Fabrication of 3D structures for the assessment of cell mechanical interactions within cell monolayers

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Recent studies highlight that the physical properties of the extracellular matrix, neglected before, modulates many cellular processes such as cell growth[1], cellular differentiation[2] or gene expression[3]. These studies show that adhering cells actively probe the physical properties of the extracellular matrix by pulling onto it through its adhesive regions[4], and respond by modulating their adhesion or their migration activity for instance[5,6,7,8].

A key issue is now to elucidate the stresses that cells transmit to each other in tissues. Measuring the forces at play in cell/cell adhesion is challenging since the use of a force sensor in a cell monolayer leads to the emergence of cell/extracellular matrix adhesions. Few attempts have been done in this direction[9,10] using cell monolayer plated onto 2D substrates like arrays of elastomeric micro-fabricated pillars, but their analysis lacks knowledge on the correlation between the two types of adhesions. Recently, isolated cells were plated onto 2D substrates with micro-fabricated pillars coated with cell/cell adhesion proteins[11], or with pillars able to stress the cell as would do neighbouring cells[12]. However, cell geometry is known to influence gene expression, and consequently cell adhesion. We suggest to use a new experimental setup that simulates more accurately the 3D environment of cells in tissues using stretchable hexagonal mono-cellular 3D structures, both connected in series and parallel distributions.

We show here a way of fabrication of these hexagonal bio-sensors, which are made of biocompatible Poly-Di-Methyl-Siloxane (PDMS). The whole fabrication process consists in silicon mould fabrication (using optical lithography & plasma etching), PDMS filling up, mechanical PDMS planarization, PDMS residual thickness etching, and then silicon master etching. This chain of simple processes allows the making of hexagonal PDMS units (cf. figure 1) using a self-demoulding technology. This demoulding strategy also allows the use of a selective etching of the silicon substrate for the selective functionalization of both the inner faces (for cells attachment, see figure 2) and the outer faces (repulsive areas for cells) of these hexagonal structures.

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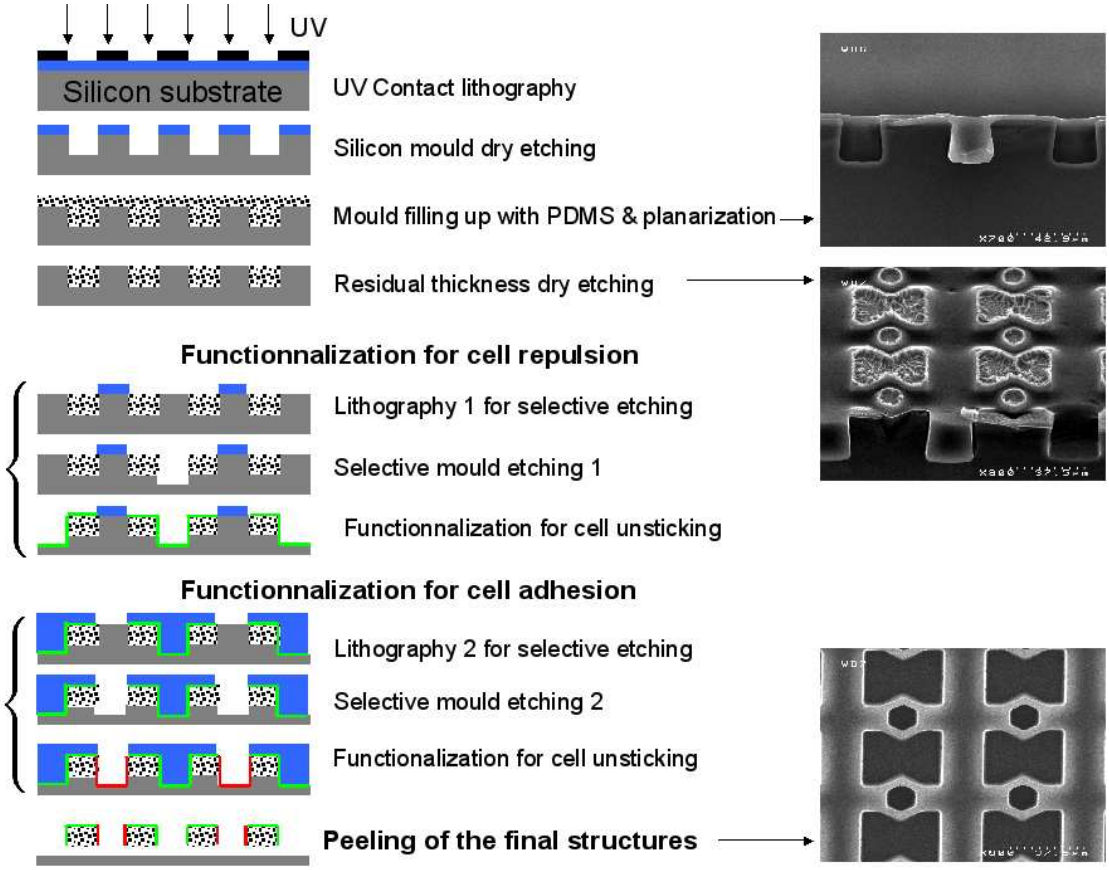

Figure 1: schematic view of the processes chain followed to fabricate the hexagonal cellular force sensor.

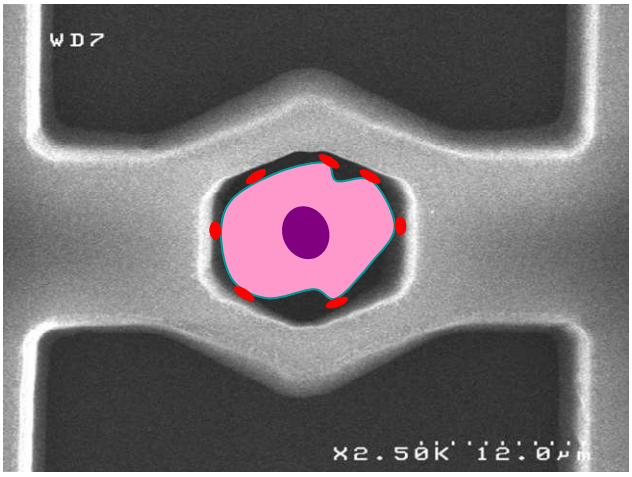

Figure 2: example of final PDMS hexagonal structures,
with a diameter of 20µm, 3µm wall width, and 20 µm height.