ESI
An Integrated Opto-Centrifugo-Microfluidic Platform for Arraying, Analysis, Identification and Manipulation of Individual Cells

R. Burger, D. Kurzbuch, R. Gorkin, G. Kijanka, M. Glynn, C. McDonagh and J. Ducrée

Fabrication of microfluidic chips

All devices used in this work have been manufactured using Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning GmbH, Germany). Molds were fabricated by lithography using a combination of SU8-3025 (Microchem, USA) and WBR2100 dry film resist (DuPont, USA). The following protocol was used:

1. Spin coat 25 µm SU8-3025 on a blank 4-inch silicon wafer
2. Soft bake at 95°C for 14 minutes
3. UV exposure with 220 mJ cm^{-2} to generate fluidic network and V-cup arrays
4. Post exposure bake at 95°C for 3 minutes
5. SU8 developing
6. Hard bake at 150°C for 5 minutes
7. Surface activation in O_2 plasma (700 mTorr, 29.6 W)
8. Lamination of one layer (100 µm) of WBR2100 DFR, roll temperature 95°C
9. UV exposure with 300 mJ cm^{-2} to generate fluidic reservoirs
10. Post exposure bake at 100°C for 55 s
11. Developing in solution of 1.6% K_2CO_3

In order to facilitate the release of PDMS from the mold a hydrophobic surface coating was applied:

1. Immersing wafer for 2 hours in 3-mM Octadecyltrichlorosilane in Heptane
2. Sonicate wafer for 5 minutes in pure Heptane
3. Rinse with Methanol
4. Bake on hotplate at 100°C for 1 hour

The full device was made from an assembly of PDMS casts containing the microfluidic network and a PDMS coated disc that served as the substrate for centrifugal actuation. In order to bond the PDMS part containing the microfluidic structures to the PMMA disc, the bonding protocol described by Thorsen *et al.* was used [1]. PDMS was mixed in a ratio of 5:1 (base to curing agent by weight) and poured in the mold. The subsequent degassing step under vacuum for 20 minutes removed entrapped air before the PDMS was placed in an oven for 30 minutes at 85°C for crosslinking. Afterwards the PDMS was peeled off the mold, cut to the appropriate size, and inlet holes were punched. Additionally, a PDMS coated disc (1.5 mm PMMA, RS Components, Ireland) was produced by mixing a ratio of 20:1 (base to curing agent), followed by spin coating the mixture on the PMMA. The PDMS was subsequently cured at 85°C. The PDMS cast containing the fluidic network was then placed on the PDMS coated disc; for bonding, this assembly was baked for 3 hours in an oven at 85°C. These discs were used for the cell staining and discrimination experiments.

For particle manipulation experiments with optical tweezers, the spacing between the microscope objective and the particle must fall below 200 µm. As the previously mentioned PMMA discs display a thickness of 1.5 mm, an alternative manufacturing method had to be established: A mold was prepared as described above. PDMS was mixed in a ratio of 10:1 (base to curing agent by weight), poured on the mold and degassed under vacuum for 20 min. The PDMS was cross-linked in the oven at 85°C for 30 min. The cured PDMS was then released from the mold, the fluidic chips cut to size, and access holes were punched. The microfluidic chip was then placed on a microscope cover slide (60 × 25 mm^2, thickness #1, Sigma-Aldrich, Ireland) fixed to a PMMA frame.

Cell assay protocol

The following protocol was used for identifying ESR-1 positive cells:

1. Fixing of cells with 8 µl of 4% paraformaldehyde (Sigma-Aldrich, Ireland) at a rotation speed of 22 Hz, incubation for 30 min
2. Washing twice with 8 µl PBS containing 0.01% Tween 20 (PBS-T) at f = 32 Hz
3. Cell permeabilization using 8 µl PBS-T containing 0.05% Triton X-100, f = 32 Hz, incubate for 10 min
4. Wash twice with 8 µl PBS-T at f = 32 Hz
5. Loading 8 µl PBS-T containing 5% BSA for blocking, f = 32 Hz, incubation for 30 min
6. Loading 8 µl of anti-ESR-1 antibodies from mouse (sc-8002, Santa Cruz, USA) diluted 1:50 in PBS-T with 1% BSA, spin at f = 32 Hz, incubate for 60 min
7. Washing twice with 8 µl PBS-T at f = 32 Hz
8. Loading 8 µl of anti-mouse IgG conjugated with Alexa Fluor 488 (Molecular Probes Inc., USA) diluted 1:200 in PBS-T with 1% BSA, at f = 32 Hz incubate for 15 min.
9. Washing twice with 8 µl PBS-T at f = 32 Hz
10. Loading 8 µl Propidium Iodide (PI) (4 µL / mL) (Invitrogen, Ireland) at f = 32 Hz, incubate for 20 min
11. Washing twice with 8 µl PBS-T at f = 32 Hz

In order to perform the EpCAM based assays, the above mentioned protocol was modified by removing steps 3 and 4. These steps are not necessary since PI enters fixed cells without permeabilization and EpCAM is expressed on the cell surface. Furthermore, the antibodies in steps 6 and 8 where replaced by anti-EpCAM antibodies from a goat source (BAF960, R&D Systems, USA) and Fluorescein labelled anti-goat antibodies (F0109, R&D Systems, USA), respectively.

[1] T. Thorsen, S. J. Maerkl, and S. R. Quake, “Microfluidic Large-Scale Integration,” *Science (80)*, vol. 298, pp. 580–584, 2002.