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Selective and tunable gradient device for cell culture and chemotaxis study

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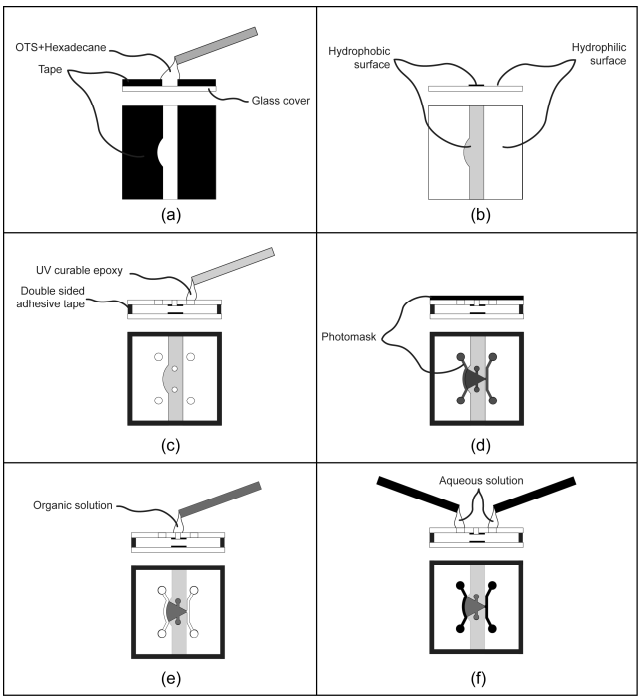
Supplemental materials



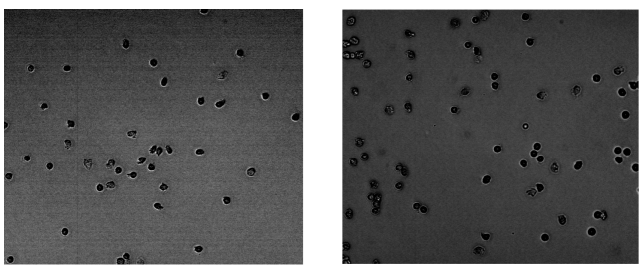
Supplemental Figure S1. Cross sectional and top views of fabrication process. (a) An adhesive tape was attached to two microscope glass cover slips (only one is shown here). Part of the tape covering the observation chamber area was removed. An OTS + hexadecane mixture was applied to the observation chamber area for 5 min. (b) The OTS formed a hydrophobic SAM on the observation chamber area. (c) One of the glass cover slips was cored to have six ports and attached to the other glass cover slip with a double-sided adhesive tape at the boundary after the patterned sides were aligned to face each other. The cavity was filled with UV curable epoxy. (d) A film photomask with the design was placed on top of the device and exposed to UV light. (e) An organic solution was introduced to the observation chamber through the cell inlets. (f) An aqueous solution was introduced to the sink and source channels.

Fabrication

The device was prepared using *in situ* liquid phase photopolymerization (LP^3) [17, 18]. Scotch tape (Scotch® Magic™ Tape 810, 3M, St. Paul, MN) was attached to two microscope glass cover slips. A part of the tape covering the observation chamber area was removed using a razor blade (Supplemental Figure S1a). An OTS (octadecyl trichlorosilane) + hexadecane mixture was applied to the observation chamber area for 5 min (Supplemental Figure 1a). The OTS formed a hydrophobic SAM (self-assembled monolayer) on the observation chamber area while other areas remained hydrophilic (Supplemental Figure S1b). To establish the passive pump [23], the other sides of the microscope glass cover slips were also treated similarly with the OTS + hexadecane mixture to create a complete hydrophobic surface. After removing the remaining Scotch tape, one of the microscope glass cover slips was cored to have six ports. The cored cover was attached to the other microscope glass cover slip with a double-sided adhesive tape at the boundary (125 μm thick for corresponding channel thickness) after the patterned sides were aligned to face each other. Next, the cavity was filled with the UV curable epoxy (NOA73, Norland Products, Cranbury, NJ) (Supplemental Figure S1c). A film photomask with the device design was placed on top of the device and exposed to UV light (EXFO Acticure 4000, Mississauga, Ontario, Canada) for 12 s using a wavelength of 365 nm and an intensity of 24 mW/cm^2 (Supplemental Figure S1d). After removing the unpolymerized epoxy with a peristaltic vacuum pump, the device was flushed with 4 mL of methanol and dried with nitrogen gas.

