ADVANCED MATERIALS

Supporting Information

Precise and Arbitrary Deposition of Biomolecules onto Biomimetic Fibrous Matrices for Spatially Controlled Cell Distribution and Functions

Chao Jia, Bowen Luo, Haoyu Wang, Yongqian Bian, Xueyong Li, Shaohua Li, and Hongjun Wang*

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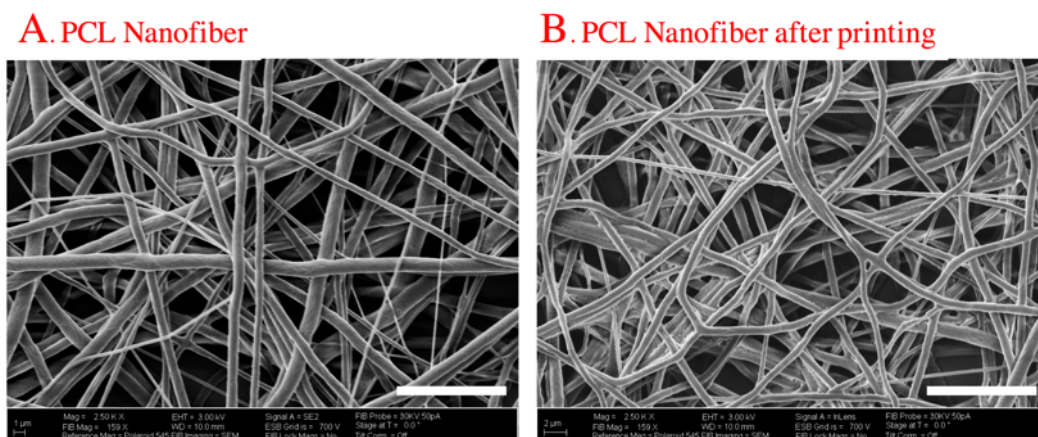
Figure S1. SEM images of PCL nanofiber matrix before (A) and after fibronectin (FN) printing (B). After printing, nanofibers coated with FN had higher charge and showed a bright edge under SEM. Scale bars: 10 μm.
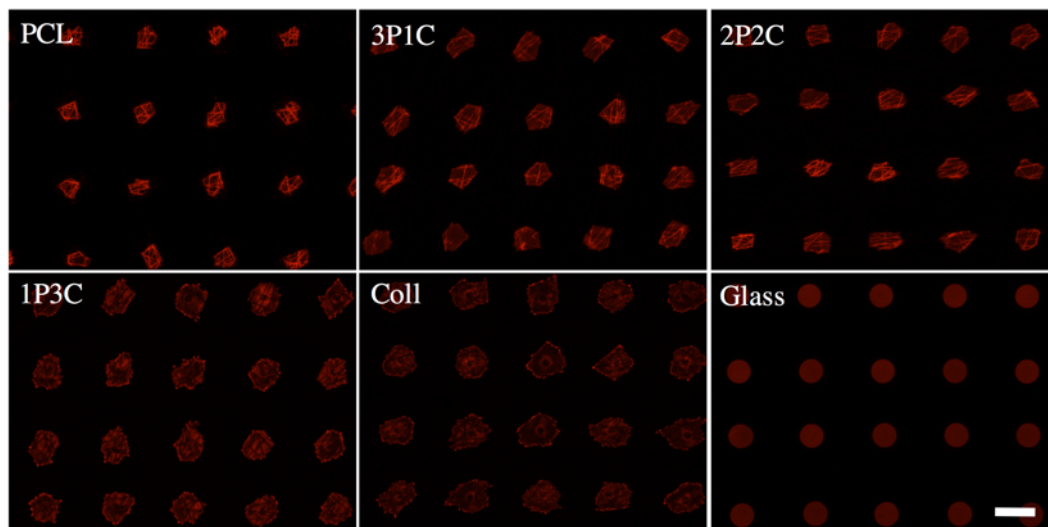

Figure S2. Fluorescence images of TRITC-BSA printed onto various nanofiber matrices [PCL, 3PCL:1collagen (3P1C), 1PCL:1collagen (2P2C), 1PCL:3collagen (1P3C), collagen (Coll) and coverglass (Glass)] using 90 μm drop distance (DD) and 6pL drop size (DS). Scale bar: 50 μm.
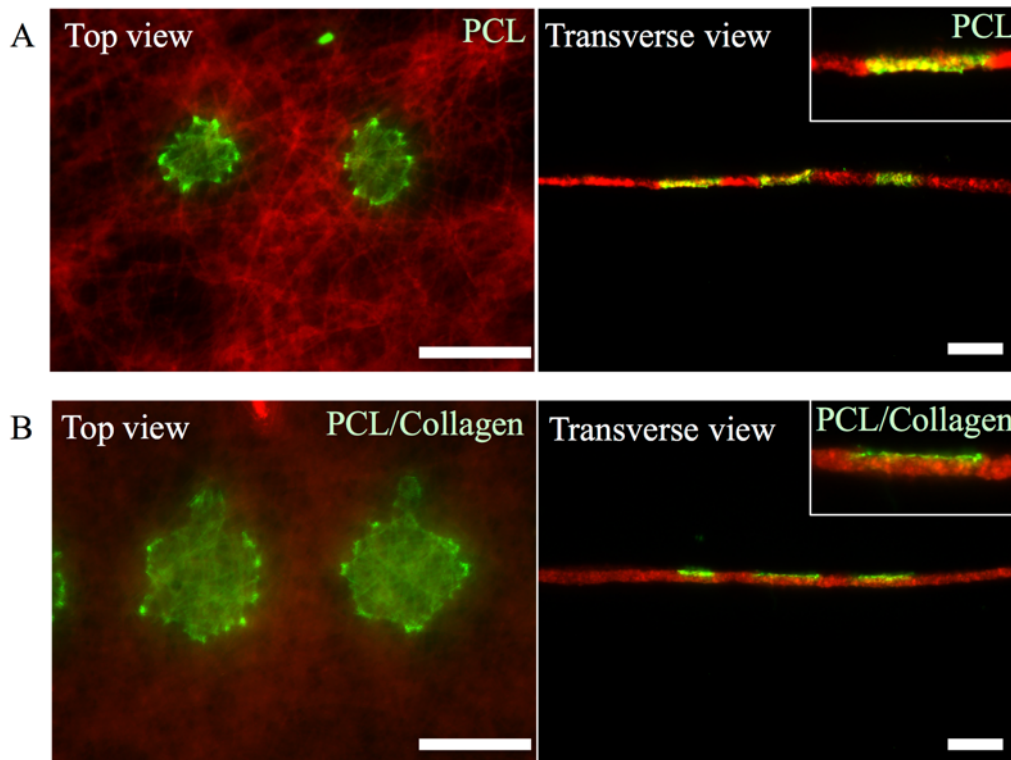
Figure S3. Top and transverse view of the fluorescence images of FITC-BSA printed onto TRITC-labeled PCL (A) or TRITC-labeled PCL/collagen (1:1 v/v) (B) nanofiber matrices (90 μm DD and 10 pL DS). Inset shows zoomed-in images of single dot. Scale bar: 50 μm.
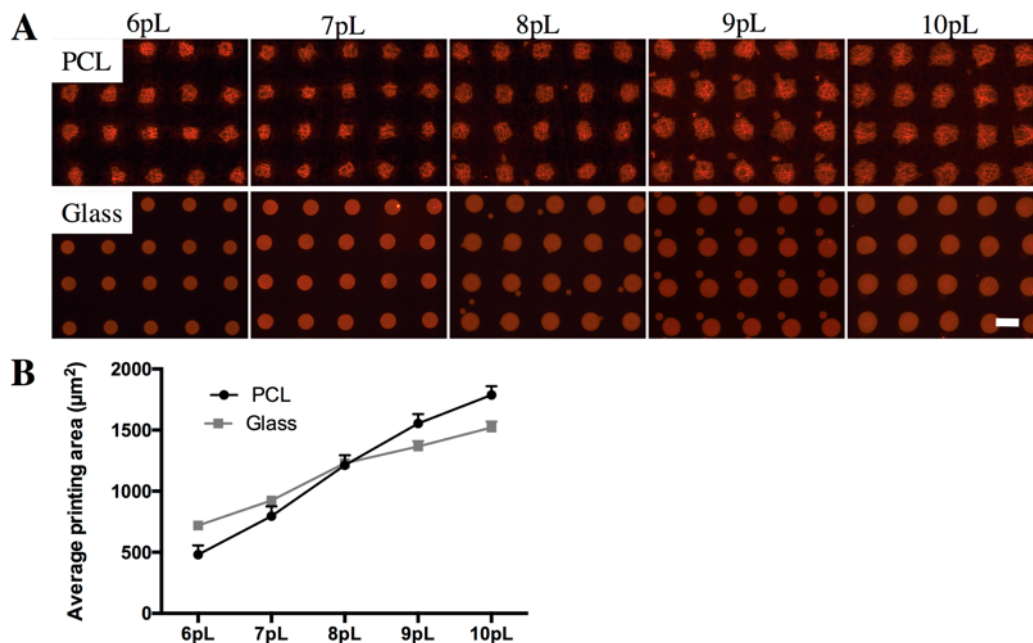

