Electronic Supplementary Material (ESI) for RSC Advances.

Fabrication of complex PDMS microfluidic structures and embedded functional substrates by one-step Injection moulding

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Supplementary S1. Fabrication details

The following supplementary information outlines the process used to produce PDMS slab components using the PDMS Injection Moulding Technique. This process is similar for both photolithographically defined and 3D printed mould structures.

- Initially PDMS pre-polymer (Sylgard 184, Dow Corning USA) is mixed with the standard 1:10 ratio (curing agent: PDMS) and degassed. Following standard degassing procedure, the pre-polymer is further degassed for approximately 30 minutes under high vacuum (~ 0.2 millibar) with a vacuum pump such as those used in many plasma treatment units. This further degassing step reduces the risk of bubbles becoming trapped between the gas-impermeable mould structures. Small bubbles that may form on feature edges during PDMS injection can be absorbed into the highly degassed PDMS pre-polymer.

- In the case of functional structure integration, the structure intended for encapsulation within the PDMS slab must be placed on the bottom mould structure, and aligned as to interface with channel structures in the desired locations.

- The top mould half is placed in contact with the bottom mould half, and aligned, either manually in the case of photolithographically defined moulds, or automatically through use mechanical alignment features integrated into 3D printed mould structures.

- Mould halves are clamped in place using a mechanical clamp structure (2 pairs of aluminium bars clamped with bolts of appropriate length and fixed with wing-nuts). Sufficient pressure must be applied to ensure firm contact between mould structure and functional structure (or spacer structure in the case of valve fabrication).

- Highly degassed PDMS pre-polymer is loaded into a lubricant free syringe. This syringe is fitted to the 4 mm Luer interface hole in the top mould, and PDMS is smoothly injected to fill the mould. PDMS injection can be continued with excess PDMS flowing out of the mould structure, injection of roughly 2 times the mould volume ensures that any bubbles that are not adhered to the mould structure can flow out from between the moulds.

- Following PDMS injection, the clamped mould structure is placed in an oven at the desired curing temperature. For photolithographically defined moulds a curing temperature of 80˚C is used to maximise effects of thermal contraction assisted mould release. In the case of 3D printed moulds, cure temperature must be kept below the heat distortion temperature of the material (<51˚C for VisiJet® SL Clear 3DSystems Rock Hill, South Carolina).

- For photolithographically defined moulds, mould separation begins to occur spontaneously during cooling when the mould is removed from the oven. The passivated bottom mould half should be removed first, this can be achieved by gentle insertion of a soft tool such as plastic tweezers or a fingernail between the mould halves. 3D printed moulds are separated in the same manner, however this is more challenging due to the lack of a passivated surface and thermal contraction driven delamination.

- Following Release of one mould half, the PDMS slab can be cut to size and peeled from the remaining mould half, the slab can then be plasma treated and permanently bonded to sealing substrates.

Supplementary S2. Cell culture protocol

The following supplementary information outlines the cell culture protocol used to achieve a co-culture in contact configuration within microfluidic devices made using the injection moulding fabrication technique. This protocol is based on our method developed for static systems [1], adapted and modified from published protocols[2] for use in flow-based microfluidic systems. Cell types used are human brain microvascular endothelial cells (ACBRI 376, Cell-Systems Corporation) and human transformed foetal astrocytes (a cell-line termed SVG[3]). Please note: all reagents and media required for these cell cultures are specified in Niego and Medcalf, *JoVE* 2013 [1].

1. Channel cleaning protocol

This initial procedure sterilizes the channel and performs an initial bubble-free fluid priming of the channel, which can be challenging when using complex geometry.

