Rat Model for Traumatic Volumetric Muscle Loss: A Platform for Testing Engineered Skeletal Muscle

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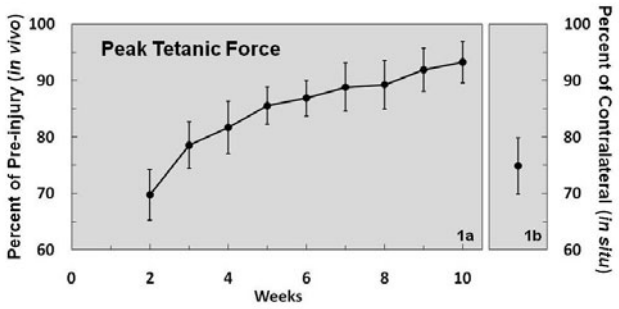
Statement of Purpose: Trauma of the head, neck, and extremities comprises 73% of battlefield injuries from the current wars [1]. Often this trauma involves the physical loss of soft tissue including skeletal muscle; termed volumetric muscle loss (VML). The surgical solutions for VML are extremely limited; in cases involving relatively small masses of muscle, muscle flaps can be used to repair the damaged area. However, this involves considerable donor site morbidity. In cases involving large muscle masses there are currently no surgical solutions. Advances in engineered muscle involving tissue engineering and regenerative medicine (TE/RM) offer great promise for the development of future solutions for VML. While the field of muscle engineering is growing, it has not advanced far beyond in vitro development. In order to advance the field there is a need for animal models of VML. Such a model should involve the creation of a defect of sufficient volume that it results in a permanent reduction in muscle function; a critical size defect. The first objective of this study was to develop a rat model of VML. Additionally, testing of any TE/RM solution must involve functional testing of the injured/repaired muscle. In vitro testing is limited to mice or very small muscles in rats. In situ testing is thus the standard for assessing function in most animal studies. However, in situ testing is time and animals intensive. In vivo muscle testing has been used in assessing other forms of muscle injury, e.g. eccentric contraction injury. It offers the advantage of allowing repeated measurements to be made in the same animal over time; thus dramatically reducing time, variability, and animal numbers. A disadvantage is that force measurements are the sum of all muscles activated by a single motor nerve; this includes the injured muscle as well as its synergists. The second objective of this study was to examine the utility of in vivo muscle testing as a means to assess VML over time.

Methods: Adult male Lewis rats were used for all experiments. The tibialis anterior muscle (TA) was used in all experiments. **Experiment 1:** Determination of critical size defect. We initially sought to determine the smallest defect that would result in permanent loss of function without disruption to major blood vessels or the motor nerve. This was done in an iterative process starting initially with a partial laceration and increasing the size and orientation of the excision until in a volume was reached that resulted in a significant, reproducible reduction of *in situ* muscle function at 28 days post injury. In situ isometric function was determined as previously described [2]. **Experiment 2:** Characterization of VML model and development of *in vivo* muscle function testing. Two surgical procedures were performed: one for electrode placement; the second for the injury. A chronic nerve cuff electrode around the peroneal nerve (innervating the dorsi flexor muscles); the leads were connected to a plug sutured between the shoulder blades [2]. In vivo muscle function was determined by placing the anesthetized rat in an apparatus modeled after Ashton-Miller [3] The Rats were allowed at least 30 days to recover and pre-injury baseline measurements were made; post-injury measurements were made at weekly intervals beginning at week 2.

Results: Experiment 1. The results demonstrate that a minimum of 120 mm x 25 mm x 1.0 mm, which corresponded to ≈ 22% of muscle mass, is required to produce a permanent loss of muscle function. Experiment 2. Partial muscle function appeared to return rapidly for the first 4 weeks following injury, from an initial 35% deficit to a 20% deficit (Fig. 1a). Over the next 6 weeks function continued to return at a slower rate, with an apparent plateau between week 8 and 10, at which point there was a deficit in function of 10%. In contrast, in situ muscle function displayed a significantly greater deficit at 10 weeks, i.e., ≈25% of the contralateral muscle (Fig. 1b).



Conclusions: We have successfully developed a small animal model of VML that can be used for testing engineered muscle. The use of in vivo muscle testing for tracking the effectiveness of repair may not be useful in this application. The reason that in vivo and in situ force measurements did not match was likely due to compensatory hypertrophy of the synergistic muscles. In situ muscle function remains the best method for testing the extent of muscle injury and recovery.

References:

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2. Walters TJ. J Appl Physiol 1991 Nov;71(5):1921-1928.
3. Ashton-Miller JA. J Appl Physiol 1992 Mar;72(3):1205-1211.