THE IMPACTS OF SEX AND MYOGENIC CELL TRANSPLANTATION ON COLLATERAL
CAPILLARY ARTERIOGENESIS

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ABSTRACT

The Impacts of Sex and Myogenic Cell Transplantation on Collateral Capillary Arteriogenesis

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Current treatments for peripheral arterial occlusive disease (PAOD) have limited success, so there is a need to develop more effective treatments. Because patients with native collaterals have a better prognosis, promoting collateral arteriogenesis is a potential PAOD treatment. Additionally, female PAOD patients have a worse response to treatment and a worse prognosis compared to males, which could be due to impaired collateralization. Cell transplantation is a potential treatment option to promote collateral arteriogenesis. Bone marrow derived stem cells are the main cell type that has been investigated, but they have had limited clinical success. Delivering a stem cell type native to the tissue like myogenic stem cells could have improved outcomes. In this study, the lateral spinotrapezius feed artery was ligated in male and female Balb/C mice to induce collateral capillary arteriogenesis, and 7 days post ligation arterialized collateral capillary (ACC) number and diameter were determined. There were no differences between sexes, which could be because young, healthy mice were used in this study rather than aged and diseased models. Because we observed no sex differences, we then assessed the effect of myogenic cell transplantation in male mice only. Immediately following ligation of the spinotrapezius feed artery, mice were treated with myogenic cells, thrombin, or vehicle, and 7 days post ligation ACC number and diameter were determined. Thrombin increased ACC number, but myogenic cells had no effect. However, myogenic cells increased ACC diameter, and both myogenic cells and thrombin decreased ACC number in the region of the muscle with the largest collateral, and increased the maximum ACC diameter. Another factor that could affect ACC formation is a pre-existing collateral (PEC), which only some Balb/C mice have, so we also separated mice into PEC and non-PEC groups for analysis. In mice with a PEC, thrombin increased ACC number, and both myogenic cells and thrombin increased ACC diameter. There was a trend toward smaller arterialized capillaries in mice with a PEC, which could be because the majority of the blood flow is redirected through the PEC, so the PEC was the main vessel to remodel. These results are consistent with previous studies that indicated that thrombin augments arteriogenesis as well as increasing V-CAM, and suggest that myogenic cells have a similar effect possibly by secreting arteriogenic factors such as VEGF and MMPs. Because myogenic cells increase arteriogenesis, and macrophages are an essential regulator of arteriogenesis, we also tested the hypothesis that myogenic cells would increase macrophage content. Macrophage number increased with ligation, but there was no difference in macrophage number between any of the treatment groups. The lack of difference in macrophage number could be because the day 7 timepoint was too late, as macrophage content peaks at day 3. Because myoblasts increased arteriogenesis, they also may have increased the number M2 macrophages, which are the main macrophage contributor to arteriogenesis, but we used a general macrophage marker and could not detect an increase in M2 macrophages. In future studies, to determine if there is an increase in M2 macrophages a stain specific to M2 macrophages like CD206 could be added. Additionally, a diabetic Balb/C strain could be used to determine if arteriogenesis is impaired in males compared to females in a diseased model.

Keywords: collateral capillary arteriogenesis, sexual dimorphism, cell transplantation

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“Just because we don't understand doesn't mean the explanation doesn't exist”
-Madeleine L'Engle

TABLE OF CONTENTS

	Page
LIST OF FIGURES	vii
INTRODUCTION	1
METHODS	6
<i>Animals</i>	6
<i>Ligation</i>	6
<i>Gelatin Preparation</i>	7
<i>Gelatin Testing</i>	8
<i>Perfusion Fixation</i>	8
<i>Staining and Imaging</i>	9
<i>Image Analysis</i>	10
<i>Data Analysis</i>	11
RESULTS	12
<i>Female versus Male Collateral Capillary Arteriogenesis</i>	12
<i>Gelatin Vehicle for Cell Transplantation</i>	14
<i>Myogenic Cell Transplantation</i>	17
DISCUSSION	26
REFERENCES	33
APPENDICES	41
<i>Appendix A: Surgery and Experimental Protocols</i>	41
<i>Appendix B: Sample Images</i>	47
<i>Appendix C: Raw Data</i>	51
<i>Appendix D: Statistics</i>	81

LIST OF FIGURES

	Page
Figure 1. Current Treatments for PAOD	1
Figure 2. Collateral arteriogenesis	2
Figure 3. Location of treatment delivery	7
Figure 4. Location of Imaging Regions	10
Figure 5. Confocal images of arterialized collateral capillaries	12
Figure 6. No difference in number of alpha-smooth muscle positive vessels between males and females	13
Figure 7. No difference between males and females in arterialized collateral capillary diameter	14
Figure 8. Cross-linked gelatin vehicle remained polymerized for 7 days in vivo	15
Figure 9. Fibroblasts have normal morphology after one day of culture on gelatin vehicle	16
Figure 10. Confocal images of arterialized collateral capillary region.....	17
Figure 11. Thrombin increases number of arterialized collateral capillaries.....	18
Figure 12. Thrombin decreases number of arterialized collateral capillaries in region with largest collateral.....	19
Figure 13. Myogenic cells increase arterialized collateral capillary size	20
Figure 14. Myogenic cells increase maximum arterialized collateral capillary diameter	21
Figure 15. Thrombin increases number of arterialized collateral capillaries in mice without PECs.....	22
Figure 16. Myogenic cells increase arterialized collateral capillary diameter in mice without PECs.....	23
Figure 17. Confocal images of CD68 positive cells	24
Figure 18. Myogenic cell transplantation has no effect on CD68 cell count.....	25
Figure 19. Preparation of sterile gelatin vehicles.....	43

INTRODUCTION

Peripheral arterial occlusive disease (PAOD) affects 8 to 12 million people in the United States and can lead to critical limb ischemia (CLI) and amputation [1]. Current treatments include stenting and bypass grafting (Figure 1), but only 50% of CLI patients are eligible, and after 1 year, only 25% of revascularization attempts in these patients are successful [2]. Amputation is the only option for ineligible patients, and within 6 months, about 40% of ineligible patients have a limb amputated and 20% die [2]. The insufficiency of current treatments suggests that promoting collateral arteriogenesis may be a desirable treatment option. Collaterals are anastomoses between two arterioles that provide an alternative route for blood flow to bypass an arterial occlusion. Patients with pre-existing collaterals in their coronary circulation have better myocardial viability, a reduced risk for a repeat cardiac event, and a lower hospital mortality rate following heart attack or revascularization [3,4,5]. In the peripheral circulation, a well-developed collateral circulation improves 6 minute walk test results, and slows performance decline over time [6,7]. Unfortunately, not all humans have a native collateral network, and about 30% of patients never develop angiographically-identifiable collaterals [8,9].

Figure 1. Current Treatments for PAOD. A) Both bypass grafting (left) and inserting a bypass vein (right) are common treatments for PAD to allow bloodflow around the occlusion. Adapted from [10] B) Stenting is also used to reopen the occluded artery. Adapted from [11]

Similar to humans, animals also have varying degrees of collateral formation in response to an occlusion. For example, mice from the C57Bl/6 strain have many pre-existing collaterals that readily enlarge, while mice from the Balb/C strain have fewer pre-existing collaterals that have impaired enlargement [12]. When pre-existing collaterals are present, an occlusion causes the pressure gradient to increase across the collateral, which elevates blood flow, increases the shear stress, activates endothelial cells, and leads to macrophage recruitment [13,14]. Macrophages secrete matrix metalloproteinases to degrade the matrix, while endothelial cells and smooth muscle cells proliferate in response to growth factors secreted by macrophages and expand the arterial diameter until the shear stress is normalized (Figure 2) [13,14]. In tissues of Balb/C mice without pre-existing arteriolar collaterals, an arterial occlusion causes arterIALIZATION in capillaries that connect ischemic an non-ischemic terminal arterioles [15]. These collateral capillaries outwardly remodel and gain a smooth muscle cell layer either by recruiting smooth muscle cells from an upstream vessel or through perivascular cell differentiation [15].

Figure 2. Collateral arteriogenesis. Before an occlusion, blood flows through both arterioles and there is a limited pressure gradient through the collaterals (left). Following an occlusion (grey), blood flow is blocked in one of the arterioles, and the pressure gradient in the collaterals increases, which leads to elevated blood flow and increased shear stress and remodeling in the collateral vessels (right). Adapted from Heil et al [16].

The first approach to target arteriogenesis as a treatment for PAOD involved delivering single factors known to contribute to arteriogenesis, which were injected either intramuscularly in the limb

requiring treatment or intravenously in a major vein [17,18]. The factors investigated included VEGF, FGF and HGF as both gene and protein therapy, and the transcription factor HIF-1 α as gene therapy [17,18,19,20,21]. In early clinical studies and animal models, VEGF, FGF, HIF-1 α and HGF effectively increased perfusion, ulcer healing, and limb salvage [17,18,19,20,21]. However, when these factors were tested in larger blinded, randomized controlled trials, the treatments had no effect on end points such as ankle-brachial index, rest pain, exercise performance, or ulcer healing [22,23,24,25,26].

The failure of single factor treatments led to the investigation of cell transplantation to stimulate arteriogenesis as a treatment for PAOD. Because arteriogenesis signaling involves multiple growth factors, delivering cells as a treatment may be more effective than delivering a single growth factor [27,28]. The first cell type investigated was bone marrow mononuclear cells (MNCs). In early clinical trials, intramuscular injections of bone marrow MNCs increased ulcer healing and amputation free survival, but had no effect on limb perfusion, pain free walking distance or ankle-brachial index [29,30,31]. These results were positive enough to perform larger clinical trials, but in randomized controlled trials, bone marrow MNCs had no effect on amputation rate, ankle-brachial index or ulcer healing [32,33,34]. Another limitation with bone marrow MNCs is that they cannot be cultured once isolated, and therefore a large volume of bone marrow must be obtained [35]. However, another bone marrow derived cell, bone marrow mesenchymal stem cells (MSCs), requires significantly less bone marrow because the cells remain potent when expanded after isolation [36]. Bone marrow MSCs also increased perfusion in the rat hindlimb more than bone marrow MNCs [37], but similar to bone marrow MNCs, they have had mixed clinical outcomes. When injected into the heart, bone marrow MSCs had no effect on left ventricle function at 18 months [38]. Additionally, intramuscular injections of bone marrow MSCs lead to a trend towards reduced amputation rates and rest pain, but had no effect on ankle brachial index [39,40]. Even though bone marrow cells have shown some promise in the clinic, they must be isolated through a bone marrow biopsy which can be painful for the patient and sometimes requires general anesthesia [41,42]. Isolation of endothelial progenitor cells (EPCs), another potential treatment for PAOD, is less painful than a bone marrow biopsy. EPCs can be isolated from the peripheral blood

after dosing with granulocyte colony stimulating factor (G-CSF), which stimulates EPCs migration into the blood [43]. In non-placebo controlled clinical trials, intramuscular injections of EPCs increased partial oxygen pressure and pain free walking distance and decreased ulcer size, but there was no difference in ankle brachial index [43,44]. When a placebo control was used, there was a trend towards a lower amputation rate at 6 and 12 months but no difference in ankle brachial index, pain free walking distance, or wound healing [45].

Although bone marrow-derived cells do not appear to be a viable alternative to revascularization treatments, exercise is already a successful PAOD treatment. Exercise treatment is as good or better than revascularization as a treatment for PAOD [46,47], and regimes that include eccentric contractions had increased walking ability [48]. However, exercise treatment is only effective when performed under supervision [49,50]. Therefore, a potential treatment for PAOD is delivering a cell type that is activated during eccentric exercise such as satellite cells [51]. Satellite cells recruit monocytes in response to muscle injury [52,53], and macrophages are more efficient at paracrine signaling in muscle tissue when satellite cells are present [54]. Satellite cells also have angiogenic properties [55,56], but their effect on arteriogenesis has not been investigated. Additionally, tissue resident stem cells may have an advantage over other cell types because they may be able to communicate within the ‘myo-vascular’ niche more effectively than non-tissue resident stem cells. For example, tissue resident mesenchymal stem cells more effectively differentiate into muscle and cartilage than bone marrow derived mesenchymal stem cells [57,58,59]. The stem cell niche is important in maintaining stem cell properties including how they communicate with surrounding cells [60,61]. Therefore, bone marrow-derived cells, which have been removed from their niche, may communicate less effectively within the ‘myo-vascular’ niche than tissue resident stem cells, which could potentially contribute to their reduced efficacy as a revascularization treatment. Another advantage of muscle derived stem cells is that the isolation only requires a muscle biopsy [62,63] rather than additional treatment with G-CSF that is required to isolate EPCs or a bone marrow biopsy that is required to isolated bone marrow MSCs and MNCs.

To develop effective new PAOD treatments, differences in efficacy of current treatments must be understood so new treatments do not have the same weaknesses. For example, women do not seem to respond to treatment as well as men. There is a higher incidence of wound complications after bypass grafting in women [64], a higher rate of hospital mortality [65], and less positive results from exercise treatment than in males [66]. Additionally, females with PAOD appear to have a worse prognosis than males, with a shorter walking distance [67], and a higher rate of critical limb ischemia [68]. The sexual dimorphism seen in humans could be explained through investigation of processes related to PAOD in animal models. For example, bone marrow MNCs reduce aortic plaque in male, but not female, ApoE^{-/-} knockout mice [69]. Impaired collateralization could be another cause of the clinical difference between males and females. In patients with an occluded coronary artery, females were less likely than males to have an angiographically identifiable collateral [70], suggesting that arteriogenesis might be impaired in females. Since the number of female patients with PAOD is at least equal to the number of males [71], and the number of women with the PAOD is expected to increase [72], it is important to understand any sex differences so women with PAOD can be treated effectively.

In summary, clinically, females appear to have a worse prognosis and response to treatment than males, which could be due to impaired collateralization in females. Additionally, myogenic stem cells have angiogenic properties [55,56], but their effect on arteriogenesis is unknown. Therefore, we tested the hypotheses that collateral capillary arteriogenesis is impaired in females compared to males, and that myogenic cell transplantation would enhance collateral capillary arteriogenesis and macrophage recruitment. To support the second hypothesis, we developed a gelatin hydrogel to use as a vehicle for myogenic cell transplantation.

METHODS

Animals

Male and Female Balb/C mice (Taconic Farms; Oxnard, CA and Jackson Labs; Sacramento, CA) between 8 and 14 weeks, and Male ICR mice (Cal Poly Vivarium) were housed 2 to 4 mice per microisolator cage with ad libetum access to food and water, plus enrichment (a mouse 'house' and a plastic tube). The cages were in a temperature controlled room with a 12-hour light/dark cycle. The male ICR mice were used during gelatin vehicle testing; Balb/C mice were used for all other experiments. To determine if collateral capillary arteriogenesis is impaired in females, mice were divided into male (n=8) and female (n=8) groups. For the myogenic cell transplantation study, only male mice were used. Mice were divided into 4 groups: myogenic cells (n=6), vehicle (n=6), thrombin (n=6), and ligation only (n=6). All procedures were approved by the California Polytechnic University Institutional Animal Care and Use Committee.

Ligation

Animals were anesthetized in an induction chamber with 5% isoflurane in oxygen flowing at 3 l·min⁻¹. After anesthetization, isoflurane was reduced to 1-3% flowing at 0.5 – 1.0 l·min⁻¹. Prior to surgery, trimming clippers and depilatory cream were used to remove hair from the dorsal area of the mouse, which was then disinfected with chlorohexidine diacetate (Nolvasan). Animals were maintained at 35°C during surgery with a heat pad and a rectal temperature probe, and ophthalmic ointment was applied to the cornea to prevent desiccation. A small incision was made parallel to the spine over the lateral edge of the spinotrapezius. The fat pad overlying the spinotrapezius was undermined using blunt dissection to expose the fat pad underlying the lateral edge of the muscle that contains the feed artery/vein pair. The artery was separated from the vein, and two single strands from 6-0 silk suture were passed under the artery. The sutures were tied around the artery in two locations, and the artery was transected between the two sutures before closing the incision using 7-0 prolene suture. On the sham side, the same procedure was repeated up to the separation of the artery from the vein.

In animals receiving either myogenic cells or vehicle, the spinotrapezius was undermined by gentle blunt dissection to create a pocket for placing the cell construct or the vehicle (Figure 1). A cylinder of the cell construct was removed with a 1.5mm biopsy punch and inserted into the pocket. For mice receiving thrombin, $1\mu\text{L}\cdot\text{g}^{-1}$ body weight of thrombin (1 NIH unit/ μL) was superfused superficially and injected deep to the muscle following ligation (Figure 3).

Figure 3. Location of treatment delivery. Following ligation of the feed artery, the spinotrapezius was undermined in the location shown (black arrow) by gentle blunt dissection to create pocket for placing the cell construct or the vehicle. For thrombin treatment delivery, $1\mu\text{L}\cdot\text{g}^{-1}$ body weight of thrombin (1 NIH unit/ μL) was superfused above and injected below the muscle in the location shown (grey area).

Gelatin Preparation

A 10% gelatin solution was prepared aseptically in an Erlenmeyer flask in a biological safety cabinet. On a hot plate outside the biological safety cabinet, the solution was boiled for 5 minutes to sterilize and solubilize the gelatin. After boiling, the gelatin solution was transferred to a 24 well plate and stored at room temperature until it polymerized. After polymerization, the gelatin was cross-linked with 6mM 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and stored overnight at 4°C . After storage, the gels were washed 3 times for 30 minutes with PBS.

Myoblasts isolated from Balb/C mice were dissociated with a Cell Dissociation Buffer (EDTA, ThermoFisher, Waltham, MA), centrifuged at 300g for 5 minutes, suspended in growth media (20% FBS, $2\mu\text{L}/\text{ml}$ media bFGF ($1\mu\text{g}/\text{ml}$), $2\mu\text{L}/\text{ml}$ media SB 203580 (10mmol) in Hams F-10 Nutrient Mixture with

1% Pen/Strep), and 100,000 cells were seeded onto one well of gelatin so that approximately 9.6×10^3 cells would be transplanted in the 1.5mm diameter punch. Gelatin vehicles were stored in the incubator overnight to allow for cell adhesion.

Gelatin Testing

Both 4% and 10% gelatin vehicles were prepared aseptically in a 24-well plate. After polymerization, vehicles were cross-linked with either 6mM or 12 mM EDC. Gelatin vehicles without cross-linker were also prepared through a similar procedure, without the addition of EDC. The vehicles were incubated overnight at 4°C to allow for crosslinking and washed 3 times for 30 minutes with phosphate buffered saline (PBS) after storage. For acute testing, vehicles were either implanted deep to the spinotrapezius or stored in the incubator overnight to determine which vehicle types remained polymerized at 37°C. To test the cytotoxicity of cross-linked gelatin, 3T3 fibroblasts were seeded onto each vehicle type. Cell morphology was observed 1, 3, and 12 hours after seeding. To test vehicle retention, both 4% and 10% vehicles cross-linked with 6mM EDC were implanted deep to the spinotrapezius (n=2). After seven days, the spinotrapezius was resected to determine if the vehicle remained in place.

Perfusion Fixation

At 7 days following ligation, mice were perfusion fixed with paraformaldehyde in preparation for immunofluorescence. Prior to fixation, overlying fascia was removed from the spinotrapezius muscles. Through a thoracotomy, a catheter was placed in the left ventricle, and 40 mL of vasodilator solution (10^{-3} SNP, 10^{-4} adenosine, and 1 U/ml heparin in PBS) was perfused through the circulation at $2 \text{ ml} \cdot \text{min}^{-1}$ using a syringe pump to displace the blood and maximally dilate the vasculature. Then 5 mL of 4% paraformaldehyde (PFA) was perfused at $1 \text{ ml} \cdot \text{min}^{-1}$ to fix the tissue. Following fixation, spinotrapezius muscles were undermined, resected, and underlying fascia was removed. Muscles were post-fixed overnight in 4% PFA at 4°C and then transferred to PBS and stored at 4°C until staining.

