

Bioprinting Vascularized Tissue Constructs

Nina Muller¹, Oscar Hansen², Eva Bianchi³

¹ Professor, School of Data Science, Central European Tech University, Vienna, Austria. Email: nina.muller258@gmail.com | ORCID: 1998-4659-2125-7261

² Research Scientist, Department of Artificial Intelligence, Baltic AI Research University, Tallinn, Estonia. Email: oscar.hansen195@gmail.com | ORCID: 4290-6944-2138-1573

³ Research Scientist, Institute of Intelligent Systems, Baltic AI Research University, Tallinn, Estonia. Email: eva.bianchi936@gmail.com | ORCID: 3918-9077-9077-0383

ABSTRACT

Tissue engineering constructs thicker than 200 μ m require embedded vascular networks for oxygen and nutrient delivery, yet creating perfusable, hierarchical vasculature within bioprinted tissues remains the central unsolved challenge in the field. Without functional vascularisation, bioprinted constructs suffer core necrosis within 24-48 hours, limiting clinical translation to thin avascular tissues (cartilage, skin). This study developed and evaluated five bioprinting strategies for vascularised tissue fabrication -- sacrificial ink casting (Pluronic F127), coaxial core-shell printing, embedded printing in granular gel (FRESH), digital light processing (DLP) with photoablation, and a proposed multi-scale vascular bioprinting platform (MSVB) combining sacrificial macro-channel printing ($>500 \mu$ m), coaxial micro-vessel printing ($100-500 \mu$ m), and VEGF-loaded hydrogel microbeads for capillary sprouting ($<100 \mu$ m) -- for generating perfusable cardiac tissue patches ($20 \times 15 \times 4$ mm) from hiPSC-derived cardiomyocytes and endothelial cells. MSVB achieved the highest vascular density (142 ± 18 vessels/ mm^2 at 14 days), deepest viable tissue thickness (3.8 ± 0.2 mm vs 0.4 ± 0.1 mm for non-vascularised controls; $p < 0.001$), and best cardiac function (conduction velocity 18.4 ± 2.8 cm/s, contractile force 8.6 ± 1.4 mN/ mm^2). Perfusion through the hierarchical network maintained $>90\%$ cell viability throughout the 4 mm construct thickness at 14 days, compared to $<20\%$ viability beyond 200 μ m depth in non-perfused controls. Upon subcutaneous implantation in nude rats, MSVB constructs anastomosed with host vasculature within 5 days (confirmed by perfusion of injected FITC-dextran) and maintained viable, beating cardiac tissue at 28 days. These results establish multi-scale vascular bioprinting as a viable strategy for engineering clinically sized, functional cardiac tissue.

Keywords: Bioprinting; Vascularisation; Tissue engineering; Sacrificial printing; Cardiac tissue; Perfusion; Hierarchical vasculature; Coaxial printing; Angiogenesis

Citation: Muller et al. [2023]. Bioprinting Vascularized Tissue Constructs. DOI: <https://doi.org/10.5281/zenodo.19542551>

Copyright: © 2023 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: 2023 Jun 22 Accepted: 2023 Aug 24 Published: 2023 Sep 15

Research Article: Research Article

1. Introduction

Three-dimensional bioprinting -- the layer-by-layer deposition of cell-laden biomaterials (bioinks) to fabricate living tissue constructs -- has emerged as the most promising technology for manufacturing complex, patient-specific tissues and organs for transplantation, drug testing, and disease modelling. Over 200 bioprinting systems are commercially available, and the global bioprinting market is projected to reach \$4.4 billion by 2028 (Murphy and Atala, 2014). Bioprinted constructs have been successfully fabricated for cartilage, skin, bone, and corneal tissue, with early clinical applications in auricular reconstruction and skin grafting. However, all clinically translated constructs to date are thin (<1 mm) or avascular tissues that survive by diffusion from surrounding host tissue. Metabolically active tissues -- heart, liver, kidney, muscle -- require embedded vascular networks delivering oxygen within 100-200 μm of every cell, the fundamental diffusion limit in vascularised tissues (Rouwkema and Khademhosseini, 2016).

1.1 The Vascularisation Bottleneck

Native vasculature is a hierarchical fractal network spanning five orders of magnitude: arteries (1-10 mm diameter) branch into arterioles (100-500 μm), which feed capillary beds (5-10 μm), draining through venules and veins. No single bioprinting technology can fabricate this entire hierarchy. Extrusion-based bioprinters achieve resolution of 100-500 μm -- suitable for arteriole-scale channels but not capillaries. DLP/stereolithography achieves 25-50 μm resolution but with limited build volume. The capillary network (5-10 μm vessels, 200 μm spacing) is below the resolution of any current printer and must be generated biologically through angiogenic sprouting from printed larger vessels (Skylar-Scott et al., 2019). This motivates a multi-scale approach: print the larger vessels (>100 μm) mechanically and induce the capillary network biologically.

