Electronic Supplementary Material (ESI) for Lab on a Chip.

ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

3D Microfluidics *via* Cyclic Olefin Polymer-Based *In-Situ* Direct Laser Writing

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Supplementary Text

COP replication using PDMS negative master molds

In this work, we micromolded a 3-mm-thick COP sheet using a Si substrate with DLW-printed patterns as the negative master mold *via* hot embossing protocols (**Fig. 1a-c**). One caveat to this approach, however, is that the high temperatures and pressures applied during hot embossing processes can limit the overall lifespan of the negative master mold. Thus, for cases that demand high numbers of COP replication steps for a single mold design, we present a secondary approach in which an additional negative master mold is fabricated using the silicone elastomer, PDMS.

The initial steps of the protocol are consistent with those depicted in **Figure 1a-d**, with a 3-mm-thick COP sheet being replicated from a DLW-patterned Si substrate. Once the micromolded COP sheet has been produced, however, it is rinsed with IPA and dried with inert N_2 gas. A 5:1 (base:curing agent) mixture of PDMS (Sylgard 184, Dow Corning, Corning, NY) is poured over the COP sheet, degassed in vacuum for 30 min, and then placed on a hot plate set at 60 °C for 2 hours (**ESI Fig. S6a**). After thermal curing, the PDMS is peeled off of the COP (**ESI Fig. S6b**). Thereafter, the molded PDMS can serve as a negative master for additional COP replication steps. Specifically, the PDMS replica can be used in place of the original DLW-patterned Si negative master mold to facilitate PDMS-based hot embossing of 3-mm-thick COP sheets at 120 °C for 5 min (**ESI Fig. S6c,d**). Fabrication results for PDMS negative master molds corresponding to various microchannel profiles are presented in **ESI Figure S6e-h**. The benefit of this approach is that the overall lifespan of the original DLW-based mold can be extended significantly, thereby limiting the time and labor associated with DLW of multiple negative master molds.

***Is*DLW inside PDMS-on-glass microchannels**

To provide insight into the differences is microfluidic sealing behavior between the presented COP-based *is*DLW approach and those based on PDMS, we also fabricated the 3D microfluidic bellow-type transistor directly inside of PDMS-to-glass microchannels (**ESI Fig. S8a**). To probe the sealing integrity of the PDMS-based print, we loaded methylene blue-dyed fluid into the gate region under an applied input pressure of 10 kPa. Experimental results revealed the inputted fluid readily permeated all areas of the microfluidic channels, including undesired flow into the source-to-drain region and around the exterior of the microfluidic transistor structure (**ESI Fig. S8b**; **ESI Movie S8**). Under applied input pressures $\geq$ 200 kPa, the PDMS channel exhibited significant expansion, with observable detachment from the microfluidic transistor (**ESI Fig. S8c**; **ESI Movie S8**). In contrast, we did not observe any such leakage or structure-to-channel sealing failures for cases in which the microfluidic transistor was *is*DLW inside of COP-COP microchannels. Rather, both brightfield (**ESI Fig. S8d-f**; **ESI Movie S9**) and fluorescence microscopy (**ESI Fig. S8g-i**) results for methylene blue and rhodamine B-dyed fluids loaded into the gate and source-to-drain regions, respectively, revealed effective isolation of the flow streams (without any observable cross-contamination) for input pressures up to 500 kPa.

