sciFLEXARRAYER Application Note
No. 08012

Microwell Preparation and Multiplexed Cell Encapsulation and Culture

Miniaturization and multiplexing of cell culture on biochip is attracting a growing interest with the development of single-cell assays and cell-based biosensors. Here, our collaborators report rapid methods for microwell preparation and controlled dispensing of different cell lines within these fabricated microwells.

Materials and methods

Gold-coated polycarbonate chips (25x12mm) were prepared by spin-coating a layer of polystyrene (500nm thick).

Microwells (8x8, 400µm diameter) were formed by solvent etching of the polystyrene layer by dispensing 3 drops of toluene (300pL/drop) in each position, using a sciFLEXARRAYER.

Each microwell was subsequently pre-loaded with 22.5nL of $CaCl_2$ (100mM) as cross-linking agent.

In each microwell position, HeLa + or eGFP-HeLa cells (55 ± 15 cells) were dispensed (from 2.10^6 cells/mL suspensions in 0.7% w/v alginate in DMEM) using 55 drops (410pL / drop).

Upon delivery in the pre-loaded microwells, cells were encapsulated by reaction of the alginate with Ca^{2+}. Following encapsulation, the biochip was culture in Ca^{2+} supplemented DMEM for 24 hrs, and then for a further 48 hrs in Ca-free DMEM in order to remove the encapsulation material.

Finally, after washing and fixation, cells were visualized and their viability was studied using a series of fluorescent stains.

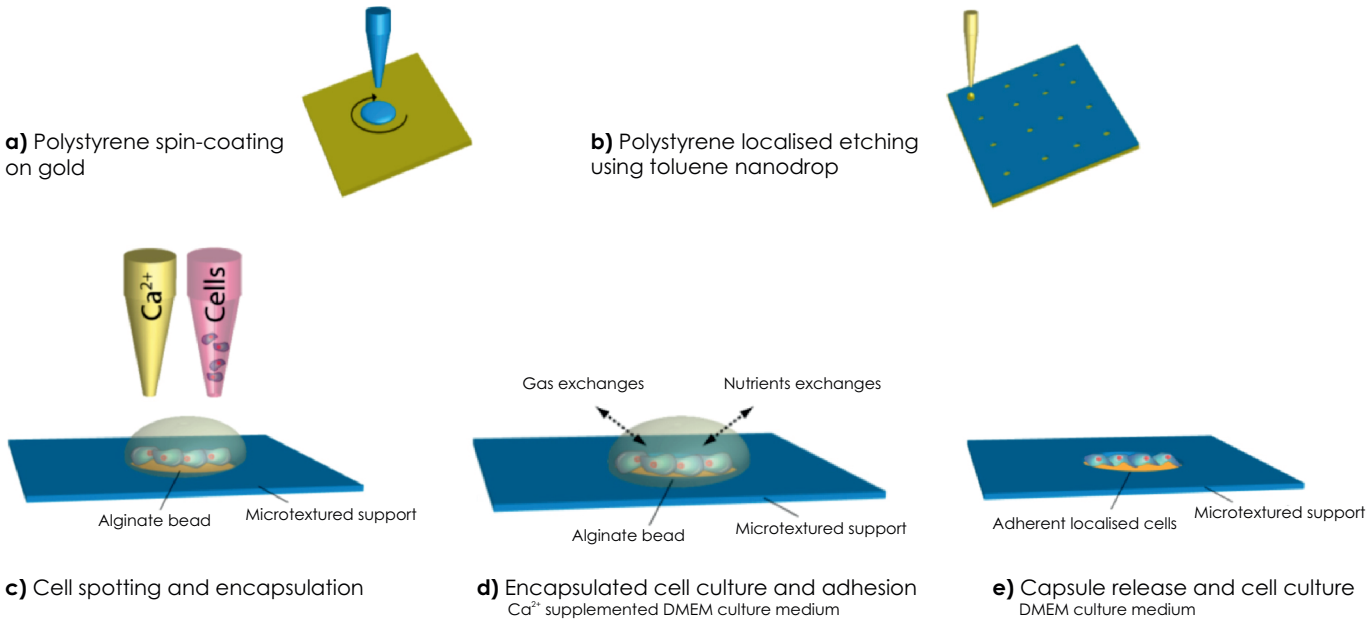

Fig. 1 Schematic illustration of microwell preparation (a, b), cell encapsulation and loading (c), and cell culturing (d, e).

Results and discussion

8x8 microwells of diameter 400µm ± 15µm were formed by etching with 3 toluene drops of 300pL each. The microwell's diameter could easily be tuned with our technology by changing the volume of each dispensed drops of toluene.

Deposition of very small volume of cell suspension is very challenging due to rapid evaporation and it typically yields poor reproducibility and low survival rates. However, the encapsulation procedure described here, allows very high viability (>99,97%) and reproducibility of the number of cells deposited into each microwell (55 ±15 cells / well).

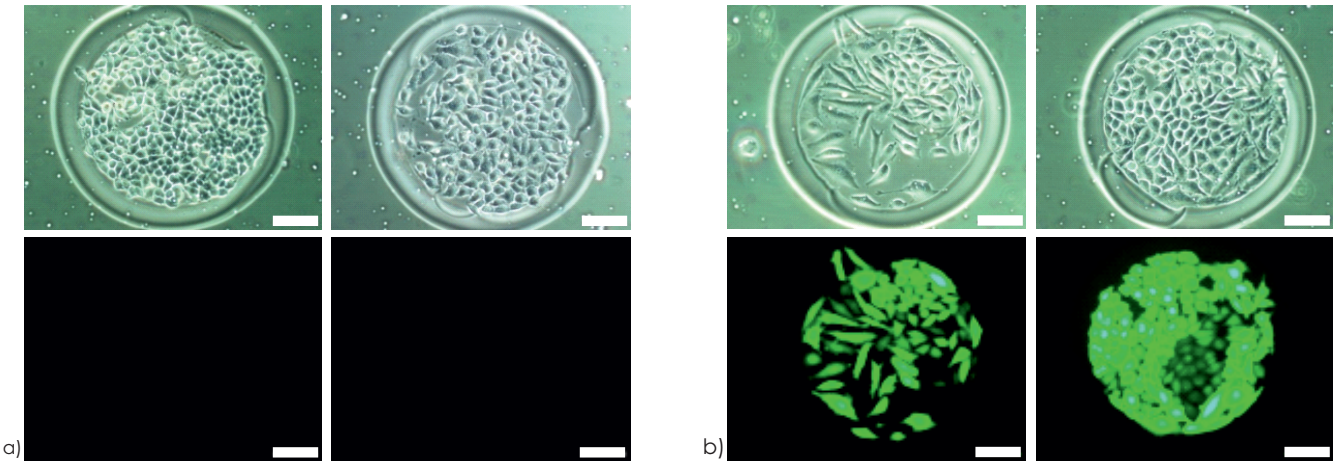

Fig. 2 Optical (top) and fluorescent (bottom) microscopy images of HeLa [+] cell (a) and HeLa–eGFP cell (b) spots after t = 72 h of culture (the white bar in the picture corresponds to 100 µm).

This novel format with different cells in gold-bottom microwells is ideally suited for further cell-based experiments or analysis such as imaging surface biomolecules interactions by surface plasmon resonance (SPRi).

Courtesy of Mrs. Ophelie Berthuy & Dr. Christophe Marquette
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Material surface engineering for multiplex cell culture in microwell Berthuy et all. J Mater Sci 2014; DOI 10.1007/s10853-014-8145-z

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