Department of Surgery, Division of Vascular Surgery

Vasculogenic Mesodermal Cells Derived From Induced Pluripotent Stem Cells Improve Limb Perfusion and Decrease Necrosis in Murine Models of Hind Limb Ischemia

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Cryptic Masons Research Fellow in Biomedical Engineering

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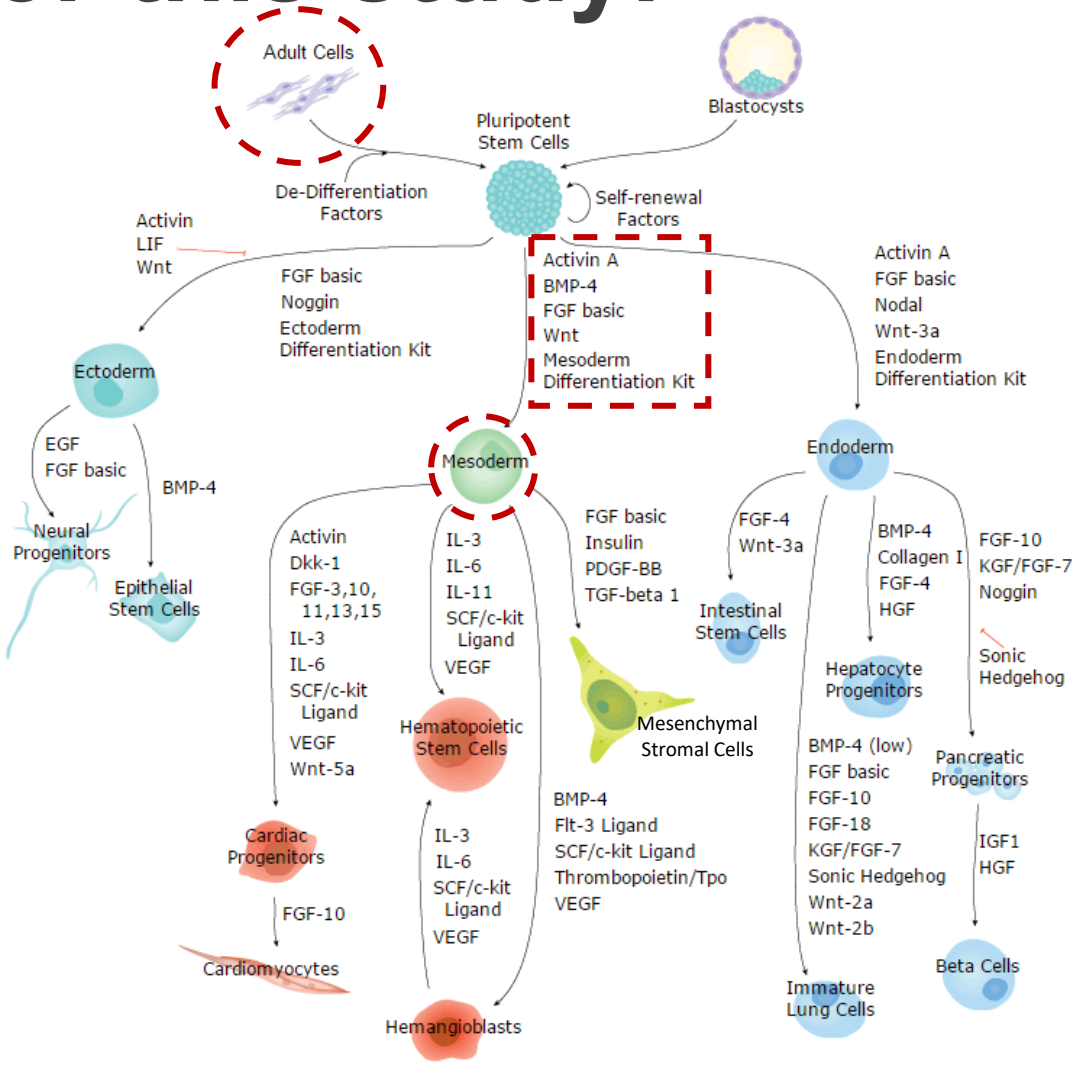
Background for this study:

