

## Supporting Information

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Direct Microextrusion Printing of a Low Viscosity Hydrogel on a Supportive Microstructured Bioprinting Substrate for the Vasculogenesis of Endothelial Cells

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#### Supplementary files include:

**Figure S1**. Structural fidelity of fibrinogen lattices printed on a glass slide, PDMS sheet, and SuBstrate.

**Figure S2.** Microscopic images of fluorescent fibrinogen lattices printed and crosslinked on a glass slide, a PDMS sheet, and a SuBstrate.

**Figure S3.** Comparison of hydrogel designs printed on a glass slide, PDMS sheet, and SuBstrate.

Figure S4. HUVEC cell viability during the bioprinting procedure.

Figure S5. Procedure for FITC–Dextran permeation out of bioprinted HUVEC lattice model.

**Captured image from Movie S1.** Compiled orthogonal images at the intersection of the lattice showing an even distribution of interconnected vasculature distributed height.

**Captured image from Movie S2.** Compiled orthogonal images at the lattice boundary illustrating lumens opening up to the exterior of the hydrogel lattice marked with yellow arrows.



**Supplementary Figure S1.** Structural fidelity of fibrinogen lattices printed on a glass slide, PDMS sheet, and SuBstrate. A) Schematics of the nozzle trajectory based on the G-code used to direct the printing head, computer-aided design (CAD) model of the SuBstrate, and their overlapped image. B) Stereomicroscopic images of a fibrinogen lattice printed on the three different substrate materials showing differences in printing fidelity. The yellow arrows point out discontinuous sections in the fibrinogen lattice printed on PDMS due to its high hydrophobicity. C) Schematics illustrating the parameters calculated to quantify the structural fidelity of the structures printed on the different substrate materials and the calculated values of E) the opening ratio (n > 5) and the opening area (n > 20).



**Supplementary Figure S2.** Microscopic images of fluorescent fibrinogen lattices printed and crosslinked on a glass slide, a PDMS sheet, and a SuBstrate.



**Supplementary Figure S3.** Comparison of hydrogel designs printed on a glass slide, PDMS sheet, and SuBstrate. A) Stereomicroscopic images of square spirals printed on the three different substrate materials using a commercial 32-gauge single-barrel nozzle. B) Stereomicroscopic images of a multi-ink hydrogel design printed on three different substrate materials using a glass capillary multibarrel nozzle showing lower and irregular resolution on bare glass and PDMS substrate materials.



**Supplementary Figure S4.** HUVEC cell viability during the bioprinting procedure. A) HUVEC cell viability in the fibrinogen bioink immediately before printing and 30 min into printing with bioink obtained from within the syringe and from the printing nozzle and B) images of the HUVECs stained with trypan blue at the three stages of printing (n = 6). Significant differences in the cell viability between the bioprinting steps were determined using a one-way ANOVA followed by Tukey's post-hoc test to show no significant differences.



**Supplementary Figure S5.** Procedure for FITC–Dextran permeation out of bioprinted HUVEC lattice model. A) Schematics outlining the steps for the outward permeation of FITC–Dextran out of the bioprinted lattice and into the washing solution. B) Schematics illustrating the side view of the bioprinted construct during each step of the procedure.

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**Captured image from Movie S1.** Compiled orthogonal images at the intersection of the lattice showing an even distribution of interconnected vasculature distributed height.



**Captured image from Movie S2.** Compiled orthogonal images at the lattice boundary illustrating lumens opening up to the exterior of the hydrogel lattice marked with yellow arrows.