Supplementary Material (ESI) for *Soft Matter*

Electronic Supporting Information (ESI)

Microfluidic Contact Printing: a Versatile Printing Platform for Patterning Biomolecules on Hydrogel Substrates

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Content:

Membrane modification

Polyvinylpyrrolidone (PVP) or PVP-free (PVPF) PCTE membranes (0.22 μm pore, GE Osmonics Labstore, Minnetonka, MN) were carefully placed in conformal contact with 1 mm thick PDMS slabs, which act as sacrificial support layers during surface modification. Care was taken to minimize scratches and deformation on the membranes throughout every step of the modification procedure, as small scratches detrimentally affect future patterning attempts. A 50 Å adhesion layer of titanium, followed by 100 Å of SiO_2 were evaporated onto the surface of the membranes, at a rate of approximately 0.1 Å/s to provide a uniform surface coverage (Temescal FC-1800 electron beam evaporator). This coverage, given the ballistic nature of the deposition method, is not conformal to the high aspect ratio pore interiors, which remain hydrophobic. Scanning electron microscopy (JEOL 6060LV General Purpose SEM, Tokyo, Japan) was used to image the membrane before and after deposition. Membrane pore sizes were measured using Image Pro Express (Media Cybernetics, Inc., Bethesda, MD) and analyzed by Microsoft Excel (Fig. S1).

Silicon master fabrication

Silicon wafers were scored into desired sizes, cleaned with piranha solution, rinsed with deionized water (Milli-Q, Millipore, Billerica, MA) and then blown dry with nitrogen. SU8-50 (MicroChem Corp., Newton, MA) was spun-coated onto the wafer pieces at 2900 rpm, with a ramping of 100 rpm/sec, for 30 seconds. The wafers were pre-baked for 5 minutes at 120 ºC and patterned by exposing to UV (MJB3 Mask Aligner, Suss Microtech, Garching, Germany) for 45 seconds. Exposed wafers were then post-baked for 5 minutes at 120 ºC and cooled, before being developed with SU8 developer (Microchem Corp.). All patterned wafers were treated with tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane

(Sigma Aldrich, St. Louis, MO) for two hours in a dessicator under vacuum, to prevent adhesion of PDMS to the fabricated masters.

Softly cured PDMS channel

Irreversible interfacing between the PDMS microchannels and supporting substrate was significantly improved when the PDMS was soft or slightly-under cured. In the fabrication process described here, the PDMS is cured long enough to be removed from the master, but can still be deformed when gently manipulated with a tweezers (i.e. a small indent on the surface can be seen versus fully cured PDMS, which cannot be deformed in such a manner). To quantitatively characterize the experimental condition of the soft-cure, the curing ratio first proposed by Go and Shoji was used.[1] The curing ratio, R, is defined as $R= t_s/t_f$, where t_s is the soft cure time and t_f is the full cure time. The full cure time, in minutes, can be calculated based on the experimental equation $\log10(t_f)= 3.4710 - 0.0158T_s$, where T_s is the soft cure temperature in degrees Celsius. For this system, samples were cured for 22 minutes at 70 °C, which gives a curing ratio, R, of 0.1. It should be noted that the soft cure time depends on the amount of PDMS being cured, so longer soft cure times may be required for larger samples.

Selective membrane exposure

A 'pen' was created to 'write' solvent in discrete places across the channel system covered by the PCTE membrane. As highlighted in Fig. S4, the 'pen' in this setup was made by connecting fused silica capillary tubing (D_{inner} = 50 μm, D_{outer} = 150 μm, Polymicro Technologies, Phoenix, AZ) to a syringe (1 ml Norm-Ject® Luer syringes, Henke Sass Wolf, Germany) loaded into a syringe pump (PHD 2000 programmable pump, Harvard Apparatus, Holliston, Massachusetts) using polyethylene tubing (PE-20, Intramedic Clay Adams® Brand, Diagnostic Systems, Sparks, MD). The capillary tubing was housed inside

an empty Sharpie marker (Sharpie®, Oak Brook, IL) shaft to facilitate handling. Prior to use, the syringe was filled with isopropyl alcohol (IPA), which served as the 'ink'.