Staining and Imaging

To determine arterialized collateral capillary (ACC) diameter, number, and smooth muscle cell coverage, the spinotrapezius muscles were stained with 1:200 1A4 clone alpha-smooth muscle actin (smooth muscle cells, Cy3, Sigma-Aldrich 1mg/mL) and 1:20 Isolectin GS-IB4 from GRI (endothelial cells, Alexa Fluor 647, Thermo Fisher Scientific 1 mg/mL). To determine macrophage number, spinotrapezius muscles were stained with 1:100 CD68 FA-11 Clone (macrophages, Alexa Fluor 488, Bio-Rad 0.05 mg/mL), which is a lysosomal/endosomal-associated membrane glycoprotein that is expressed on lysosomes and endosomes [73]. All antibodies were prepared in PBS with 0.1% Triton X-100 and 2% BSA, and incubated at 4°C for 72 hours. The muscles were then washed 3 times for 20 minutes with 0.1% Triton X-100 in PBS, and then for 30 minutes in PBS. Muscles were mounted on glass slides in a 50:50 PBS:glycerol solution.

Using a 20x objective on a confocal microscope (Olympus FV 1000, Olympus, Center Valley, Pennsylvania), three regions were imaged between ischemic and perfused arteriolar trees, where ACCs are located in operated muscle (Figure 4). To find the appropriate imaging area, the ischemic arteriolar tree was followed down to a terminal arteriole where ACCs were identified because they are similar in appearance to capillaries, but they are also surrounded by smooth muscle cells. Unlike typical capillaries, ACCs connect two terminal arterioles, are relatively straight, and their long axis is parallel to the muscle fibers. On the sham side, the appropriate imaging location was found by following the arteriolar tree that would be the ischemic tree in the operated muscle to a terminal arteriole until there was also a terminal arteriole from a different arteriolar tree in the field of view. At each location, a 150µm thick z-stack with 5µm thick slices was obtained.

Figure 4. Location of Imaging Regions. Each spinotrapezius muscle was imaged using a 20x objective on a confocal microscope. The muscles were imaged at three locations between arteriolar trees (red squares) where ACCs are located on ligated muscles. Red staining is alpha-smooth muscle actin. Blue staining is lectin. Scale bar on montage is 1mm. Scale bars on regional images are 50 μ m. Figure adapted from Mac Gabhann et al [15].

Image Analysis

To determine the number and diameter of capillaries and ACCs, the z-stacks were compressed and the channels were separated using ImageJ software. Alpha-smooth muscle positive vessels (ACCs) and lectin positive vessels (capillaries) were counted in a 200 μ m² region in the compressed image. Inner diameters of three alpha-smooth muscle positive vessels and lectin positive vessels were also measured in each region.

To determine the maximum ACC diameter, the diameter of the largest collateral was measured in each replicate. To determine ACC number in the region of the largest collateral, all alpha-smooth muscle

actin positive vessels were counted along a line drawn perpendicular to the ACCs in the region with the largest collateral.

CD68 positive cells were counted by finding large oval or elliptical areas of that were oriented around an ACC. To determine overall macrophage number, CD68 positive cells were counted within the same 200 μm^2 region as the ACCs. To determine vessel associated macrophage number, CD68 positive cells that were within 50 μm^2 of an ACC were counted along the entire length of the ACC.

Data Analysis

Differences in vessel number and diameter in males and females and in ligated and sham muscles were determined by independent t-tests. Differences in number and diameter of vessels in the four groups (myogenic cells, vehicle, thrombin, and ligation only) and in ligated and sham muscles were determined using One-Way ANOVA.

Differences in maximum diameter and ACC number in the four groups (myogenic cells, vehicle, thrombin, and ligation only) and in ligated and sham muscles were determined using One-Way ANOVA. Differences in number of CD68 positive cells in the four groups (myogenic cells, vehicle, thrombin, and ligation only) and in ligated and sham muscles were determined using One-Way ANOVA. Data are presented as mean $\pm$ SE.

RESULTS

Female versus Male Collateral Capillary Arteriogenesis

To test the hypothesis that collateral capillary arteriogenesis is impaired in female mice as compared to male mice, we used confocal microscopy (Figure 5) to count and measure the diameters of collateral capillaries and arterIALIZED collateral capillaries (ACCs) that connect ischemic and perfused arteriolar trees.

Figure 5. Confocal images of arterIALIZED collateral capillaries. Confocal images were taken of ACCs between the ligated arteriolar tree and an adjacent, perfused arteriolar tree using a 20x objective. (A,D) Alpha-smooth muscle actin positive vessels show arterioles and ACCs. (B,E) Lectin positive vessels show both capillaries and ACCs. (C,F) A composite image combining A and B or E and F. A-C are from an operated muscle and D-F are from a sham muscle. Scale bars are 50 μ m.

There was no difference in the overall number of vessels between male and female mice or between ligated and sham muscles (10.8 ± 0.4 female operated, 11.2 ± 0.3 female sham, 11.6 ± 0.6 male operated, 11.8 ± 0.5 male sham). To determine the number of ACCs, we counted the number of alpha-smooth muscle actin positive vessels in the capillary position. As expected, the number of alpha-smooth muscle actin positive vessels increased in operated muscles (zero for both male and female sham, $4.6 \pm$

0.1 female operated, 4.9 ± 0.1 male operated). Additionally, there was a trend ($p = 0.08$) towards an increase in presumptive ACCs in males (Figure 6), but qualitatively it did not seem like there was a difference between males and females. There was also limited variation in ACC number between imaging regions, which suggests that 3 regions were sufficient for imaging.

Figure 6. No difference in number of alpha-smooth muscle positive vessels between males and females. Capillaries and ACCs were counted between ischemic and perfused arteriolar trees on both male ($n=6$) and female ($n=5$) replicates at day 7 using a 20x objective on a confocal microscope. The number of alpha-smooth muscle actin positive vessels increased in operated muscles. There was no difference between males and females in the number of ACCs. There was also no difference between males and females or ligated and sham in capillary number. (*, $p<0.05$ for operated versus sham)

As expected, ACCs had a larger diameter ($10.8 \pm 0.5 \mu\text{m}$ in females and $11.2 \pm 0.3 \mu\text{m}$ in males) than capillaries ($4.7 \pm 0.2 \mu\text{m}$ in females and $4.6 \pm 0.1 \mu\text{m}$ in males), indicating outward remodeling occurred, but there was no difference in male and female ACC diameter or in capillary diameter between

ligated and sham muscles (Figure 7). There was also limited variation in ACC diameter between imaging regions, which suggests that 3 regions were sufficient for imaging.

Figure 7. No difference between males and females in arterialized collateral capillary diameter. Capillary diameter and ACC diameter were measured between ischemic and perfused trees on both male (n=6) and female (n=5) replicates at day 7 using a 20x objective on a confocal microscope. Alpha-smooth muscle actin positive vessels had an increased diameter compared to capillaries. There was no difference between males and females in ACC diameter. There was also no difference between males and females or ligated and sham in capillary diameter. (*, $p < 0.05$ for alpha-smooth muscle actin positive vessels versus capillaries)

Gelatin Vehicle for Cell Transplantation

Because there was no difference in collateral capillary arterialization in males and females, we evaluated the ability of myogenic cell transplantation to augment arteriogenesis only in male mice. Myogenic cell transplantation is a potential solution to the need for improved PAOD treatments. Previous cell transplantation attempts in our laboratory utilized type I rat tail collagen as the vehicle. Unfortunately, rat-tail collagen polymerized inconsistently- in some instances, the vehicles polymerized easily, while in others no polymerization ever occurred (*unpublished observations*). Therefore, to

transplant myogenic cells in the spinotrapezius, we needed to develop a more consistent cell vehicle. Gelatin was chosen as the vehicle material because it was easy to prepare, inexpensive, and biocompatible [74]. A gelatin solution was prepared, heated aseptically and then aliquoted into a 24-well plate for polymerization.

In addition to developing a protocol for sterile gelatin preparation, we also developed the vehicle composition. The first two iterations of the vehicle, 4% and 10% gelatin vehicles, depolymerized immediately when implanted beneath the spinotrapezius. Therefore, we added a crosslinking agent (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)) to gelatin preparations. To test the cross-linked gelatin acutely, both 4% and 10% gelatin vehicles cross-linked with either 6mM or 12mM EDC were incubated overnight at 37°C with 5% CO₂; all vehicles remained polymerized. Since both cross-linker concentrations remained polymerized, 6mM EDC was used in subsequent vehicle preparation to minimize the amount of cross-linker. With crosslinking maintaining gelatin polymerization, vehicles (4% and 10% gelatin cross-linked with 6mM EDC) were implanted deep to the spinotrapezius for 7 days (n = 2). After 7 days only the 10% gelatin vehicles remained polymerized, and there were no signs of infection or inflammation (Figure 8). Thus, we continued using the 10% gelatin cross-linked with 6mM EDC as the cell transplantation vehicle.

Figure 8. Cross-linked gelatin vehicle remained polymerized for 7 days in vivo. (A) Gelatin vehicles were punched with a 1.5mm biopsy punch and implanted under the spinotrapezius. (B) Seven days later, the spinotrapezius was excised and the vehicle (white arrow) was still intact and there was no infection or inflammation.

Finally, to assess the cytotoxicity of the cross-linked gelatin, we seeded them with fibroblasts and cultured for 12 hours, which is the same duration the myogenic cells would be cultured on the vehicle before transplantation. The 12-hour incubation time was chosen to allow the myogenic cells time to adhere to the gelatin while minimizing culture time in an attempt to possibly prevent the cells from differentiating any further because myogenic stem cells differentiate when they are cultured outside of their niche. After 12 hours, the fibroblasts had normal morphology and appeared adherent to the gelatin (Figure 9).

Figure 9. Fibroblasts have normal morphology after one day of culture on gelatin vehicle. Fibroblasts were cultured on the gelatin vehicle to evaluate cytotoxicity. After 24 hours, the cells had normal morphology and they appeared to have adhered to the vehicle.

Myogenic Cell Transplantation

To test the hypothesis that myogenic cell transplantation increases collateral capillary arterIALIZATION we used confocal microscopy (Figure 10) to count and measure the diameter of collateral capillaries and ACCs between ischemic and perfused arteriolar trees.

Figure 10. Confocal images of arterIALIZED collateral capillary region. Confocal images were taken of ACCs between the ligated arteriolar tree and an adjacent arteriolar tree using a 20x objective. (A,D) Alpha-smooth muscle actin positive vessels show arterioles and ACCs. (B,E) Lectin positive vessels include both capillaries and ACCs. (C,F) Composite images combining A and B or D and E. A-C are images of an operated muscle and D-F are images of a sham muscle. Scale bars are 50 μ m.

There was no difference in the total number of vessels between treatment groups (12.4 ± 0.6 vessels with thrombin, 13.6 ± 0.5 vessels with myogenic cells, 12.5 ± 0.3 with vehicle, 13.7 ± 0.6 vessels with ligation only, and 13.6 ± 0.3 vessels in sham muscles) (Figure 11). To determine if myogenic cell transplantation increases ACC number, we counted alpha-smooth muscle actin positive vessels. Thrombin, which enhances collateral capillary arteriogenesis [75], increased the ACC number (9.3 ± 0.5 vessels versus 7.1 ± 0.4 vessels with myogenic cells, 6.6 ± 0.1 vessels with vehicle, and 7.5 ± 0.3 vessels

with ligation only) (Figure 11). There was limited variation in ACC number between imaging regions, which suggests that 3 regions were sufficient for imaging.

Figure 11. Thrombin increases number of arterIALIZED collateral capillaries. Capillaries and ACCs were counted between ischemic and perfused trees in myogenic cells (n=6), vehicle (n=6), thrombin (n=5), ligation only (n=5), and sham (n=24) replicates at day 7 using a 20x objective on a confocal microscope. Thrombin increased the number of ACCs. There was no difference in the total number of vessels between groups. (*, $p < 0.05$ for a-SMA positive vessels in thrombin treated versus all other groups)

Previously, thrombin decreased the number of ACCs in the region with the largest collateral [75]. To determine if myogenic cells have a similar effect, ACCs were counted in the region of the muscle with the largest collateral. While the previous ACC numbers were determined by counting ACCs in regions with average diameter ACCs (Figure 11), these measurements were determined by finding each replicate's largest ACC and counting the ACCs in that region. Both thrombin and myogenic cells decreased ACC number in this region (8.5 ± 1.2 vessels and 9.7 ± 1.2 vessels versus 14.8 ± 0.9 vessels with vehicle and 13.7 ± 0.9 vessels with ligation only) (Figure 12).

Figure 12. Thrombin decreases number of arterIALIZED collateral capillaries in region with largest collateral. ACCs were counted between ischemic and perfused trees in mice without PECs in myogenic cells (n=4), vehicle (n=3), thrombin (n=3), and ligation only (n=3) replicates at day 7 using a 20x objective on a confocal microscope. (A) Example image of a region of average diameter ACCs (B) Example image of ACCs in the region with the largest collateral. (C) Myogenic cells and thrombin decreased the number of ACCs in the region with the largest collateral. (*, $p < 0.05$ vs vehicle and ligation only)

The decrease in vessel number suggests that myogenic cells could be affecting only selected collaterals, allowing them to enlarge slightly more than surrounding collaterals which increases the shear stress in that collateral and allows it to continue to enlarge faster than its neighbors. Therefore, to determine if myogenic cell transplantation increases overall ACC diameter, alpha-smooth muscle actin positive vessel diameters were measured. Myogenic cells increased ACC diameter ($12.1 \pm 0.8 \mu\text{m}$ and $11.9 \pm 0.5 \mu\text{m}$) compared to the vehicle ($10.1 \pm 0.4 \mu\text{m}$) and ligation only ($10.5 \pm 0.4 \mu\text{m}$), but not compared to thrombin ($11.9 \pm 0.5 \mu\text{m}$) (Figure 10). There was no difference in capillary size between the ligated and sham muscles or between treatment groups (Figure 13). There was also limited variation in ACC diameter between imaging regions, which suggests that 3 regions were sufficient for imaging.

Figure 13. Myogenic cells increase arterIALIZED collateral capillary size. Capillary and ACC diameters were measured between two arterial trees in myogenic cells (n=6), vehicle (n=6), thrombin (n=5), and ligation only (n=5) replicates at day 7 using a 20x objective on a confocal microscope. Myogenic cells increased ACC diameter compared to vehicle and ligation only. There was no difference in capillary size between ligated and sham muscles or between treatment groups. (*, $p < 0.05$ versus vehicle and ligation only)

Even though thrombin did not increase the average ACC diameter, previous work demonstrated that thrombin increases the maximum ACC diameter [75], so we determined if myogenic cells have a similar effect. Both myogenic cells and thrombin increased the maximum ACC diameter ($29.9 \pm 3.4 \mu\text{m}$ with myogenic cells and $27.9 \pm 2.0 \mu\text{m}$ with thrombin versus $17.9 \pm 1.7 \mu\text{m}$ with vehicle and $18.9 \pm 1.1 \mu\text{m}$ with ligation only) (Figure 14).

Figure 14. Myogenic cells increase maximum arterialized collateral capillary diameter. The largest ACC diameter was measured in myogenic cells (n=4), vehicle (n=4), thrombin (n=3), and ligation only (n=3) replicates at day 7 using a 20x objective on a confocal microscope. Myogenic cells and thrombin replicates increased the maximum diameter ACC compared to the other two groups. (*, p<0.05 vs gelatin and ligation only)

Some Balb/C mice have pre-existing collaterals (PECs), which may affect arteriogenesis because blood flows along the path of least resistance [76], and arteriogenesis occurs in response to increased shear stress due to increased blood flow. Therefore, it is possible that a PEC ‘steals’ flow and the majority of the remodeling happens in the PEC. To determine if PECs influenced the number of ACCs, mice were analyzed in two groups: mice with PECs (n = 2 for all groups) and mice without PECs (n = 4 for

myogenic cells and vehicle, $n = 3$ for thrombin and ligation only). In mice without PECs, thrombin increased the number of ACCs (9.9 ± 0.5 vessels versus 7.1 ± 0.5 vessels with myogenic cells, 6.5 ± 0.2 vessels with vehicle, and 7.7 ± 0.1 vessels with ligation only) (Figure 15). There was no difference in the number ACCs between mice without PECs and mice with PECs for all groups (Figure 15).

Figure 15. Thrombin increases number of arterIALIZED collateral capillaries in mice without PECs. ACCs were counted between ischemic and perfused trees in myogenic cells (PEC: $n=2$, No PEC: $n=4$), vehicle (PEC: $n=2$, No PEC: $n=4$), thrombin (PEC: $n=2$, No PEC: $n=3$), and ligation only (PEC: $n=2$, No PEC: $n=3$) replicates at day 7 using a 20x objective on a confocal microscope. There was no difference in the number of ACCs in mice with a PEC and mice without a PEC. In mice without a PEC, thrombin increased the number of ACCs. (*, $p<0.05$ versus all other groups)

To determine if PECs influence ACC size, ACC diameters were compared in mice with PECs and in mice without PECs. Mice without PECs trended towards a larger ACC diameter than mice without PECs (Figure 16). Additionally, in mice without a PEC, myogenic cells increased ACC diameter compared to other treatment groups ($13.7 \pm 1.2 \mu\text{m}$ with myogenic cells, $10.5 \pm 0.5 \mu\text{m}$ with vehicle, 13.4

$\pm 0.6 \mu\text{m}$ with thrombin, and $12.0 \pm 0.5 \mu\text{m}$ with ligation only), and thrombin increased diameter compared to vehicle (Figure 16).

Figure 16. Myogenic cells increase arterIALIZED collateral capillary diameter in mice without PECs. Capillary and ACC diameters were measured between two arterial trees in myogenic cells (PEC: n=2, No PEC: n=4), vehicle (PEC: n=2, No PEC: n=4), thrombin (PEC: n=2, No PEC: n=3), and ligation only (PEC: n=2, No PEC: n=3) replicates at day 7 using a 20x objective on a confocal microscope. There was a trend toward an increased ACC size in mice without a PEC. Myogenic cell transplantation increased ACC diameter. (#, $p < 0.05$ versus vehicle, thrombin, and ligation only; *, $p < 0.05$ versus vehicle)

Because myogenic cells increase arteriogenesis and macrophages are an important contributor to arteriogenesis [77,78,79], we tested the hypothesis that myogenic cells increase ACC diameter by increasing macrophage recruitment. We used confocal microscopy to count the number of CD68 positive cells in the region in which ACCs form anastomoses between the ischemic and perfused arteriolar trees as well as to count the number of vessel associated CD68 cells (Figure 17).

Figure 17. Confocal images of CD68 positive cells. Confocal images were taken of CD68 positive cells between the ligated arteriolar tree and an adjacent arteriolar tree using a 20x objective. (A,D) Alpha-smooth muscle actin positive vessels show arterioles and ACCs. (B,E) CD68 positive cells indicate macrophages. (C,F) Composite images combining A and B or D and E. A-C are images of an operated muscle and D-F are images of a sham muscle. Scale bar is 50 μ m.

Ligation increased CD68 positive cells (Figure 18), but myogenic cells and thrombin did not enhance this effect (16.0 ± 0.5 cells with myogenic cells, 15.8 ± 0.4 cells with vehicle, 15.3 ± 0.5 with thrombin, and 15.5 ± 0.5 with ligation only) (Figure 18). Myogenic cells also did not affect the number of vessel associated cells (16.9 ± 0.8 cells with myogenic cells, 14.6 ± 0.7 cells with vehicle, 16.1 ± 0.7 with thrombin, and 17.1 ± 0.8 with ligation only) (Figure 18). There was limited variation in CD68 content between imaging regions, which suggests that 3 regions were sufficient for imaging.

