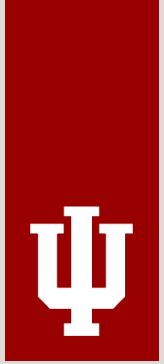

Department of Surgery

Human Induced Pluripotent Stem Cell-derived Mesenchymal Stromal Cells Promote Muscle Regeneration in a Diabetic Mouse Model of Critical Limb Threatening Ischemia

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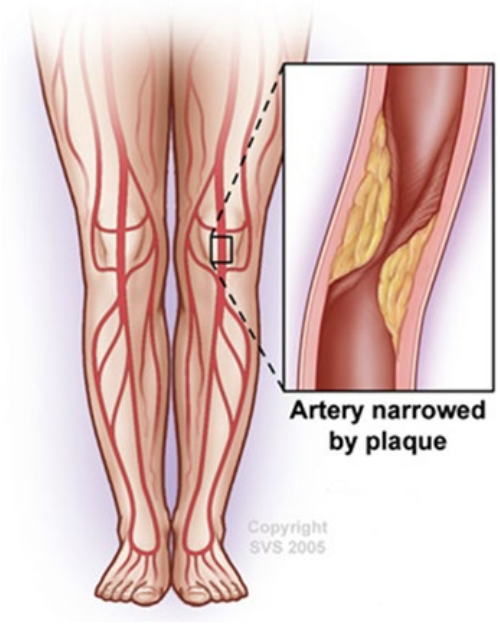
Division of Vascular Surgery

Critical Limb Threatening Ischemia (CLTI)

CLTI, the end stage of PAD, is a major healthcare priority because a 50% increase in non-traumatic major amputations has been observed since 2010.

AHA Policy Statement: Time to get to our feet – Reducing nontraumatic lower-extremity amputations 20% by 2030



Background

- Clinical data from our group have shown that allogeneic bone marrow-derived mesenchymal stromal cells (BMD-MSC) from young, healthy donors stimulate angiogenesis in diabetic CLTI patients.
- However, bone marrow provides relatively low numbers of MSC which results in high variability between preparations, and such cells are susceptible to senescence and phenotypic shift.
- Human induced pluripotent stem cell (iPSC) derived MSC may be generated in unlimited numbers, are resistant to senescence, and may be genetically modified, making them a more consistent and effective MSC type for treating CLTI.

Study Objective

Utilize a diabetic, ischemic hindlimb mouse model of CLTI to elucidate mechanisms by which iPSC-MSC may stimulate angiogenesis and muscle regeneration.

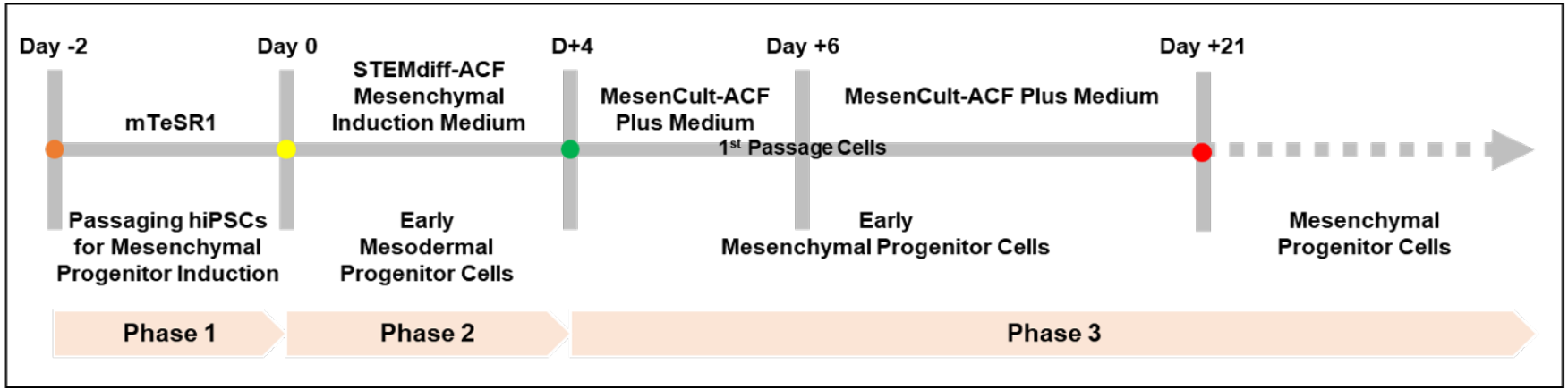
Part 1. Effects on blood perfusion, muscle regeneration, and angiogenesis

- Pathology (Adipocytes, fiber size, central nuclei, etc.)
- Fibrosis (collagen deposition)
- Limb blood perfusion (LDPI)
- Muscle function (energy and fatigue resistance)
- Capillary density (angiogenesis)

Part 2. Mechanism of action – markers for:

- Muscle regeneration
- Angiogenesis
- Macrophage phenotype
- T regulatory cell

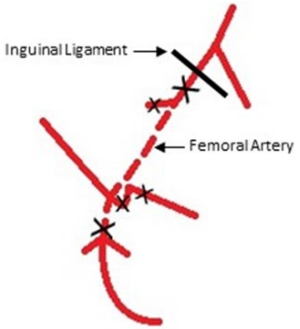
Derivation of MSCs from human iPSCs

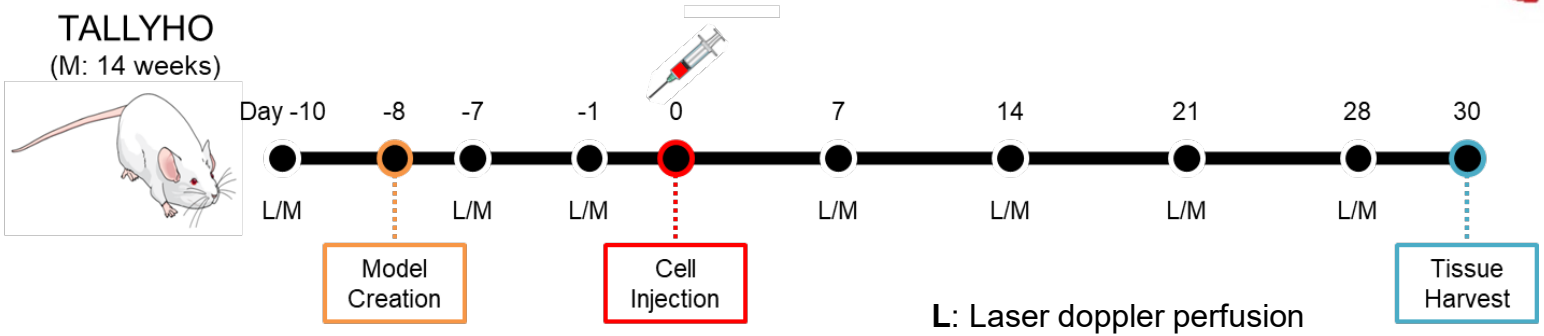


Human adult fibroblast cells are the source for deriving iPSC

The Diabetic TALLYHO Mouse Model of Critical Limb Threatening Ischemia

- The TALLYHO is a polygenic diabetic mouse model
- CLTI models were created by ligation/excision of the common femoral artery
- iPSC-MSC (500K cells) or vehicle control injected into the gracilis muscle 7 days after model creation