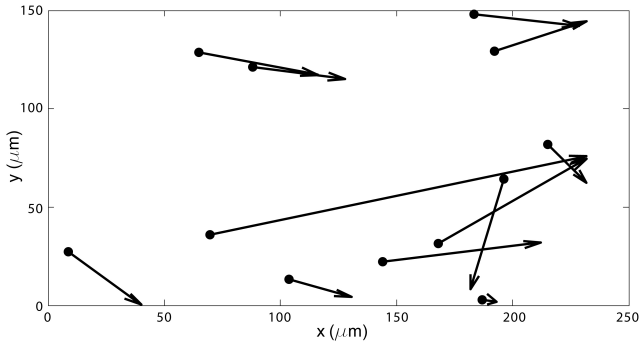
An organic solution (0.006 mL Adipoyl-chloride in 1 mL Toluene) was introduced to the observation chamber through the cell inlets (Supplemental Figure S1e). An aqueous solution (0.01 mL 1,6-diaminohexane 60% solution in 1 mL DI water) was introduced to the sink and source channels (Supplemental Figure S1f). The diaminohexane and adipoyl chloride reacted at the interface of the solutions to form membranes. After a 15 min reaction time, the organic solution was removed and the observation chamber was washed with toluene. The aqueous solution was also removed and channels were washed with methanol. The entire device was washed with methanol and dried with nitrogen gas as the final washing step [19-21].

All chemicals were obtained from Acros Organics (Geel, Belgium) and used ‘as is’ unless otherwise indicated.

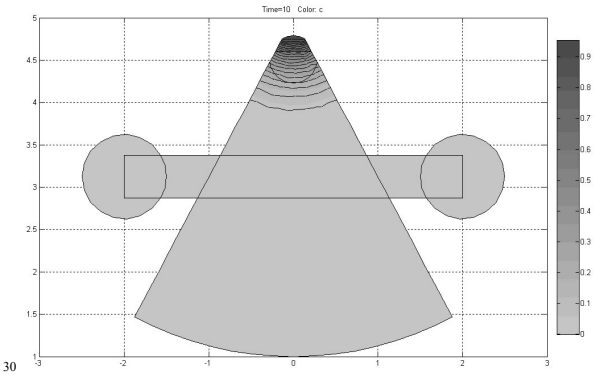


(a) Uncoated polystyrene (b) OTS-treated glass

Supplemental Figure S2. Preliminary cell motility test on polystyrene and OTS-treated glass surfaces. Cell spreading was similar on both surfaces, suggesting the motility on both surfaces was also comparable.



Supplemental Figure S3. Displacement vectors of the 12 neutrophils in the filed of view. Eleven out of twelve neutrophils migrated along the positive *x* direction which was aligned toward the source of the chemoattractants.



Supplemental Figure S5. Normalized concentration profile of the model during the first 10 min computed using MatLab PDETool when the diffusion coefficient was 0.2×10^{-9} m^2/s.

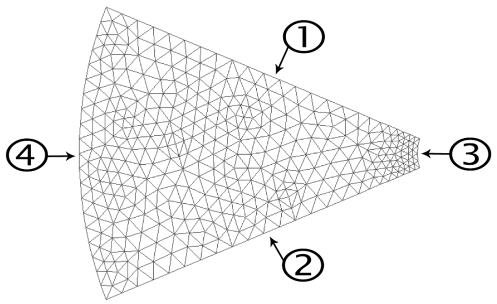
Theoretical model

As a governing equation to predict the concentration gradient at a certain time point in the fan-shaped chamber, the diffusion equation in cylindrical coordinates reduces to:

$$\frac{\partial c}{\partial t} = \frac{D}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c}{\partial r}\right) \quad (1)$$

where c is the density of the diffusing material, t is time, D is the collective diffusion coefficient, and r is the radial coordinate if no gradient along azimuthal and height axes is assumed.

Equation (1) can be solved using the MatLab PDETool (Mathworks, Natick, MA, USA). A geometrical model of the fan-shaped chamber is shown in Supplemental Figure S4. Edges 1 and 2 were assigned to have a Neumann boundary condition with no flux so that they maintain the cylindrical property. Edges 3 and 4 were assigned to have a Dirichlet condition with normalized concentrations 1 (infinite source) and 0 (infinite sink), respectively.



Supplemental Figure S4. Geometrical model of the fan-shaped chamber used for the finite element method (FEM). Edges 1 and 2 were assigned to have a Neumann boundary condition with no flux. Edges 3 and 4 were assigned to have a Dirichlet condition with normalized concentrations 1 and 0, respectively.