2017

Perivascular stem cells diminish muscle atrophy following massive rotator cuff tears in a small animal model

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et al

Perivascular Stem Cells Diminish Muscle Atrophy Following Massive Rotator Cuff Tears in a Small Animal Model

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Background: Rotator cuff tears are a common cause of shoulder pain and often necessitate operative repair. Muscle atrophy, fibrosis, and fatty infiltration can develop after rotator cuff tears, which may compromise surgical outcomes. This study investigated the regenerative potential of 2 human adipose-derived progenitor cell lineages in a murine model of massive rotator cuff tears.

Methods: Ninety immunodeficient mice were used (15 groups of 6 mice). Mice were assigned to 1 of 3 surgical procedures: sham, supraspinatus and infraspinatus tendon transection (TT), or TT and denervation via suprascapular nerve transection (TT + DN). Perivascular stem cells (PSCs) were harvested from human lipoaspirate and sorted using fluorescence-activated cell sorting into pericytes (CD146$^+$ CD34$^-$ CD45$^-$ CD31$^-$) and adventitial cells (CD146$^-$ CD34$^+$ CD45$^-$ CD31$^-$). Mice received no injection, injection with saline solution, or injection with pericytes or adventitial cells either at the time of the index procedure ("prophylactic") or at 2 weeks following the index surgery ("therapeutic"). Muscles were harvested 6 weeks following the index procedure. Wet muscle weight, muscle fiber cross-sectional area, fibrosis, and fatty infiltration were analyzed.

Results: PSC treatment after TT (prophylactic or therapeutic injections) and after TT + DN (therapeutic injections) resulted in less muscle weight loss and greater muscle fiber cross-sectional area than was demonstrated for controls (p < 0.05). The TT + DN groups treated with pericytes at either time point or with adventitial cells at 2 weeks postoperatively had less fibrosis than the TT + DN controls. There was less fatty infiltration in the TT groups treated with pericytes at either time point or with adventitial cells at the time of surgery compared with controls.

Conclusions: Our findings demonstrated significantly less muscle atrophy in the groups treated with PSCs compared with controls. This suggests that the use of PSCs may have a role in the prevention of muscle atrophy without leading to increased fibrosis or fatty infiltration.

Clinical Relevance: Improved muscle quality in the setting of rotator cuff tears may increase the success rates of surgical repair and lead to superior clinical outcomes.

Peer Review: This article was reviewed by the Editor-in-Chief and one Deputy Editor, and it underwent blinded review by two or more outside experts. It was also reviewed by an expert in methodology and statistics. The Deputy Editor reviewed each revision of the article, and it underwent a final review by the Editor-in-Chief prior to publication. Final corrections and clarifications occurred during one or more exchanges between the author(s) and copyeditors.

Rotator cuff tears are common shoulder injuries that often result in pain, disability, and diminished quality of life. Rotator cuff repair is the most common shoulder surgery in the United States, with >75,000 procedures performed annually[1].

Massive rotator cuff tears account for 10% to 40% of all rotator cuff injuries[2]. These injuries are associated with poor clinical outcomes, including decreased range of motion, strength, and patient satisfaction[3]. Despite advances in surgical

technique, reported repair failure rates range from 11% to as high as 94%[4-7]. Degenerative muscle changes, including atrophy, fibrosis, and fatty infiltration, can develop after massive rotator cuff tears and compromise surgical repair. Fibroadipogenic degeneration and atrophy decrease compliance of the musculotendinous unit[8], lead to increased tension at the repair site[9], and may contribute to repair failure and poor clinical outcomes[3,6,8,10-12].

Several animal models have been used to characterize the etiology of muscle degeneration following rotator cuff tears[13-16]. Liu et al. developed a mouse model demonstrating consistent muscle atrophy, fibrosis, and fatty infiltration following transection of the supraspinatus and infraspinatus tendons (TT), denervation via suprascapular nerve transection (DN), and a combined procedure (TT + DN)[15]. These findings suggest that this small animal model demonstrates pathological changes similar to those observed in human rotator cuff tears.

Human perivascular stem cells (PSCs), a source of resident mesenchymal stem cells (MSCs), have demonstrated robust myogenic potential in other models of muscle injury[17-19]. In the current study, we evaluated 2 adipose-derived PSC populations—pericytes residing in small blood vessels and adventitial cells residing in large blood vessels—as potential regenerative therapies to reduce the fibroadipogenic changes seen following massive rotator cuff tears[17]. PSCs are highly myogenic, both in culture and in vivo[18,20], and have also demonstrated the ability to recover functional deficits following muscle injury[17,21,22]. These multipotent cells have distinct translational advantages. They can be obtained in high numbers from adipose tissue and isolated via cell surface markers, potentially without the need for culture, providing expanded availability for autologous regenerative therapies. In addition to their inherent regenerative potential, PSCs may act in a trophic fashion by secreting paracrine growth factors and cytokines, which enhance native stem cell recruitment and myogenic differentiation[23]. While the myogenic potential of pericytes is well documented, the potential of adventitial cells to differentiate into skeletal myofibers is still under investigation[17-19,24]. However, the proliferative advantage of adventitial cells over pericytes in long-term cultures makes them better therapeutic candidates[17], warranting further investigation into their myogenic potential[17-19,24].

This study investigated the regenerative potential of 2 human adipose-derived progenitor cell lineages in a murine model of massive rotator cuff tears. We specifically studied these cells in the absence of rotator cuff repair to assess their potential as a perioperative adjuvant. Regenerative potential was assessed by measuring changes in muscle atrophy, fibrosis, and fatty infiltration following the index surgery as well as cell survival. We hypothesized that (1) the administration of PSCs would diminish muscle atrophy and fatty infiltration, (2) pericytes would have a more robust regenerative effect than adventitial cells, and (3) this therapeutic effect would be greater when cells were delivered in the presence of early fibroadipogenic muscle changes.

Materials and Methods

Mice

Immunodeficient mice (JAX 001303; Jackson Laboratories) were used. Mice were 10 weeks old at the date of the procedure and were housed under specific pathogen-free conditions in the Animal Barrier Facility of the University of California, Los Angeles. All experiments were approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles.

Rotator Cuff Injury Model

Rotator cuff injury was induced in sex-matched mice. Animals were assigned to 1 of 3 surgical procedures: sham, TT, or TT + DN. Procedures were performed utilizing methods previously described[15].

