Supporting Information

Polydopamine-Based Interfacial Engineering of Extracellular Matrix Hydrogels for Construction and Long-Term Maintenance of Living Three-Dimensional Tissues

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Experimental Section

Fabrication of PDMS culture wells and microfluidic devices

Polymeric masters fabricated by stereolithographic 3D-printing were used to generate PDMS cell culture wells described in Figures 1, 2, and 3. Master molds for microfluidic devices shown in Figure 4 were fabricated using SU-8 2100 (MicroChem, USA) negative photoresist to create relief structures on prime-grade 4-inch silicon wafers (Wafer World Inc., USA) per manufacturer recommended protocols. Briefly, the wafer was spin-coated with SU-8 2100 to a nominal 250 µm thickness, pre-baked for 90 min at 95 ºC, exposed with hard contact through a quartz photomask at 300 mJ/cm^2 on a SUSS MA-6 Gen3 mask aligner (SUSS Microtek, Germany), post-exposure baked at 95ºC for 30 min, and allowed to cool. This process was repeated for any additional layers. Development was performed in SU-8 Developer (MicroChem, USA) according to manufacturer protocols (manual agitation during immersion for 60 minutes, followed by a 10-minute immersion in clean SU-8 developer, after which the wafer was rinsed with isopropyl alcohol and then air dried with nitrogen).

The PDMS wells and microdevices used in our study were created using conventional soft lithography. Sylgard 184 silicone elastomer base (Dow Corning, USA) and its curing agent were mixed at a ratio of 10:1 (w/w) and poured over the master wafer or 3D printed mold. Air bubbles were removed by degassing under vacuum for 30 minutes, after which the PDMS was heated for polymerization in a convection oven for 24 h at 65°C. Cured PDMS was cut from the wafer or mold using a scalpel and then peeled. Tubing access ports or wells were punched out from the patterns wherever required using disposable biopsy punches (Integra Miltex, USA). 6 mm punches (Miltex cat. 33-36) were used to generate circular PDMS wells and media reservoirs for microfluidic cell culture. We also used a 1 mm disposable biopsy punch with a plunger (Miltex cat. 33-31AA-P) to create access ports for injection of hydrogel solutions into our microfluidic cell culture device. Subsequently, the PDMS substrates were bonded to blank PDMS slabs to form open wells or enclosed chambers using uncured PDMS as a mortar/glue,

which was prepared by mixing PDMS base with a curing agent at a ratio of 10:3 (w/w). The bonded layers were then cured at 65°C for 2 h. PDMS wells and microdevices were stored at room temperature and UV-sterilized for 30 min prior to PDA coating.

PDA-based surface modification of PDMS substrates

For surface modification, the culture surfaces of PDMS wells and microchannels were incubated with a 2 mg/ml dopamine solution (Sigma Aldrich, USA) prepared in 10 mM Tris-HCl (pH 8.5) for 2 h at room temperature. During this period, the initially clear dopamine solution turned dark brown due to pH-induced oxidation, and this noticeable color change was used as a visual indicator of reaction progress. After 2 hours of incubation, the dopamine solution was gently aspirated, and the PDMS surfaces were washed twice with sterile filtered deionized (DI) water to remove unbound PDA molecules. The washed substrates were kept sterile at room temperature until use.

Characterization of PDMS surface wettability

The wettability of PDA-treated and untreated PDMS substrates was evaluated by measuring static water contact angles. Briefly, 10 µl of DI water was carefully dispensed onto the top surface of a horizontally oriented flat PDMS substrate to form a small drop. We then used a high-resolution monochrome camera (Axiocam 506 mono, Carl Zeiss, Germany) to image the shape of the water drop. For any given substrate, images taken at 6 different spots were used to determine an average static water contact angle using the static sessile drop tangent method.