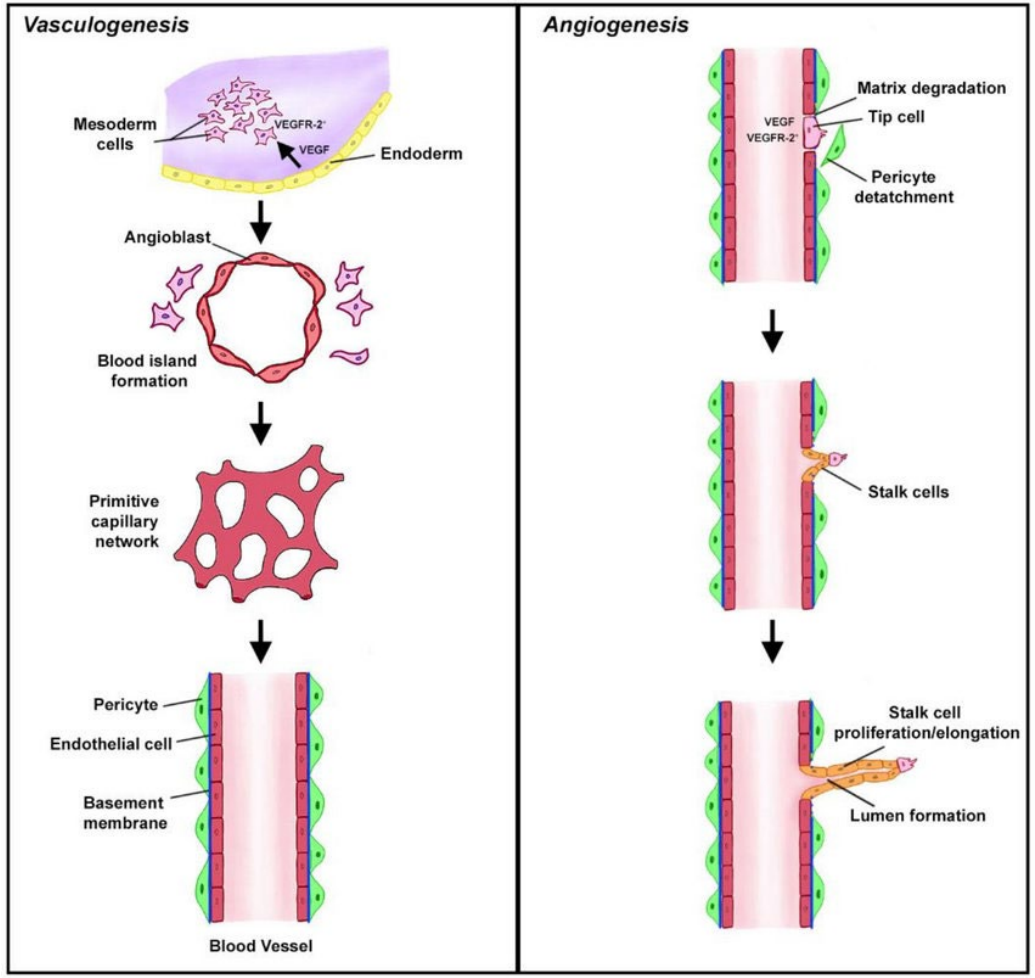
- Critical Limb Threatening Ischemia (CLTI) is a major healthcare priority as a
  - 50% increase in non-traumatic major amputations has been observed from 2010-2018[1]
- 40% of CLTI patients are not candidates for revascularization
  - Many factors play into this, namely anatomy and comorbidities
- Many cell products are limited in that they are only able to secrete *pro-angiogenic* growth factors
- We hypothesize that IM injection of human iPSC mesodermal cells (VSC100) will participate in de-novo *vasculogenesis* and improve limb perfusion and mitigate tissue necrosis in a murine model of hind limb ischemia

