

The Angiogenic Effect of Micropuncture on Arterial and Venous Endothelial Cells

Mohammad Hossein Asgardoon, MD, MPH, Shailaja Agrawal, PhD, Mary Landmesser, BS , Maryam Abdelaal, BS, Jazzmyn Dawes, BS, Dino Ravnic, DO, MPH

Pennsylvania State University, College of Medicine, Hershey, PA, USA



Introduction

- Scaffolds are cornerstone to reconstructive surgery and tissue engineering.
- Their success is limited by slow vascularization following implantation.
- Micropuncture (MP) is a novel surgical approach that can be used to induce angiogenesis and expedite adjacent scaffold vascularization.
- In MP, we use a 60 μm -diameter needle (**Figure 1A**) to precisely perforate the blood vessel wall at defined intervals to stimulate microvascular growth¹.
- In vivo* testing suggests that venous MP (**Figure 1B**) expedites capillary formation compared to arterial MP (**Figure 1C**), but the reason is uncertain.
- We hypothesize that MP augments the initial release of pro-angiogenic factors from venous endothelial cells (EC).

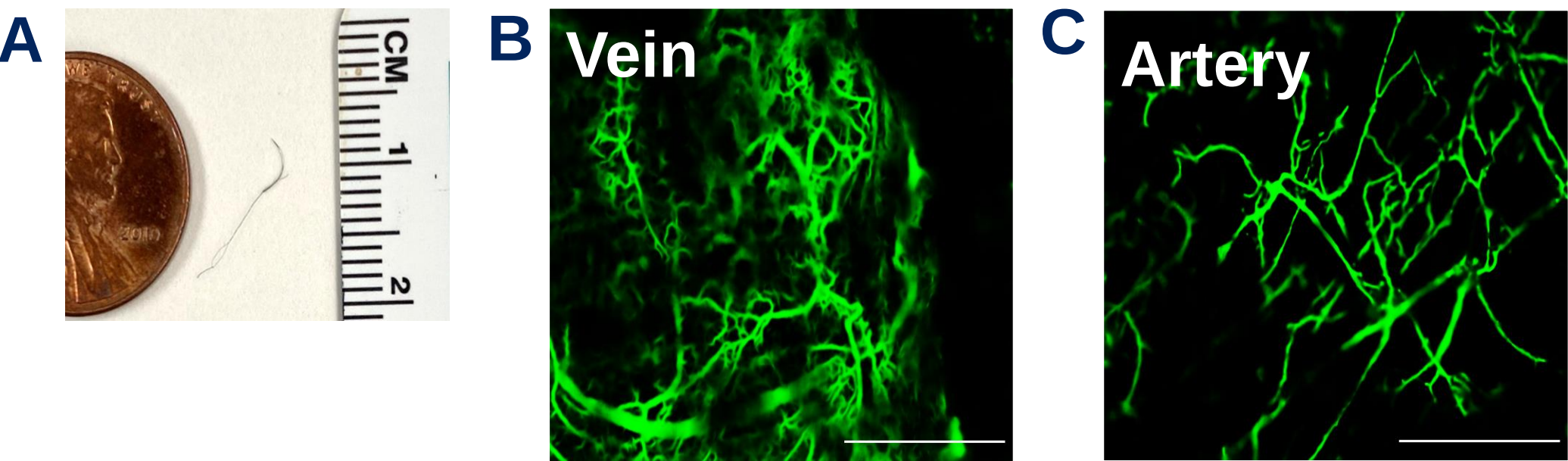


Figure 1. (A) Representative image of the needle used in MP. Angiograms from *in vivo* studies detected by artificial intelligence (AI) show enhanced microvascular growth following venous MP (B) compared to arterial MP (C).

Methods

- Experimental design is summarized in **Figure 2**.
- Arterial and venous ECs were extracted from the rat aorta and vena cava, respectively.
- Cells were seeded (1×10^5) onto a cell culture insert (0.9 cm^2 area).
- Following confluence, 20 MPs were delivered to each insert.
- Controls did not receive MPs.
- At 24 hours, tube formation was quantified using AI (MetaVi Labs, Texas), the supernatant analyzed using a 20-protein antibody array, and mRNA sequencing performed.
- The antibody array data were quantified with Image J and analyzed with GraphPad Prism.
- Gene expression analysis was performed using R programming.
- Fold change was defined as the ratio of MP to non-MP controls.
- A p-value of < 0.05 was considered statistically significant.