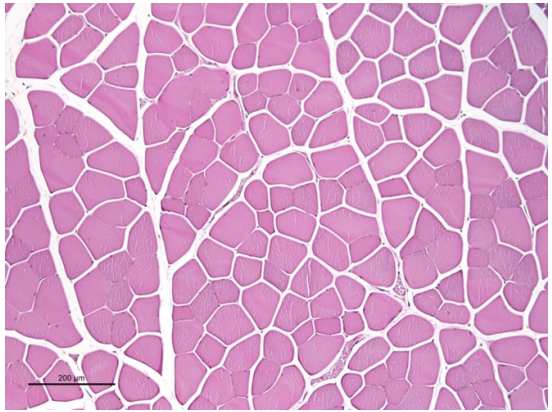




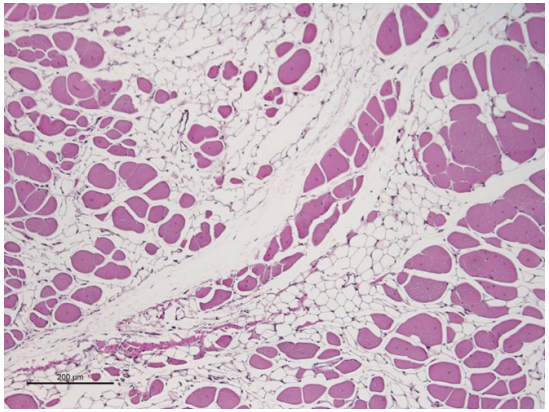
L: Laser doppler perfusion imaging (LDPI)
M: Plantar flexion muscle testing

iPSC-MSC Administration Reduces Pathology in Gastrocnemius Muscle

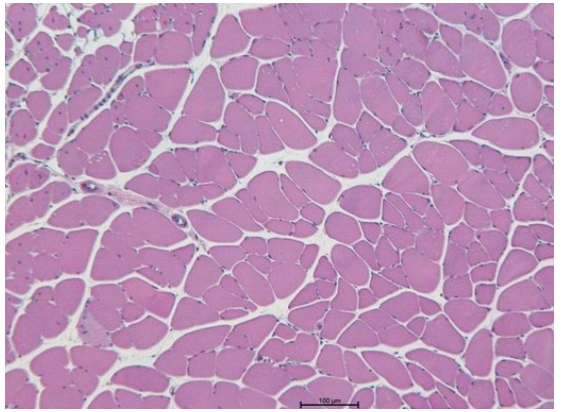
Non-ischemic

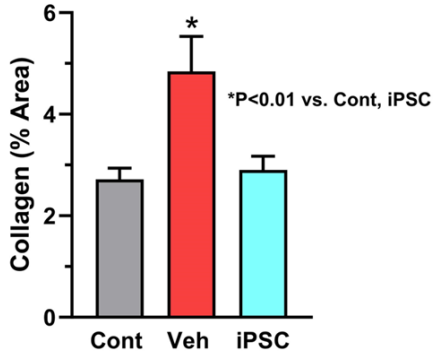


Ischemic, Vehicle



Ischemic, iPSC-MSC

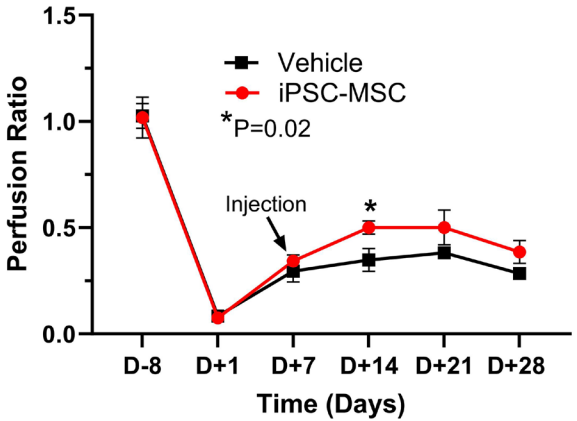




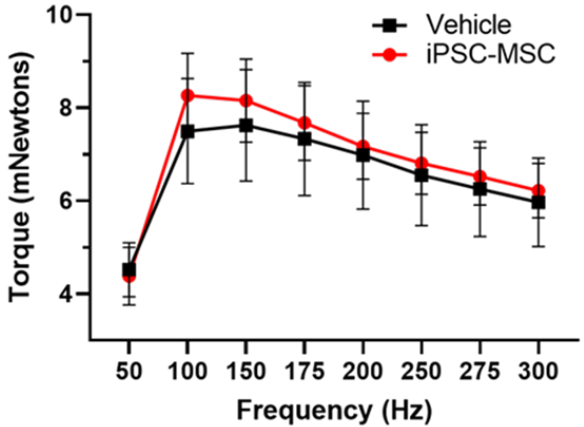
Representative images of H&E- stained paraffin sections of gastrocnemius muscle (100X). Administration of iPSC-MSC significantly reduced muscle fiber loss and decreased fibrosis and adipose formation as compared to the ischemic, vehicle treated hindlimb.

iPSC-MSC Increase Blood Perfusion, Muscle Energy, and Capillary Density

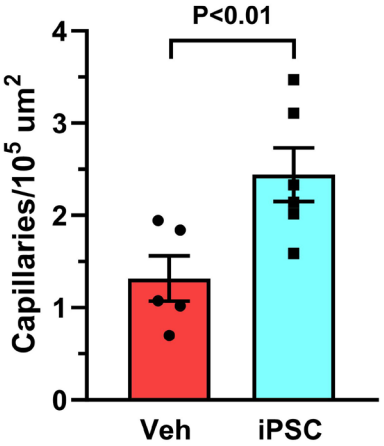
Hindlimb Blood Perfusion (LDPI)