Figure S4. A) Fluorescence images of TRITC-BSA printed onto PCL nanofiber matrices and glass surfaces using 90 μm DD but different DS from 6pL to 10pL. Scale bar: 50 μm. B) Quantification of the resulting printed area showed a nearly linear correspondence between DS and average dot area printed onto PCL ES matrices and glass.
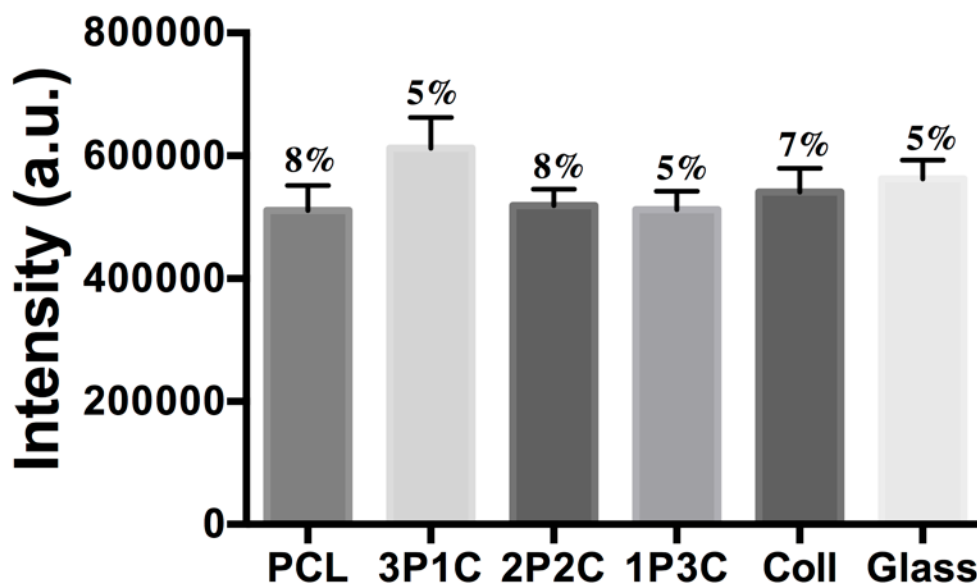
Figure S5. Fluorescence intensity measurement shows minimal dot-to-dot variation (<8%) for those dots of TRITC-BSA printed onto different surfaces [PCL (8%), 3P1C (5%), 2P2C (8%), 1P3C (5%), Coll (7%) and Glass (5%)] using 90 μm DD and 10pL DS.
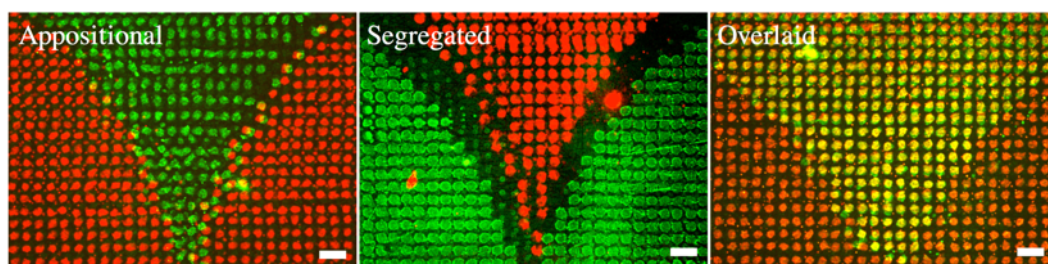

