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


# Two-level submicron high porosity membranes (2LHPM) for the capture and release of white blood cells (WBCs)

## – Supporting Information –

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### 1. Vacuum-assisted UV micro-molding (VAUM) fabrication process

The process is described in detail elsewhere.[1] Briefly, a micropillar array was replicated from molds obtained by standard photolithography and DRIE. The structure was then closed using a polyester film coated with a UV-curable resin to form an enclosed 3D microcavity. By using a vacuum chamber, the enclosed mold can be fully filled by the UV-curable Fluorolink® MD 700 resin (80 µL are usually enough to fill most molds), afterwards, it's cured by a 2 min UV exposure (2000-EC Series UV curing flood lamp, DYMAX). Finally, the cover is peeled off, and the molds are placed in an acetone bath during 15-20 minutes, allowing the membranes to self-de-mold from the pillar array. Membrane pores as small as 3.2 µm in diameter have been successfully produced with this fabrication process.

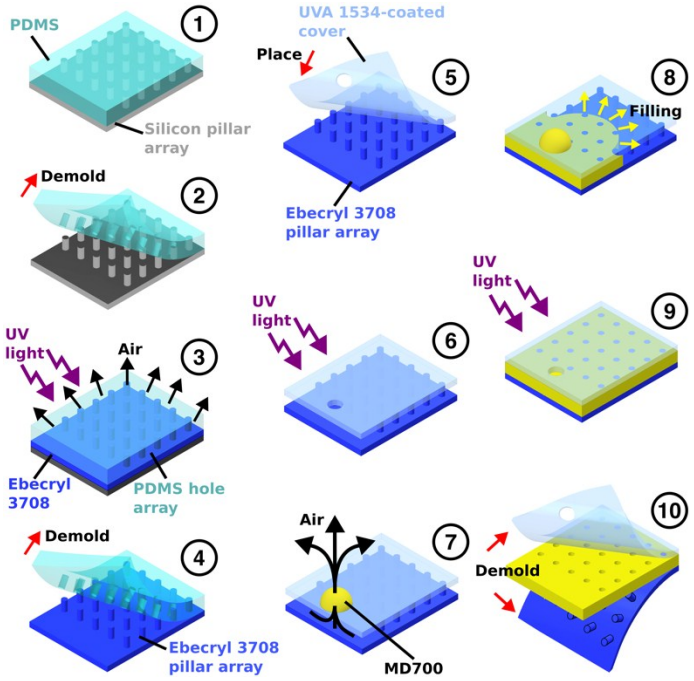


*Figure S1 – Schematic of the VAUM fabrication process. An enclosed mold is fabricated using a sacrificial pillar array replicated on a UV-curable resin (Ebecryl 3708), and a polystyrene cover coated with a partially cured second resin (UVA 1534). The membrane building material is introduced with the help of a vacuum process, by degassing the mold, a pressure difference is established, which drives the MD700 material inside the mold and allows for a complete filling. After curing, defect-free, freestanding, microporous polymer membranes are obtained.*

## 2. SEM images of PET mesh filter

The mesh materials used in PluriStrainer® 1 µm filter is polyethylene terephthalate. It is formed by interlaced by PET wires in diameter of about 40 µm and packed with three layers vertically, resulting in a mesh filter with thickness of about 100 µm. The pores are formed at the crossing point among the wires as shown by the elliptical circles in the SEM images depicted in Figure S2. The pores are not perfectly uniform in terms of the height (1-3 µm) and width (20-30 µm in lateral direction).

