

How Flow Dynamics Influence Angiogenic Factor Release in Endothelial Cells Undergoing Micropuncture

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

- Poor vascularization is a critical factor limiting graft survival in reconstructive surgery.
- Micropuncture (MP) is a novel surgical technique that can be used to enhance angiogenesis out of a targeted macrovasculature¹.
- In MP, we use a 60 μ m-diameter needle (**Figure 1A**) to precisely perforate the blood vessel wall at defined intervals to stimulate microvascular growth (**Figure 1B**).
- Static *in vitro* testing suggests that venous MP expedites microvascular formation compared to arterial MP via angiogenic factor release but the significance of flow to the process is unknown.
- We hypothesize that flow further augments angiogenic factor secretion following MP from both arterial and venous endothelial cells (ECs).

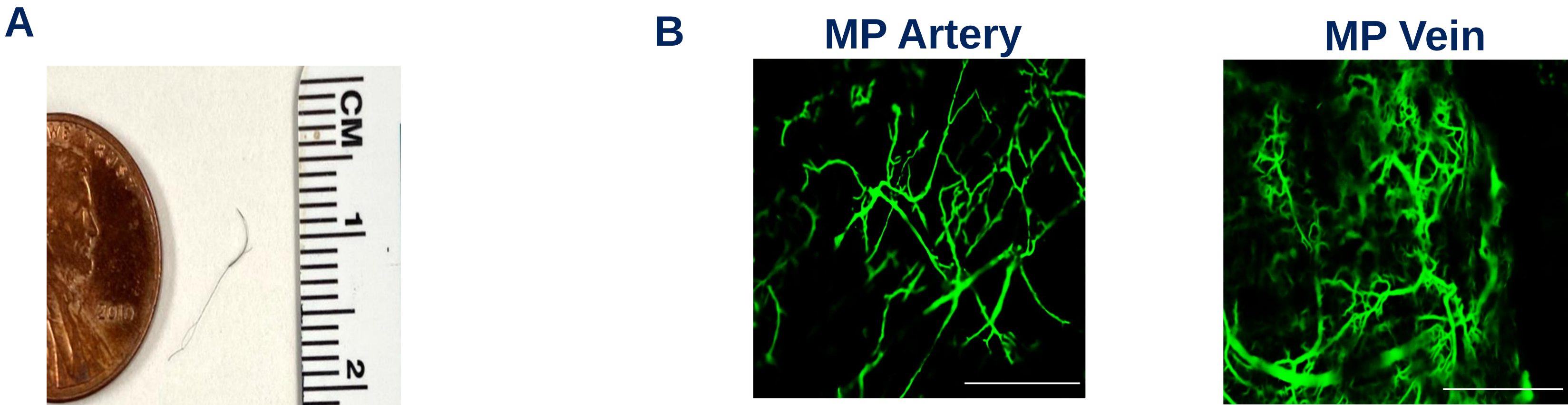


Figure 1. (A) Representative image of the needle used in MP. **(B)** Angiograms from in vivo studies, detected by artificial intelligence (MetaVi Labs, TX), show enhanced angiogenesis.

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 cell culture inserts (area = 4.2 cm²).
- Following confluence, 40 MPs were delivered to each insert.
- Non-MP arterial and venous ECs served as baseline controls.
- ECs were then transferred to a microslide channel and connected to a continuous infusion system (flow rate = 1.5 ml/min).
- At 24 hours, supernatant was analyzed using a semi-quantitative antibody array, with results compared to static culture conditions.
- All tests were run in duplicate. ImageJ and GraphPad Prism were used for analysis.
- Fold change was defined as the ratio of secreted factors in the MP group to the non-MP control.

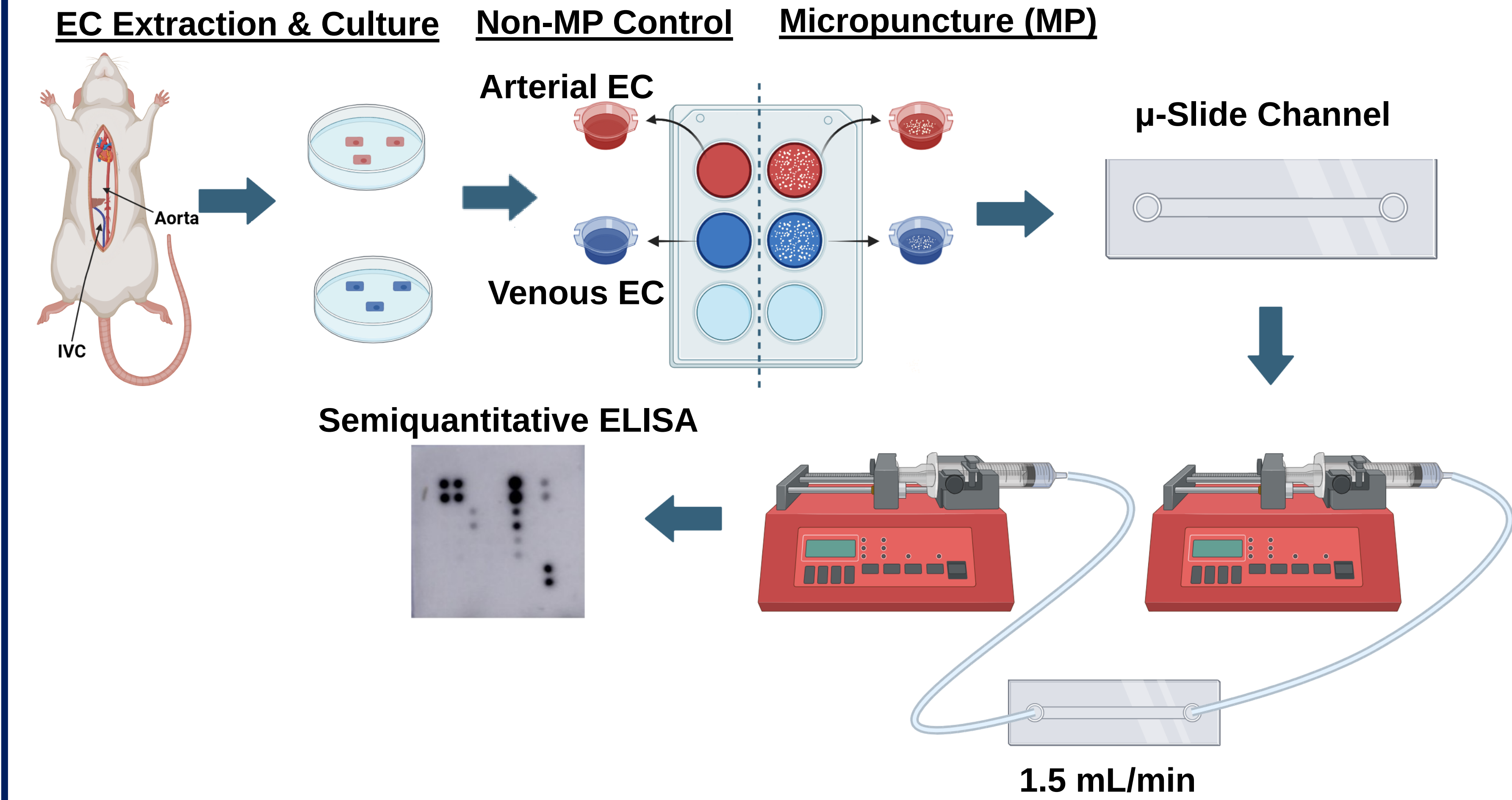


Figure 2. Schematic of experimental design.

Results

- Under static conditions, MP was associated with >1-fold change of 17/20 (85%) angiogenic factors when venous ECs were tested but only 11/20 (55%) factors when arterial ECs were tested (**Table 1**).
- The same trend was observed under dynamic culture.
- Furthermore, flow amplified the rise of venous angiogenic factors following MP with 8/20 (40%) demonstrating >1.5-fold change when compared to static conditions (5/20, 25%, denoted by ★ ; **Table 1**)
- Most notable was the effect that flow had on the release of PDGF from micropunctured venous ECs.

Conclusions

- MP leads to pro-angiogenic factor release from ECs.
- It appears venous ECs respond differently to MP compared to arterial ECs.
- These changes are further amplified under flow conditions with venous ECs having a heightened factor release profile.
- Subsequent investigations will determine how these findings can be translated clinically.

Table 1. Fold change in angiogenic factor secretion in arterial and venous ECs at 24 hours following MP.

Angiogenic Factors	Static Culture		Dynamic Culture	
	Arterial ECs	Venous ECs	Arterial ECs	Venous ECs
Angiogenin	1.0	1.1	1.8 ★	1.9 ★
EGF	0.8	1.1	0.9	1.2
ENA-78	0.8	1.2	1.5 ★	1.5 ★
bFGF	0.2	1.2	1.0	1.4
GRO	1.6	0.6	1.5 ★	1.3
IFN-gamma	1.5	1.5	2.2 ★	1.2
IGF-I	1.0	1.8	1.4	1.3
IL-6	1.3	1.5	2.2 ★	0.2
IL-8	1.1	1.4	1.2	1.3
Leptin	0.5	1.4	1.1	1.3
MCP-1	0.7	1.2	0.8	1.5 ★
PDGF-BB	0.4	1.2	0.8	6.2 ★
PIGF	2.5	1.1	0.9	1.1
RANTES	1.8	2.0	1.5	1.5 ★
TGF-beta 1	1.9	1.6	0.9	0.9
TIMP-1	1.5	1.1	1.2	0.8
TIMP-2	1.4	1.1	0.7	2.4 ★
Thrombopoietin	0.5	1.4	0.8	0.8
VEGF	1.5	1.0	0.6	1.7 ★
VEGF-D	3.0	0.8	0.1	1.9 ★

Reference

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