A Configurable Periosteum-Inspired Platform Orchestrates Spatiotemporal Osteogenic-Vasculogenic Coupling

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Introduction

The periosteum couples osteogenesis and vasculogenesis during bone regeneration — its removal reduces new bone formation by ~73% and neovascularization 10-fold [1, 2]. Yet existing *in vitro* models fail to recapitulate sustained, compartment-resolved osteogenic–vasculogenic coupling [3, 4]. Here, we developed a periosteum-inspired tri-layer platform integrating melt electrowriting (MEW) and 3D bioprinting to systematically interrogate this coupling.

Osteogenic Module
Ost-F

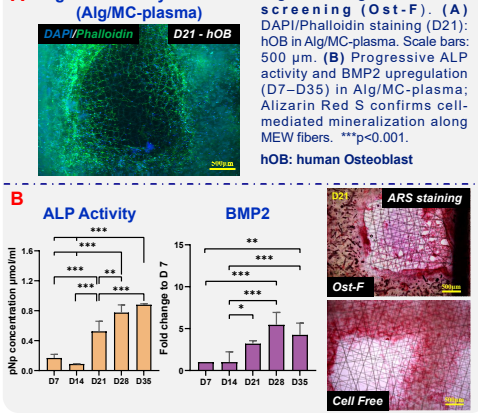


Fig.1 Osteogenic module screening (Ost-F). **(A)** DAPI/Phalloidin staining (D21): hOB in Alg/MC-plasma. Scale bars: 500 μm. **(B)** Progressive ALP activity and BMP2 upregulation (D7–D35) in Alg/MC-plasma; Alizarin Red S confirms cell-mediated mineralization along MEW fibers. ***p<0.001.

hOB: human Osteoblast

MEW Fibrous Scafflod
MEW-Uni / MEW-Bi

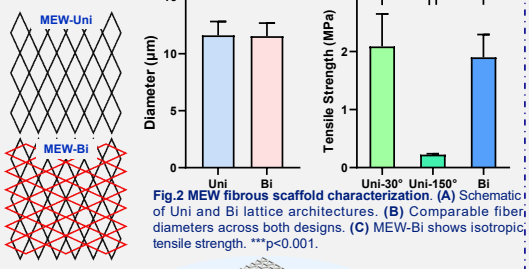


Fig.2 MEW fibrous scaffold characterization. **(A)** Schematic of Uni and Bi lattice architectures. **(B)** Comparable fiber diameters across both designs. **(C)** MEW-Bi shows isotropic tensile strength. ***p<0.001.

Vasculogenic Module
Vas-F

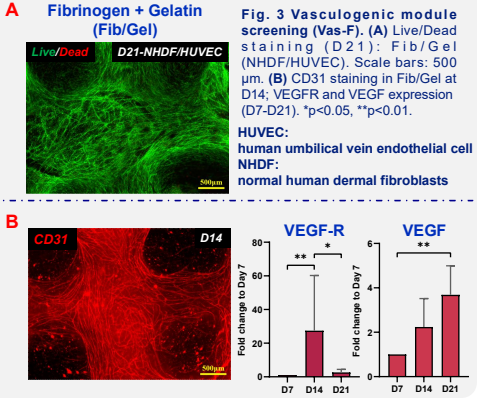
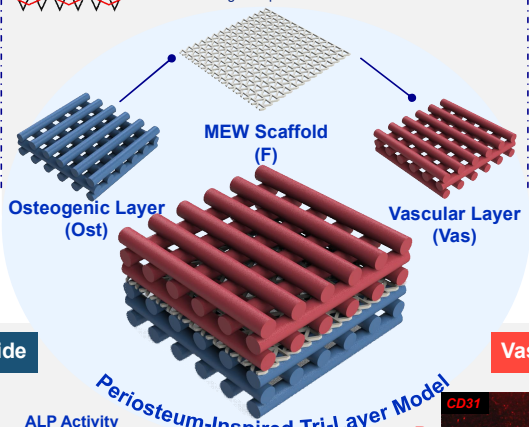


Fig. 3 Vasculogenic module screening (Vas-F). **(A)** Live/Dead staining (D21): Fib/Gel (NHDF/HUVEC). Scale bars: 500 μm. **(B)** CD31 staining in Fib/Gel at D14; VEGFR and VEGF expression (D7-D21). *p<0.05, **p<0.01.