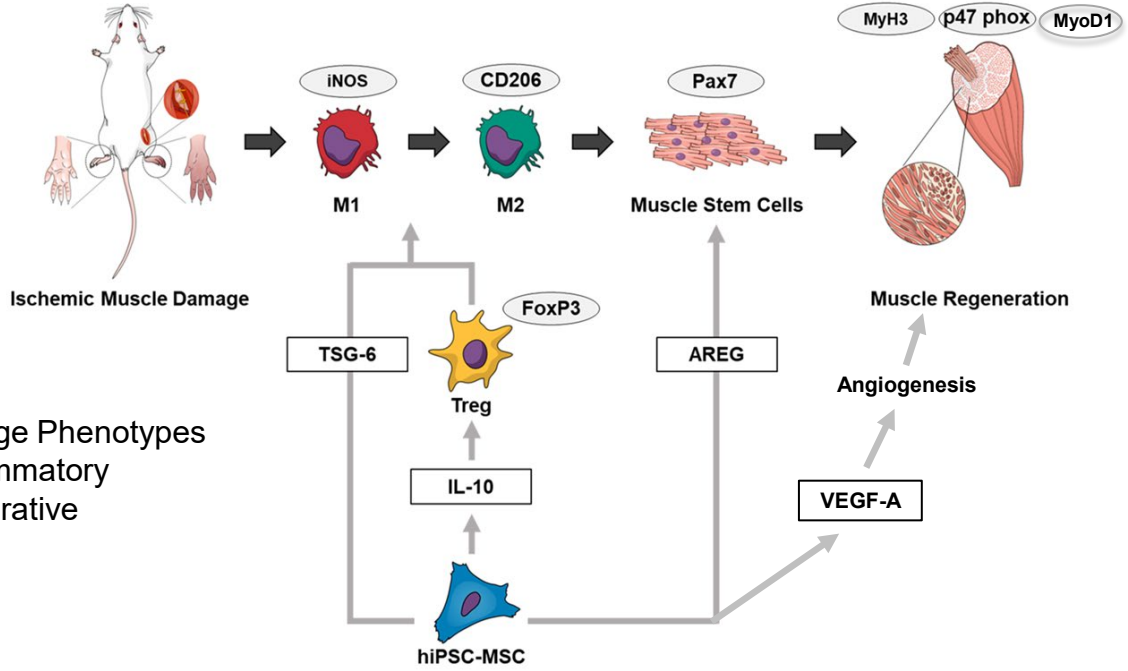
Plantar Flexion Muscle Testing



Angiogenesis



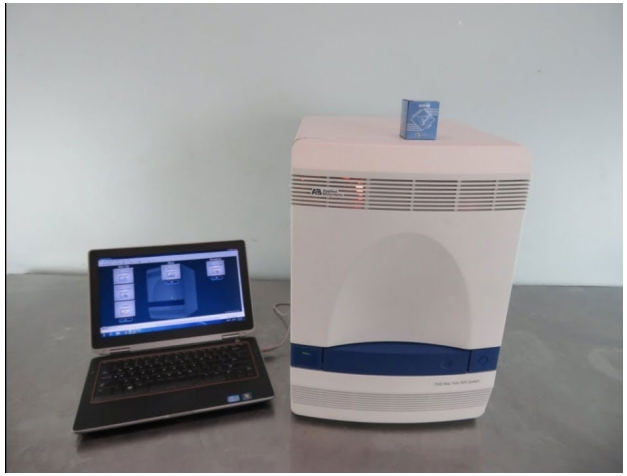
Determining the Mechanism of iPSC-MSC Mediated Muscle Regeneration



Macrophage Phenotypes
M1 - Inflammatory
M2 - Reparative

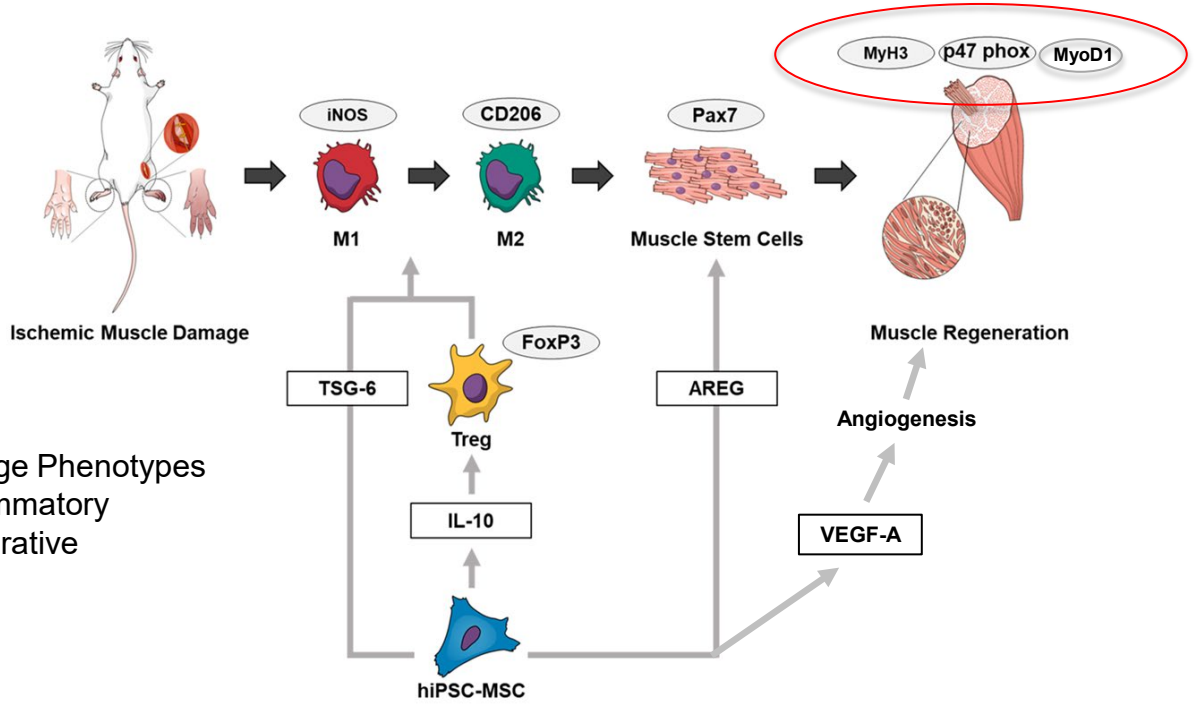
Determination of mRNA Expression by Real Time Quantitative Polymerase Chain Reaction

- Isolation/purification of total RNA from muscle
- Reverse transcription of RNA into cDNA
- Amplification of specific cDNA by PCR
- Detection and analysis of product



