Geometrically complex microfluidic devices for the study of cell migration

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Method Article

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Abstract

This method will create polydimethylsiloxane \(PDMS) microfluidic devices for the study of cell migration in geometrically complex environments.

Introduction

Cell migration is a fundamental process in biology and essential for almost all types of immune responses. In recent years, dendritic cells \(DCs) have become a widely used model system for rapidly migrating leukocytes[2,4]. DCs represent the pivotal link between innate and adaptive immunity. They reside in peripheral organs of the body, such as the skin, in an immature state where they constantly scan their environment for antigens. Upon antigen uptake, DCs start to mature, become highly migratory and follow chemokine gradients guiding them towards the next lymphatic vessel. From there, DCs travel to the draining lymph node and present the acquired antigen to T cells, eventually triggering the adaptive immune response[1]. On their way from the periphery to lymph nodes, DCs are challenged with varying environments of different complexities. In recent years, three-dimensional \(3D) collagen matrices have been successfully used to mimic this complexity and to study DC cell migration in vitro[2]. However, the complexity in the z-plane in these assays makes it very challenging to correlate e.g. cell speed or persistence with parameters like cell shape or the distribution of certain molecules. To this end we established a technique to manufacture geometrically complex microfluidic devices. At the same time, cells are confined in the z dimension. This enables the study of cell migration under full optical control in well-defined, geometrically complex environments.

Reagents

• Sylgard 184 1kg PDMS kit \(Ellsworth Adhesives) • 4” silicon wafer \(Si-Mat.com Germany) • Microchem SU-8 2005 \(Microresist technologies Germany) • Trichloro\(1H,1H,2H,2H-perfluorooctyl)silane 97%\(Sigma-Aldrich) • 70% Ethanol • Silicone aquarium glue • R10 medium \(consisting of RPMI1640 supplemented with 10% FCS, 2mM L-Glutamin, 100U/ml Penicillin, 100µg/ml Streptomycin and 50µM 2-Mercaptoethanol) • CCL19 chemokine in R10 \(2.5µg/ml, Peprotech) • Bone marrow derived dendritic cells[3]

Equipment

• 4” diameter casting disk 1 cm thick • Mixer-Defoamer, ARE-250 \(Thinky) • Spin Coater - WS-650-23B \(Laurell Technologies Corporation. PA, USA) • Harrick Plasma Cleaner, pdc-002 \(Harrick Plasma NY) • 200g Laboratory scale 0.01g resolution • LINKCAD, Coreldraw X18 or Autocad software • Quartz 5” Photomask Class 4, \(http://www.jd-photodata.co.uk/ ,England) • EVG Mask Aligner 610 \(EVG group, Austria) • 2mm Harris Unicore biopsy puncher • Hot plate with 1°C resolution, \(Digital hotplate SD 160 Carl Roth) • Sonicator \(Elma, Elmasonic S30) • Quadratic petri dishes 120mmx120mmx17mm \(Carl

Roth) • Falcon easy grip 60x15mm tissue culture dishes with ø17mm hole in the middle \(home made) • #1.5 cover slips

