Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information for

Coaxial bioprinting of cell-laden vascular constructs using a gelatin-tyramine bioink

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Contents:

Equation. S1 The percentage of alamarBlue ® metabolic activity was calculated using the following equation:

$$\text{Percentage of alamarBlue metabolic activity} = \frac{(O2 \times A1) - (O1 \times A2)}{(R1 \times N2) - (R2 \times N1)} \times 100$$

where:

O1 = molar extinction coefficient of oxidized alamarBlue at 570 nm

O2 = molar extinction coefficient of oxidized alamarBlue at 600 nm

R1 = molar extinction coefficient of reduced alamar Blue at 570 nm

R2 = molar extinction coefficient of reduced alamar Blue at 600 nm

A1 = absorbance of samples at 570 nm

A2 = absorbance of samples at 600 nm

N1 = absorbance of negative control at 570 nm

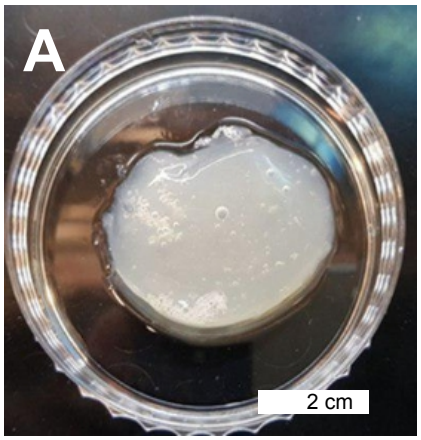
N2 = absorbance of negative control at 600 nm

Protocol. S1

Quantification from LIVE/DEAD® assay using ImageJ software (NIH)

Digital images of stained cells were captured using confocal microscopy and were processed in ImageJ in the following manner: (*1*) Using *Image ❍ Color ❍ Split Channels,* channels were separated into live and dead fluorescence channels. (*2*) Using *Image ❍ Adjust ❍ Threshold,* a constant threshold level was set across a set of conditions to ensure that sufficient signal was present to identify viable or dead cells. (*3*) Using *Process ❍ Binary ❍ Watershed,* a segmentation analysis was performed to allow for adequate automated tabulation. (*4*) Using *Analyze ❍ Analyze Particles,* a constant area exclusion filter was applied to ensure that tiny signal specs, likely from debris or instrument artifacts, were excluded from the final counts of live or dead cells. (5) Green-stained live cells in the PBS solution tends to be stained red during fluorescence imaging. Cells co-stained with green and red were counted as live cells in the merged images. (6) Cell viability was expressed as the ratio of the number of live cells to the sum of the number of live cells and the number of dead cells.

Figure S1. Gelled GPT-50 hydrogels for cell culture. (a) Hydrogel in tissue culture plate. (b) Hydrogels on glass slide.



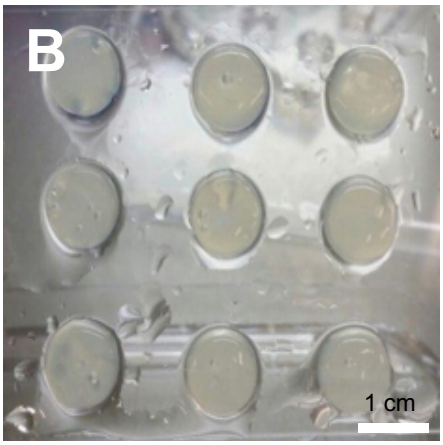


Figure S2. (A, B) Printed GPT hydrogel strand structures on fibrin gel. (C) GPT-50 hydrogel covered with 5 mg/ml fibrin.

