Electronic Supplementary Material (ESI) for Lab on a Chip
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Micropatterned porous substrates for combinatorial cell-based assays.

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Supplementary Information – ESI-1

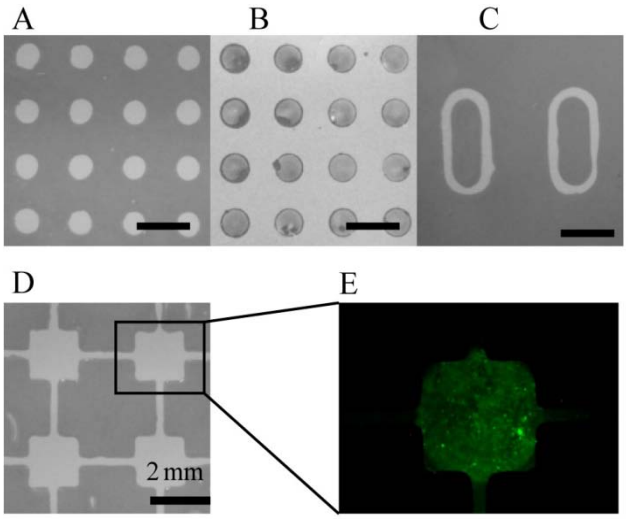

Figure S1. Polycarbonate membrane (Isopore, Millipore) printed with various PDMS patterns. (A) An array of 1-mm porous disks. The dark areas correspond to non porous areas. (B) An array of 1-mm non porous disks. Rings (C) and squares connected by lines (D) are other examples of the potential of this method to create any patterns of porosity. The lines are close to the resolution limit of contact printing with PDMS. This is evidenced by the presence of undulations on the border of the patterns. (E) MDCK cells treated with calcein-AM using a µPM with the pattern depicted in (D). Scale bar is 2 mm.

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Supplementary Information – ESI-2