The mice that underwent TT or TT + DN received no injection, injection with saline solution, or injection with pericytes or adventitial cells either at the time of the index procedure (as a "prophylactic" injection) or at 2 weeks following the index surgery (as a "therapeutic" injection). The mice that underwent the sham procedure received either an injection of pericytes or adventitial cells at the time of surgery or saline solution injection (Table I). Surgeries were performed on the animals' right shoulders, with the contralateral shoulders left intact as controls. All mice received buprenorphine injections for postoperative pain management.

Power analysis was performed using an estimated 15% difference in muscle weight loss between control groups and cell-injection groups, on the basis of our previous experience utilizing this model and these PSCs[15], with $\beta = 0.80$ and $\alpha = 0.05$. We determined that 6 mice per group would be sufficient to demonstrate significant differences in muscle atrophy between the control and treatment groups.

TABLE I Experimental Groups

Group	Surgical Procedure	Intervention
I	TT + DN	No injection
II	TT + DN	Saline solution injection
III	TT + DN	Adventitial cells at time of surgery (prophylactic)
IV	TT + DN	Adventitial cells 2 wk postop. (therapeutic)
V	TT + DN	Pericytes at time of surgery (prophylactic)
VI	TT + DN	Pericytes 2 wk postop. (therapeutic)
VII	TT	No injection
VIII	TT	Saline solution injection
IX	TT	Adventitial cells at time of surgery (prophylactic)
X	TT	Adventitial cells 2 wk postop. (therapeutic)
XI	TT	Pericytes at time of surgery (prophylactic)
XII	TT	Pericytes 2 wk postop. (therapeutic)
XIII	Sham	Adventitial cells at time of surgery
XIV	Sham	Pericytes at time of surgery
XV	Sham	Saline solution injection

Cell Culture

PSCs were isolated from human white adipose tissue (WAT)[18]. These tissues were obtained from cosmetic procedures and de-identified of any patient-specific information prior to their use, rendering them institutional review board-exempt. Pericytes and adventitial cells were harvested from WAT using methods previously described[18]. Briefly, bloody tumescent tissue was removed from lipoaspirate samples via centrifugation. Adipose tissue was digested using Dulbecco's Modified Eagle Medium (Gibco), 1× penicillin-streptomycin-amphotericin B (Anti-Anti [antibiotic-antimycotic]; Invitrogen), and collagenase type II (Sigma). Cell pellets were separated from mature adipocytes and resuspended in an erythrocyte lysis buffer. Using fluorescence-activated cell sorting (FACS), PSCs were divided into pericytes (CD146$^+$ CD34$^-$ CD45$^-$ CD31$^-$) and adventitial cells (CD146$^-$ CD34$^+$ CD45$^-$ CD31$^-$), as previously described[19]. The following commercial antibodies were used for FACS: CD146-FITC (AbD Serotec), CD31-PE (BD Pharmingen), CD45-APC-H7 (BD Biosciences), and CD34-APC (BD Biosciences). These purified cells were expanded in vitro and cultured in endothelial cell growth medium (Lonza). The medium was exchanged every 3 to 4 days. All injections were performed using passage-5 cells.

PSC Injections

For PSC injections, 5×10^5 cells were suspended in 30 μL of phosphate-buffered saline (PBS) solution and delivered in 2 equal aliquots into 2 locations (distal and proximal) in the supraspinatus muscle using a 29-gauge insulin syringe. The injections were administered either at the time of surgery ("prophylactic" injection) or at 2 weeks following the index procedure ("therapeutic" injection) (Fig. 1). For therapeutic injections, the mice were anesthetized, a skin incision was made, and the trapezius muscle was separated to allow for visualization of the supraspinatus muscle during cell injections.

Wet Muscle Weight

Both supraspinatus (SS) muscles were harvested from each animal 6 weeks following the index procedure. Muscle atrophy was assessed by measuring the percent difference in wet supraspinatus muscle weight—right (SS_R) versus left (SS_L)—for each animal. The percent change in wet muscle weight was calculated using the following equation: $([SS_R - SS_L]/SS_L) \times 100$. Mean wet muscle weight was calculated for each experimental group.

Histology

Fresh tissue specimens were frozen by immersion in cooled isopentane and embedded in Tissue-Plus OCT (optimal cutting temperature) freezing medium (Fisher Scientific). Sections were cut at 10-μm thickness on a cryostat (Microm) and fixed with 50% acetone. Hematoxylin and eosin (H & E) (Fisher Scientific), oil red O (ORO) (Sigma), and picrosirius red (PSR) (Polysciences) staining was completed according to manufacturer instructions. Slides were mounted using Cytoseal XYL (Fisher Scientific) or Aqua-Mount (Thermo Scientific Shandon) mounting medium and observed on a bright field microscope (Axio Imager; Zeiss).

Histomorphometric analyses of H & E, PSR, and ORO-stained sections were performed using ImageJ (National Institutes of Health). For all histo-

logical analyses, cross-sections from the mid-belly of the supraspinatus muscle were used. To calculate muscle fiber cross-sectional area, random regions of interest were selected using customized macros. All muscle fibers within each region of interest were measured and averaged for each sample. The area fraction of collagen was measured by dividing the area of PSR staining by the entire sample area. Similarly, the area fraction of fat was measured by dividing the area of ORO staining by the entire sample area.

In Vivo Imaging

Luciferase-expressing human PSCs were established using lentiviral infection with MSCV-luciferase-RFP-TK virus (murine stem cell virus-luciferase-red fluorescent protein-thymidine kinase virus) (System Biosciences) to enable in vivo vital and longitudinal cell tracking. Prior to injection, luciferase-expressing cells were removed from the culture dish and co-labeled with the cell tracker CM-Dil (Molecular Probes) according to the manufacturer instructions. Cells were injected 2 weeks following the sham, TT, or TT + DN procedures. At various intervals post-implantation, mice were anesthetized and received intraperitoneal injection of luciferin (30 mg/kg). Bioluminescence was recorded using the IVIS Lumina II imaging system (PerkinElmer). Data were analyzed with Living Image software (version 4.5.2; PerkinElmer). Rabbit anti-human and mouse α-smooth muscle actin (α-SMA) (Abcam) and goat anti-rabbit-Alexa-488 (Molecular Probes) antibodies were used for immunohistochemistry. Fluorescence imaging was performed using an Axio Imager 2 microscope (Zeiss).
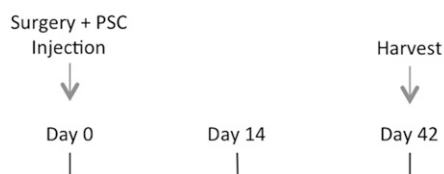
Statistical Analysis

Student t tests were used to evaluate differences in the mean wet muscle weight, area fraction of collagen, and area fraction of fat. Paired Kruskal-Wallis tests were used to calculate differences in muscle fiber cross-sectional area. All histological analyses were performed in a blinded fashion by 2 independent observers. Two-way, random-effects, interclass correlation coefficients (ICCs) with absolute agreement were determined for each histological quantification method. Differences between groups were evaluated using a significance level of 0.05. All statistical analyses were performed using Stata (version 13; StataCorp).