Figure S6. A) Fluorescence images of two-color printing on PCL nanofiber matrices with FITC-BSA (green) and TRITC-BSA (red). By modulating the AutoCAD design and printer cartridge, the patterns with appositional, segregated or overlaid arrangement of two types of biomolecules could be achieved. Scale bars: 100 μm.
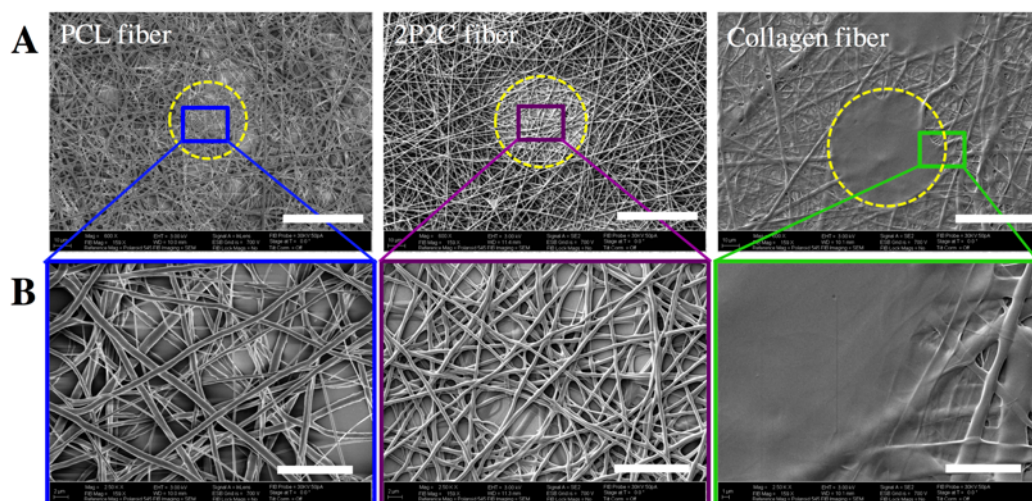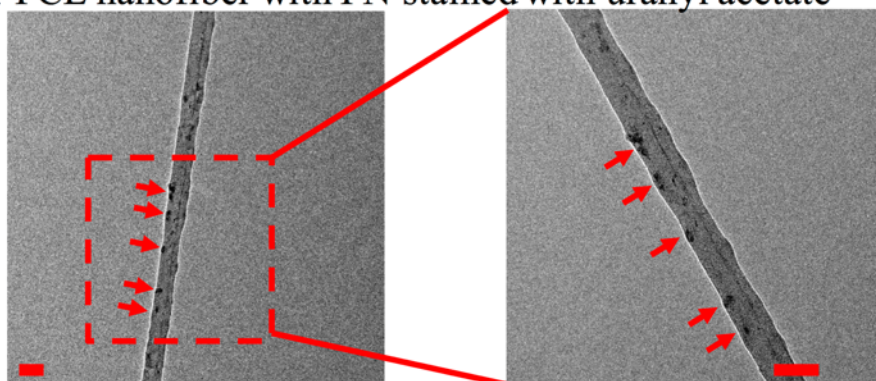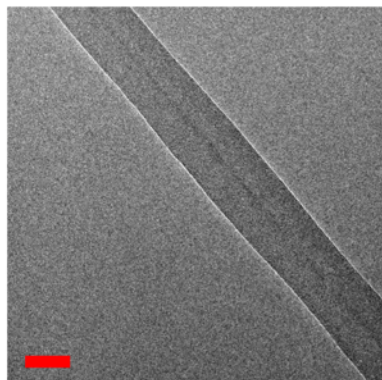
Figure S7. A) SEM images of the morphology of nanofiber matrices after fibronectin (FN) printing. Dashed yellow circles highlighted the overview of the printed drop. Scale bars: 50 μm. B) Zoomed-in SEM micrograph of the marked rectangular area of (A) revealed that PCL and 2P2C ES matrices maintained their original fibrous morphology and fiber dimension after printing while the fibers of printed area in pure collagen fiber matrices fused into a continuous film. Scale bars: 10 μm.
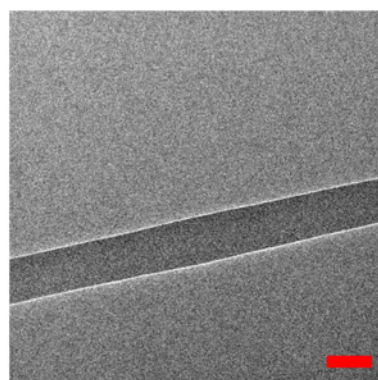

Figure S8. A) TEM images of fibronectin (FN) clusters stained with uranyl acetate (red arrows) on PCL nanofibers. Zoomed-in image of the red dashed box was highlighted and shown on the right side. B) TEM image of PCL nanofiber with FN coating but without uranyl acetate staining. C) TEM image of PCL nanofiber without FN coating but with uranyl acetate staining. All scale bars: 100 nm.
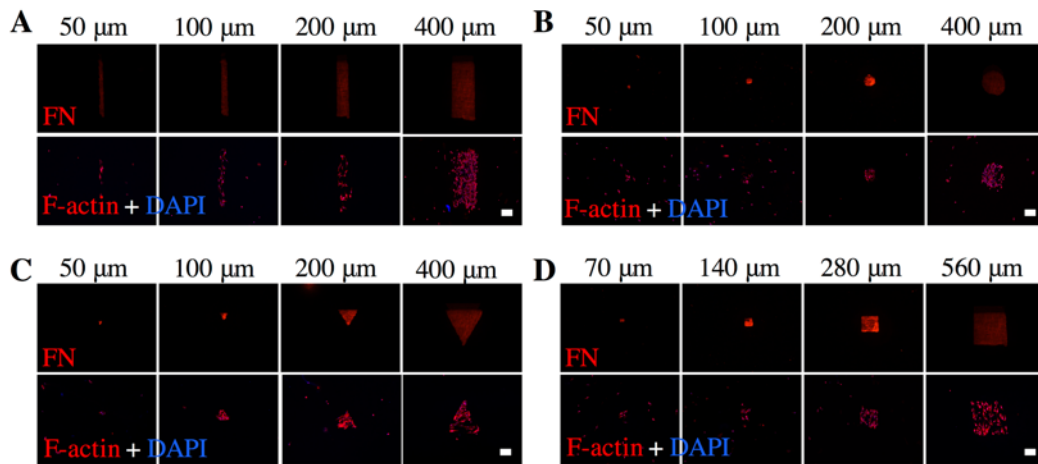
Figure S9. Fluorescence images of fibronectin printed onto PCL nanofiber matrices after staining with four different shapes: (A) rectangle, (B) circle, (C) triangle and (D) square (first row) and their corresponding MOVAS cell patterns (second row). Cells were stained with Phalloidin-TRITC and DAPI. Scale bars: 200 μm.
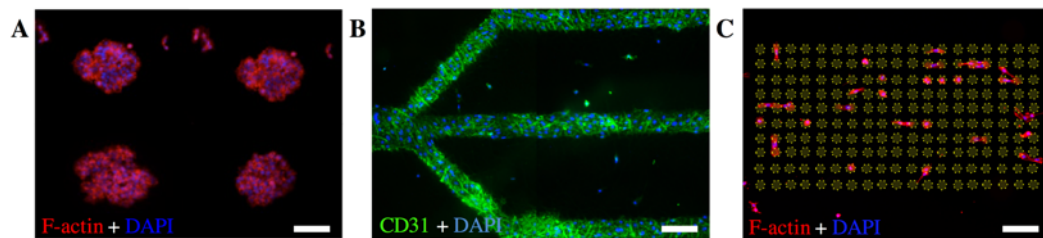
Figure S10. Fluorescence images of MCF-7 cells attached onto 100-μm FN dots (A), MS-1 cells formed microvessel-like patterns (B) after 2 days, and MS-1 cells attached to an array of 25-μm FN dots on PCL nanofiber matrices by stretching two or three dots or sticking to one dot. MCF-7 cells were stained with Phalloidin-TRITC and DAPI in (A); MS-1 cells were stained for CD31 and DAPI in (B); MS-1 cells were stained with Phalloidin-TRITC and DAPI in (C). Scale bars: 100 μm.

Figure S11. The effect of cell seeding density on the accuracy of cell patterning. A-i) and B-i) Bright field images of MS-1 cells attached onto the FN lines on nanofiber

meshes for 5 minutes after cell seeding. A-ii and iii) and B-ii and iii) Fluorescence images of MS-1 cells attached to the FN lines for 1 day. Cells were stained with Phalloidin-TRITC and DAPI. Scale bars: 100 μm.

