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


# Fabrication of large-area polymer microfilter membranes and their application for particle and cell enrichment

## – Supplementary Information –

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### 1. Characterization of the bead populations

The characterization of the beads was performed using a Millipore Scepter Automated Cell Counter, which yields information regarding the size of a population of cells or beads. We were particularly interested in the characteristics of the 8.27 µm beads, since approximately only 40% of them were captured during our bead isolation experiments. The graphs in Figure S1 show the results obtained from these measurements.

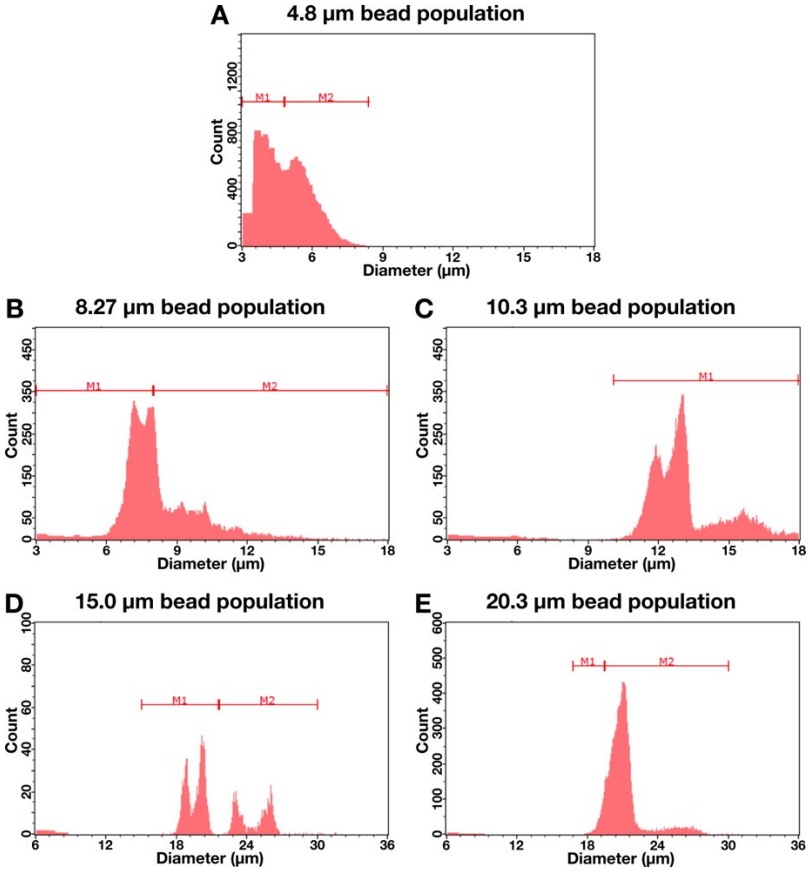


***Figure S1*** *– Bead population analysis results. (A) Population of the 4.8 µm beads used as background for all the experiments, approximately 65% of the beads have a diameter < 5µm (M1), while the rest are between 5 and 8.5 µm. (B) In the case of the 8.27 µm beads, approximately 60% of them were smaller than 8 µm (M1), while the rest were larger (M2), this helps explain the capture rates obtained in the bead isolation experiments. (C) and (D) For the 10.3 and 15.0 µm beads, respectively, close to 99% of the beads counted had a diameter larger than 10 or 15 µm. (E) Finally, in the case of the 20.3 µm beads, approximately 96% of them were bigger than 20 µm (M2).*

## 2. Additional examples of large area thin open-through hole membranes fabricated using VAUM

Using the VAUM method, we were also able to fabricate membranes with various geometric characteristics, such as membranes as thin as 9.3 µm with pores as small as 3.2 µm, membranes with higher porosity (~60%).

