Functional Analysis of Limb Recovery with Autograft Treatment following Volumetric Muscle Loss in the Quadriceps Femoris

INTRODUCTION
Severe traumatic injuries to the extremities often involve damage to both hard tissues and soft tissues. Traditionally, musculoskeletal research of traumatic injuries has focused on bone healing and infection reduction. However, injuries involving volumetric muscle loss (VML) have been shown to severely impair and/or delay the functional recovery of the limb, resulting in decreased quality of life for patients [1]. Skeletal muscle regenerative strategies for VML have mostly been neglected due to the lack of pre-clinical animal models that could be used to test such strategies. Our objective was to develop and characterize a VML rat model in which muscle regeneration and restoration of limb function can be quantitatively analyzed. In addition to the empty defect, we characterized limb recovery with muscle autograft treatment, which is the current gold standard clinical treatment. We hypothesized that a full thickness defect through the quadriceps femoris would result in a significant loss of muscle strength and limb function, and that the autografts would be able to significantly restore muscle function.

METHODS
All procedures were reviewed and approved by the Georgia Tech IACUC. Unilateral surgeries were performed on age-matched 13-week-old female Sprague-Dawley rat. The muscle defect comprised of the use of an 8-mm biopsy punch to create a full-thickness defect through the medial anterior portion of the quadriceps femoris, resulting in damage to the rectus femoris, vastus medialis, and vastus intermedius muscles. The defect was then left EMPTY, treated with a WHOLE tissue autograft, or treated with a MORSELIZED autograft (n=6 in each group at each time point). The removed biopsy tissue was used for the autograft treatments. In the MORSELIZED autograft group, biopsied muscle tissue was cut with a scalpel into cubes approximately 2.5mm in length. Animals were euthanized at 2- and 4-weeks post-injury.

Muscle regeneration was quantitatively analyzed by measuring muscle strength, muscle mass, and histology to determine areas of regenerating muscle. Transverse muscle sections taken approximately from the middle of the muscle were stained with hematoxylin & eosin (H&E) for morphological analysis as well as Masson’s trichrome to determine the extent of fibrosis. To measure muscle strength, a nerve cuff was used to stimulate the femoral nerve, and a force transducer measured the maximal isometric tetanic torque production about the knee. Limb function was analyzed by monitoring gait with the Noldus CatWalk system, conducted at 2 and 4 weeks for all surviving animals.

Forthcoming data will include magnetic resonance imaging (MRI) of rat legs in order to determine the size of the muscle injury. Histological sections will also be used with immunohistochemistry (IHC) in order to determine presence and/or location of hypoxia-inducing factors, vascular endothelial growth factor receptors, and myogenic regulatory factors.

RESULTS
Muscle regeneration was assessed by measuring muscle strength and mass (Fig. 1). In all groups, the injured muscle mass was only at 80-90% of its contralateral control at 2 weeks post-injury, and mass decreased further by 4 weeks post-injury, though this decrease from 2 to 4 weeks was not significant (Fig. 1, right). Muscle strength, on the other hand, was much lower in the injured limb, at 20 to 40% of the contralateral leg strength at 2 weeks. Between 2 and 4 weeks, muscle strength increased significantly for the MORSELIZED autograft group (Fig. 1, left). To quantify general limb function, static and dynamic gait parameters were assessed (Fig. 2). Print area, a static gait parameter, decreased at 2 and 4 weeks compared to baseline, though this was significant only in the EMPTY defect group (Fig. 2, left). Duty cycle, a dynamic gait parameter, also significantly decreased at 2 and 4 weeks in the EMPTY and WHOLE groups (Fig. 2, right). Representative histological images of the muscle from all 3 groups showed extensive fibrosis, as indicated by blue-stained tissue in the sections stained with Mason’s trichrome (Fig. 3, top). H&E stained sections showed regenerative muscle fibers (indicated by centrally-located nuclei) near the edges of the fibrotic tissue (Fig. 3, bottom). Qualitatively, the WHOLE autograft and EMPTY defect groups seemed to have more fibrotic tissue than the MORSELIZED autograft group.

DISCUSSION
Volumetric muscle loss in a limb can prevent full restoration of limb function after a traumatic injury. We have developed a novel VML model in the quadriceps femoris, in which autograft treatment did not result in full recovery of muscle function. Muscle mass decreased from 2 to 4 weeks post-injury while gait function did not seem to fully recover. This suggests that the injured leg may have atrophied, possibly due to the animal adapting its gait to minimize the use of the injured leg. Histology showed extensive fibrosis throughout the length of the muscle, not just at the injury site (data not shown). This indicates that damaged muscle fibers did not recover and underwent necrosis, after which fibrotic tissue may have replaced the necrosed muscle tissue. The muscle mass may have consisted of a large percentage of fibrotic tissue rather than functional muscle tissue, which would explain the large loss of function without a comparable loss in mass. The MORSELIZED autograft group experienced more muscle recovery than the WHOLE and EMPTY defect groups. This may be due to release of myogenic growth factors from the morcelized tissue. IHC may reveal further details about the mechanisms behind this difference in healing response.

SIGNIFICANCE
To date, clinical treatment of VML does not result in a full recovery of limb use. This presents a significant clinical problem that can be further studied in this model. Our VML model also provides a means to test muscular regenerative strategies that aid in functional limb recovery.

REFERENCE

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