INTRODUCTION:
Post-traumatic heterotopic ossification (HO) is a condition characterized by ectopic bone formation during muscle regeneration that occurs following orthopaedic injury. However, traumatic HO occurs much more frequently in patients that have experienced high-energy extremity injury and polytrauma. More than 60% of the patients that sustained blast-related trauma during Operation Enduring Freedom and Operation Iraqi Freedom were diagnosed with HO within one year of their injury [1]. Given the high incidence of this disease in the military population, there is a substantial need to characterize the microenvironment of the injured muscle tissue to identify factors that may be dysregulating the process of regeneration and to propose treatment options that will minimize the formation of ectopic bone. The roles of bone morphogenetic proteins (BMPs) are of particular interest, as they have recently been associated with a variety of functions in healthy and injured muscle tissue [2,3], and they are also capable of inducing osteogenic differentiation.

Our laboratory has previously identified a population of multipotent mesenchymal progenitor cells (MPCs) that are present in regenerating muscle tissue after high-energy trauma [4]. Although these cells are capable of osteogenic differentiation, they also perform many of the functions that are characteristic of bone-marrow-derived mesenchymal stem cells, specifically promoting tissue regeneration by enhancing angiogenesis, regulating fibrogenesis and modulating inflammation [5]. The effect of BMPs secreted by the traumatized muscle tissue on MPC function and the precise role of these cells in ectopic bone formation is unknown. Therefore, the goal of this study is to better define the interactions between MPCs and the BMPs that are present in the microenvironment of injured muscle tissue to identify factors that may be dysregulating the process of regeneration and to propose treatment options that will minimize the formation of ectopic bone. The roles of BMPs and BMP-4 are of particular interest, as they have recently been associated with a variety of functions in healthy and injured muscle tissue [2,3], and they are also capable of inducing osteogenic differentiation. Our laboratory has previously identified a population of multipotent mesenchymal progenitor cells (MPCs) that are present in regenerating muscle tissue after high-energy trauma [4]. Although these cells are capable of osteogenic differentiation, they also perform many of the functions that are characteristic of bone-marrow-derived mesenchymal stem cells, specifically promoting tissue regeneration by enhancing angiogenesis, regulating fibrogenesis and modulating inflammation [5].

METHODS:
Muscle samples were obtained from the zone of injury following blast trauma according to an IRB approved protocol. Control muscle tissue was obtained from patients undergoing elective orthopaedic reconstruction. Muscle fragments approximately 0.2 cc in volume were taken from each sample. One fragment was homogenized to determine the gene expression of BMPs and BMP-4 with qPCR. Protein-level measurements of BMP were determined using the other fragments by determining their wet-weight and placing them in 12-well trans-well plates containing 2 ml of growth medium (DMEM supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin). The growth medium was collected every 3 days, and the concentration of BMP-2, -4 and -6 was measured in the collected medium with ELISAs (R&D Systems).

MPCs were derived from the debrided muscle samples by following a previously described protocol [4]. The cells were seeded in 12-well plates to adhere overnight, and then treated with either BMP-2, -4 or -6 over the range of concentrations that was observed during the BMP release kinetics experiment. The cell supernatants were collected after 72 hours. Finally, the effect of the BMPs on the ability of MPCs to regulate fibrogenesis was assayed by measuring the concentration of MMP-1 in the cell supernatants with ELISA. The 4 different BMPs that were isoexpressed from traumatized muscle tissue were exposed to varying concentrations of BMP-4, and the supernatant was then collected at 72 hours. An ELISA for MMP1 was then performed on the supernatant.

RESULTS:
We measured substantial gene-level expression of BMP2, BMP4, BMP5 and BMP6 in control muscle (Figure 1), although the gene expression of BMP2 and BMP4 was significantly lower in the traumatized muscle tissue (p<0.015, Student’s t-tests). Protein-level expression BMP was detected in the explant supernatants for up to 21 days (Figure 2). We also measured a significant increase in the amount of MMP -1 expressed by the MPCs that were cultured with BMP-4 (p<0.05, Repeated Measures ANOVA with n=3; Figure 3).

DISCUSSION:
It is unclear whether traumatized-muscle-derived MPCs have a direct or an indirect role in the formation of ectopic bone following high-energy extremity injury. The MPCs are capable of osteogenic induction; although other wound healing cells have also been implicated in the formation of HO [6]. Alternatively, the MPCs may also play an indirect role in promoting HO formation by changing the biochemical microenvironment favor fibrosis and ectopic osteogenesis over muscle regeneration.

Although BMP-4 gene expression is down regulated in response to injury, we detected an increase in BMP-4 accumulation in the explant. BMP-4 could be released from the extracellular matrix sue during the early remodeling stages of wound healing. BMP-4 has previously been shown to promote anti-fibrotic muscle regeneration by down-regulation of TGF-β1 and up-regulation of MMPs [7]. We found that increased BMP concentration resulted in a corresponding increase in MMP-1 concentration, which has been associated with decreased fibrosis [8].

Our results suggest that there may be a window following injury, during which functional muscle regeneration is promoted via BMP-4 release from the muscle tissue. In cases where the injury is severe and the process of muscle regeneration extends beyond this window, the regenerative response may shift to form fibrotic tissue, thus generating an osteoinductive environment susceptible to HO formation.

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