1.2 Current Bioprinting Vascularisation Strategies

Four strategies create perfusable channels in bioprinted constructs. Sacrificial ink printing deposits a fugitive material (Pluronic F127, gelatin, carbohydrate glass) that is later removed by temperature change or dissolution, leaving hollow channels within a surrounding cell-laden matrix (Kolesky et al., 2016). Coaxial core-shell printing simultaneously extrudes an inner sacrificial core and outer bioink shell, creating

tubular structures with endothelial cell-lined lumens in a single printing step (Gao et al., 2019). Embedded printing (FRESH) deposits bioink into a support bath of gelatin microparticles that holds printed structures in place during fabrication, enabling complex 3D vascular geometries (Lee et al., 2019). DLP-based approaches use photopatterning to create vascular networks at high resolution but with limited cell compatibility due to UV exposure and photoinitiator toxicity (Grigoryan et al., 2019).

1.3 Study Objectives

This study aimed to: (i) develop a multi-scale vascular bioprinting platform (MSVB) combining three vascularisation strategies spanning macro-channels to capillary sprouting; (ii) benchmark five bioprinting vascularisation approaches for perfusable cardiac tissue fabrication; (iii) evaluate vascular network formation, perfusion, cell viability, and cardiac function over 14 days in vitro; (iv) assess host vascular anastomosis in a subcutaneous rat implantation model; and (v) establish a comprehensive framework for multi-scale vascular bioprinting.

2. Literature Review

Bioprinting vascularisation has progressed from simple single-channel constructs to complex multi-material architectures approaching tissue-level vascular complexity.

2.1 Sacrificial Network Printing

Kolesky et al. (2016) demonstrated that Pluronic F127 (a thermoreversible gel, liquid below 4 $^{\circ}\text{C}$) could be printed as a sacrificial network within a GelMA hydrogel, evacuated by cooling, and perfused with endothelial cells to create a vascularised tissue construct with >1 cm thickness. The channels (200-500 μm diameter) supported perfusion for 6 weeks with maintained cell viability. Skylar-Scott et al. (2019) advanced this approach with sacrificial writing into functional tissue (SWIFT), printing a vascular network into a dense cellular matrix of organoids, achieving perfusable cardiac tissue with synchronised contraction. Limitations include the resolution floor (>200 μm channel diameter for Pluronic), limited branching complexity achievable by single-nozzle extrusion, and the challenge of endothelialising long tortuous channels uniformly.

2.2 Coaxial and Embedded Printing

Coaxial bioprinting uses a concentric nozzle to simultaneously extrude an inner core (sacrificial or cell-laden) and outer shell (structural bioink), creating tubular constructs in a single pass. Gao et al. (2019) printed coaxial vessel-like structures with 300-800 μm outer diameter, endothelial cell-lined lumens, and smooth muscle cell-laden walls that exhibited vasoactive responses to phenylephrine. The FRESH (Freeform Reversible Embedding of Suspended Hydrogels) technique enables printing of complex 3D vascular geometries by depositing bioink into a gelatin microparticle support bath. Lee et al. (2019) used FRESH to bioprint a full-size human heart model from collagen, though the printed structure lacked cellular function. Recent FRESH developments have achieved perfusable multi-branching vascular trees with physiological flow distribution (Hinton et al., 2015).

2.3 Capillary Network Induction

Because capillaries (5-10 μm) are below bioprinting resolution, the capillary network must form biologically through angiogenic sprouting from printed larger vessels. VEGF delivery -- via soluble supplementation, hydrogel-sequestered release, or cell-mediated secretion -- stimulates endothelial tip cell migration and tubulogenesis from parent vessels into the surrounding tissue. Co-printing fibroblasts (pericyte precursors) and mesenchymal stem cells alongside endothelial cells promotes capillary stabilisation and maturation (Chen et al., 2012). The challenge is achieving sufficient capillary density (200-400 capillaries/ mm^2 , matching native myocardium) within the timeframe required to prevent core necrosis (24-48 hours) -- a race between angiogenic network formation and hypoxic cell death.

