Supporting Information

Generation of Cost-Effective Paper-Based Tissue Models through Matrix-Assisted Sacrificial 3D Printing

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Materials and Methods. *Preparation of the bacterial cellulose hydrogel matrix.* The bacterial cellulose membranes (Hainan Yide Industry Co. Ltd., China) were cut into small pieces and washed by deionized water several times to remove residual mannitol agar medium and impurity.[1] The small pieces of bacterial cellulose were soaked in 8 w/v% NaOH solution at room temperature and stirred for 4 h to further purify the material.[2] Afterwards, the membranes were washed with plenty of deionized water to remove the remaining NaOH until neutral. Subsequently, the washed membranes were pulped with a mechanical homogenizer (Hamilton Beach Brands, USA) at the speed of 10,000 rpm and diluted with deionized water to obtain a slurry of 0.45-0.9 w/v % bacterial cellulose hydrogel.

Preparation of the petroleum jelly-liquid paraffin-based fugitive ink. The fugitive ink was prepared by mixing petroleum jelly (CVSHealth, USA) and liquid paraffin (Walgreens, USA) at different volume ratios (4:4, 3:4, 2:4, or 1:4). The blend was heated at 70 ºC, vigorously stirred for 10 min, and the bubbles were eliminated during heating. To aid visualization, 40 nM of rhodamine 6G (Sigma-Aldrich, USA) was mixed with the petroleum jelly-liquid paraffin inks.

Rheological properties of the bacterial cellulose hydrogels and the inks. The rheological properties of bacterial cellulose hydrogels and the fugitive inks with different compositions were characterized using a hybrid rheometer (HR-3, Waters, USA). The bacterial cellulose hydrogel matrices were kept stirring at room temperature pre-test to prevent sedimentation, while the fugitive inks were vigorously stirred at 70 ºC and then maintained still to remove bubbles immediately pre-test. Rheological measurements were performed for the bacterial cellulose matrices and the inks within 20 min. The different concentrations of bacterial cellulose in the matrices and different ratios of petroleum jelly and liquid paraffin in the inks

were assessed in a shear ramp mode running from 0.01 to 1000 s^{-1} with a 1000-μm gap size at 25 °C.

Matrix-assisted 3D printing. The 3D structures were prepared by extruding the petroleum jelly-liquid paraffin ink directly into a bacterial cellulose hydrogel matrix using a commercial bioprinter (Allevi I, Allevi Inc, USA). The 3D patterns were designed using SolidWorks (Dassault Systèmes, France) and converted to G-code by the bioprinter slicing software (Repetier-Host V1.6.1, Hot-World GmbH & Co. KG, Germany). The melted petroleum jelly-liquid paraffin as the fugitive ink was loaded into a 10mL syringe at 70 °C and cooled at 4 °C for 1 h before printing. The thickness of the extruded lines was controlled by extrusion pressure and translational speed of the printhead. The concentrations of the bacterial cellulose hydrogel matrices were explored in the range of 0.45-0.9 w/v %, and the ratios of petroleum jelly-liquid paraffin ink were examined at 4:4, 3:4, 2:4, and 1:4 volume ratios to optimize the 3D printing conditions.

Fabrication of 3D perfusable microchannels in paper devices. The paper-based devices containing perfusable microchannels were obtained by removing the fugitive inks. First, the fugitive ink was printed inside of the bacterial cellulose hydrogel matrix and the entire matrix along with the ink was air-dried to form a paper-like membrane. Second, the dried paper membrane was heated to 70 °C in an oven for 5-10 min to melt the ink, and the melted ink was removed by blowing air through the microchannels. Third, the membrane was washed with *n*-hexane at 70 °C for 2 h and centrifuged at 3000 rpm for 40 min to remove *n*-hexane from the microchannels. Then, the membrane was washed with ethanol and centrifuged at 3000 rpm triple times. Finally, the membrane was cleaned with distilled water for three times and air-dried again at room temperature to form a clean paper device containing hollow, perfused microchannels. To visualize the microchannels in different layers, fluorescent microbeads were

injected. Microchannels and the cross-sections were imaged using a fluorescence microscope (Olympus BX51, Japan).

Porosity and water retention of paper devices. The porosity of the bacterial membrane was determined by the fluid replacement method.[3, 4] Ethanol was used as the displacement shrinkage liquid since it can easily penetrate into the pores of the membrane but do not cause shrinkage or swelling. The bacterial cellulose membrane was soaked in absolute ethanol until saturated. The masses of the bacterial membrane were measured before and after soaking. The porosity (P) was calculated according to the following equation.

$$P = \frac{W_m - W_0}{\rho V_{BC}} \times 100\% \qquad (1)$$

In brief, W_m and W_0 are the weight of the membranes before and after immersion in ethanol, respectively. V_{BC} is the volume of the bacterial cellulose membrane before immersion in ethanol, which was measured by the formula of length × width × height of the membrane. ρ is the density of ethanol (0.789 g cm^{-3}). The value was expressed as the mean of three replicates for each bacterial cellulose membrane.

