MULTIPLEXED CELLS MICROPATTERNING USING CAPILLARY ASSEMBLY

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ABSTRACT

This paper reports a novel technology to localize the immobilization of different types of particles or cells on a surface with a sub-micrometric precision in order to study their interactions. The process uses capillary assembly on arrays of crescent-shaped structures with different orientations. Successive assemblies in different substrate orientations with different types of particles allow for the creation of imbricated arrays with sub-micrometer particle positioning accuracy. Using this process, antibody coated microparticles were assembled on substrates and used as capture patterns for the creation of complex cell networks.

KEYWORDS

Capillary assembly, colloids, multiplexing, biopatterning, cell capture.

INTRODUCTION

Several teams have already succeeded in assembling locally bacteria [1][2], yeasts[3] or mammal cells[4][5] on surfaces, but both the control of the number of cells and the deposition of different types of cells on the same area in a deterministic and precise way remain a challenge.

In our approach we extended the capabilities of capillary assembly to organize multiplexed arrays of functionalized particles on surfaces. These particles were further used as traps for the selective capture of living cells. Capillary assembly is a powerful technique to pattern structured surfaces with high precision using micro or nano-object suspended in a liquid. A colloidal suspension is dragged along a surface topographically patterned with cavities or obstacles. [6]

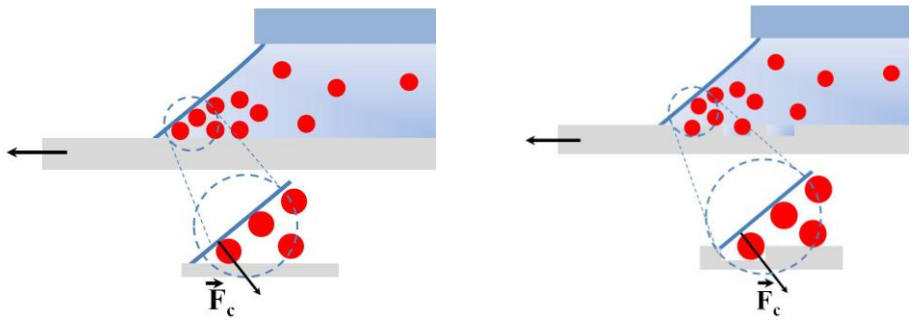

Figure 1. Principle of the capillary assembly process. A colloidal suspension is dragged over a patterned surface. Beads are carried away on flat surface by the capillary force F_c, but are selectively trapped in the recessed or protruding area. The assembly occurs for contact angle typically between 20° and 60°.

Particles are selectively trapped into the recessed or protruding structures while on the flat areas, particles are carried away by the capillary forces exerted by the meniscus and no deposition occurs. This process allows for the creation of dense or sparse arrays of particles over large areas at high speed, up to 1mm/s.

MULTIPLEXED PARTICLES ARRAY

The principle of the multiplexed capture is depicted in figure 2. Our approach relies on the selectivity of the assembly process regarding the size, shape and especially orientation of the capture patterns relative to the meniscus direction. In this work we investigated arrays of crescent-shaped structures with four different orientations on patterned Polydimethylsiloxane (PDMS) samples. We proceeded to successive capillary assemblies by rotating the sample 90° before each deposition. By this process four different types of microparticles could be selectively trapped in the corresponding structures. The surface was dried after each deposition to ensure particles adhesion to the substrate and limit potential release of the already immobilized beads during each assembly step. With this method, imbricated arrays of individual objects could be created, with a sub-micrometer placement accuracy. Figure 2 also shows examples of micrometric polymer particles with four different colors assembled in a deterministic way on a patterned PDMS surface. In this experiment, capillary assembly was done at 20 µm.s^{-1} at room temperature using the suspensions of beads in a 50/50 (v/v) mixture of a 10 mM Sodium Dodecyl Sulfate (SDS) solution and a 0.1% (w/v) Triton X-45 solution.