Preparation of hydrogel precursor solutions

To prepare a collagen hydrogel solution, 20 µl of 10X phosphate buffered saline (PBS) was mixed with 4.2 µl of 1 N NaOH and 55.4 µl of DI water in a sterile 1.5 ml tube. Subsequently, 120.4 µl of collagen type I (#354236, Corning, USA) stock solution (stock density: 3.32 mg/ml)

was added to the tube and mixed thoroughly to generate a hydrogel precursor solution with a final concentration of 2 mg/ml at pH 7.4. A fibrin gel solution was prepared by mixing fibrinogen (10 mg/ml; F8630, Sigma) with thrombin (1 U/ml; Sigma) in PBS. To generate Matrigel scaffolds, Corning Matrigel Basement Membrane Matrix (354234, Corning) was used in this study.

3D cell culture

Normal human lung fibroblasts (hLFs; cc-2512, Lonza, CH), human hepatic stellate cells (hHSCs; #5300, ScienCell Laboratories, USA), and mouse skeletal myoblasts (C2C12; CRL-1772, ATCC, USA) used in our study were cultured and maintained in hLF media supplemented with growth factors included in the FGM-2 BulletKit (CC-3131 & CC-4126, Lonza, CH), stellate cell media (5301, ScienCell, USA), and Dulbecco's Modified Eagle's Medium (DMEM, Gibco, ThermoFisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS, PAA Laboratories), respectively. These cells were used between passage 4 and 6. Prior to use, the cells were trypsinized following the protocols provided by the vendors and resuspended in a hydrogel precursor solution. To generate cell-laden collagen hydrogel constructs, the cells were first mixed with a 2 mg/ml collagen solution at a final density of 1.0×10^6 cells/ml. 100 µl of the mixture solution was injected into each well, which was then placed in a humidified cell culture incubator at 5% CO_2 and 37°C to induce gelation. Following 30-min incubation, culture media were added to the open wells containing solidified collagen hydrogel constructs. For cell culture in fibrin gel, a cell suspension solution containing fibrinogen (10 mg/ml in PBS), thrombin (1 U/ml in PBS), and primary human lung fibroblasts (1×10^6 cells/ml) was prepared. 100 µl of the mixture solution was pipetted into each well and incubated in a humidified cell culture incubator for gelation, after which culture media were added to the open wells.

Construction of microengineered *in vitro* vascular model

The three-lane microfluidic device depicted in Figure 4A was fabricated in PDMS using a replica-molding technique described above. To form cell-laden hydrogel scaffolds in this device, hLFs and red fluorescent protein (RFP)-expressing human umbilical vein endothelial cells (HUVECs) were suspended each at 3 x 10^6 cells/ml in a mixture of fibrinogen (10 mg/ml in PBS) and thrombin (1 U/ml in PBS), and injected into the center lane. The seeded device was then placed in a 37 ºC incubator with 5% CO_2 for 30 min, during which fibrinogen was enzymatically converted to fibrin. Afterwards, adjacent media channels alongside of the central lane were filled with Endothelial Cell Growth Medium (EGM)-2 (C-22011, PromoCell GmbH) containing HUVECs suspended in EGM-2 at 1 X 10^6 cells/ml. During microfluidic culture, media in the reservoirs were replaced every other day. Vascular perfusion in our model was tested using 70 kDa FITC-Dextran (46945-100MG-F, Sigma-Aldrich, USA) injected into the side channels using a Chemyx Fusion 200 syringe pump (Chemyx Inc., USA) driven at 10 µl/min. Confocal microscopy of vascular self-assembly and perfusion was performed on a Zeiss LSM 800 (Carl Zeiss, Germany) confocal microscope with a 10X 0.25 Long Working Distance objective.