1. Policy Statement From the American Heart Association. *Circulation*. 2021 Apr 27;143(17)

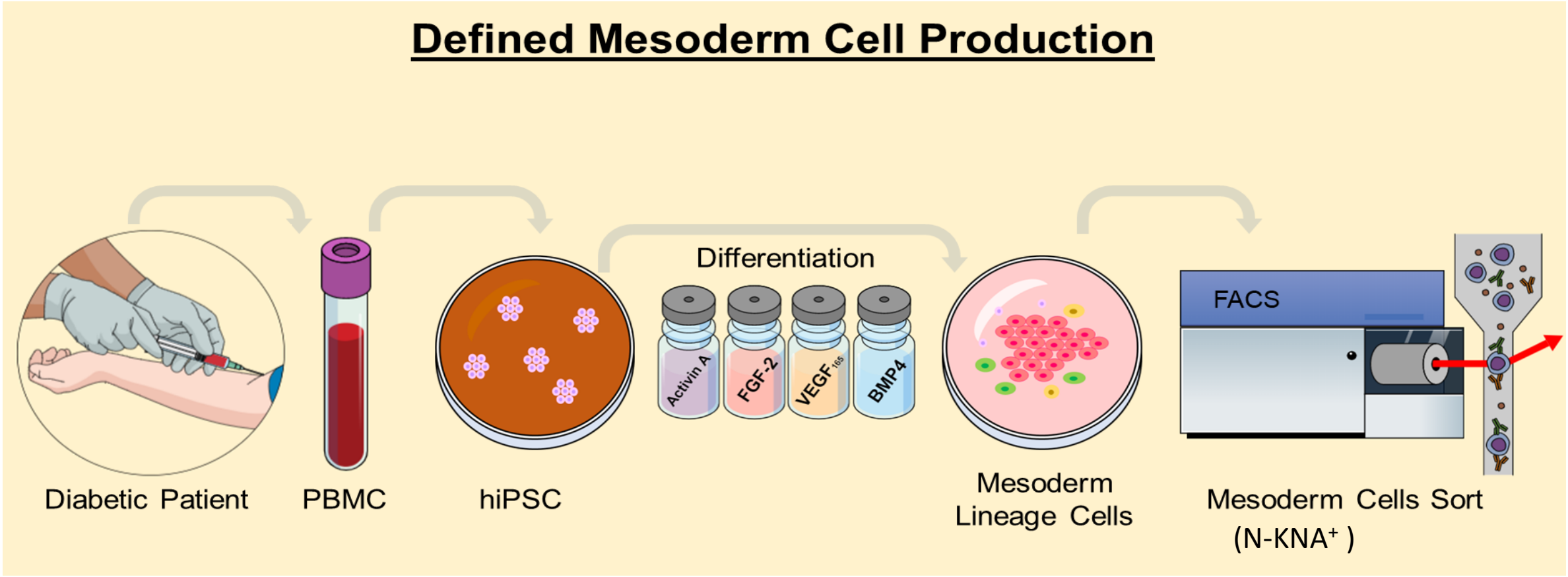
Background for this study:



Vasculogenesis (Stromal Cells) vs Angiogenesis (Non-Stromal)



