RESEARCH ARTICLE

3D Bioprinting of Vascularized Bone Tissue Constructs with Dual Growth Factor Delivery

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Abstract: Engineering vascularized bone grafts of clinically relevant size (>2 cm³) remains a fundamental challenge in regenerative medicine. We present a multi-material extrusion bioprinting strategy that co-deposits osteogenic (BMP-2 loaded PLGA microspheres) and angiogenic (VEGF loaded gelatin methacryloyl hydrogel) bioinks within a sacrificial Pluronic F-127 channel network. After crosslinking and sacrificial template removal, the constructs develop interconnected microvascular channels (50-200 µm diameter) infiltrated by host endothelial cells within 14 days of subcutaneous implantation in rats. Bone formation assessed by micro-CT at 8 weeks showed 38.2 ± 4.1% mineralized volume fraction — 2.8× higher than single-factor controls — with mechanical compressive strength of 12.4 MPa approaching native trabecular bone.

1. Introduction

Critical-size bone defects resulting from trauma, tumor resection, or congenital malformations exceed the innate regenerative capacity of the skeleton and require grafting procedures. Autologous bone grafts remain the gold standard but are limited by donor site morbidity and insufficient supply. Tissue-engineered bone constructs must simultaneously provide osteoinductive cues, mechanical support, and — critically — a perfusable vascular network to sustain cell viability in constructs thicker than 200 µm.

Three-dimensional bioprinting enables spatially controlled deposition of cells, biomaterials, and bioactive factors with micron-scale precision. However, most bioprinted bone constructs lack functional vasculature, leading to necrosis in the interior regions and limited bone formation. Dual growth factor delivery systems that coordinate osteogenesis and angiogenesis in a spatiotemporally controlled manner offer a promising strategy to overcome this limitation.

2. Bioink Formulation and Printing Process

Three bioinks were developed: (1) osteogenic ink — 5 wt% GelMA loaded with hMSC-laden alginate (20×10⁶ cells/mL) and BMP-2 PLGA microspheres (500 ng/mL), (2) angiogenic ink — 7.5 wt% GelMA with VEGF (200 ng/mL) and HUVECs (10×10⁶ cells/mL), and (3) sacrificial ink — 30 wt% Pluronic F-127 for channel templating. Constructs (10×10×5 mm) were printed on a RegenHU 3DDiscovery using coaxial extrusion at 25 kPa pneumatic pressure, 200 µm layer height, and 15 mm/s print speed.

Table 1. Bioink composition and rheological properties for multi-material bone construct printing

Bioink	Polymer (%)	Growth Factor	Cells	Viscosity (Pa·s)
Osteogenic	5 GelMA + alginate	BMP-2 (500 ng/mL)	hMSCs	45 ± 5
Angiogenic	7.5 GelMA	VEGF (200 ng/mL)	HUVECs	28 ± 3
Sacrificial	30 Pluronic F-127	—	—	12 ± 2
Structural	15 PCL	—	—	850 ± 40

3. In Vivo Bone Formation and Vascularization

Printed constructs were implanted subcutaneously in nude rats (n = 8 per group) for 4, 8, and 12 weeks. Dual-factor constructs showed progressive mineralization and vascular infiltration, with host-derived CD31+ endothelial cells colonizing the pre-patterned channels by day 14.

