Intramuscular transplantation and survival of freshly isolated bone marrow cells following skeletal muscle ischemia-reperfusion injury

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BACKGROUND: Delayed treatment cellular therapies offer an attractive means to treat extremity injuries involving acute skeletal muscle ischemia-reperfusion injury (I/R). Bone marrow is a rich source of stem and progenitor cells with the potential to improve skeletal muscle regeneration. The extent to which bone marrow cells (BMCs) may be useful for I/R is not known. The purposes of this study were twofold: (1) to evaluate BMC survival following intramuscular injection 0, 2, 7, and 14 days after injury and (2) to determine whether BMCs improve functional recovery following I/R.

METHODS: Magnetic-activated cell sorting was used to isolate lineage-negative (Lin^-) BMCs and enrich for stem and progenitor cells. To evaluate in vivo cell survival following I/R, Lin^- BMCs were injected intramuscularly 0, 2, 7, and 14 days after I/R, and bioluminescent imaging was performed for up to 28 days after cell injections. To assess their ability to improve muscle regeneration, intramuscular injections were performed 2 days after injury, and in vivo muscle function was assessed 14 days later.

RESULTS: Lin^- BMCs survived throughout the study period regardless of the timing of delivery. Intramuscular injection of Lin^- BMCs did not improve maximal isometric torque (300 Hz); however, both saline-injected and Lin^- BMC–injected muscles exhibited an increase in the twitch-tetanus ratio, suggesting that damage incurred with the intramuscular injections may have had deleterious consequences for functional recovery.

CONCLUSION: Although BMCs injected intramuscularly survived cell transplantation, they failed to improve muscle function following I/R. The ability of BMCs to persist in injured muscle following I/R lends to the possibility that with further development, their full potential can be realized. (*J Trauma Acute Care Surg*. 2013;75: S142–S149. Copyright © 2013 by Lippincott Williams & Wilkins)

KEY WORDS: Ischemia; reperfusion; stem cell; skeletal muscle; mice.

Extremity injuries, especially those caused by explosive munitions, can cause fractures, tissue loss, and vascular injury, all of which place skeletal muscle at risk of ischemia-reperfusion injury (I/R) and/or compartment syndrome. Numerous interventional strategies have been shown to reduce I/R when administered before ischemia, while others have shown promise when administered before or immediately following reperfusion.[1,2] Regardless, these are largely impractical in a trauma setting owing to the inability to predict an event causing I/R and the priority to control life-threatening injuries over performing elaborate procedures to treat extremity injuries immediately following severe trauma.[3] More specifically, although the importance of the early management of extremity injuries is becoming increasingly appreciated, the practice of damage-control surgery does not currently support early definitive treatment for muscle injury.[3] A more realistic and practical strategy would be to treat I/R after patient stabilization, which can be accomplished using cell-based therapies.

The number of stem and progenitor cells that are emerging as plausible candidates for cell-based therapies for musculoskeletal trauma is rapidly growing. The use of purified subpopulations of stem and progenitor cells is encouraging and may eventually lead to dramatic leaps forward in regenerative medicine; however, regulatory and logistical hurdles currently limit their widespread use in the trauma setting. Bone marrow is a recognized source of a variety of stem and progenitor cells for muscle injury, which includes, but is not limited to, mesenchymal stem cells (MSCs), hematopoietic stem cells, and endothelial progenitor cells.[4,5] Cell therapies using unseparated bone marrow mononuclear cells consisting of a heterogenous mixture of stem and progenitor cells, as opposed to purified and selected subpopulations, have clinical relevance for the treatment of skeletal muscle injury as supported by their safety and restorative potential in patients with critical limb ischemia.[6–8] Lending further to their clinical relevance is the availability of devices capable of concentrating bone marrow mononuclear cells at the bedside (point-of-care), allowing for an autologous

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transplantation procedure, in addition to the capability to perform for an early intervention.[9–12]