Results

Wet Muscle Weight

The mice in the TT groups that were treated with either prophylactic or therapeutic PSCs had significantly less wet weight loss at the final 6-week evaluation than did the TT controls (groups VII and VIII in Table I) ($p < 0.05$) (Fig. 2). The TT + DN groups treated with therapeutic injections (groups IV and VI) also had significantly less weight loss than their respective controls (groups I and II) ($p < 0.05$); however, the TT + DN groups treated with prophylactic injections (groups III and V) demonstrated no significant differences in weight loss compared with controls (groups I and II).

A. Prophylactic schematic

Surgery + PSC
Injection   Harvest

↓   ↓

Day 0   Day 14   Day 42

B. Treatment schematic

Surgery   PSC Injection   Harvest

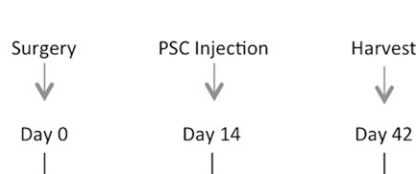↓   ↓   ↓

Day 0   Day 14   Day 42

Fig. 1
Schematics showing the timeline for "prophylactic" injections (**Fig. 1-A**) and treatment ("therapeutic") injections (**Fig. 1-B**).
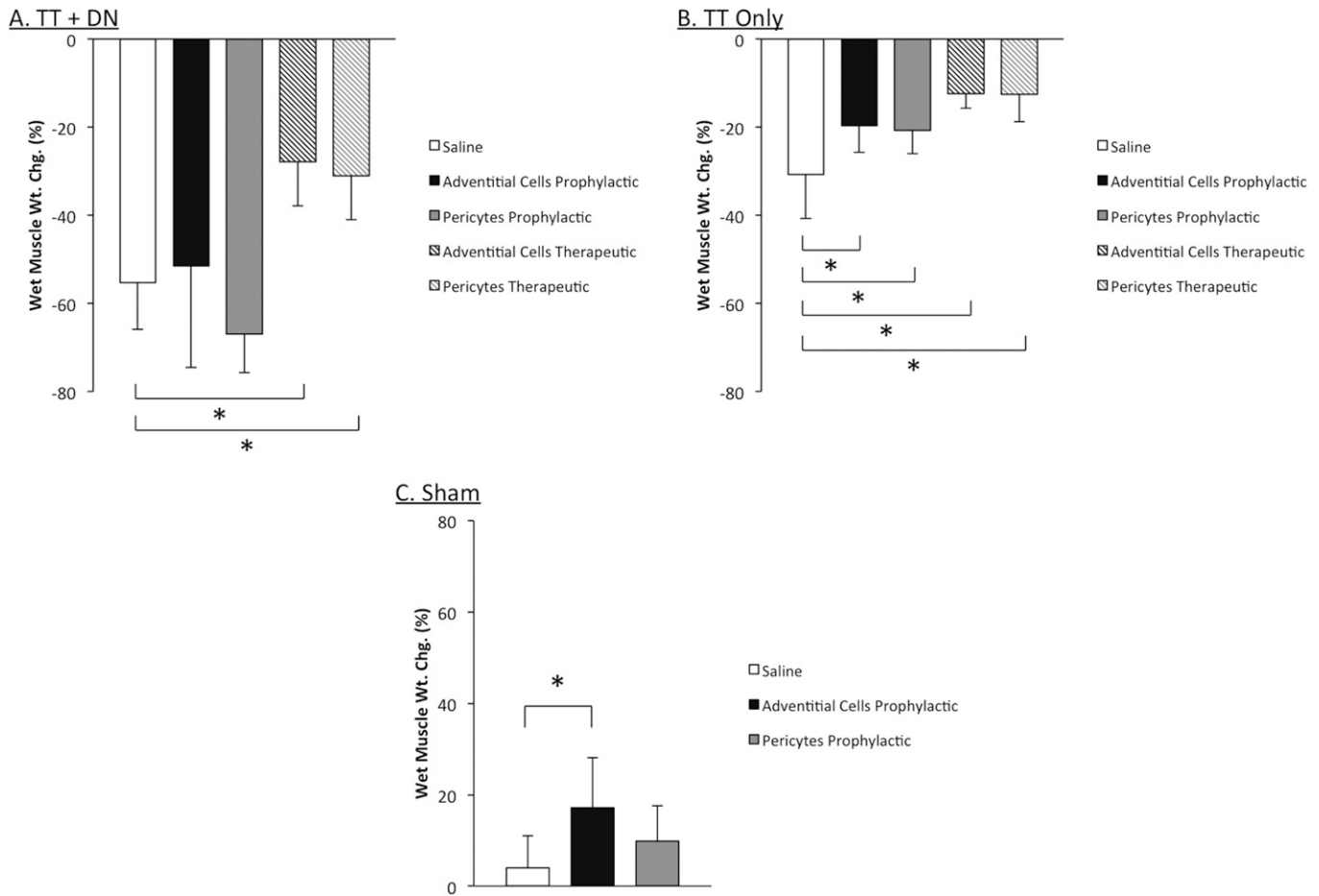
Fig. 2

Figs. 2-A, 2-B, and 2-C Percent wet muscle weight change. *P < 0.05. Error bars indicate the standard deviation. **Fig. 2-A** In the TT + DN groups, there was significantly less weight loss in both therapeutic PSC groups compared with the saline solution control group. **Fig. 2-B** In the TT groups, there was significantly less weight loss in all PSC groups compared with the saline solution control group. **Fig. 2-C** In the sham groups, there was significantly more weight gain in the adventitial cell injection group compared with the saline solution control group.