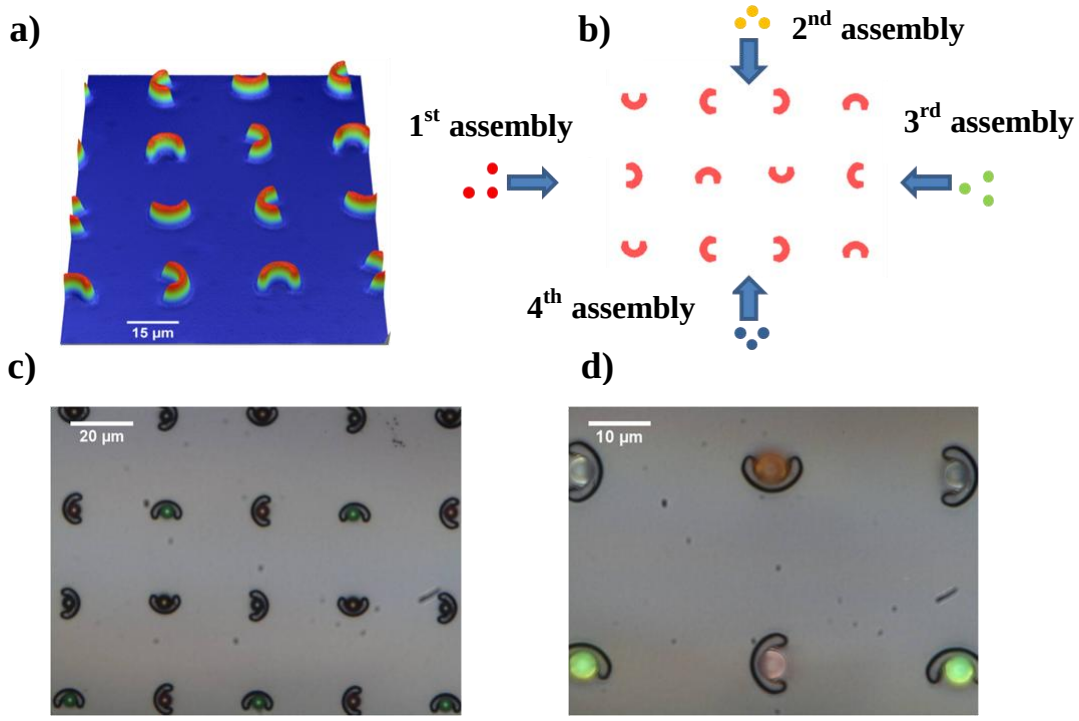

Figure 2. a) 3D profilemeter scan of crescent- shaped structures used for the immobilization of particles. The structure dimension is adapted to the size of the beads. The height of the structures is around 2µm. b) Principle of the multiplexed assembly process. A first assembly is performed by dragging the meniscus perpendicular to the first pattern orientation. The sample is then dried, rotated by 90° and another suspension is dragged over the surface. By repeating this process, four different types of particles can be selectively immobilized on the surface. c)-d) Bright field optical micrographs of an array of dyed beads assembled on a patterned PDMS surface.

MULTIPLEXED CELLS ARRAY

In another set of experiments, antibody coated particles were assembled and used as anchor points for the specific attachment of two types of cancer cells through surface antigen interactions: Human Ovarian Carcinoma cells (Ovcar) and Leukemia cells (Jurkat). A cells suspension was incubated on the surface and further washed by successive dilutions in buffer to remove unbound cells. Cells were captured with a high yield. Another suspension is incubated and thus a second kind of cells is captured. The principle of the technique is depicted on figure 3.