Figure 18. Myogenic cell transplantation has no effect on CD68 cell count. CD68 positive cells were counted between two arterial trees in myogenic cells (n=6), vehicle (n=6), thrombin (n=5), and ligation only (n=5) replicates replicates at day 7 using a 20x objective on a confocal microscope. There was no difference in the number of CD68 cells between groups. (*, $p < 0.05$ vs sham)

DISCUSSION

Pre-existing collaterals improve the prognosis in patients with ischemic diseases, but some patients never develop angiographically-identifiable collateral vessels [3,4,5,8,9]. Additionally, females appear to have a worse prognosis than males for peripheral arterial occlusive disease (PAOD) [64,65,66], possibly due to impaired collateral development [70]. Collateral vessels develop through arteriogenesis in response to increased shear stress that triggers a cascade of events involving endothelial cell activation, macrophage recruitment, matrix degradation, and smooth muscle/endothelial cell proliferation. Arteriogenesis results in a collateral arteriole with an expanded diameter that helps restore blood flow to the ischemic area. Because current treatments for severe PAOD have limited positive outcomes [2], alternative treatments to promote arteriogenesis are needed. Cell transplantation is a promising treatment for PAOD, but there has been limited clinical success for multiple cell types including bone marrow mononuclear cells, bone marrow mesenchymal cells, and endothelial progenitor cells [29,30,39,40,44,45]. Tissue resident stem cells, such as myogenic stem cells, have not been investigated as a treatment for PAOD, but they have potential to promote arteriogenesis [46,51,55,56]. Therefore, we determined if arterialized collateral capillary (ACC) formation was impaired in female mice compared to male mice, developed a myogenic cell transplantation protocol, and tested the hypotheses that myogenic cell transplantation enhanced arteriogenesis and macrophage recruitment.

Seven days post ligation, ACCs on the operated muscle were significantly larger in diameter than collateral capillaries in the sham muscle, demonstrating that outward remodeling occurred. There was also no difference in total number of vessels in the ligated and sham muscles, demonstrating that the ACCs developed through capillaries outwardly remodeling and recruiting smooth muscle cells, as observed previously [15,80]. Interestingly, there was no difference between males and females in ACC diameter or number. The lack of difference between male and female mice could have occurred because the mice used in this study were healthy, and clinical sex differences are observed in patients who are often elderly with comorbidities and other risk factors [65,66,67]. Unfortunately, we do not know of any arteriogenesis investigations in female mice with ischemic disease comorbidities, but arteriogenesis is

impaired in both aged and diabetic mice compared to healthy models [81,82], and female ApoE^{-/-} mice exhibit endothelial dysfunction compared to male mice [83,84,85] and have larger atherosclerotic lesions [86,87]. To determine if comorbidities affect arteriogenesis in female mice, a diabetic or aged mouse model could be investigated.

Seven days post ligation, thrombin increased the number of ACCs compared to all other treatment groups, including myogenic cells. However, the increase in ACC number with thrombin treatment is not necessarily an advantage because it may not result in a more effective restoration of blood flow. In the mouse hindlimb ischemia model, the density of blood vessels in the hindlimb peaks seven days post ligation, and as the number of vessels decreased, the hindlimb perfusion stayed the same [88], suggesting that the increased number of vessels is not necessary to restore bloodflow. With other arteriogenic factors, such as Fibroblast Growth Factor (FGF) and Vascular Endothelial Growth Factor (VEGF), the initial response to treatment is accelerated [89,90,91], but at later timepoints, there was no difference in perfusion [92], and the excess vessels formed during the initial accelerated development appear to be leaky and therefore not functional [92]. Previous work has demonstrated that thrombin augments the remodeling process at early timepoints similar to FGF and VEGF [75,93]. The administration of thrombin may promote the growth of blood vessels that are unnecessary to restore shear stress in the collateral zone, and therefore will regress at a later timepoint. Although myogenic cells did not increase in the number of ACCs, increasing the number of ACCs may not be necessary for ischemic recovery. To determine if thrombin's effect is temporary, a study with longer endpoints such as 14 or 28 days could be performed.

Even though myogenic cells had no effect on the number of ACCs, both myogenic cells and thrombin increased ACC diameter. The maximum ACC diameter was also larger with both thrombin and myogenic cells. Additionally, in the region of the muscle with the largest collateral, both treatments decreased the number of ACCs compared to the other two treatment groups. Previous work has demonstrated that thrombin augments the remodeling of only certain collaterals [75]. The results from this study suggest that myogenic cell transplantation has a similar effect to thrombin injections, which

supports our hypothesis that myogenic cells would augment arteriogenesis. Myogenic cells secrete factors such as matrix metalloproteinases (MMPs) and VEGF [94,95] which are involved in arteriogenesis signaling and could be secreting these factors to accelerate arteriogenesis. VEGF is responsible for endothelial cell proliferation during arteriogenesis [96,97,98], and MMPs are responsible for degrading the matrix during vessel remodeling [14]. In a future study, to determine if myogenic cells are increasing arteriogenesis by secreting arteriogenic factors, we could analyze conditioned media to determine what proteins the myoblasts are secreting.

Myogenic cells are not the only factor that can affect arteriogenesis in certain collaterals, pre-existing collaterals (PECs), which are present in some mice from the Balb/C strain, could also affect which collaterals experience arteriogenesis. To determine if PECs affect collateral capillary arterialization, mice with PECs were analyzed separately from mice without PECs. There was a trend towards smaller ACCs in mice with PECs. Blood flows along the path of least resistance [76], so after a ligation blood flow would be redirected mainly through the PEC since it is the lowest resistance vessel. Because the PEC has the largest increase in blood flow and therefore the largest increase in shear stress, the PEC would be the main arteriole to undergo remodeling. While mice with a PEC appeared to have a trend towards reduced collateral capillary arteriogenesis, they did experience some collateral capillary arteriogenesis. Because the PEC was smaller than the original feed artery, it is possible that it could not normalize shear stress on its own, so some of the capillaries could have experienced an increase in shear stress causing some collateral capillary arteriogenesis [15]. In mice with a PEC, the PEC could have been the main collateral and therefore could have experienced the majority of the remodeling. While PECs may have an effect on ACC formation, including mice with a PEC in the initial vessel count and vessel diameter analysis may not have affected the results because the same conclusions about the effects of myogenic cells were drawn when mice with a PEC were excluded. To determine if the PEC experiences the largest increase in blood flow following ligation, we could utilize an imaging technique that allows for blood flow quantification such as laser speckle flowmetry [99]. In future studies, more replicates would

be included to determine if there are any statistical differences between mice with PECs and mice without PECs.

In addition to secreting arteriogenic factors, myoblasts also recruit white blood cells [52,53]. To determine if myogenic cell transplantation increased macrophage recruitment, the number of CD68 positive cells were counted. As expected, ligation increased the number of macrophages in the region with ACCs. Following ligation, the increased shear stress in the collateral vessel activates the endothelium, causing it to secrete MCP-1 which activates monocytes, enhancing their adhesion to the endothelium [100]. Once activated, monocytes extravasate into the tissue and become macrophages [100]. Without macrophage recruitment, there is minimal arteriogenesis [77,78,79], so it was expected that the ligated muscle would see an increase in macrophages. However, both myogenic cells and thrombin had no effect on macrophage recruitment. The lack of difference between the groups could have occurred because the seven day timepoint is too long after ligation to see macrophage number differences. Macrophages are recruited to the collateral site early in arteriogenesis, and the number of macrophages peaks three days after ligation and is significantly decreased by day 7 [100,101]. Additionally, in thrombin treated mice, at three days post ligation there is only a trend towards an increase in the number of macrophages when compared to a control injection [75]. This suggests that arteriogenic agents such as thrombin or myogenic cells may be able to increase macrophage recruitment. Therefore, there may have been a difference in the number of macrophages between groups at 3 days post ligation that was gone by the 7 day timepoint used in this study. In a future study, to determine if myogenic cells increase macrophage recruitment earlier than 7 days post ligation, a 3 day timepoint could be added.

Another possible reason for the failure of myogenic cells to affect macrophage number is that there may be differences in the numbers of each type of macrophage. Macrophages exist on a spectrum that ranges from classically activated to alternatively activated [102]. Classically activated macrophages are also referred to as M1 macrophages and are pro-inflammatory, while alternatively activated macrophages are also referred to as M2 macrophages and are involved in tissue remodeling [102]. M2 macrophages are the main contributor to arteriogenesis and the main type of macrophage at the collateral

site [103,104]. Because a general macrophage marker, CD68, was used in this study, the number of each type of macrophage could not be determined. It is possible that myogenic cells increased the number of M2 macrophages, but this increase could not be observed. To determine if there are more M2 macrophages a stain like CD206 that is specific to M2 macrophages [73] could be used in addition to the CD68 stain.

There were a few limitations to this study. The sample size was small both for comparing mice with PECs to mice without PECs, and for comparisons made with only mice without PECs. When comparing mice with PECs to mice without PECs, only general trends could be observed. In the future, to draw statistical conclusions about the effect of a PEC on ACCs, a sample size of 6 mice with a PEC would be needed to have a power of 0.8. In addition, 6 replicates would also be needed to have power of 0.8 for comparisons between treatment groups that were made only in mice without PECs, such as the maximum collateral diameter and the number of ACCs in the region with the largest collateral.

Since this study was the first cell transplantation study in the spinotrapezius performed in this lab, the protocol could still be optimized. It is possible that myogenic cells did not increase the number of ACCs like thrombin because the number of cells transplanted was not optimized. In this study, 9.3×10^3 cells was chosen based on results from previous work done in the lab in the mouse hindlimb, which used more cells but had disorganized blood vessel growth [105,106], as well as the number of cells available at the time of surgery. This study was the first cell transplantation study performed in the spinotrapezius, so a cell dose response study may be necessary to determine the optimal number of cells and to determine if changing the number of cells affects the number of ACCs. To determine the optimal number of cells to transplant, we could perform a dose response study with 1,000 to 1 million cells based on previous cell transplantation studies [88,107,108].

The vehicle could also be optimized for future cell transplantation studies. Gelatin was chosen for this initial cell transplantation study because it is biocompatible, easy to prepare, and inexpensive [74]. However, there are some disadvantages to gelatin. Ideally, the gelatin vehicle would have a modulus close to the modulus of muscle so that the myogenic cells are in an environment similar to their native ECM

[109], but because gelatin must be boiled, it is difficult to create gelatin vehicles with a consistent gelatin concentration and therefore a consistent modulus. We chose the concentration of gelatin and EDC for the vehicles based on literature that measured the gelatin modulus [110,111,112,113,114], but were unable to determine if the modulus of the final vehicle was close to that of muscle. Another disadvantage to the gelatin vehicles was the use of a biopsy punch to create appropriately sized vehicles. Thick gelatin vehicles were used because they worked with the biopsy punch and thinner gels did not, but thinner gels may have been more beneficial. Also, because the biopsy punch was used during surgery when the cells were already on the gelatin vehicles, it was difficult to obtain an accurate cell count. In future studies, extra punches could be prepared and stained with BBI or a myogenic stem cell marker to estimate the number of transplanted myogenic stem cells. Additionally, during the follow-up procedure, the punches could be explanted and stained with the same stains to determine how many myogenic stem cells remained in the vehicle. It is also possible that the biopsy punch and the use of the forceps to place the gelatin vehicles beneath the spinotrapezius damaged some of the cells, despite performing both steps gently. To determine if implanting the vehicle damaged the cells, we could simulate the vehicle implantation and then use a live/dead stain on the vehicle. However, a construct that requires less interaction with surgical instruments would be ideal. A solution to the problems encountered with gelatin is the use of a thermoreversible hydrogel, which can be engineered with a specific modulus [115,116,117], and the cells can be added to the exact injected volume of vehicle. Since the vehicle would be injected directly into the mouse, it can be designed such that the syringe minimally damages the cells during construct delivery [118]. Additionally, with the thermoreversible gel the cells would be dispersed throughout the gel rather than on the surface as they are with the gelatin. Since the cells would be throughout the gel, they may be more protected from damage during transplantation than they are with gelatin, but any secreted factors would have to diffuse through the gel to reach the muscle.

Myogenic cells increased ACC diameter and maximum ACC diameter, and decreased the number of ACCs in the region surrounding the maximum diameter ACC. This supports the hypothesis that myogenic cell transplantation is a potential arteriogenesis-enhancing treatment for PAOD. However,

myogenic cells did not affect macrophage recruitment. The methods used in this study still need optimization to determine if myogenic cells affect macrophage recruitment at earlier time points or in specific populations. Additionally, we found no differences in arteriogenesis between males and females. In future studies, diseased animal models can be utilized to determine if impaired arteriogenesis is involved in the clinical sex differences seen with PAOD.

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APPENDICES

Appendix A: Surgery and Experimental Protocols Spinotrapezius Ligation Protocol

Date _____

Spinotrapezius Ligation

Initials _____

Mouse Information

DOB: _____

Sex: _____

Tag: _____

Genotype/strain: _____

Cage: _____

Weight: _____

Materials

Instruments

- ____ 1. Standard Pattern Forceps (1)
- ____ 2. Iris Scissors (1)
- ____ 3. S&T(1)
- ____ 4. 5/45 (1)
- ____ 5. Dumont #7's (1)
- ____ 6. Microdissection scissors (1)
- ____ 7. Castro Viejos (1)

Pre-sterilize in autoclave

- ____ 8. Cotton gauze (4)
- ____ 9. Cotton swabs (4)
- ____ 10. 6.0 silk suture (2x1 in)
- ____ 11. 7-0 Prolene Suture
- ____ 12. Surgical Drape

Obtained in surgery suite

- ____ 13. Petri dish w/ sterile saline
- ____ 14. Gloves (non-sterile and sterile)
- ____ 15. FST heat pad w/ rectal probe
- ____ 16. Recovery bin and weigh boat
- ____ 17. Depilatory cream
- ____ 18. non-sterile cotton swabs
- ____ 19. non-sterile cotton gauze
- ____ 20. Isolation mask & cap
- ____ 21. Analgesic (buprenorphine)

Surgery Preparation

- ____ 22. Spray surgery area with Nolvasan
- ____ 23. Weigh animal in weight boat
- ____ 24. Place animal in anesthesia box
- ____ 25. Open the oxygen cylinder and set anesthesia-machine flow meter to $\sim 3 \text{ l} \cdot \text{min}^{-1}$
- ____ 26. Anesthetize animal w/ 5% isoflurane
- ____ 27. Reduce flow rate to $0.5\text{-}1.0 \text{ l} \cdot \text{min}^{-1}$ and the isoflurane to 1-3%
- ____ 28. Lay animal prone with nose in nose-cone
- ____ 29. Remove hair on anterior dorsal aspect of the animal with clippers and depilatory cream.
- ____ 30. Apply veterinary ointment to eyes to avoid drying during the procedure
- ____ 31. Give animal subcutaneous injection of

buprenorphine

- ____ 32. Lay animal prone on heat pad (w/ 4x4 on top) w/ nose in nose-cone
- ____ 33. Insert rectal probe and set thermo-controller to 35°C
- ____ 34. Adjust focus, lighting, and center mouse
- ____ 35. Don isolation mask, cap, and scrubs
- ____ 36. Open sterile surgical instrument pack
- ____ 37. Open sterile pack and dump in surgical instrument field
- ____ 38. Place sterile petri dish in sterile field and fill with sterile saline
- ____ 39. Don sterile gloves
- ____ 40. Place surgical drape on the animal and arrange the items in sterile field

Surgery

- ____ 41. Make 5mm incision parallel to spine at intersection of fat pad and muscle
- ____ 42. Dissect skin overlying the spinotrapezius muscle
- ____ 43. Search for lateral feed artery vein pair jumping from the underlying fat pad to the spinotrapezius
- ____ 44. Blunt dissect fat pad to reveal arteriole-venule pair, using papavrine as a vasodilator if necessary
- ____ 45. Blunt dissect arteriole away from venule
- ____ 46. Pass a separated strand from 6-0 silk suture below the arteriole and tie off
- ____ 47. Repeat again about 1mm above previous ligature
- ____ 48. Cut arteriole between the two ligatures with micro dissection scissors
- ____ 49. Suture incision with 7-0 prolene suture
- ____ 50. Repeat for contralateral side to perform sham, but do not tie off ligature

Post-Surgical

- ____ 51. Give the animal a subcutaneous injection of buprenorphine
- ____ 52. Place the animal in the recovery bin, on a blue bench cover, above a heat pad and allow to recover
- ____ 53. Turn the flow meter down to 0, turn off the isoflurane, and close the oxygen cylinder
- ____ 54. Indicate surgery on cage card

Notes

Sterile Gelatin Preparation Protocol

Date: _____

Sterile Gelatin Preparation

Initials: _____

Sterile Materials

- 25ml Erlenmeyer flask
- Rubber stopper + tubing
- Rubber stopper
- Spatula
- Distilled H₂O
- Gelatin powder
- 10mL syringe
- Syringe filter
- Pipettes
- 50 mL conicals
- 24 Well Plate

Non-Sterile Materials

- Hot plate
- Analytical balance
- 6mM 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)

Procedure

1. Turn hot plate on to 200°C
2. In the hood, open the sterile packs with the Erlenmeyer flask and the rubber stopper
3. Weigh empty 25ml Erlenmeyer flask + rubber stopper on an analytical balance and zero the balance
4. Bring the Erlenmeyer flask back to the hood, and bring the gelatin into the hood
5. Open the sterile pack with the spatula and use to put some gelatin powder into the Erlenmeyer flask
6. Put the rubber stopper back on the flask and weigh it on the analytical balance
7. Add enough distilled H₂O to the flask to make a 10% gelatin solution (by weight)
8. Open the sterile pack with the rubber stopper + tubing and put it on the flask
9. Bring the flask to the hot, wait for it to reach a boil, and then allow it to boil for 5 minutes
10. Bring the flask back into the hood and put 0.3mL of the gelatin solution in each well
11. While the gelatin is solidifying use the syringe filters to filter the 6mM EDC
12. Once the gelatin has solidified, add 0.5mL of EDC to each well
13. Leave the gelatin in the EDC solution overnight at 4°C to allow for crosslinking
14. The next day aspirate the EDC solution and wash the gel 3x30 minutes with sterile H₂O

Development of Gelatin Boiling Technique

We tried a couple techniques for sealing and venting the gelatin solution before developing the final solution. The first attempt utilized a foam stopper in an Erlenmeyer flask, but this method did not allow sufficient ventilation. The pressure buildup in the flask prevented the foam stopper from sealing the flask throughout the entire boiling process. The second attempt was to use a side arm distilling head and a round bottom flask borrowed from the chemistry department, which successfully sealed the flask and allowed ventilation. However, during the glassware testing a simpler solution was developed, so we decided to continue with the simpler solution for the final protocol. This solution used an Erlenmeyer flask with a vented rubber stopper and a piece of curved tubing attached with a tubing connector (Figure 19).

Figure 19. Preparation of sterile gelatin vehicles. Sterile gelatin was prepared by weighing gelatin powder in a cell culture hood and adding sterile water to make a 10% gelatin solution. The solution was then boiled outside the hood while sealed and vented, as shown, and aliquoted into a 24-well plate to solidify.