The frequency at which stem and progenitor cells are found in unseparated bone marrow mononuclear cells is relatively low owing to a preponderance of committed hematopoietic cells (lineage-positive cells, Lin^+), thereby diluting the number of stem cells delivered in a fixed volume. Since stem and progenitor cells are found within the lineage-negative (Lin^-) fraction of bone marrow, the use of lineage depletion techniques to enrich for these putatively therapeutic (Lin^-) populations of cells has been recognized as an effective regenerative cell therapy.[13] In support of this idea, magnetic-activated cell sorting (MACS) has previously been used to deplete highly differentiated cells. This depletion enriches for stem and progenitor cells and has been applied to a variety of disease and injury models.[14–18] In the current study, the use of lineage-depleted bone marrow cells (Lin^- BMCs) was explored as a treatment option for improving recovery following I/R. Although not an absolute requisite for successful treatment outcomes, prolonged survival of transplanted cells may positively influence the overall effectiveness of a cell therapy. To this end, in the first study, Lin^- BMCs were injected intramuscularly during specific healing phases following I/R (0, 2, 7, and 14 days after I/R), and cell survival was measured in vivo for up to 28 days after transplantation to assess the importance of the timing of delivery on cell survival. Upon finding that Lin^- BMCs survived up to 28 days after transplantation in a manner virtually irrespective of the time of injection, a second study was performed to evaluate the impact of BMC therapy on the recovery of muscle function in animals that were injected 2 days after injury.

MATERIALS AND METHODS

This study has been conducted in compliance with the Animal Welfare Act and the Implementing Animal Welfare Regulations and in accordance with the principles of the *Guide for the Care and Use of Laboratory Animals* and was conducted in the animal facility at the US Army Institute of Surgical Research. FVB-L2G85 transgenic and FVB mice (The Jackson Laboratory, Bar Harbor, ME) were housed individually in a temperature-controlled environment with a 12-hour light-dark cycle.

Experimental Design

To determine the importance of timing of injection on BMC survival following I/R, tibialis anterior (TA) muscles of FVB mice were injected with Lin^- BMCs derived from FVB-L2G85 transgenic mice 0 (30 minutes, n = 8), 2 (n = 6), 7 (n = 7), and 14 (n = 8) days after I/R. Cell survival was assessed 0, 1, 3, 7, 14, 21, and 28 days after cell injection. To determine the impact of BMCs on skeletal muscle regeneration following I/R, in study two mice were allotted to one of three experimental groups: (1) injured with no treatment (no treatment, n = 4), (2) injured with intramuscular injection of saline (saline, n = 9), and (3) injured with intramuscular injection of saline and Lin^- BMCs (saline + Lin^- BMCs, n = 10). Intramuscular injections occurred 2 days after injury. Muscle regeneration was assessed by measuring the functional capacity of the injured musculature in vivo.

Study 1: Survival of BMCs Following I/R

Isolation and Assessment of Lineage-Depleted BMCs

The tibiae and femurs of FVB-L2G85 transgenic mice were flushed, and BMC suspensions underwent MACS with lineage depletion. Cells from these transgenic animals express firefly luciferase, thereby allowing for in vivo measurements of survival over time when used in combination with bioluminescent imaging.[19,20] Briefly, BMCs were labeled with a cocktail of biotin-conjugated antibodies against lineage-specific antigens (CD5, CD45R (B220), CD11b, Gr-1 [Ly-6G/C], 7-4, and Ter-119) and subsequently magnetically labeled with Anti-Biotin MicroBeads (Miltenyi Biotec Inc., Auburn, CA). The lineage-positive cells (T cells, B cells, monocytes/macrophages, granulocytes, erythrocytes, and their committed precursors) were depleted by retaining them on a MACS column in the magnetic field of the autoMACS Pro Separator, while unlabeled lineage-negative cells pass through. In a subset of animals (n = 5), flow cytometry was used to assess the presence of mesenchymal and hematopoietic stem cells within the Lin^- BMCs. Lin^- BMCs were blocked with FcR blocking reagent for 10 minutes and then labeled with phycoerythrin or allophycocyanin-conjugated monocolonal antibodies (CD105, CD117, Sca-1; Miltenyi Biotec Inc., Auburn, CA.) or isotype control antibodies (BD Biosciences, San Jose, CA). Live gating was performed with propidium iodide (Miltenyi Biotec Inc., Auburn, CA). Flow cytometry analyses were performed using MacsQuant analyzer and MacsQuantify software 2.4 (Miltenyi Biotec Inc., Auburn, CA).