Applied Biosystems 7500 PCR System

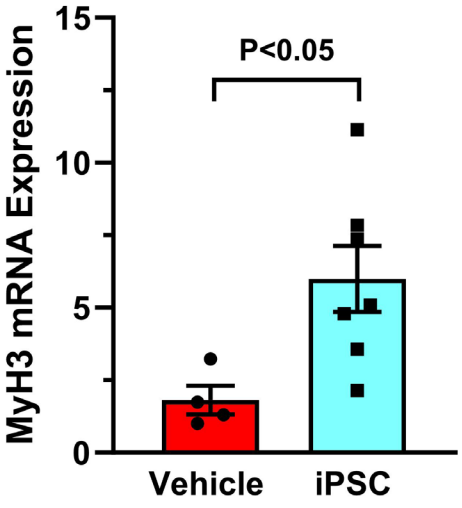
Determining the Mechanism of iPSC-MSC Mediated Muscle Regeneration



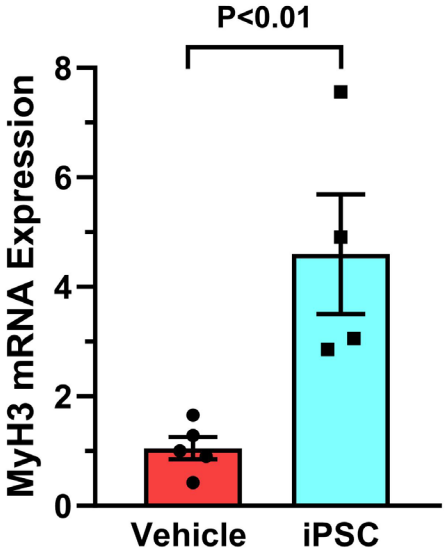
Macrophage Phenotypes
M1 - Inflammatory
M2 - Reparative

Molecular Markers for Muscle Regeneration

Embryonic myosin heavy chain (MyH3)

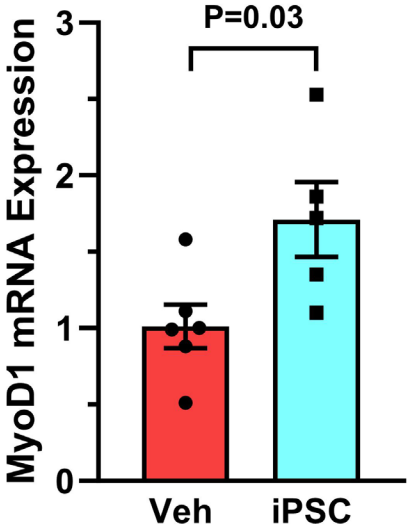


Tibialis



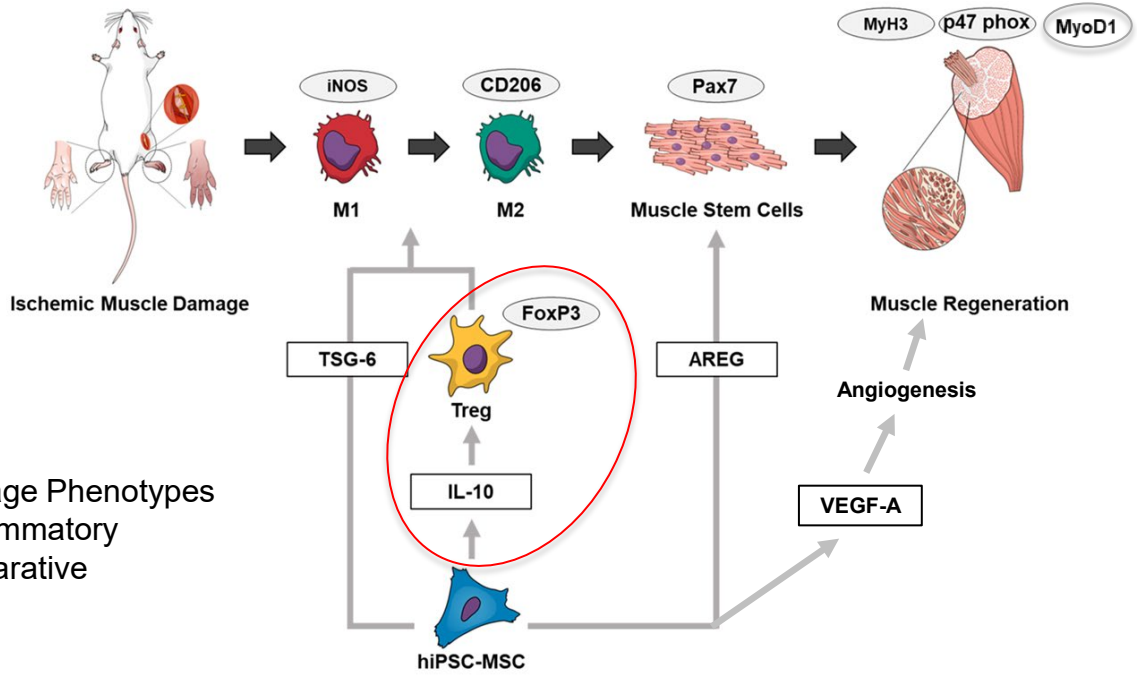
Gastrocnemius

Myoblast determination protein 1 (MyoD1)



Gastrocnemius

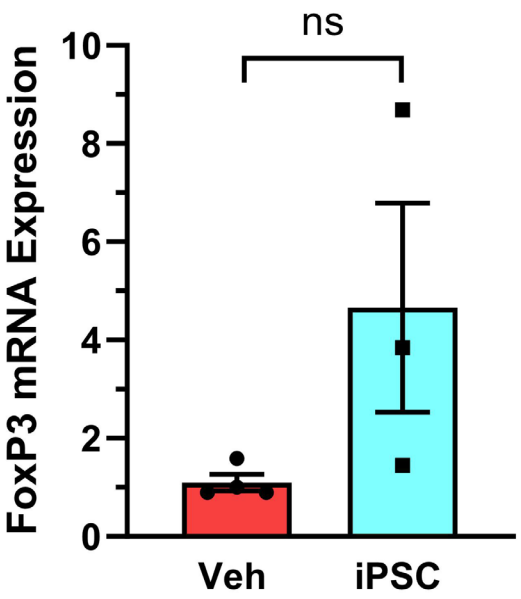
Investigating the Mechanism of iPSC-MSC Mediated Muscle Regeneration



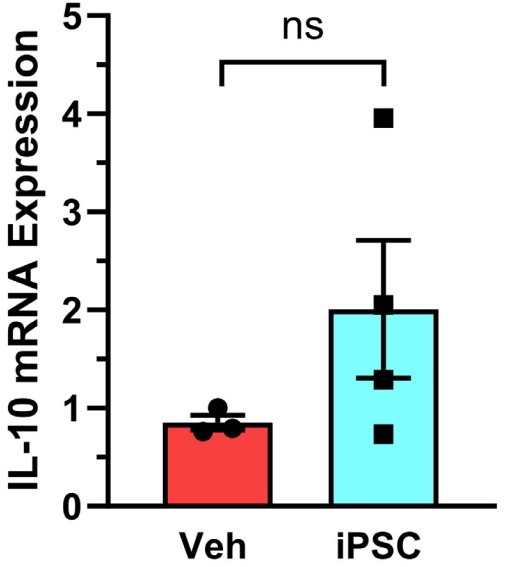
Macrophage Phenotypes
M1 - Inflammatory
M2 - Reparative