A PDMS channel device, fabricated as described above, was submerged in a petri dish of deionized water. Submerging the device in water while 'writing' allows for a greater working time to pattern the device, as the 'ink' will quickly evaporate when exposed to air. The submerged device was placed on a sample stage that can be precisely controlled laterally and vertically. A stereoscope (SZX7, Olympus, Melville, NY) mounted on an articulating arm boom stand was used to observe the writing process.

Before the writing process, the syringe pump was set to 1 μl/min, while flow was allowed to stabilize. The stage was slowly brought up until the water covered the tip of the capillary tubing. At this point, the stereoscope was used to observe the progress of IPA through the 'pen' tip. When the IPA reached a steady perfusion state through the tip, a change in the refractive index between the IPA and the water can be observed. At this time, the dish was slowly brought up further, until an interface formed between the surface of the device and tip. It is important that the capillary tip not touch the top of device, as it will tear and/or deform the membrane as it rasters along the surface. Once the interface has formed, the desired pattern can be written on the PDMS device, by carefully moving the stage in the x- and y-directions. After drawing is complete, devices are submerged in deionized water. A sequence of images that demonstrates this process can be seen in Fig. S6.

Image background correction

All images used, either in the text or for image analysis, were background corrected in the following manner, modified from method described by Model *et al*.[2] A standard slide was created by placing 0.1 mM fluorescein solution between a coverslip and a microscope slide,

which was subsequently imaged using fluorescence microscopy. These images could then be used to highlight fluorescent intensity heterogeneity related to the microscope and camera. Matlab (The MathWorks, Natick, MA) was used to background correct the raw images. Using this software, RGB images were first converted to gray scale and then each was divided by an image of the reference; corrected images were then outputted with false coloring.

Hippocampal neuron culture and imaging

Prior to use in culture, patterned polylysine gel slabs were sterilized by exposure to 0.1% Gentamicin (Invitrogen) PBS solution for 2 hours [3] before being transferred to a petri dish of fresh sterile PBS, where they were stored overnight at 4 ºC.

The cell plating procedure used is similar to one published previously.[4] Hippocampal neurons were isolated from post-natal day one (P1) Long-Evans rats. All experiments were conducted under protocols approved by the UIUC Institutional Animal Care and Use Committee of the Vice Chancellor for Research, and under continuous supervision of the campus veterinarian staff. The gels were plated at an initial density of approximately 150 cells/mm^2. Neuronal samples were maintained in a humidified environment at 37 ºC with 5% CO_2 and supplemented with Neurobasal media twice weekly for one week.

After 7 days in culture, neuronal samples were prepared for fluorescent imaging using the same method presented previously.[4] Samples were treated with DAPI (Invitrogen) to stain the DNA in the nucleus blue, while rhodamine-phalloidin (Invitrogen) was used to stain the actin in the cytoskeleton red. All fluorescent microscopy was carried out using a Zeiss Axiovert 200M inverted research-grade microscope. A Dapi/Hoechts/AMCA filter (Chroma Technology, Rockingham, VT) was used for the DAPI imaging, a Special Yellow

Rhodamine/Cy3/Texas Red filter (Chroma Technology) was used for the rhodamine imaging, and a Piston/GFP filter (Chroma Technology) was used to image the fluorescein.

Testing the device reusability and pattern reproducibility

The reusability of the device was demonstrated by patterning gels using the same device at different time points over several days. The device, in this case, was based on serpentine channel (width = 50 μm) that connected nine dots (diameter = 350 μm), forming a 3x3 array as shown in Fig. 1d. Fluorescein labeled biotinylated dextran (i.e., mini-emerald) was injected at 1 μl/min and the stamping time between the hydrogel and the device was held constant at 5 minutes. Using the same device, 11 patterned gels were created. The first 5 gels were printed on day 1, while the following 6 were printed three days later (day 4). In between printing cycles on day 1 and 4, the device was stored at 4 ºC in deionized water. Patterned gels were imaged, background corrected, and analyzed using Image Pro Express. For each image, the pixels in nine representative areas, corresponding to the nine dots patterned directly from the underlying channel design, were sampled. The mean intensity of pixels within each circular area was measured to represent the area intensity.