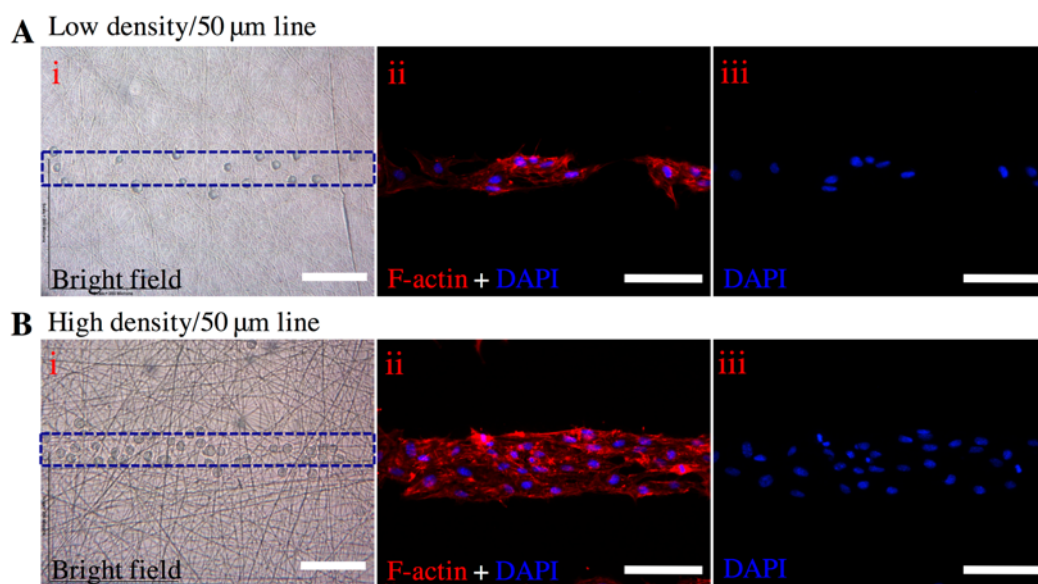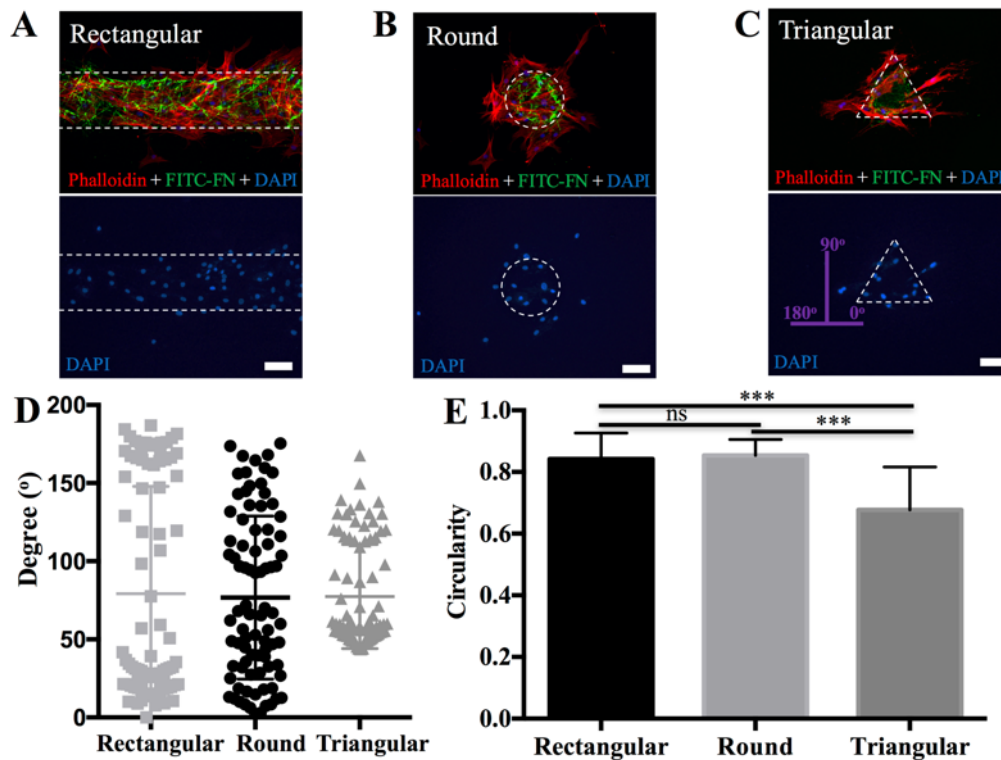Figure S12. Fluorescence images of human normal dermal fibroblasts attached onto 100-μm FITC-FN rectangular print (A), round print (B) and triangular print (C). Cells were stained with TRITC-Phalloidin for F-actin and DAPI for nuclei. Dashed white shapes indicates the pattern shapes Scale bars: 100 μm. D) The distribution of the angel of nuclei on three FN patterns shown in A, B and C. The orientation of cell nuclei was calculated according to the coordinates labeled in C of DAPI image. The angel distribution was plotted into a scatter chart with average and standard deviations (n=90). E) Column chart with standard deviations showed the average nuclei circularity of cells cultured on rectangular, round and triangular patterns (n=90, ***p<0.001, ns: Not significant).

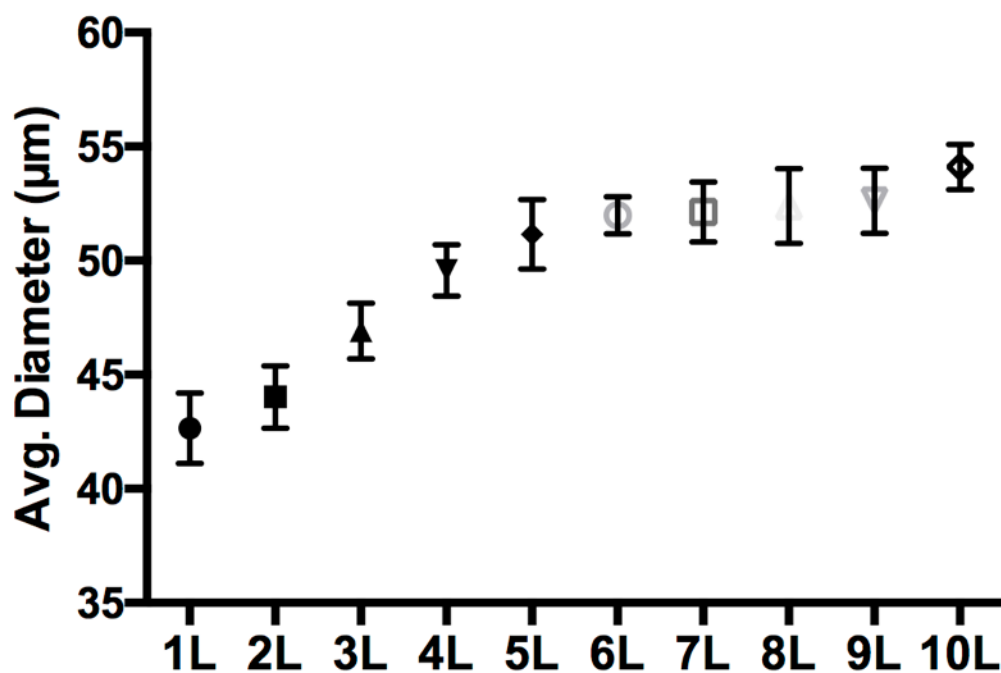Figure S13. The correlation between individual printed dot area and the number of repeated printing layers of TRITC-BSA using 10-pL DS.
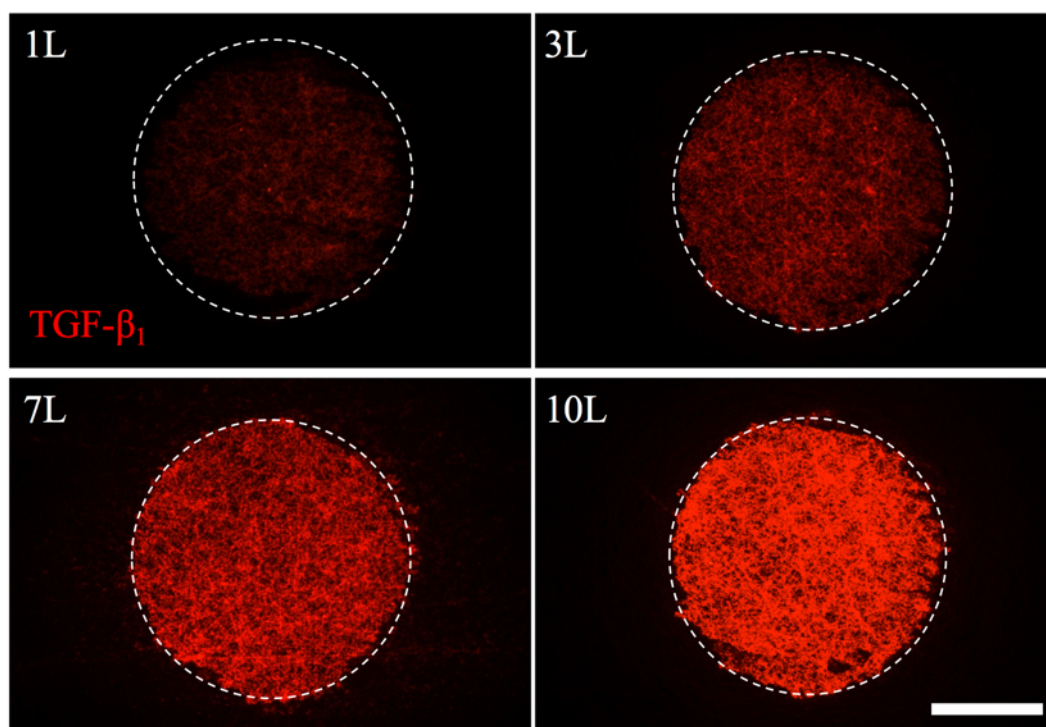