Molecular markers for MSC-mediated Treg Function

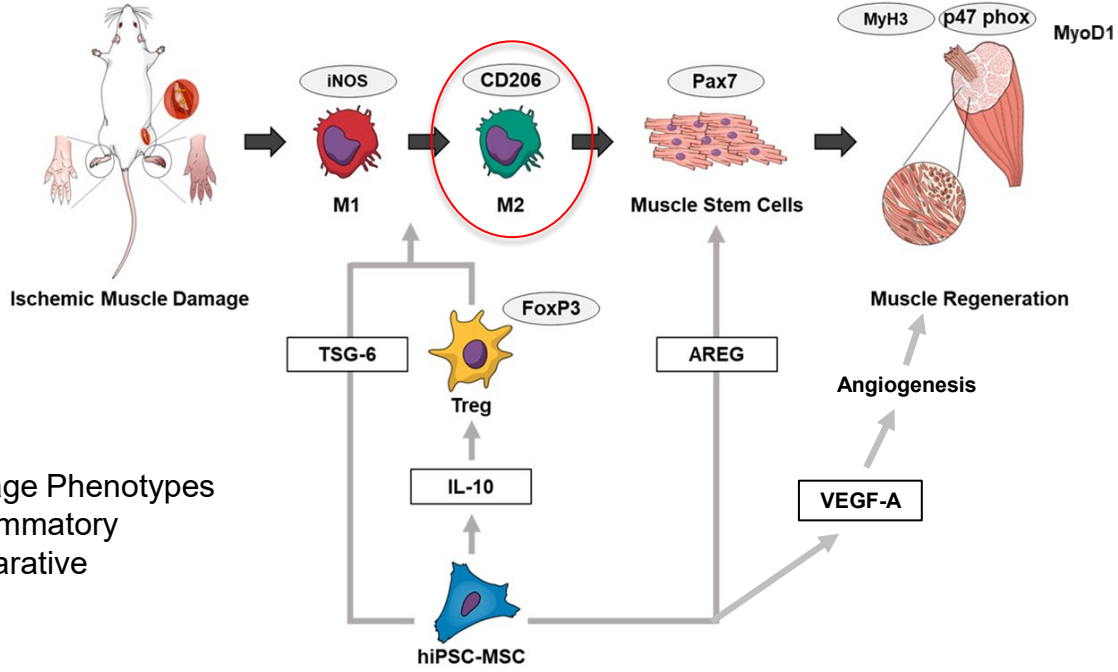
Forkhead Box P3 protein (FoxP3)



Interleukin 10 (IL-10)



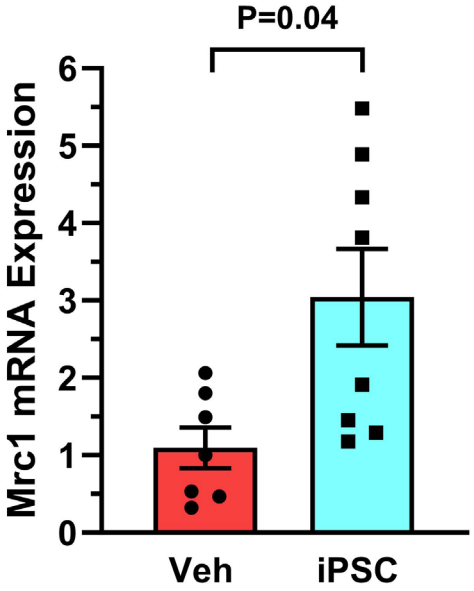
Investigating the Mechanism of iPSC-MSC Mediated Muscle Regeneration

