

Droplet Microarray: Facile Formation of Arrays of Microdroplets and Hydrogel Micropads for Cell Screening Applications

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Electronic Supplementary Information

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1. Superhydrophilic-Superhydrophobic Pattern Preparation

a. Glass Surface Modification

To achieve covalent attachment of the polymer layer, the glass surfaces are first activated and then functionalized with an anchor group for methacrylates.

Activation of glass slides: Clean glass slides are immersed in 1 M NaOH for 1 h and afterwards washed with deionized water, and then immersed in 1 M HCl for 30 min, washed with deionized water, and dried with a nitrogen gun.

Modification of glass slides: Several drops of a solution containing 20 v/v% 3-(trimethoxysilyl)propyl methacrylate in ethanol are dropped on an activated glass slide. The plate is covered with another activated glass slide. The solution is reapplied after 30 min. After another 30 min, the slides are washed with acetone and dried with a nitrogen gun.

Fluorination of glass slides: An activated glass slide is placed in a vacuumed desiccator together with a vial containing several drops of tridecafluoro-(1,1,2,2)-tetrahydrooctyltrichlorosilane over night.

b. Polymerization Procedure and Photografting

Polymerization: Two strips of Teflon film (American Durafilm Co.), defining the thickness of the polymer layer, are placed at the edges of a fluorinated glass slide and a modified glass slide is clamped on top of it. The polymerization mixture is injected in between the mold and irradiated for 15 min at 12 mW/cm² with a 260 nm UV light. The mold is then carefully opened using a scalpel.

An inert, fluorinated glass slide is used as the bottom plate. The inability of covalent attachment of the growing polymer to the fluorinated glass slide allows for the whole polymer to stick to the top, modified glass slide during the separation process. The fluorinated glass slide can be reused several times. The resulting nonporous superficial layer can be easily removed by applying and rapidly removing adhesive film (Scotch tape) immediately after separating the plates while the layer is still wetted with porogen. The plate is washed extensively with methanol and kept in methanol for several hours to remove unreacted monomers and porogens.

Polymerization mixture: 2-hydroxyethyl methacrylate (24 wt %), ethylene dimethacrylate (16 wt %), 1-decanol (12 wt %), cyclohexanol (48 wt %), and 2,2-dimethoxy-2-phenylacetophenone (1 wt % with respect to monomers).

Photografting: The polymer layer is wetted with the photografting mixture and covered with a fluorinated glass slide. A quartz chromium photomask (Rose Fotomasken, Germany) is placed on top and it is irradiated for 30 min at 12 mW/cm² with a 260 nm UV light. The obtained pattern is washed extensively with methanol and stored in methanol for several hours to remove excess monomer and porogen, and for sterilization before cell culture.

Photografting mixture: 2,2,3,3,3-pentafluoropropyl methacrylate (20 wt %), ethylene dimethacrylate (1.3 wt %), 1:3 (v/v) mixture of water:*tert*-butanol (78 wt %), benzophenone as the initiator (0.33 wt %).

The superhydrophobic barriers allow different volumes of solution to be printed per spot, while still confining the solution within the defined superhydrophilic area. Thus, volume and concentration adjustments can be made without changing the footprint of the printed spot.

2. Microdroplet Formation with Methyl Green Solution

Figure S1 shows an array of squares and hexagons immersed in a methyl green water solution and slowly pulled out to form separated droplets in each superhydrophilic spot. After allowing the droplets to dry in air, methyl green is present only in the superhydrophilic spots and not on the superhydrophobic barriers. This demonstrates that the DropletMicroarray method can be used to deposit substances in one step into thousands of superhydrophilic spots of different geometries.

