

The Early Angiogenic Effects of Arterial and Venous Micropuncture Using *in vivo* and *in vitro* Models

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Introduction

- Attempts at engineered tissue replacements are limited by slow vascularization upon implantation.
- Recently, we described a microsurgical approach called micropuncture (MP) to induce rapid scaffold vascularization.
- In MP, the blood vessel wall is precisely disrupted to provide an immediate route for inflammatory and endothelial cell (EC) extravasation leading to subsequent capillary formation.
- Our initial *in vivo* studies¹ demonstrated the utility of MP when applied concurrently to the femoral artery and vein (**Figure 1**). However, the specific contributions of each vessel remains unclear.
- We hypothesize that early MP-induced angiogenesis is dependent on concurrent arterial and venous targeting.

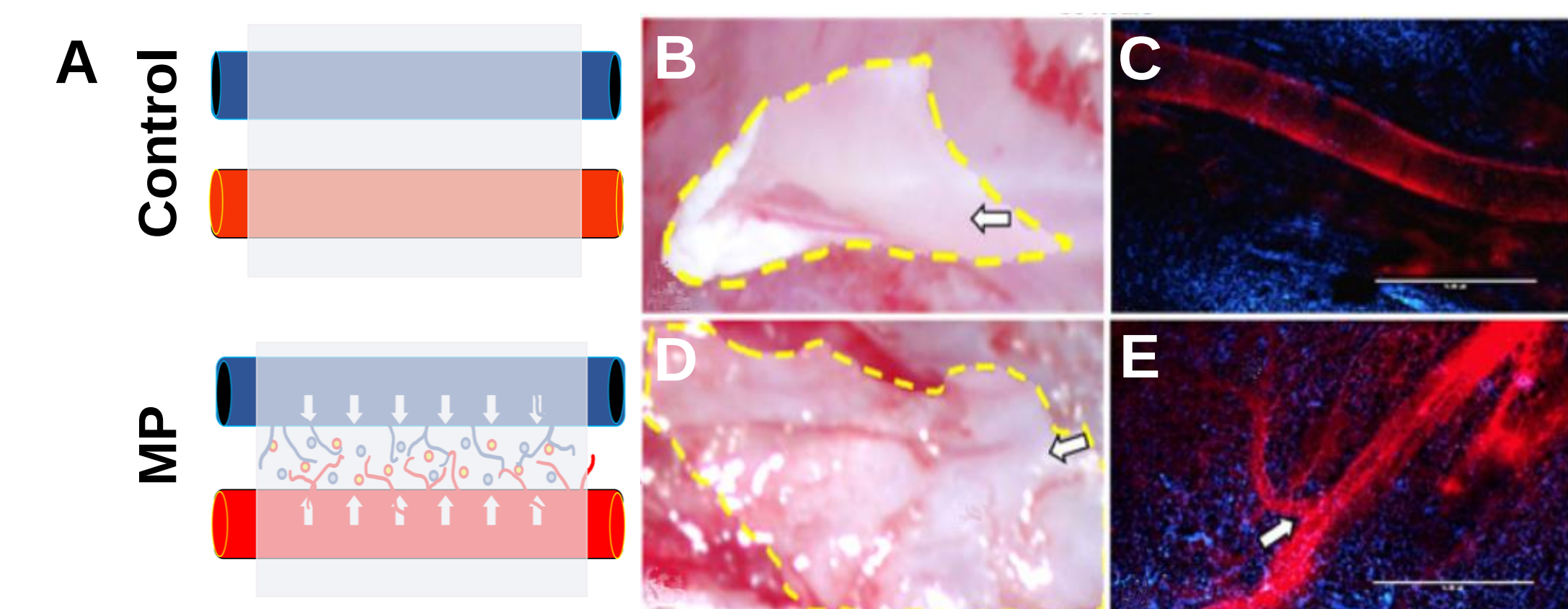


Figure 1 Schematic of prior *in vivo* studies comparing non-MP control with MP (**A**). Collagen scaffolds at post-operative day 4 showed enhanced capillary growth in MP group (**D**) compared to control (**B**). Angiograms also showed higher vascular density in MP group (**E**) compared to control group (**C**).