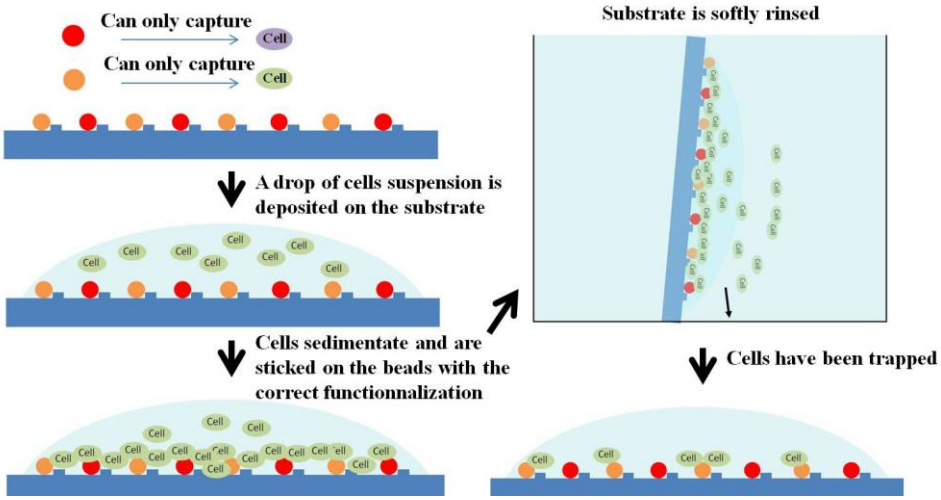

Figure 3. Multiplexed cell arrays: A cell suspension is deposited on a surface patterned with antibody coated beads. Cells sediment on the surface and are captured selectively on the particles. Then the substrate is washed and another cell suspension is deposited. The process can be restarted several times to capture several kinds of cells.

Figure 4 shows an array of Ovcar cells and a fluorescence image of an array of Jurkat (green) and Ovcar (blue) cells, both made by this technique.

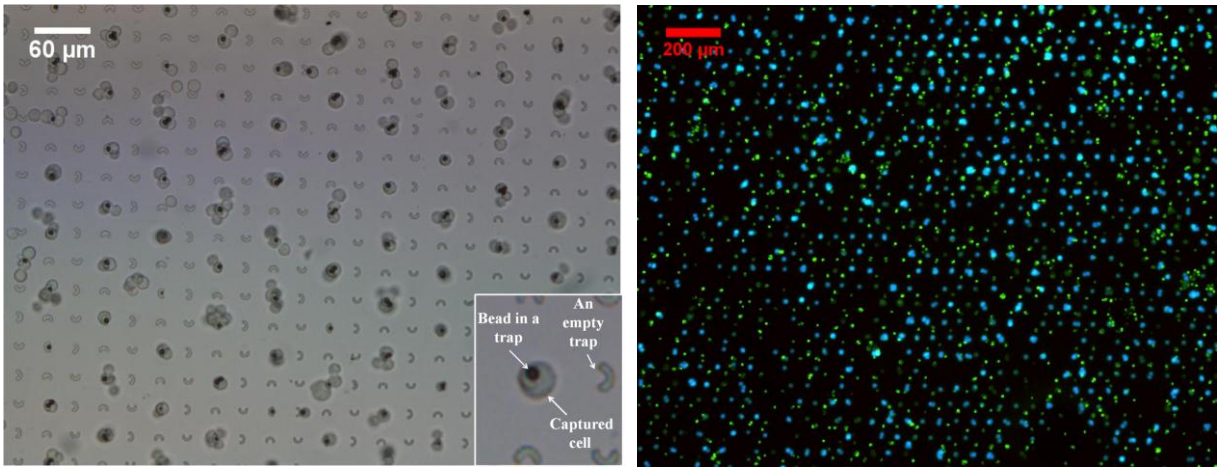

Figure 4. Left: Ovcar cells have been selectively trapped on 4.5 µm anti-CD19 coated particles, thus creating an hexagonal cells network. Cells remain viable for culture or molecular typing. The capture yield is around 90%. Right: Fluorescence image of an array of stained Jurkat (green, stained with Wheat germ agglutinine, Oregon Green® 488 conjugate) and Ovcar (blue, stained with Hoechst™ 33342 trihydrochloridetrihydrate).

By this method, imbricated arrays of cells can be easily prepared with a high specificity. The method can be extended to any arbitrary arrangement with a sub-micrometric resolution. Moreover, once captured, the cells remain viable for culture or any further molecular characterization. We are convinced that the easiness, low cost, effectiveness of this cell patterning approach is an enabling tool to develop new high-throughput screening technologies for cell-cell interactions studies.

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