I/R

I/R injury was induced in mice using similar methodology to that which has been described previously for mice[21] and rats.[22,23] While under anesthesia with isofluorane gas (1.5–2.5%), the right hind limb of FVB syngeneic mice was elevated, and a pneumatic digit tourniquet (D.E. Hokanson, Inc., Bellevue, WA) (modified for mice) was placed as proximal as possible around the left upper hind limb and inflated to the pressure of 250 mm Hg for a duration of 2 hours using a Rapid Cuff Inflator (D.E. Hokanson, Inc.). Mice received buprenorphine (0.1 mg/kg, subcutaneously) 30 minutes before tourniquet release and every 12 hours thereafter for the first 24 hours.

Cell Injection

At the indicated time point after injury, Lin^- BMCs ($0.2–0.25 \times 10^6$) were resuspended in saline (40–50 μL) for cell injections using a tuberculin syringe (Tyco Healthcare, Mansfield, MA). For intramuscular injections, while under anesthesia with isofluorane gas (1.5–2.5%), the needle (29.5 gauge) was inserted parallel to the long axis into the TA muscle of FVB syngeneic mice, and the cells were injected as the needle was withdrawn.

Bioluminescence Imaging of Cell Survival

At the indicated time point after injection, mice were anesthetized and injected intraperitoneally with D-luciferin (Promega) (15 mg per kilogram of body weight). Photons emitted from the luciferase-expressing cells were collected by a

CCCD camera using a 15-minute exposure beginning 5 minutes after injection. A 1.75 × 0.75-cm region of interest was drawn over the ischemic limb and quantified using Living Image 3.0 software (Caliper Life Sciences Inc., Hopkington, MA). TA and extensor digitorum longus (EDL) muscles were excised and weighed following the completion of bioluminescent imaging.

Study 2: Effect of Lin$^-$ BMC Therapy on Skeletal Muscle Function

I/R was induced, and Lin$^-$ BMCs were injected intramuscularly 2 days after injury as described previously. The presence of cells on the day of injection and on the day of euthanasia was verified by bioluminescent imaging as described in Study 1.

In Vivo Muscle Function

Anterior crural muscle (i.e., TA and EDL muscles) isometric torque was measured as a function of stimulation frequency (20–200 Hz) in anesthetized mice (isoflurane, 1.5%, 1.5 L/min) within 1 week before and 16 days after I/R (14 days after intramuscular injection of Lin$^-$ BMCs). This postinjury time point was chosen because (1) preliminary experiments indicated that muscle function in this I/R model was reduced by approximately 30% 2 weeks after injury but was fully recovered by 1 month after injury; (2) in a rat I/R model, consistent with this time course of functional recovery, evidence of muscle fiber damage and regeneration was still present at 2 weeks after injury;[23] and (3) a 2-week interval was considered sufficient time to observe a cell therapy–mediated improvement in functional recovery. Contractile function (i.e., torque-frequency relationship) of the left anterior crural muscles was measured in vivo as previously described.[24] Anesthetized mice were placed on a heated platform to maintain core body temperature at approximately 37°C. The left knee was clamped, and the left foot was secured to a footplate that was attached to the shaft of a 300C servomotor (Aurora Scientific, Ontario, CA). Both the knee and the ankle were positioned at right angles. Sterilized needles were inserted through the skin for stimulation of the left common peroneal nerve. Stimulation voltage and needle electrode placement were optimized with 5 to 15 isometric contractions (200-millisecond train of 0.1-millisecond pulses at 300 Hz). Contractile function of the anterior crural muscles was assessed by measuring maximal isometric torque as a function of stimulation frequency (20–200 Hz). Core body temperature was monitored continuously during all functional testing with a mouse rectal thermocouple.

Torque-frequency relationships were modeled with the following equation:

$$\text{Torque}(x) = T_{min} + (T_{max} - T_{min})/[1 + (x/\text{Freq}_{50})^{\text{Hill}}]. \quad \text{Eq. 1}$$

where T_{min} and T_{max} are the smallest (i.e., twitch) and largest (i.e., peak tetanic) respective torques estimated; $Freq_{50}$ is the stimulation frequency at which half the amplitude of force ($T_{max} - T_{min}$) is reached; and Hill is the coefficient describing the slope of the steep portion of the curve.

TA and EDL muscles were excised and weighed following the completion of functional measurements.