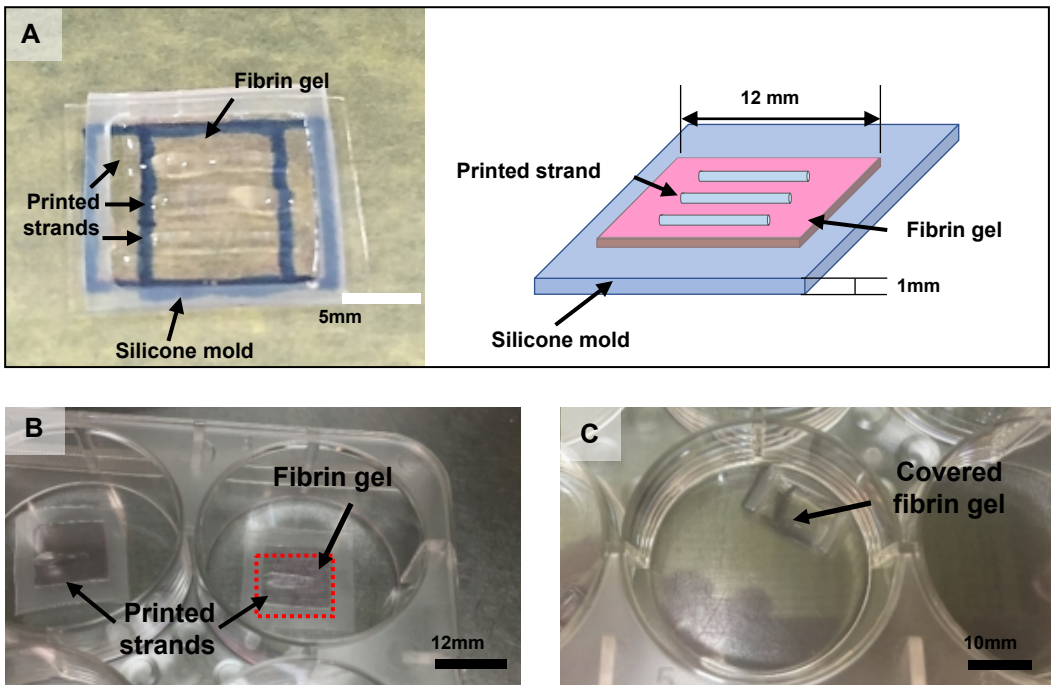


Figure S3. ^{1}H-NMR spectra of gelatin-PEG-Tyramine (GPT) bioink. The amino groups in tyramine and PEG were conjugated with gelatin: tyramine (δ 6.6–6.8 ppm) and PEG (δ 3.2–3.6 ppm). The results demonstrated that the GPT was synthesized successfully using NHS-PEG-NHS.

