INTRODUCTION:
Extremity trauma constitutes the majority of war wounds, and is a significant problem in civilian medicine¹. A large portion of these involve muscle trauma in the form of ischemia reperfusion injury (I/R) caused by vascular trauma, tourniquet use and/or compartment syndrome. I/R results in muscle atrophy and loss of muscle function. Past research by others has focused on reducing the progression of I/R using pretreatments or treatments administered immediately after ischemia. However, these strategies are not practical on trauma settings, especially when a life over limb approach is necessary². Transplantation of muscle progenitor cells (MPCs) has been shown to improve muscle function in animal models of muscular diseases, denervation, toxins, cryo-injuries, and volumetric muscle loss, and has been used to treat Duchenne dystrophy and cardiovascular diseases in clinical trials³⁻⁴. However, it is unclear whether delayed MPC transplantation can improve muscle function following I/R. Here we report the results of the treatment of tourniquet induced I/R with injection of MPCs at 48 hours after tourniquet application in a rat model.

METHODS:
Muscle progenitor cell isolation and culture: Hind limb muscles of adult male Lewis rats were isolated, minced, and digested with 1.25 mg/ml pronase (Sigma) in PBS at 37°C for 1 hour. The pronase and tissue debris were then removed through differential centrifugation, and cells were pre-plated on 150 mm tissue culture-treated dishes in DMEM medium with 10% FBS for 2 hours. After pre-plating, cells were seeded onto matrigel-coated 100 mm tissue culture-treated dishes in growth medium (F-10 medium, 20% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 40 µg/ml gentamicin).

Adenoviral infection of MPCs: Adenoviral vector encoding LacZ gene was purchased from Vector BioLabs (Philadelphia, PA). MPCs were infected with adenoviruses at a multiplicity of infection of 500 plaque-forming units/cell overnight. Adenoviral infection of MPCs: Adenoviral vector encoding LacZ gene was purchased from Vector BioLabs (Philadelphia, PA). MPCs were infected with adenoviruses at a multiplicity of infection of 500 plaque-forming units/cell overnight. Ischemia reperfusion and muscle contractile property tests: To induce injury, animals were anesthetized with 1.5% to 2.5% isofluorane, a pneumatic digit tourniquet (TK) was placed around rat thigh and inflated to a pressure of 250 mmHg for 3 hours. Forty-eight hours after TK application, animals were randomly assigned into 2 treatment groups, receiving injections with either saline containing MPCs (MPC) or saline alone as vehicle control (Veh). MPC injections contained a total of 10⁶ MPCs were injected at a perpendicular angle into the injured tibialis anterior (TA) muscle at 3 sites for each muscle. The contralateral TA muscle was used as a non-injured control.

Two weeks after tourniquet application, animals were anesthetized (see above), and the peroneal nerve was exposed for placement of a cuff electrode for stimulation. Experimental limbs were stabilized by a transverse Steinmann pin drilled through the femur. The distal TA tendon was cut and secured to the lever of a dual-mode muscle lever system (Aurora Scientific, Mod. 309b). The muscle lever was controlled, and data was acquired using a custom designed LabView (National Instruments) based program. Peak twitch force (Pt) and peak tetanic force (Po) were measured at optimal muscle length. Following mechanical measurements the muscle length was determined, muscles were excised, weighed and muscle cross-sectional area (mm²) was calculated⁶.

Tissue processing and histology: Serial transverse frozen sections (10 µm) were collected from the snap-frozen TA and then stained with H&E, collagen, CD68, desmin, and X-gal. Image-pro Plus software was used for quantifying collagen and CD68 immunoreactivity in tissue sections.

Statistical analysis: SPSS software was used for all statistical analysis. ANOVA followed by Tukey posthoc analysis was used to determine differences among non-injured controls, Veh and MPCs. Difference is considered significant when p < 0.05. All values are presented as mean ± SEM.

RESULTS:
The mass of the muscles were not different between treatment groups. While I/R resulted in dramatic reductions in absolute muscle force, the declines were less in the MPC group. Pt was reduced by 68% and 40% (Veh vs. MPC) (p=0.244) and Po was reduced by 72% and 62% (Veh vs. MPC) (p=0.233). Specific force (N/cm²) was reduced in both groups, but the decline was significantly less in MPC group (48%) compared to Veh group (57%) (p=0.034) (Fig. 1).

Histological results showed that the transplanted cells formed muscle fibers (Fig. 2). A large number of small muscle fibers with central nuclei were evident in the injured muscles in both treatment groups, indicating an ongoing regeneration process (data not shown). Significant inflammation and collagen deposition was observed in I/R muscle, and MPC treatment slightly reduced inflammation (Fig. 3).

DISCUSSION:
Our results indicate that transplanted MPCs can improve recovery of muscle function through improvement of muscle quality (specific force). Transplanted cells differentiated and formed muscle fibers which could potentially contribute to force generation. In addition, I/R was associated with significant inflammation and collagen deposition, and MPC treatment ameliorated inflammation, which could contribute to improved specific force.

REFERENCES: