



Intraoperative Bioprinting Meets Microsurgery: Engineering Functional Vasculature in 3D-Printed Tissue Scaffolds

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Introduction

- Intraoperative bioprinting (IOB) of scaffolds offers a new frontier in reconstructive surgery.
- Scaffold success relies on rapid vascularization, which can be enhanced through endothelial cell (EC) seeding.
- We have previously described micropuncture (MP)¹, a surgical technique in which the recipient macrovasculature is precisely disrupted to facilitate microvascular outgrowth and expedite adjacent scaffold vascularization.
- In this study we examine the potential synergistic effects of MP and EC-seeding in a collagen-based bioink at short- and long-term timepoints.
- We hypothesize that the combination of MP and EC-seeding will promote scaffold vascularization that persists with time.

Methods

- Rat femoral vessels underwent MP on one hindlimb while the other served as a non-MP control (**Figure 1 A**).
- Bioink scaffolds composed of collagen and chitosan with or without rat aortic ECs (cell) were printed over the exposed vessels using an extrusion bioprinter (**Figure 1 B**).
- On POD 10 or 40, fluorescence angiography was performed, and vascular metrics were evaluated with artificial intelligence.

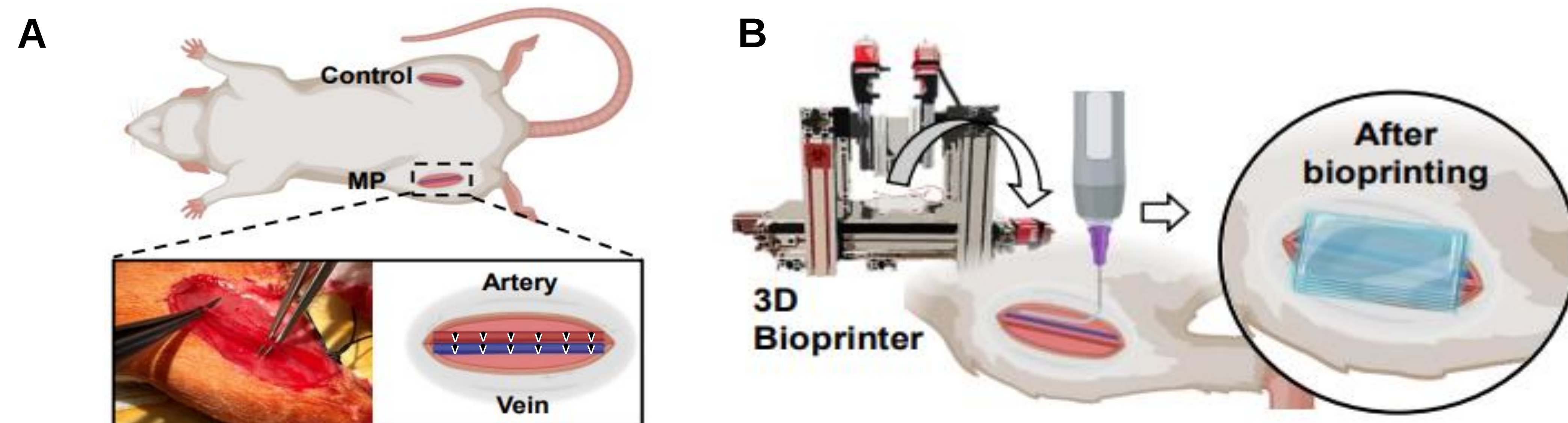


Figure 1. Micropuncture + Bioprinting. **A)** Rat femoral vessels underwent MP on one hindlimb while the contralateral side served as a non-MP control. **B)** Immediately after an extrusion bioprinter was brought into operative field to deposit collagen/chitosan scaffolds with or without ECs directly over the exposed vessels.