Immunofluorescence imaging

Cell-containing hydrogel constructs in PDMS wells and microdevices were rinsed twice with PBS and fixed using 4% paraformaldehyde (w/v in 1x PBS, Sigma Aldrich) at room temperature for 15 min. The fixed samples were then washed twice with PBS and incubated with 0.1% Triton X-100 (v/v in 1X PBS; ThermoFisher Scientific) for 3 min at room temperature. Subsequently, the constructs were washed with PBS twice and immersed in 3% bovine serum albumin (w/v in 1x PBS; Sigma, USA) overnight at 4°C. This step was followed by two washes with PBS and overnight incubation at 4°C with Phalloidin-iFluor 488 reagent-Cytopainter (ab176753, abcam, USA; 1:1000). After two washing steps with PBS, the culture wells and the reservoirs of our microfluidic devices were filled with fresh PBS and placed on a rocking shaker overnight at 4°C.

For immunofluorescence imaging, we used a laser scanning confocal microscope (LSM 800; Carl Zeiss, Germany) to obtain confocal micrographs with a resolution of 1024X1024. The captured images were processed using ZEN 2012 (Zeiss, Germany) and ImageJ (National Institutes of Health, USA). At least 5 images (n = 5) were used for analysis of each group.

Directionality Analysis

The orientation angle of image features was calculated in Figure 3D by using the OrientationJ toolbox in the FIJI distribution of ImageJ. For Figure 3E (top, middle), the orientation of cell bodies was quantified in ImageJ by identifying the major axis of the cell body with one-segment line ROIs and measuring the angle to the horizontal axis (corresponding to the direction of stretch). A cell was defined as being aligned to the stretch axis if it had a cell body major axis within ± 20º of the stretch axis.

Quantification of cell proliferation

Cell nuclei were stained with DAPI and enumerated in 1 mm^2 micrographs using a pipeline in CellProfiler.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed for between-group comparisons except for Figure 3B, 3D, and 3E, in which two-way repeated-measures ANOVA followed by Tukey's post-hoc test, the Kolmogorov-Smirnov test, and unpaired t test with Welch's correction were employed, respectively. Results are presented as mean ± standard error of the mean (SEM) except in Figure 3, where data are presented as mean ± standard deviation (SD).