Supplementary Figures

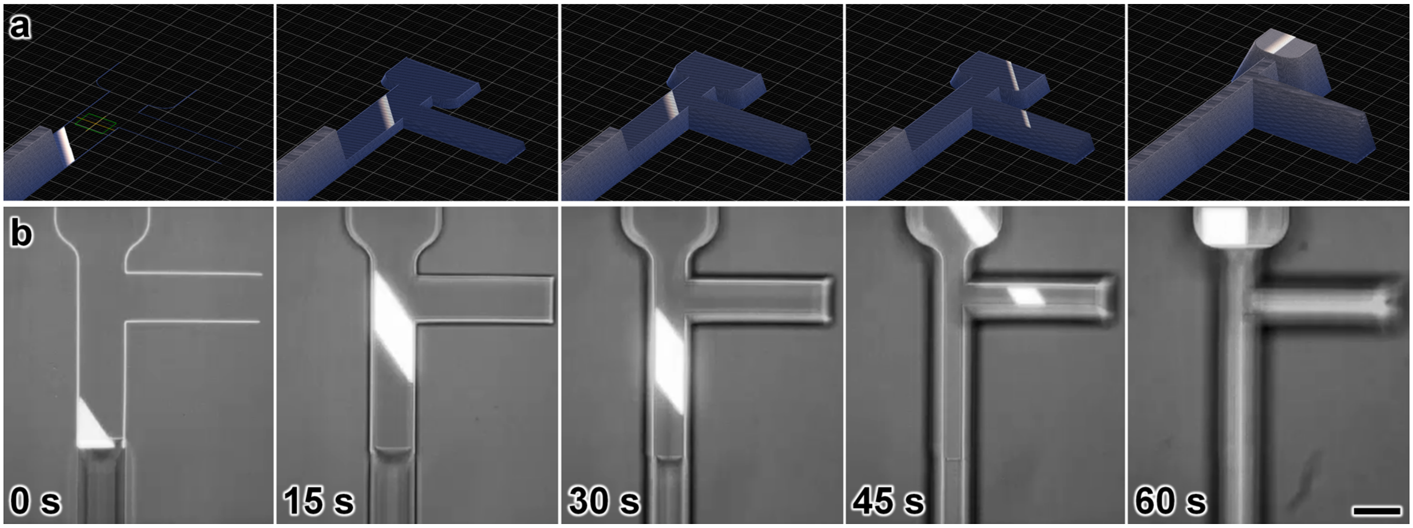

Fig. S1 Fabrication of microchannel negative master molds *via* “stitching”-based DLW. (**a**) Sequential computer-aided manufacturing (CAM) simulations of printing a new 280 μm $\times$ 280 μm area of the mold that is connected to a previously fabricated part of the mold. (**b**) Corresponding sequential brightfield micrographs of results for the DLW fabrication process. Scale Bar = 100 μm (*see also* **ESI Movie S1**).