Statistical Analysis

Muscle weights (two-way analysis of variance), bioluminescent imaging results (two-way analysis of variance with repeated measures), and body weight and functional measurements (one-way analysis of variance) were analyzed using SPSS (version 18.0, SPSS Inc., Chicago, IL) or SigmaPlot (version 12.0, Systat Software Inc., San Jose, CA) software followed by Fisher LSD post hoc analyses where appropriate. Differences were considered significant when $p < 0.05$. All values are presented as mean ± SEM.

RESULTS

Assessment of BMCs

Lin$^-$ BMCs were characterized using flow cytometry and putative markers for MSCs and hematopoietic stem cells after lineage depletion, respectively. The percentage of CD117 (48.5 ± 4.7), Sca-1 (15.8 ± 1.6), and CD105 (53.4 ± 3.1) are comparable with that previously reported for lineage-depleted mouse BMCs.[16,25,26]

Study 1: Survival of BMCs Following I/R

Twenty-eight days after cell injection, there was no difference in body weight among any of the groups (Table 1). However, the wet weight of the TA muscles was less in the 0-day group as compared with all other injured TAs, and the TAs in the 2-day and 7-day group were greater than their contralateral controls (Table 1). The wet weights of the EDL muscles were greater in the 2-day group as compared with all other groups and with their contralateral controls (Table 1). There was no difference among any of the groups for the TAs and EDLs from the contralateral limb.

Cells were delivered intramuscularly following I/R and tracked by bioluminescent imaging as an index of in vivo cell survival for up to 4 weeks after injection. Representative images of the bioluminescent imaging experiment are shown in Figure 2*A*. Quantification of the bioluminescent imaging resulted in a main effect of imaging day with 3 days and 7 days after injection greater than Days 0, 1, 21, and 28 and a main effect of injection day after I/R with Day 0 being different from Days 2 and 14 and Day 7 different from Day 14 (Fig. 2*B*, $p < 0.05$). The increase in bioluminescence imaging values on Days 3 and 7 after injection suggest that there is a transient cell expansion between the timing of injection and early days following intramuscular delivery. The lack of a significant difference between Day 0 and 28 after injection supports the contention that Lin$^-$ BMC survival following I/R was substantial regardless of the timing of injection.

Study 2: Effect of BMC Therapy on Skeletal Muscle Function

Body weights at the time of functional assessment either before injury or 16 days after injury were not different among groups (Table 1). Sixteen days after I/R, injured TA muscle wet weight was reduced by 20% compared with contralateral uninjured muscles for all experimental groups. There was no difference in preinjury isometric torque parameters among groups, so they were pooled and compared with postinjury outcomes for each of the treatment groups. Sixteen days after

TABLE 1. The Absolute Body Weights and Muscle Weights of the TA and EDL Taken From Injured and Control Limbs on the Last Day of Imaging (28 Days After Cell Injection) or Before or at the Conclusion of Muscle Function Measurements (14 Days After Cell Injection) for the Animals in Study 2

Study 1. Effect of Timing on Lin$^-$ BMC Survival

		Muscle Weight 28 d After Injection, mg			
		TA		EDL	
Group	Body Weight, g	Injured	Control	Injured	Control
0	22.81 ± 0.21	39.83 ± 2.69*	38.43 ± 2.69	8.75 ± 0.65	7.30 ± 1.01
2	23.55 ± 0.45	51.73 ± 1.57**	38.83 ± 1.38	11.9 ± 1.70*,**	8.45 + 0.56
7	22.39 ± 0.15	49.59 ± 2.58**	39.90 ± 0.57	8.88 ± 0.81	7.24 ± 0.14
14	22.93 ± 0.68	46.48 ± 2.50	41.28 ± 1.78	8.70 ± 0.53	8.09 ± 0.57

Study 2. Effect of Lin$^-$ BMC Therapy on Muscle Function

			Muscle Weight 14 d After Injection, mg			
	Body Weight, g		TA		EDL	
Group	Before	After	Injured	Control	Injured	Control
No treat	19.8 ± 0.3†	21.1 ± 0.8	34.3 ± 3.9†	37.9 ± 1.7	8.0 ± 0.4	6.8 ± 0.7
Saline	20.7 ± 0.3†	22.4 ± 0.3	31.3 ± 2.0†	39.9 ± 1.0	6.6 ± 0.6	7.5 ± 0.3
Saline+ Lin$^-$ BMCs	21.2 ± 0.4†	22.1 ± 0.4	31.3 ± 1.3†	40.4 ± 0.6	7.0 ± 0.4	7.7 ± 0.1