HUVEC:
human umbilical vein endothelial cell
NHDF:
normal human dermal fibroblasts



Ost-F-Vas^{cell-}

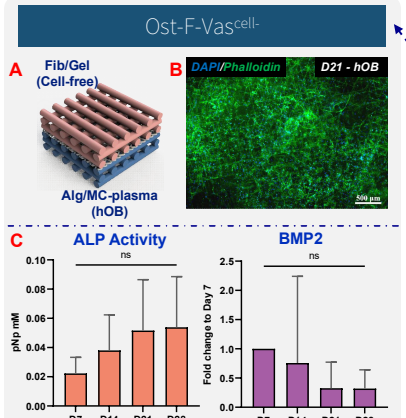


Fig. 4 Osteogenic maturation without HUVEC (Ost-F-Vas^{cell-}). **(A)** Schematic: osteogenic layer with cell-free vasculogenic compartment. **(B)** DAPI/Phalloidin staining at D21. **(C)** ALP activity and BMP2 expression showed no significant changes over D7–D28. ns = no significance.

Ost-F-Vas

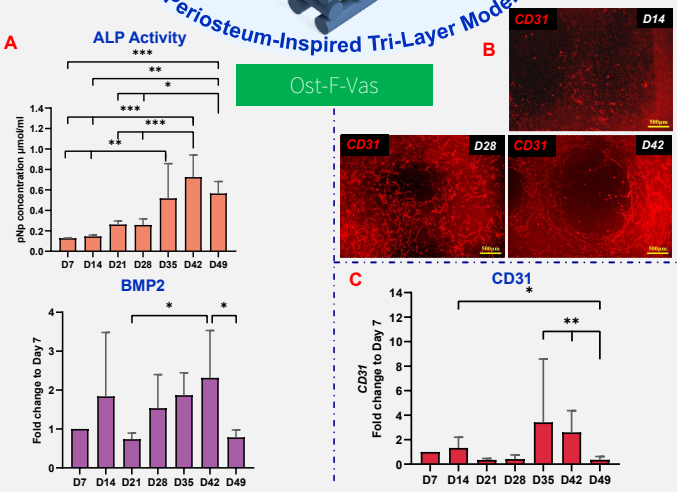


Fig. 5 Spatiotemporal osteogenic–vasculogenic coupling in the integrated construct (Ost-F-Vas). **(A)** Ost side: progressive ALP activity and BMP2 upregulation over D7–D49. **(B–C)** Vas side: CD31 gene expression peaked at D35–D42; CD31 staining from D14 to D42, demonstrating delayed but sustained vascular maturation. *p<0.05, **p<0.01, ***p<0.001.

Ost^{cell-}-F-Vas

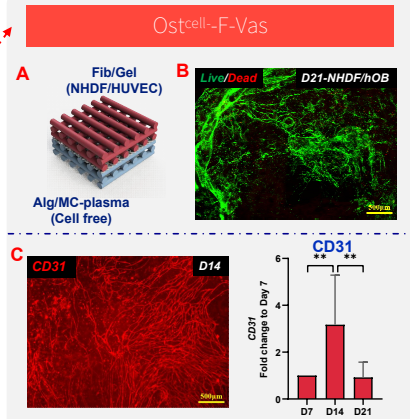


Fig.6 Vasculogenic maturation without hOB (Ost^{cell-}-F-Vas). **(A)** Schematic: vasculogenic layer with cell-free osteogenic compartment. **(B)** Live/Dead staining (D21): elongated network morphology. **(C)** CD31 staining at D14; CD31 gene expression (D7-D21). **p<0.01.

Conclusion:

1. This platform provides a standardized human cell-based *in vitro* framework for interrogating periosteum-inspired design variables under defined conditions.

2. The vasculogenic module enhances osteogenesis, while the osteogenic module delays vascular network formation — revealing time-dependent osteogenic-vasculogenic coupling.

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References:

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