Typically, in a microfluidic channel, there is a drop in pressure as the distance from the channel inlet increases.[5] Thus, it might be expected that the intensity of a transferred pattern would be directly related to the underlying location in the microchannel. In an effort to address this phenomenon, the nine circular areas were analyzed separately by grouping corresponding areas of the 11 consecutively printed patterns together (so there were 9 groups in total). For each group, the mean intensity of the 11 circular areas was determined to attain a normalization factor. This factor was then used to generate a normalized mean intensity for the corresponding area of the pattern. The plot given in Fig.

S8 gives the normalized mean intensity for each of the 9 regions of the pattern; standard deviation of the intensity for each region was used to generate error bars.

Testing the time dependence relationship on transferred pattern intensity and size

The printed pattern fidelity, in terms of diameter in comparison to the original channel design, was investigated using the channel design (Fig. 1d) and setup described in the pattern transfer reproducibility/device reusability section. In this analysis, the only parameter that was changed was the stamping time which ranged from 0.5 to 10 minutes. . Specifically, the relationship between printing time and pattern intensity and diameter were considered. The AOI (Area of Interest) function was used to fit the 9 transferred dots. The normalization factor that was generated for the pattern reproducibility study (overall mean intensity for dots 1-9 printed at 5 minutes) was used as a baseline. The 5 minute overall intensity was used as a baseline, as this time was most often used for dextran patterning. The individual mean intensity for each dot was divided by this normalization factor, to allow for strict comparison between time and intensity. Using this ratio, the percent change from the baseline or the most typically used printing parameters can be visualized. This data is presented in Fig. S7, with error bars representing the standard deviation. As can be seen from the highlighted plot, the intensity of the transferred pattern increases approximately linearly with stamping time.

The dependence of time on the size of the resulting patterns (in relation to the original channel design) was investigated similarly. The AOI function was used to determine the area in pixels of each of the 9 dots. An optical image of the original channel design was loaded into Image Pro, the actual radius determined (~35 pixels) which was used to determine the area. The determined area was subsequently used as a normalization factor.

Each individually measured area was divided by the normalization factor, to get a ratio of the actual dot size in relation to the transferred dot. These data are plotted in Fig. S7, with error bars representing the standard deviation. We found that the diameter of the printed circles increased linearly with printing ranging from 0.5 to 10 minutes. For example, the diameter of the 10 minute transfer is almost twice as large as that of 5 minute transfer. The size of the transferred pattern depends on the diffusion kinetics of the dextran inside the gel, the capture efficiency of the streptavidin for the biotinylated dextran, as well as the size and conformation of the dextran itself.

References

1. J. S. Go and S. Shoji, *Sensor Actuat. A-Phys.*, 2004, **114**, 438-444.
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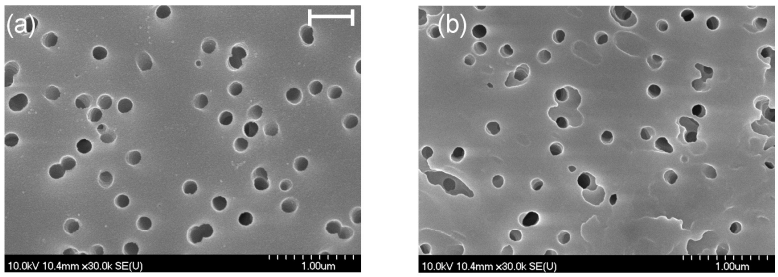
3. M. R. Hynd, J. P. Frampton, N. Dowell-Mesfin, J. N. Turner and W. Shain, *J. Neurosci. Meth.*, 2007, **162**, 255-263.
4. J. N. Hanson, M. J. Motola, M. L. Heien, M. Gillette, J. V. Sweedler and R. G. Nuzzo, *Lab Chip*, 2009, **9**, 122-131.
5. S. K. Sia and G. M. Whitesides, *Electrophoresis* 2003, **24**, 3563–3576.



Statistic	Before Deposition	After Deposition
Min. diameter (nm)	76	96
Max. diameter (nm)	196	194
Mean diameter(nm)	154	140
Standard Deviation	13	11
Sample size	40	47

Fig. S1 SEM micrographs of track-etched polycarbonate membrane before (a) and after (b) Ti/SiO_2 deposition. The table presents statistical information of the pore size analysis. The average diameter of the pore decreased about 14 nm after Ti/SiO_2 deposition. Scale bar is 500 nm.