Supplementary Figures

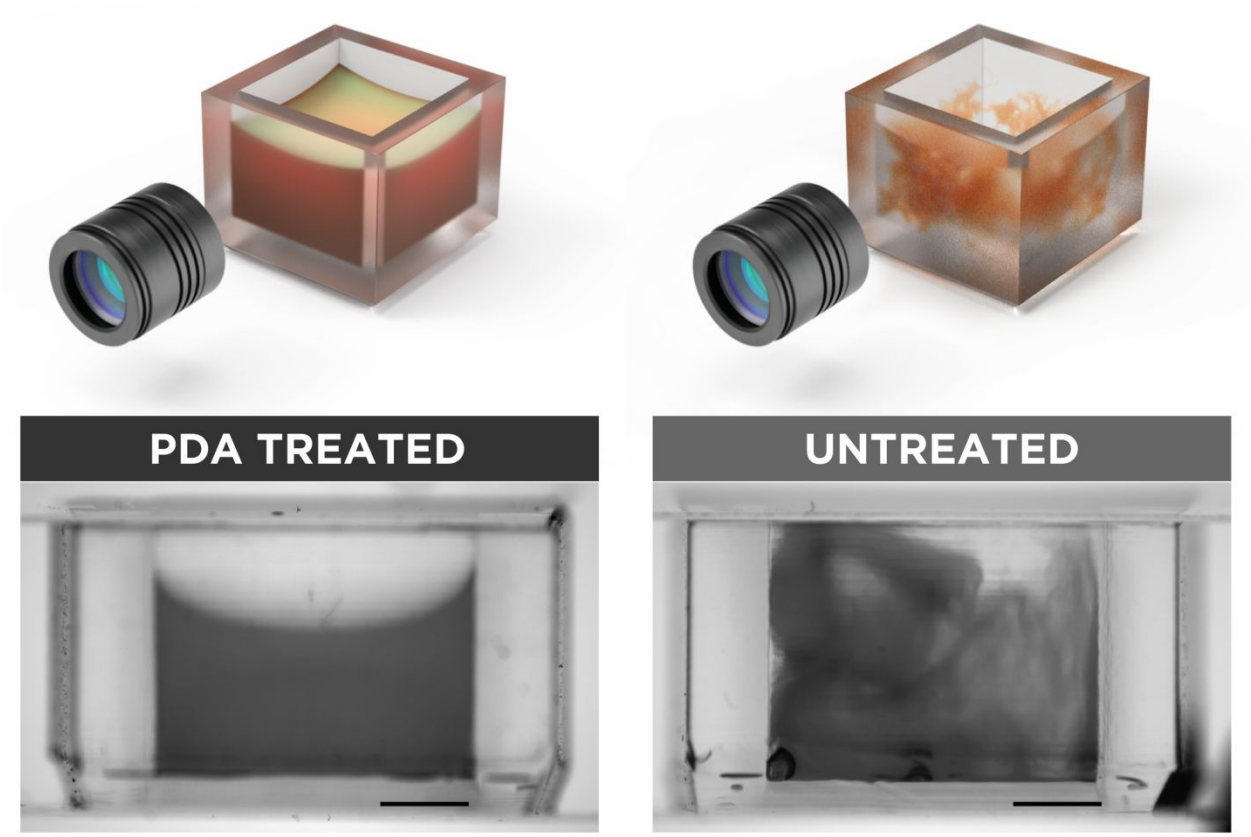

Figure S1. Side view of 2 mg/ml collagen gels containing 1 million/ml normal human lung fibroblasts (NHLFs) in PDA-treated (left) and untreated (right) PDMS chambers. Images captured after 3 days of culture demonstrate well-maintained hydrogel structure along the entire thickness of the construct in the PDA-treated chamber, whereas the hydrogel detaches from the vertical walls of the untreated chamber and undergoes structural distortion. Scale bars, 3 mm.