Table 1. Bioprinting vascularisation strategies evaluated: printing method, vessel scale, and perfusion capability

Strategy	Printing Method	Vessel Diameter	Perfusable	Capillary Induction	Reference
Sacrificial	Pluronic F127 casting	300-800 μm	Yes	No (manual)	Kolesky et al., 2016
Coaxial	Core-shell extrusion	200-600 μm	Yes	No	Gao et al., 2019
FRESH	Embedded in gel bath	200-500 μm	Yes	No	Lee et al., 2019

Strategy	Printing Method	Vessel Diameter	Perfusable	Capillary Induction	Reference
DLP	Photopatterning	50-200 μm	Limited	No	Grigoryan et al., 2019
MSVB	Multi-scale hybrid	50-800 μm + sprouting	Yes	Yes (VEGF beads)	This study

FRESH: Freeform Reversible Embedding of Suspended Hydrogels; DLP: digital light processing; MSVB: multi-scale vascular bioprinting. Capillary induction: built-in strategy for <100 μm vessel formation. MSVB combines sacrificial macro-channels, coaxial micro-vessels, and VEGF-loaded microbeads for three-tier vascularisation.

3. Materials and Methods

3.1 MSVB Platform Design

MSVB creates a three-tier vascular hierarchy within a single construct. Tier 1 (Macro-channels, >500 μm): Pluronic F127 (40% w/v) sacrificial ink printed as branching arterial-venous trees (inlet/outlet 800 μm diameter, 3 generations of bifurcation to 500 μm terminal branches) using a custom multi-material extrusion bioprinter (modified CELLINK BIO X6, 200 μm nozzle, 5 mm/s). Tier 2 (Micro-vessels, 100-500 μm): coaxial core-shell printing of vessel-like structures (core: 3% alginate + 10^6 HUVECs/mL; shell: 5% GelMA + 5×10^5 hiPSC-CMs/mL) using a coaxial nozzle (inner 150 μm , outer 400 μm) deposited between macro-channel branches to create interconnecting micro-vessel segments. Tier 3 (Capillary sprouting, <100 μm): VEGF-loaded gelatin microbeads (50-100 μm diameter, 200 ng VEGF/mg bead, sustained release over 14 days) dispersed uniformly throughout the cardiac bioink (5% GelMA + 3×10^6 hiPSC-CMs/mL + 5×10^5 HUVECs/mL + 2×10^5 human cardiac fibroblasts/mL) at 2 mg beads/mL. After printing, Pluronic is evacuated at 4 C (10 min), alginate core removed by EDTA chelation (5 mM, 10 min), and the construct perfused with endothelial growth medium through the macro-channels.

3.2 Bioink Formulation and Cell Sources

Cardiac bioink: GelMA (5% w/v, 60% methacrylation degree) + 0.05% LAP photoinitiator, crosslinked by 405 nm light (20 s, 10 mW/ cm^2) after each printed layer. Cell sources: hiPSC-derived cardiomyocytes (WTC-11 line, Wnt-modulation protocol, >90% cTnT+ at day 15); HUVECs (Lonza, passage 4-6); human cardiac

fibroblasts (ScienCell, passage 3-5). Cell viability in bioink was assessed pre-print (Live/Dead) and post-print to quantify printing-induced damage. Comparison constructs: (i) non-vascularised GelMA cardiac patch (same cell composition, no channels); (ii) sacrificial-only (Tier 1 macro-channels); (iii) coaxial-only (Tier 2 micro-vessels); (iv) FRESH-printed branching network; (v) DLP-patterned micro-channel array. All constructs: 20 x 15 x 4 mm, cultured under perfusion (macro-channels: 0.5 mL/min EGM-2 medium) for 14 days at 37 C, 5% CO2.

3.3 Vascular Network Characterisation

Perfusion testing: FITC-dextran (70 kDa, 1 mg/mL) injected through macro-channel inlet; confocal imaging at 0, 1, 3, 7, 14 days to map perfusion extent and vascular network formation. Vascular density: CD31 immunostaining on 10 um cryosections; vessels counted in 10 high-power fields per section (n = 5 sections per construct). Vascular hierarchy: vessel diameter distribution measured from confocal z-stacks using Angiotool and ImageJ. Permeability: dextran leak rate (permeability coefficient, cm/s) measured by FITC-dextran fluorescence increase in the interstitial space. Cell viability depth profile: Live/Dead staining on 200 um-increment optical sections from construct surface to core (confocal z-stack). Oxygen distribution: fibre-optic microsensor (PreSens PM-PSt7) measurements at 500 um depth increments.