There was no difference in body weight among any of the groups. All values are mean ± SEM.
*Statistically different from all other groups within injured.
**Statistical difference between injured and control within a group.
†Main effect for time (body weight) or injury (TA).

injury (14 days after cell injection), I/R resulted in a similar 31%, 31%, and 37% reduction in maximal tetanic isometric torque (T_{max}) for no treatment, saline-only, and saline + Lin$^-$ BMC groups, respectively, compared with preinjury values ($p < 0.001$). In addition, all experimental groups exhibited a similar leftward shift in the torque-frequency curve ($Freq_{50}$, Table 2), wherein greater fusion occurred at lower stimulation frequencies. Lastly, the twitch-tetanus ratio (T_{min}/T_{max}) was significantly greater only for muscles receiving an intramuscular injection (saline or saline + Lin$^-$ BMC groups), compared with preinjury or no-treatment group values (Fig. 2*C* at 20 Hz, Table 2).

DISCUSSION

The overall objective of this study was to determine the feasibility for using freshly isolated BMCs for the delayed treatment of I/R. The benefits of using freshly isolated BMCs include the availability and use of clinical grade MACS systems, the potential for cell delivery within hours of the isolation, and the delivery of a heterogenous mixture of stem cells. Herein, we demonstrate that Lin$^-$ BMCs survive up to 28 days after transplantation when injected intramuscularly at a very early (0 day, approximately 30 minutes after I/R) or delayed (14 days) time after injury, indicating a wide time range for delivery for therapeutic application. Although functional improvements were not realized in this study, the ability of transplanted cells to survive for up to a month following injection provides enthusiasm for further exploration.

There are a number of cell types that may be useful for the treatment of I/R. We previously reported a functional improvement when muscle progenitor cells were injected intramuscularly using a rat I/R model, despite limited cell survival.[22] However, given the burgeoning research emphasis and clinical[27,28] therapeutic development of bone marrow–derived cells, the application of freshly isolated BMCs under clinically relevant conditions for the treatment for I/R was explored in the current study. The efficacy of use of BMCs for I/R is partially based on animal models of chronic partial ischemia demonstrating therapeutic benefits.[19,29] In addition, MSCs from the marrow (which are component of Lin$^-$ BMCs) have been shown to improve regeneration in a variety of organs (e.g., kidney and liver) following I/R.[30,31] Specific to skeletal muscle, bone marrow–derived stem cells have been shown to possess many of the salient characteristics thought to be required to improve regeneration of traumatized skeletal muscle. For example, bone marrow–derived stem cells have been shown to directly participate (i.e., myogenic differentiation) in skeletal muscle regeneration, albeit at a relatively low level, which can be enhanced with the selection of specific subpopulations and even further augmented in the context of muscle injury.[14,32,33] Moreover, aside from the direct conversion of bone marrow–derived cells to muscle fibers, the paracrine secretory effects of stem cells resident in bone marrow, including MSCs, cannot be overlooked and have the potential to contribute to overall muscle regeneration.[34] Lastly, MSC transplantation has been shown to improve skeletal muscle regeneration with concomitant improvements in muscle function[35,36] in animal studies of crush injury. Collectively, these findings support the use of bone marrow–derived cells as a logical therapy for I/R.

As an ultimate assessment of muscle regeneration, the benefit of Lin$^-$ BMCs on muscle functional recovery was determined in the current study. Previously, we[22,23] and others[37,38] have demonstrated that the induction of I/R results in an immediate and prolonged functional deficit that is the result of neural, vascular, and muscular damage. The recovery

of which is mediated by the well-characterized degenerative and regenerative response conserved among various modes of skeletal muscle injuries (e.g., I/R, eccentric contractions, freeze injury). In an effort to expedite tissue regeneration and thus functional recovery, Lin^- BMCs were transplanted intramuscularly 2 days after I/R, reflecting a clinically relevant timing of treatment. Despite the clear presence of Lin^- BMCs at the time of functional assessment (14 days after I/R for 2-day group, Fig. 1*A* and *B*) and for up to 28 days after injury (Fig. 1*A* and *B*), this cell therapy did not