Procedure

Stage 1: Photolitography 1. Design photomask in Coreldraw X18 or Autocad. 2. Convert file to Gerber format using Linkcad 3. Order class 4 photomask, 5” square quartz, 1µ resolution from JD Photo data 4. For best results follow the Microchem protocol for SU8-2005 5. Take a 4” wafer and bake at 110°C for 5 min. to remove water. 6. Spin coat SU-8 2005. Ramp to the final speed over 10 sec. and then spin coat at the final speed for 30sec. For 5µm high structures use 3000 RPM, for 4µm high structures use 5000 RPM, and for 3.5µm structures use 8200 RPM 7. Soft bake on hotplate at 95°C for 2 min. 8. Expose 90 – 105 mJ/cm2 on the EVG mask aligner 9. Post exposure bake 95°C for 3 min. 10. Develop SU-8 in SU-8 Developer **Stage 2: Silanization of wafers** 1. Place the wafers in a wafer carrier. Place inside a vacuum desiccator inside a fume hood, together with a 15ml Falcon tube. Pipette 20µl of the silane into the falcon tube and eject the tip into it. Be careful with the silane, it is hazardous. 2. Apply vacuum on the dessicator, at least 0.1 bar. 3. Seal the desiccator and turn off the vacuum pump to allow the silane to vaporize 4. Wait 1h. 5. Vent the desiccator then put the cap back on the falcon tube and dispose as hazardous waste. 6. Wafers are now hydrophobic and coated with a monolayer of silane and PDMS will peel off easily. **Stage 3: Curing PDMS devices** 1. Make a 6”x6” square of aluminum foil. 2. Shape the aluminium foil around the wafer to make a circular tray. Place the wafer inside the tray and the tray inside a square petri dish. 3. Pour 10:1 PDMS \(approximately 50g total) inside a Thinky mixer cup. 4. Mix the PDMS in Thinky mixer. Mix: 2000 RPM, 2 min., defoam: 2000 RPM, 2min. 5. Pour the mixed PDMS onto the wafer. 6. Put wafer in the dish in a vacuum desiccator. Pump out the remaining bubbles. Vent as necessary to pop the bubbles. 7. Cover the petri dish and place in oven at 80°C overnight. **Stage 4: Dicing PDMS and plasma bonding** 1. Cut off all PDMS and foil off the back of the wafer using a razor blade. 2. Peel off the PDMS carefully. Work with it features facing upward and avoid touching the surface. 3. Cut the PDMS into small pieces according to the design. 4. Punch holes in the PDMS with a 2mm Harris Unicore biopsy punch. Alternatively use a 12 gauge blunt sharpened needle. 5. Clean the PDMS with scotch tape. 6. Place PDMS devices into beaker containing 70% ethanol 7. Sonicate beaker for 5 min., sweep 8. Devices are now ready to be plasma cleaned. 9. Setup a hotplate at approximately 85°C 10. Dry PDMS with an airgun. It is critical to make sure you remove all traces of ethanol. Ethanol will inhibit bonding of devices to glass. 11. Place the PDMS features face up along with #1.5 glass cover slips in plasma cleaner. 12. Start the vacuum pump and pump down until the plasma appears on high power for 2 min. 13. Turn off the equipment and remove the glass and PDMS from the plasma machine 14. Place cover slips on hot plate. For bonding, slightly press PDMS, features facing downward, onto cover slip. Wait 2h, 85°C. 15. For additional bonding one can place the bonded devices in the oven overnight at 80°C. 16. Glue cover slip bound PDMS device to Petri dish with hole using aquarium glue. Let the glue harden over night. **Stage 5: Introduction of cells** 1. Place dish with glued PDMS device into plasma cleaner. Remove the lid of the dish. Apply vacuum for 2 min., and then switch on plasma cleaner to high for another 2 min. 2. Pipette ~3ml of warm R10 on the PDMS device. Additionally flush the device

through the punched holes. Make sure that there are no air bubbles inside the device. Incubate for 1h in a humidified incubator at 37°C/ 5% CO2. 3. Remove medium from the top of the PDMS device to ensure that liquids pipetted into the holes do not mix on top of the device. 4. Designate one hole for the chemokine, the other for the cells 5. Carefully remove 5µl of medium from the cell hole 6. Remove 10µl from the chemokine hole and replace with the same volume of chemokine 7. Carefully pipette 5µl of cell suspension \(50.000 cells) into the hole for the cells 8. Put back into incubator. Cells will start to migrate and reach the maze after 2-3h. 9. Start imaging at microscope of choice.