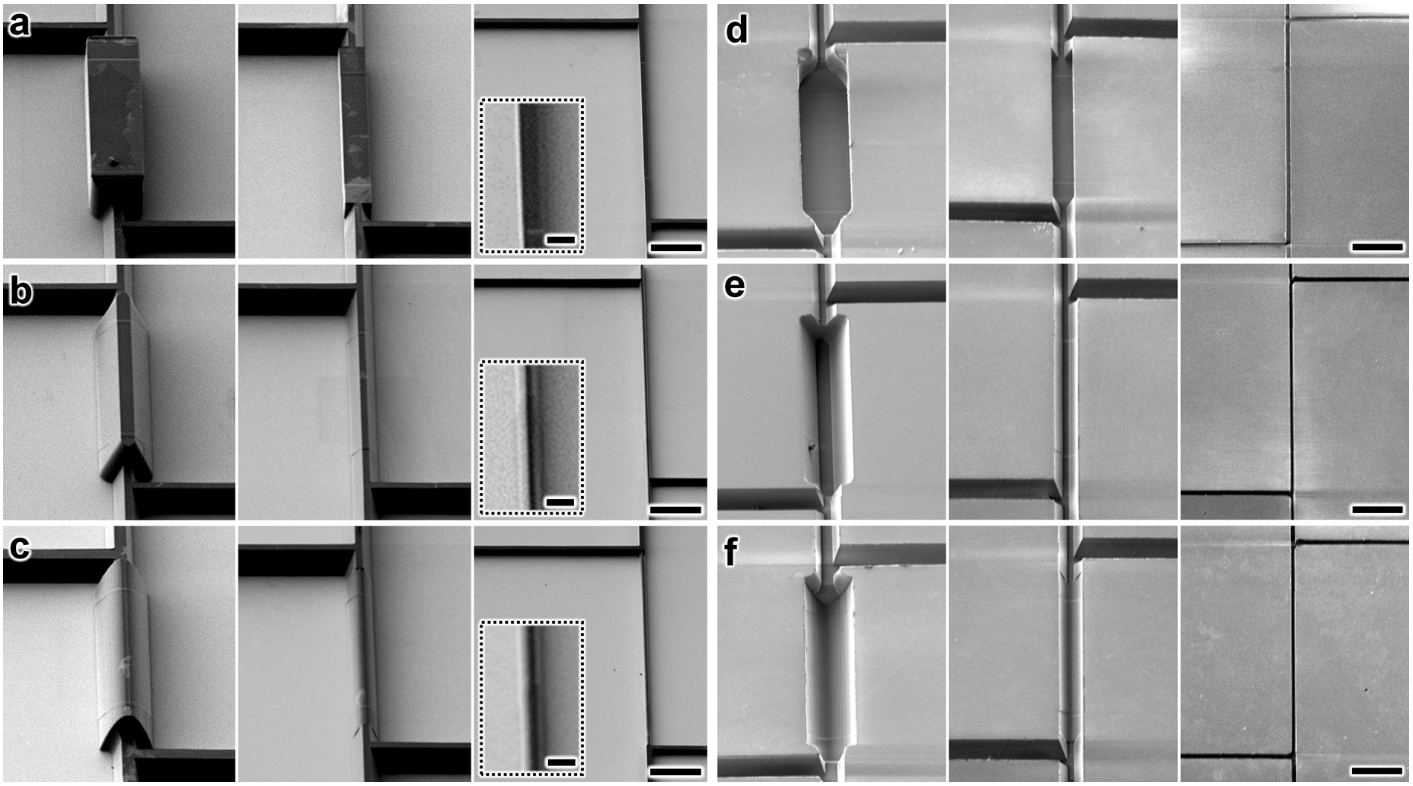

Fig. S2 Fabrication results for (**a-c**) DLW-printed negative master molds and (**d-f**) hot embossing-replicated COP corresponding to channel heights of: (*Left*) 100 μm, (*middle*) 50 μm, and (*right*) 10 μm. (**a**) Rectangular channel molds. (**b**) Trapezoidal (20°) channel molds. (**c**) Semi-ovular channel molds. (**d**) Replicated rectangular channels. (**e**) Replicated trapezoidal channels. (**f**) Replicated semi-ovular channels. Scale bars = 100 μm; Inset scale bars = 10 μm