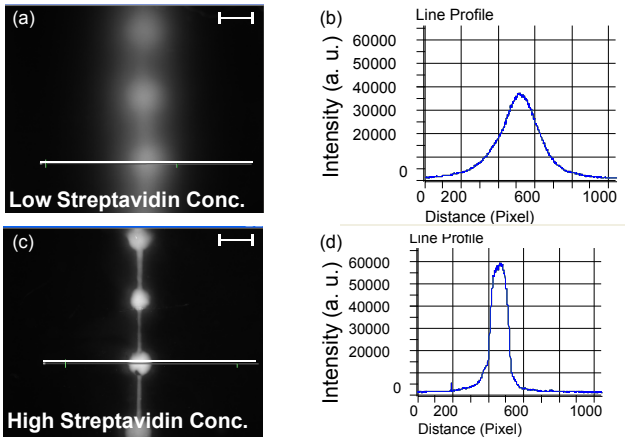


Fig. S2 Initial printing results for biotin-polylysine-FITC on polyacrylamide hydrogels. Hydrogels in (a) and (c) were made with prepolymer solution containing 50 µg/ml and 1mg/ml streptavidin acrylamide, respectively. Line scans in (b), (d) highlight differences in pattern resolution related to streptavidin concentration. Scale bars are 500 µm.

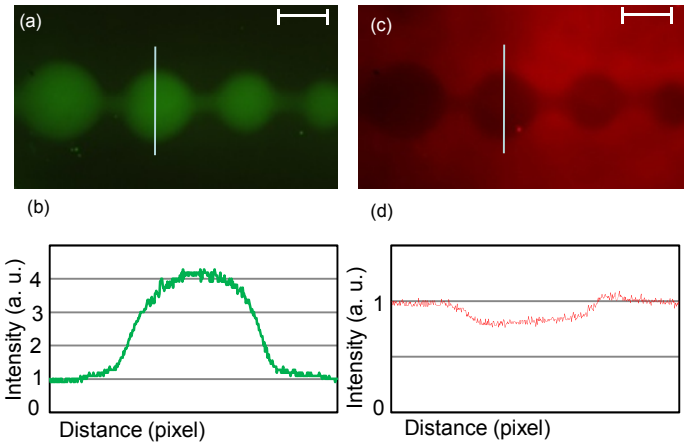


Fig. S3 (a) Fluorescent micrograph highlights initial patterning of biotin-PDL-FITC, while (c) shows secondary exposure of biotin-BSA-Rhodamine. The line scans in (b) and (d) quantitatively describe the initial patterning and the orthogonal exposure, respectively. Scale bars are 500 μm.

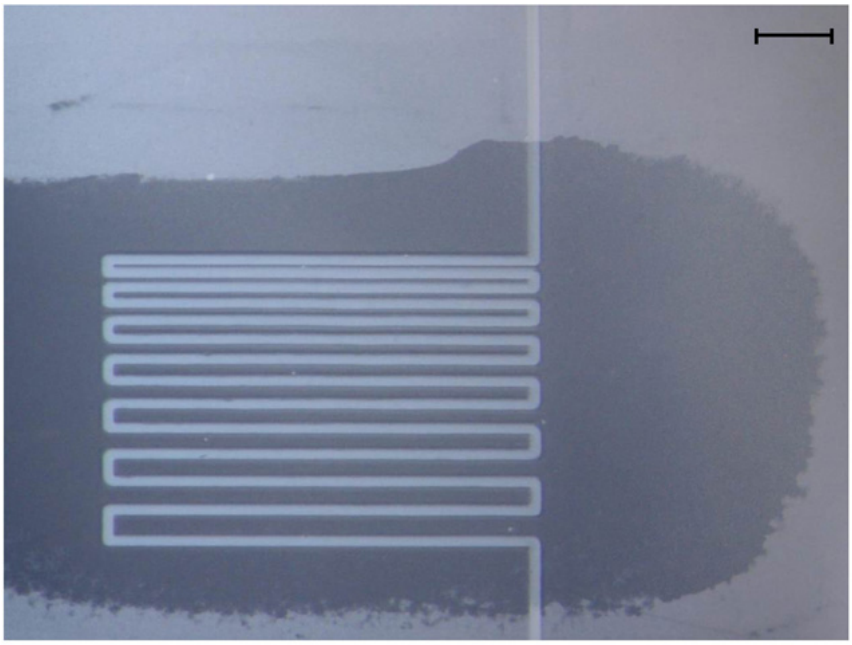


Fig. S4 The underlying channel design was wetted with isopropyl alcohol using a cotton swab. The wetted device was immediately submerged in water to prevent solvent evaporation. The color of the PCTE membrane changed from white to translucent after wetting. Scale bar is 500 μm.