Muscle Fiber Cross-Sectional Area
The TT + DN groups treated with PSCs (groups III, IV, V, and VI) had significantly greater mean muscle fiber cross-sectional areas than their respective controls (groups I and II) (p < 0.05).

Similarly, TT groups treated with PSCs (groups IX, X, XI, and XII) had significantly greater mean muscle fiber cross-sectional areas than their respective controls (groups VII and VIII) (p < 0.05) (Table II). Two independent observers calculated the

TABLE II Mean Muscle Fiber Cross-Sectional Area by Intervention*			
Intervention	TT + DN (μm^2)	TT (μm^2)	Sham (μm^2)
No injection	879 ± 531	1,762 ± 358	
Saline solution	954 ± 445	1,838 ± 644	2,031 ± 434
Adventitial cells at time of surgery (prophylactic)	1,040 ± 569†	2,526 ± 919†	2,208 ± 432
Pericytes at time of surgery (prophylactic)	1,901 ± 814†	2,673 ± 421†	2,912 ± 388†
Adventitial cells 2 wk postop. (therapeutic)	1,305 ± 963†	2,231 ± 218†	
Pericytes 2 wk postop. (therapeutic)	1,626 ± 439†	2,856 ± 674†	

*N = 6 for each group. The values are given as the mean and the standard deviation. †A significant difference (adjusted p value of <0.001) compared with respective controls on the basis of paired Kruskal-Wallis tests.
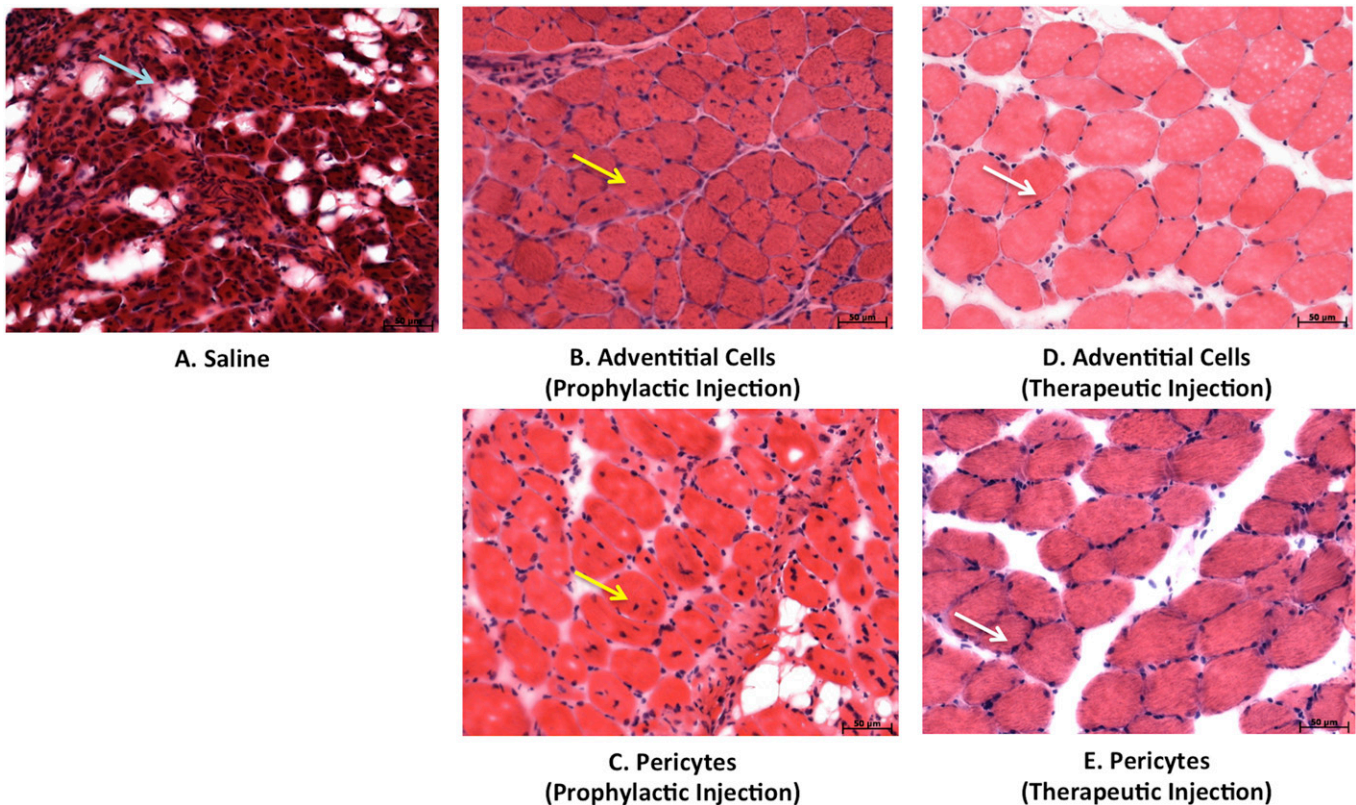
Fig. 3

Figs. 3-A through 3-E Representative images from the TT + DN groups in assessing muscle fiber cross-sectional area (H & E, ×20; bright-field microscope). **Fig. 3-A** The blue arrow indicates an area of adipocyte formation within the supraspinatus muscle. **Figs. 3-B and 3-C** The yellow arrow indicates centrally located nuclei within skeletal myofibers, indicative of fiber regeneration. **Figs. 3-D and 3-E** The white arrow indicates peripheral nuclei, suggestive of regenerated muscle.

muscle fiber cross-sectional area measurements; the ICC was 0.886 (95% confidence interval [CI], 0.828 to 0.924). Representative histological images from each group are displayed in Figures 3, 4, and 5.

Fibrosis

As measured by the mean area fraction of collagen, there was no significant difference in fibrosis among any of the TT groups. However, the TT + DN group treated with therapeutic adventitial cells (group IV) and the TT + DN groups treated with either prophylactic or therapeutic pericytes (groups V and VI) had significantly less fibrosis than their respective controls (groups I and II) (p < 0.05) (Fig. 6). Two independent observers performed the analysis; the ICC was 0.868 (95% CI, 0.706 to 0.930).