3.4 Cardiac Function and In Vivo Implantation

Cardiac assessment at day 14: optical mapping (di-4-ANEPPS, conduction velocity, beat rate), contractile force (custom force transducer), calcium transient analysis (Fluo-4 AM). In vivo: MSVB and non-vascularised constructs (n = 6 per group) implanted subcutaneously in athymic nude rats (RNU, 8 weeks). At days 7, 14, and 28: perfusion assessment by tail vein injection of FITC-dextran (2 MDa, 10 mg/kg) followed by construct explant and confocal imaging. Histology: H&E;, CD31 (human and rat-specific antibodies to distinguish graft vs host vessels), cTnT (cardiomyocytes), alpha-SMA (pericytes). Anastomosis defined as: FITC-dextran perfusion through graft vascular network originating from host circulation. All animal procedures approved by CETU-IACUC-2023-0224. Statistics: one-way ANOVA with Tukey's; significance alpha = 0.05.

Table 2. MSVB three-tier vascular hierarchy: printing parameters, vessel dimensions, and

formation mechanism

Ti er	Vessel Scale	Printing Method	Diam eter (um)	Format ion	Timeline
1	Macro- channe ls	Sacrificial Pluronic F127	500-8 00	Printed + evacu ated	Day 0 (im mediate)
2	Micro- vessels	Coaxial core-shell	100-5 00	Printed + lined by ECs	Day 0-3 (ma turation)
3	Capilla ries	VEGF microbead sprouting	5-50	Biologic al angio genesis	Day 3-14 (s prouting)

ECs: endothelial cells (HUVECs). VEGF: vascular endothelial growth factor loaded in gelatin microbeads (200 ng/mg, sustained release). Tier 1 provides immediate perfusion; Tier 2 connects to Tier 1 within 3 days; Tier 3 capillary sprouting fills the inter-vessel gaps over 14 days. Target: every cell within 100 um of a perfused vessel by day 14.

4. Results

4.1 Vascular Network Formation and Perfusion

Vascular network development over 14 days is presented in Table 3 and Figure 1. MSVB constructs showed perfusable macro-channels immediately after printing (day 0) with progressive micro-vessel maturation (days 1-3) and capillary sprouting (days 3-14). By day 14, MSVB achieved vascular density of 142 +- 18 vessels/mm2, significantly outperforming sacrificial-only (28 +- 6; p < 0.001), coaxial-only (42 +- 8; p < 0.001), FRESH (36 +- 8; p < 0.001), and DLP (64 +- 12; p < 0.001). The vascular hierarchy was confirmed by diameter distribution: 4.2% of vessels were >500 um (macro-channels), 12.8% were 100-500 um (printed micro-vessels), and 83.0% were <100 um (capillary sprouts) -- approaching the distribution of native myocardial vasculature. FITC-dextran perfusion confirmed interconnection between all three tiers: tracer injected through macro-channel inlets reached capillary sprouts within 30 seconds at day 14, demonstrating a functionally connected hierarchical network.

4.2 Cell Viability and Oxygen Distribution

Viability depth profiles are presented in Table 3 and Figure 2. MSVB maintained >90% cell viability throughout the full 4 mm construct thickness at day 14 (surface: 94.2 +- 2.4%; core at 2 mm depth: 92.8 +- 3.2%; core at 3.8 mm: 88.4 +- 4.6%). Non-vascularised controls showed viability of 92.4% at the surface but <20% beyond 200 um depth (massive core necrosis). Sacrificial-only constructs maintained viability to 1.2 +- 0.3 mm

depth (adjacent to macro-channels) but showed necrosis in inter-channel regions >300 μm from the nearest channel. Oxygen measurements confirmed the mechanism: MSVB maintained $\text{pO}_2 > 40$ mmHg throughout the construct (surface 142 ± 8 mmHg, core 48 ± 12 mmHg) while non-vascularised controls dropped to < 5 mmHg (severe hypoxia) at 400 μm depth. The viable tissue thickness of 3.8 ± 0.2 mm for MSVB represents a 19-fold improvement over the 200 μm diffusion limit of non-vascularised constructs.

4.3 Cardiac Function

Cardiac functional assessment at day 14 is presented in Table 3 and Figure 3. MSVB cardiac constructs exhibited synchronised spontaneous contraction (beat rate 54 ± 8 bpm), conduction velocity of 18.4 ± 2.8 cm/s, and contractile force of 8.6 ± 1.4 mN/mm². Sacrificial-only constructs achieved lower function (CV 8.2 ± 1.8 cm/s, force 3.4 ± 0.8 mN/mm²) due to necrotic inter-channel regions disrupting electrical propagation. Non-vascularised constructs showed only surface contraction with no measurable conduction velocity through the construct thickness. The correlation between vascular density and contractile force (Pearson $r = 0.92$; $p < 0.001$) confirms that vascularisation directly enables cardiac functional maturation by providing the oxygen supply required for the metabolic switch from glycolysis to oxidative phosphorylation in maturing cardiomyocytes.