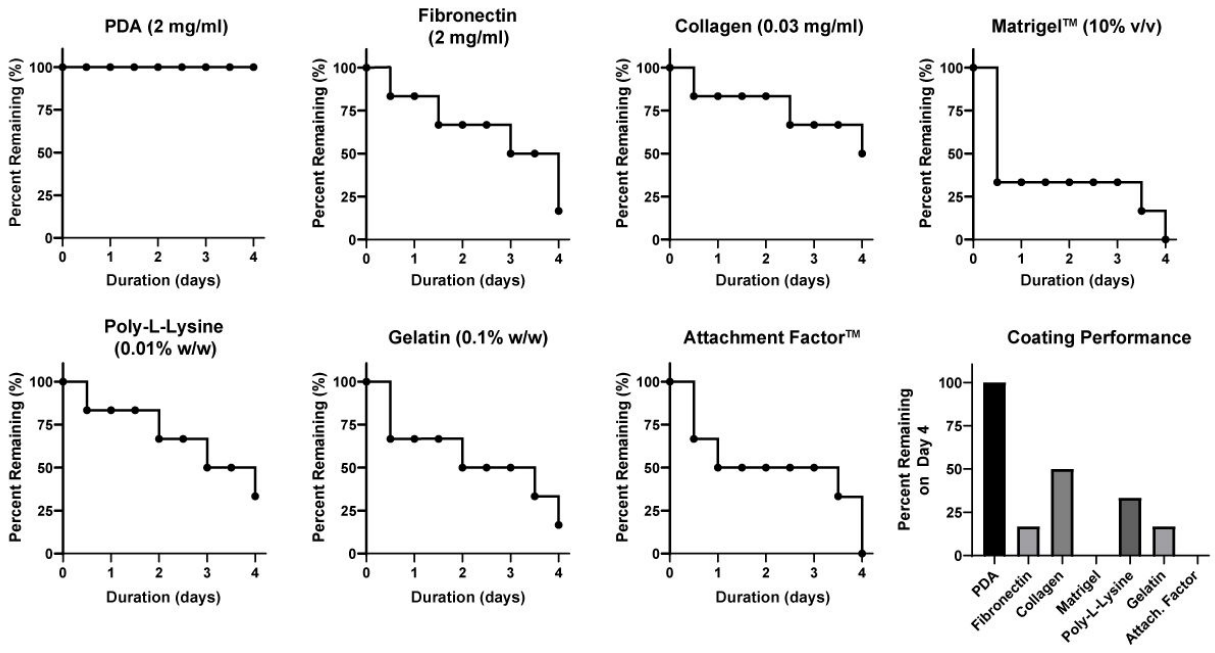

Figure S2. ECM hydrogel adhesion test in PDMS cell culture wells treated with various coating materials. After surface treatment, each PDMS well was filled with 2 mg/ml collagen gel containing 1 million/ml primary human lung fibroblasts. The percent fraction of ECM hydrogel constructs that remain attached to the culture chambers was plotted with respect to the number of days in culture.

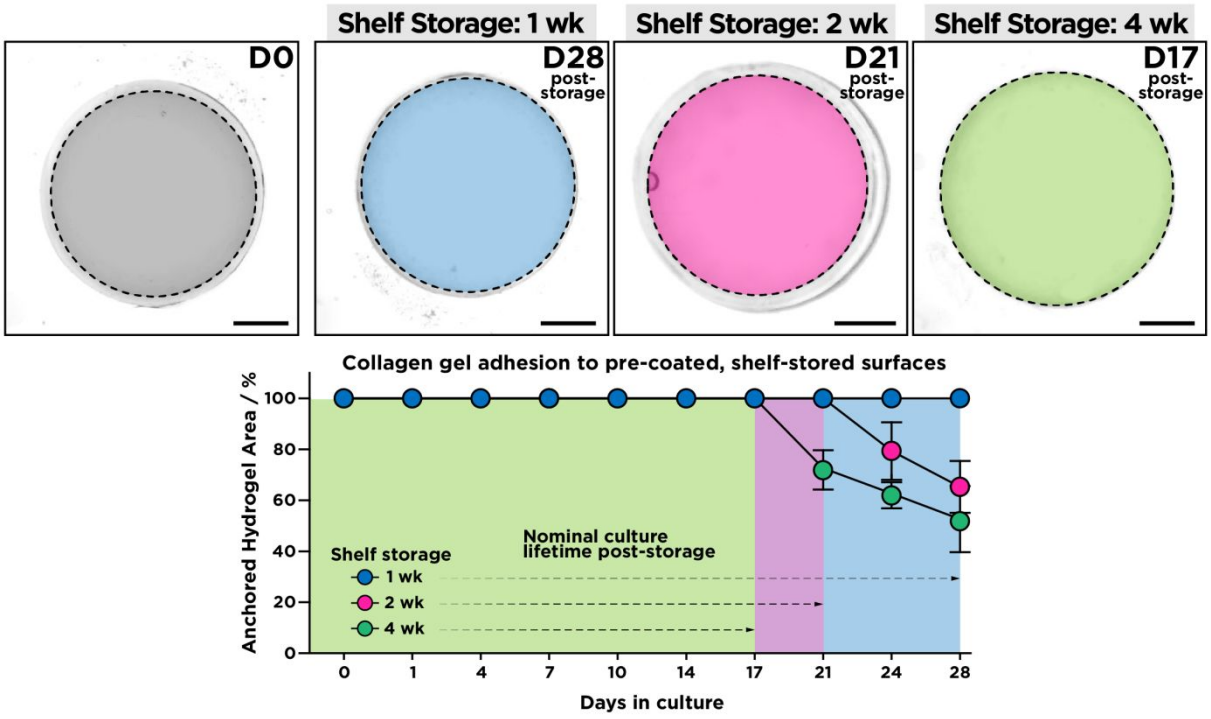

Figure S3. PDA coating remains effective after shelf-storage. The graph shows the quantification of the projected surface area of fibroblast-laden hydrogel constructs maintained in circular PDMS wells with respect to days in culture. Scale bars, 2 mm. Data are presented as mean ± SEM (*n* = 6).