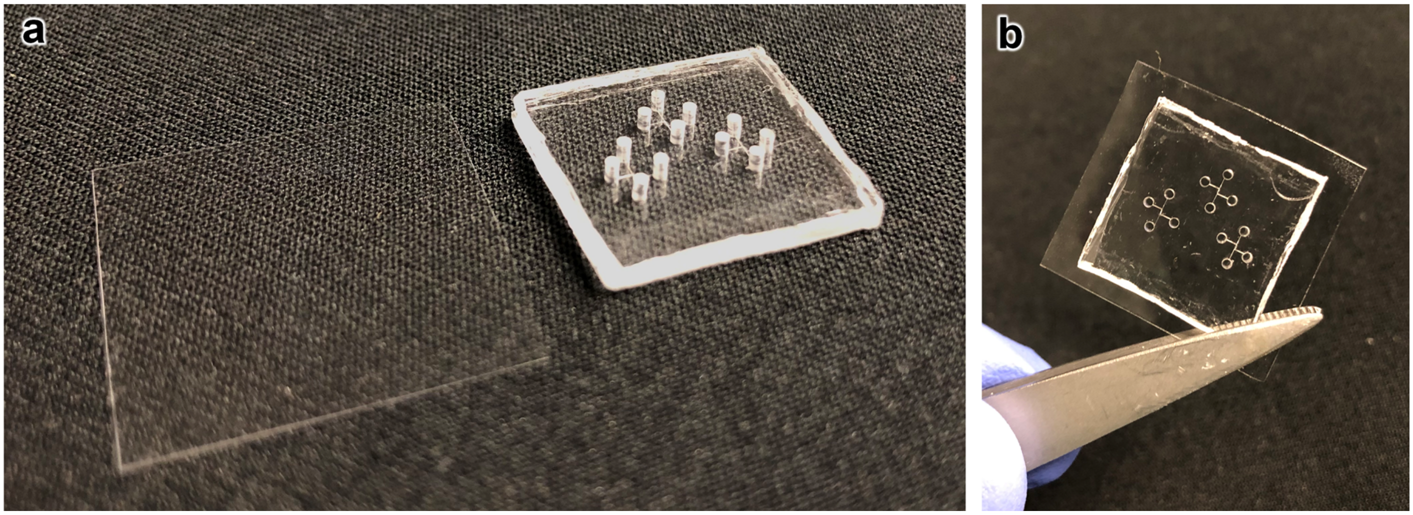

Fig. S3 Fabrication results for the COP-COP microfluidic device. (**a**) Image of COP components prior to the bonding process. (*Left*) A 100-μm-thick COP sheet. (*Right*) Micromolded COP with access ports at inlet and outlet locations. (**b**) Image of a COP-COP microfluidic device following the vapor-phase solvent bonding process.

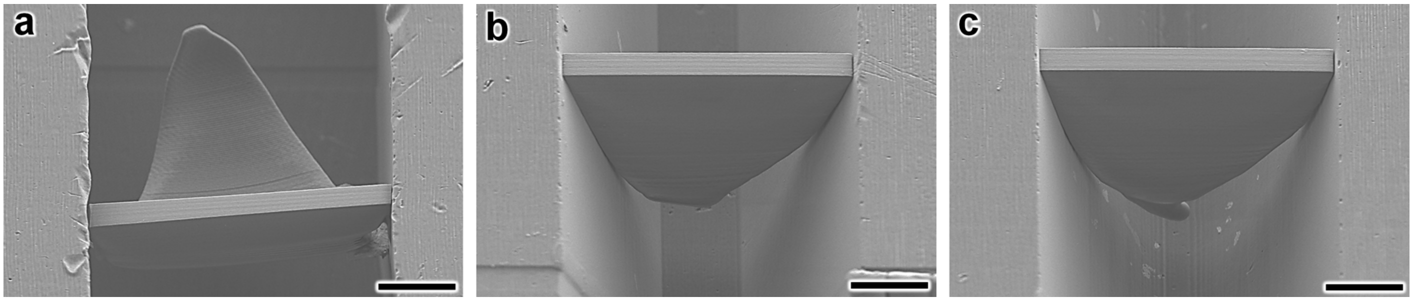

Fig. S4 Micrographs of fabrication results for *is*DLW of 10-μm-thick fluidic barrier structures in 100-μm-tall microchannels of various profiles (aspect ratio = 1) corresponding to a constant laser power (20 mW) and laser scanning speed (10 mm s^{-1}). (**a**) Rectangular cross-sectional profile. (**b**) Trapezoidal (20°) cross-sectional profile. (**c**) Semi-elliptical cross-sectional profile. Scale bars = 20 μm