Methods

- In vivo*, 60 μ m MP was performed on the rat femoral artery (MPA), vein (MPV), or both (MPAV) before collagen scaffold implantation (**Figure 2**). Controls did not receive MP.
- Angiogenesis was assessed at Day 3 via *in situ* fluorescence angiography.
- EC and macrophage (M ϕ) infiltration was evaluated using CD-31, F4/80 and DAPI staining.
- CD-31 and F4/80 expression were quantified with ImageJ and analyzed with GraphPad Prism.
- In vitro*, ECs were isolated from rat femoral artery (ECA) and vein (ECV) (**Figure 3**).
- ECA, ECV, or their coculture (ECAV, 1:1) were seeded (1×10^5 cells) on collagen scaffolds and punctured (20 MP/well). Non-MP controls were included.
- Conditioned media was collected at 24 h for cytokine analysis (ECA or ECV alone vs. non-MP controls).
- Tube formation was assessed at 72 h post-MP using ≥ 10 images per well.
- mRNA sequencing at 24 h was analyzed using R.
- Angiograms and tube formation were quantified using AI (MetaVi Labs, TX).
- P < 0.05 was considered statistically significant.

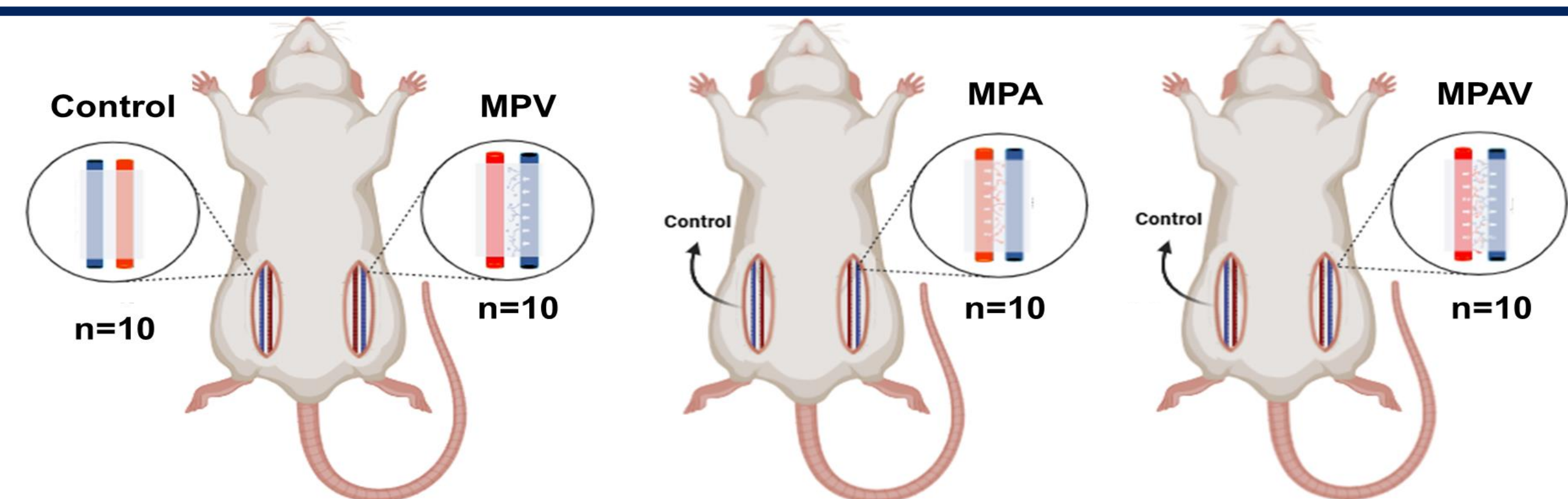


Figure 2 Schematic representation of *in vivo* experimental design

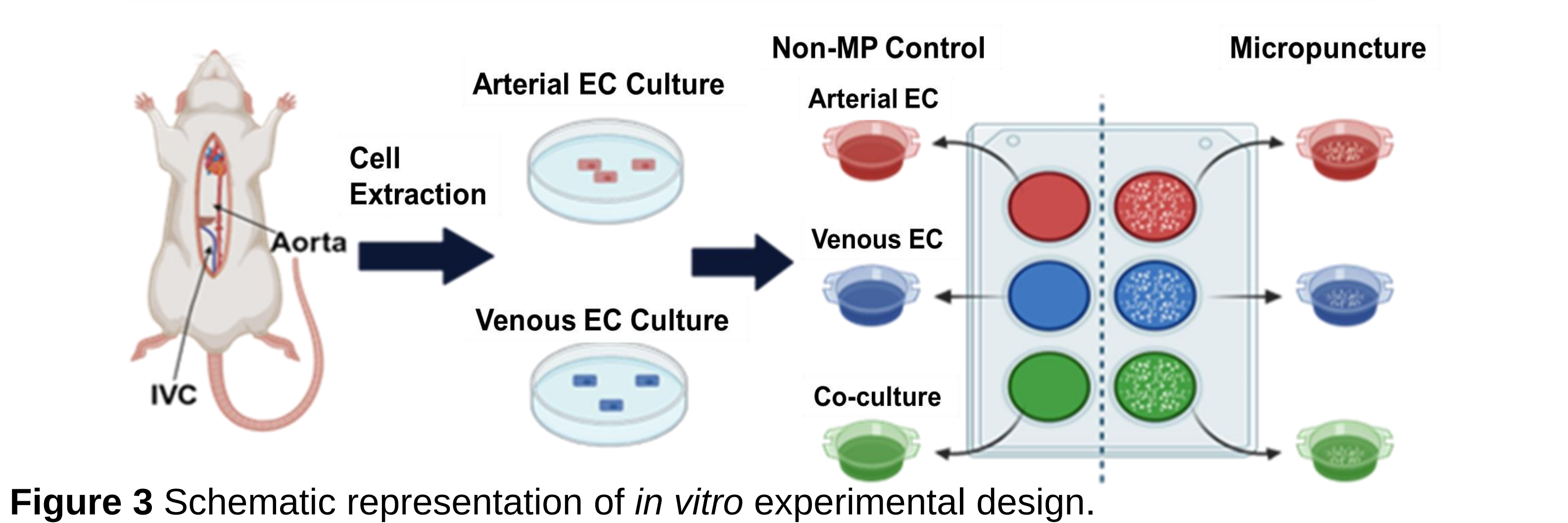


Figure 3 Schematic representation of *in vitro* experimental design.

