practice 6:

PREPARATION AND TESTING OF MICROFLUIDIC CHIP

location: Loschmidt Laboratories, Kamenice 5/A13, 2nd floor, room 208
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I. WORKFLOW

- preparation microfluidic chip
- basic chip operation
- droplet microfluidics and microscopy

II. MOTIVATION

Microfluidics can be defined as the science and technology manipulating and analysing fluid flow in sub-millimeter dimensions. It is becoming important technology for many emerging applications and disciplines, especially in the fields of chemistry, biology and medicine. Concrete application examples are biosensor devices for molecular diagnostics, polymerase chain reaction chips, high-throughput screening, controlled drug delivery systems, drug discovery methods, forensic analysis instruments, and so on (1).

III. THEORETICAL BACKGROUND

Formation of water in oil droplets in microfluidic chip has several benefits when compared to standard high-throughput technology. Amongst such benefits belong low volume of reagents consumed, chip modularity, low cost and simple fabrication. When all pros combined properly one may encounter drop costs of screening million fold (2, 3).

In this practice students will put hands on microfluidic chip fabrication on their own. During the lesson they will go through cutting out and assembling a chip applying oxygen plasma bonding. Prepared chips are to be used for water in oil droplet generation (4). An example will follow with encapsulation of single E.coli BL21 DE3 cells to droplets. Finally, there will be observation of single cells in emulsions generated.

IV. DESIGN OF EXPERIMENT

Solutions and reagents:

- Dow Corning Sylgard® 184
- HFE-7500 oil
- Trichloro(*1H,1H,2H,2H*-perfluorooctyl)silane
- 1.5M NaCl
- deionized distilled H2O

– *E.coli* BL21 DE3 or other bacterial cells
– Percoll ®
– isopropanol

Equipment:

– Chemyx Fusion 200 syringe pumps
– gas-tight syringes (various volumes)
– PTFE tubing
– razor
– biopsy puncher
– glass slide
– USB-microscope

Protocol for PDMS chip assembling:

1. using razor carefully cut PDMS with chip out of plate
2. with the help of ruler cut out single chip and place them bottom up
3. punch holes for inlet and outlet holes with biopsy puncher
4. clean glass slide and PDMS chip with isopropanol
5. place chip on the tray and put inside the oxygen plasma
6. run oxygen plasma for 3 minutes at 50% generator power at oxygen flow around 5scsm at 200 mTorr
7. immediately after oxygen plasma process ends, bond microfluidic chip and glass cover and press with your fingers; NOTE: try to avoid having bubbles between PDMS and glass
8. place bonded chip on hot plate at 60°C for another 5 minutes
9. let chip cool down to room temperature
10. flush channels with 1 % silane solution in HFE-7500, after 3 minutes replace by pure HFE-7500 oil and air

Droplet formation protocol:

1. load syringes with HFE-7500 oil and 150 mM NaCl, 25 % (v/v) Percoll and properly diluted cells
2. attach PTFE tubing to the syringe and remove any bubbles
3. put syringes into the syringe pumps, lock tight and set proper syringe diameter
4. connect syringe via tubing into the chip
5. set liquid flow – 300 µL.h-1 for oil phase and 30 µL.h-1 for the aqueous phase
6. observe droplet formation under microscope at various magnification
7. verify cell occupation in emulsions on inverted microsope

V. HOMEWORK

1. Estimate volume in pico-/femto- litres for monodisperse droplet formed at channel having dimensions 5, 10 and 20 µm, respectively (assume square cross-section forms spherical droplets of the same diameter).

2. For calculated droplet volumes estimate approximate cell density in $x.10^{y}$ per mL, to put single cell per droplet. There is approximately 1.10^{8} cells in medium with OD_{600} 0.5. Cultivated cells have OD_{600} 4.8. In case that grown culture has insufficient density, calculate the factor for thickening cell media to sufficient level. Account for 10 per cent pipetting error.

VI. LITERATURE

1. Dittrich, P. S., and Manz, A. (2006) Lab-on-a-chip: microfluidics in drug discovery., Nat. Rev. Drug Discov. 5, 210–8.
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3. Yin, H., and Marshall, D. (2012) Microfluidics for single cell analysis., Curr. Opin. Biotechnol. 23, 110–9.
4. Teh, S.-Y., Lin, R., Hung, L.-H., and Lee, A. P. (2008) Droplet microfluidics., Lab Chip, The Royal Society of Chemistry 8, 198–220.