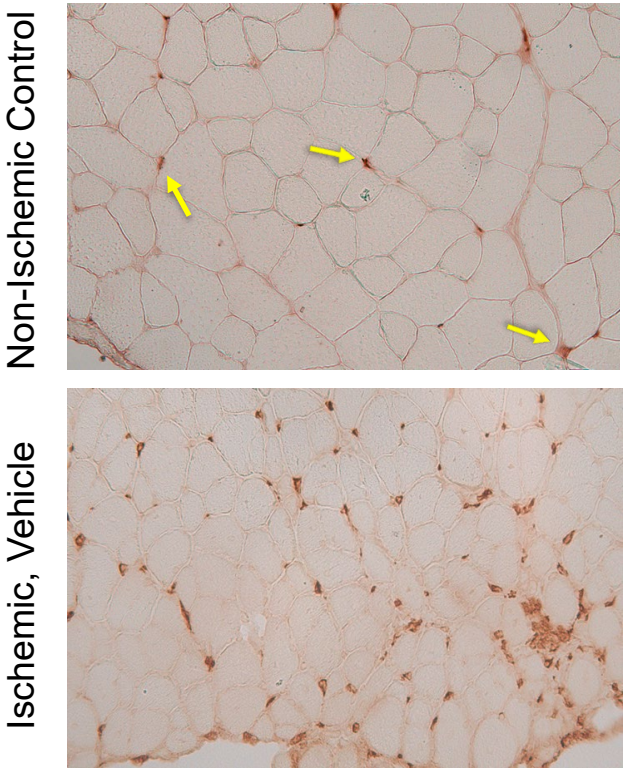


Macrophage Phenotypes
M1 - Inflammatory
M2 - Reparative

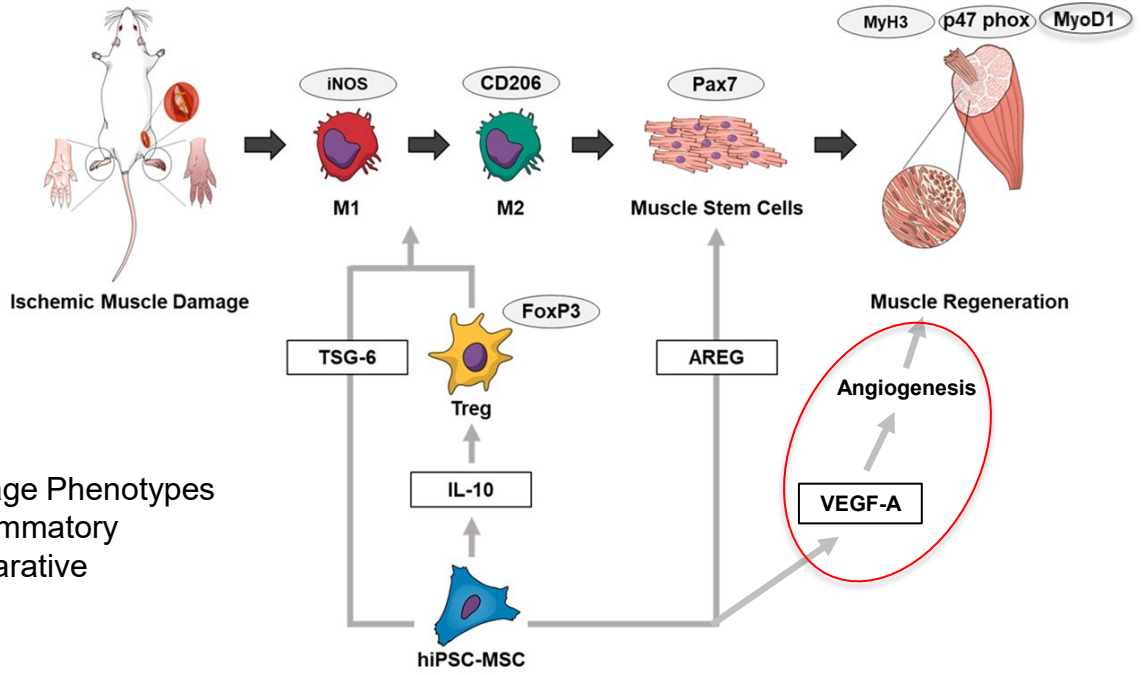
Molecular Markers for M2-biased Macrophage Phenotype (CD206)

Mannose receptor C - Type 1 (Mrc1)





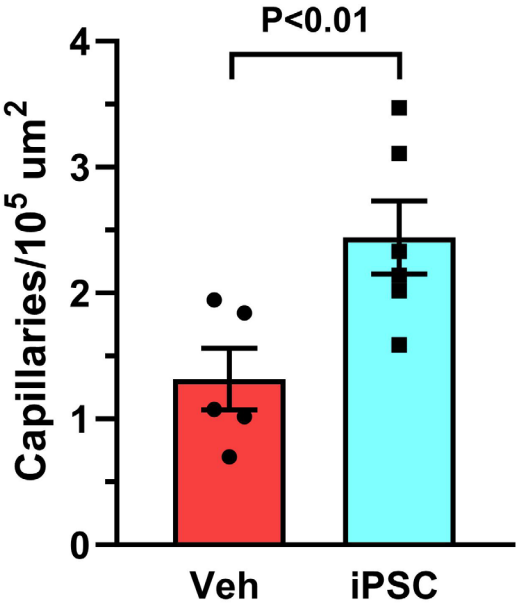
Investigating the Mechanism of iPSC-MSC Mediated Muscle Regeneration



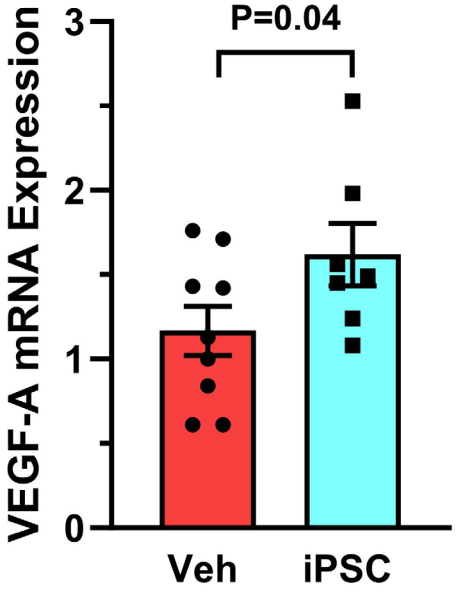
Macrophage Phenotypes
M1 - Inflammatory
M2 - Reparative

Molecular Markers for Angiogenesis (Capillary Growth)

Angiogenesis (Capillary Density)



Vascular endothelial cell growth factor (VEGF)

