MICROCONTACT PRINTING OF PROTEINS ON MODIFIED SUBSTRATES FOR PROTEIN ARRAYS

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Enhancement of protein array sensitivities is an important goal, especially because many molecular targets within patient tissues are of low abundance. The ideal array substrate will have a high protein-binding affinity and low intrinsic signal. To date glass has provided an effective substrate for protein binding in the microarray format when using fluorimetric detection systems. Other substrate materials as silicon with and without modifications have been studied [1] and several solutions [2] have been addressed in order to solve the low signal-to-noise ratio and poor repeatability of results.

Currently micropatterning of proteins is still under development unlike DNA microarrays. It is known that proteins can be transferred to a substrate without the loss of biological activity using microcontact printing techniques [3]. The low cost of fabrication, as well as the simplicity of transferring proteins to substrates when compared to other techniques such as lithography, pin arrayer, printing robots or ink jet printers, makes microcontact printing fabricated arrays very attractive.

In this work, via chemical modification, reactive groups: amino groups, Glutaraldehyde and Streptavidin have been added to the glass surface for comparison of relative protein binding. All substrates have been studied by fluorescence microscopy, Raman spectroscopy and contact angle measurements in order to be able to select the most appropriate for protein microarrays obtained by microcontact printing.

The biotin-streptavidin system has been studied comparing adsorbed proteins via conventional chemistry and microcontact printing on those modified substrates. As expected, the glutaraldehyde substrates showed the highest protein binding both with biotin and streptavidin. Surprisingly, both proteins are adsorbed on the amino groups by microcontact printing whereas no or small reaction occurs in solution.

It can be concluded that aminated substrates have the lowest fluorescence signal, lowest contact angles and are a good candidate for microcontact printing of protein arrays. It can also be concluded that with a 5min process a high density of proteins is bound to the substrates. On Fig.1 a comparison of the spots created on the different substrates.

[1] A. Jasper Nijdam et al, "Physicochemically modified silicon as a substrate for protein microarrays", Biomaterials 28 (2007) 550-558
[2] Y. Zhang "Micropatterning of proteins on nanospheres", Colloids and Surfaces B: Interfaces 48 (2006) 95-100
[3] HD Inerowicz et al "Multiprotein Immunoassay Arrays fabricated by microcontact printing" Langmuir 2002, 18, 5263-5268

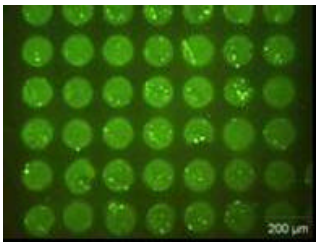
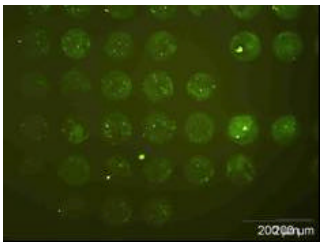
a) Aminated surface	b) Glutaraldehyde-modified surface	c) Streptavidin-modified surface
		

Fig1. Microcontact printing of biotin fluorescein on substrates with different functionalizations: a) amine groups; b) glutaraldehyde and c) streptavidin.