Myoblast Passing Protocol

Date: _____

Primary Myoblast Expansion Protocol

Initials: _____

Purpose: To expand primary mouse myoblasts from live Extensor Digitorum (EDL) muscle fibers

Necessary Materials:

- Growth Media (20% FBS in Base Media)
- SB 203580 working solution (10 mmol)
- bFGF working solution (1 μ g/ml in PBS)
- Cell Dissociation Solution - EDTA (Fisher 13151014)
- Trypsin/EDTA (0.05% Invitrogen 25300-062)
- PBS (-/-, Ca⁺², Mg⁺²)
- ECM coated flask

1. ___ Obtain an ~80% confluent culture vessel
 - a. myoblasts will begin to differentiate and form myotubes as they approach confluency
2. ___ Clean Phase Contrast Microscope with 70% v/v Isopropanol (IPA)
3. ___ Image save pictures of the culture with a phase contrast microscope
4. ___ Determine passage ratio to be used
 - a. myoblasts should not be split at a ratio higher than 1:3
5. ___ Choose passaging method based upon goals and culture conditions
 - a. use Trypsin for:
 - i. for better cell distribution, single cell suspension
 - ii. for a confluent flask with low overall confluency (1:1 pass)
 - b. use EDTA for:
 - i. preservation of surface receptors (integrins)
 - ii. selective passaging (more differentiated cells will more strongly adhere)

Trypsin Passaging

1. ___ Warm Trypsin and Growth Media in 37C H₂O bath immediately before use
2. ___ Aspirate media from culture vessel
3. ___ Wash with PBS (-/-) and aspirate
4. ___ Add warm trypsin
 - a. 0.04 ml/cm² surface (T12.5 - 0.5 ml, T75 - 3 ml)
5. ___ Immediately place in incubator for ~2 min
6. ___ Observe with phase contrast microscope
 - a. cells should begin to ball up and become phase bright
7. ___ Hit the flask repeatedly to dislodge weakly adherent cells
8. ___ Observe under the phase contrast microscope to ensure detachment of all cells
 - a. If cells are still attached, return them to the incubator for an additional min and repeat
9. ___ Add the same volume of Growth Media as trypsin to stop enzymatic cleavage
10. ___ Wash the surface of the culture vessel with the cell suspension
11. ___ Label a 15 ml conical tube and transfer the cell suspension
12. ___ Centrifuge at 300g for 5 min to create a cell pellet
13. ___ Aspirate media from the conical
 - a. be careful to avoid aspirating the pellet
14. ___ Resuspend in the volume of growth media (+ factors) appropriate for the chosen passage ratio
15. ___ Repeatedly pipette up and down to break of cell clumps
16. ___ Rock the culture vessel repeatedly: forward/back and left/right to evenly disperse the cells
 - a. Even cell distribution can be checked under the phase contrast microscope
17. ___ Place in incubator
 - a. close the door gently to prevent uneven cell distribution

Perfusion Fixation Protocol

Date _____

Perfusion Fixation

Initials _____

Mouse Information

DOB: _____

Sex: _____

Tag: _____

Genotype/strain: _____

Cage: _____

Weight(g): _____

Materials

Non-Sterilize Dissection Instruments

- ____ 1. Forceps (1)
- ____ 2. Fine forceps (2)
- ____ 3. Bone scissors (1)
- ____ 4. Curved Iris scissors (1)
- ____ 5. Microdissection scissors (1)
- ____ 6. Vascular clamp (1)
- ____ 7. Castroviejos

Obtained in surgery suite

- ____ 8. Tape
- ____ 9. 20 mL syringes (2)
- ____ 10. 5 mL syringe (1)
- ____ 11. Syringe pump
- ____ 12. Petri-dish
- ____ 13. Bench cover
- ____ 14. Depilatory cream
- ____ 15. Clippers
- ____ 16. Veterinary ointment
- ____ 17. Heating pad
- ____ 18. Catheter
- ____ 19. Non-sterile saline
- ____ 20. Cotton swabs
- ____ 21. Gauze sponges
- ____ 22. Saran wrap

Vasodilator Cocktail Preparation

- ____ 23. Turn on water bath to 37°C
- ____ 24. 400 μ L heparin
- ____ 25. 1mL SNP(orange)
- ____ 26. 600 μ L Adenosine(clear)
- ____ 27. 38mL PBS solution
- ____ 28. 5 mL 4% Paraformaldehyde (PFA)
- ____ 29. Thaw SNP, Adenosine and PFA
- ____ 30. Add heparin, SNP, Adenosine, and PBS solution together in a 50mL conical
- ____ 31. Place vasodilator cocktail in water bath

Procedure Preparation

- ____ 32. Obtain saline filled petri-dish, cotton swab, and instruments

Fixation

- ____ 33. Remove hair on back by shaving & depilation
- ____ 34. Expose muscles of interest and blunt dissect to aid in removal post-fixation, then cover with saran wrap
- ____ 35. Tape animal in supine position to 4X4 gauze sponge over heating pad
- ____ 36. Fill 10mL syringe with 10 mL warm Vaso D, load into syringe pump and attach catheter
- ____ 37. Flow liquid through the catheter to the tip to prevent air from being injected into circulatory system
- ____ 38. Lift skin from muscle in abdominal region and cut a window over the sternum
- ____ 39. Lift sternum and cut connective tissue under
- ____ 40. Use bone scissors to quickly cut through the ribs to the armpit on both sides
- ____ 41. Clamp sternum with castroviejos and reflect towards mouse's head
- ____ 42. Cut diaphragm with curved iris scissors to open chest cavity
- ____ 43. Cut away excess tissue around the heart
- ____ 44. Make a small incision in the apex of the heart
- ____ 45. Insert catheter and clamp with vascular clamp and cut right atrium
- ____ 46. Inject Vaso D solution into animal approximately 4x 10mL (2 mL/min, 14.43 mm diameter), soaking up excess blood and fluids with gauze sponges
- ____ 47. Inject 5 mL PFA (1 mL/min, 11.99 mm diameter)
- ____ 48. Dissect out muscles of interest using fine forceps and microdissection scissors
- ____ 49. Turn off water bath, cover scope, turn off oxygen, turn off isoflurane, and clean instruments

Notes

Staining Protocol

Date: _____ **Alpha-Smooth Muscle Actin, Lectin, and CD68 Staining Protocol** Initials: _____

Materials

- 24-well culture plates (Cat#: 3738, Corning Incorporated)
- PBS
- 0.1% Triton-X (Cat#: T8787, Sigma-Aldrich)
- 2% Bovine Serum Albumin (Cat# B6917, Sigma Aldrich)
- Monoclonal Anti-Alpha Smooth Muscle Actin, Cy3 Conjugate (Cat#: C6198, Sigma-Aldrich)
- Isolectin GS-IB4 from GRI DyLight 649 (Cat #: DL-1208, Vector Laboratories)
- Rat Anti Mouse CD68 FA-11 Clone Alexa Fluor 488 (Cat #: MCA1957A488T, Bio-Rad)
- Glass Slides
- Glass Coverslips
- Parafilm
- Nail Polish
- Aluminum foil

Staining

- ___ 1. Using forceps, remove muscle from PBS (stored in microcentrifuge tube at 4°C) and place in a single well of a 24-well culture plate.
- ___ 2. Prepare antibody solution containing 1:200 1A4 clone (alpha-smooth muscle actin, Cy3 conjugate), 1:100 Isolectin GS-IB4 from GRI (Alexa Fluor 647), and 1:200 Rat Anti Mouse CD68 FA-11 Clone (Alexa Fluor 488) in 0.1% Triton-X, 2% BSA (reconstituted in PBS) in PBS, using 0.3mL of solution per muscle.
- ___ 3. Wrap the 24-well plate in parafilm and incubate muscle in antibody solution at 4°C for 72 hours for the Spinotrapezius or 7 days for the Gracilis.
- ___ 4. Wash in 0.1% Triton-x in PBS 3x for 20 minutes at room temperature. Cover plate with foil during each wash.
- ___ 5. Wash in plain PBS for 30 minutes at room temperature. Cover with foil during wash.
- ___ 6. Place 1-2 drops of 50/50 PBS and Glycerol onto slide.
- ___ 7. Remove muscle from well using forceps and place on a slide. Depression slides are best for the gracilis because the muscle is thick and the coverslip will not lie flat on a flat slide.
- ___ 8. Add 1-2 drops of 50/50 PBS and Glycerol to the top of the muscle and place coverslip over the muscle.
- ___ 9. Paint edges of coverslip with clear nail polish to create a seal and prevent tissue desiccation.
- ___ 10. Store slides at 4°C wrapped in foil or an opaque container between imaging.
- ___ 11. Wait 24 hours before imaging to allow the glycerol to clear and the nail polish to dry.

Imaging

- ___ 12. Image between three arteriolar trees using the 20x objective on the confocal microscope.
- ___ 13. Use the following lasers: Alexa Fluor 488 for CD68, Alexa Fluor 559 for alpha-smooth muscle actin, Alexa Fluor 647 for lectin

Appendix B: Sample Images

Key

Stain Color	Meaning
Red	α -SMA vessels (ACCs)
Blue	Lectin vessels (capillaries)
Green	CD68 Cells (macrophages)

Female Ligated

Female Sham

Male Ligated

Male Sham

Myogenic Cells Ligated

Myogenic Cells Sham

Vehicle Ligated

Vehicle Sham

Thrombin Ligated

Thrombin Sham

Ligation Only Ligated

Ligation Only Sham

Appendix C: Raw Data

Male and Female Vessel Count Data

Replicate	Sex	Muscle	Region	Total Vessels	Capillaries	α-SMA Vessels
1	Male	Ligated	Region 1	PEC	PEC	PEC
1	Male	Ligated	Region 2	PEC	PEC	PEC
1	Male	Ligated	Region 3	PEC	PEC	PEC
2	Male	Ligated	Region 1	9	4	5
2	Male	Ligated	Region 2	10	6	4
2	Male	Ligated	Region 3	10	4	5
3	Male	Ligated	Region 1	12	7	5
3	Male	Ligated	Region 2	10	5	5
3	Male	Ligated	Region 3	8	3	5
4	Male	Ligated	Region 1	11	6	5
4	Male	Ligated	Region 2	11	7	4
4	Male	Ligated	Region 3	10	6	4
5	Male	Ligated	Region 1	17	12	5
5	Male	Ligated	Region 2	17	12	5
5	Male	Ligated	Region 3	15	10	5
6	Male	Ligated	Region 1	13	8	5
6	Male	Ligated	Region 2	13	8	5
6	Male	Ligated	Region 3	14	9	5
7	Male	Ligated	Region 1	10	4	6
7	Male	Ligated	Region 2	10	5	5
7	Male	Ligated	Region 3	9	3	6
8	Male	Ligated	Region 1	PEC	PEC	PEC
8	Male	Ligated	Region 2	PEC	PEC	PEC
8	Male	Ligated	Region 3	PEC	PEC	PEC
1	Male	Sham	Region 1	10	10	0
1	Male	Sham	Region 2	12	12	0
1	Male	Sham	Region 3	10	10	0
2	Male	Sham	Region 1	10	10	0
2	Male	Sham	Region 2	8	8	0
2	Male	Sham	Region 3	10	10	0
3	Male	Sham	Region 1	10	10	0
3	Male	Sham	Region 2	10	10	0
3	Male	Sham	Region 3	12	12	0

4	Male	Sham	Region 1	16	16	0
4	Male	Sham	Region 2	9	9	0
4	Male	Sham	Region 3	12	12	0
5	Male	Sham	Region 1	12	12	0
5	Male	Sham	Region 2	19	19	0
5	Male	Sham	Region 3	14	14	0
6	Male	Sham	Region 1	10	10	0
6	Male	Sham	Region 2	15	15	0
6	Male	Sham	Region 3	12	12	0
7	Male	Sham	Region 1	16	16	0
7	Male	Sham	Region 2	9	9	0
7	Male	Sham	Region 3	11	11	0
8	Male	Sham	Region 1	14	14	0
8	Male	Sham	Region 2	11	11	0
8	Male	Sham	Region 3	11	11	0
1	Female	Ligated	Region 1	PEC	PEC	PEC
1	Female	Ligated	Region 2	PEC	PEC	PEC
1	Female	Ligated	Region 3	PEC	PEC	PEC
2	Female	Ligated	Region 1	12	7	5
2	Female	Ligated	Region 2	10	6	4
2	Female	Ligated	Region 3	9	5	4
3	Female	Ligated	Region 1	9	4	5
3	Female	Ligated	Region 2	10	6	4
3	Female	Ligated	Region 3	13	8	5
4	Female	Ligated	Region 1	PEC	PEC	PEC
4	Female	Ligated	Region 2	PEC	PEC	PEC
4	Female	Ligated	Region 3	PEC	PEC	PEC
5	Female	Ligated	Region 1	11	7	4
5	Female	Ligated	Region 2	11	6	5
5	Female	Ligated	Region 3	12	7	5
6	Female	Ligated	Region 1	PEC	PEC	PEC
6	Female	Ligated	Region 2	PEC	PEC	PEC
6	Female	Ligated	Region 3	PEC	PEC	PEC
7	Female	Ligated	Region 1	PEC	PEC	PEC
7	Female	Ligated	Region 2	PEC	PEC	PEC
7	Female	Ligated	Region 3	PEC	PEC	PEC
8	Female	Ligated	Region 1	10	5	5
8	Female	Ligated	Region 2	11	7	4
8	Female	Ligated	Region 3	11	6	5

1	Female	Sham	Region 1	12	12	0
1	Female	Sham	Region 2	9	9	0
1	Female	Sham	Region 3	11	11	0
2	Female	Sham	Region 1	10	10	0
2	Female	Sham	Region 2	10	10	0
2	Female	Sham	Region 3	11	11	0
3	Female	Sham	Region 1	12	12	0
3	Female	Sham	Region 2	11	11	0
3	Female	Sham	Region 3			0
4	Female	Sham	Region 1	11	11	0
4	Female	Sham	Region 2	13	13	0
4	Female	Sham	Region 3	14	14	0
5	Female	Sham	Region 1	12	12	0
5	Female	Sham	Region 2	11	11	0
5	Female	Sham	Region 3	11	11	0
6	Female	Sham	Region 1	11	11	0
6	Female	Sham	Region 2	9	9	0
6	Female	Sham	Region 3	12	12	0

PEC indicates that the muscle had a pre-existing collateral and measurements were not taken

Male and Female Vessel Diameter Data

Replicate	Sex	Muscle	Region	Vessel	α -SMA Vessel Diameter (μm)	Lectin Vessel Diameter (μm)
1	Male	Ligated	Region 1	Vessel 1	PEC	PEC
1	Male	Ligated	Region 1	Vessel 2	PEC	PEC
1	Male	Ligated	Region 1	Vessel 3	PEC	PEC
1	Male	Ligated	Region 2	Vessel 1	PEC	PEC
1	Male	Ligated	Region 2	Vessel 2	PEC	PEC
1	Male	Ligated	Region 2	Vessel 3	PEC	PEC
1	Male	Ligated	Region 3	Vessel 1	PEC	PEC
1	Male	Ligated	Region 3	Vessel 2	PEC	PEC
1	Male	Ligated	Region 3	Vessel 3	PEC	PEC
2	Male	Ligated	Region 1	Vessel 1	12.5	6.1
2	Male	Ligated	Region 1	Vessel 2	12.6	4.8
2	Male	Ligated	Region 1	Vessel 3	10.2	4.4
2	Male	Ligated	Region 2	Vessel 1	14.8	
2	Male	Ligated	Region 2	Vessel 2	12.0	
2	Male	Ligated	Region 2	Vessel 3	8.5	
2	Male	Ligated	Region 3	Vessel 1	12.1	4.6
2	Male	Ligated	Region 3	Vessel 2	10.8	4.7
2	Male	Ligated	Region 3	Vessel 3	10.9	4.0
3	Male	Ligated	Region 1	Vessel 1	9.4	3.8
3	Male	Ligated	Region 1	Vessel 2	7.5	3.9
3	Male	Ligated	Region 1	Vessel 3	10.8	5.3
3	Male	Ligated	Region 2	Vessel 1	9.7	4.7
3	Male	Ligated	Region 2	Vessel 2	12.1	4.3
3	Male	Ligated	Region 2	Vessel 3	12.4	4.1
3	Male	Ligated	Region 3	Vessel 1	11.4	4.6
3	Male	Ligated	Region 3	Vessel 2	9.8	4.5
3	Male	Ligated	Region 3	Vessel 3	8.6	4.2
4	Male	Ligated	Region 1	Vessel 1	11.8	4.4
4	Male	Ligated	Region 1	Vessel 2	7.2	3.8
4	Male	Ligated	Region 1	Vessel 3	11.1	4.1
4	Male	Ligated	Region 2	Vessel 1	10.9	4.5
4	Male	Ligated	Region 2	Vessel 2	10.6	3.1
4	Male	Ligated	Region 2	Vessel 3	11.2	5.1
4	Male	Ligated	Region 3	Vessel 1	11.2	5.4
4	Male	Ligated	Region 3	Vessel 2	13.0	4.4

4	Male	Ligated	Region 3	Vessel 3	13.4	5.0
5	Male	Ligated	Region 1	Vessel 1	11.6	4.5
5	Male	Ligated	Region 1	Vessel 2	12.5	4.7
5	Male	Ligated	Region 1	Vessel 3	6.9	4.5
5	Male	Ligated	Region 2	Vessel 1	8.8	4.5
5	Male	Ligated	Region 2	Vessel 2	8.6	4.8
5	Male	Ligated	Region 2	Vessel 3	11.7	3.8
5	Male	Ligated	Region 3	Vessel 1	15.3	4.1
5	Male	Ligated	Region 3	Vessel 2	6.7	3.6
5	Male	Ligated	Region 3	Vessel 3	12.5	4.8
6	Male	Ligated	Region 1	Vessel 1	10.7	4.8
6	Male	Ligated	Region 1	Vessel 2	14.0	5.0
6	Male	Ligated	Region 1	Vessel 3	14.2	4.9
6	Male	Ligated	Region 2	Vessel 1	10.9	3.7
6	Male	Ligated	Region 2	Vessel 2	9.9	5.3
6	Male	Ligated	Region 2	Vessel 3	10.6	3.9
6	Male	Ligated	Region 3	Vessel 1	14.9	3.4
6	Male	Ligated	Region 3	Vessel 2	11.0	4.0
6	Male	Ligated	Region 3	Vessel 3	15.4	5.4
7	Male	Ligated	Region 1	Vessel 1	12.3	4.4
7	Male	Ligated	Region 1	Vessel 2	13.5	5.5
7	Male	Ligated	Region 1	Vessel 3	11.4	4.5
7	Male	Ligated	Region 2	Vessel 1	9.8	5.3
7	Male	Ligated	Region 2	Vessel 2	9.4	5.7
7	Male	Ligated	Region 2	Vessel 3	8.0	6.1
7	Male	Ligated	Region 3	Vessel 1	11.1	5.0
7	Male	Ligated	Region 3	Vessel 2	14.0	5.3
7	Male	Ligated	Region 3	Vessel 3	13.9	4.4
8	Male	Ligated	Region 1	Vessel 1	PEC	PEC
8	Male	Ligated	Region 1	Vessel 2	PEC	PEC
8	Male	Ligated	Region 1	Vessel 3	PEC	PEC
8	Male	Ligated	Region 2	Vessel 1	PEC	PEC
8	Male	Ligated	Region 2	Vessel 2	PEC	PEC
8	Male	Ligated	Region 2	Vessel 3	PEC	PEC
8	Male	Ligated	Region 3	Vessel 1	PEC	PEC
8	Male	Ligated	Region 3	Vessel 2	PEC	PEC
8	Male	Ligated	Region 3	Vessel 3	PEC	PEC
1	Male	Sham	Region 1	Vessel 1	N/A	5.3
1	Male	Sham	Region 1	Vessel 2	N/A	5.2