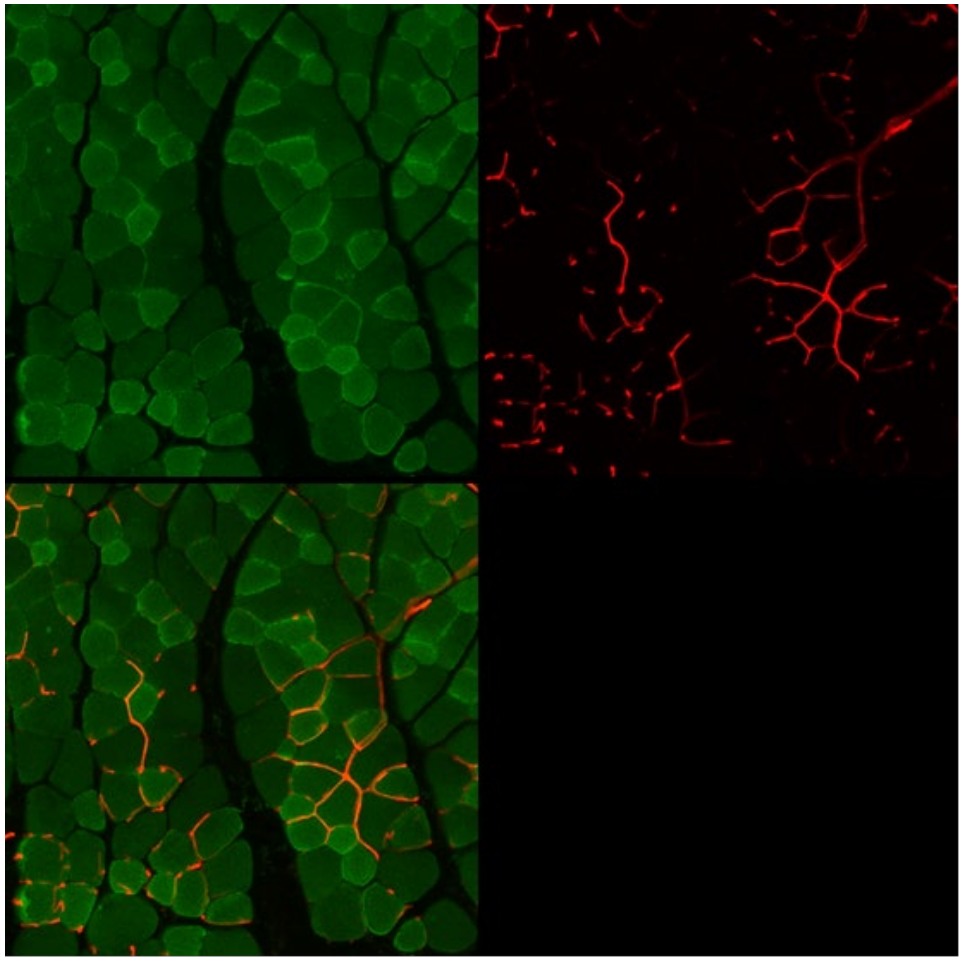


SUMMARY - Effects of iPSC-MSC Administration

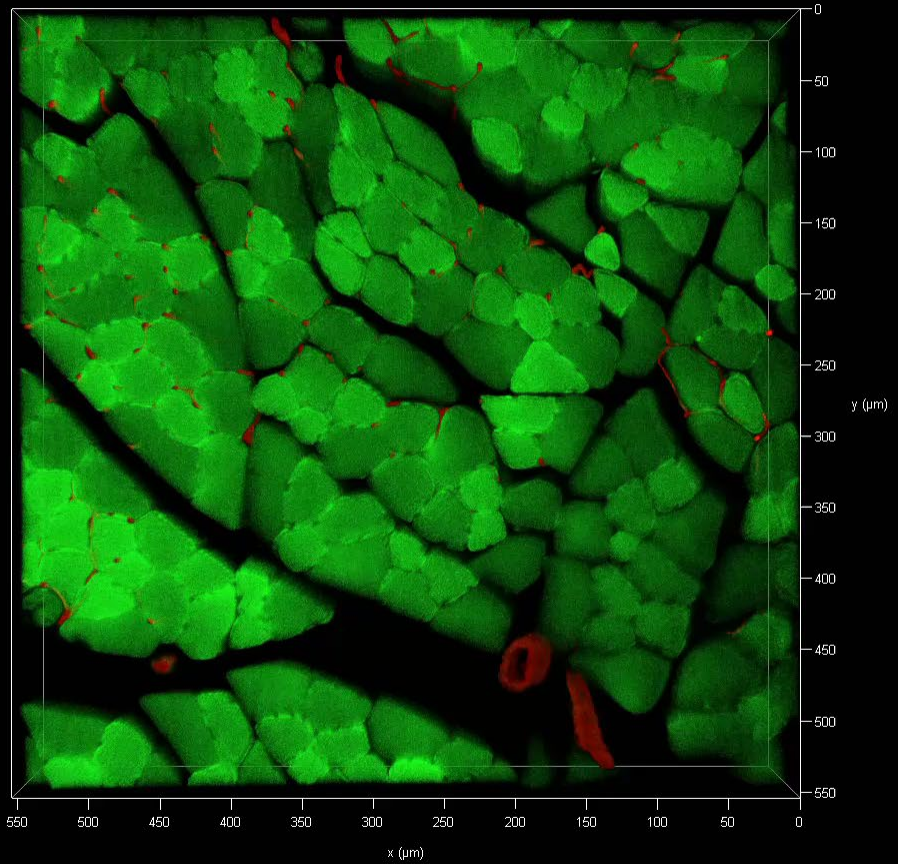
- Reverses pathology/muscle fibrosis, stimulates regeneration
- Increases limb perfusion and peak muscle energy
- Stimulates angiogenesis (increased capillary density)
- Increases expression of molecular markers consistent with muscle regeneration via Treg promotion of the M1-M2 macrophage phenotypic shift

Projects & Methods in Progress

- Alginate encapsulation of iPSC-MSC: in vitro and mouse experiments
- Analysis of changes in hindlimb vascularity using microCT
- Analysis of angiogenesis (capillaries) using confocal microscopy



Confocal Image of a 50-micron Tibialis Muscle Section Perfused with the Lipophilic Fluorescent Dye DiI


0
50
100
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550
y (µm)
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450
400
350
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200
150
100
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x (µm)

Acknowledgements

Lab Personnel

Michael P. Murphy, MD
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Nic Moldovan, PhD
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Students

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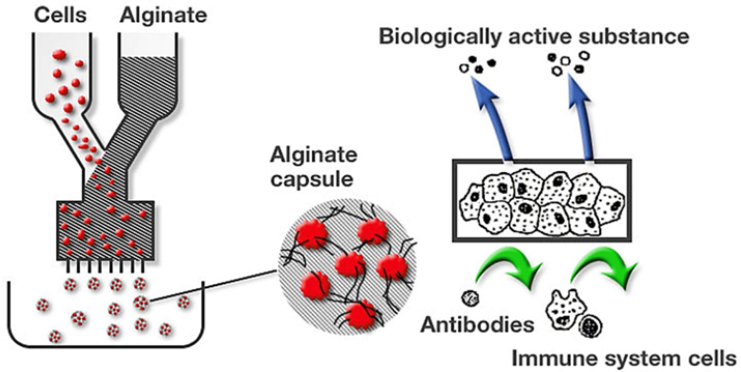
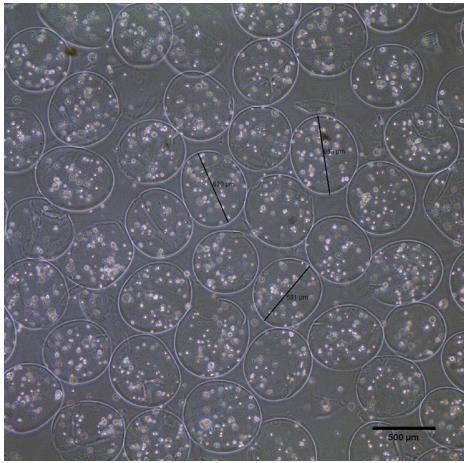
- Veterans Administration Directorship Award

Cell Encapsulation

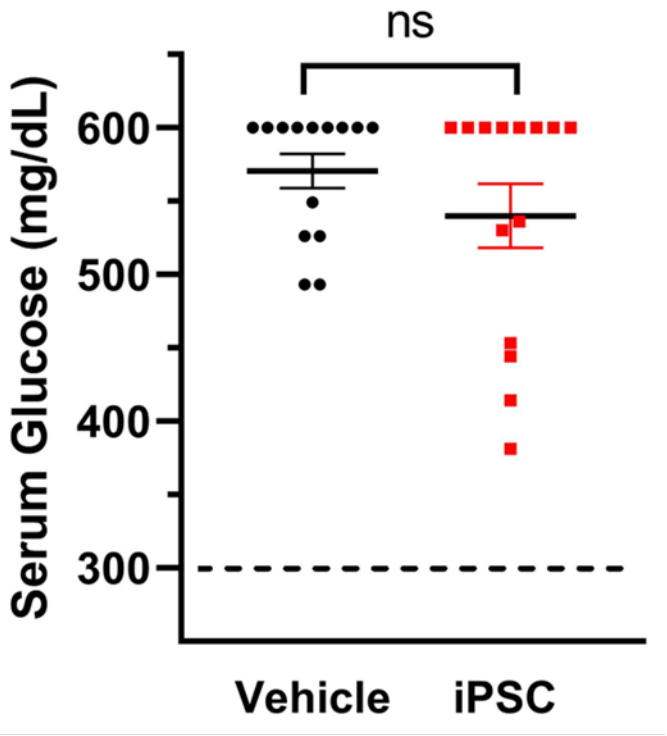
- Alginate hydrogel
- Prolongs MSC lifespan
- Protects cells from immune system
- Alters paracrine secretome
- Greater quantity/type of cytokines and growth factors are expressed and secreted



