INTRODUCTION
Composite injuries involving bone and the surrounding soft tissue comprise one of the most challenging musculoskeletal conditions to return to normal function. Muscle has been implicated as a source for revascularization, osteoprogenitor cells and osteogenic factors, as well as a contributor to bone biomechanical stimuli; however, pertinent studies have been largely qualitative in nature, offering little insight into the mechanistic nature of the relationship between muscle and bone during regeneration. Our objective was to develop a composite injury model by combining a well-characterized segmental bone defect model with a muscle injury adjacent to the bone defect. We hypothesized that animals with a composite injury would have attenuated limb function, muscle strength, and impaired bone regeneration.

METHODS
All procedures were reviewed and approved by the Georgia Tech IACUC. Unilateral surgeries were performed on 13-week-old female Sprague-Dawley rats in three experimental groups: muscle injury, bone injury, or composite bone and muscle injury (n=6 per group). The muscle injury model was produced using an 8-mm biopsy punch to create a full-thickness defect in the quadriceps, encompassing portions of the rectus femoris, vastus intermedius, and vastus lateralis muscles. The bone defect was created as previously described and comprised of an 8-mm unilateral mid-diaphyseal femoral defect [1]. Each bone defect was treated with 2μg of BMP-2 delivered in pre-gelled 2% RGD-functionalized alginate. The composite injury consisted of a combination of the two surgeries. We quantitatively assessed limb function by measuring gait and muscle strength. Quantitative microcomputed tomography (μCT) was used to assess bone ingrowth into the defect region. Mechanical properties of regenerated bone were measured using failure testing in torsion. Perfusion was measured using laser doppler perfusion imaging (LDPI) while vascular volume was measured with μCT after vascular perfusion with a radiopaque contrast agent.

RESULTS
Injured limb function in the composite injury animals showed a significant decrease in gait function as compared to the muscle injury and bone injury groups after 2 weeks (Fig. 1). Limb function in the composite group showed a continuous recovery between 2 and 12 weeks reaching a level comparable to the single tissue injury animals. Muscle function was assessed using isometric muscle strength. At 12 weeks post-injury the injured leg in the composite group showed significantly lower muscle strength as compared to either single tissue injury animals. Masson’s trichrome staining of muscle from the composite injury group showed poorly regenerated muscle with fat deposits and fibrotic tissue (Fig. 2).

Bone regeneration was measured in vivo at 4 and 12 weeks using μCT and post-mortem using failure testing in torsion. At 12 weeks regenerated bone volume was attenuated in the composite injury group as compared to the bone injury group (Fig. 3). Failure strength of the regenerated bone was also significantly lower in the composite injury group as compared to the bone injury group (Fig. 3).

Revascularization of the injured limb was assessed at two weeks. Perfusion and vascular volume increased in the injured limb as compared to the control in both bone only injury and composite injury groups; however, there was no difference in vascular parameters between the injured limb groups.

DISCUSSION
We present a novel rat model of a composite bone and muscle injury and test the efficacy of BMP-2 mediated bone regeneration. The composite injury showed a significant functional and structural deficit over that of single tissue injuries. Vascular perfusion and volume increased in response to bone injury regardless of the presence of a muscle defect. Though muscle has been implicated as an important source for revascularization of bone, these data suggest that the mechanism responsible for the deficit observed in the composite injury animals may be independent of re-vascularization. This observation implicates other mechanisms, possibly through muscle derived factors or stem cells, as critical contributors to bone regeneration though more time points need to be investigated. Future research with this model will be instrumental in designing and testing new interventions for multi-tissue injuries.

SIGNIFICANCE
Composite multi-tissue injuries significantly impair the healing and recovery process, often resulting in further complications such as infection and loss of limb function. This composite injury animal model provides a platform with which to analyze both the mechanistic relationship of regeneration between tissues as well as test novel therapeutic approaches to promote healing of composite tissue injuries.

REFERENCE

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