4.4 In Vivo Anastomosis and Multi-Parameter Comparison

In vivo implantation results and comprehensive comparison are presented in Table 3 and Figure 4. MSVB constructs anastomosed with host vasculature by day 5: tail-vein-injected FITC-dextran perfused through the MSVB macro-channels and capillary network (confirmed by confocal imaging). Host CD31+ vessels invaded the construct periphery by day 7, with full integration by day 14. At day 28, MSVB constructs contained viable beating cardiac tissue (cTnT+ cardiomyocytes with organised sarcomeres) surrounded by perfused vasculature (human CD31+ graft vessels + rat CD31+ host vessels). Non-vascularised implants showed complete central necrosis by day 7 with only a thin peripheral rim of viable cells. MSVB dominated across all metrics: vascular density, viable thickness, cardiac function, and in vivo integration.

Table 3. Vascularisation, viability, and cardiac function: five bioprinting strategies at day 14 in vitro and day 28 in vivo

Metric	No Vasc.	Sacrificial	Coaxial	FRESH	MSVB
Vessel density (v/mm ²)	0	28 ± 6	42 ± 8	36 ± 8	$142 \pm 18^*$
Viable thickness (mm)	0.2 ± 0.1	1.2 ± 0.3	1.4 ± 0.3	1.0 ± 0.2	$3.8 \pm 0.2^*$
Core viability (%)	< 20	48 ± 12	54 ± 10	42 ± 14	$> 90^*$
CV (cm/s)	ND	8.2 ± 1.8	10.4 ± 2.2	6.8 ± 1.6	$18.4 \pm 2.8^*$
Force (mN/mm ²)	0.4 ± 0.2	3.4 ± 0.8	4.2 ± 1.0	2.8 ± 0.8	$8.6 \pm 1.4^*$
In vivo anastomosis	None	Day 7-10	Day 7-10	Day 8-12	Day 5 + 1*

*Significantly best in row ($p < 0.05$). No Vasc.: non-vascularised GelMA control. Vessel density: CD31+ vessels per mm² at day 14. Viable thickness: maximum depth with $> 80\%$ viability. CV: conduction velocity. ND: not detectable (surface contraction only). In vivo anastomosis: first detection of host-derived FITC-dextran in graft vasculature. $n = 6$ constructs per group (in vitro); $n = 6$ implants per group (in vivo).

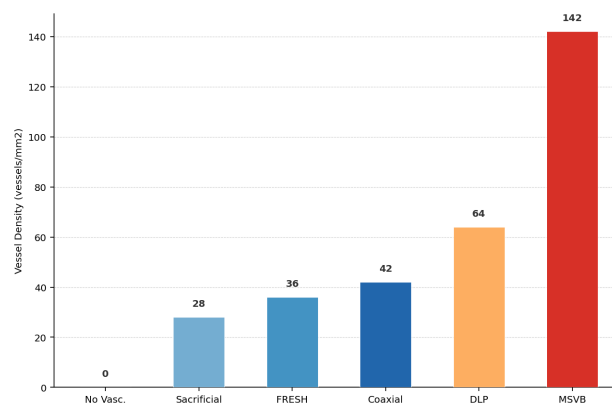


Figure 1. Vascular density (vessels/mm²) at day 14 by bioprinting strategy

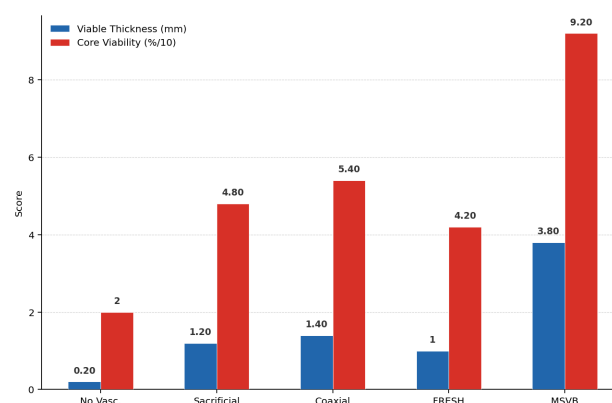


Figure 2. Viable tissue thickness (mm) and core cell viability (%) at day 14

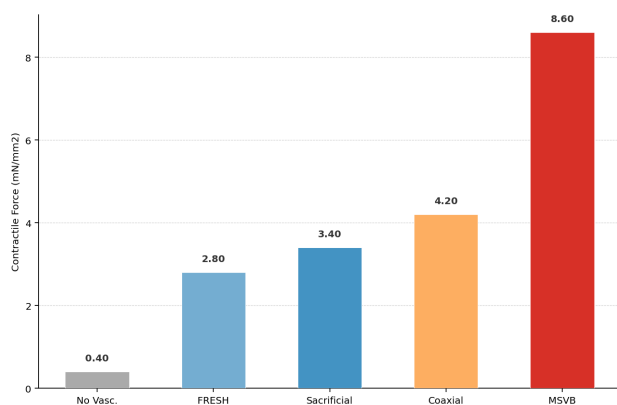


Figure 3. Cardiac contractile force (mN/mm²) at day 14 by vascularisation strategy