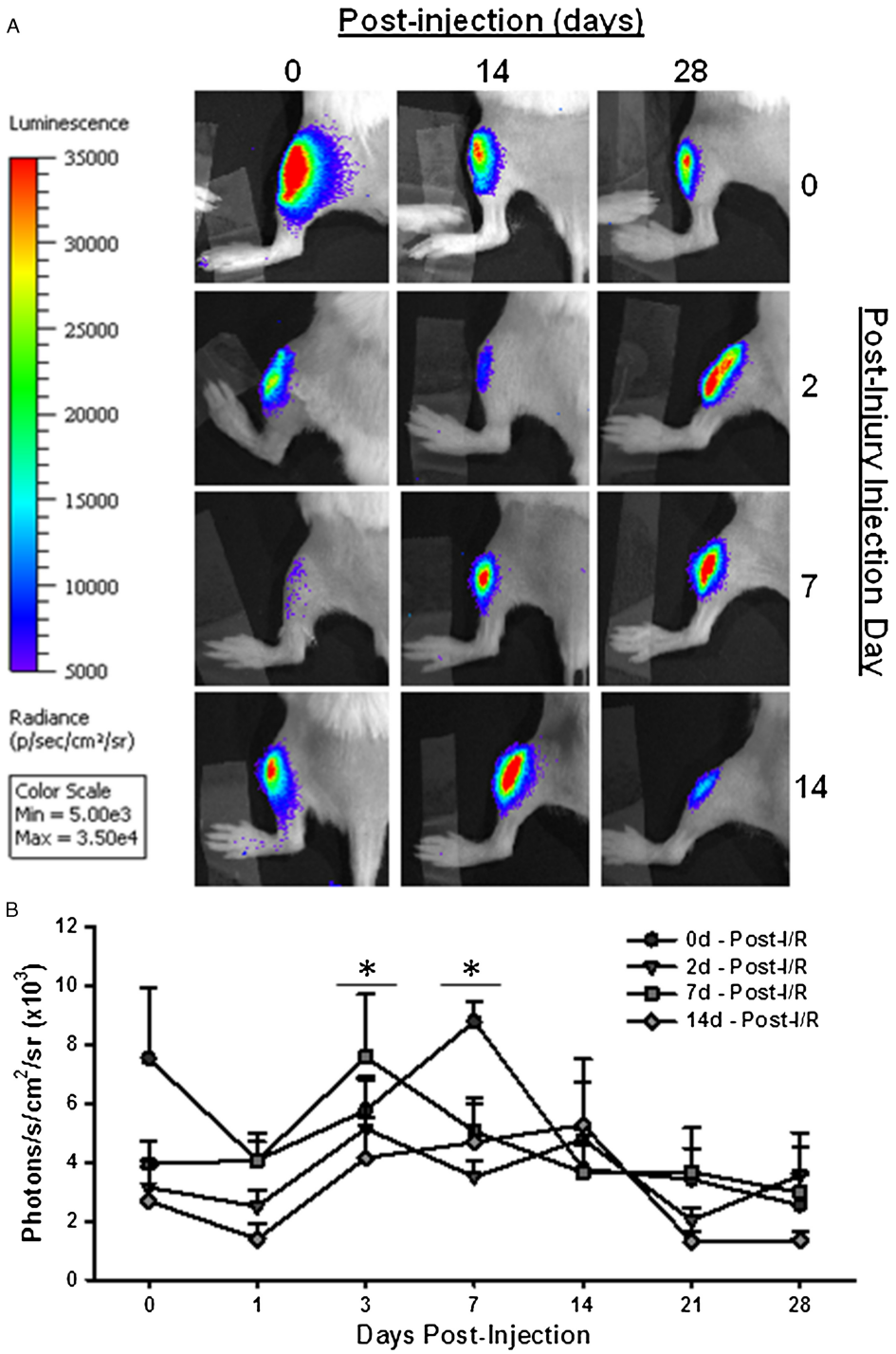

Figure 1. Representative images (*A*) and quantification (*B*) of bioluminescent imaging following intramuscular injection of cells 0, 2, 7, or 14 days after injury. Bioluminescent imaging was performed 0, 1, 3, 7, 14, 21, and 28 days after cell injection to estimate cell survival. *Main effect of imaging day with statistically significantly difference from Day 0, 1, 21, and 28 ($p < 0.05$). Values are mean ± SEM.