Figur S14. Fluorescence images of the 500-µm TGF-β1 dots printed onto PCL nanofiber matrices with various printing layers after staining: 1, 3, 7 and 10 layers. The fluorescence intensity increases over the repeated printing. All scale bars: 200 µm.
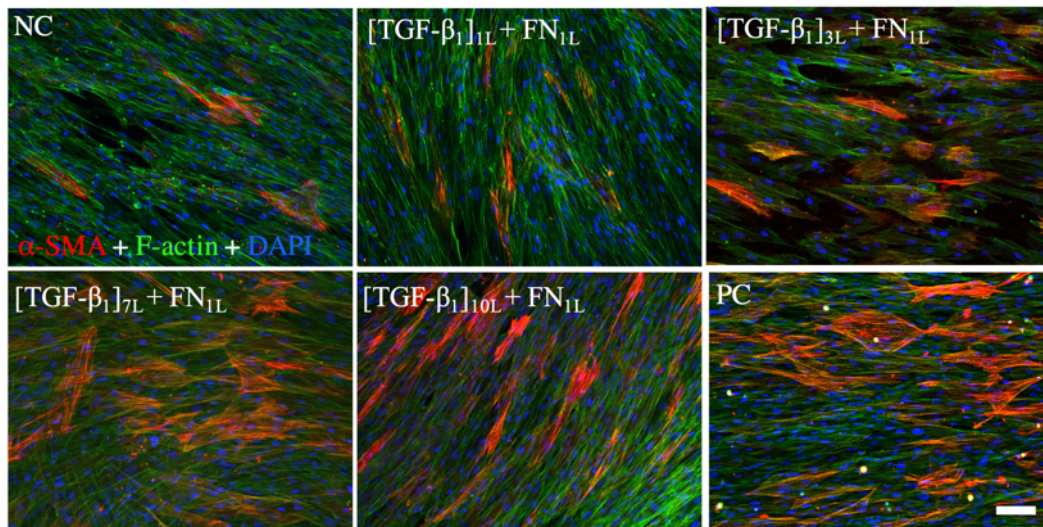
Figure S15. Fluorescence images of human dermal fibroblasts cultured on PCL nanofiber matrices without FN and TGF-β_1 (NC), or with 1 (1L), 3 (3L), 7 (7L), 10 (10L) repeated printing layers of TGF-β_1 and 1 layer of FN but without TGF-β_1 in medium and PCL nanofiber with no FN or TGF-β_1 printing but with 10ng/ml TGF-β_1 in medium (PC). Scale bar: 100 μm.
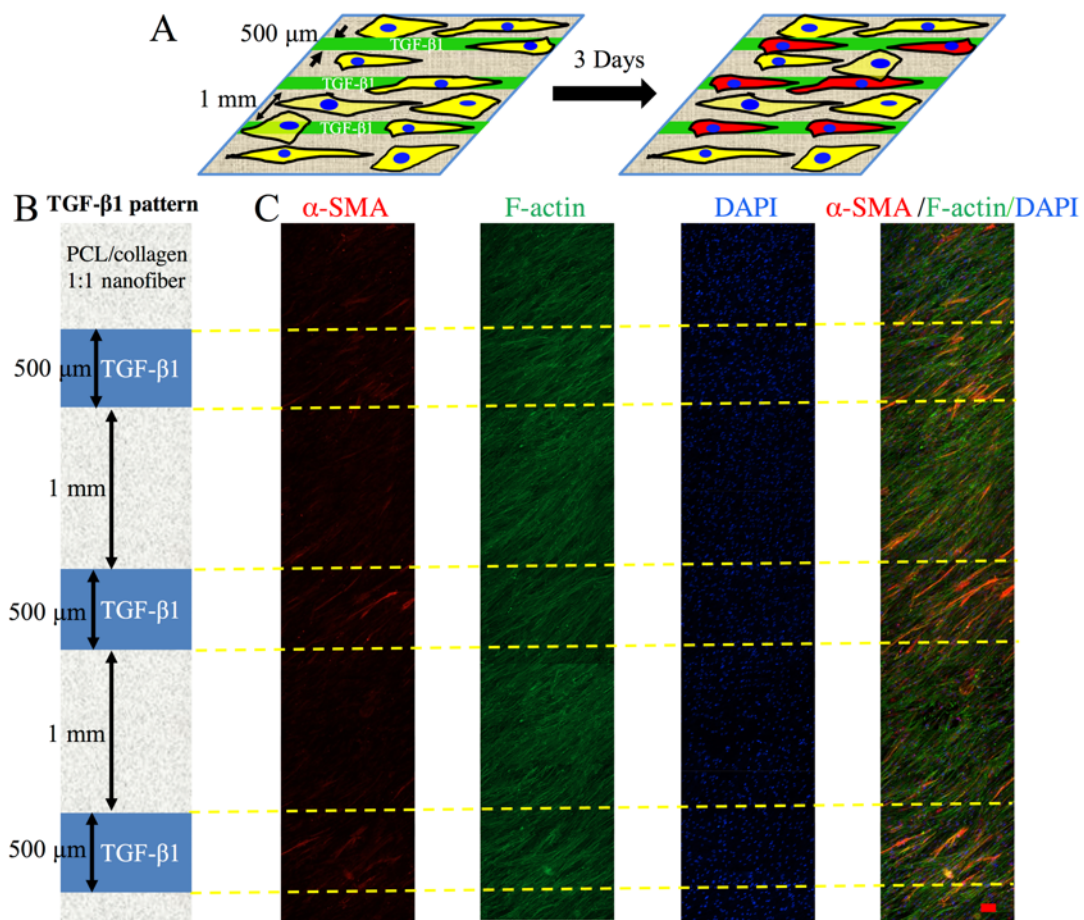
Figure S16. A) Schematic illustration on the experimental design for studying the effect of TGF-β1 printing on fibroblasts. 500-μm parallel lines of TGF-β1 are printed onto PCL/collagen nanofiber matrices and then the entire nanofiber matrices are seeded with human normal dermal fibroblasts (HNDFs). TGF-β1 can induce more