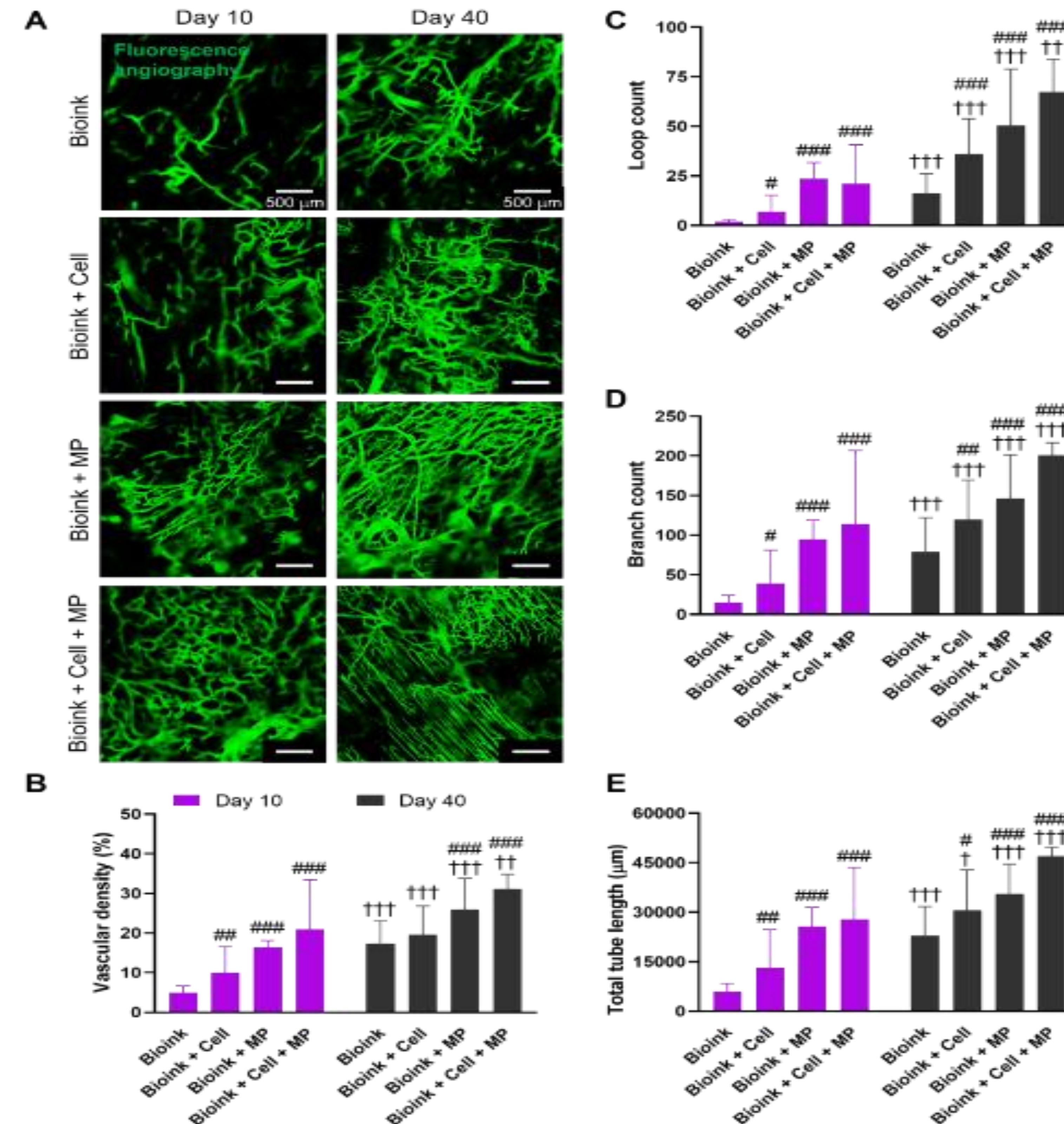


Figure 2. In-vivo evaluation of vascularization via fluorescence angiography. **A)** Angiogram images taken of whole mount scaffolds (n = 3). **B)** Percentage of vascular density. Quantification of **C)** loop counts, **D)** branch counts, and **E)** total tube length. One-on-one comparison between the Bioink (control) and experimental groups was conducted via independent-samples T test ($p^{\#} < 0.05$, $p^{\#\#} < 0.01$, $p^{####}$). One-on-one comparison between two timepoints within the same group was conducted via paired-samples T test ($p^{\dagger} < 0.05$, $p^{\dagger\dagger} < 0.01$, $p^{\dagger\dagger\dagger} < 0.001$).

Results

- All experimental groups demonstrated significantly increased vascular density compared to the POD10 bioink only control, and these results were augmented with time (**Figure 2 A, B**).
- The microvascular architecture evolved over time as illustrated by loop counts, branch counts, and total tube length (**Figure 2 C-E**).
- The greatest vascularization was observed in the Bioink+Cell+MP group at POD40.
- The addition of MP alone also significantly augmented microvascular development.

Conclusions

- This study introduces a new approach that combines MP and IOB to synergistically create vascularized scaffolds.
- The engineered microvasculature persists with time.
- Independently, both MP and EC-seeding also show potential as effective pro-angiogenic strategies.
- Future research will focus on utilizing these techniques to develop in-situ flaps for vascularized tissue reconstruction.

Reference

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

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