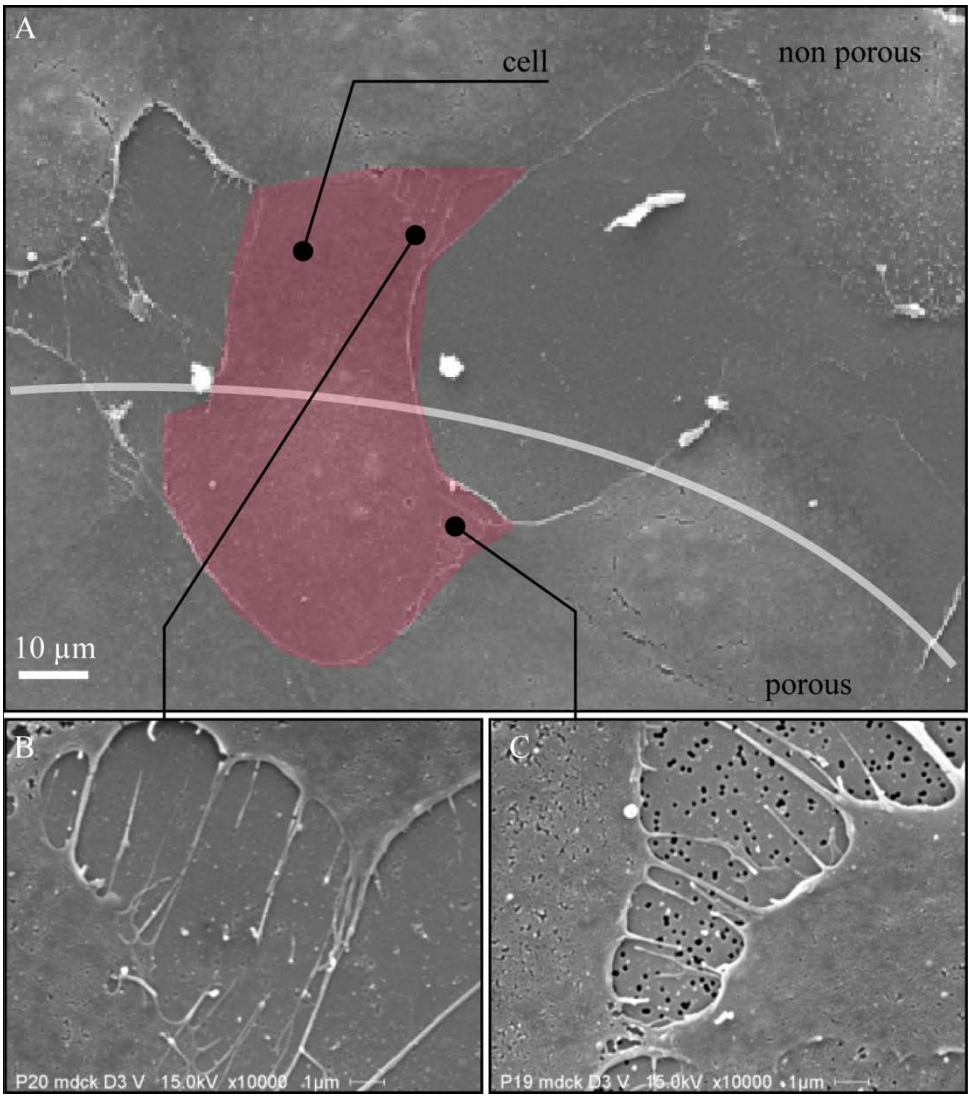

Figure S2. SEM images of MDCK cells grown on a collagen-coated verso side of a µPM. (A) Cells are spreading across both PDMS-printed and porous regions of the membrane. Images taken at higher magnification around filipodia did not show any differences in cell adhesion between non porous (B) *versus* porous region (C). **Methods:** Cell cultures were rinsed twice with PBS then fixed in 4% paraformaldehyde at 4 °C overnight, rinsed three times with PBS and dehydrated by successive 10 min immersions in 30, 50, 70, 90, 95 and 100% (twice) ethanol. They were dried using the critical point drying technique before SEM observation.