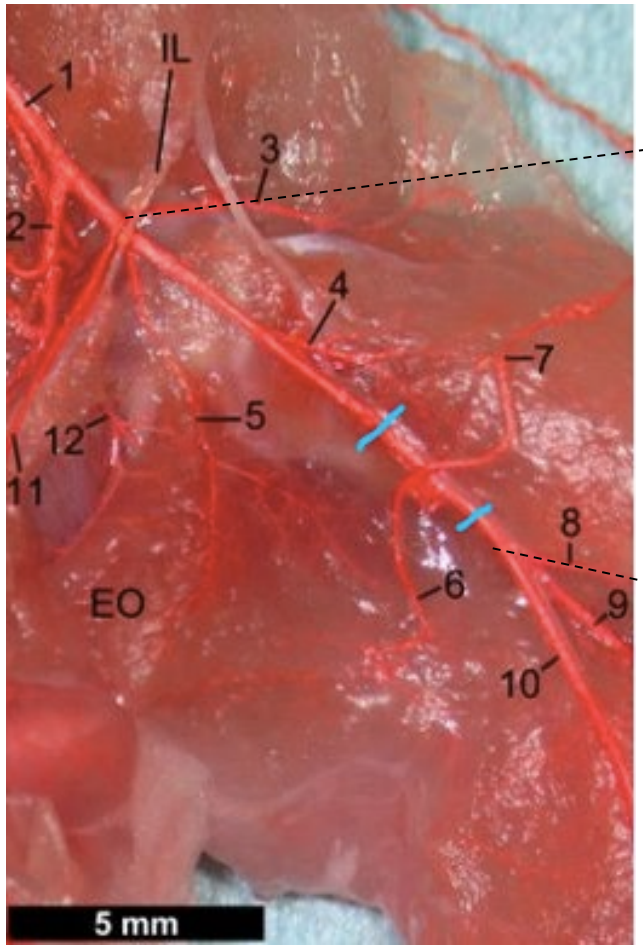
Methods – iPSC Differentiation



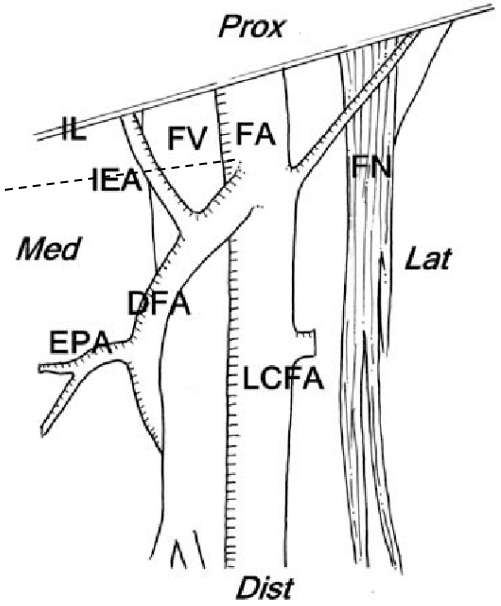
Sci Adv. 2022 Mar 4;8(9)

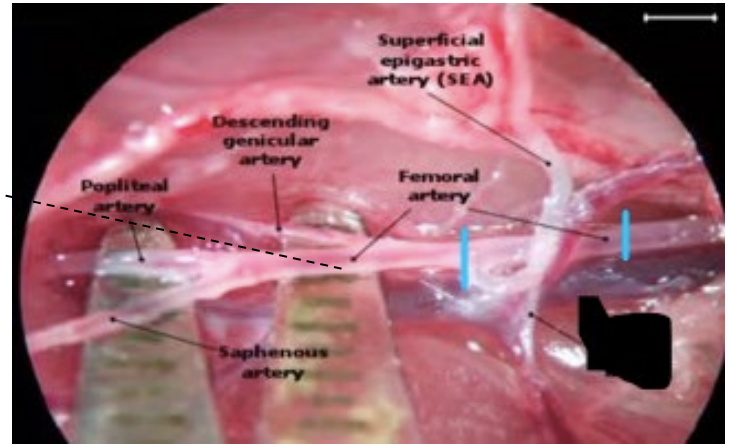
Methods – CLTI Model

- Murine CLTI created via excision of Superficial Femoral Artery.
- Male BALB-C nude mice (6-8 weeks old) randomized to $1x10^6$ VSC100 cells labeled with td-tomato (red fluorescence) or vehicle control injected into the gastrocnemius and gracilis muscles (N=10/group) at an average of 10 days postinduction of ischemia.
- Limb perfusion measured with Laser Speckled Contrast Imaging (LSCI) up to day 64.
- Tissue loss was quantified using a validated necrosis score.
- Qualitative and quantitative analysis of VSC100 differentiation into capillaries was assessed with immunohistochemical (IHC) staining for ***human*** CD31, Isolectin B4 and confocal microscopy to detect TD tomato.