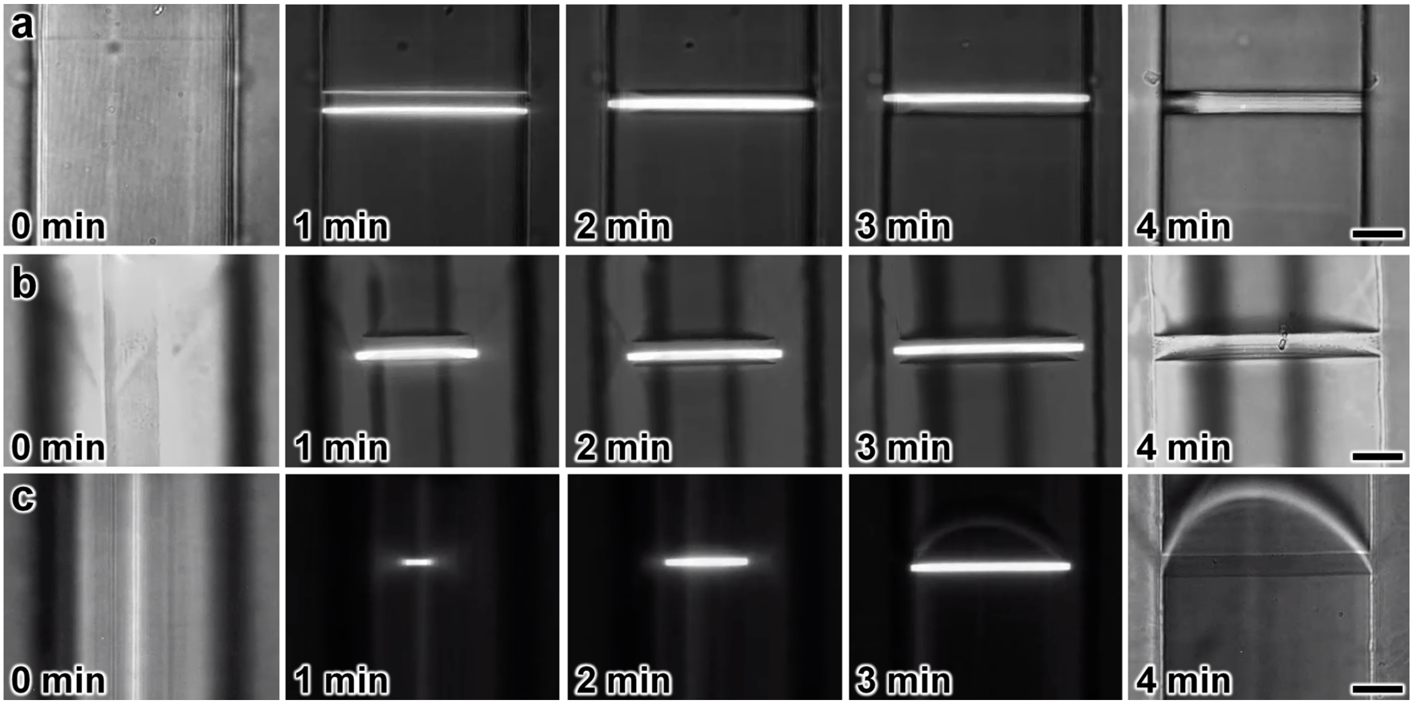

Fig. S5 Sequential micrographs of isDLW-printing of 10-μm-thick fluidic barrier structures inside 100-μm-tall channels by varying the laser power with height. (**a**) Rectangular cross-sectional profile. (**b**) Trapezoidal cross-sectional profile. (**c**) Semi-elliptical cross-sectional profile. Scale bars = 25 μm (*see also* **ESI Movie S2**).