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Supplementary Information – ESI-3

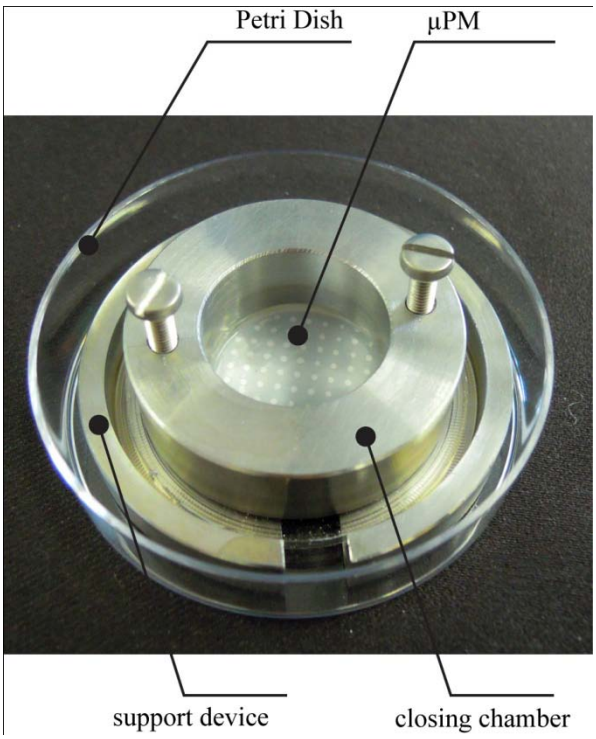

Figure S3. Photography of the holding device used to support the µPM during chemical treatments. It fits into a 35mm Petri dish and is composed of two parts, the bottom part is here to support the µPM and to ensure that culture medium can be circulated below the µPM if needed. The closing chamber is used to prevent drop spilling from the *recto* of the µPM into the culture medium and to maintain the µPM in a fixed position.

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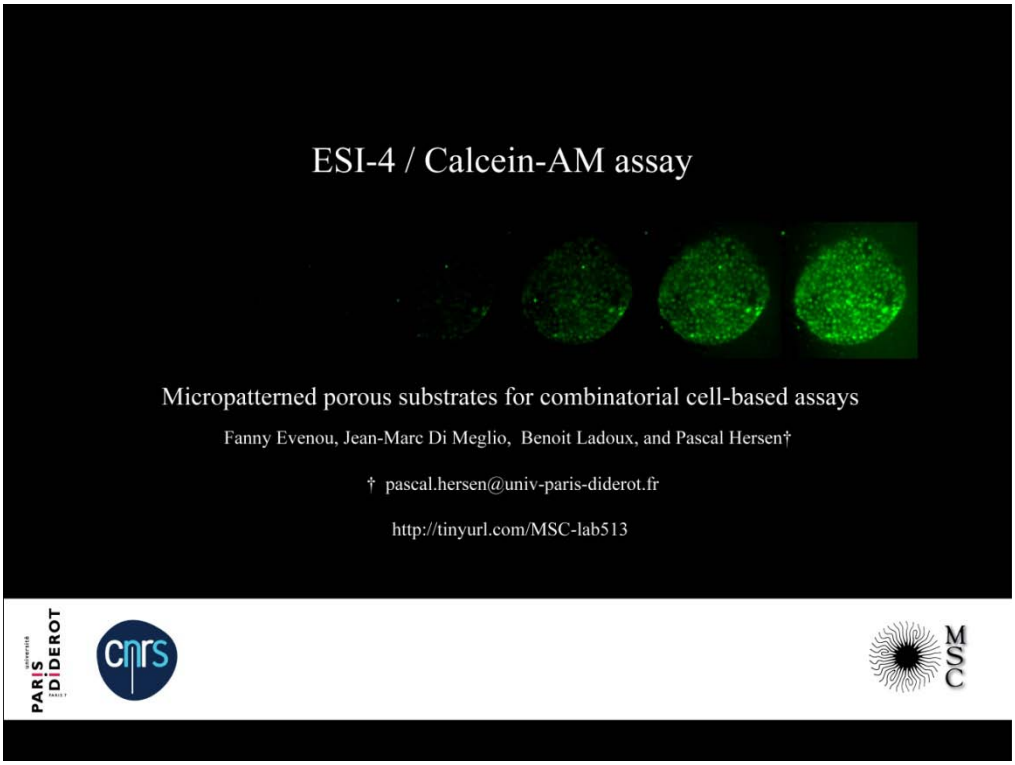
Supplementary Information – ESI-4