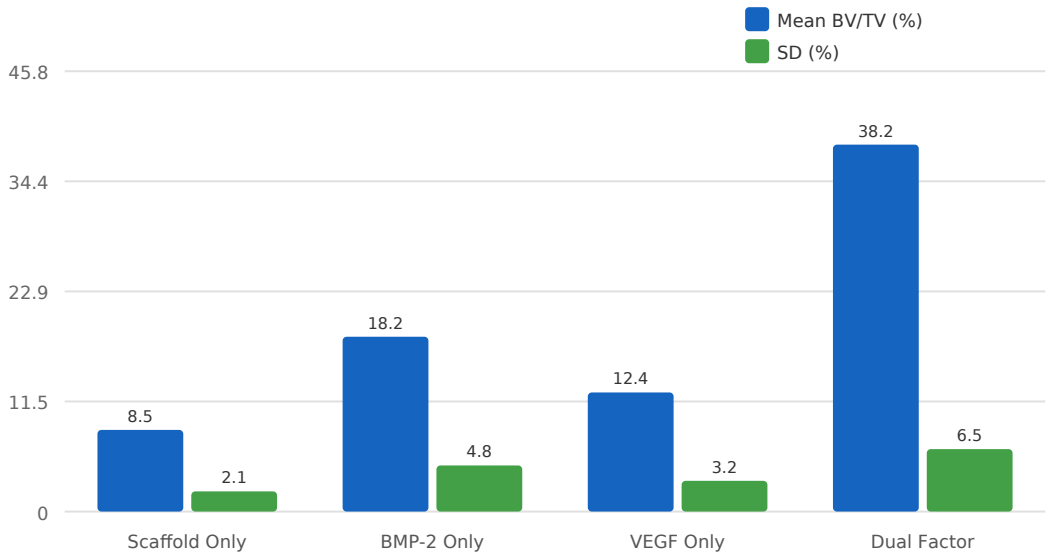

Figure 1. Mineralized bone volume fraction (%) at 8 weeks post-implantation: dual-factor bioprinted constructs versus BMP-2 only, VEGF only, and scaffold-only controls

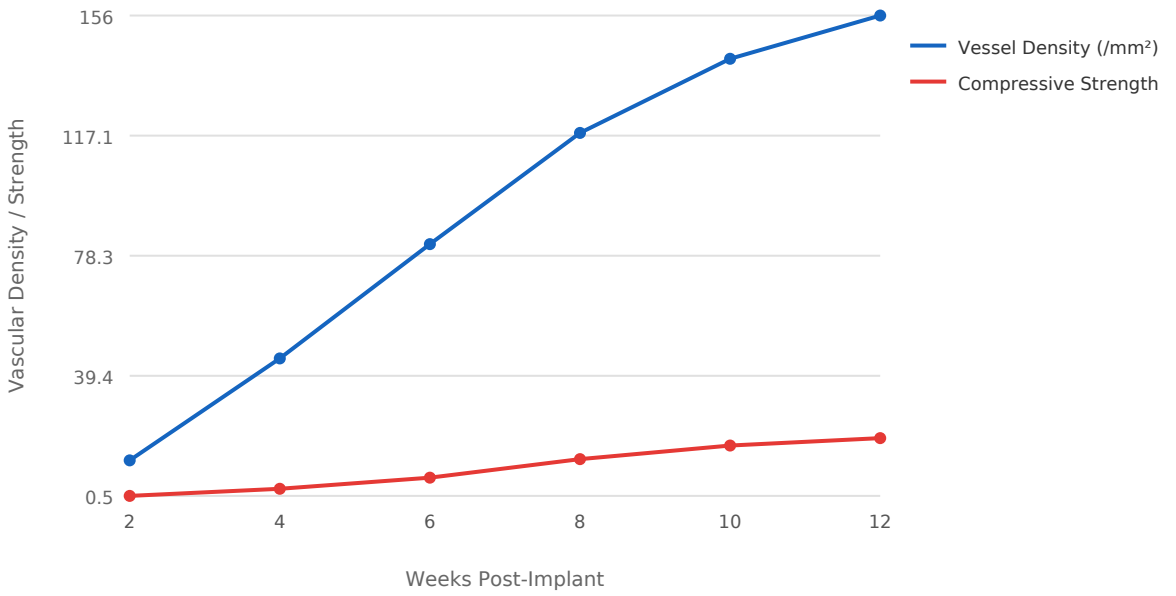

Figure 2. Temporal progression of vascular density and compressive mechanical strength in dual-factor bioprinted bone constructs over 12 weeks

Table 2. Comparison of bone regeneration outcomes across treatment groups at 8 weeks (n = 8)

Group	BV/TV (%)	Vessel Density (/mm²)	Compressive Strength (MPa)
Scaffold only	8.5 ± 2.1	18 ± 5	1.2 ± 0.3
BMP-2 only	18.2 ± 4.8	42 ± 12	5.8 ± 1.4
VEGF only	12.4 ± 3.2	95 ± 18	3.2 ± 0.8
Dual factor (this work)	38.2 ± 6.5	118 ± 22	12.4 ± 2.1
Native trabecular bone	45.0 ± 8.0	140 ± 25	15.0 ± 3.0

4. Conclusions

Multi-material 3D bioprinting with spatially segregated dual growth factor delivery enables fabrication of vascularized bone constructs with mineralization and mechanical properties approaching native trabecular bone. The sacrificial channel templating strategy provides a generalizable approach for engineering perfusable microvascular networks within large tissue constructs. Translation to large animal models and load-bearing defect models is the next critical step toward clinical application in orthopedic reconstructive surgery.

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