To evaluate the water retention rate, membrane samples soaked with double deionized water were transferred in a centrifuge tube and centrifuged at 3500 rpm for 3 min to remove excessive water. The water retention rate was measured with the following equation.

$$WR = \frac{M_h - M_0}{M_0} \times 100\% \qquad (2)$$

, where M_h is mass of the membrane after centrifugation, and M_0 is the initial dry mass of the membrane. The value was mean of three replicates for each membrane.

Cell culture. According to experimental requirements, two different types of cells were used, GFP-tagged HUVECs and MCF-7 breast cancer cells. GFP-tagged HUVECs were cultured in endothelial growth medium (EGM, Lonza, USA) supplemented with 1 v/v % penicillin-streptomycin (ThermoFisher, USA). MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, ThermoFisher) supplemented with 10 v/v % fetal bovine serum (FBS, ThermoFisher) and 1 v/v % penicillin-streptomycin. The cells were cultivated at 37 °C and 5% CO_2 until 70% confluency. Prior to cell seeding, the paper-based devices containing perfusable microchannels were autoclaved for 15 min to rehydrate as well as to sterilize. Subsequently, 100 μL of fibronectin (50 ng mL^{-1}) was injected into the microchannels and the devices were maintained at 37 °C for 1 h. HUVECs were seeded into the microchannels at a density of 10×10^6 cells mL^{-1} for 30 min, and the devices were flipped on the side for another 30 min to ensure uniform cell attachment. After 2 h or culture, MCF-7 cells (2×10^6 cells mL^{-1}) were further inoculated onto the surface of the paper-based device for 30 min and this was repeated on the reverse surface.

Cell viability and morphology analyses. A Live/Dead® Cell Viability Kit (ThermoFisher) was used to evaluate of cell viability according to the manufacturer's instructions at days 1, 3, 7, and 14 after cell seeding in the paper-based devices. The samples were stained with calcein-AM (green)/ethidium homodimer-1 (red), followed by observation under an inverted fluorescence microscope (Nikon Ti-E, Japan). At least six images from different areas were randomly selected to calculate the number of live and dead cells using ImageJ (National Institutes of Health, USA), and the cell viability was expressed as the percentage of the number of live cells to the total cell number. Cell proliferation of paper-based devices were determined by PrestoBlue (ThermoFisher) to evaluate the metabolic activity. A mouse sourced antibody against basement membrane protein collagen type IV (Abcam, USA) was diluted at a 1:100 ratio in PBS (ThermoFisher) containing 5 v/v % goat serum (ThermoFisher) and 0.2 v/v %

Triton X-100 (Sigma-Aldrich) for 24 h at 4 ºC. After being washed with PBS, the samples were incubated with Alexa Fluor 594-goat anti-mouse secondary antibody (ThermoFisher) for 12 h at 4 ºC. Confocal images were taken using a Zeiss Confocal Microscope (Zeiss LSM 880 with Airyscan, Germany) and reconstructed using image J.

Tamoxifen treatment. 10 µM of tamoxifen was injected into the microchannels after 24 h of culture of the vascularized breast tumor models in the paper devices and the perfusion was continued for another 48 h. Cell viability was subsequently measured as described above.

Figure S1. Photograph showing flexibility of the bacterial cellulose film.

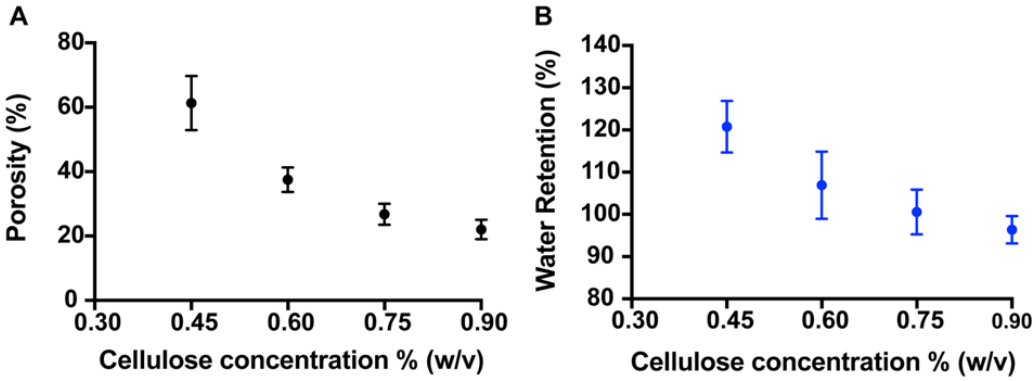

Figure S2. (A) Porosity and (B) water retention of the paper devices.