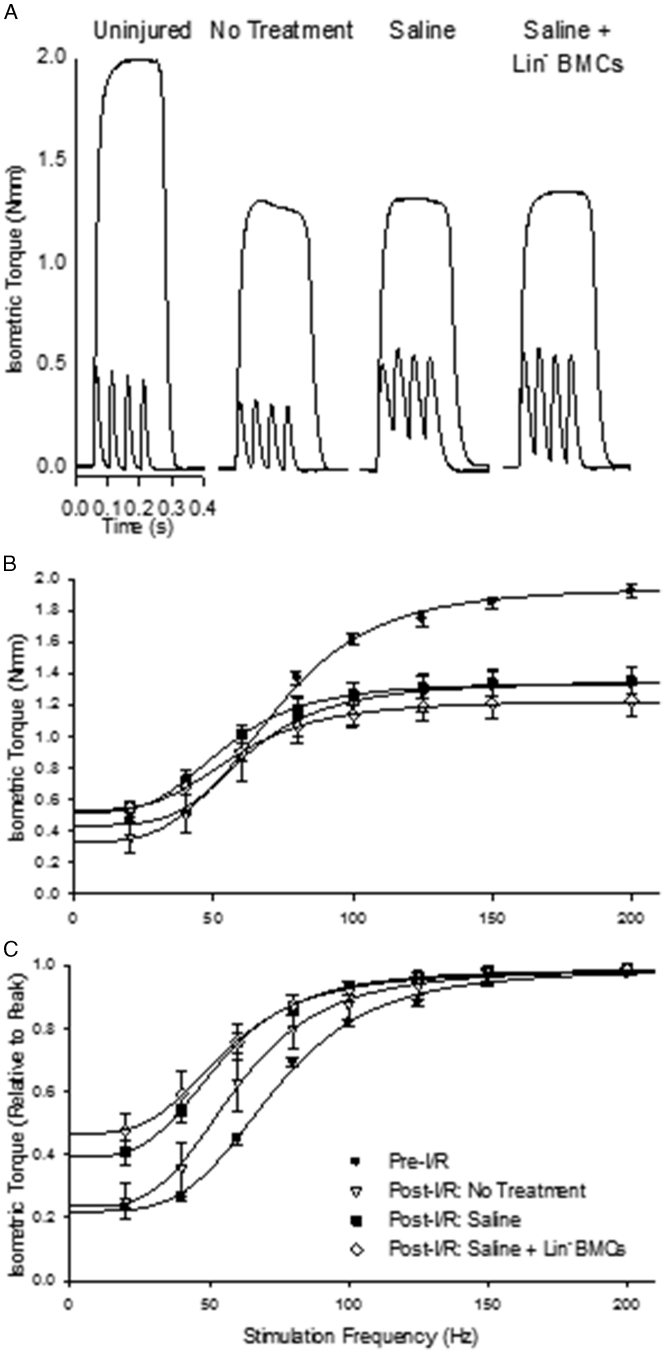

Figure 2. In vivo anterior crural muscle functional assessment. Representative torque tracings (*A*) are presented at 300 (tetani) and 20 Hz for each group. Note the greater fusion at 20 Hz for the saline and saline + Lin^- BMC groups. Isometric torque was measured as function of common peroneal nerve stimulation frequency (20–200 Hz). Group mean ± SEM at each frequency is presented as absolute torque (*B*) or torque normalized to maximal torque (*C*). Absolute values for torque and torque normalized to maximal torque as well as statistical comparisons are presented in Table 2.

improve peak isometric torque (Fig. 2*A* and *B*). This is in contrast to our previous report in which we showed that intramuscular injection of muscle progenitor cells significantly improved muscle function 2 weeks after injury despite a low level of cell engraftment.[22] These discordant findings are likely related to a number of different experimental factors including cell type (current study vs. Chen et al.,[22] Lin^- BMC vs. muscle progenitor cell), culture status (freshly isolated vs. culture expanded); host species (mouse vs. rat, both immune competent strains); and the degree of injury (2 weeks after I/R, approximately 30% vs. approximately 70%). It is important to note that both the previous rat model and the mouse I/R model used in this study are recoverable; therefore, functional improvements at an intermediate point (e.g., 14 days) would indicate an improvement in the rate of recovery. As more studies aimed at developing cell therapies for I/R are performed, a greater understanding of the underlying boundary conditions for such therapies will be gained.