1	Male	Sham	Region 1	Vessel 3	N/A	4.4
1	Male	Sham	Region 2	Vessel 1	N/A	5.5
1	Male	Sham	Region 2	Vessel 2	N/A	4.9
1	Male	Sham	Region 2	Vessel 3	N/A	4.8
1	Male	Sham	Region 3	Vessel 1	N/A	5.3
1	Male	Sham	Region 3	Vessel 2	N/A	5.2
1	Male	Sham	Region 3	Vessel 3	N/A	5.5
2	Male	Sham	Region 1	Vessel 1	N/A	8.4
2	Male	Sham	Region 1	Vessel 2	N/A	5.9
2	Male	Sham	Region 1	Vessel 3	N/A	6.5
2	Male	Sham	Region 2	Vessel 1	N/A	6.2
2	Male	Sham	Region 2	Vessel 2	N/A	6.9
2	Male	Sham	Region 2	Vessel 3	N/A	5.7
2	Male	Sham	Region 3	Vessel 1	N/A	5.8
2	Male	Sham	Region 3	Vessel 2	N/A	5.1
2	Male	Sham	Region 3	Vessel 3	N/A	4.7
3	Male	Sham	Region 1	Vessel 1	N/A	5.1
3	Male	Sham	Region 1	Vessel 2	N/A	4.8
3	Male	Sham	Region 1	Vessel 3	N/A	6.6
3	Male	Sham	Region 2	Vessel 1	N/A	7.2
3	Male	Sham	Region 2	Vessel 2	N/A	7.1
3	Male	Sham	Region 2	Vessel 3	N/A	5.2
3	Male	Sham	Region 3	Vessel 1	N/A	5.5
3	Male	Sham	Region 3	Vessel 2	N/A	7.0
3	Male	Sham	Region 3	Vessel 3	N/A	5.7
4	Male	Sham	Region 1	Vessel 1	N/A	5.8
4	Male	Sham	Region 1	Vessel 2	N/A	4.4
4	Male	Sham	Region 1	Vessel 3	N/A	3.7
4	Male	Sham	Region 2	Vessel 1	N/A	3.8
4	Male	Sham	Region 2	Vessel 2	N/A	4.1
4	Male	Sham	Region 2	Vessel 3	N/A	3.1
4	Male	Sham	Region 3	Vessel 1	N/A	6.0
4	Male	Sham	Region 3	Vessel 2	N/A	5.1
4	Male	Sham	Region 3	Vessel 3	N/A	5.4
5	Male	Sham	Region 1	Vessel 1	N/A	4.8
5	Male	Sham	Region 1	Vessel 2	N/A	4.0
5	Male	Sham	Region 1	Vessel 3	N/A	5.8
5	Male	Sham	Region 2	Vessel 1	N/A	5.8
5	Male	Sham	Region 2	Vessel 2	N/A	4.7

5	Male	Sham	Region 2	Vessel 3	N/A	6.9
5	Male	Sham	Region 3	Vessel 1	N/A	7.4
5	Male	Sham	Region 3	Vessel 2	N/A	6.6
5	Male	Sham	Region 3	Vessel 3	N/A	7.6
6	Male	Sham	Region 1	Vessel 1	N/A	4.3
6	Male	Sham	Region 1	Vessel 2	N/A	5.5
6	Male	Sham	Region 1	Vessel 3	N/A	6.2
6	Male	Sham	Region 2	Vessel 1	N/A	6.4
6	Male	Sham	Region 2	Vessel 2	N/A	4.4
6	Male	Sham	Region 2	Vessel 3	N/A	6.8
6	Male	Sham	Region 3	Vessel 1	N/A	6.2
6	Male	Sham	Region 3	Vessel 2	N/A	5.4
6	Male	Sham	Region 3	Vessel 3	N/A	5.0
7	Male	Sham	Region 1	Vessel 1	N/A	4.7
7	Male	Sham	Region 1	Vessel 2	N/A	4.4
7	Male	Sham	Region 1	Vessel 3	N/A	4.7
7	Male	Sham	Region 2	Vessel 1	N/A	5.1
7	Male	Sham	Region 2	Vessel 2	N/A	3.6
7	Male	Sham	Region 2	Vessel 3	N/A	4.3
7	Male	Sham	Region 3	Vessel 1	N/A	4.4
7	Male	Sham	Region 3	Vessel 2	N/A	5.0
7	Male	Sham	Region 3	Vessel 3	N/A	5.3
8	Male	Sham	Region 1	Vessel 1	N/A	4.9
8	Male	Sham	Region 1	Vessel 2	N/A	4.0
8	Male	Sham	Region 1	Vessel 3	N/A	4.7
8	Male	Sham	Region 2	Vessel 1	N/A	4.5
8	Male	Sham	Region 2	Vessel 2	N/A	4.1
8	Male	Sham	Region 2	Vessel 3	N/A	3.7
8	Male	Sham	Region 3	Vessel 1	N/A	4.6
8	Male	Sham	Region 3	Vessel 2	N/A	4.1
8	Male	Sham	Region 3	Vessel 3	N/A	5.0
1	Female	Ligated	Region 1	Vessel 1	PEC	PEC
1	Female	Ligated	Region 1	Vessel 2	PEC	PEC
1	Female	Ligated	Region 1	Vessel 3	PEC	PEC
1	Female	Ligated	Region 2	Vessel 1	PEC	PEC
1	Female	Ligated	Region 2	Vessel 2	PEC	PEC
1	Female	Ligated	Region 2	Vessel 3	PEC	PEC
1	Female	Ligated	Region 3	Vessel 1	PEC	PEC
1	Female	Ligated	Region 3	Vessel 2	PEC	PEC

1	Female	Ligated	Region 3	Vessel 3	PEC	PEC
2	Female	Ligated	Region 1	Vessel 1	9.9	3.3
2	Female	Ligated	Region 1	Vessel 2	11.2	3.4
2	Female	Ligated	Region 1	Vessel 3	12.4	4.1
2	Female	Ligated	Region 2	Vessel 1	7.7	4.2
2	Female	Ligated	Region 2	Vessel 2	7.3	5.2
2	Female	Ligated	Region 2	Vessel 3	11.8	5.1
2	Female	Ligated	Region 3	Vessel 1	7.8	5.8
2	Female	Ligated	Region 3	Vessel 2	9.2	5.7
2	Female	Ligated	Region 3	Vessel 3	10.1	4.7
3	Female	Ligated	Region 1	Vessel 1	9.6	5.0
3	Female	Ligated	Region 1	Vessel 2	12.3	4.3
3	Female	Ligated	Region 1	Vessel 3	13.2	6.2
3	Female	Ligated	Region 2	Vessel 1	12.1	7.5
3	Female	Ligated	Region 2	Vessel 2	11.0	5.9
3	Female	Ligated	Region 2	Vessel 3	8.3	5.7
3	Female	Ligated	Region 3	Vessel 1	10.9	5.5
3	Female	Ligated	Region 3	Vessel 2	13.5	6.0
3	Female	Ligated	Region 3	Vessel 3	11.8	4.3
4	Female	Ligated	Region 1	Vessel 1	PEC	PEC
4	Female	Ligated	Region 1	Vessel 2	PEC	PEC
4	Female	Ligated	Region 1	Vessel 3	PEC	PEC
4	Female	Ligated	Region 2	Vessel 1	PEC	PEC
4	Female	Ligated	Region 2	Vessel 2	PEC	PEC
4	Female	Ligated	Region 2	Vessel 3	PEC	PEC
4	Female	Ligated	Region 3	Vessel 1	PEC	PEC
4	Female	Ligated	Region 3	Vessel 2	PEC	PEC
4	Female	Ligated	Region 3	Vessel 3	PEC	PEC
5	Female	Ligated	Region 1	Vessel 1	19.3	4.9
5	Female	Ligated	Region 1	Vessel 2	16.3	4.5
5	Female	Ligated	Region 1	Vessel 3	8.3	3.6
5	Female	Ligated	Region 2	Vessel 1	10.4	3.8
5	Female	Ligated	Region 2	Vessel 2	7.6	4.2
5	Female	Ligated	Region 2	Vessel 3	10.1	4.9
5	Female	Ligated	Region 3	Vessel 1	9.8	3.4
5	Female	Ligated	Region 3	Vessel 2	10.9	3.3
5	Female	Ligated	Region 3	Vessel 3	8.6	3.0
6	Female	Ligated	Region 1	Vessel 1	PEC	PEC
6	Female	Ligated	Region 1	Vessel 2	PEC	PEC

6	Female	Ligated	Region 1	Vessel 3	PEC	PEC
6	Female	Ligated	Region 2	Vessel 1	PEC	PEC
6	Female	Ligated	Region 2	Vessel 2	PEC	PEC
6	Female	Ligated	Region 2	Vessel 3	PEC	PEC
6	Female	Ligated	Region 3	Vessel 1	PEC	PEC
6	Female	Ligated	Region 3	Vessel 2	PEC	PEC
6	Female	Ligated	Region 3	Vessel 3	PEC	PEC
7	Female	Ligated	Region 1	Vessel 1	PEC	PEC
7	Female	Ligated	Region 1	Vessel 2	PEC	PEC
7	Female	Ligated	Region 1	Vessel 3	PEC	PEC
7	Female	Ligated	Region 2	Vessel 1	PEC	PEC
7	Female	Ligated	Region 2	Vessel 2	PEC	PEC
7	Female	Ligated	Region 2	Vessel 3	PEC	PEC
7	Female	Ligated	Region 3	Vessel 1	PEC	PEC
7	Female	Ligated	Region 3	Vessel 2	PEC	PEC
7	Female	Ligated	Region 3	Vessel 3	PEC	PEC
8	Female	Ligated	Region 1	Vessel 1	13.5	4.5
8	Female	Ligated	Region 1	Vessel 2	12.8	4.6
8	Female	Ligated	Region 1	Vessel 3	12.7	4.3
8	Female	Ligated	Region 2	Vessel 1	12.8	4.8
8	Female	Ligated	Region 2	Vessel 2	8.6	4.1
8	Female	Ligated	Region 2	Vessel 3	12.0	5.3
8	Female	Ligated	Region 3	Vessel 1	9.6	4.7
8	Female	Ligated	Region 3	Vessel 2	6.6	5.6
8	Female	Ligated	Region 3	Vessel 3	6.4	5.1
1	Female	Sham	Region 1	Vessel 1	N/A	5.0
1	Female	Sham	Region 1	Vessel 2	N/A	4.5
1	Female	Sham	Region 1	Vessel 3	N/A	4.7
1	Female	Sham	Region 2	Vessel 1	N/A	6.4
1	Female	Sham	Region 2	Vessel 2	N/A	4.3
1	Female	Sham	Region 2	Vessel 3	N/A	6.1
1	Female	Sham	Region 3	Vessel 1	N/A	5.0
1	Female	Sham	Region 3	Vessel 2	N/A	5.2
1	Female	Sham	Region 3	Vessel 3	N/A	5.2
2	Female	Sham	Region 1	Vessel 1	N/A	4.4
2	Female	Sham	Region 1	Vessel 2	N/A	5.2
2	Female	Sham	Region 1	Vessel 3	N/A	5.8
2	Female	Sham	Region 2	Vessel 1	N/A	5.8
2	Female	Sham	Region 2	Vessel 2	N/A	6.9

2	Female	Sham	Region 2	Vessel 3	N/A	4.6
2	Female	Sham	Region 3	Vessel 1	N/A	6.0
2	Female	Sham	Region 3	Vessel 2	N/A	5.2
2	Female	Sham	Region 3	Vessel 3	N/A	5.5
3	Female	Sham	Region 1	Vessel 1	N/A	4.2
3	Female	Sham	Region 1	Vessel 2	N/A	3.4
3	Female	Sham	Region 1	Vessel 3	N/A	4.5
3	Female	Sham	Region 2	Vessel 1	N/A	4.5
3	Female	Sham	Region 2	Vessel 2	N/A	6.4
3	Female	Sham	Region 2	Vessel 3	N/A	5.2
4	Female	Sham	Region 1	Vessel 1	N/A	5.7
4	Female	Sham	Region 1	Vessel 2	N/A	5.6
4	Female	Sham	Region 1	Vessel 3	N/A	5.5
4	Female	Sham	Region 2	Vessel 1	N/A	4.7
4	Female	Sham	Region 2	Vessel 2	N/A	6.7
4	Female	Sham	Region 2	Vessel 3	N/A	6.1
4	Female	Sham	Region 3	Vessel 1	N/A	4.4
4	Female	Sham	Region 3	Vessel 2	N/A	4.5
4	Female	Sham	Region 3	Vessel 3	N/A	5.5
5	Female	Sham	Region 1	Vessel 1	N/A	4.5
5	Female	Sham	Region 1	Vessel 2	N/A	5.5
5	Female	Sham	Region 1	Vessel 3	N/A	5.0
5	Female	Sham	Region 2	Vessel 1	N/A	5.3
5	Female	Sham	Region 2	Vessel 2	N/A	5.4
5	Female	Sham	Region 2	Vessel 3	N/A	4.6
5	Female	Sham	Region 3	Vessel 1	N/A	7.0
5	Female	Sham	Region 3	Vessel 2	N/A	5.7
5	Female	Sham	Region 3	Vessel 3	N/A	6.3
6	Female	Sham	Region 1	Vessel 1	N/A	5.1
6	Female	Sham	Region 1	Vessel 2	N/A	4.9
6	Female	Sham	Region 1	Vessel 3	N/A	5.3
6	Female	Sham	Region 2	Vessel 1	N/A	4.8
6	Female	Sham	Region 2	Vessel 2	N/A	4.7
6	Female	Sham	Region 2	Vessel 3	N/A	5.2
6	Female	Sham	Region 3	Vessel 1	N/A	4.9
6	Female	Sham	Region 3	Vessel 2	N/A	4.2
6	Female	Sham	Region 3	Vessel 3	N/A	4.6

PEC indicates that the muscle had a pre-existing collateral and measurements were not taken

Cell Transplantation Vessel Count Data

Replicate	Treatment	PEC or No PEC	Muscle	Region	Total Vessels	Capillaries	α-SMA Vessels
1	Myogenic Cells	PEC	Ligated	Region 1	15	7	8
1	Myogenic Cells	PEC	Ligated	Region 2	15	7	8
1	Myogenic Cells	PEC	Ligated	Region 3	15	8	7
2	Myogenic Cells	No PEC	Ligated	Region 1	13	7	6
2	Myogenic Cells	No PEC	Ligated	Region 2	9	6	3
2	Myogenic Cells	No PEC	Ligated	Region 3	14	9	5
3	Myogenic Cells	PEC	Ligated	Region 1	15	8	7
3	Myogenic Cells	PEC	Ligated	Region 2	16	10	6
3	Myogenic Cells	PEC	Ligated	Region 3	15	8	7
4	Myogenic Cells	No PEC	Ligated	Region 1	15	7	8
4	Myogenic Cells	No PEC	Ligated	Region 2	14	5	9
4	Myogenic Cells	No PEC	Ligated	Region 3	11	2	9
5	Myogenic Cells	No PEC	Ligated	Region 1	8	0	8
5	Myogenic Cells	No PEC	Ligated	Region 2	13	6	7
5	Myogenic Cells	No PEC	Ligated	Region 3	14	5	9
6	Myogenic Cells	No PEC	Ligated	Region 1	14	7	7
6	Myogenic Cells	No PEC	Ligated	Region 2	15	8	7
6	Myogenic Cells	No PEC	Ligated	Region 3	14	7	7
1	Vehicle	PEC	Ligated	Region 1	12	6	6
1	Vehicle	PEC	Ligated	Region 2	14	7	7
1	Vehicle	PEC	Ligated	Region 3	13	6	7
2	Vehicle	PEC	Ligated	Region 1	13	6	7
2	Vehicle	PEC	Ligated	Region 2	13	6	7
2	Vehicle	PEC	Ligated	Region 3	12	5	7
3	Vehicle	No PEC	Ligated	Region 1	11	5	6
3	Vehicle	No PEC	Ligated	Region 2	13	6	7
3	Vehicle	No PEC	Ligated	Region 3	10	5	5
4	Vehicle	No PEC	Ligated	Region 1	11	11	
4	Vehicle	No PEC	Ligated	Region 2	11	4	7
4	Vehicle	No PEC	Ligated	Region 3	15	8	7
5	Vehicle	No PEC	Ligated	Region 1	13	6	7
5	Vehicle	No PEC	Ligated	Region 2	12	5	7
5	Vehicle	No PEC	Ligated	Region 3	13	7	6
6	Vehicle	No PEC	Ligated	Region 1	13	6	7
6	Vehicle	No PEC	Ligated	Region 2	13	7	6

6	Vehicle	No PEC	Ligated	Region 3	13	6	7
1	Thrombin	No PEC	Ligated	Region 1	14	4	10
1	Thrombin	No PEC	Ligated	Region 2	14	5	9
1	Thrombin	No PEC	Ligated	Region 3	15	7	8
2	Thrombin	PEC	Ligated	Region 1	13	2	11
2	Thrombin	PEC	Ligated	Region 2	8	-3	11
2	Thrombin	PEC	Ligated	Region 3	14	4	10
3	Thrombin	No PEC	Ligated	Region 1	14	4	10
3	Thrombin	No PEC	Ligated	Region 2	14	3	11
3	Thrombin	No PEC	Ligated	Region 3	12	4	8
4	Thrombin	No PEC	Ligated	Region 1	11	0	11
4	Thrombin	No PEC	Ligated	Region 2	14	2	12
4	Thrombin	No PEC	Ligated	Region 3	11	1	10
5	Thrombin	PEC	Ligated	Region 1	12	7	5
5	Thrombin	PEC	Ligated	Region 2	12	5	7
5	Thrombin	PEC	Ligated	Region 3	8	2	6
1	Ligation Only	PEC	Ligated	Region 1	13	6	7
1	Ligation Only	PEC	Ligated	Region 2	12	5	7
1	Ligation Only	PEC	Ligated	Region 3	15	6	9
2	Ligation Only	No PEC	Ligated	Region 1	13	3	10
2	Ligation Only	No PEC	Ligated	Region 2	11	3	8
2	Ligation Only	No PEC	Ligated	Region 3	16	6	10
3	Ligation Only	No PEC	Ligated	Region 1	16	10	6
3	Ligation Only	No PEC	Ligated	Region 2	12	5	7
3	Ligation Only	No PEC	Ligated	Region 3	12	4	8
4	Ligation Only	No PEC	Ligated	Region 1	12	5	7
4	Ligation Only	No PEC	Ligated	Region 2	13	7	6
4	Ligation Only	No PEC	Ligated	Region 3	11	4	7
5	Ligation Only	PEC	Ligated	Region 1	18	10	8
5	Ligation Only	PEC	Ligated	Region 2	16	10	6
5	Ligation Only	PEC	Ligated	Region 3	15	8	7
1	Myogenic Cells	PEC	Sham	Region 1	14	14	0
1	Myogenic Cells	PEC	Sham	Region 2	14	14	0
1	Myogenic Cells	PEC	Sham	Region 3	14	14	0
2	Myogenic Cells	No PEC	Sham	Region 1	10	10	0
2	Myogenic Cells	No PEC	Sham	Region 2	15	15	0
2	Myogenic Cells	No PEC	Sham	Region 3	12	12	0
3	Myogenic Cells	PEC	Sham	Region 1	17	17	0
3	Myogenic Cells	PEC	Sham	Region 2	15	15	0

3	Myogenic Cells	PEC	Sham	Region 3	13	13	0
4	Myogenic Cells	No PEC	Sham	Region 1	16	16	0
4	Myogenic Cells	No PEC	Sham	Region 2	19	19	0
4	Myogenic Cells	No PEC	Sham	Region 3	18	18	0
5	Myogenic Cells	No PEC	Sham	Region 1	16	16	0
5	Myogenic Cells	No PEC	Sham	Region 2	14	14	0
5	Myogenic Cells	No PEC	Sham	Region 3	16	16	0
6	Myogenic Cells	No PEC	Sham	Region 1	13	13	0
6	Myogenic Cells	No PEC	Sham	Region 2	13	13	0
6	Myogenic Cells	No PEC	Sham	Region 3	15	15	0
1	Vehicle	PEC	Sham	Region 1	11	11	0
1	Vehicle	PEC	Sham	Region 2	15	15	0
1	Vehicle	PEC	Sham	Region 3	17	17	0
2	Vehicle	PEC	Sham	Region 1	12	12	0
2	Vehicle	PEC	Sham	Region 2	14	14	0
2	Vehicle	PEC	Sham	Region 3	12	12	0
3	Vehicle	No PEC	Sham	Region 1	8	8	0
3	Vehicle	No PEC	Sham	Region 2	11	11	0
3	Vehicle	No PEC	Sham	Region 3	8	8	0
4	Vehicle	No PEC	Sham	Region 1	10	10	0
4	Vehicle	No PEC	Sham	Region 2	12	12	0
4	Vehicle	No PEC	Sham	Region 3	12	12	0
5	Vehicle	No PEC	Sham	Region 1	15	15	0
5	Vehicle	No PEC	Sham	Region 2	13	13	0
5	Vehicle	No PEC	Sham	Region 3	13	13	0
6	Vehicle	No PEC	Sham	Region 1	17	17	0
6	Vehicle	No PEC	Sham	Region 2	15	15	0
6	Vehicle	No PEC	Sham	Region 3	14	14	0
1	Thrombin	No PEC	Sham	Region 1	14	14	0
1	Thrombin	No PEC	Sham	Region 2	12	12	0
1	Thrombin	No PEC	Sham	Region 3	11	11	0
2	Thrombin	PEC	Sham	Region 1	14	14	0
2	Thrombin	PEC	Sham	Region 2	10	10	0
2	Thrombin	PEC	Sham	Region 3	10	10	0
3	Thrombin	No PEC	Sham	Region 1	12	12	0
3	Thrombin	No PEC	Sham	Region 2	14	14	0
3	Thrombin	No PEC	Sham	Region 3	14	14	0
4	Thrombin	No PEC	Sham	Region 1	12	12	0
4	Thrombin	No PEC	Sham	Region 2	14	14	0

