FABRICATION OF A MICROFLUIDIC CELL MADE OF THIOL-ENE FOR MICROARRAY APPLICATIONS

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ABSTRACT

We report on the fabrication of a microfluidic cell completely made of thiol-ene for microarray applications. To overcome the issues related to the hydrophobic nature of the thiol-ene material we have also introduced a new polymeric coating based of *N,N*-dimethylacrylamide (DMA) which is able to reduce the material hydrophobicity, to avoid aspecific protein binding and, thanks to the functional monomer propinyl mathacrylate, it is able to bind, in an oriented way, azido modified biomolecules.

KEYWORDS: Microfluidics, thiol-ene, coatings, microarray, antibody oriented immobilization

INTRODUCTION

Thiol-ene formulations have gained a considerable interest in the field of micro and nano fluidics, because of their physico-chemical properties and their polymerization characteristics. Recently this newly introduced UV curable system has been proposed as a new material for rapid prototyping of micro and nano fluidic systems [1]. Here we report on the fabrication of a disposable microfluidic cell completely made of thiol-ene for microarray applications. To overcome the issues related to the hydrophobic nature of this material, which represents a considerable drawback to its application in micro and nano fluidics [2, 3], we have also introduced a new copolymer able to form a stable coating on thiol-ene. The copolymer backbone is made of *N,N*-dimethylacrylamide (DMA) bearing either silanols (MAPS) and -yne (PMA) groups which has the double function of grafting to the thiol groups on the surface by a UV assisted reaction and to bind azido-modified biomolecules via a *click chemistry* reaction.

EXPERIMENTAL

A microfluidic cell is obtained by pouring thiol-ene monomer mix into a PDMS mould. The mix is formulated so to have an excess of 40% of thiol groups on the surface. As shown in Figure 1, the cell is composed by two parts: a flat surface where the biomolecules are immobilized and a patterned lid with connectors for the introduction of the assay reagents and for the washing steps. The two parts are bonded and sealed by a simple finger pressure and, at the end of the assay, the microfluidic cell can be easily opened and the flat part is analysed by a fluorescence scanner.

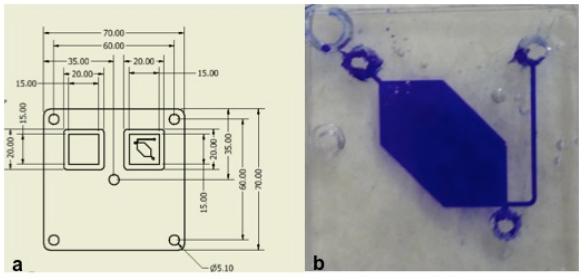

Figure 1: a) Schematic representation of the two part of the thiol-ene disposable microfluidic cell b) assembled thiol-ene microfludic cell filled with bromophenol solution

Both the two parts have been coated with a new copolymer composed of *N,N*-dimethylacrylamide (DMA), propinylmethacrylate (PMA) and 3-(trimethoxysilyl) propyl methacrylate (MAPS), poly(DMA-PMA-MAPS) (Figure 2), by a simple *dip and rinse* process assisted by UV light (365 nm). After the coating step, biomolecules have been immobilized on the flat surface by means of a piezoelectric spotter. In fact, the functional monomer of this copolymer (PMA) allows the oriented immobilization of azido-modified biomolecules via a *click chemistry* reaction (Figure 2).

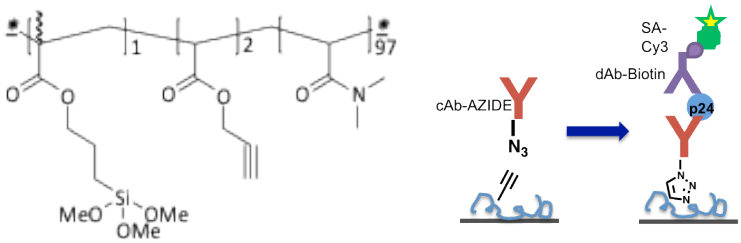

Figure 2. (Left) Structure of p-(DMA-PMA-MAPS). The coating is performed by immersing the slides into a 1% w/v aqueous solution of the polymer in presence of a photoinitiator (Irgacure 2959), exposed to UV light for 30 minutes and then cured at 80°C for 30 minutes under vacuum.(Right) Schematic representation of the microarray assay performed in the microfluidic cell using an oriented immobilization of the antibody

We ran a classic sandwich assay to detect antigen p-24 wich is an early marker of infections of HIV. The antibody against HIV-p24 (cAb-p24) is modified using the SiteClick™ antibody labeling kit [4], which introduces an azido group only onto the heavy chain of the antibody, far from antigen-binding domain. A classical sandwich assay is completely performed into the chamber: after the biomolecules immobilization, the cell is sealed and the antigen p24 is injected into the cell with a flow rate of 0.2 uL/min for 90 minutes. Subsequently, a biotin-labeled detection antibody against p24 is injected, followed by a cyanine-3 labeled streptavidin, using the same flow rate.