Andersen et al. PMID:27600217



iPSC-MSC did not alter serum glucose concentrations

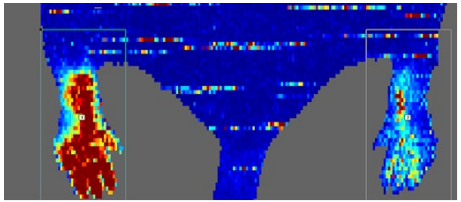


Average serum glucose concentrations determined via a glucometer in the untreated (Vehicle) and human iPSC-MSC treated (iPSC) mice were 570.5±11.6 and 539.9±21.9 mg/dL, respectively. Dashed line indicates threshold for murine diabetes.

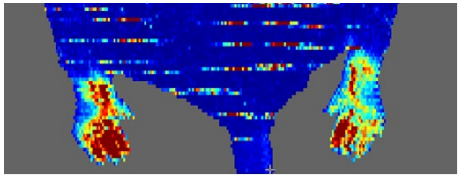
iPSC-MSC Administration Increases Limb Perfusion

Laser Doppler Perfusion Imaging

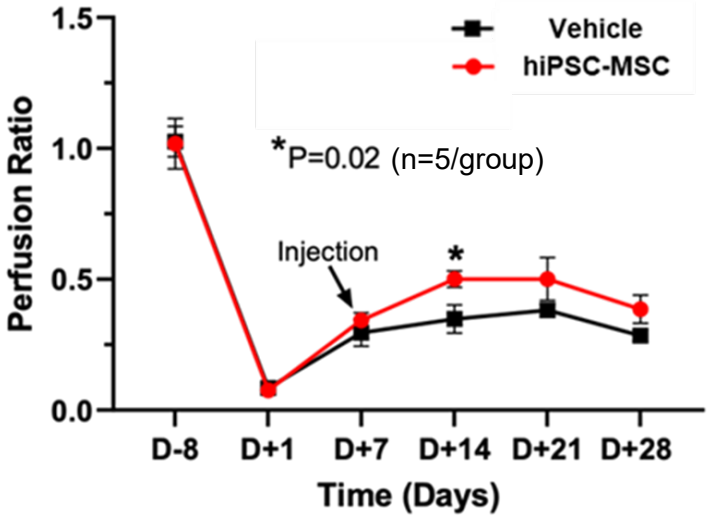
Vehicle



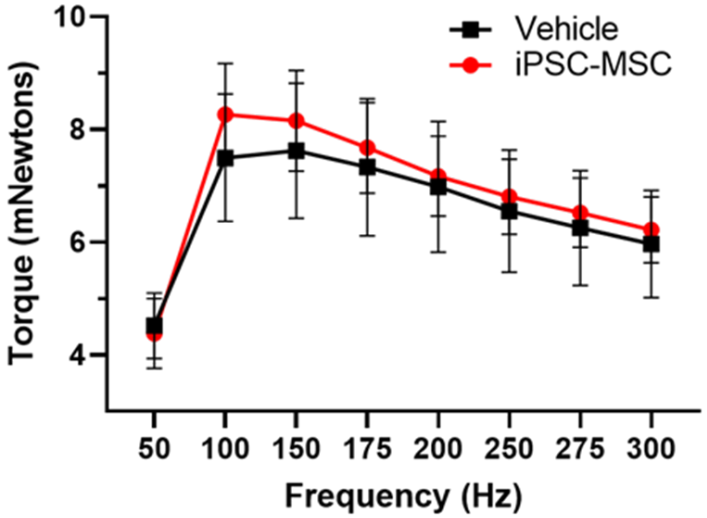
vBA-MSC



Left – control limb
Right – Ligated limb



iPSC-MSC Increase Muscle Energy

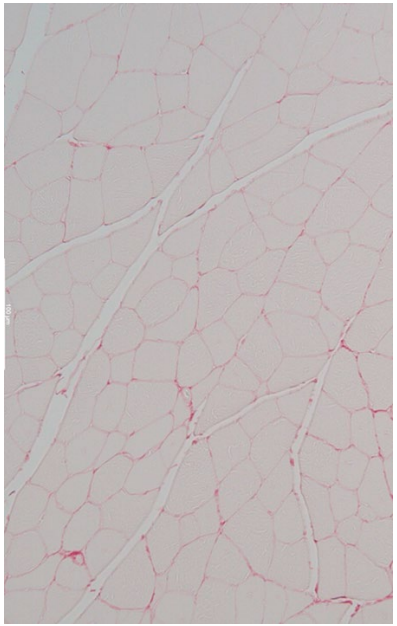


Analysis of plantar flexion muscle torque over a range of stimulation frequencies showed that iPSC-MSC treated ischemic muscle tended to have greater peak torque than control, vehicle treated ischemic muscle 28 days post-cell injection (n=5/group).

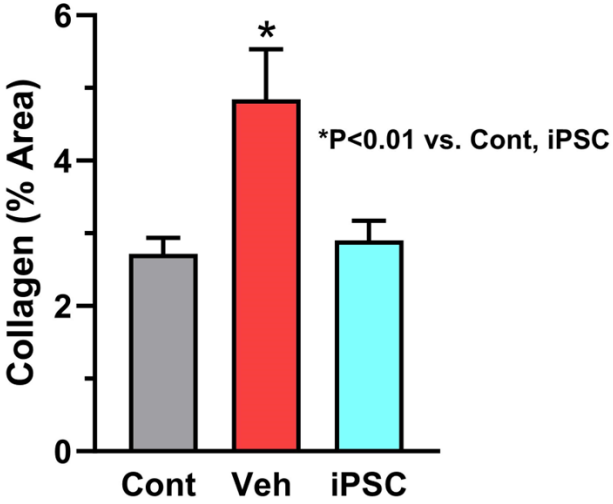
iPSC-MSC Reverse Fibrosis in Ischemic Gastrocnemius

Non-ischemic, Control

Ischemic, Vehicle



Ischemic, vBA-MSC



Mouse Hindlimb Muscle Anatomy