differentiate of fibroblasts into α-SMA positive cells. B) Illustration of the TGF-β1 printing pattern. C) Fluorescence images of human dermal fibroblasts covered the entire nanofiber matrix surface after 3-day culture and the cells within TGF-β1 printing lines exhibited more α-SMA positive cells. Scale bar: 200 μm.
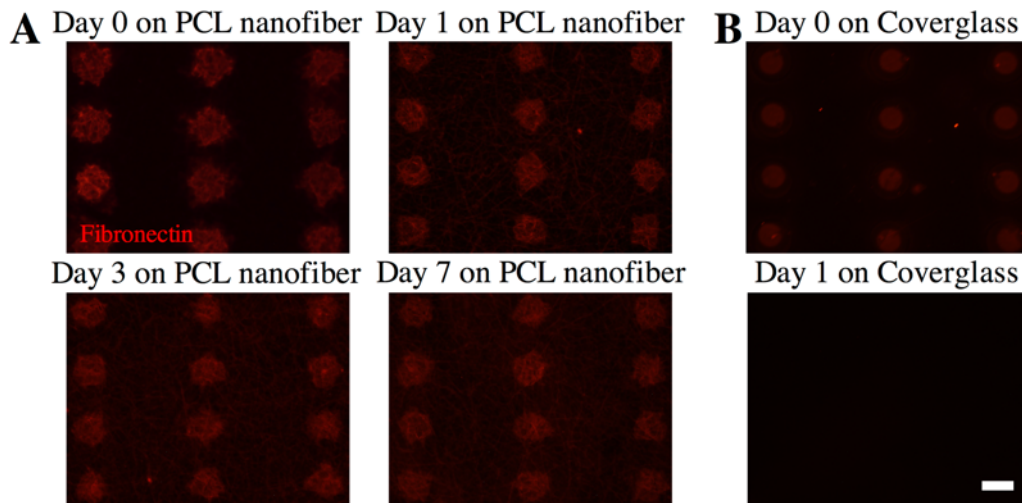

Figure S17. Fluorescence images showed the retention of printed FN on the PCL nanofiber matrices after incubation in culture media for 1, 3 and 7 days (A). In contrast, FN dots printed on the glass coverslip surface disappeared after 1-day incubation (B). All the dots were immunostained with anti-fibronectin antibody at each time point. All the printed samples were incubated in the typical fibroblast culture media (DMEM with 10% FBS) before staining. Scale bar: 50 μm.
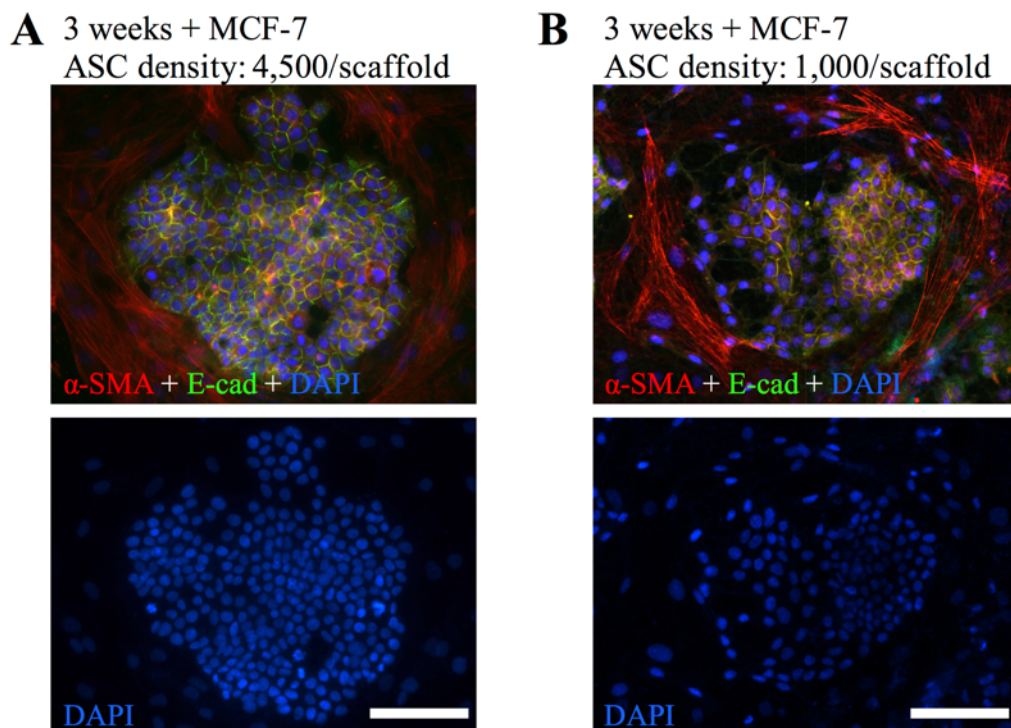
Figure S18. Fluorescence images of the co-cultured ASCs and MCF-7 cells stained for α-SMA, E-cadherin and nuclei (DAPI) after 3-week culture. Two different ASC seeding densities were evaluated: A). 4,500 cells/scaffold. B) 1,000 cells/scaffold. Scale bar: 100 μm.
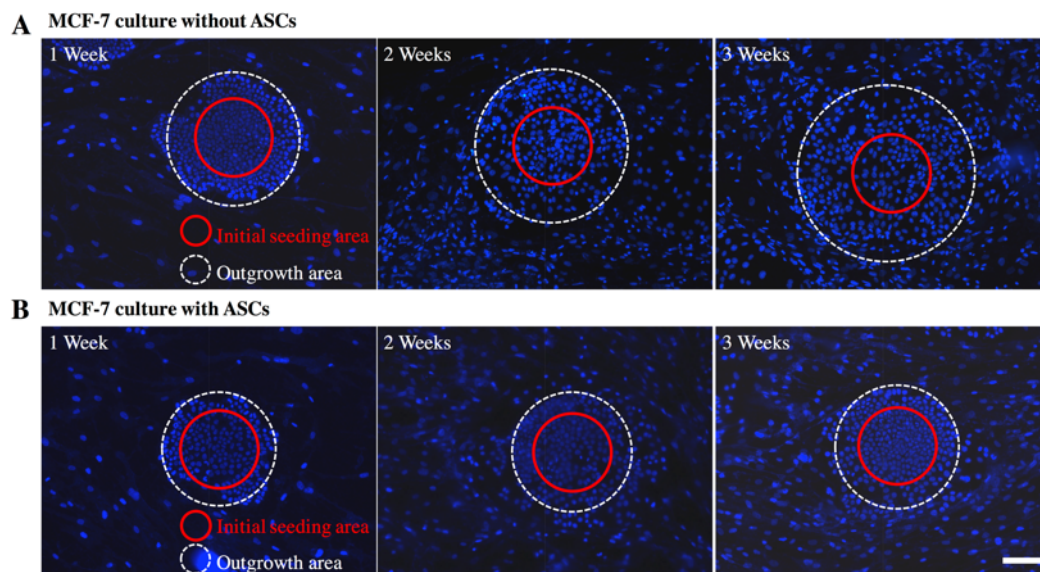
Figure S19. Fluorescence images of the co-cultured ASCs and MCF-7 cells stained for cell nuclei with DAPI showed the effect of ASCs on time-resolved changes of MCF-7 clusters. A) MCF-7 alone culture showed the cluster/colony formation after 1-week culture but significant cell migration out of the original clusters was observed as early as two weeks. B) Co-culture of MCF-7 cells with ASCs could help keep MCF-7 cells in clusters. Red circles indicate the initial seeding area of MCF-7 cells and dashed white circles indicate the outgrowth of MCF-7 after 1, 2, and 3 weeks. Scale bar: 100 μm.

