal muscle with engineered bone anchors, myotendinous junctions, and entheses, which can produce force both spontaneously, and in response to electrical stimulation. Though phenotypically immature in vitro, we have shown that following one week of implantation in an ectopic site, our muscle constructs develop vascularization and innervation, an epimysium-like outer layer of connective tissue, an increase in muscle fiber content, and dramatically increased force production. These findings suggest that our engineered muscle tissue survives implantation and develops the necessary interfaces needed to advance the phenotype toward adult muscle. However, these constructs have not yet been used for muscle repair. Thus, the purpose of this experiment was to assess the efficacy of our SMUs for muscle replacement in a VML model.

Methods: Satellite cells and fibroblasts were isolated from enzymatically-dissociated rat soleus muscles and bone marrow stromal cells from rat femurs to fabricate our engineered muscle and bone tissues, respectively, as previously described. The engineered tissues were then combined to form our multiphasic SMUs (Figure 1). Thirty percent of the tibialis anterior (TA) muscles was removed in the VML model, and single SMUs were used to repair the VML sites. The rats were then allowed 28 days for recovery. At 28 days, the contractile forces of the repaired TAs were measured and histological analysis was performed to examine the development of the SMUs and assess their effectiveness for VML repair.

Results: Histological evaluation of the SMUs showed that the engineered muscle remodeled in vivo, developing small, distinct, aligned muscle fibers with an average cross-sectional area of 7.4% that of the native TA fibers (Figure 2A&B). These fibers had advanced sarcomere structure, similar to that of native muscle fibers (Figure 2C). The mass of the regenerated tissue was less than 5% of the total TA tissue and its contribution to overall force production was not appreciable; however, specific forces of the repaired muscles were 24.9 ± 0.9 N/cm², indicating that implantation of the SMUs did not adversely affect the contractile function of the host muscles.

Figure 1: A SMU prior to implantation
Figure 2: (A) H&E staining of the explanted SMUs indicates that they have developed into small but distinct and aligned fibers. (B) Immunohistochemical evaluation of the SMU muscle fibers indicates that they express myosin II heavy chains (MF-20, red), and (C) have advanced sarcomere organization (α-actinin, red).

Discussion: The purpose of this study was to evaluate the potential of our SMUs to restore muscle tissue to sites of acute VML. We have shown that our SMUs mature in vivo, and may provide additional muscle fibers to damaged muscles. We hypothesize that these grafted fibers will continue to hypertrophy with greater recovery periods, allowing for tissue engineering replacement of lost muscle tissue. We conclude from this study that our SMUs mature and remodel dramatically in vivo, and have the potential to restore lost tissue volume in cases of VML.

Significance: We have developed a method of using tissue engineering to add small but aligned muscle fibers to damaged muscle. This addition of muscle tissue may be used to treat VML.

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