Fatty Infiltration

There was significantly less fatty infiltration in the TT groups treated with pericytes at either time point (groups XI and XII) or with prophylactic adventitial cells (group IX) when compared with their respective controls (groups VII and VIII) (p < 0.05). There were no significant differences in fatty infiltration between the cell injection groups and controls for either TT + DN or

sham procedures (Fig. 7). Two independent observers performed the analysis; the ICC was 0.859 (95% CI, 0.618 to 0.932).

In Vivo Imaging

Representative bioluminescence images can be found in Figure 8-A. Bioluminescent signal was observed up to 4 weeks following PSC injection for all surgical groups, indicating the persistence of injected cells over this interval. Transplanted CM-Dil prelabeled PSCs are shown aligning with myofibers in Figure 8-B. The absence of α-SMA expression demonstrates that CM-Dil PSCs did not adopt a fibrotic phenotype despite the environmental adipofibrotic cues (Fig. 9).

Discussion

Modest progress has been made in utilizing regenerative adjuvants to improve rotator cuff healing and repair. To date, most investigative efforts have focused on improving healing at the bone-tendon interface and have yielded mixed results[25-30]. Less attention has been focused on the roles of muscle atrophy and fatty infiltration and their effect on the healing of rotator cuff tears. Given the efficacy of MSCs in regenerating injured tissue in other musculoskeletal applications, we hypothesized that perivascular progenitor cells could be utilized in the
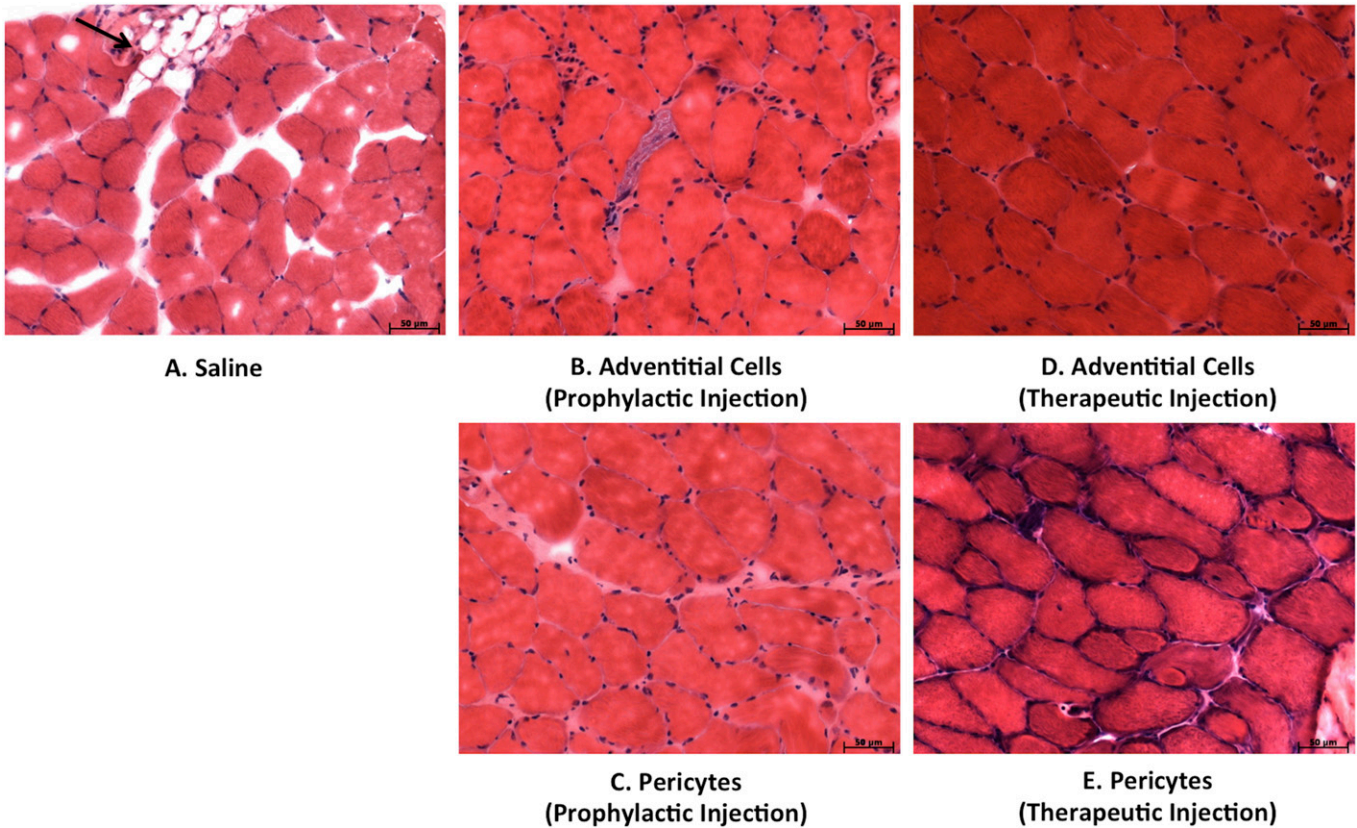
Fig. 4

Figs 4-A through 4-E Representative images from the TT groups in assessing muscle fiber cross-sectional area (H & E, ×20 magnification; bright-field microscope). **Fig. 4-A** The black arrow indicates an area of adipocyte formation within the supraspinatus muscle. **Figs. 4-B through 4-E** A larger cross-sectional area of myofibers was observed compared with the saline solution control.

treatment of massive rotator cuff tears to diminish fibroadipogenic degeneration.

Utilizing a mouse model of massive rotator cuff tears, our study demonstrated that there was significantly less muscle atrophy in the groups treated with PSCs compared with the respective controls for both TT and TT + DN. These findings were supported by both wet muscle weight and muscle fiber cross-sectional area data.