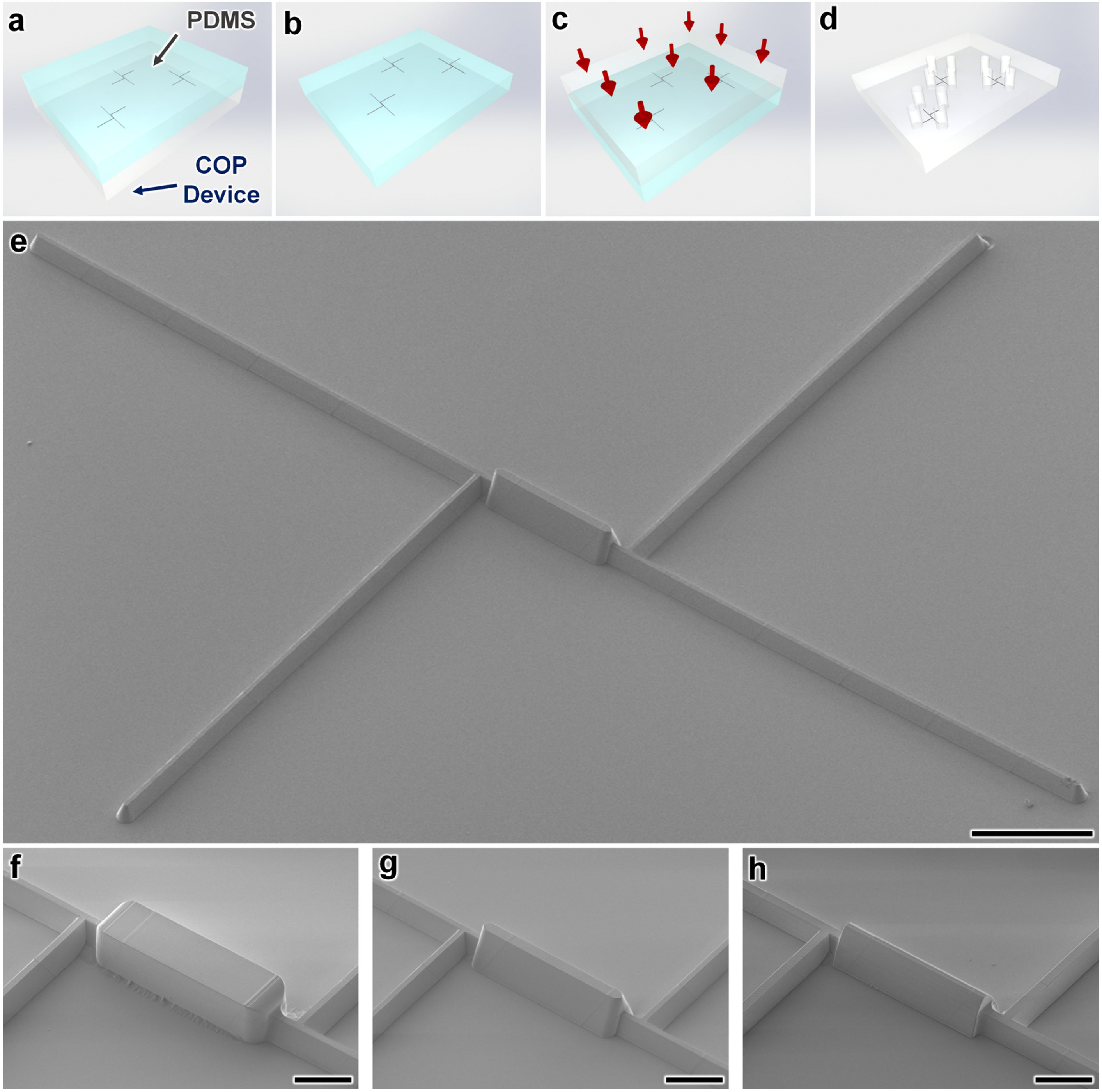

Fig. S6 COP replication using PDMS negative master molds. (**a-d**) Conceptual illustrations. (**a**) Replication of PDMS using a micromolded COP sheet. (**b**) Fabricated PDMS negative master mold. (**c**) Hot embossing-based replication of micromolded COP using PDMS mold. (**d**) Micromolded COP. (**e-h**) Micrographs of fabrication results for PDMS molds (100 μm in height) with various channel profiles. (**e**) Expanded view of the PDMS mold for the trapezoidal cross-sectional profile. Scale bar = 300 μm. Close-up views of results corresponding to the (**f**) rectangular, (**g**) trapezoidal, and (**h**) semi-elliptical cross-sectional profiles. Scale bars = 100 μm