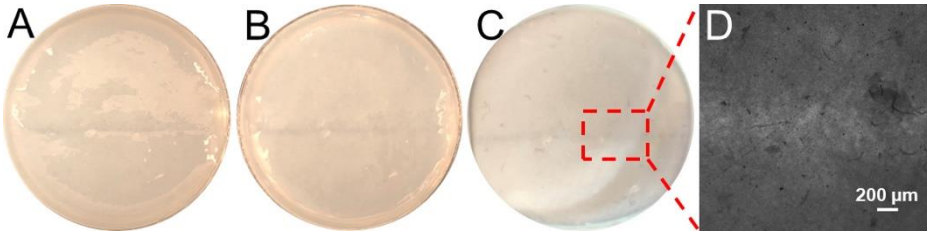

Figure S3. (A-C) Photographs showing self-healing ability of the bacterial cellulose hydrogel matrix during the air-drying process after needle scratch (without the ink deposition) at (A) 0 h, (B) 12 h, and (C) 24 h (completely dried). (D) Optical micrograph (transmission mode) of the paper device indicating the slightly thinner cellulose nanofibers in the region of scratch.

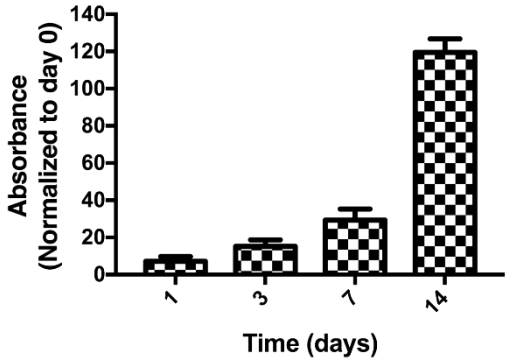

Figure S4. Quantification of proliferation of the cells over the 14 days cultured.

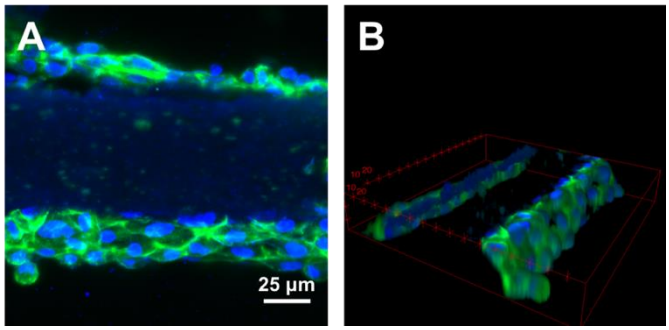

Figure S5. Confocal microscopy images showing (A) cross-sectional view and (B) reconstructional view of the paper device after MCF-7 culture for 7 days.

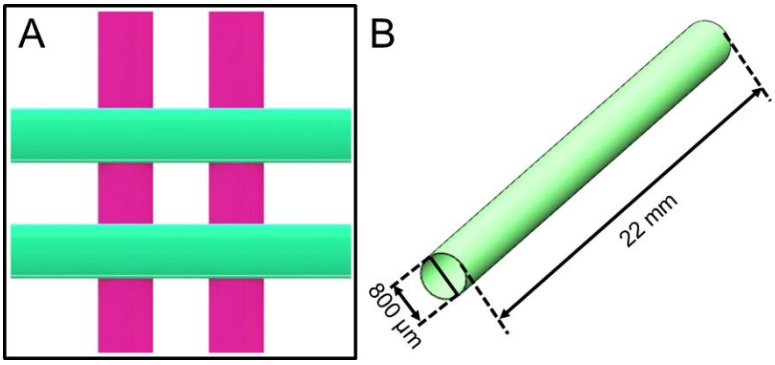

Figure S6. The dimensions of a typical paper device used for generating our *in vitro* tumor models. (A) Schematic diagram of the paper device containing four microchannels in two different layers. (B) Dimensions of the microchannel in the paper device.

Table S1. Material cost analysis of a single piece of the paper device generated with matrix-assisted sacrificial printing.

Ink component	Unit Cost (USD/mL)	Volume (mL) [#]	Cost (USD)
Bacterial cellulose[†]	0.0063	5.0000	0.0313
Petroleum jelly[‡]	0.0110	0.1894	0.0021
Liquid paraffin[‡]	0.0127	0.2526	0.0032
Total cost			0.0366

[#] Volumes based on the dimensions of a paper device as shown Figure S6

[†] Calculation based on mass ratio of bacterial cellulose solution at 0.6 %

[‡] Calculation based on the ratio of petroleum jelly-liquid paraffin at 3:4

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