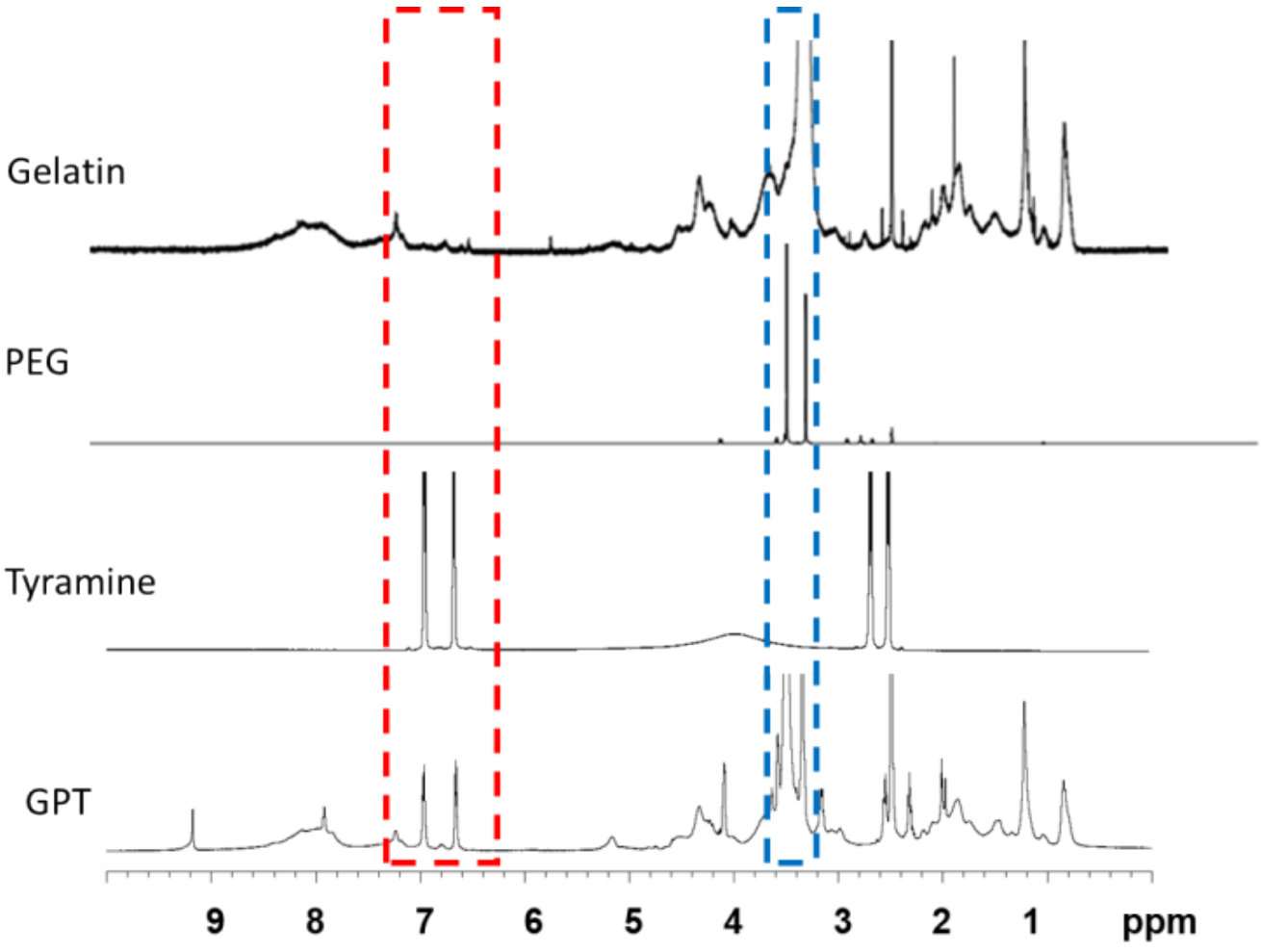


Figure S4. Results of rheological oscillation amplitude tests to evaluate storage modulus (n=3). Linear viscoelastic region of GPT hydrogels. (a) GPT-5, (b) GPT-20, (c) GPT-50. A 20 mm parallel steel plate was employed for the rheology experiment at 0.1Hz frequency under 25±0.02 °C.

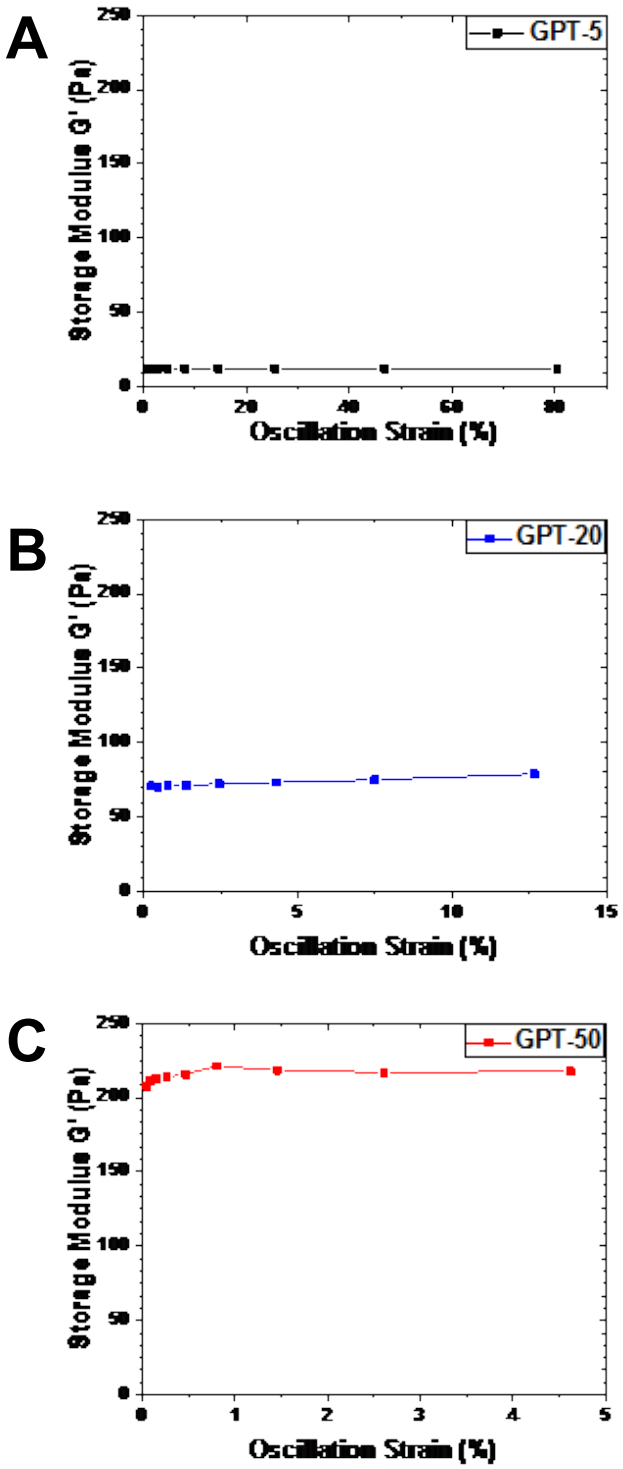


Figure S5. Fibroblasts (HDFn) and endothelial cells (HUVEC) in the gelatin-based bioink. (A) Schematic diagram of a cell culture experiment on the bioink (2D) and within the bioink (3D). Viabilities of fibroblasts (HDFn) and endothelial cells (HUVEC) (B1-B4) on the gelatin-based bioink (2D) or (B5-B8) in the gelatin-based bioink on day 3. Green: Live cells, Red: Dead cells.