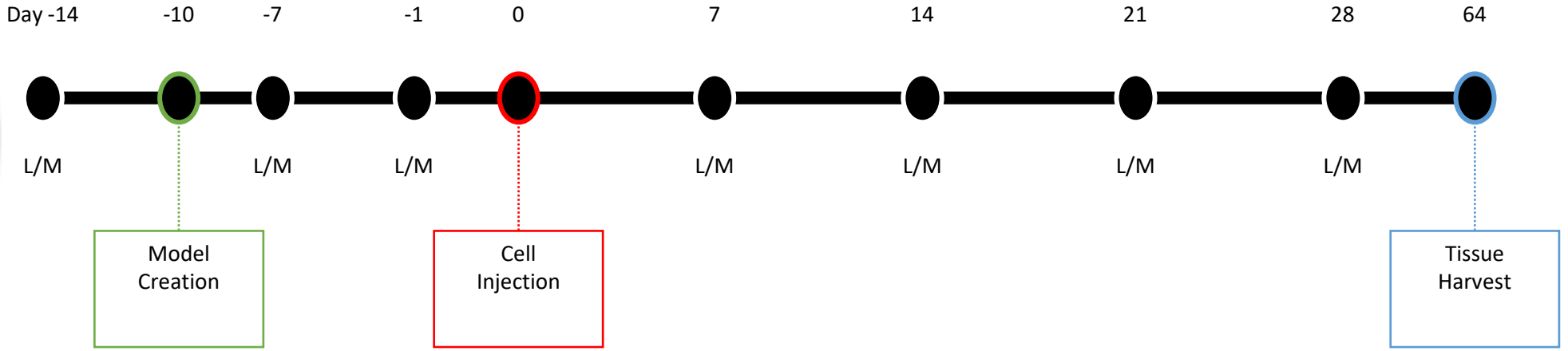
(1) common iliac artery, (2) internal iliac artery, (3) iliacofemoral artery, (4) lateral circumflex femoral artery, (5) deep femoral artery, (6) proximal caudal femoral artery, (7) superficial caudal epigastric artery, (8) medial proximal genicular artery, (9) popliteal artery, (10) saphenous artery, (11) external pudendal artery, (12) obturator artery



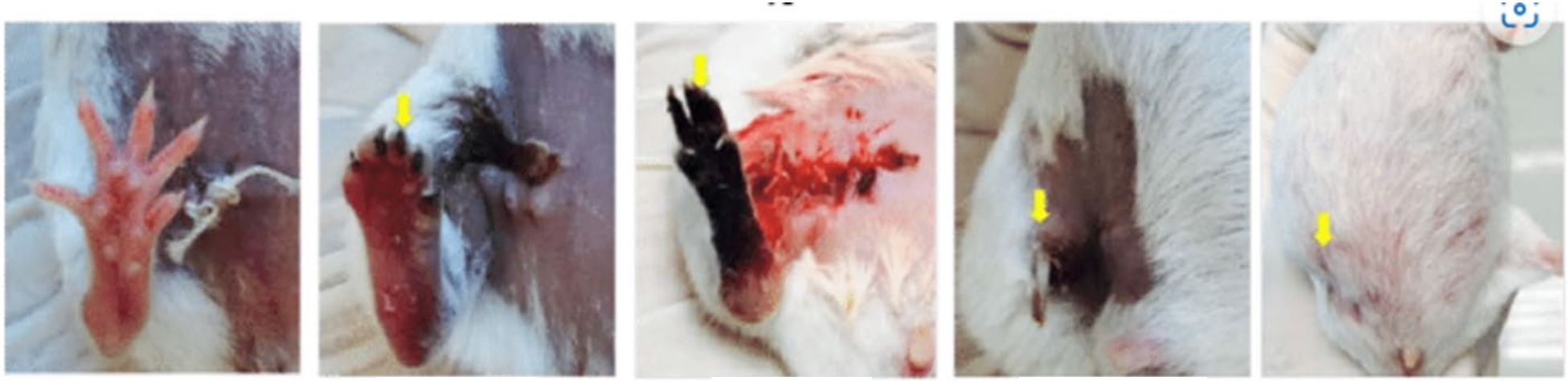


Methods – Cell Injection Scheme

BALB-C Nude
(6-8 weeks old)



Methods – Necrosis Scoring



Grade 0 Grade I Grade II Grade III Grade IV

The scoring based only on the severity of necrosis.

Scored 0 - IV, with 0 indicating no necrosis and IV limb loss

Results – Limb Perfusion

- Treated mice showed significant increase in limb perfusion compared to controls.
- Average percent blood flow relative to contralateral limb showed ratio increases of 45% compared to 25% in controls (P<0.001) at day 64.