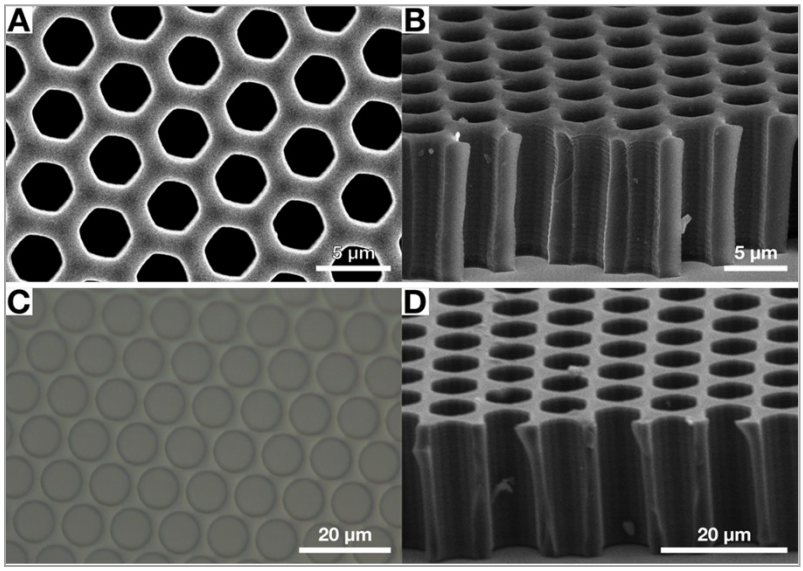


*Figure S2 – Optical and SEM pictures of different membranes fabricated using VAUM in MD 700. (A) and (B) show SEM images of a membrane with pores of about 3.2 µm in diameter and a thickness of just 9.3 µm. The slightly hexagonal shape of the pores is a defect introduced during the photolithography process. It can be clearly seen that the pores are open-through. (C) and (D) show pictures of a high porosity membrane (~ 60%) with pores of 8 µm in diameter fabricated in MD 700.*

## 3. Cell viability

For control experiments, MDA-MB-231 cells were harvested from flasks and cultured in Petri dishes overnight. For positive controls, cells were kept alive in their culture medium in the incubator. For negative controls, just prior viability assay, dead cells were prepared by incubation in 70 % methanol during 45 minutes.

Cells were washed with PBS then stained by incubation in the staining solution consisting in 4.0 µmol L-1 of EthD-1 and 2.0 µmol $L^{-1}$ of calcein AM diluted in PBS, during 45 minutes at room temperature. Viability was determined using fluorescence microscopy on 10 images per samples and averaged on three replicated experiments (Excitation/emission wavelengths: 485/530 and 530/645 nm for calcein AM and EthD-1 respectively.) Cell viability was found to be 96.5 ± 0.9 %, highlighting the non-toxicity of the filter and that the filtration conditions are soft enough to maintain cells alive.

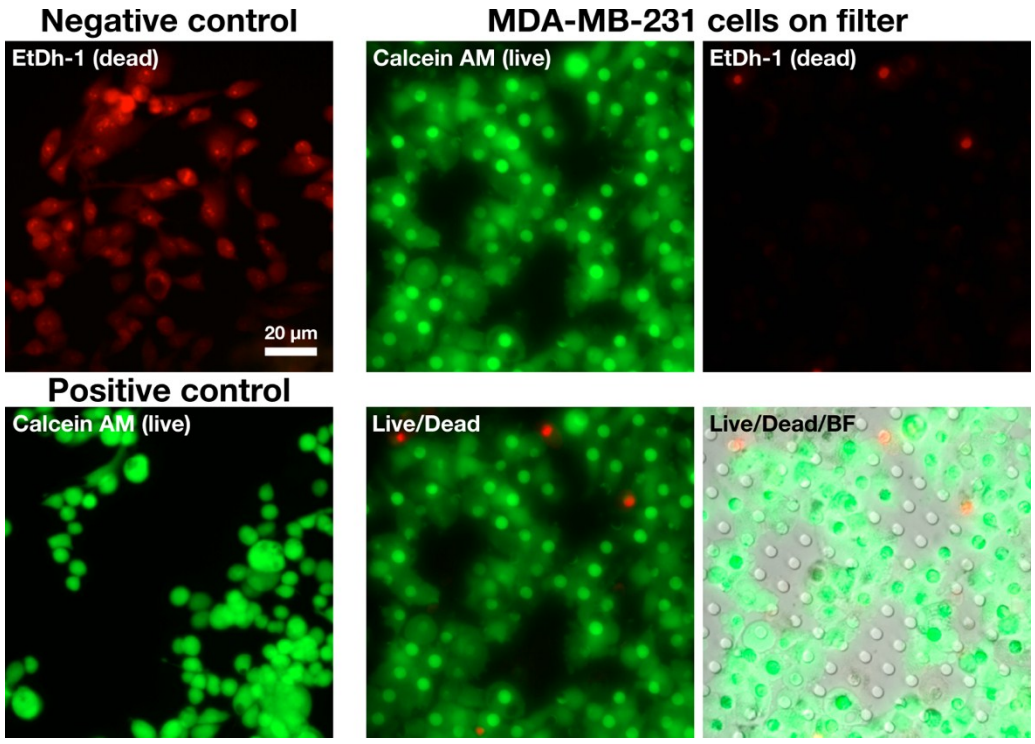


*Figure S3 - Cell viability after filtration. Representative images of dead (negative control) and live (positive control) MDA-MB-231 cells. Cells for controls were cultured overnight in Petri dishes. Dead cells were prepared by incubation 30 minutes in 70 % methanol. Viability of captured cells was determined after filtration, directly on filter. All cells were stained with calcein AM (green, live cells) and EthD-1 (red, dead cells).*