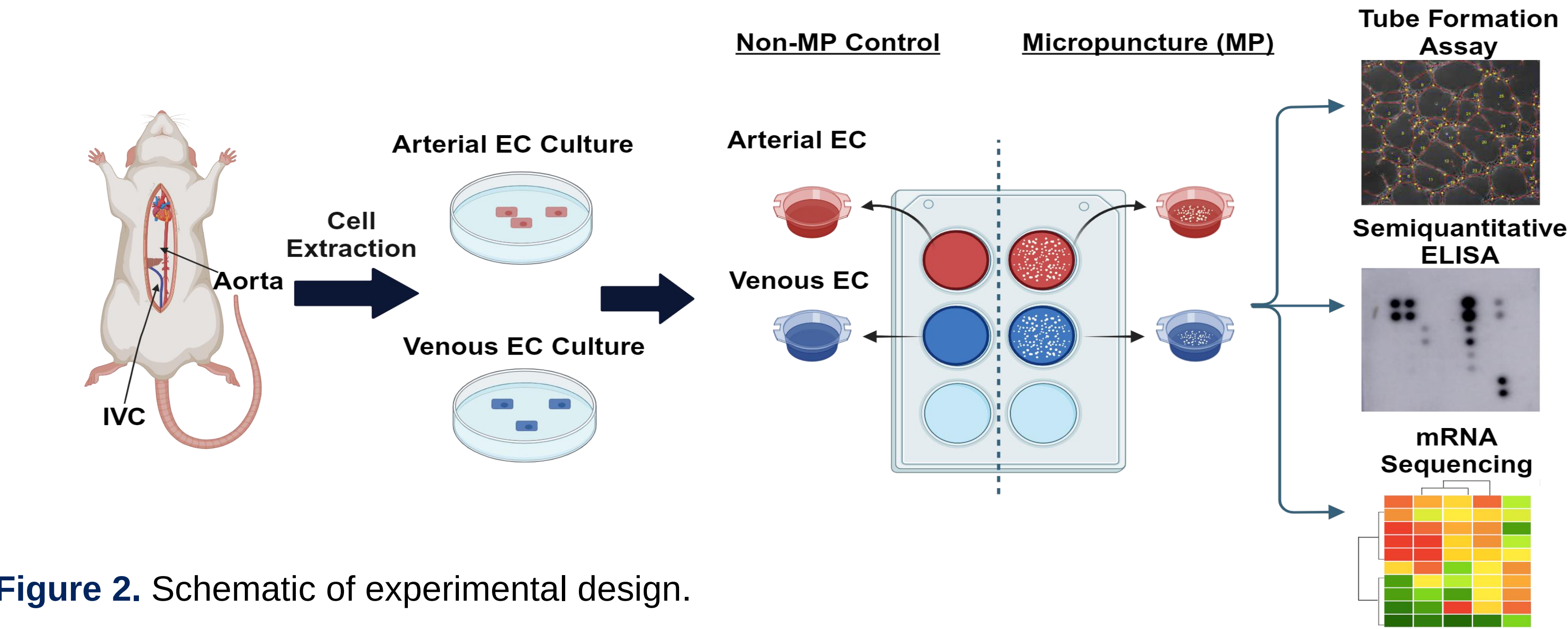


Figure 2. Schematic of experimental design.