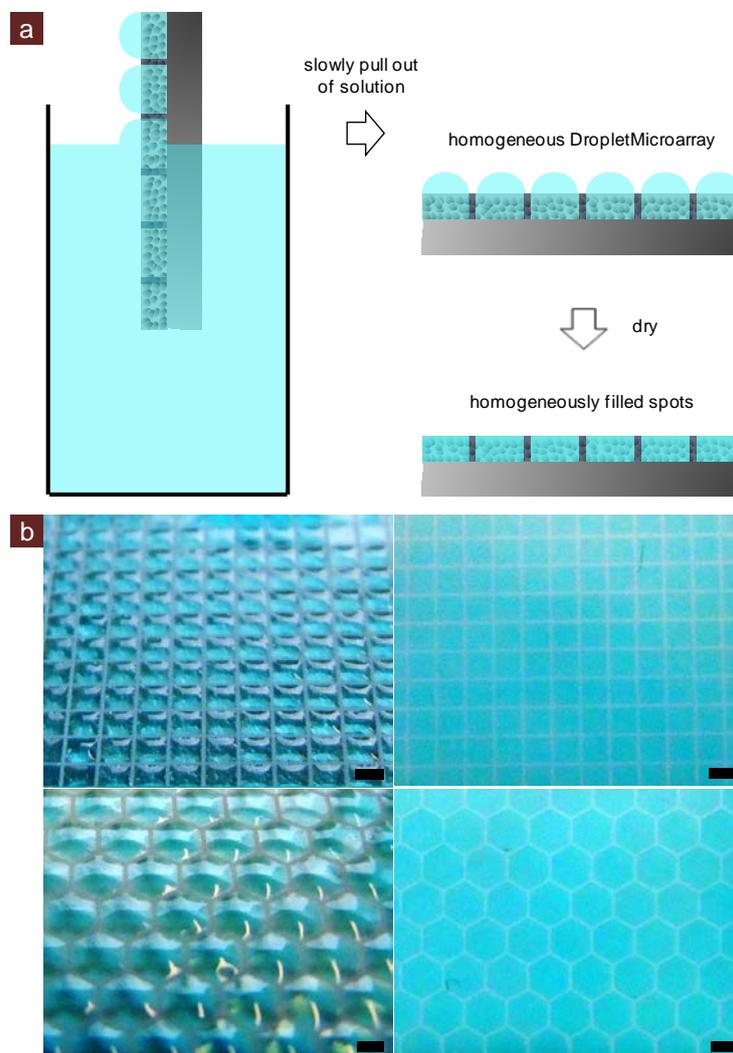


Fig. S1 Application of the DropletMicroarray for deposition of substances; here, a methyl green dye solution. a) Schematic representation of the droplet deposition method. The superhydrophilic-superhydrophobic patterned substrate is immersed in an aqueous solution and pulled out of the solution to form homogeneous droplets in the superhydrophilic spots. b) Microdroplets formed using a methyl green solution (left) and then dried (right). Scale bars are 1 mm.

3. Droplet Volumes for Different Pattern Geometries

With our superhydrophilic-superhydrophobic patterning technique, we had a smaller and larger droplet size limitation. The smallest microdroplets that could be produced had a volume of approximately 0.7 nl for a $200 \times 200 \mu\text{m}^2$ square pattern. This was mainly due to the limitations of the resolution using this patterning technique. The volume of large droplets is more difficult to control because of the increasingly important role of gravity. We observe this effect for $3 \times 3 \text{ mm}^2$ square patterns and larger.

Method for measuring volumes of water droplets:

1. Measure mass of water or other liquid that will be applied ($20 \mu\text{l}$, m_0).
2. Roll the droplet across the pattern of interest. Leftover solution is aspirated by pipette. Measure the mass of the leftover solution (m_1).
3. Determine the mass of the solution in a spot by subtracting the leftover mass from the initial mass and dividing it by the number of spots (N), $m = (m_0 - m_1) / N$.
4. Calculate the volume of the droplet.

Table S1 Dependence of the water droplet volume on the geometry and area of the superhydrophilic spot. All superhydrophobic barriers are 100 μm .

Geometry	Area of the superhydrophilic spot, $10^6 \mu\text{m}^2$	Volume of droplet, nl
Triangle (1 mm side length)	0.433	14 \pm 10
Circle (1 mm diameter)	0.785	72 \pm 5
Square (1 mm side length)	1.000	67 \pm 10
Hexagons (1 mm side length)	2.598	184 \pm 50
Circle (3 mm diameter)	7.069	2815 \pm 323

Table S2 The water droplet volume is greatly affected by the size of the superhydrophilic spot for a given geometry (here, square geometry). All superhydrophobic barriers are 100 μm . DropletMicroarrays were produced by the rolling drop method.

Side length of the superhydrophilic square, μm	Area of the superhydrophilic spot, $10^4 \mu\text{m}^2$	Volume of droplet, nl
100	1	0.2 \pm 0.1
200	4	0.7 \pm 0.1
500	25	8 \pm 2
650	42.25	18 \pm 4
800	64	29 \pm 7
1000	100	67 \pm 10

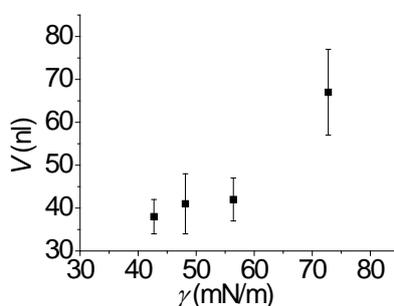


Fig. S2 Dependence of the water droplet volume (V) in a single spot of square geometry (1000 μm side length, 100 μm barrier) on the surface tension (γ) of the liquid (surface tension of ethanol-water mixtures are from reference S1).^{S1}

4. Hydrogel Array Images

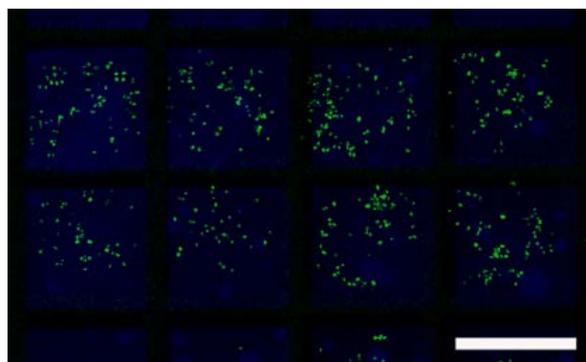


Fig. S3 Fluorescent HT1080-eGFP cells encapsulated in hydrogel micropads stained with 7-diethylamino-3-(4-maleimidophenyl)-4-methylcoumarin (blue) after hydrogel crosslinking and immersion in medium. Scale bar is 500 μm .