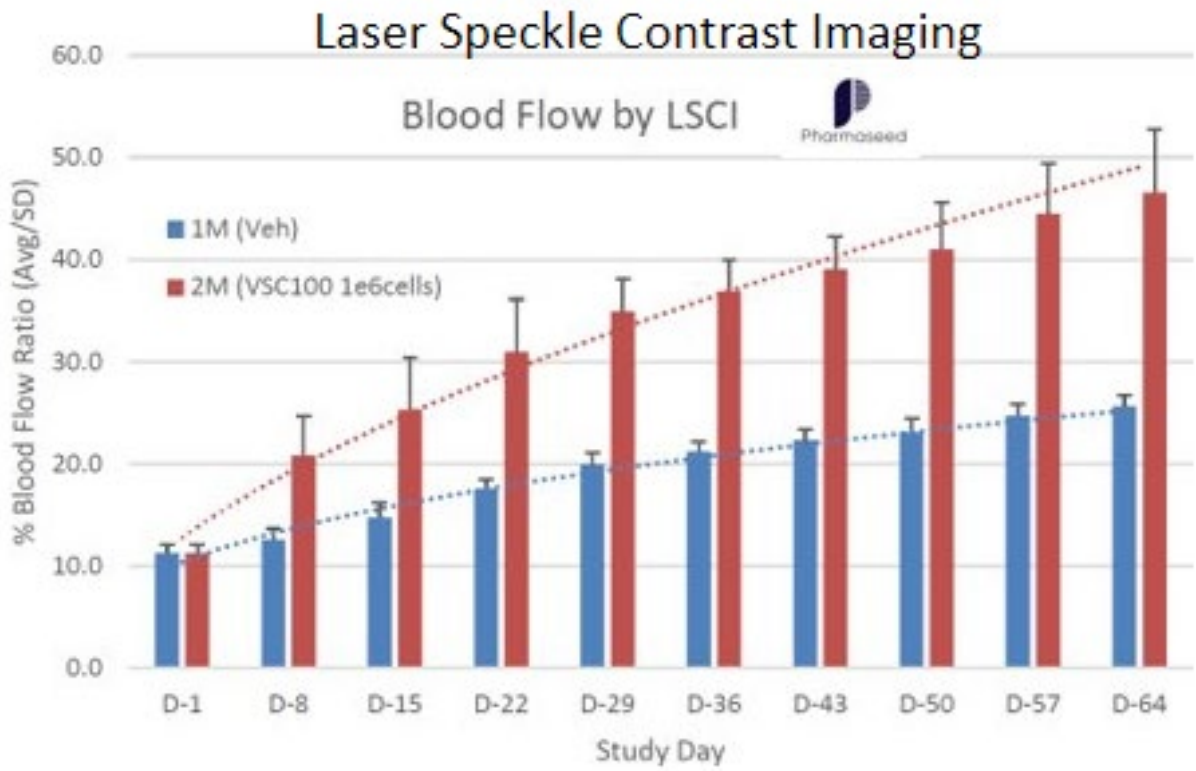