Timing

Stage 1: _Photolitography._ 1 hour **Stage 2:** _Wafer silanization._ 1 hour **Stage 3:** _Curing the PDMS devices._ Overnight **Stage 4:** _Dicing PDMS and plasma bonding._ Overnight **Stage 5:** _Introduction of cells._ 4-5 hours until starting of imaging.

Troubleshooting

Stage 4: _Dicing PDMS and plasma bonding._ In case of defective bonding make sure that the PDMS is dry and completely free of Ethanol before plasma cleaning. In some cases it might also help to clean the cover slips before plasma cleaning. Immerse cover slips into 70% Ethanol, followed by sonication and drying with an airgun. **Stage 5:** _Introduction of cells._ It has been observed that cells will not enter the device from the hole where they have been injected when their concentration is too low. The concentration of the cell suspension used for injection should be between 5-10x106 cells/ml. If the distance between the chemokine- and cell hole is too big it will take very long until the chemokine has diffused far enough to be sensed by the cells. As a rule of thumb, the distance between the two holes should not be much more than the diameter of the biopsy puncher \(2mm).

Anticipated Results

The protocol will produce high-resolution microfluidic devices of various desired designs \(see Figure 1. for examples) to study cell migration in a controlled, defined and complex environment. The devices have been designed and tested for dendritic cell migration but will also be useful to study any other cell type.

References

1. Banchereau, J., & Steinman, R. M. \(1998). Dendritic cells and the control of immunity. Nature, 392\(6673), 245–52. http://doi.org/10.1038/32588 2. Lämmermann, T., Bader, B. L., Monkley, S. J., Worbs, T., Wedlich-Söldner, R., Hirsch, K., … Sixt, M. \(2008). Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature, 453\(7191), 51–5. http://doi.org/10.1038/nature06887 3. Lutz, M. B., Kukutsch, N., Ogilvie, a L., Rössner, S., Koch, F., Romani, N., & Schuler, G. \(1999). An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. Journal of Immunological Methods, 223\(1), 77–92. 4. Renkawitz, J., Schumann, K., Weber, M., Lämmermann, T.,

Pflicke, H., Piel, M., … Sixt, M. \(2009). Adaptive force transmission in amoeboid cell migration. Nature Cell Biology, 11\(12), 1438–43. http://doi.org/10.1038/ncb1992

Figures

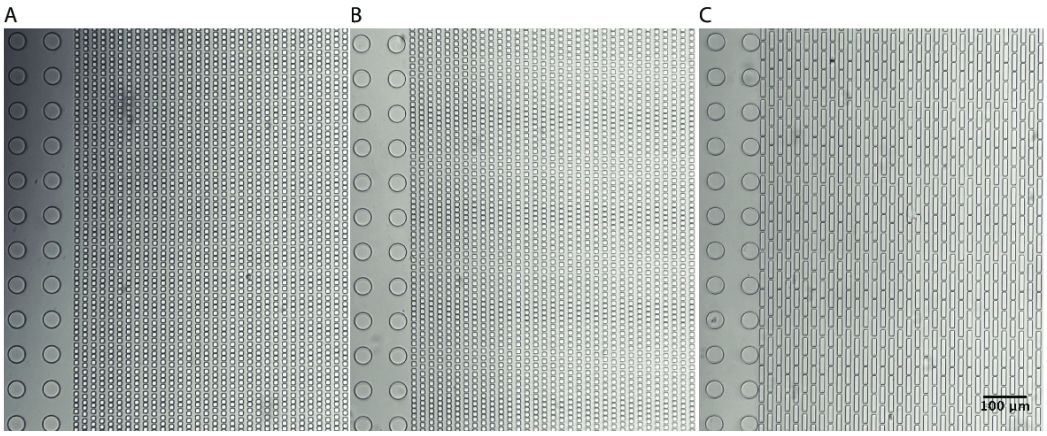

Figure 1

Exemplary designs *(A-C)* Three different examples of microfluidic devices that have been produced with the described method.

Supplementary Files

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