A. MCF-7 clusters on both sides of endothelial cell line
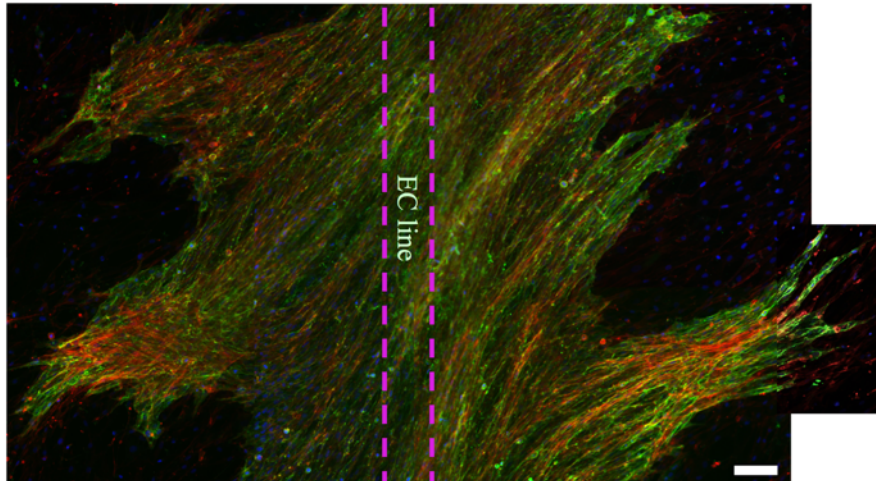
B. MCF-7 clusters on left side of endothelial cell line
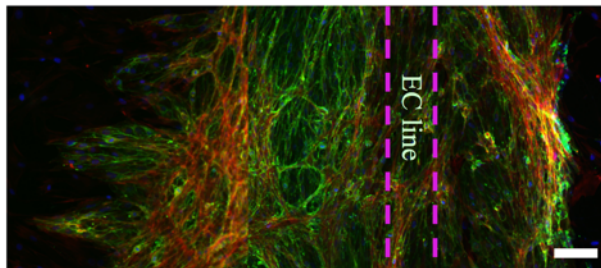
C. No MCF-7 clusters
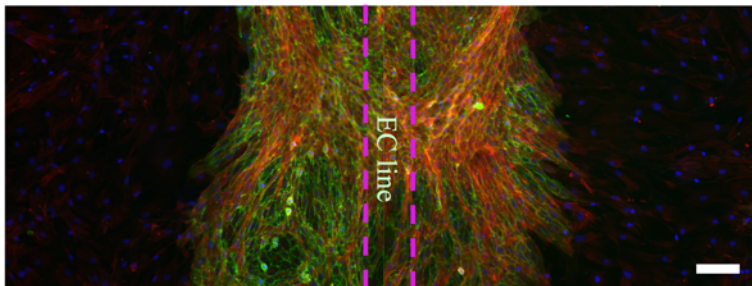
Figure S20. Fluorescence images of endothelial cells immunostained for CD31 and all cells stained with Phalloidin-TRITC and DAPI showed the endothelial sprouting with cancer clusters on both sides (A), on left side (B) and no cancer clusters (C). The dashed lines indicated the initial endothelial cells attached to FN lines. Scale bar: 100 μm.
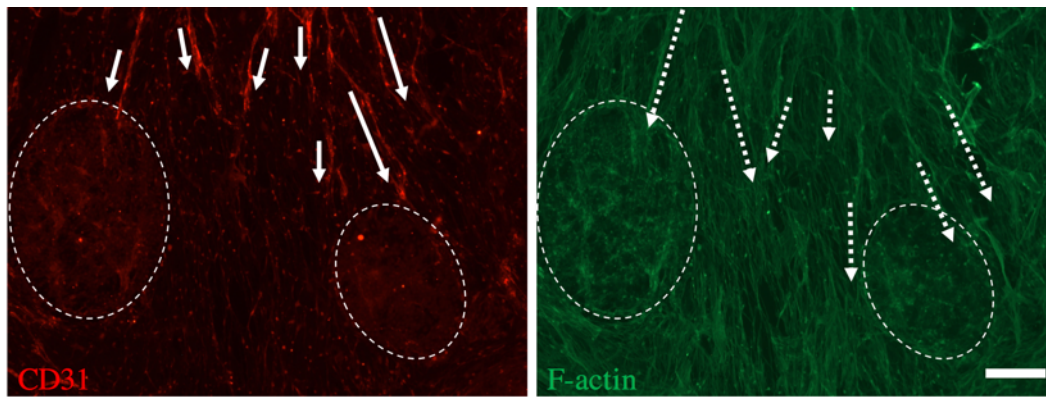
Figure S21. Fluorescence images of endothelial cells immunostained for CD31 and ASCs stained with Phalloidin-FITC showed the directional correlation between endothelial sprouting and the orientation of ASCs. White arrows on the left indicate the endothelial sprouting direction and the dashed arrows on the right showed the ASC orientation. The dashed white circles indicate the MCF-7 clusters. Scale bar: 100 μm.
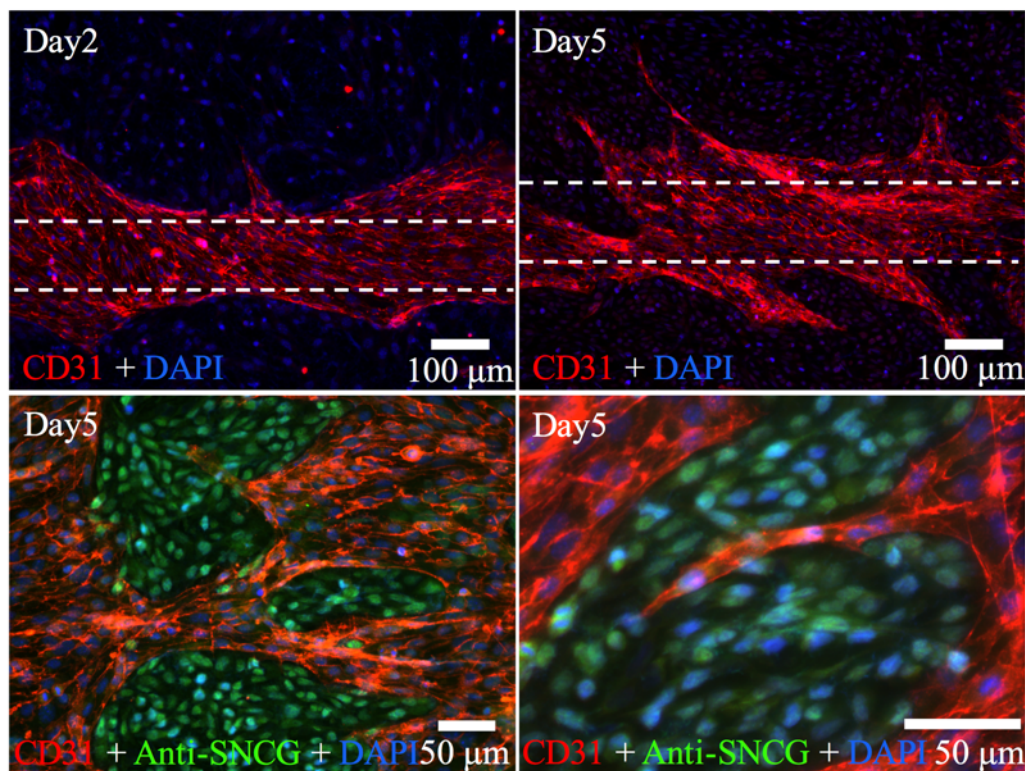
Figure S22. Fluorescence images of tumor angiogenesis, in which endothelial cells (MS-1) formed 100-μm wide lines for 2 days and then human breast cancer cells (MDA-MB 231) were seeded to occupy all the remaining area. After culture for another 3 days, cells were stained with anti-CD31, anti-SNCG and DAPI. Endothelial cells sprouted into the surrounding cancer domains, indicating the early angiogenesis.