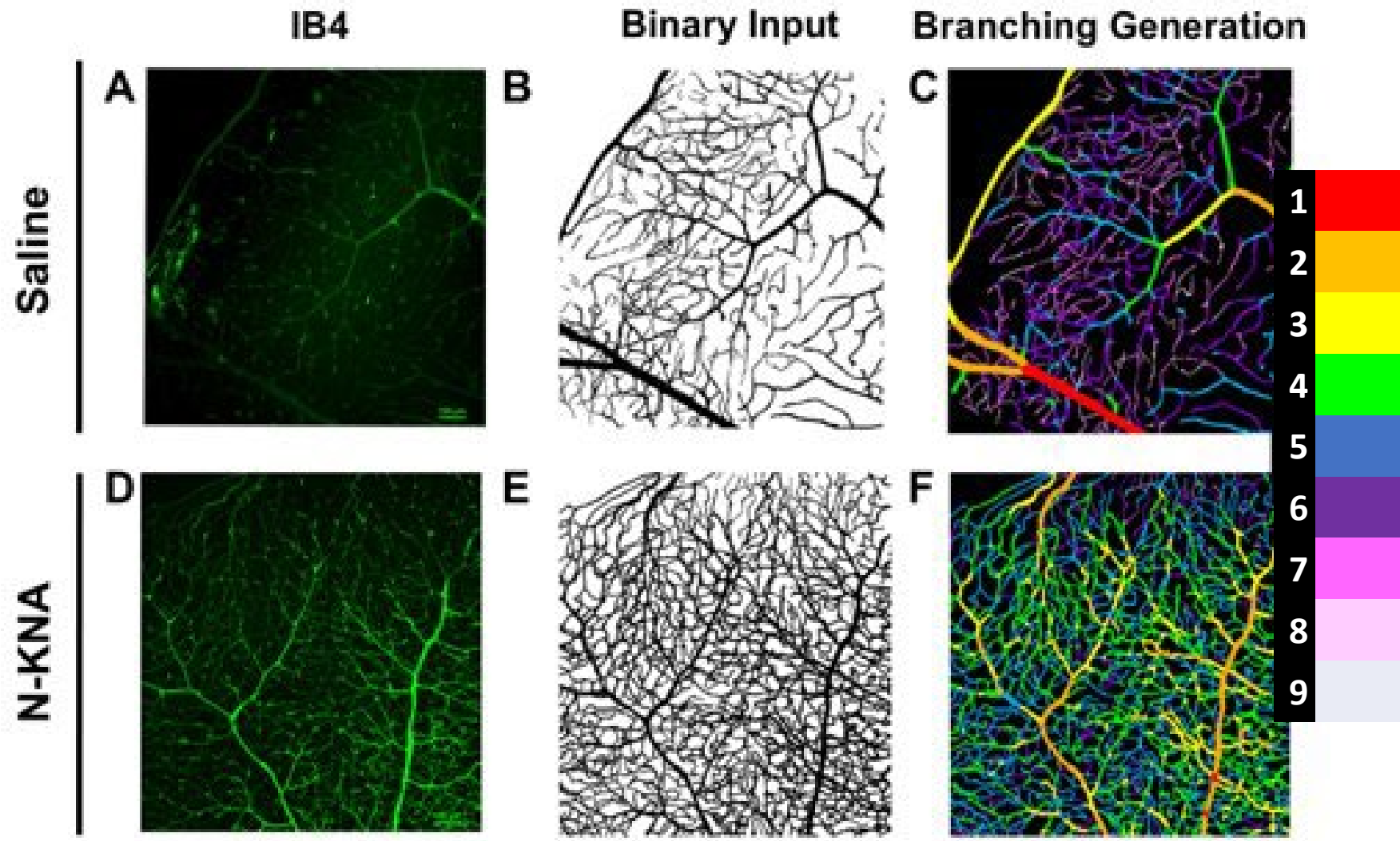