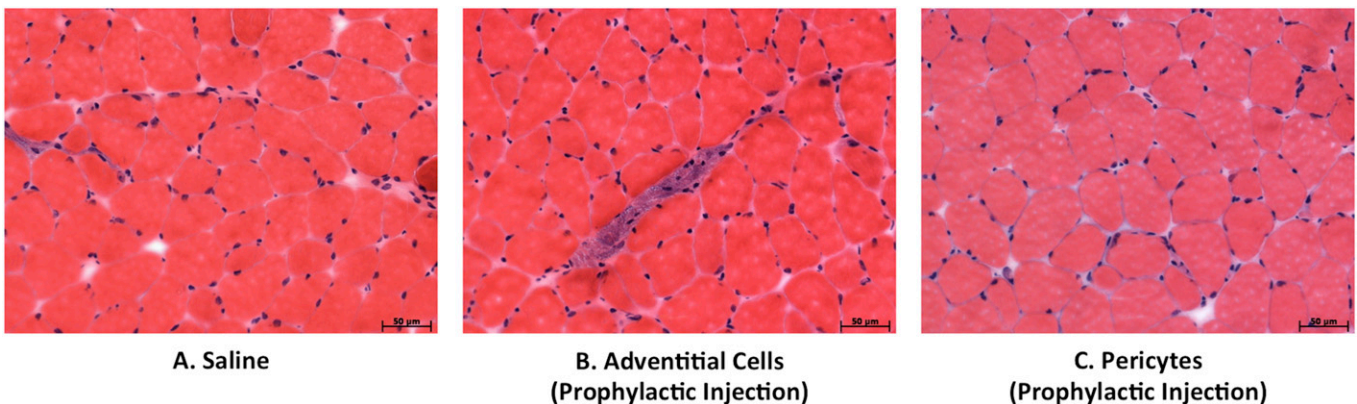The reduction in muscle atrophy observed with PSC treatment could be explained by 2 phenomena. PSCs may have

Fig. 5

Figs. 5-A, 5-B, and 5-C Representative images from the sham groups in assessing muscle fiber cross-sectional area (H & E, ×20; bright-field microscope). A slight increase in myofiber cross-sectional area was seen in the pericyte injection group compared with the saline solution control and adventitial injection groups.
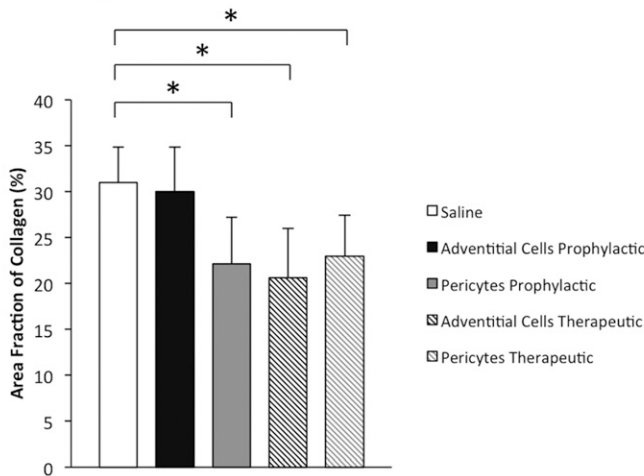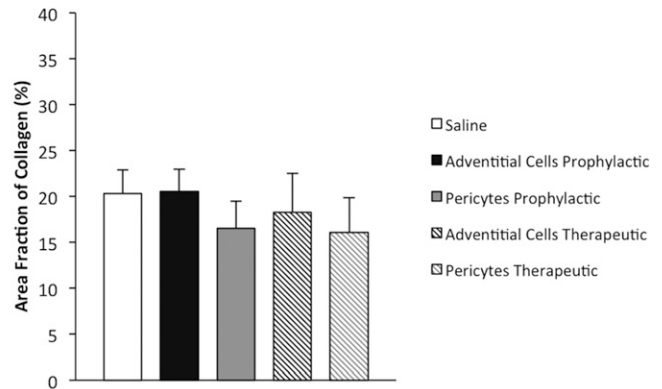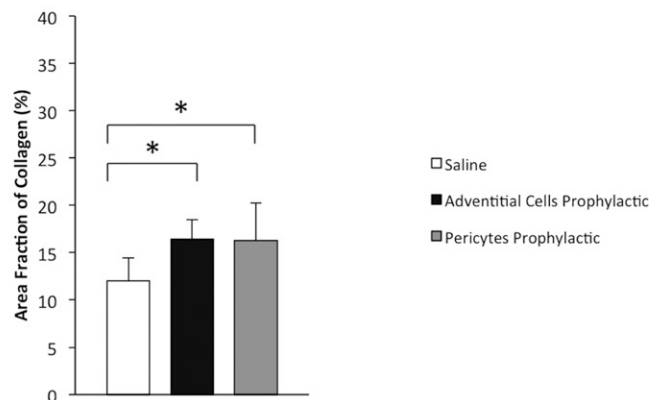
Fig. 6

Figs. 6-A, 6-B, and 6-C Mean area fraction of collagen. *P < 0.05. The error bars indicate the standard deviation. **Fig. 6-A** In the TT + DN groups, there was significantly less fibrosis for both the prophylactic and therapeutic pericyte groups and the therapeutic adventitial cell group compared with the saline solution control group. **Fig. 6-B** In the TT groups, there were no significant differences in fibrosis between the PSC groups and the saline solution control group. **Fig. 6-C** In the sham groups, there was significantly more fibrosis in the PSC groups compared with the saline solution control group.

a regenerative effect in which they fuse to, or differentiate into, native myofibers. This would be consistent with recent evidence suggesting that progenitor cells can contribute to myogenesis either by differentiating and fusing to growing myofibers or by entering the satellite cell pool[22]. At the 2-week time point in the current study, injected PSCs were found to align with native myofibers of the injured rotator cuff. Furthermore, our co-localization immunohistochemistry studies suggested that CM-Dil PSCs did not adopt a fibrotic phenotype, given the lack of expression of the myofibroblast marker α-SMA. Lastly, RNA-sequencing analysis demonstrated that pericytes and adventitial cells express myogenesis-related genes such as FGF2 (fibroblast growth factor 2), FST (follistatin), HGF (hepatocyte growth factor), IGF1 (insulin-like growth factor 1), and IL6 (interleukin 6) (see Appendix).

PSCs may also have a prolonged trophic effect on the native muscle via growth-factor expression. The ability of these cell populations to produce growth factors known to enhance tissue repair, such as heparin-binding epidermal growth factor

(HB-EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF)-BB, and vascular endothelial growth factor (VEGF), has been previously described[23]. Additionally, we found that secretion of VEGF, a growth factor known to promote angiogenesis and enhance MSC survival[31,32], was high in cultured PSCs (see Appendix). Therefore, one or multiple processes may contribute to the clinical and histological differences in muscle atrophy observed in the current study.