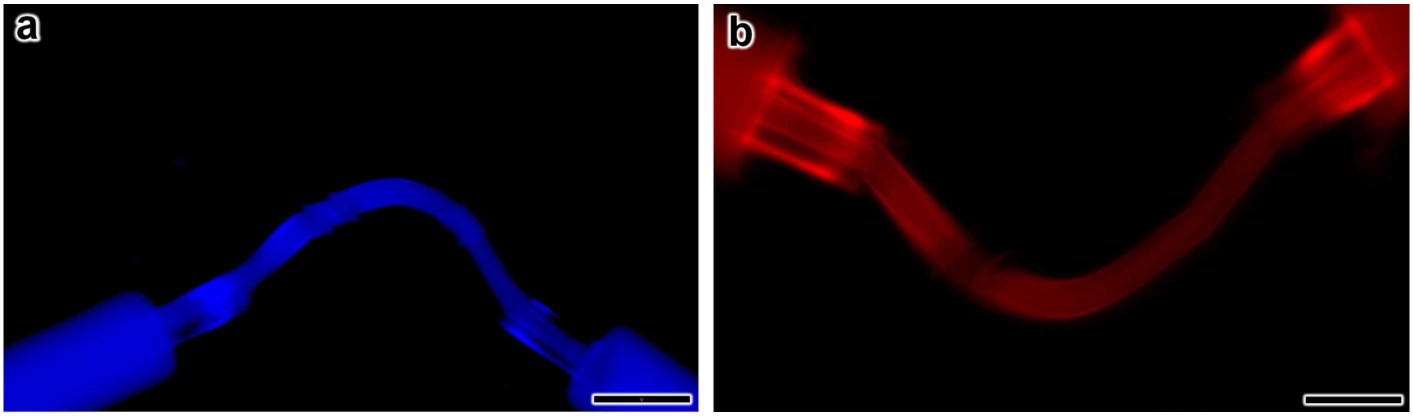

Fig. S7 Fluorescence micrographs of the interwoven microvessel structure filled with distinctly labelled fluids. (**a**) Methylene Blue. (**b**) Rhodamine B. Scale bars = 50 μm