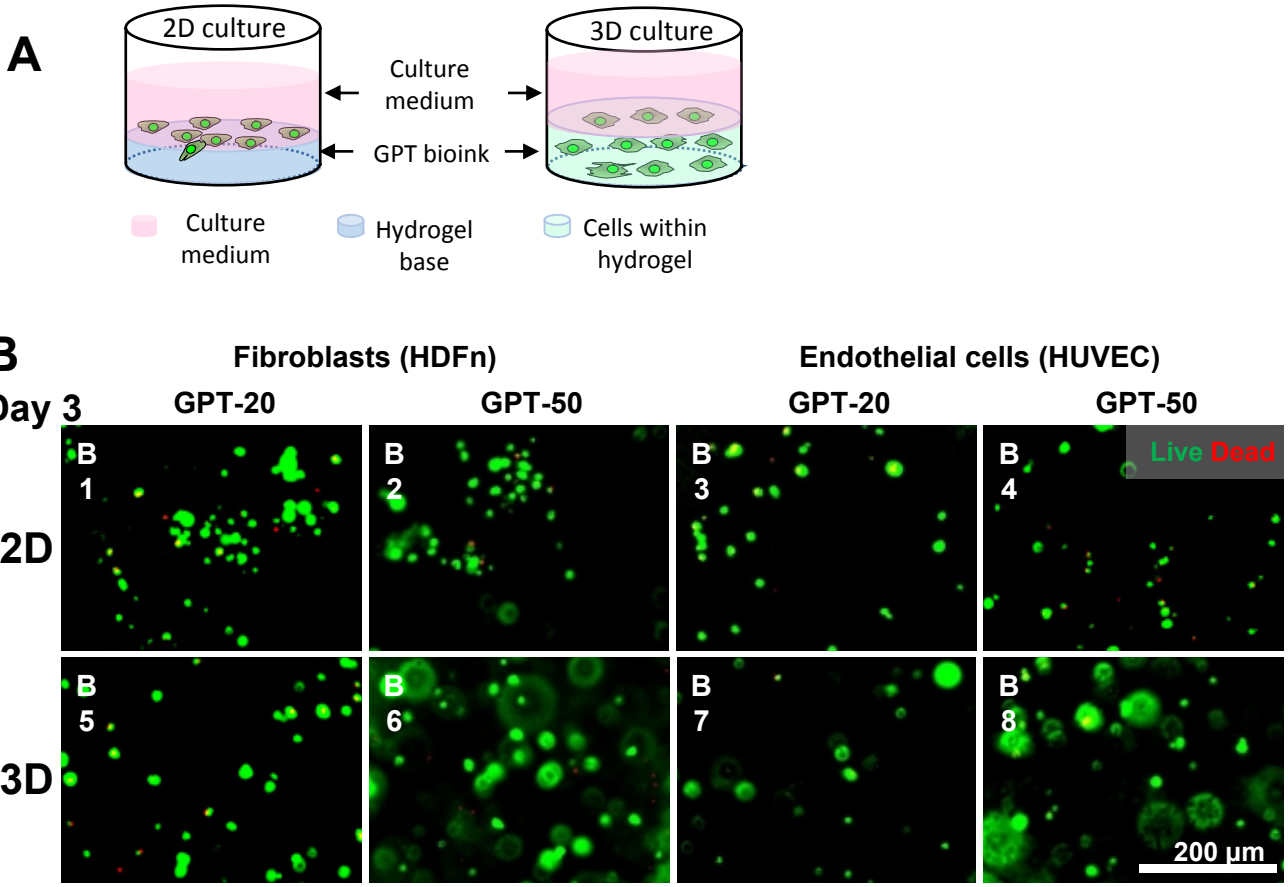


Figure S6. Fluorescence microscope images of cultured vessel structure with (A) RFP-HUVEC, (B) GFP-HDF, and (C) an overlap of both cell types on day 3 Fibroblasts (Red: RFP-HUVEC, Green: GFP-HDF) (Scale bars are 400 μm). The dashed line shows the printed strand.