Additionally, we found that PSC injections did not contribute to increased fibrosis in the setting of rotator cuff tears. However, the TT + DN groups that received either prophylactic or therapeutic pericyte injections or therapeutic adventitial cells had less fibrosis than TT + DN controls. The absence of increased fibrosis is promising, as a potential complication with these injections is that the cells may follow a profibrotic pathway, leading to adverse outcomes[33-35]. However, the decreased fibrosis seen in the TT + DN groups suggests that the cells may inhibit these profibrotic processes. This is
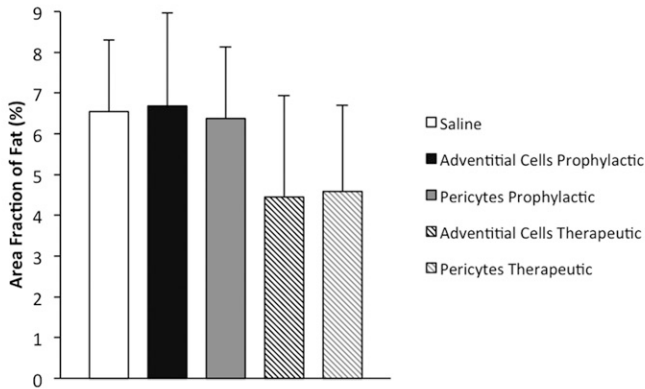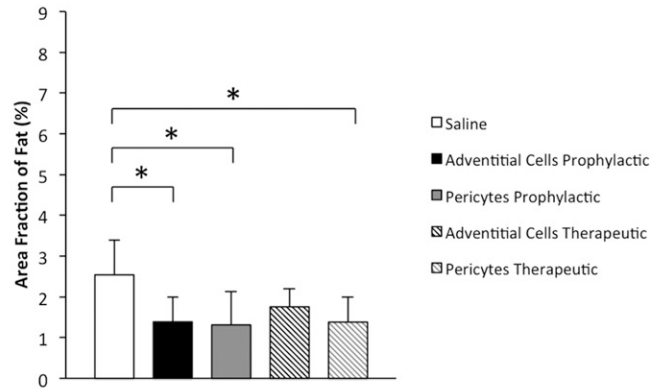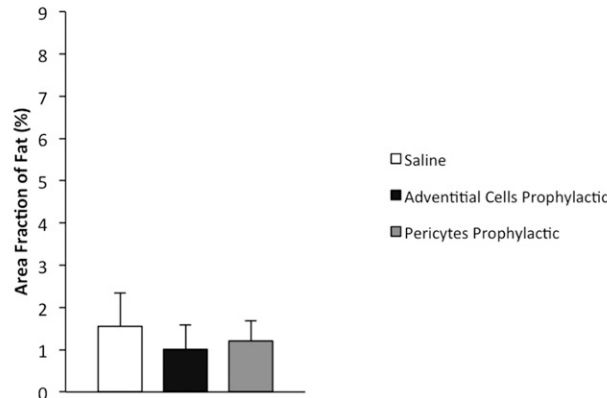
Fig. 7

Figs. 7-A, 7-B, and 7-C Mean area fraction of fat. *P < 0.05. The error bars indicate the standard deviation. **Fig. 7-A** In the TT + DN groups, there were no significant differences in fatty infiltration between the PSC groups and the saline solution control group. **Fig. 7-B** In the TT groups, there was significantly less fatty infiltration in the prophylactic and therapeutic pericyte groups and the prophylactic adventitial cell group compared with the saline solution control group. **Fig. 7-C** In the sham groups, there were no significant differences in fatty infiltration between the PSC groups and the saline solution control group.

consistent with the results reported by Chen et al., utilizing pericytes in a mouse model of myocardial infarction[21].

Some of our histomorphometric results differed between the prophylactic and therapeutic treatment groups. Specifically, wet muscle weight change for the therapeutic groups following TT + DN was significantly less than for the respective controls, while the prophylactic injection groups demonstrated no such change. These findings may be attributable to an altered microenvironment and increases in myogenic transcription factor expression following rotator cuff tears[36]. A previous study by Frey et al. demonstrated increased expression of the myogenic transcription factor Myf-5 in the weeks following rotator cuff tenotomy in an ovine model[37], which could explain the more robust clinical response observed in our therapeutic treatment groups, by which the cells were delivered into a biological milieu primed to drive myogenic differentiation.

Finally, there was a modest though significant difference in fatty infiltration in the TT groups treated with PSCs compared with controls, but no differences in fatty infiltration among the TT + DN groups. The similarities in fatty infiltration among the TT + DN groups may be secondary to denervation, which has been shown to play an important role in the development of fatty infiltration[14,38,39]. Furthermore, while there was significantly less fatty infiltration in the TT groups, given the small absolute difference (~1%), it is likely not clinically relevant. Nonetheless, our results suggest that the use of PSCs may contribute to the prevention of muscle atrophy by aiding in the maintenance of muscle bulk without leading to increased fibroadipogenesis.

There were limitations to our study, many of which are inherent to using animal models to replicate human pathology. Suprascapular nerve transection was required to produce massive fat infiltrate, consistent with the original model developed by Liu et al.[15]. While there are data demonstrating that suprascapular nerve palsy may contribute to fatty infiltration following rotator cuff injury[40,41], complete transection of the suprascapular nerve rarely occurs in human disease. Thus, while several aspects of human rotator
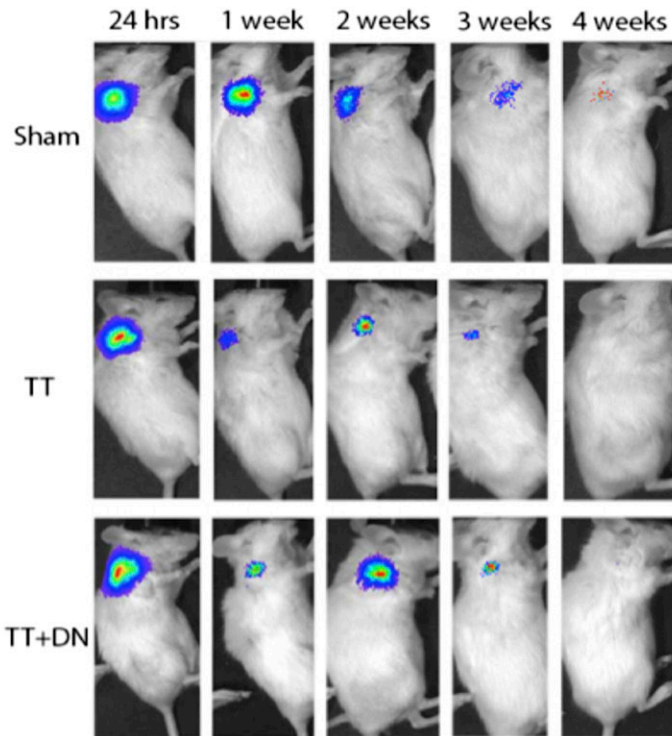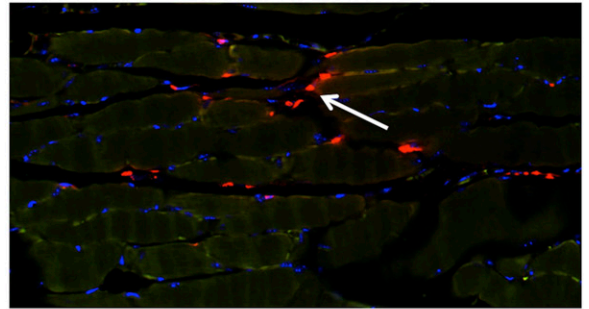
Fig. 8