In this regard, the intramuscular injection may have affected unidentified aspects of the injury, as evidenced by both intramuscular injection groups exhibiting an increased twitch-tetanus ratio (Table 2; Fig. 2*C*). An increase in the twitch-tetanus ratio has been observed at acute periods in a variety of self-healing muscle injury models[23,36,39] and reflects a general diminution in the functional capacity of the injured musculature. It should be noted that the injection volume (50 μL) used was consistent with other studies involving similar size muscles in mice.[14] These observations indicate that consideration should be given to the injection strategy in determining experimental design (i.e., including appropriate cell delivery controls) and to functional measurements (i.e., submaximal and maximal torque measurements) when assessing treatment outcomes. Furthermore, these findings support the exploration of systemic delivery of Lin^- BMCs for treatment of I/R. Because the ability of stem cells to home to sites of injury is well established,[19,40] it is logical that systemic Lin^- BMC delivery would allow for cell migration to the appropriate focal areas of injury while avoiding potential secondary damage by intramuscular injections.

Since this procedure involves the use of freshly isolated bone marrow–derived cells, an additional objective was to gain useful information that may help inform the use of other technologies that support the use of freshly isolated bone marrow aspirate. As one example, the timing of cell administration represents a significant knowledge gap with regard to devices currently available. Based on the results of the current study, the timing of administration had little effect on cell survival regardless of whether intramuscular delivery was performed acutely during a primarily degenerative and inflammatory phase (0 and 2 days), when inflammation is subsiding and regeneration is fully initiated (7 days), or when muscle regeneration predominates (14 days).[21,23] Although the timing of injection on cell survival was investigated, only the 2-day injection time point was used for the determination of functional outcomes. Despite the lack of difference between the overall cell survival among the injection days used in the current study, it remains to be determined whether the intramuscular injection of cells improves functional recovery when delivered earlier or later than 2 days after I/R. It is important to note that this is a spontaneously healing injury model; therefore, whether the timing of injection plays a bigger role in cell survival in a more challenging injury insult also requires

TABLE 2. In Vivo Isometric Torque Parameters

		Postinjury (16 d)		
	Preinjury	**Nontreated**	**Saline**	**Saline + Lin$^-$ BMCs**
Sample size, n	23	4	9	10
T_{max}, N mm	1.97 ± 0.04	1.36 ± 0.09*	1.36 ± 0.08*	1.25 ± 0.11*
T_{min}, N mm	0.47 ± 0.02	0.36 ± 0.09	0.54 ± 0.05**	0.55 ± 0.03**
T_{min}/T_{max}	0.24 ± 0.01	0.25 ± 0.06	0.41 ± 0.04*,**	0.48 ± 0.05*,**
$Freq_{50}$, Hz	73 ± 1	60 ± 5*	53 ± 2*	53 ± 4*
Hill coefficient	4.3 ± 0.1	4.0 ± 0.3	3.8 ± 0.2	4.1 ± 0.2

Values are means ± SEM. Parameters are defined in the Materials and Methods section. Within each parameter, * ≠ Preinjury and ** ≠ Nontreated ($p < 0.05$).

further study. Incomplete muscle regeneration after traumatic injury with residual functional deficiencies remains a severe problem in military and civilian orthopedics and traumatology, with impacts ranging from reduced quality of life to unsuccessful limb salvage. Cell-based therapies have historically shown great promise for addressing these issues, and continued work in this area remains a critical area of research. The finding that Lin$^-$ BMCs survive in an I/R model are encouraging; however, the lack of a functional improvement suggests that there is a need for further modification for the potential of this approach to be fully realized.

AUTHORSHIP

B.T.C. collected and analyzed the data, participated in the study design and interpretation, and wrote and critically revised the manuscript. J.C.W. participated in the study design and interpretation and critically revised the manuscript. T.J.W. participated in the study design and interpretation and critically revised the manuscript. C.R.R. collected and analyzed the data, participated in the study design and interpretation, and wrote and critically revised the manuscript.

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DISCLOSURE

The authors declare no conflict of interest.

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