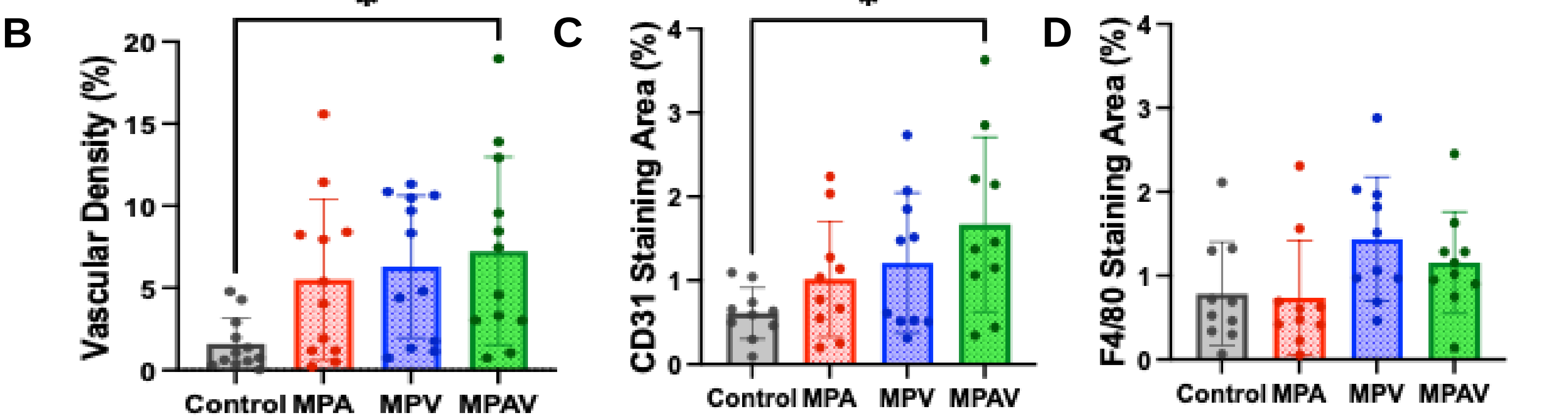
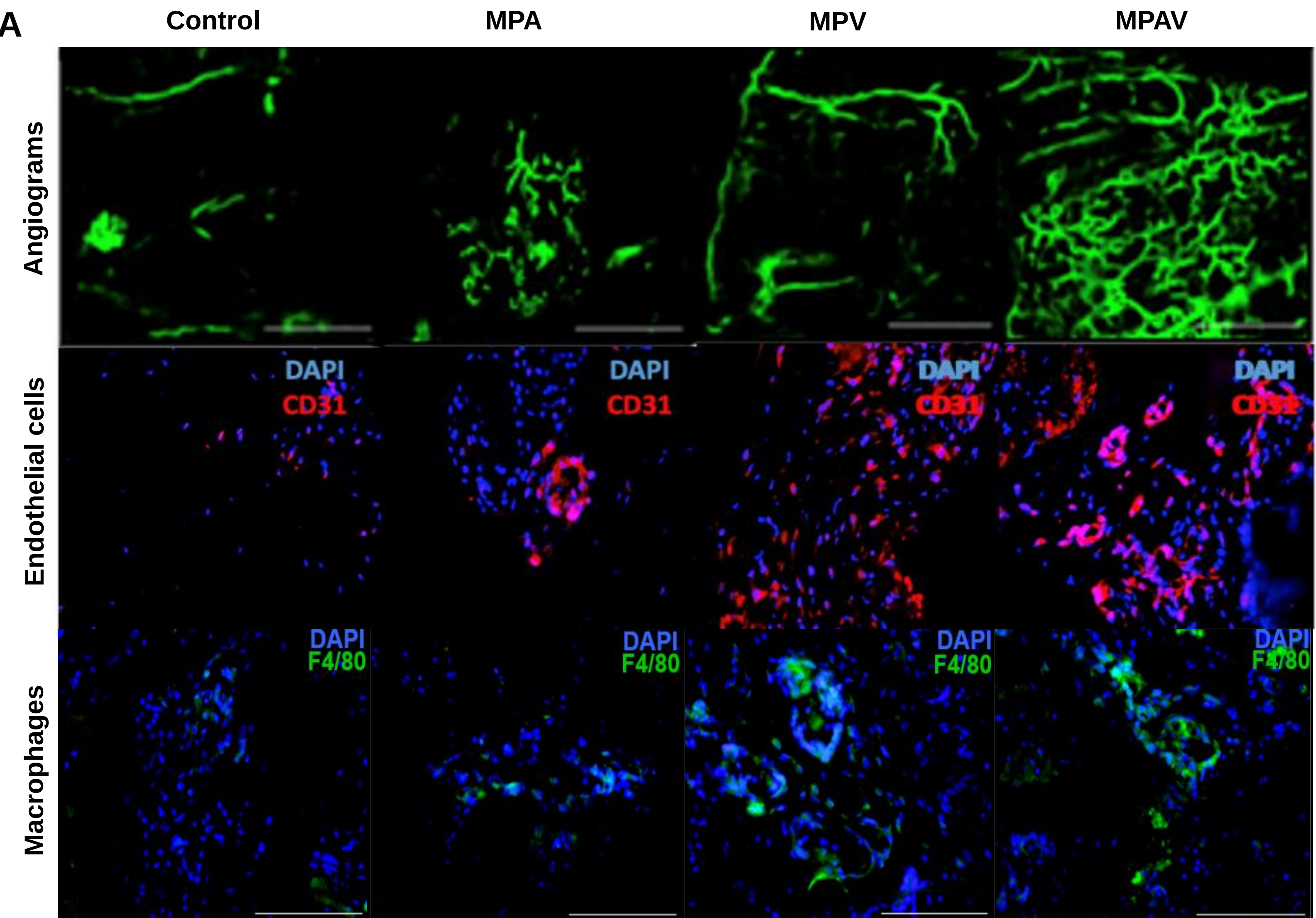


Figure 4 *in vivo* findings. **A**) Representative images of *in vivo* angiography, CD-31 and M ϕ expression. **B-D**) Quantification of vascular density, CD-31 and M ϕ expression.