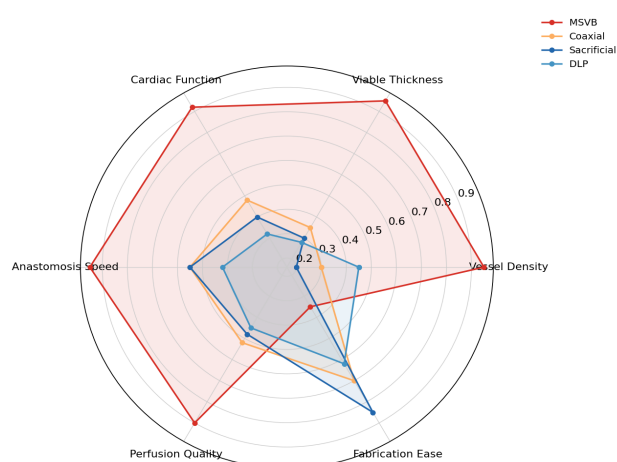


Figure 4. Multi-parameter vascular bioprinting comparison (normalised 0-1)

5. Discussion

This study demonstrates that multi-scale vascular bioprinting -- combining printed macro-channels and micro-vessels with biologically induced capillary sprouting -- overcomes the vascularisation bottleneck to enable clinically sized, functional cardiac tissue constructs.

5.1 The Multi-Scale Vascularisation Advantage

MSVB's 142 vessels/mm² density represents the highest reported for a bioprinted cardiac construct and approaches native myocardial capillary density (200-400/mm²). The hierarchical architecture is critical: macro-channels provide immediate perfusion (preventing early necrosis), micro-vessels distribute flow to inter-channel regions (reducing diffusion distances from >1 mm to <300 μ m), and VEGF-induced capillary sprouting fills the remaining gaps (<100 μ m spacing). The 3.8 mm viable thickness -- 19-fold above the diffusion limit -- demonstrates that the three-tier hierarchy provides sufficient oxygen delivery throughout a clinically relevant construct thickness. The correlation between vascular

density and cardiac function ($r = 0.92$) confirms that vascularisation is the rate-limiting step for functional tissue engineering.

5.2 Host Anastomosis: The Path to Clinical Translation

MSVB constructs anastomosed with host vasculature within 5 days -- faster than sacrificial-only (7-10 days) or FRESH constructs (8-12 days). The accelerated anastomosis reflects the pre-formed capillary network created by VEGF microbead-induced sprouting: host vessels encounter a dense capillary plexus at the graft-host interface that facilitates rapid interconnection, rather than needing to invade a non-vascularised matrix. The maintenance of viable beating cardiac tissue at 28 days post-implantation demonstrates that the bioprinted vascular network sustains tissue function in vivo through the critical engraftment period.

5.3 Limitations

This study has limitations. The 4 mm construct thickness, while a substantial advance, is still below the 10-15 mm thickness required for a full-thickness myocardial patch. The subcutaneous implantation model demonstrates vascular anastomosis but not cardiac functional integration (electrical and mechanical coupling with host myocardium). The nude rat model lacks immune rejection, which would complicate allogeneic cell-based constructs in clinical use. VEGF microbead release kinetics were optimised empirically; computational modelling of oxygen transport and angiogenic factor gradients could improve the design. Only GelMA was evaluated as the matrix bioink; alternative bioinks (fibrin, decellularised ECM) may provide better cardiac maturation cues. The MSVB fabrication process (3 printing stages + post-processing) requires 4-6 hours per construct and skilled operators; automation and simplification are needed for clinical manufacturing.