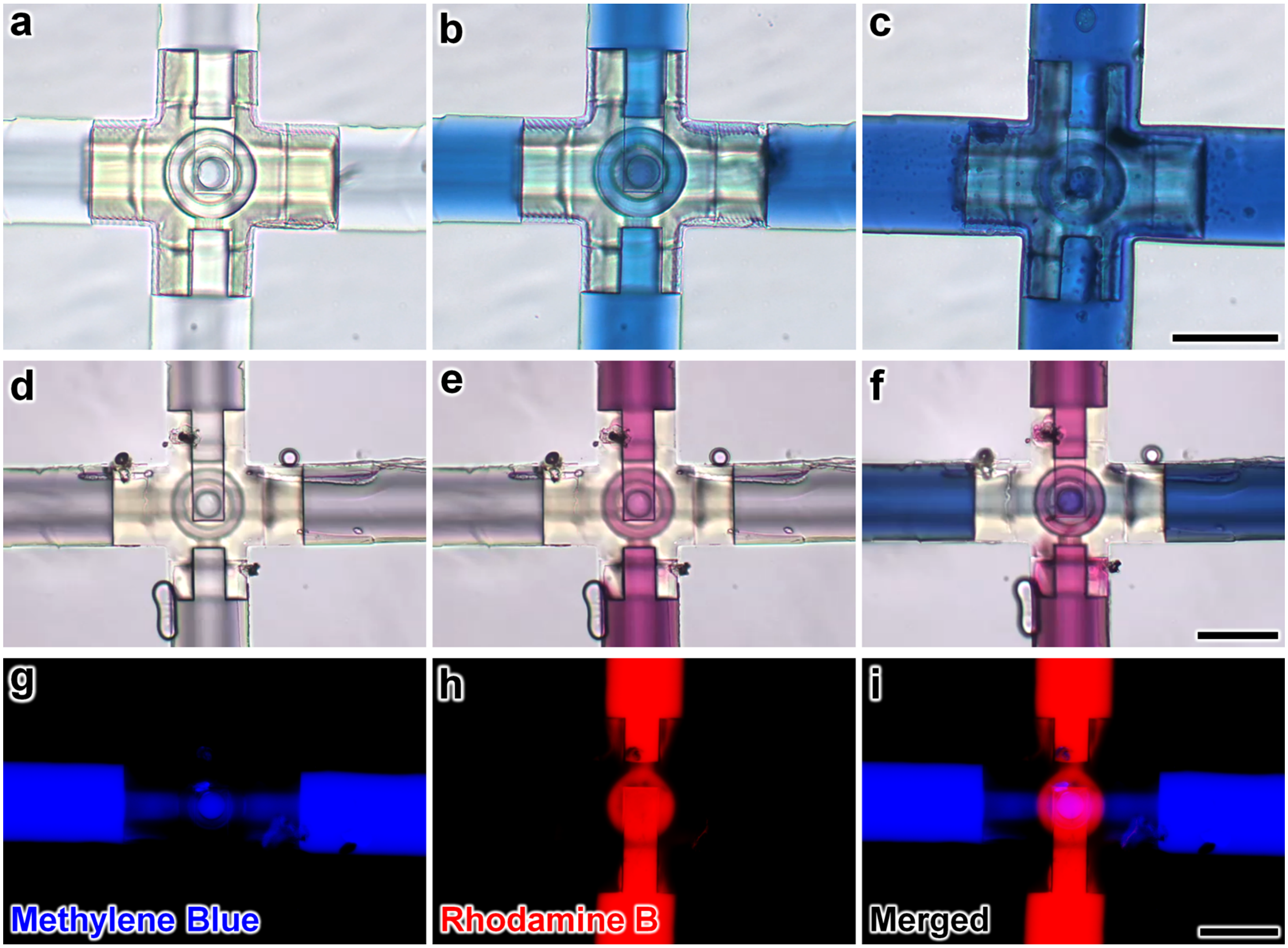

Fig. S8 Experimental results for comparison of (**a-c**) PDMS-based *is*DLW and (**d-i**) COP-based *is*DLW. **(a-c)** Micrographs of a 3D microfluidic bellow-type transistor *is*DLW-printed inside of a PDMS-on-glass device corresponding to microfluidic loading of methylene blue-dyed fluid into the gate region at input pressures of: (**a**) 0 kPa, (**b**) 10 kPa, and (**c**) 200 kPa. Scale bar = 30 μm (*see also* **ESI Movie S8**). (**d-f**) Micrographs of a 3D microfluidic bellow-type transistor *is*DLW-printed inside of a COP-COP device: (**d**) before loading of fluids, (**e**) after loading of a rhodamine B-dyed fluid (*pink*) into the source-to-drain region, and then (**f**) after loading of a methylene blue-dyed fluid (*blue*) into the gate region. Scale bar = 50 μm (*see also* **ESI Movie S9**). (**g-i**) Fluorescence micrographs of the 3D microfluidic bellow-type transistor *is*DLW-printed inside of a COP-COP device corresponding to distinct fluorescence signatures in the source-to-drain and gate regions. Scale bar = 50 μm

Supplementary Movie Captions

Movie S1. Fabrication of a 100-μm-tall trapezoidal microchannel negative master mold pattern onto a Si substrate *via* “stitching”-based DLW. Video speed = 30×; Scale bar = 100 μm.

Movie S2. Fabrication results for *is*DLW-printing of a 10-μm-thick fluidic barrier structures inside a 100-μm-tall channel with (**a**) rectangular, (**b**) trapezoidal, and (**c**) semi-elliptical cross-sectional profiles by varying the laser power with height. Video speed = 10×; Scale bar = 25 μm.

Movie S3. CAM simulations (*left*) and corresponding fabrication results (*right*) for the *is*DLW printing process of interwoven microvessel-inspired structures. Video speed = 30×; Scale bar = 50 μm.

Movie S4. Experimental results for COP-based *is*DLW-printed microvessel-inspired structures during loading of rhodamine B-labelled fluid (*pink*) and methylene blue-labelled fluid (*blue*). Scale bar = 50 μm.

Movie S5. Results for 3D COMSOL Multiphysics fluid-structure interaction (FSI) simulations of fluid velocity field (*colored arrows*) and displacement distributions for the 3D microfluidic bellow-type transistor with P_S = 10 kPa and P_G increasing from 0 kPa to 90 kPa.

Movie S6. CAM simulations (*left*) and corresponding fabrication results (*right*) for the *is*DLW printing process of the 3D microfluidic bellow-type transistor. Video speed = 15×; Scale bar = 50 μm.

Movie S7. Experimental results for the 3D microfluidic bellow-type transistor during dynamic operation of the gate. Video speed = 5×; Scale bar = 50 μm.

Movie S8. Microfluidic loading of a methylene blue-labelled fluid into the gate region of a 3D microfluidic bellow-type transistor *is*DLW-printed inside of a PDMS-on-glass device. Scale bar = 50 μm.

Movie S9. Microfluidic loading of a rhodamine B-labelled fluid and a methylene blue-labelled fluid into the source-to-drain region and gate region, respectively, of a 3D microfluidic bellow-type transistor *is*DLW-printed inside of a COP-COP device. Scale bar = 50 μm.