4	Thrombin	No PEC	Sham	Region 3	16	16	0
5	Thrombin	PEC	Sham	Region 1	19	19	0
5	Thrombin	PEC	Sham	Region 2	16	16	0
5	Thrombin	PEC	Sham	Region 3	15	15	0
1	Ligation Only	PEC	Sham	Region 1	10	10	0
1	Ligation Only	PEC	Sham	Region 2	12	12	0
1	Ligation Only	PEC	Sham	Region 3	16	16	0
2	Ligation Only	No PEC	Sham	Region 1	12	12	0
2	Ligation Only	No PEC	Sham	Region 2	14	14	0
2	Ligation Only	No PEC	Sham	Region 3	11	11	0
3	Ligation Only	No PEC	Sham	Region 1	15	15	0
3	Ligation Only	No PEC	Sham	Region 2	16	16	0
3	Ligation Only	No PEC	Sham	Region 3	13	13	0
4	Ligation Only	No PEC	Sham	Region 1	13	13	0
4	Ligation Only	No PEC	Sham	Region 2	14	14	0
4	Ligation Only	No PEC	Sham	Region 3	18	18	0
5	Ligation Only	PEC	Sham	Region 1	13	13	0
5	Ligation Only	PEC	Sham	Region 2	14	14	0
5	Ligation Only	PEC	Sham	Region 3	14	14	0

Cell Transplantation Vessel Diameter Data

Replicate	Treatment	PEC or No PEC	Muscle	Region	Vessel	α-SMA Vessel Diameter (μm)	Lectin Vessel Diameter (μm)
1	Myogenic Cells	PEC	Ligated	Region 1	Vessel 1	9.0	5.0
1	Myogenic Cells	PEC	Ligated	Region 1	Vessel 2	8.6	4.2
1	Myogenic Cells	PEC	Ligated	Region 1	Vessel 3	7.7	5.7
1	Myogenic Cells	PEC	Ligated	Region 2	Vessel 1	10.6	4.1
1	Myogenic Cells	PEC	Ligated	Region 2	Vessel 2	8.0	3.5
1	Myogenic Cells	PEC	Ligated	Region 2	Vessel 3	12.3	5.1
1	Myogenic Cells	PEC	Ligated	Region 3	Vessel 1	7.9	6.2
1	Myogenic Cells	PEC	Ligated	Region 3	Vessel 2	9.2	4.4
1	Myogenic Cells	PEC	Ligated	Region 3	Vessel 3	8.1	3.7
2	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 1	16.0	5.7
2	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 2	18.9	5.3
2	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 3	18.7	5.4
2	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 1	28.2	4.9
2	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 2	26.3	5.3
2	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 3	43.4	4.9
2	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 1	10.7	8.0
2	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 2	15.8	6.9
2	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 3	12.9	7.4
3	Myogenic Cells	PEC	Ligated	Region 1	Vessel 1	10.1	6.6
3	Myogenic Cells	PEC	Ligated	Region 1	Vessel 2	10.3	5.8
3	Myogenic Cells	PEC	Ligated	Region 1	Vessel 3	7.7	6.0
3	Myogenic Cells	PEC	Ligated	Region 2	Vessel 1	12.4	8.7
3	Myogenic Cells	PEC	Ligated	Region 2	Vessel 2	9.9	7.5
3	Myogenic Cells	PEC	Ligated	Region 2	Vessel 3	11.9	8.6
3	Myogenic Cells	PEC	Ligated	Region 3	Vessel 1	14.0	6.6
3	Myogenic Cells	PEC	Ligated	Region 3	Vessel 2	13.7	4.9
3	Myogenic Cells	PEC	Ligated	Region 3	Vessel 3	10.4	6.4
4	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 1	11.5	4.7
4	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 2	8.4	4.1
4	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 3	8.9	4.1
4	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 1	9.0	4.8
4	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 2	8.1	5.3
4	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 3	9.1	5.0
4	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 1	10.5	4.4

4	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 2	8.3	3.9
4	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 3	10.7	3.6
5	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 1	12.2	4.8
5	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 2	14.5	5.6
5	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 3	13.9	4.7
5	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 1	19.9	4.7
5	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 2	20.5	4.7
5	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 3	20.7	5.1
5	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 1	10.4	3.6
5	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 2	10.9	4.0
5	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 3	10.4	3.9
6	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 1	18.9	4.9
6	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 2	16.2	4.1
6	Myogenic Cells	No PEC	Ligated	Region 1	Vessel 3	17.9	5.8
6	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 1	11.3	5.2
6	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 2	14.6	5.1
6	Myogenic Cells	No PEC	Ligated	Region 2	Vessel 3	11.0	4.5
6	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 1	18.0	5.0
6	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 2	15.2	5.4
6	Myogenic Cells	No PEC	Ligated	Region 3	Vessel 3	15.4	5.5
1	Vehicle	PEC	Ligated	Region 1	Vessel 1	15.6	6.1
1	Vehicle	PEC	Ligated	Region 1	Vessel 2	13.3	5.9
1	Vehicle	PEC	Ligated	Region 1	Vessel 3	14.8	5.2
1	Vehicle	PEC	Ligated	Region 2	Vessel 1	14.9	4.7
1	Vehicle	PEC	Ligated	Region 2	Vessel 2	12.5	4.6
1	Vehicle	PEC	Ligated	Region 2	Vessel 3	7.6	4.4
1	Vehicle	PEC	Ligated	Region 3	Vessel 1	7.3	5.0
1	Vehicle	PEC	Ligated	Region 3	Vessel 2	7.6	5.0
1	Vehicle	PEC	Ligated	Region 3	Vessel 3	6.9	7.3
2	Vehicle	PEC	Ligated	Region 1	Vessel 1	6.8	4.6
2	Vehicle	PEC	Ligated	Region 1	Vessel 2	6.3	3.5
2	Vehicle	PEC	Ligated	Region 1	Vessel 3	4.5	5.1
2	Vehicle	PEC	Ligated	Region 2	Vessel 1	9.6	5.0
2	Vehicle	PEC	Ligated	Region 2	Vessel 2	9.2	4.6
2	Vehicle	PEC	Ligated	Region 2	Vessel 3	8.8	4.7
2	Vehicle	PEC	Ligated	Region 3	Vessel 1	9.5	3.9
2	Vehicle	PEC	Ligated	Region 3	Vessel 2	5.9	4.8
2	Vehicle	PEC	Ligated	Region 3	Vessel 3	8.4	5.5
3	Vehicle	No PEC	Ligated	Region 1	Vessel 1	11.7	7.4

3	Vehicle	No PEC	Ligated	Region 1	Vessel 2	7.8	5.7
3	Vehicle	No PEC	Ligated	Region 1	Vessel 3	7.3	6.8
3	Vehicle	No PEC	Ligated	Region 2	Vessel 1	11.5	4.6
3	Vehicle	No PEC	Ligated	Region 2	Vessel 2	9.6	5.9
3	Vehicle	No PEC	Ligated	Region 2	Vessel 3	7.6	3.5
3	Vehicle	No PEC	Ligated	Region 3	Vessel 1	8.4	6.0
3	Vehicle	No PEC	Ligated	Region 3	Vessel 2	8.0	6.3
3	Vehicle	No PEC	Ligated	Region 3	Vessel 3	7.7	7.6
4	Vehicle	No PEC	Ligated	Region 1	Vessel 1		5.5
4	Vehicle	No PEC	Ligated	Region 1	Vessel 2		5.1
4	Vehicle	No PEC	Ligated	Region 1	Vessel 3		4.4
4	Vehicle	No PEC	Ligated	Region 2	Vessel 1	9.4	5.1
4	Vehicle	No PEC	Ligated	Region 2	Vessel 2	16.9	4.8
4	Vehicle	No PEC	Ligated	Region 2	Vessel 3	8.0	4.9
4	Vehicle	No PEC	Ligated	Region 3	Vessel 1	11.3	5.9
4	Vehicle	No PEC	Ligated	Region 3	Vessel 2	8.9	6.1
4	Vehicle	No PEC	Ligated	Region 3	Vessel 3	11.6	5.7
5	Vehicle	No PEC	Ligated	Region 1	Vessel 1	11.0	7.8
5	Vehicle	No PEC	Ligated	Region 1	Vessel 2	8.2	4.1
5	Vehicle	No PEC	Ligated	Region 1	Vessel 3	9.5	5.2
5	Vehicle	No PEC	Ligated	Region 2	Vessel 1	8.3	4.3
5	Vehicle	No PEC	Ligated	Region 2	Vessel 2	9.5	5.0
5	Vehicle	No PEC	Ligated	Region 2	Vessel 3	9.6	7.5
5	Vehicle	No PEC	Ligated	Region 3	Vessel 1	12.6	4.3
5	Vehicle	No PEC	Ligated	Region 3	Vessel 2	14.3	4.8
5	Vehicle	No PEC	Ligated	Region 3	Vessel 3	12.1	5.1
6	Vehicle	No PEC	Ligated	Region 1	Vessel 1	14.0	4.3
6	Vehicle	No PEC	Ligated	Region 1	Vessel 2	8.7	4.0
6	Vehicle	No PEC	Ligated	Region 1	Vessel 3	11.5	4.1
6	Vehicle	No PEC	Ligated	Region 2	Vessel 1	9.0	3.4
6	Vehicle	No PEC	Ligated	Region 2	Vessel 2	14.9	4.1
6	Vehicle	No PEC	Ligated	Region 2	Vessel 3	13.2	3.2
6	Vehicle	No PEC	Ligated	Region 3	Vessel 1	10.0	3.7
6	Vehicle	No PEC	Ligated	Region 3	Vessel 2	10.6	4.1
6	Vehicle	No PEC	Ligated	Region 3	Vessel 3	12.5	5.0
1	Thrombin	No PEC	Ligated	Region 1	Vessel 1	11.2	4.7
1	Thrombin	No PEC	Ligated	Region 1	Vessel 2	13.8	4.5
1	Thrombin	No PEC	Ligated	Region 1	Vessel 3	15.0	5.6
1	Thrombin	No PEC	Ligated	Region 2	Vessel 1	22.3	3.5

1	Thrombin	No PEC	Ligated	Region 2	Vessel 2	15.8	3.3
1	Thrombin	No PEC	Ligated	Region 2	Vessel 3	11.2	4.5
1	Thrombin	No PEC	Ligated	Region 3	Vessel 1	12.1	5.0
1	Thrombin	No PEC	Ligated	Region 3	Vessel 2	12.9	4.3
1	Thrombin	No PEC	Ligated	Region 3	Vessel 3	15.4	4.5
2	Thrombin	PEC	Ligated	Region 1	Vessel 1	11.3	4.1
2	Thrombin	PEC	Ligated	Region 1	Vessel 2	13.8	4.4
2	Thrombin	PEC	Ligated	Region 1	Vessel 3	9.7	4.2
2	Thrombin	PEC	Ligated	Region 2	Vessel 1	16.2	5.9
2	Thrombin	PEC	Ligated	Region 2	Vessel 2	11.5	4.8
2	Thrombin	PEC	Ligated	Region 2	Vessel 3	13.3	4.9
2	Thrombin	PEC	Ligated	Region 3	Vessel 1	9.0	4.0
2	Thrombin	PEC	Ligated	Region 3	Vessel 2	11.7	4.1
2	Thrombin	PEC	Ligated	Region 3	Vessel 3	8.6	4.6
3	Thrombin	No PEC	Ligated	Region 1	Vessel 1	15.7	4.8
3	Thrombin	No PEC	Ligated	Region 1	Vessel 2	14.4	5.1
3	Thrombin	No PEC	Ligated	Region 1	Vessel 3	16.7	5.0
3	Thrombin	No PEC	Ligated	Region 2	Vessel 1	10.3	4.7
3	Thrombin	No PEC	Ligated	Region 2	Vessel 2	10.1	5.2
3	Thrombin	No PEC	Ligated	Region 2	Vessel 3	11.0	5.2
3	Thrombin	No PEC	Ligated	Region 3	Vessel 1	11.5	4.5
3	Thrombin	No PEC	Ligated	Region 3	Vessel 2	9.2	4.9
3	Thrombin	No PEC	Ligated	Region 3	Vessel 3	7.0	4.9
4	Thrombin	No PEC	Ligated	Region 1	Vessel 1	14.2	5.2
4	Thrombin	No PEC	Ligated	Region 1	Vessel 2	11.8	5.3
4	Thrombin	No PEC	Ligated	Region 1	Vessel 3	14.1	3.7
4	Thrombin	No PEC	Ligated	Region 2	Vessel 1	14.9	3.8
4	Thrombin	No PEC	Ligated	Region 2	Vessel 2	11.9	5.6
4	Thrombin	No PEC	Ligated	Region 2	Vessel 3	11.8	4.9
4	Thrombin	No PEC	Ligated	Region 3	Vessel 1	15.0	4.6
4	Thrombin	No PEC	Ligated	Region 3	Vessel 2	14.1	5.5
4	Thrombin	No PEC	Ligated	Region 3	Vessel 3	19.2	4.1
5	Thrombin	PEC	Ligated	Region 1	Vessel 1	7.5	4.5
5	Thrombin	PEC	Ligated	Region 1	Vessel 2	5.0	5.5
5	Thrombin	PEC	Ligated	Region 1	Vessel 3	6.5	3.0
5	Thrombin	PEC	Ligated	Region 2	Vessel 1	8.8	5.1
5	Thrombin	PEC	Ligated	Region 2	Vessel 2	7.3	2.6
5	Thrombin	PEC	Ligated	Region 2	Vessel 3	6.8	4.8
5	Thrombin	PEC	Ligated	Region 3	Vessel 1	7.3	4.5

5	Thrombin	PEC	Ligated	Region 3	Vessel 2	8.5	5.5
5	Thrombin	PEC	Ligated	Region 3	Vessel 3	10.5	4.7
1	Ligation Only	PEC	Ligated	Region 1	Vessel 1	13.2	4.0
1	Ligation Only	PEC	Ligated	Region 1	Vessel 2	14.6	4.3
1	Ligation Only	PEC	Ligated	Region 1	Vessel 3	4.6	3.8
1	Ligation Only	PEC	Ligated	Region 2	Vessel 1	7.7	3.3
1	Ligation Only	PEC	Ligated	Region 2	Vessel 2	6.9	4.6
1	Ligation Only	PEC	Ligated	Region 2	Vessel 3	9.1	4.0
1	Ligation Only	PEC	Ligated	Region 3	Vessel 1	6.4	5.9
1	Ligation Only	PEC	Ligated	Region 3	Vessel 2	7.0	4.1
1	Ligation Only	PEC	Ligated	Region 3	Vessel 3	4.3	3.2
2	Ligation Only	No PEC	Ligated	Region 1	Vessel 1	12.1	4.9
2	Ligation Only	No PEC	Ligated	Region 1	Vessel 2	13.5	5.9
2	Ligation Only	No PEC	Ligated	Region 1	Vessel 3	14.6	4.3
2	Ligation Only	No PEC	Ligated	Region 2	Vessel 1	13.4	6.2
2	Ligation Only	No PEC	Ligated	Region 2	Vessel 2	12.3	5.6
2	Ligation Only	No PEC	Ligated	Region 2	Vessel 3	11.5	3.7
2	Ligation Only	No PEC	Ligated	Region 3	Vessel 1	9.0	3.5
2	Ligation Only	No PEC	Ligated	Region 3	Vessel 2	9.3	4.4
2	Ligation Only	No PEC	Ligated	Region 3	Vessel 3	6.4	4.9
3	Ligation Only	No PEC	Ligated	Region 1	Vessel 1	15.2	5.1
3	Ligation Only	No PEC	Ligated	Region 1	Vessel 2	10.9	5.2
3	Ligation Only	No PEC	Ligated	Region 1	Vessel 3	7.8	5.2
3	Ligation Only	No PEC	Ligated	Region 2	Vessel 1	13.0	6.0
3	Ligation Only	No PEC	Ligated	Region 2	Vessel 2	15.2	6.1
3	Ligation Only	No PEC	Ligated	Region 2	Vessel 3	10.1	4.8
3	Ligation Only	No PEC	Ligated	Region 3	Vessel 1	12.1	7.0
3	Ligation Only	No PEC	Ligated	Region 3	Vessel 2	10.6	7.0
3	Ligation Only	No PEC	Ligated	Region 3	Vessel 3	10.5	5.8
4	Ligation Only	No PEC	Ligated	Region 1	Vessel 1	14.7	5.4
4	Ligation Only	No PEC	Ligated	Region 1	Vessel 2	11.0	4.8
4	Ligation Only	No PEC	Ligated	Region 1	Vessel 3	10.6	5.6
4	Ligation Only	No PEC	Ligated	Region 2	Vessel 1	9.6	6.1
4	Ligation Only	No PEC	Ligated	Region 2	Vessel 2	13.3	6.3
4	Ligation Only	No PEC	Ligated	Region 2	Vessel 3	14.2	5.8
4	Ligation Only	No PEC	Ligated	Region 3	Vessel 1	17.4	3.8
4	Ligation Only	No PEC	Ligated	Region 3	Vessel 2	15.5	5.9
4	Ligation Only	No PEC	Ligated	Region 3	Vessel 3	10.3	4.8
5	Ligation Only	PEC	Ligated	Region 1	Vessel 1	6.4	5.1