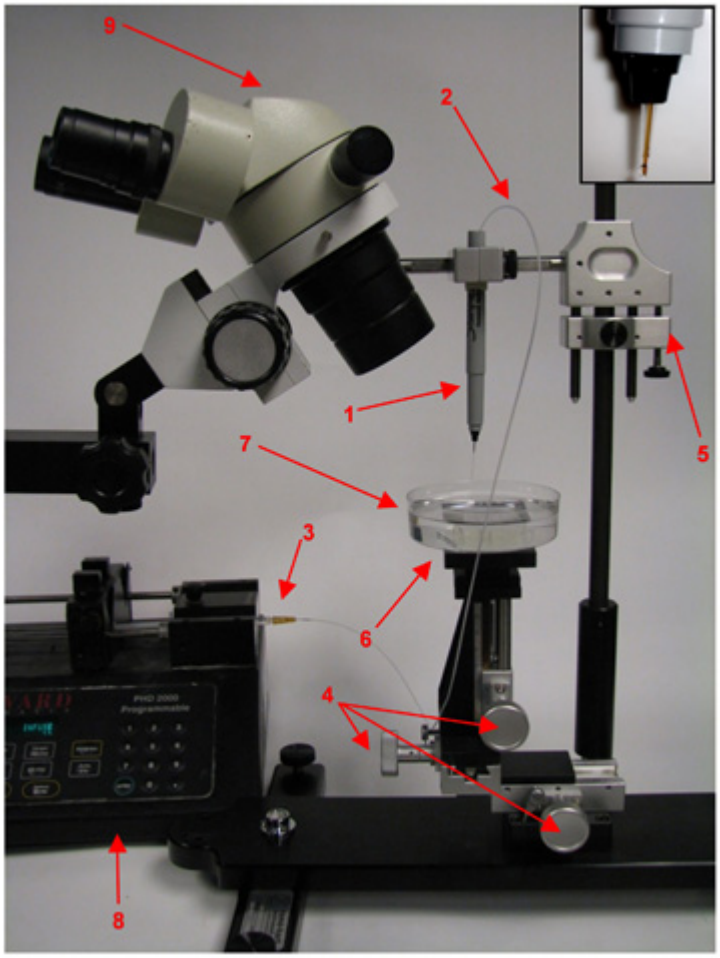


Fig. S5 Experimental set-up of the writing station for selective membrane wetting. (1) "Pen", an empty Sharpie® marker shaft contains fused silica capillary tubing (D_{inner} = 50 µm, D_{outer} = 150 µm, see inset for an enlarged view); (2) PE-20 tubing; (3) 1 ml syringe, loaded with isopropyl alcohol; (4) Control of lateral, vertical movement of stage; (5) Adjustable clamp; (6) Sample stage; (7) Petri dish, with submerged device in water; (8) Syringe pump; (9) Stereoscope.