- Perfuse 1 ml of 70 % ethanol at 300 µl/min
- Wait 10 min to sterilize
- Perfuse 1 ml sterile distilled water at 300 µl/min
- Wait 10 min to allow complete ethanol diffusion out of PDMS
- Perfuse 1 ml distilled water at 300 µl/min
- Perfuse 500 µl PBS at 300 µl/min

2. Fibronectin coating

This critical step prepares the channel surface for cell adhesion; from this point onwards fluids can be drawn backwards through the chip to minimize waste of valuable reagents, however care must be take to avoid bubble infiltration.

- Dilute fibronectin in PBS to make 100 µl at 10 µg/ml concentration approx. 50 µl or as required
- Draw minimum volume into the chip at 100-200 µl/min (accounting for tube length + channel volume + diffusion margin approx. 25 µl)
- Wait 45 min for adsorption
- Flush with equal volume to fibronectin treatment (approx. 25µl) of PBS or media at 200 µl/min

3. Endothelial cell seeding

Introduce cells suspended in culture media at concentration of 10 million per ml

- Prepare endothelial cell suspension at a concentration of 10 million cells per ml
- Draw cells into the chip in orientation which allows gravitational sedimentation of cells onto the desired face of the membrane / channel surface
    - Perfuse cell suspension at 100-200 µl/min (a minimum suspension volume needs to account for tube length + channel volume + diffusion margin)
- Clamp tubing to ensure static conditions (i.e. no movement of fluids) for optimal cell adhesion
- Place chip in incubator and allow cells to adhere in static conditions for 2-6 hours

4. Astrocyte Seeding

Astrocytes are cultured on the opposite face of the porous membrane, allowing the two cell types to form physical contact unique to this blood-brain barrier (BBB) model.

- Prepare astrocyte cell suspension at a concentration of 8 million cells per ml
- Flip the microfluidic device upside down
- Draw astrocytes into the second channel. Astrocytes will sink onto the opposite surface of the membrane (and the entire channel length)
- Perfuse astrocyte suspension at 100-200 µl/min (account for minimum volume as mentioned above)
- Clamp tubing to ensure static conditions for cell adhesion
- Place chip in incubator and initiate flow of media to endothelial channel only. Calculate flow to induce a shear rate of 1 dyn/cm^2.

6. Cell growth period

Cultivate the co-culture for 24-48 hours in low shear rates (1 dyn/cm^2; equivalent to physiological shear rates in venules). During this low shear period cells proliferate and form a confluent monolayer. When confluence is reached, the microfluidic BBB contact model is ready for experimentation.

Supplementary S3. Experimental verification of valve functionality

Functionality of valves fabricated using the injection moulding technique was qualitatively verified using a simple experimental setup involving merging of dye coloured water with a stream of clear water. Clear water was fed through a central micro channel using a syringe pump. Intersecting dye channels are maintained at a slight positive pressure by pressurising external fluid reservoirs, and interface to this primary channel via valve gates, which are actuated manually through compression or expansion of gas within a connected syringe [4]. Application of negative pressure lifts these valve gates, while application of slight positive pressure ensures sealing of the valve gate upon closing. Control of the valve gate position is used to modulate the fluidic resistance of the gated channel. Pressure applied to the actuation chambers of the valves is varied between ambient and a relative pressure, to actively close the valve, and between ambient and relative negative pressure to regulate flow rates of the auxiliary dye channels.

These valves are shown in operation in **supplementary Video 1**. This video shows operation of the valves to regulate flow rate of coloured dyes into primary flow of clear water, followed by application of valves in this configuration used to dose droplets with varying concentrations of dye.

References:

1. B. Niego and R. L. Medcalf, *JoVE (Journal of Visualized Experiments)*, 2013, e50934-e50934.
2. R. Booth and H. Kim, *Lab on a chip*, 2012, 12, 1784-1792.
3. E. O. Major, A. E. Miller, P. Mourrain, R. G. Traub, E. De Widt and J. Sever, *Proceedings of the National Academy of Sciences*, 1985, 82, 1257-1261.
4. D. Irimia and M. Toner, *Lab on a Chip*, 2006, 6, 345-352.