Figs. 8-A and 8-B In vivo imaging and histology of prelabeled human PSCs after injection. **Fig. 8-A** The survival of transplanted human perivascular cells. Mice were imaged after intraperitoneal injection of luciferin at the indicated time points following cell injection 2 weeks post-operation. **Fig. 8-B** Fluorescent image (×200) demonstrating CM-Dil-prelabeled PSCs, which appear red. The white arrow indicates PSCs aligning with myofibers post-injection.

cuff pathology are recreated in this small animal model, it may not completely mirror the human disease process. While mice of similar age, weight, and sex were used in this study, normal variation in rotator cuff muscle weight may be present in this strain. We attempted to control for this variation by comparing the treatment effect in the experimental limb with values noted for the untreated limb in the same animal.

Additionally, our study utilized a mature, but relatively young, population of mice. While rotator cuff tears are more commonly observed in an older human population, mice of this age were chosen because young mice demonstrated degenerative muscle changes similar to those seen in humans in this previously validated model[15]. While the number of native progenitor cells has been shown to decrease with age[42,43], the
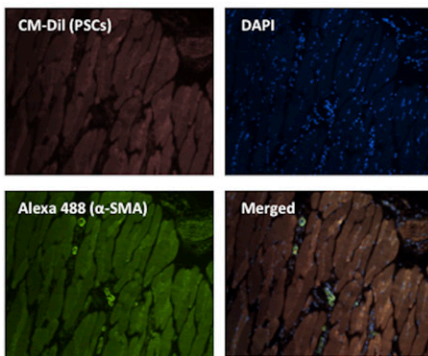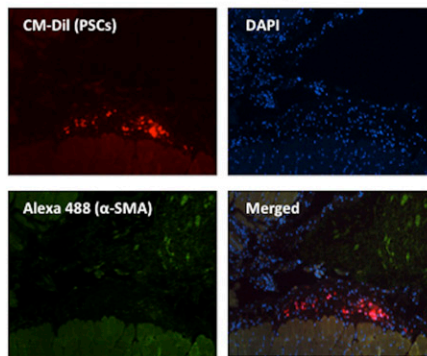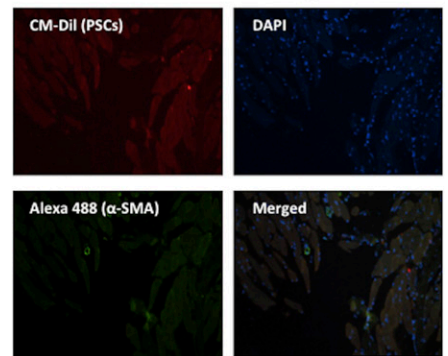
Fig. 9

Figs. 9-A, 9-B, and 9-C CM-Dil-prelabeled PSCs (×100) with α-SMA co-staining. **Fig. 9-A** α-SMA positive control demonstrates staining of native murine blood vessels. **Fig. 9-B** Sham sample 2 weeks post-injection. CM-Dil-prelabeled PSCs can be seen. There is no co-staining of α-SMA. **Fig. 9-C** TT + DN sample 2 weeks post-injection. CM-Dil-prelabeled PSCs can be seen with no co-staining of α-SMA.

myogenic potential and intrinsic functionality of satellite cells appear to be age-independent[44-46]. However, additional studies in aged animals are necessary to further characterize the role of PSCs in reducing muscle atrophy.

Finally, to validate the use of human PSCs preclinically, we utilized immunodeficient mice as PSC recipients. While the complex interplay of immune responses following rotator cuff injury has not been fully elucidated[47-50], it is possible that this model does not mirror the muscle changes that would be seen in an immunocompetent model. However, a study by Brzóska et al. suggested that skeletal muscle regeneration in severe combined immunodeficient [SCID] mice is similar to that in immuno-competent mice with respect to muscle fiber size, fibrosis, and fatty infiltration[51]. Nonetheless, additional studies investigating the role of inflammatory responses following rotator cuff tears would augment our understanding of fibroadipogenesis.

In summary, we demonstrated that 2 PSC populations—adventitial cells and pericytes—exhibit regenerative potential in the setting of massive rotator cuff tears. Currently, there is great heterogeneity among methods of stem cell procurement, methods of application, and cell types utilized in rotator cuff repair studies. Ultimately, the goal is to characterize and standardize a cell population with the greatest ease and efficacy of clinical translation. PSCs provide the distinct advantages of having an abundant and readily available source in adipose tissue and of being easily isolated via cell surface markers. Further investigation is necessary to fully elucidate the regenerative capacity of these cells and to better characterize their mechanism of action. Nonetheless, these results, which demonstrate the regenerative potential of these cells in a model of massive rotator cuff tears with no significant increases in fatty infiltration or fibrosis, suggest that they may serve as a viable perioperative therapy.

Appendix

*e*A Tables detailing the PSC transcript expression of growth factors related to muscle cell growth and/or differentiation and PSC secretion of VEGF as demonstrated in RNA-sequencing analysis are available with the online version of this article as a data supplement at jbjs.org (http://links.lww.com/JBJS/A10). ∎

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