Experimental sections

Materials. Polycaprolactone (PCL, Mw=80,000), fluorescein isothiocyanate (FITC), and tetramethylrhodamine (TRITC) were purchased from Sigma-Aldrich (St. Louis, MO) and type I collagen was obtained from Elastin Products Inc. (Owensville, MO). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was acquired from Oakwood Products, Inc. (West Columbia, SC). Fetal bovine serum (FBS) was obtained from Atlantic Biologicals (Atlanta, GA). All other reagents and solutions were from Thermo Fisher (Waltham, MA) unless otherwise indicated.

Electrospinning (ES) nanofiber meshes. PCL and collagen solution were separately prepared in HFIP at 8% (w/v). Solutions were stirred thoroughly overnight before mixed to prepare the blended solution of PCL and collagen at the ratio of 3:1, 1:1 and 1:3, respectively. All the solutions were thoroughly mixed for at least 12 hours before ES. For ES, the solution was transferred to a 5-mL syringe attached with a 23G tip-blunt nozzle. The solution was pumped through the nozzle at a steady flow rate of 10μL/min. To pull the solution into nanofibers, a high voltage of 15kV was applied between the nozzle and the grounded collector with a distance of 15 cm. Morphology and microstructure of the obtained nanofiber were evaluated by using the LEO 982 FEG scanning electron microscope (SEM) (Carl Zeiss SMT Inc., Peabody, MA) after gold-sputter coating.

Inkjet printing. The aqueous solutions of fibronectin (bovine plasma) (100 μg/mL), FITC- (10 μg/mL) or TRITC-conjugated BSA (10 μg/mL) were used as the ink, respectively. The ink was loaded into the cartridge and printed onto ES matrices following the pre-drawn AutoCAD patterns. Optimization of the printing parameters

was carried out by using 90 drop distance (DD) and 1 drop printing with different drop size (DS) on the ES matrices of various material combinations. The resulting patterns upon printing were observed by the Nikon Ti-E epifluorescence microscopy. The pattern size was calculated using ImageJ. Except for the parameter optimization experiments, otherwise the printing setup (10pL DS, 20 DD, 1 drop) was adopted for all other printings. The resulting pattern was also examined by the epifluorescence microscopy.

Cell seeding and culture. Mouse pancreatic islet endothelial cells (MS-1, ATCC), mouse smooth muscle cells (MOVAS, ATCC) and human breast cancer cells (MB-MDA-231, ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, 4.5g/L glucose) supplemented with 10% FBS and 1% penicillin-streptomycin. Human breast cancer cells (MCF-7, ATCC) were cultured in DMEM (1g/L glucose) supplemented with 10% FBS and 1% penicillin-streptomycin. Human adipose stromal cell (ASC, Coriell Institute of Medical Research) were cultured in the media composed of DMEM: MCDB 201: MCDB 131 (60:20:20) and supplemented with 0.5% FBS, 0.2% bovine serum albumin (BSA), 100μM L-ascorbate-2-phosphate, 100μM β-mercaptoethanol, 10ng/ml rhEGF, 5ng/ml rhPDGF-BB, 1nM dexamethasone. To seed the cells onto the nanofiber matrices with fibronectin patterns, the matrices were sterilized with UV irradiation for 20 minutes and then rinsed twice with culture media. In the experiments for cell patterning with different shapes and sizes, 150μl MS-1 or MOVAS cell suspension (1 million cells per ml) were seeded onto the fiber surface for 2 min and then rinsed thoroughly for 3 times using the culture media. The seeded matrices were continuously cultured at 37ºC with 95% humidity and 5% CO_2. To form the cancer cluster patterns and the blood vessel-

like patterns, MDA-MB-231, MCF-7 cells and MS-1 cells were seeded onto the fibronectin-printed fibrous matrices with a concentration of 1 million cells/ml. ASC cells were seeded onto the area without cells at the concentration of 1.0×10^5 cells per scaffold. All the culture was kept at 37ºC with 95% humidity and 5% CO_2 and media was refreshed every two days.

Cell patterning and function characterization. All the collected samples were fixed in 4% paraformaldehyde (PFA), permeabilized with 0.1% Triton X-100 and blocked with 3% BSA/PBS. Endothelial cells were stained with anti-CD31 antibody. MOVAS and ASC cells were stained with Phalloidin-TRITC. The cancer-activated stromal cells were stained with anti-smooth muscle α-actin antibody. Cell nuclei were stained with DAPI. The fluorescence images were obtained with the Nikon Ti-E epifluorescence microscope. Quantification of activated stromal cells and endothelial cell outgrowth were done using ImageJ.

Transmission electron microscopy. TEM images were obtained using an FEI CM20 FEG S/TEM. PCL fibers were electron-spun on 300-mesh copper grids and printed with FN. 5 μl of 1 wt% aqueous solution of uranylacetate (Ted Pella, Inc.) were deposited on the top of the spun fibers for staining for 5 mins, followed by droplet removal by a filter paper, and subsequent deposition and removal of 5 uL droplets of water for rinsing. The samples were then dried and analyzed by a TEM operated at 200 kV.

Statistical analysis. The results are all from at least 6 groups of experiment data and experiments were separately repeated three time. Statistical analysis was performed

using one-way ANOVA and $p < 0.05$ was considered statistically significant. NS indicated not significant, * indicated $p<0.05$, ** indicated $p<0.001$.