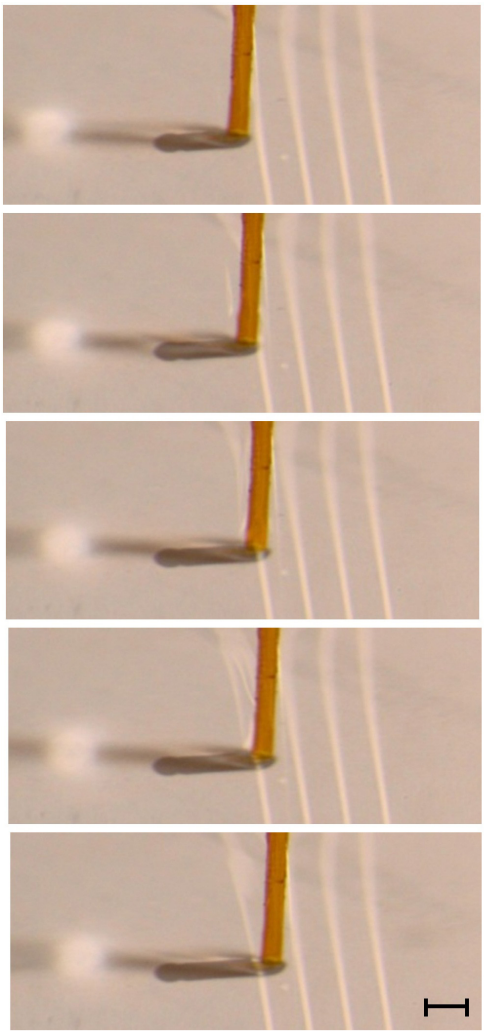


Fig. S6 Image sequences highlights selective wetting of the PCTE membrane with isopropyl alcohol using a capillary 'pen', gently rastered across the membrane surface. Membrane color changes from white to translucent after IPA exposure. Scale bar is 300 μm.

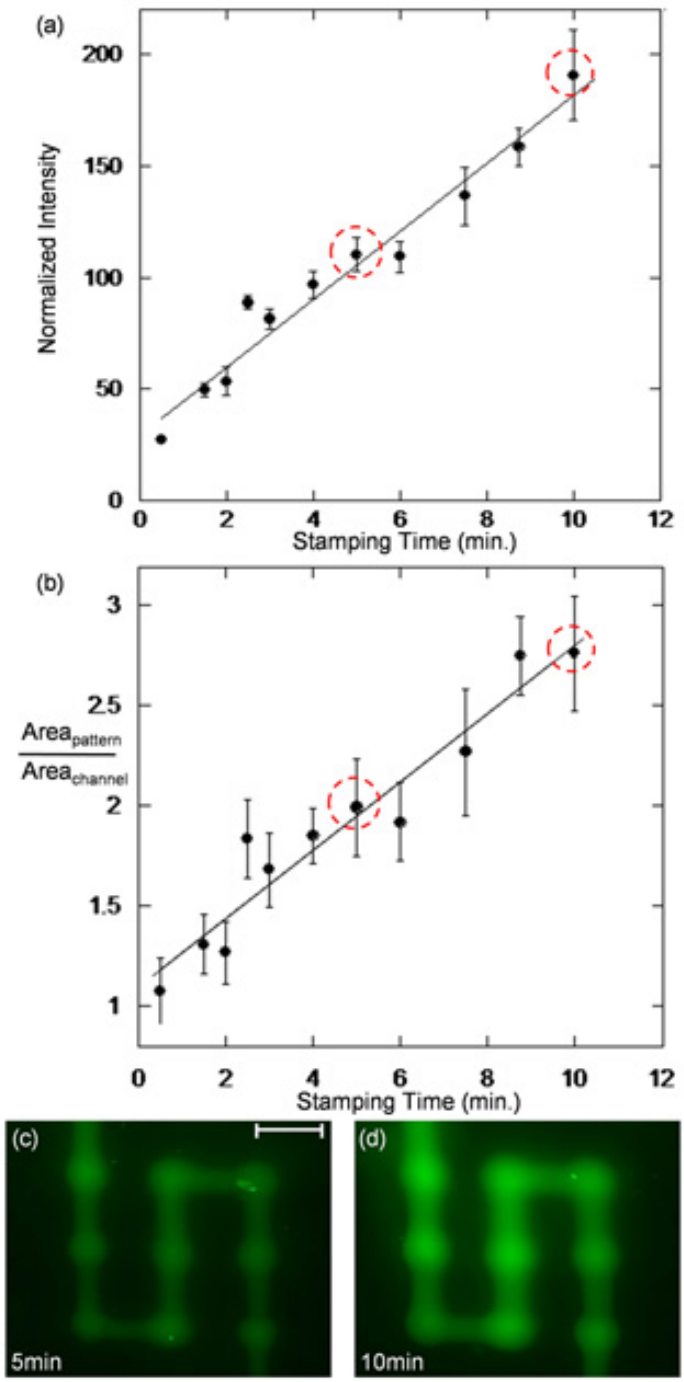


Fig. S7 The fluorescence intensity (a) and diameter (b) of the printed pattern linearly increases, through a stamping time range of 0.5-10 minutes. In (c) a micrograph of the printed pattern at 5 minutes is given, while the printed pattern after 10min is given in (d). Scale bar is 500 μm.