SOFT LITHOGRAPHY A USEFUL TECHNIQUE FOR BIOLOGY AND MEDICINE

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For many applications of biochip technology for medical diagnosis or drug screening, it is necessary to fix probe molecules on a surface at registered position. This technological process is called biopatterning. The spatial resolution and the quality of the arrangement of the probe molecules on the surface are of crucial importance for both the density of the chip and its sensitivity. I will show in the presentation why soft-lithography and more specifically Micro-Contact printing (µCP) exhibit specific advantages for advanced biopatterning.

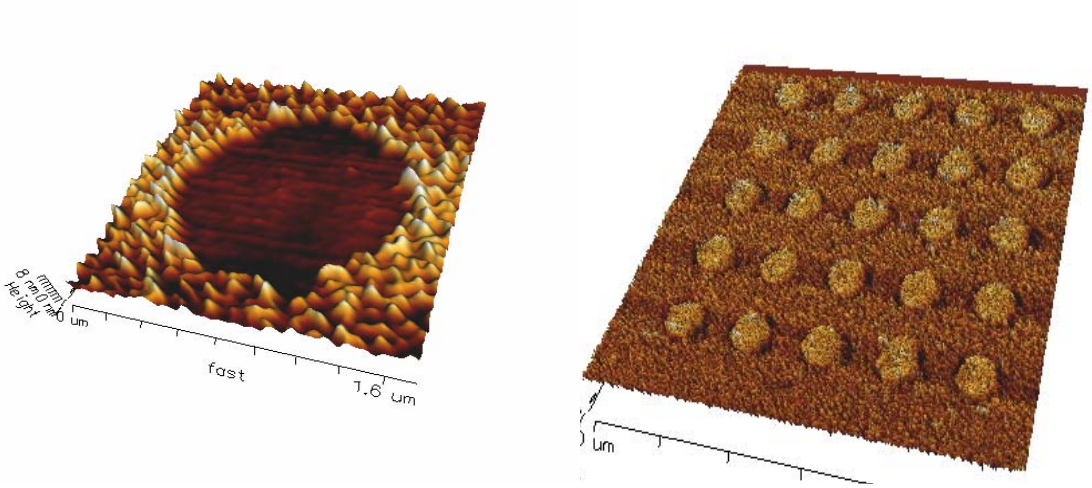
In a first part, I will show how µCP can be used for the fabrication of DNA micro array [1]. We have shown that inking times and contact times of less than 30 seconds give high quality and high resolution arrays. Moreover, we have found that the fluorescence signal emitted by DNA hybridization spots created by µCP is systematically higher compared to similar spots created by conventional tip deposition. In order to interpret the rapid and efficient adsorption of DNA molecules on freshly cured PDMS stamp as well as the improvement of the fluorescence signal of printed DNA spots, we have investigated the possible role of free PDMS fragments present at the stamp surface. Our results show that in the case of DNA printing, the presence of PDMS fragments in the elastomeric stamp improves a lot the quality of the transfer of the molecules [2].

In a second part I will address the key issue of printing different molecules in one step. Two methods have been developed. The first one is capable of generating self-aligned patterns of different biomolecules using PDMS stamps exhibiting several levels of topography. The principle is based on the deformability of the PDMS stamp, used for bringing into contact with the surface the different levels of the stamp which have been selectively inked with the different biomolecules [3]. The second one is adapted to the production of advanced microarrays. We have developed and patented a novel method for Multiplexing the µCP technique which targets the deposition of 100-1000 different molecules in one step. We have fabricated stamps containing millimetric dots arranged in a periodic array compatible with titration plates. Through a very simple procedure of molding, each of these dots includes micro and nanoscale features on its surface. Then by inking in a single step each dot with a different ink through a titration plate, it becomes possible to print different inks in one step. Our latest results show that without any trace of contamination, up to 800 different biological inks can be printed in one step.

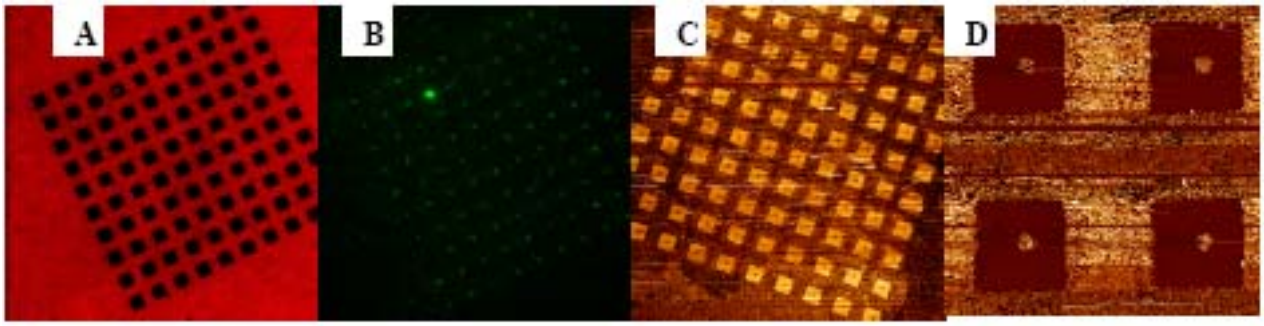
In a last part, I will present the fabrication of submicronic domains of lipidic bilayers using soft-lithography and self-assembly [3]. This method is designed for the investigation of membrane proteins and the assembly of nanomachines like the flagellar nanomotor of bacteria. Along this route, that combines nanopatterning with bottom-up assembly, we have used microcontact printing (µCP) for fabricating self assembled supported phospholipids membranes that can mimic cell membranes and their compartments due to the ability of the technique to shape domains at the micro and nano scale. I will also present some new results showing the versatility of the method for organized DNA combing or immobilization of single living bacteria [4].

References:

[1] C. Thibault, V. Leberre, S. Casimirius, E. Trévisiol, J.M. François and C. Vieu, , Journal of Nanobiotechnology 3:7, (2005) (http://www.jnanobiotechnology.com/qc/content/3/1/7)
[2] C. Thibault, C. Séverac, A.F. Mingotaud, C. Vieu and M. Mauzac, Langmuir, 23 (21), 10706 -10714 (2007)
[3] Chalmeau J, Salome L, Thibault C and Vieu C, MICROELECTRONIC ENGINEERING Volume: 84 Issue: 5-8 Pages: 1754-1757 (2007)
[4] Aline Cerf, Jean-Christophe Cau and Christophe Vieu, Accepted in Colloid and Surfaces (2008)



AFM images in liquid medium of sub micronic domains of supported lipidic membranes. The lipidic bilayer is patterned into disks of 1 µm (left-side) and 500 nm (right side). The surrounding areas correspond to BSA proteins that have been printed by µCP and prevent the fusion of liposome on the glass surface.



Self aligned patterns of BSA (red) and Streptavidine (green) printed in one step. A) Epifluorescence of BSA patterns, B) Epifluorescence of Strepatavidin patterns, C) AFM view of a large number of printed patterns, D) AFM view of four elementary patterns of 1 µm wide dots of Streptavidin aligned in the middle of 5 µm wide square surrounded by BSA meshes.