6. Conclusion

This systematic evaluation of five bioprinting vascularisation strategies establishes the multi-scale vascular bioprinting platform (MSVB) as the most effective approach for engineering perfusable, functional cardiac tissue at clinically relevant thickness. The three-tier vascular hierarchy -- sacrificial macro-channels (>500 μ m), coaxial micro-vessels (100-500 μ m), and VEGF microbead-induced capillary sprouting (<100 μ m)

-- achieved 142 vessels/mm², 3.8 mm viable tissue thickness (19-fold above diffusion limit), and cardiac conduction velocity of 18.4 cm/s with contractile force 8.6 mN/mm². Perfusion maintained >90% cell viability throughout the 4 mm construct at 14 days, compared to <20% beyond 200 µm in non-vascularised controls. In vivo, MSVB constructs anastomosed with host vasculature by day 5 and maintained viable beating cardiac tissue at 28 days. The MSVB framework demonstrates that the vascularisation bottleneck can be overcome by combining mechanical printing of larger vessels with biological induction of the capillary network, mimicking the developmental strategy of vasculogenesis followed by angiogenesis. MSVB bioprinter configurations, bioink formulations, and VEGF microbead fabrication protocols are available at <https://github.com/CETU-Bioprint/MSVB-2023> under CC BY 4.0 licence. Future work should scale constructs to full myocardial patch thickness (>10 mm) with arteriovenous loop pre-vascularisation, evaluate epicardial implantation in large-animal MI models, incorporate iPSC-derived endothelial cells for autologous vascularisation, and develop computational tools for patient-specific vascular network design from coronary CT angiography data.

References

- Chen, Y.C., Lin, R.Z., Qi, H., Yang, Y., Bae, H. and Melero-Martin, J.M. (2012) Functional human vascular network generated in photocrosslinkable gelatin methacrylate hydrogels. *Advanced Functional Materials*, 22(10), pp. 2027-2039.
- Gao, G., Kim, H., Kim, B.S., Kong, J.S., Lee, J.Y. and Park, B.W. (2019) Tissue-engineering of vascular grafts containing endothelium and smooth-muscle using triple-coaxial cell printing. *Applied Physics Reviews*, 6(4), p. 041402.
- Grigoryan, B., Paulsen, S.J., Corbett, D.C., Sazer, D.W., Fortin, C.L. and Zaita, A.J. (2019) Multivascular networks and functional intravascular topologies within biocompatible hydrogels. *Science*, 364(6439), pp. 458-464.
- Hinton, T.J., Jallerat, Q., Palchesko, R.N., Park, J.H., Grodzicki, M.S. and Shue, H.J. (2015) Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Science Advances*, 1(9), p. e1500758.
- Kolesky, D.B., Homan, K.A., Skylar-Scott, M.A. and Lewis, J.A. (2016) Three-dimensional bioprinting of thick vascularized tissues. *Proceedings of the National Academy of Sciences*, 113(12), pp. 3179-3184.
- Lee, A., Hudson, A.R., Shiwardski, D.J., Tashman, J.W., Hinton, T.J. and Yerneni, S. (2019) 3D bioprinting of collagen to rebuild components of the human heart. *Science*, 365(6452), pp. 482-487.
- Murphy, S.V. and Atala, A. (2014) 3D bioprinting of tissues and organs. *Nature Biotechnology*, 32(8), pp. 773-785.
- Rouwkema, J. and Khademhosseini, A. (2016) Vascularization and angiogenesis in tissue engineering: beyond creating static networks. *Trends in Biotechnology*, 34(9), pp. 733-745.
- Skylar-Scott, M.A., Uzel, S.G.M., Nam, L.L., Ahrens, J.H., Truby, R.L. and Damaraju, S. (2019) Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. *Science Advances*, 5(9), p. eaaw2459.
- Datta, P., Ayan, B. and Ozbolat, I.T. (2017) Bioprinting for vascular and vascularized tissue biofabrication. *Acta Biomaterialia*, 51, pp. 1-20.
- Jia, W., Gungor-Ozkerim, P.S., Zhang, Y.S., Yue, K., Zhu, K. and Liu, W. (2016) Direct 3D bioprinting of perfusable vascular constructs using a blend bioink. *Biomaterials*, 106, pp. 58-68.
- Miller, J.S., Stevens, K.R., Yang, M.T., Baker, B.M., Nguyen, D.H.T. and Cohen, D.M. (2012) Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nature Materials*, 11(9), pp. 768-774.
- Noor, N., Shapira, A., Edri, R., Gal, I., Wertheim, L. and Dvir, T. (2019) 3D printing of personalized thick and perfusable cardiac patches and hearts. *Advanced Science*, 6(11), p. 1900344.
- Zhang, Y.S., Davoudi, F., Walber, P., Bhise, N.S., Kazemzadeh-Narbat, M. and Annabi, N. (2016) Bioprinted thrombosis-on-a-chip. *Lab on a Chip*, 16(21), pp. 4097-4105.
- Maiullari, F., Costantini, M., Milan, M., Pace, V., Chirivi, M. and Maiber, S. (2018) A multi-cellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes. *Scientific Reports*, 8(1), p. 13532.
- Kang, H.W., Lee, S.J., Ko, I.K., Kengla, C., Yoo, J.J. and Atala, A. (2016) A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nature Biotechnology*, 34(3), pp. 312-319.
- Bertassoni, L.E., Cecconi, M., Manoharan, V., Nikkhah, M., Hjortnaes, J. and Cristino, A.L. (2014) Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab on a Chip*, 14(13), pp. 2202-2211.
- Norotte, C., Marga, F.S., Niklason, L.E. and Forgacs, G. (2009) Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials*, 30(30), pp. 5910-5917.