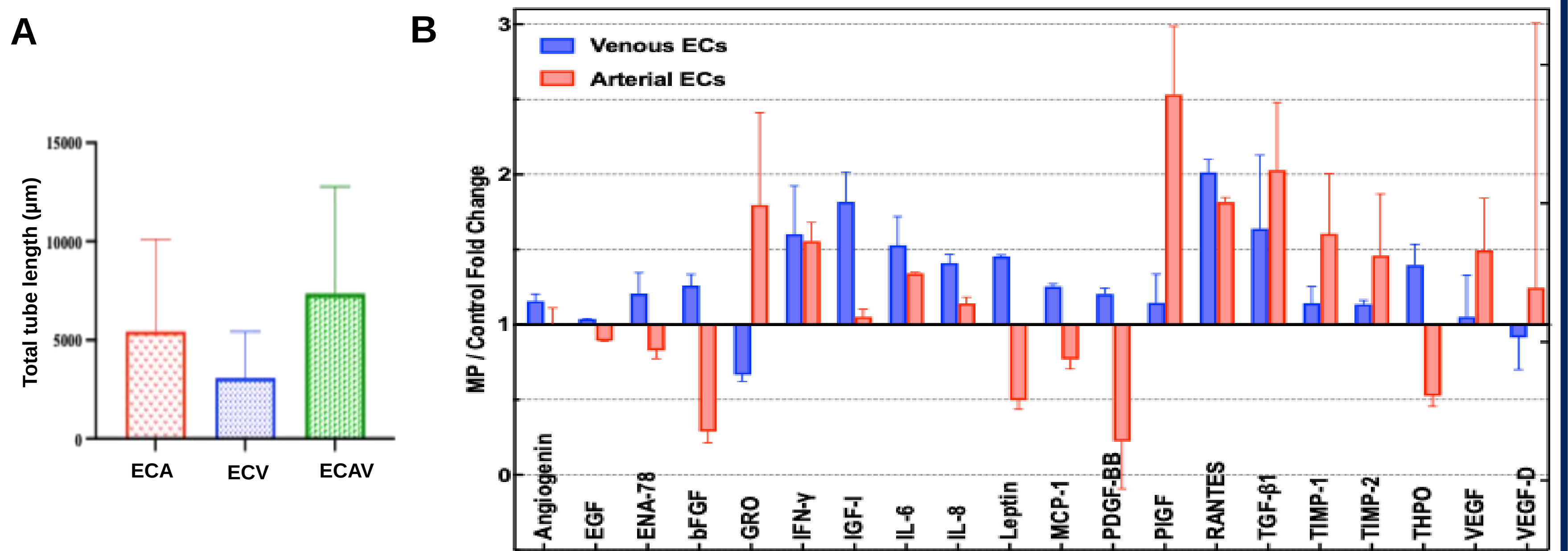


Figure 5 *in vitro* findings. **A**) Quantification of tube formation. **B**) Semi-quantitative ELISA - Fold change was defined as the ratio of MP to non-MP controls.

Results

- In vivo*, MPAV profoundly increased scaffold vascularization (**Figure 4A**).
- MPV alone showed greater vascular density compared to MPA, confirmed with CD-31 expression (**Figure 4B, C**).
- M ϕ infiltration into the scaffold was also enhanced with MP and appeared to originate primarily from the vein (**Figure 4D**).
- However, the interaction between ECs and M ϕ s during MP-induced angiogenesis remains unclear.
- In vitro*, ECAV formed more tubes compared to the non-MP combined cohort (**Figure 5A**).
- Semi-quantitative ELISA analysis revealed substantial alterations in angiogenic protein secretion following MP (**Figure 5B**).
- Specifically, inflammatory cytokines such as IL-6, MCP-1, GRO, IFN- γ , and RANTES were higher in ECV compared to ECA (**Figure 5B**).
- mRNA sequencing demonstrated that cytokine genes such as IL-6 and CCL2 were upregulated in MPV and MPAV, however the level of upregulation was greater in the MPV.

Conclusion

- MP appears to stimulate rapid capillary formation through differential protein expression in arterial and venous ECs.
- While arterial and venous MP alone can trigger microvascular formation, the angiogenic effects are most profound when they are targeted concurrently.
- Further refinement of microsurgical therapies to induce angiogenesis could play an important role in advancing tissue engineering.
- Future *in vitro* studies will aim to mechanistically determine the role of macrophages in MP-induced angiogenesis under both static and dynamic conditions.

Reference

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