Figure 1: Laser Speckled Contrast Imaging. Date of injection was D-0 (ligation occurring prior). IPSC injected into both Gracilis and Gastrocnemius, analyzed via ANOVA (P<0.001%) (N=10/group).

Results - Vascularity

- iPSC-VPC: Enhanced Vascularity
  - Organization of capillaries into microvascular networks (D)
  - Integrate into murine blood vessels (C-F)



Results – Necrosis Scores

- VSC100 treated mice also showed positive results
- Necrosis scores were statistically reduced in treated vs control groups (P<0.005).

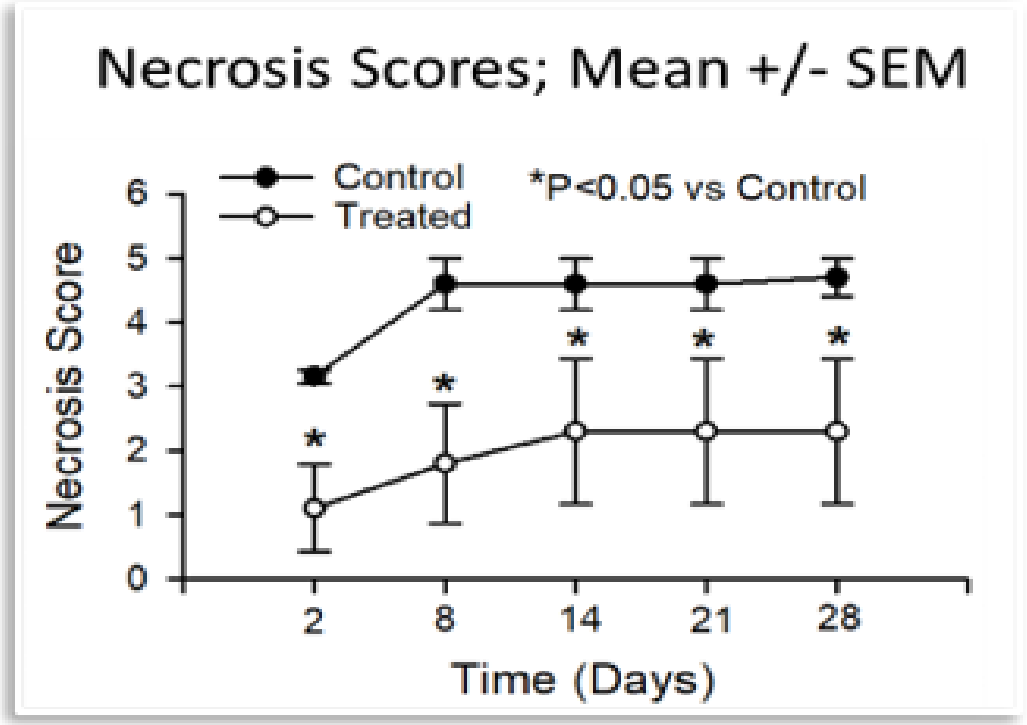


Figure 2: Clinical scoring ratio of CLTI in control vs. Treated groups, Mouse population same as in figure 1 (N=10/group).

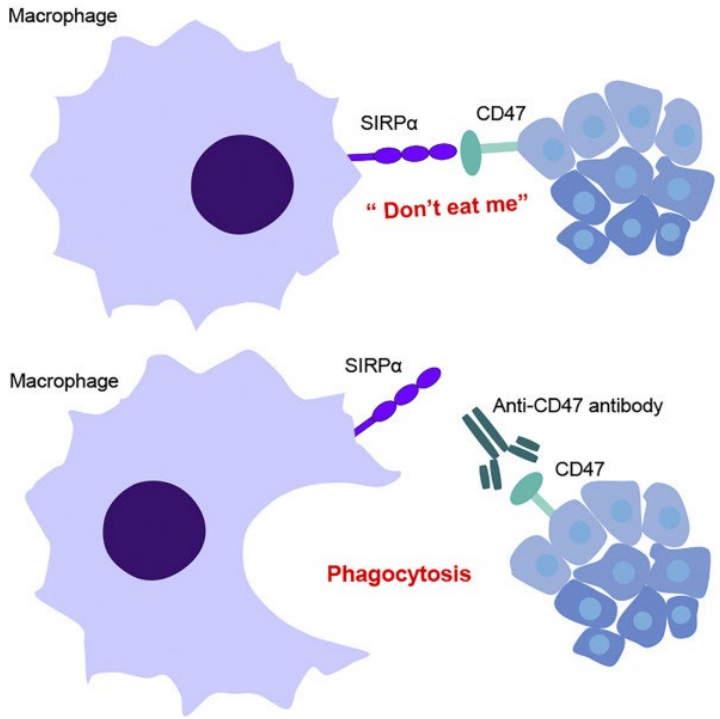
Conclusion

- iPSC derived VSC100 is a novel approach to CLTI treatment.
- Cells can form functioning blood vessels in ischemic muscle. A profound advance in the field of cell therapy.
- Can lead to improved tissue perfusion and a mitigation of tissue loss, which is the primary event leading to lower extremity amputation.

Future Directions

- Developing an iPSC cell line, genetically modified in which we've deleted MHC-I/II genes so cells won't be detected by host immune system and increase survival. Alongside upregulation of CD47 (the "Don't Eat Me" Antigen) helping cells escape phagocytosis by macrophages

- Translational work into human clinical trials



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