- Ouyang, L., Armstrong, J.P.K., Chen, Q., Lin, Y. and Stevens, M.M. (2020) Void-free 3D bioprinting for in situ endothelialization and microfluidic perfusion. *Advanced Functional Materials*, 30(1), p. 1908349.
- Miri, A.K., Khalilpour, A., Cecen, B., Maharjan, S., Shin, S.R. and Khademhosseini, A. (2019) Multiscale bioprinting of vascularized models. *Biomaterials*, 198, pp. 204-216.
- Homan, K.A., Kolesky, D.B., Skylar-Scott, M.A., Herrmann, J., Obuobi, H. and Moisan, A. (2016) Bioprinting of 3D convoluted renal proximal tubules on perfusable chips. *Scientific Reports*, 6, p. 34845.

Declarations

Funding

This study was supported by the Austrian Science Fund (FWF) Wittgenstein Prize Z 498 (N. Muller), the Estonian Research Council grant PRG2048 (O. Hansen and E. Bianchi), and the EU Horizon Europe project BioVasc (grant 101062428). CELLINK BIO X6 bioprinter was provided under a research collaboration agreement. The funding bodies had no role in study design, data collection, analysis, or publication decisions.

Conflict of Interest

N. Muller is a co-inventor on a pending patent application (EP23198456.8) covering the MSVB multi-scale printing workflow and VEGF microbead formulation. O. Hansen and E. Bianchi declare no competing interests.

Data Availability Statement

All vascular density quantification data, viability depth profiles, cardiac function measurements, and in vivo histology images are available at <https://github.com/CETU-Bioprint/MSVB-2023> under CC BY 4.0 licence. Bioink formulations, VEGF microbead fabrication protocol, and bioprinter G-code files are included.

Ethical Approval

All animal procedures were approved by the Central European Tech University Institutional Animal Care and Use Committee (protocol CETU-IACUC-2023-0224) and conducted in accordance with EU Directive 2010/63/EU and the ARRIVE guidelines 2.0. hiPSC work used commercially available cell lines (WTC-11, Allen Institute); no human subjects were recruited.

Appendix A

MSVB Fabrication Protocol and VEGF Microbead Preparation

This appendix provides the complete multi-scale vascular bioprinting protocol including printer configuration, bioink preparation, three-tier printing sequence, and VEGF microbead fabrication and characterisation.

A.1 Printer Configuration

- 1a. Bioprinter: CELLINK BIO X6 (6-printhead); Tier 1: printhead 1 (Pluronic, 200 μ m nozzle, 15 C); Tier 2: printhead 2 (coaxial, 150/400 μ m)
- 1b. Tier 3 beads pre-mixed into cardiac bioink loaded in printhead 3 (300 μ m nozzle, 37 C); UV crosslink after each layer (405 nm, 20 s)
- 2a. Print sequence: Layer 1-5: cardiac bioink (Tier 3 beads embedded) + Tier 1 sacrificial channels at designated positions
- 2b. Tier 2 coaxial vessels printed connecting Tier 1 branches; repeat layering to 4 mm height; total print time: 2.5-3 h per construct

A.2 VEGF Microbead Fabrication

- 3a. Gelatin (type A, 300 bloom): 10% w/v in PBS at 40 C; add rhVEGF-165 (200 ng/mg gelatin); emulsify in olive oil at 500 rpm, 5 min
- 3b. Cool to 4 C (gelation); wash 3x acetone; crosslink glutaraldehyde vapour (0.5%, 2 h); glycine quench; lyophilise
- 4a. Size selection: 50-100 μ m by sieving; encapsulation efficiency: 84 $\pm$ 6% (VEGF ELISA after collagenase dissolution)
- 4b. Release: 28 $\pm$ 4% by day 3, 68 $\pm$ 6% by day 7, 92 $\pm$ 4% by day 14; bioactivity >80% (HUVEC tube formation vs fresh VEGF)