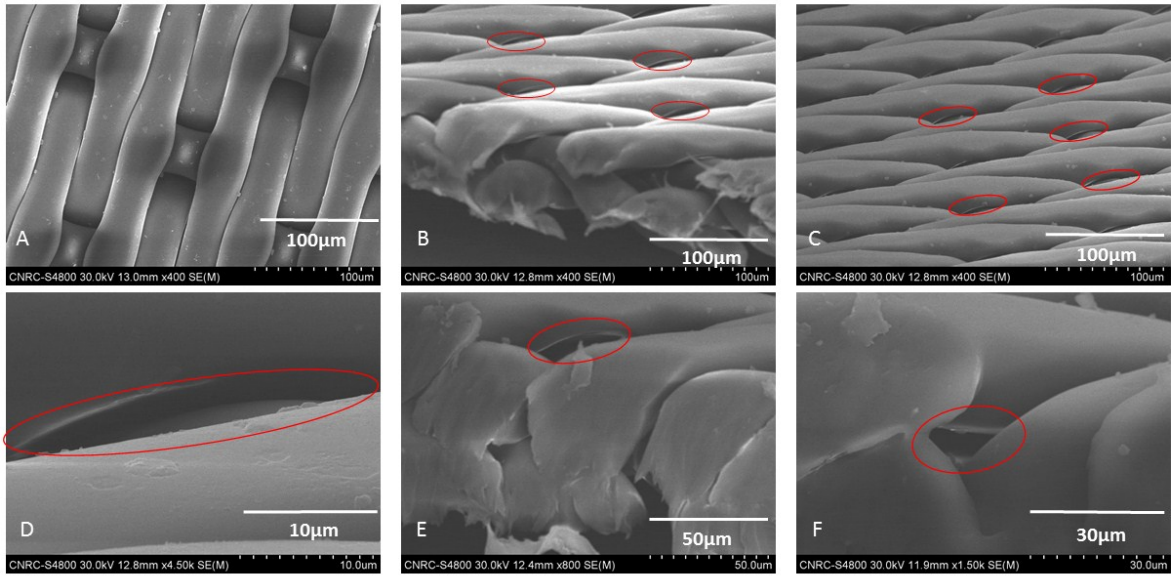


*Figure S2 – SEM images of the PluriStrainer® mesh filters.*

### 3. WBC capture and staining without centrifugation steps

For the experiments presented in the main text of this manuscript, some centrifugation steps were done prior to flowing the cell solutions through the filter membranes. This was done to remove harsh buffers while the cells were being counted and the cartridges primed for the filtration protocol. However, it's possible to do without these centrifugation steps while still maintaining the same capture efficiency stated in the text.

Some WBC capture experiments were performed by running the samples through the filter cartridge right after the RBC lysis step, no filter clogging issues were encountered. Successful capture of the WBCs, buffer exchange, and multi-step staining were also validated, as shown in Figure S3. A capture efficiency of ≈ 96% was measured for this experiment, which is in accordance to the results obtained in the tests presented in the main text.

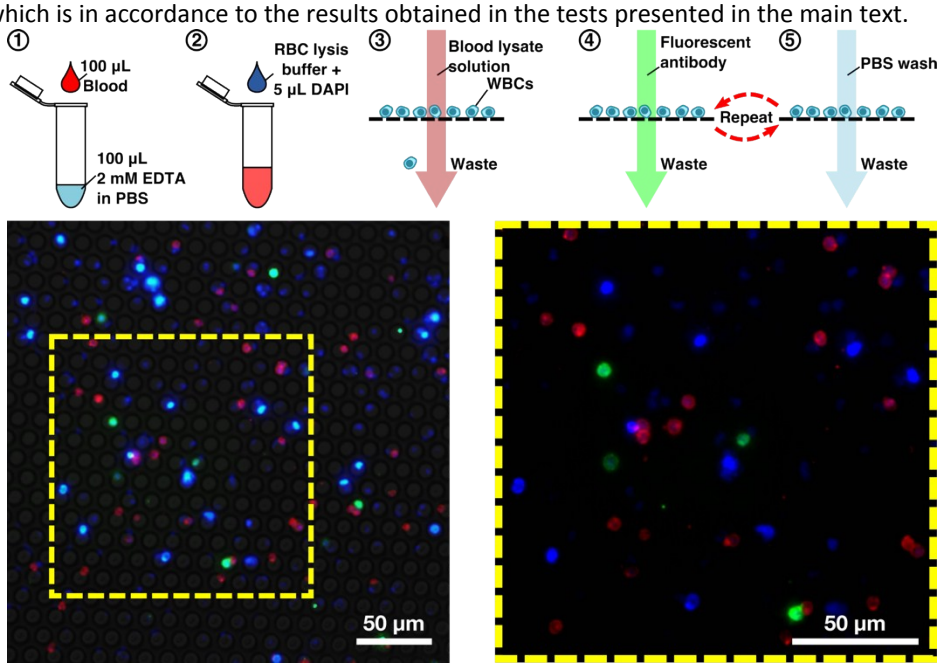


*Figure S3 – WBC capture and staining from solution right after RBC lysis. The only difference between this experiment and the ones for WBC capture and release presented in the manuscript is the removal of the centrifugation and resuspension step to remove the RBC lysis buffer before filtration. Cells were stained for DAPI (blue), CD4 (red), and CD20 (green).*

**References**

1. J. A. Hernández-Castro, K. Li, A. Meunier, D. Juncker and T. Veres, *Lab. Chip*, 2017, **17**, 1960–1969.