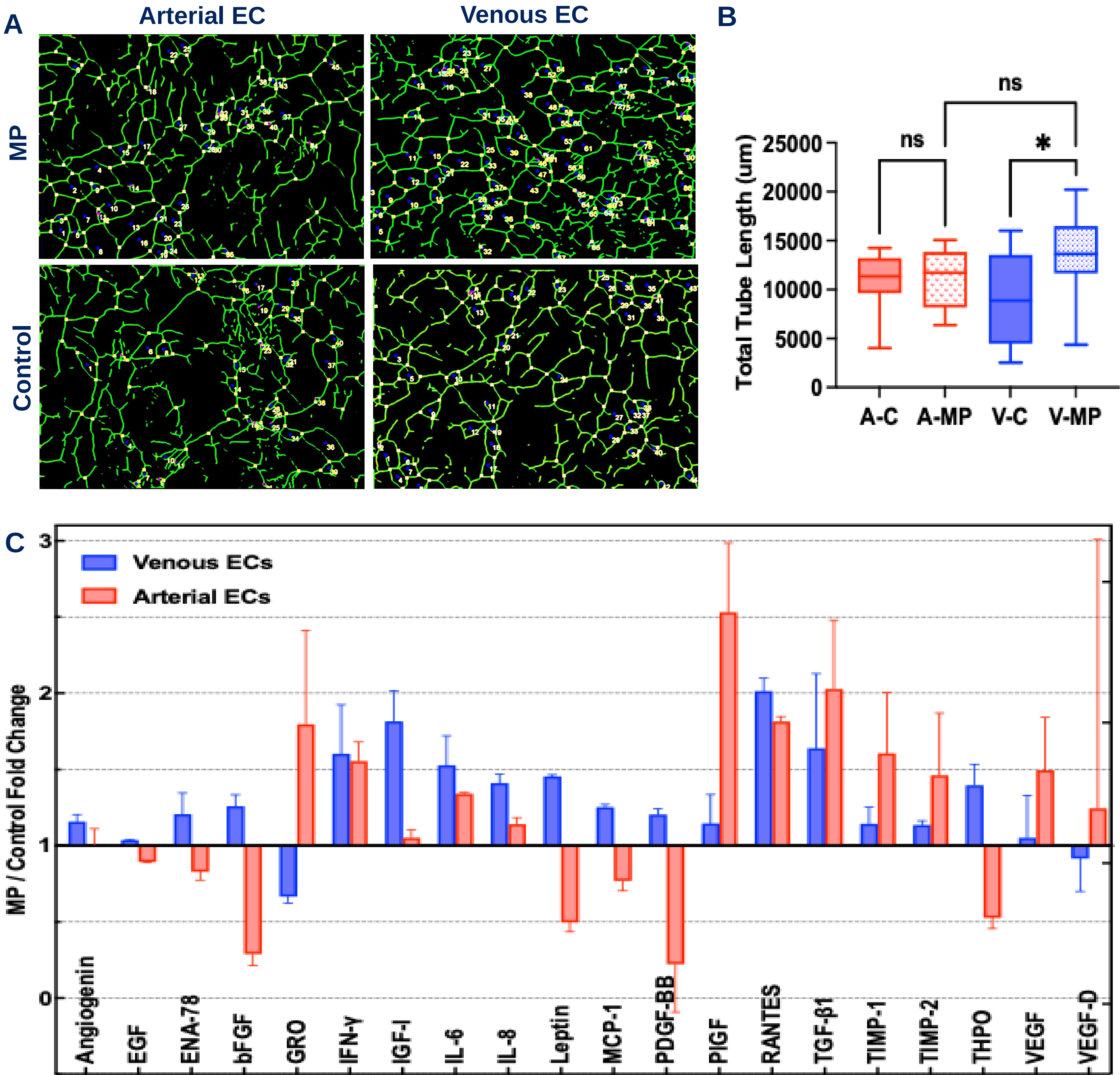


Figure 3. (A) AI detection of tube formation with (B) quantification. (C) Array of supernatant.

Results

- Venous MP appeared to elicit more tube formation compared to arterial MP (**Figure 3A**).
- Venous MP increased total tube length compared to non-MP controls but there was no difference across arterial cells (**Figure 3B**).
- Array analysis showed venous MP was associated with a more pro-angiogenic microenvironment compared to arterial MP (18/20 versus 13/20 proteins increased; **Figure 3C**).
- The most upregulated proangiogenic genes following MP in venous ECs were Ccl2, Timp1, Il6, and Lep (**Figure 4**).
- Venous MP was associated with downregulation of Cxcr2, Pgf, Egf, Cxcl1 and Ccl5 (**Figure 4**).