5	Ligation Only	PEC	Ligated	Region 1	Vessel 2	9.6	5.4
5	Ligation Only	PEC	Ligated	Region 1	Vessel 3	5.2	5.2
5	Ligation Only	PEC	Ligated	Region 2	Vessel 1	10.6	4.5
5	Ligation Only	PEC	Ligated	Region 2	Vessel 2	8.1	5.0
5	Ligation Only	PEC	Ligated	Region 2	Vessel 3	7.7	4.2
5	Ligation Only	PEC	Ligated	Region 3	Vessel 1	6.7	5.0
5	Ligation Only	PEC	Ligated	Region 3	Vessel 2	9.0	4.9
5	Ligation Only	PEC	Ligated	Region 3	Vessel 3	8.9	6.2
1	Myogenic Cells	PEC	Sham	Region 1	Vessel 1	N/A	4.3
1	Myogenic Cells	PEC	Sham	Region 1	Vessel 2	N/A	4.5
1	Myogenic Cells	PEC	Sham	Region 1	Vessel 3	N/A	4.8
1	Myogenic Cells	PEC	Sham	Region 2	Vessel 1	N/A	7.1
1	Myogenic Cells	PEC	Sham	Region 2	Vessel 2	N/A	4.8
1	Myogenic Cells	PEC	Sham	Region 2	Vessel 3	N/A	7.2
1	Myogenic Cells	PEC	Sham	Region 3	Vessel 1	N/A	4.5
1	Myogenic Cells	PEC	Sham	Region 3	Vessel 2	N/A	5.5
1	Myogenic Cells	PEC	Sham	Region 3	Vessel 3	N/A	5.2
2	Myogenic Cells	No PEC	Sham	Region 1	Vessel 1	N/A	6.0
2	Myogenic Cells	No PEC	Sham	Region 1	Vessel 2	N/A	4.6
2	Myogenic Cells	No PEC	Sham	Region 1	Vessel 3	N/A	5.7
2	Myogenic Cells	No PEC	Sham	Region 2	Vessel 1	N/A	5.1
2	Myogenic Cells	No PEC	Sham	Region 2	Vessel 2	N/A	4.0
2	Myogenic Cells	No PEC	Sham	Region 2	Vessel 3	N/A	4.1
2	Myogenic Cells	No PEC	Sham	Region 3	Vessel 1	N/A	4.2
2	Myogenic Cells	No PEC	Sham	Region 3	Vessel 2	N/A	4.3
2	Myogenic Cells	No PEC	Sham	Region 3	Vessel 3	N/A	5.0
3	Myogenic Cells	PEC	Sham	Region 1	Vessel 1	N/A	5.0
3	Myogenic Cells	PEC	Sham	Region 1	Vessel 2	N/A	4.6
3	Myogenic Cells	PEC	Sham	Region 1	Vessel 3	N/A	4.4
3	Myogenic Cells	PEC	Sham	Region 2	Vessel 1	N/A	4.7
3	Myogenic Cells	PEC	Sham	Region 2	Vessel 2	N/A	4.6
3	Myogenic Cells	PEC	Sham	Region 2	Vessel 3	N/A	4.0
3	Myogenic Cells	PEC	Sham	Region 3	Vessel 1	N/A	4.7
3	Myogenic Cells	PEC	Sham	Region 3	Vessel 2	N/A	4.4
3	Myogenic Cells	PEC	Sham	Region 3	Vessel 3	N/A	3.8
4	Myogenic Cells	No PEC	Sham	Region 1	Vessel 1	N/A	4.2
4	Myogenic Cells	No PEC	Sham	Region 1	Vessel 2	N/A	4.8
4	Myogenic Cells	No PEC	Sham	Region 1	Vessel 3	N/A	4.1
4	Myogenic Cells	No PEC	Sham	Region 2	Vessel 1	N/A	4.9

4	Myogenic Cells	No PEC	Sham	Region 2	Vessel 2	N/A	5.7
4	Myogenic Cells	No PEC	Sham	Region 2	Vessel 3	N/A	4.4
4	Myogenic Cells	No PEC	Sham	Region 3	Vessel 1	N/A	5.9
4	Myogenic Cells	No PEC	Sham	Region 3	Vessel 2	N/A	5.5
4	Myogenic Cells	No PEC	Sham	Region 3	Vessel 3	N/A	5.3
5	Myogenic Cells	No PEC	Sham	Region 1	Vessel 1	N/A	4.4
5	Myogenic Cells	No PEC	Sham	Region 1	Vessel 2	N/A	4.2
5	Myogenic Cells	No PEC	Sham	Region 1	Vessel 3	N/A	3.9
5	Myogenic Cells	No PEC	Sham	Region 2	Vessel 1	N/A	5.8
5	Myogenic Cells	No PEC	Sham	Region 2	Vessel 2	N/A	5.9
5	Myogenic Cells	No PEC	Sham	Region 2	Vessel 3	N/A	5.4
5	Myogenic Cells	No PEC	Sham	Region 3	Vessel 1	N/A	6.4
5	Myogenic Cells	No PEC	Sham	Region 3	Vessel 2	N/A	4.7
5	Myogenic Cells	No PEC	Sham	Region 3	Vessel 3	N/A	5.0
6	Myogenic Cells	No PEC	Sham	Region 1	Vessel 1	N/A	5.6
6	Myogenic Cells	No PEC	Sham	Region 1	Vessel 2	N/A	5.1
6	Myogenic Cells	No PEC	Sham	Region 1	Vessel 3	N/A	4.7
6	Myogenic Cells	No PEC	Sham	Region 2	Vessel 1	N/A	5.6
6	Myogenic Cells	No PEC	Sham	Region 2	Vessel 2	N/A	5.3
6	Myogenic Cells	No PEC	Sham	Region 2	Vessel 3	N/A	6.0
6	Myogenic Cells	No PEC	Sham	Region 3	Vessel 1	N/A	5.5
6	Myogenic Cells	No PEC	Sham	Region 3	Vessel 2	N/A	4.7
6	Myogenic Cells	No PEC	Sham	Region 3	Vessel 3	N/A	4.4
1	Vehicle	PEC	Sham	Region 1	Vessel 1	N/A	4.2
1	Vehicle	PEC	Sham	Region 1	Vessel 2	N/A	3.6
1	Vehicle	PEC	Sham	Region 1	Vessel 3	N/A	3.9
1	Vehicle	PEC	Sham	Region 2	Vessel 1	N/A	4.6
1	Vehicle	PEC	Sham	Region 2	Vessel 2	N/A	5.3
1	Vehicle	PEC	Sham	Region 2	Vessel 3	N/A	5.8
1	Vehicle	PEC	Sham	Region 3	Vessel 1	N/A	5.5
1	Vehicle	PEC	Sham	Region 3	Vessel 2	N/A	5.0
1	Vehicle	PEC	Sham	Region 3	Vessel 3	N/A	5.0
2	Vehicle	PEC	Sham	Region 1	Vessel 1	N/A	5.6
2	Vehicle	PEC	Sham	Region 1	Vessel 2	N/A	5.0
2	Vehicle	PEC	Sham	Region 1	Vessel 3	N/A	4.3
2	Vehicle	PEC	Sham	Region 2	Vessel 1	N/A	4.1
2	Vehicle	PEC	Sham	Region 2	Vessel 2	N/A	4.3
2	Vehicle	PEC	Sham	Region 2	Vessel 3	N/A	4.4
2	Vehicle	PEC	Sham	Region 3	Vessel 1	N/A	4.0

2	Vehicle	PEC	Sham	Region 3	Vessel 2	N/A	4.6
2	Vehicle	PEC	Sham	Region 3	Vessel 3	N/A	4.9
3	Vehicle	No PEC	Sham	Region 1	Vessel 1	N/A	4.1
3	Vehicle	No PEC	Sham	Region 1	Vessel 2	N/A	5.0
3	Vehicle	No PEC	Sham	Region 1	Vessel 3	N/A	3.9
3	Vehicle	No PEC	Sham	Region 2	Vessel 1	N/A	6.1
3	Vehicle	No PEC	Sham	Region 2	Vessel 2	N/A	6.3
3	Vehicle	No PEC	Sham	Region 2	Vessel 3	N/A	4.6
3	Vehicle	No PEC	Sham	Region 3	Vessel 1	N/A	3.5
3	Vehicle	No PEC	Sham	Region 3	Vessel 2	N/A	6.4
3	Vehicle	No PEC	Sham	Region 3	Vessel 3	N/A	5.9
4	Vehicle	No PEC	Sham	Region 1	Vessel 1	N/A	5.4
4	Vehicle	No PEC	Sham	Region 1	Vessel 2	N/A	5.6
4	Vehicle	No PEC	Sham	Region 1	Vessel 3	N/A	5.1
4	Vehicle	No PEC	Sham	Region 2	Vessel 1	N/A	5.4
4	Vehicle	No PEC	Sham	Region 2	Vessel 2	N/A	7.5
4	Vehicle	No PEC	Sham	Region 2	Vessel 3	N/A	5.6
4	Vehicle	No PEC	Sham	Region 3	Vessel 1	N/A	4.8
4	Vehicle	No PEC	Sham	Region 3	Vessel 2	N/A	6.2
4	Vehicle	No PEC	Sham	Region 3	Vessel 3	N/A	4.8
5	Vehicle	No PEC	Sham	Region 1	Vessel 1	N/A	4.8
5	Vehicle	No PEC	Sham	Region 1	Vessel 2	N/A	6.3
5	Vehicle	No PEC	Sham	Region 1	Vessel 3	N/A	4.9
5	Vehicle	No PEC	Sham	Region 2	Vessel 1	N/A	6.1
5	Vehicle	No PEC	Sham	Region 2	Vessel 2	N/A	5.5
5	Vehicle	No PEC	Sham	Region 2	Vessel 3	N/A	5.4
5	Vehicle	No PEC	Sham	Region 3	Vessel 1	N/A	5.8
5	Vehicle	No PEC	Sham	Region 3	Vessel 2	N/A	6.0
5	Vehicle	No PEC	Sham	Region 3	Vessel 3	N/A	6.0
6	Vehicle	No PEC	Sham	Region 1	Vessel 1	N/A	4.9
6	Vehicle	No PEC	Sham	Region 1	Vessel 2	N/A	4.5
6	Vehicle	No PEC	Sham	Region 1	Vessel 3	N/A	4.8
6	Vehicle	No PEC	Sham	Region 2	Vessel 1	N/A	6.1
6	Vehicle	No PEC	Sham	Region 2	Vessel 2	N/A	4.5
6	Vehicle	No PEC	Sham	Region 2	Vessel 3	N/A	4.4
6	Vehicle	No PEC	Sham	Region 3	Vessel 1	N/A	5.3
6	Vehicle	No PEC	Sham	Region 3	Vessel 2	N/A	5.0
6	Vehicle	No PEC	Sham	Region 3	Vessel 3	N/A	4.1
1	Thrombin	No PEC	Sham	Region 1	Vessel 1	N/A	5.8

1	Thrombin	No PEC	Sham	Region 1	Vessel 2	N/A	4.8
1	Thrombin	No PEC	Sham	Region 1	Vessel 3	N/A	4.4
1	Thrombin	No PEC	Sham	Region 2	Vessel 1	N/A	6.6
1	Thrombin	No PEC	Sham	Region 2	Vessel 2	N/A	6.3
1	Thrombin	No PEC	Sham	Region 2	Vessel 3	N/A	5.0
1	Thrombin	No PEC	Sham	Region 3	Vessel 1	N/A	4.4
1	Thrombin	No PEC	Sham	Region 3	Vessel 2	N/A	4.9
1	Thrombin	No PEC	Sham	Region 3	Vessel 3	N/A	4.9
2	Thrombin	PEC	Sham	Region 1	Vessel 1	N/A	5.2
2	Thrombin	PEC	Sham	Region 1	Vessel 2	N/A	5.2
2	Thrombin	PEC	Sham	Region 1	Vessel 3	N/A	4.3
2	Thrombin	PEC	Sham	Region 2	Vessel 1	N/A	3.9
2	Thrombin	PEC	Sham	Region 2	Vessel 2	N/A	5.5
2	Thrombin	PEC	Sham	Region 2	Vessel 3	N/A	4.4
2	Thrombin	PEC	Sham	Region 3	Vessel 1	N/A	7.0
2	Thrombin	PEC	Sham	Region 3	Vessel 2	N/A	7.1
2	Thrombin	PEC	Sham	Region 3	Vessel 3	N/A	5.0
3	Thrombin	No PEC	Sham	Region 1	Vessel 1	N/A	5.9
3	Thrombin	No PEC	Sham	Region 1	Vessel 2	N/A	4.9
3	Thrombin	No PEC	Sham	Region 1	Vessel 3	N/A	5.2
3	Thrombin	No PEC	Sham	Region 2	Vessel 1	N/A	3.8
3	Thrombin	No PEC	Sham	Region 2	Vessel 2	N/A	4.5
3	Thrombin	No PEC	Sham	Region 2	Vessel 3	N/A	5.6
3	Thrombin	No PEC	Sham	Region 3	Vessel 1	N/A	4.1
3	Thrombin	No PEC	Sham	Region 3	Vessel 2	N/A	5.2
3	Thrombin	No PEC	Sham	Region 3	Vessel 3	N/A	3.7
4	Thrombin	No PEC	Sham	Region 1	Vessel 1	N/A	4.8
4	Thrombin	No PEC	Sham	Region 1	Vessel 2	N/A	4.7
4	Thrombin	No PEC	Sham	Region 1	Vessel 3	N/A	5.5
4	Thrombin	No PEC	Sham	Region 2	Vessel 1	N/A	4.3
4	Thrombin	No PEC	Sham	Region 2	Vessel 2	N/A	5.6
4	Thrombin	No PEC	Sham	Region 2	Vessel 3	N/A	4.6
4	Thrombin	No PEC	Sham	Region 3	Vessel 1	N/A	4.6
4	Thrombin	No PEC	Sham	Region 3	Vessel 2	N/A	4.7
4	Thrombin	No PEC	Sham	Region 3	Vessel 3	N/A	4.2
5	Thrombin	PEC	Sham	Region 1	Vessel 1	N/A	5.2
5	Thrombin	PEC	Sham	Region 1	Vessel 2	N/A	4.0
5	Thrombin	PEC	Sham	Region 1	Vessel 3	N/A	5.0
5	Thrombin	PEC	Sham	Region 2	Vessel 1	N/A	5.3

5	Thrombin	PEC	Sham	Region 2	Vessel 2	N/A	3.7
5	Thrombin	PEC	Sham	Region 2	Vessel 3	N/A	4.1
5	Thrombin	PEC	Sham	Region 3	Vessel 1	N/A	4.7
5	Thrombin	PEC	Sham	Region 3	Vessel 2	N/A	4.7
5	Thrombin	PEC	Sham	Region 3	Vessel 3	N/A	4.4
1	Ligation Only	PEC	Sham	Region 1	Vessel 1	N/A	5.2
1	Ligation Only	PEC	Sham	Region 1	Vessel 2	N/A	5.0
1	Ligation Only	PEC	Sham	Region 1	Vessel 3	N/A	4.6
1	Ligation Only	PEC	Sham	Region 2	Vessel 1	N/A	6.0
1	Ligation Only	PEC	Sham	Region 2	Vessel 2	N/A	6.6
1	Ligation Only	PEC	Sham	Region 2	Vessel 3	N/A	4.9
1	Ligation Only	PEC	Sham	Region 3	Vessel 1	N/A	4.9
1	Ligation Only	PEC	Sham	Region 3	Vessel 2	N/A	4.2
1	Ligation Only	PEC	Sham	Region 3	Vessel 3	N/A	6.2
2	Ligation Only	No PEC	Sham	Region 1	Vessel 1	N/A	4.3
2	Ligation Only	No PEC	Sham	Region 1	Vessel 2	N/A	5.3
2	Ligation Only	No PEC	Sham	Region 1	Vessel 3	N/A	5.8
2	Ligation Only	No PEC	Sham	Region 2	Vessel 1	N/A	5.7
2	Ligation Only	No PEC	Sham	Region 2	Vessel 2	N/A	5.7
2	Ligation Only	No PEC	Sham	Region 2	Vessel 3	N/A	5.0
2	Ligation Only	No PEC	Sham	Region 3	Vessel 1	N/A	5.2
2	Ligation Only	No PEC	Sham	Region 3	Vessel 2	N/A	6.7
2	Ligation Only	No PEC	Sham	Region 3	Vessel 3	N/A	8.4
3	Ligation Only	No PEC	Sham	Region 1	Vessel 1	N/A	3.9
3	Ligation Only	No PEC	Sham	Region 1	Vessel 2	N/A	5.5
3	Ligation Only	No PEC	Sham	Region 1	Vessel 3	N/A	4.8
3	Ligation Only	No PEC	Sham	Region 2	Vessel 1	N/A	5.6
3	Ligation Only	No PEC	Sham	Region 2	Vessel 2	N/A	5.7
3	Ligation Only	No PEC	Sham	Region 2	Vessel 3	N/A	5.6
3	Ligation Only	No PEC	Sham	Region 3	Vessel 1	N/A	5.4
3	Ligation Only	No PEC	Sham	Region 3	Vessel 2	N/A	4.8
3	Ligation Only	No PEC	Sham	Region 3	Vessel 3	N/A	6.1
4	Ligation Only	No PEC	Sham	Region 1	Vessel 1	N/A	4.9
4	Ligation Only	No PEC	Sham	Region 1	Vessel 2	N/A	5.2
4	Ligation Only	No PEC	Sham	Region 1	Vessel 3	N/A	5.6
4	Ligation Only	No PEC	Sham	Region 2	Vessel 1	N/A	4.9
4	Ligation Only	No PEC	Sham	Region 2	Vessel 2	N/A	5.1
4	Ligation Only	No PEC	Sham	Region 2	Vessel 3	N/A	7.1
4	Ligation Only	No PEC	Sham	Region 3	Vessel 1	N/A	4.3

4	Ligation Only	No PEC	Sham	Region 3	Vessel 2	N/A	5.9
4	Ligation Only	No PEC	Sham	Region 3	Vessel 3	N/A	4.6
5	Ligation Only	PEC	Sham	Region 1	Vessel 1	N/A	4.0
5	Ligation Only	PEC	Sham	Region 1	Vessel 2	N/A	4.9
5	Ligation Only	PEC	Sham	Region 1	Vessel 3	N/A	5.0
5	Ligation Only	PEC	Sham	Region 2	Vessel 1	N/A	6.7
5	Ligation Only	PEC	Sham	Region 2	Vessel 2	N/A	5.7
5	Ligation Only	PEC	Sham	Region 2	Vessel 3	N/A	6.3
5	Ligation Only	PEC	Sham	Region 3	Vessel 1	N/A	4.9
5	Ligation Only	PEC	Sham	Region 3	Vessel 2	N/A	5.9
5	Ligation Only	PEC	Sham	Region 3	Vessel 3	N/A	6.2

Cell Transplantation Maximum Diameter Data

Replicate	Treatment	Muscle	Max α-SMA Vessel Diameter (μm)	Number of Vessels in Region with Max Diameter Vessel
1	Myogenic Cells	Ligated	PEC	PEC
2	Myogenic Cells	Ligated	44.1	5
3	Myogenic Cells	Ligated	PEC	PEC
4	Myogenic Cells	Ligated	17.7	12
5	Myogenic Cells	Ligated	28.7	8
6	Myogenic Cells	Ligated	28.9	9
1	Vehicle	Ligated	PEC	PEC
2	Vehicle	Ligated	PEC	PEC
3	Vehicle	Ligated	9.3	15
4	Vehicle	Ligated	18.0	12
5	Vehicle	Ligated	23.6	16
6	Vehicle	Ligated	20.9	16
1	Thrombin	Ligated	25.0	10
2	Thrombin	Ligated	PEC	PEC
3	Thrombin	Ligated	23.0	8
4	Thrombin	Ligated	35.6	11
5	Thrombin	Ligated	PEC	PEC
1	Ligation Only	Ligated	PEC	PEC
2	Ligation Only	Ligated	19.1	12
3	Ligation Only	Ligated	15.2	15
4	Ligation Only	Ligated	22.5	14
5	Ligation Only	Ligated	PEC	PEC

Cell Transplantation CD68 Data

Replicate	Treatment	Muscle	Region	Overall CD68 Cell Count	Vessel Associated CD68 Cells
1	Myogenic Cells	Ligated	Region 1	19	14
1	Myogenic Cells	Ligated	Region 2	15	11
1	Myogenic Cells	Ligated	Region 3	16	16
2	Myogenic Cells	Ligated	Region 1	15	21
2	Myogenic Cells	Ligated	Region 2	19	24
2	Myogenic Cells	Ligated	Region 3	16	15
3	Myogenic Cells	Ligated	Region 1	15	15
3	Myogenic Cells	Ligated	Region 2	14	13
3	Myogenic Cells	Ligated	Region 3	14	18
4	Myogenic Cells	Ligated	Region 1	14	20
4	Myogenic Cells	Ligated	Region 2	14	14
4	Myogenic Cells	Ligated	Region 3	15	20
5	Myogenic Cells	Ligated	Region 1	15	23
5	Myogenic Cells	Ligated	Region 2	20	16
5	Myogenic Cells	Ligated	Region 3	19	18
6	Myogenic Cells	Ligated	Region 1	16	16
6	Myogenic Cells	Ligated	Region 2	17	13
6	Myogenic Cells	Ligated	Region 3	15	18
1	Vehicle	Ligated	Region 1	15	16
1	Vehicle	Ligated	Region 2	15	16
1	Vehicle	Ligated	Region 3	16	17
2	Vehicle	Ligated	Region 1	16	14
2	Vehicle	Ligated	Region 2	14	16
2	Vehicle	Ligated	Region 3	15	12
3	Vehicle	Ligated	Region 1	18	14
3	Vehicle	Ligated	Region 2	21	20
3	Vehicle	Ligated	Region 3	15	12
4	Vehicle	Ligated	Region 1	14	12
4	Vehicle	Ligated	Region 2	17	13
4	Vehicle	Ligated	Region 3	17	13
5	Vehicle	Ligated	Region 1	16	17
5	Vehicle	Ligated	Region 2	15	19
5	Vehicle	Ligated	Region 3	17	11
6	Vehicle	Ligated	Region 1	14	18
6	Vehicle	Ligated	Region 2	14	11