RESULTS AND DISCUSSION

Poly(DMA-PMA-MAPS) forms a stable coating onto the microfluidic cell surfaces because of the UV assisted reaction between the triple bond of PMA and the thiol groups of thiol-ene. The presence of PMA allows also the oriented immobilization of azido modified biomolecules onto the flat part of the cell, via a *click chemistry* reaction, in order to run a classic sandwich microarray experiment. Figure 3 shows the spotting scheme and the fluorescence signals obtained analyzing the flat part of the cell using a fluorescence scanner: the signal is obtained only where the specific azido modified antibody against the antigen p-24 (B) was immobilized. The same antibody which has not been modified with the azido group (A) did not give any signal, demonstrating the specificity of the binding to the surface; similarly, no signal was obtained where an azido modified negative control was immobilized (Z), demonstrating the specificity of the assay.

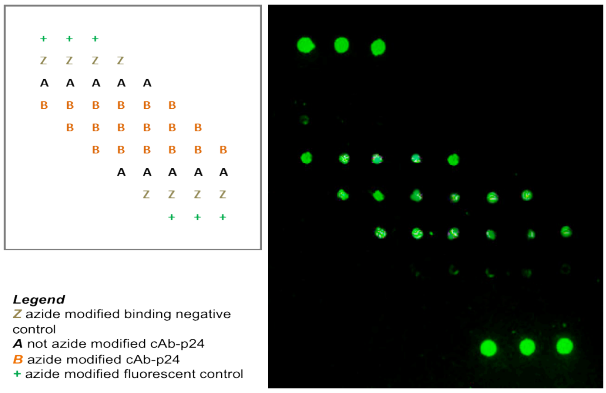

Figure 3. Spotting scheme and fluorescent result of the assay

The presence of an azide group on the heavy chain of the antibody, far from antigen-binding domain allows the perfect orientation of the biomolecule on the surface, thus improving the performance of the entire assay.
Figure 4 shows a comparison of the relative fluorescence intensity between a classic static incubation and the assay performed into the cell demonstrating the feasibility of the assay in a fluidic system which requires less reagent volumes that can be easily automated.

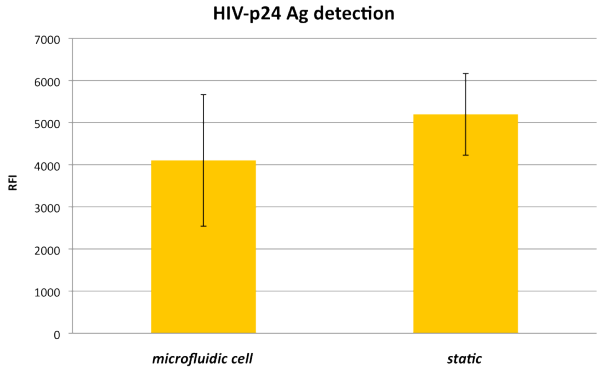

Figure 4. Comparison of relative fluorescence intensity between a classic static incubation and an assay performed in the microfluidic cell. Intensities are detected with laser power 70% and PMT gain 70%.

CONCLUSION

A disposable microfluidic cell completely made of thiol-ene has been fabricated and coated with a new polymer, poly(DMA-PMA-MAPS). The functional monomer of this polymer allows to form a stable film on the thiol-ene surface and to bind in an oriented way azido modified biomolecules. A sandwich assay to detect HIV p-24 protein was entirely perfomed within the cell demonstrating the feasibility of a completely automated process that requires less reagent volumes (4 µL) respect to a classic static protocol.

ACKNOWLEDGEMENTS

This work was supported by the EU through FP7 NADINE (NAnosystems for early DIagnosis of NEurodegenerative diseases) project contract n° 246513.

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