Heatmap of Selected Genes (Renormalized)

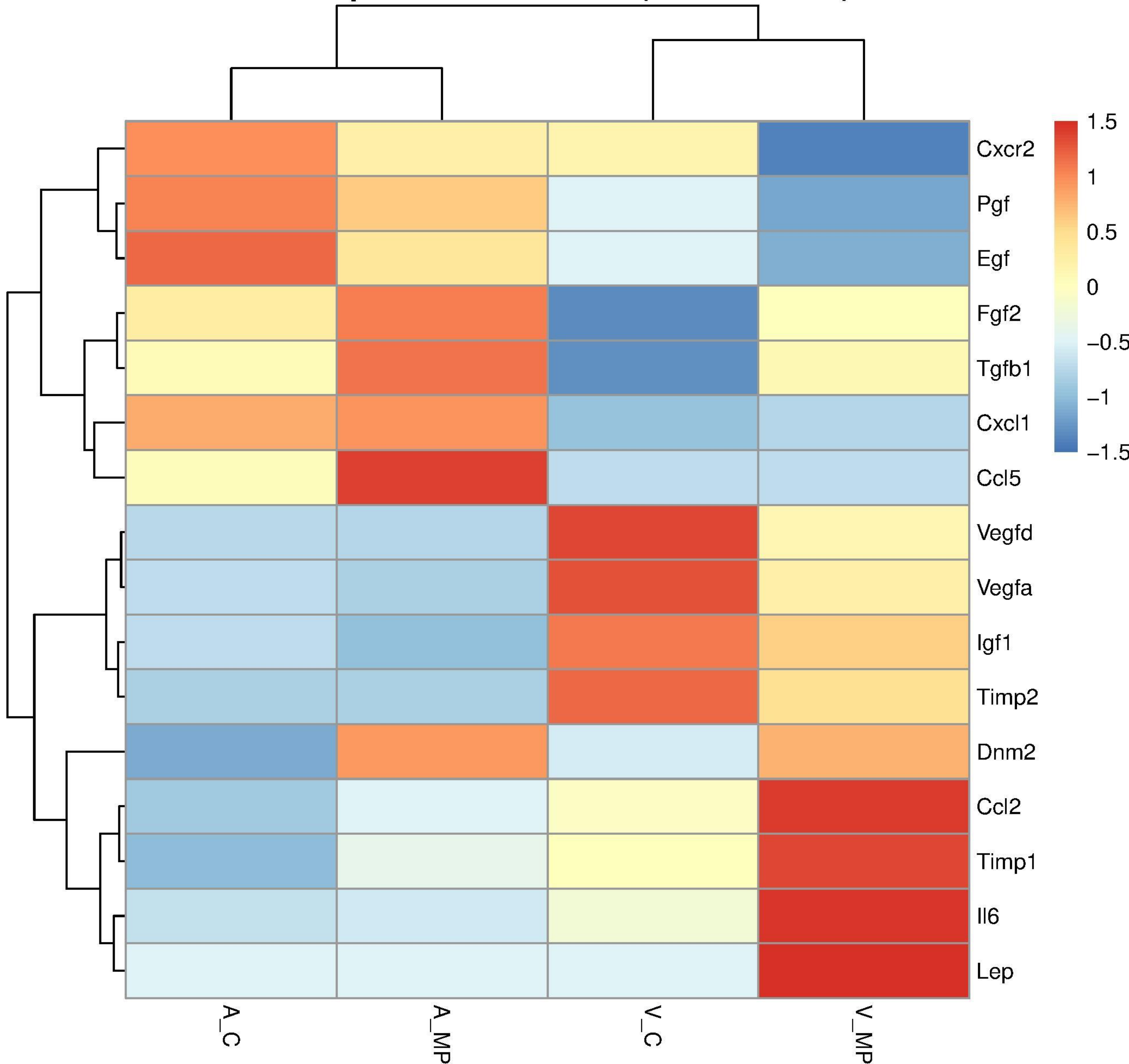


Figure 4. Heat map of selected genes. A_C: Arterial EC Control; A_MP: Arterial EC MP; V_C: Venous EC Control; V_MP: Venous EC MP ; * $p < 0.05$

Conclusions

- MP stimulates angiogenesis differently depending on if the artery or vein is targeted.
- It appears that venous ECs secrete more pro-angiogenic factors immediately after MP resulting in augmented capillary formation.
- Future work will determine which proteins are most relevant and how they can be leveraged to promote vascularization.

References

- Hancock PC, Koduru SV, Sun M, Ravnic DJ. Induction of scaffold angiogenesis by recipient vasculature precision micropuncture. Microvascular Research. 2021 Mar; 134:104121.