Movie S4. Calcein-AM assay. 1 mL-drop of calcein-AM solution was spread over the *recto* of a µPM with a 8×8 array of 1 mm-diameter disks, 1 mm-distant from each other, and with MDCK cells cultured on its *verso*. Calcein-AM is progressively hydrolyzed into green fluorescent calcein inside cells directly below porous regions of the µPM. The total duration of the movie is one hour.

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Supplementary Information – ESI-5

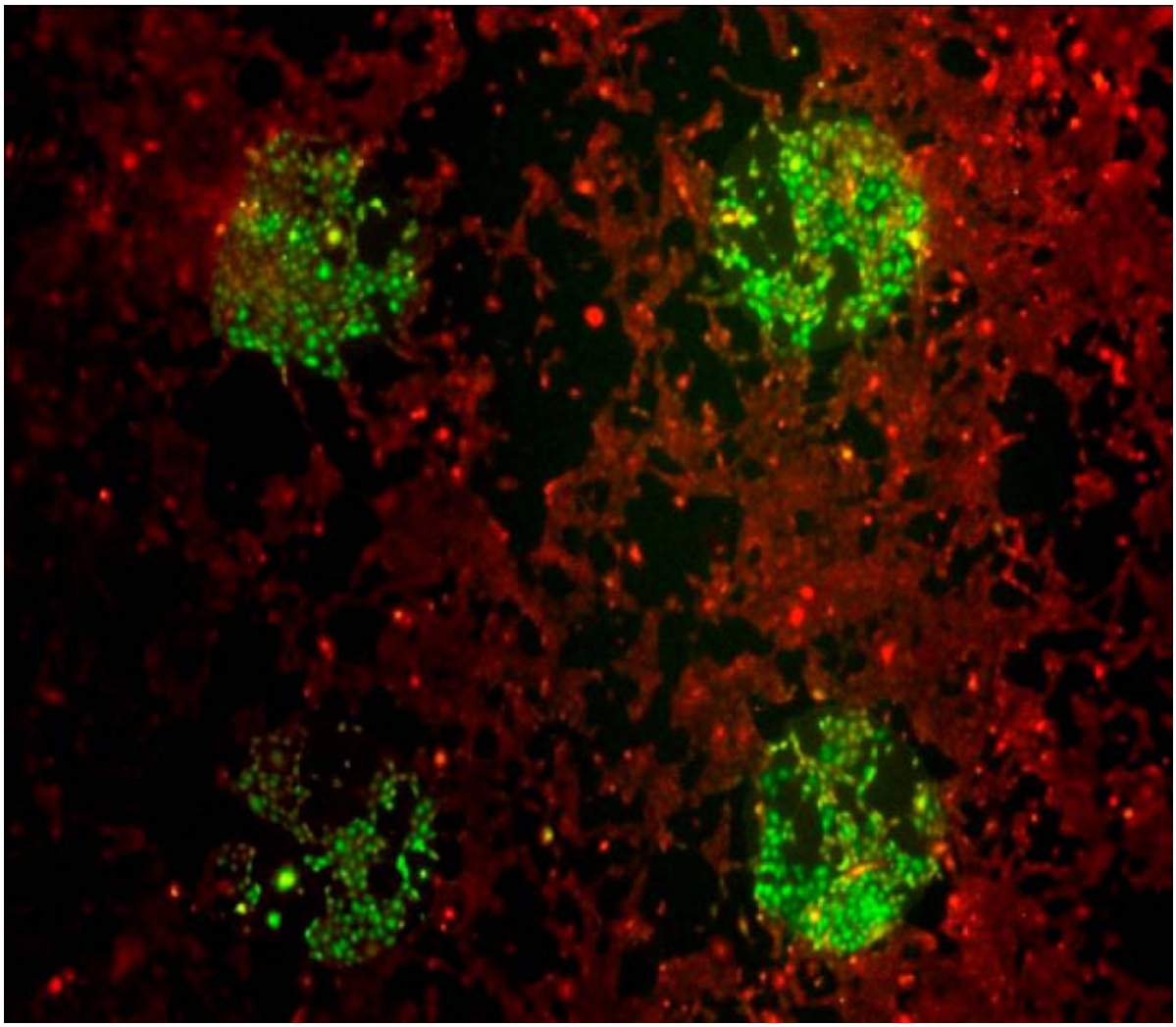

Figure S5. Calcein-AM diffusion assay using a µPM with a 8×8 array of 1 mm-diameter disks, 1 mm-distant from each other. MDCK cells expressing actinin-RFP were cultured on the *verso* to near confluence and 1 mL-drop of calcein-AM was deposited on the recto side. Experiment duration is 1 hour. The localized expression of fluorescent calcein (GFP) was observed in four adjacent disks at small magnification (x2).

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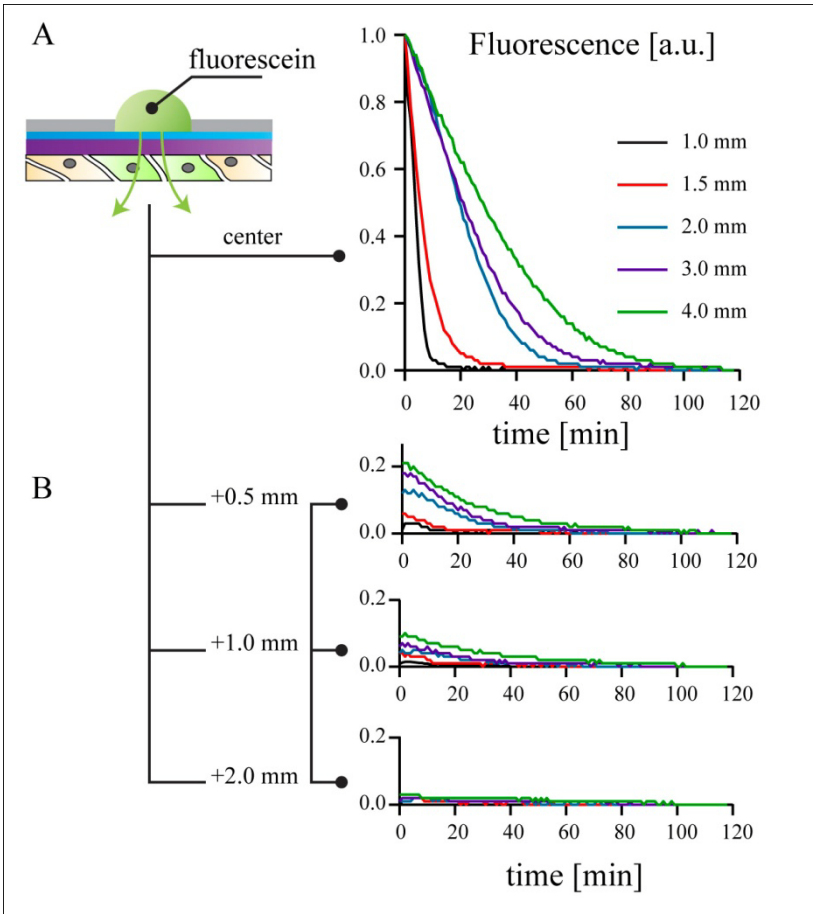

Figure S6. Diffusion of fluorescein through a µPM patterned with a single central hole. A series of membranes were patterned with a single disk of increasing diameter: 1, 1.5, 2, 3 or 4 mm. Fluorescein was used to study the diffusion kinetics through the porous area in the presence of a confluent monolayer of MDCK cells on the *verso*. (A) Drops of 3 µM fluorescein were deposited on the porous disks on the *recto*. The deposited volumes varied according to disk size and were calculated considering drops as hemispheres with a diameter matching the one of the disk. Fluorescein diffusion was analyzed by time-lapse fluorescence imaging using an inverted fluorescence microscope. (B) Time course of fluorescence intensity at the center of the disk and at 0.5 mm, 1 mm and 2 mm away outside of the disk. The fluorescence intensity was normalized by the maximum intensity inside the porous area at start. For all disk sizes, the fluorescence was mainly detected immediately below the pattern (porous regions of the µPMs). Its intensity progressively decreased over time as fluorescein diffused through the membranes and got diluted in the culture medium. Fluorescence intensity outside the porous regions was very low. For disks of 1 mm in diameter, there was less than 6% of fluorescence after 0.5 mm from the edge of the disk (compared to 100% in the center of the porous area). It was less than 2% after 1 mm-distance.