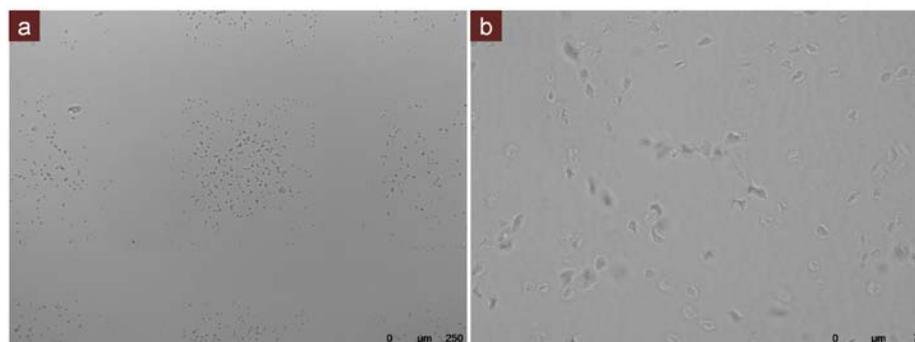


Fig. S4 Fixed It-NES cells encapsulated in hydrogel micropads on day 6. Low (a) and high (b) magnification. DAPI channel image shown in Figure 4e.

Adhesion between the hydrogel micropads and the substrate was strong enough to handle standard cell fixation and permeabilization procedures, immunofluorescent staining procedures, and several washing steps without any indication of hydrogel detachment. Of course, physical detachment of the hydrogel micropad using stronger mechanical force is possible. In addition, when we used the thiol-reactive probe, 7-diethylamino-3-(4-maleimidophenyl)-4-methylcoumarin, in the PEG-crosslinker mixture and visualized the hydrogel micropads with a confocal microscope, we saw blue fluorescence inside the thin polymer substrate, suggesting successful crosslinking of the gel within the supporting polymer film and physical attachment of the hydrogel micropads to the substrate.

5. Cell Culture

Human cervical tumor cells stably expressing green fluorescent protein (HeLa-GFP) were cultured in DMEM (Gibco, Cat. #41966) supplemented with 10% FBS (PAA Laboratories, Cat. #A15-151) and 1% penicillin/streptomycin in a humid incubator at 37°C with 5% CO₂, and were split every two to three days. For selection of GFP-positive cells, 10 µg/ml of Blasticidin was used. A cell suspension containing 2×10^6 cells/ml was used to form the DropletMicroarray in Fig. 3b and imaged on a Leica MZ10 F stereo microscope. A MI-PVA-cell solution containing 16,000 cells/µl was used to form the cell-encapsulated hydrogel micropads in Fig. 3e and imaged on a Leica DM5500 B microscope.

Human fibrosarcoma cells stably expressing enhanced green fluorescent protein (HT1080-eGFP) were cultured in RPMI Medium 1640 (Gibco, Cat. #A10491) supplemented with 10% FBS (PAA Laboratories, Cat. #A15-151) in a humid incubator at 37°C with 5% CO₂, and were split every two to three days. For selection of eGFP-positive cells, 1 mg/ml of G418 was used. A MI-PVA-cell solution containing 16,000 cells/µl was used to form the cell-encapsulated hydrogel micropads in Figs. 4d and S3 and imaged on a Leica TCS SP5 confocal microscope and Leica DM5500 B microscope, respectively.

Long-term self-renewing neuroepithelial like stem cells (It-NES cells) derived from hESC lines H9 were cultured in DMEM/F12 containing N2 supplement (PAA Laboratories), 10 ng/ml fibroblast growth factor 2 (FGF2), 10 ng/ml epidermal growth factor (EGF) (both R&D Systems), 1:1000 B27 supplement (Invitrogen), and 1.6 mg/l glucose in a humid incubator at 37°C with 5% CO₂. Cells were fixed in 4% paraformaldehyde in PBS for 20 min, permeabilized with 0.1% Triton X-100 in PBS, and stained with DAPI (Sigma-Aldrich). A MI-PVA-cell solution containing 40,000 cells/µl was used to form the cell-encapsulated hydrogel micropads in Fig. 3e and S4 and imaged on a Leica DM5500 B microscope.

Human breast adenocarcinoma cells (MDA-MB-231) were cultured in 1:1 F12:DMEM (Gibco, Cat. #21765 and #A10491) supplemented with 10% FBS (PAA Laboratories, Cat. #A15-151) in a humid incubator at 37°C with 5% CO₂, and were split every two to three days. A MI-PVA-cell solution containing 4,000 cells/µl was used to form the cell-encapsulated hydrogel micropads in Figs. 5a,b and imaged on a Keyence BZ-9000 fluorescent microscope (Japan).

References for ESI

S1 G. Vazquez, E. Alvarez and J. M. Navaza, *J. Chem. Eng. Data*, 1995, **40**, 611-614.