6	Vehicle	Ligated	Region 3	15	12
1	Thrombin	Ligated	Region 1	19	14
1	Thrombin	Ligated	Region 2	16	15
1	Thrombin	Ligated	Region 3	17	15
2	Thrombin	Ligated	Region 1	15	16
2	Thrombin	Ligated	Region 2	14	15
2	Thrombin	Ligated	Region 3	16	15
3	Thrombin	Ligated	Region 1	10	12
3	Thrombin	Ligated	Region 2	16	20
3	Thrombin	Ligated	Region 3	16	16
4	Thrombin	Ligated	Region 1	16	19
4	Thrombin	Ligated	Region 2	16	19
4	Thrombin	Ligated	Region 3	16	22
5	Thrombin	Ligated	Region 1	14	13
5	Thrombin	Ligated	Region 2	15	17
5	Thrombin	Ligated	Region 3	14	13
1	Ligation Only	Ligated	Region 1	15	16
1	Ligation Only	Ligated	Region 2	18	15
1	Ligation Only	Ligated	Region 3	17	17
2	Ligation Only	Ligated	Region 1	19	20
2	Ligation Only	Ligated	Region 2	13	17
2	Ligation Only	Ligated	Region 3	17	18
3	Ligation Only	Ligated	Region 1	15	19
3	Ligation Only	Ligated	Region 2	15	22
3	Ligation Only	Ligated	Region 3	15	20
4	Ligation Only	Ligated	Region 1	13	19
4	Ligation Only	Ligated	Region 2	16	20
4	Ligation Only	Ligated	Region 3	13	16
5	Ligation Only	Ligated	Region 1	15	12
5	Ligation Only	Ligated	Region 2	15	12
5	Ligation Only	Ligated	Region 3	16	14
1	Myogenic Cells	Sham	Region 1	8	N/A
1	Myogenic Cells	Sham	Region 2	7	N/A
1	Myogenic Cells	Sham	Region 3	10	N/A
2	Myogenic Cells	Sham	Region 1	5	N/A
2	Myogenic Cells	Sham	Region 2	7	N/A
2	Myogenic Cells	Sham	Region 3	7	N/A
3	Myogenic Cells	Sham	Region 1	7	N/A
3	Myogenic Cells	Sham	Region 2	8	N/A

3	Myogenic Cells	Sham	Region 3	8	N/A
4	Myogenic Cells	Sham	Region 1	8	N/A
4	Myogenic Cells	Sham	Region 2	7	N/A
4	Myogenic Cells	Sham	Region 3	6	N/A
5	Myogenic Cells	Sham	Region 1	7	N/A
5	Myogenic Cells	Sham	Region 2	8	N/A
5	Myogenic Cells	Sham	Region 3	8	N/A
6	Myogenic Cells	Sham	Region 1	6	N/A
6	Myogenic Cells	Sham	Region 2	6	N/A
6	Myogenic Cells	Sham	Region 3	6	N/A
1	Vehicle	Sham	Region 1	7	N/A
1	Vehicle	Sham	Region 2	7	N/A
1	Vehicle	Sham	Region 3	6	N/A
2	Vehicle	Sham	Region 1	8	N/A
2	Vehicle	Sham	Region 2	11	N/A
2	Vehicle	Sham	Region 3	8	N/A
3	Vehicle	Sham	Region 1	9	N/A
3	Vehicle	Sham	Region 2	8	N/A
3	Vehicle	Sham	Region 3	6	N/A
4	Vehicle	Sham	Region 1	6	N/A
4	Vehicle	Sham	Region 2	7	N/A
4	Vehicle	Sham	Region 3	6	N/A
5	Vehicle	Sham	Region 1	6	N/A
5	Vehicle	Sham	Region 2	6	N/A
5	Vehicle	Sham	Region 3	6	N/A
6	Vehicle	Sham	Region 1	7	N/A
6	Vehicle	Sham	Region 2	6	N/A
6	Vehicle	Sham	Region 3	8	N/A
1	Thrombin	Sham	Region 1	7	N/A
1	Thrombin	Sham	Region 2	6	N/A
1	Thrombin	Sham	Region 3	7	N/A
2	Thrombin	Sham	Region 1	10	N/A
2	Thrombin	Sham	Region 2	9	N/A
2	Thrombin	Sham	Region 3	6	N/A
3	Thrombin	Sham	Region 1	6	N/A
3	Thrombin	Sham	Region 2	7	N/A
3	Thrombin	Sham	Region 3	8	N/A
4	Thrombin	Sham	Region 1	7	N/A
4	Thrombin	Sham	Region 2	6	N/A

4	Thrombin	Sham	Region 3	7	N/A
5	Thrombin	Sham	Region 1	6	N/A
5	Thrombin	Sham	Region 2	6	N/A
5	Thrombin	Sham	Region 3	7	N/A
1	Ligation Only	Sham	Region 1	8	N/A
1	Ligation Only	Sham	Region 2	9	N/A
1	Ligation Only	Sham	Region 3	8	N/A
2	Ligation Only	Sham	Region 1	10	N/A
2	Ligation Only	Sham	Region 2	6	N/A
2	Ligation Only	Sham	Region 3	5	N/A
3	Ligation Only	Sham	Region 1	5	N/A
3	Ligation Only	Sham	Region 2	6	N/A
3	Ligation Only	Sham	Region 3	5	N/A
4	Ligation Only	Sham	Region 1	7	N/A
4	Ligation Only	Sham	Region 2	7	N/A
4	Ligation Only	Sham	Region 3	7	N/A
5	Ligation Only	Sham	Region 1	7	N/A
5	Ligation Only	Sham	Region 2	8	N/A
5	Ligation Only	Sham	Region 3	7	N/A

Appendix D: Statistics

Sample Independent t-test Outputs

Male versus Female α -SMA vessel count

Estimation for Difference

Difference	95% CI for Difference
0.3611	(-0.0426, 0.7648)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
1.85	24	0.0772

Male versus Female α -SMA vessel diameter

Estimation for Difference

Difference	95% CI for Difference
0.4907	(-0.5765, 1.5580)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.92	63	0.3617

Sample One-Way ANOVAs with Tukey comparisons

Cell Transplantation α -SMA vessel counts

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	61.458	20.4859	9.89	<0.0001
Error	61	126.327	2.0709		
Total	64	187.785			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.43907	32.73%	29.42%	23.37%

Means

Group	N	Mean	StDev	95% CI
Cells	18	7.1111	1.4907	(6.4329, 7.7894)
Gel	17	6.6471	0.6063	(5.9491, 7.3450)
Ligation Only	15	7.5333	1.3020	(6.7903, 8.2763)
Thrombin	15	9.2667	2.0517	(8.5237, 10.0097)

Pooled StDev = 1.43907

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Thrombin	15	9.2667	A
Ligation Only	15	7.5333	B
Cells	18	7.1111	B
Gel	17	6.6471	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gel-Cells	-0.4641	0.4867	(-1.7512, 0.8230)	-0.95	0.7761
Ligation Only-Cells	0.4222	0.5031	(-0.9083, 1.7527)	0.84	0.8356
Thrombin-Cells	2.1556	0.5031	(0.8251, 3.4861)	4.28	0.0004
Ligation Only-Gel	0.8863	0.5098	(-0.4619, 2.2344)	1.74	0.3130
Thrombin-Gel	2.6196	0.5098	(1.2714, 3.9678)	5.14	<0.0001
Thrombin-Ligation Only	1.7333	0.5255	(0.3437, 3.1230)	3.30	0.0086

Individual confidence level = 98.96%

Cell Transplantation Sham Vessel Count

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	34.307	11.4357	2.09	0.1102
Error	62	338.678	5.4625		
Total	65	372.985			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.33721	9.20%	4.80%	0.00%

Means

Group	N	Mean	StDev	95% CI
Cells	18	14.6667	2.1693	(13.5655, 15.7679)
Gel	18	12.7222	2.6078	(11.6210, 13.8234)
Ligated Only	15	13.6667	2.0587	(12.4604, 14.8730)
Thrombin	15	13.5333	2.4456	(12.3270, 14.7396)

Pooled StDev = 2.33721

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Cells	18	14.6667	A
Ligated Only	15	13.6667	A
Thrombin	15	13.5333	A
Gel	18	12.7222	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gel-Cells	-1.9444	0.7791	(-3.9992, 0.1104)	-2.50	0.0705
Ligated Only-Cells	-1.0000	0.8171	(-3.1551, 1.1551)	-1.22	0.6142
Thrombin-Cells	-1.1333	0.8171	(-3.2884, 1.0218)	-1.39	0.5122
Ligated Only-Gel	0.9444	0.8171	(-1.2107, 3.0995)	1.16	0.6566
Thrombin-Gel	0.8111	0.8171	(-1.3440, 2.9662)	0.99	0.7541
Thrombin-Ligated Only	-0.1333	0.8534	(-2.3843, 2.1176)	-0.16	0.9986

Individual confidence level = 98.95%

Cell Transplantation Total Vessel Count

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	4	35.274	8.81843	1.84	0.1252
Error	127	608.696	4.79288		
Total	131	643.970			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.18927	5.48%	2.50%	0.00%

Means

Group	N	Mean	StDev	95% CI
Cells	18	13.6111	2.1731	(12.5900, 14.6322)
Gel	18	12.5000	1.2005	(11.4789, 13.5211)
Ligation Only	15	13.6667	2.1602	(12.5481, 14.7852)
Sham	66	13.6515	2.3955	(13.1183, 14.1848)
Thrombin	15	12.4000	2.1647	(11.2814, 13.5186)

Pooled StDev = 2.18927

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Ligation Only	15	13.6667	A
Sham	66	13.6515	A
Cells	18	13.6111	A
Gel	18	12.5000	A
Thrombin	15	12.4000	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gel-Cells	-1.1111	0.7298	(-3.1287, 0.9065)	-1.52	0.5498
Ligation Only-Cells	0.0556	0.7654	(-2.0605, 2.1717)	0.07	1.0000
Sham-Cells	0.0404	0.5821	(-1.5691, 1.6499)	0.07	1.0000
Thrombin-Cells	-1.2111	0.7654	(-3.3272, 0.9050)	-1.58	0.5114
Ligation Only-Gel	1.1667	0.7654	(-0.9494, 3.2828)	1.52	0.5487
Sham-Gel	1.1515	0.5821	(-0.4580, 2.7610)	1.98	0.2827
Thrombin-Gel	-0.1000	0.7654	(-2.2161, 2.0161)	-0.13	0.9999
Sham-Ligation Only	-0.0152	0.6262	(-1.7465, 1.7162)	-0.02	1.0000
Thrombin-Ligation Only	-1.2667	0.7994	(-3.4769, 0.9435)	-1.58	0.5101
Thrombin-Sham	-1.2515	0.6262	(-2.9829, 0.4798)	-2.00	0.2727

Individual confidence level = 99.35%

Cell Transplantation α -SMA Vessel Diameters

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	373.97	124.655	6.87	0.0002
Error	191	3465.09	18.142		
Total	194	3839.06			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.25932	9.74%	8.32%	6.07%

Means

Group	N	Mean	StDev	95% CI
Cells	54	13.4971	6.2256	(12.3538, 14.6403)
Gel	51	10.0868	2.7971	(8.9104, 11.2632)
Ligation Only	45	10.4503	3.2423	(9.1979, 11.7027)
Thrombin	45	11.9054	3.5585	(10.6530, 13.1578)

Pooled StDev = 4.25932

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Cells	54	13.4971	A
Thrombin	45	11.9054	A B
Ligation Only	45	10.4503	B
Gel	51	10.0868	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gel-Cells	-3.4103	0.8317	(-5.5686, -1.2520)	-4.10	0.0004
Ligation Only-Cells	-3.0467	0.8597	(-5.2778, -0.8157)	-3.54	0.0028
Thrombin-Cells	-1.5917	0.8597	(-3.8227, 0.6394)	-1.85	0.2528
Ligation Only-Gel	0.3636	0.8711	(-1.8971, 2.6242)	0.42	0.9755
Thrombin-Gel	1.8186	0.8711	(-0.4420, 4.0793)	2.09	0.1608
Thrombin-Ligation Only	1.4551	0.8979	(-0.8752, 3.7853)	1.62	0.3696

Individual confidence level = 98.98%

Cell Transplantation Mice without a PEC Vessel Count

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	56.258	18.7527	14.33	<0.0001
Error	34	44.505	1.3090		
Total	37	100.763			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.14410	55.83%	51.93%	44.36%

Means

Group	N	Mean	StDev	95% CI
Cells	9	7.8889	0.9280	(7.1139, 8.6639)
Gel	11	6.5455	0.6876	(5.8444, 7.2465)
Ligation Only	9	7.6667	1.5000	(6.8916, 8.4417)
Thrombin	9	9.8889	1.3642	(9.1139, 10.6639)

Pooled StDev = 1.14410

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Thrombin	9	9.8889	A
Cells	9	7.8889	B
Ligation Only	9	7.6667	B
Gel	11	6.5455	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gel-Cells	-1.3434	0.5142	(-2.7325, 0.0456)	-2.61	0.0610
Ligation Only-Cells	-0.2222	0.5393	(-1.6790, 1.2346)	-0.41	0.9760
Thrombin-Cells	2.0000	0.5393	(0.5432, 3.4568)	3.71	0.0040
Ligation Only-Gel	1.1212	0.5142	(-0.2678, 2.5102)	2.18	0.1493
Thrombin-Gel	3.3434	0.5142	(1.9544, 4.7325)	6.50	<0.0001
Thrombin-Ligation Only	2.2222	0.5393	(0.7654, 3.6790)	4.12	0.0013

Individual confidence level = 98.93%

Cell Transplantation Vessel Count in the Region with the Maximum Diameter ACC

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	78.019	26.0064	9.46	0.0038
Error	9	24.750	2.7500		
Total	12	102.769			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.65831	75.92%	67.89%	50.75%

Means

Group	N	Mean	StDev	95% CI
Cells	3	9.667	2.082	(7.501, 11.833)
Gel	4	14.7500	1.8930	(12.8743, 16.6257)
Ligation Only	3	14.3333	0.5774	(12.1675, 16.4992)
Thrombin	3	9.6667	1.5275	(7.5008, 11.8325)

Pooled StDev = 1.65831

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Gel	4	14.7500	A
Ligation Only	3	14.3333	A
Thrombin	3	9.6667	B
Cells	3	9.667	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gel-Cells	5.083	1.267	(1.125, 9.042)	4.01	0.0133
Ligation Only-Cells	4.667	1.354	(0.435, 8.898)	3.45	0.0307
Thrombin-Cells	0.000	1.354	(-4.232, 4.232)	0.00	1.0000
Ligation Only-Gel	-0.417	1.267	(-4.375, 3.542)	-0.33	0.9869
Thrombin-Gel	-5.083	1.267	(-9.042, -1.125)	-4.01	0.0133
Thrombin-Ligation Only	-4.667	1.354	(-8.898, -0.435)	-3.45	0.0307

Individual confidence level = 98.78%

Cell Transplantation Maximum Diameter Vessel

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	1216.26	405.421	8.14	0.0003
Error	38	1892.38	49.799		
Total	41	3108.64			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.05687	39.13%	34.32%	26.64%

Means

Group	N	Mean	StDev	95% CI
Cells	12	29.854	10.204	(25.730, 33.978)
Gelatin	12	17.932	5.803	(13.808, 22.056)
Ligation ONLY	9	18.906	3.336	(14.144, 23.668)
Thrombin	9	27.860	5.995	(23.098, 32.622)

Pooled StDev = 7.05687

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Cells	12	29.854	A
Thrombin	9	27.860	A
Ligation ONLY	9	18.906	B
Gelatin	12	17.932	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gelatin-Cells	-11.922	2.881	(-19.663, -4.181)	-4.14	0.0010
Ligation ONLY-Cells	-10.948	3.112	(-19.309, -2.587)	-3.52	0.0060
Thrombin-Cells	-1.994	3.112	(-10.355, 6.368)	-0.64	0.9181
Ligation ONLY-Gelatin	0.974	3.112	(-7.388, 9.335)	0.31	0.9892
Thrombin-Gelatin	9.928	3.112	(1.567, 18.290)	3.19	0.0145
Thrombin-Ligation ONLY	8.954	3.327	(0.016, 17.893)	2.69	0.0494

Individual confidence level = 98.94%

Cell Transplantation Overall CD68)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	4.489	1.49630	0.43	0.7328
Error	62	216.178	3.48674		
Total	65	220.667			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.86728	2.03%	0.00%	0.00%

Means

Group	N	Mean	StDev	95% CI
Cells	18	16.0000	1.9704	(15.1202, 16.8798)
Gel	18	15.7778	1.7675	(14.8980, 16.6576)
Ligation Only	15	15.4667	1.7674	(14.5029, 16.4304)
Thrombin	15	15.3333	1.9518	(14.3696, 16.2971)

Pooled StDev = 1.86728

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Cells	18	16.0000	A
Gel	18	15.7778	A
Ligation Only	15	15.4667	A
Thrombin	15	15.3333	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Gel-Cells	-0.2222	0.6224	(-1.8639, 1.4194)	-0.36	0.9843
Ligation Only-Cells	-0.5333	0.6528	(-2.2551, 1.1885)	-0.82	0.8462
Thrombin-Cells	-0.6667	0.6528	(-2.3885, 1.0551)	-1.02	0.7377
Ligation Only-Gel	-0.3111	0.6528	(-2.0329, 1.4107)	-0.48	0.9640
Thrombin-Gel	-0.4444	0.6528	(-2.1662, 1.2773)	-0.68	0.9040
Thrombin-Ligation Only	-0.1333	0.6818	(-1.9317, 1.6650)	-0.20	0.9973

Individual confidence level = 98.95%

Cell Transplantation Vessel Associated CD68 Cells

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Group	3	68.596	22.8653	2.39	0.0775
Error	62	593.889	9.5789		
Total	65	662.485			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.09497	10.35%	6.02%	0.00%

Means

Group	N	Mean	StDev	95% CI
Cells	18	16.9444	3.5887	(15.4862, 18.4027)
Ligation	15	17.1333	2.9968	(15.5359, 18.7307)
Thrombin	15	16.0667	2.8402	(14.4693, 17.6641)
Vehicle	18	14.6111	2.8313	(13.1529, 16.0693)

Pooled StDev = 3.09497

Grouping Information Using the Tukey Method and 95% Confidence

Group	N	Mean	Grouping
Ligation	15	17.1333	A
Cells	18	16.9444	A
Thrombin	15	16.0667	A
Vehicle	18	14.6111	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Ligation-Cells	0.189	1.082	(-2.665, 3.043)	0.17	0.9981
Thrombin-Cells	-0.878	1.082	(-3.732, 1.976)	-0.81	0.8489
Vehicle-Cells	-2.333	1.032	(-5.054, 0.388)	-2.26	0.1184
Thrombin-Ligation	-1.067	1.130	(-4.047, 1.914)	-0.94	0.7814
Vehicle-Ligation	-2.522	1.082	(-5.376, 0.332)	-2.33	0.1020
Vehicle-Thrombin	-1.456	1.082	(-4.309, 1.398)	-1.35	0.5381

Individual confidence level = 98.95%

Mice with a PEC Sample Size Power Analysis Graph

Mice without a PEC Sample Size Power Analysis Graph