ENZYME IMMOBILIZATION INSIDE BIOMEMS USING PLASMA POLYMERIZED FILMS

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Abbas, Abdennour[1,2,3]; Treizebre, Anthony[1]; Vercaigne-Marko, Dominique[3]; Guillochon, Didier[3]; Vivien, Céline[2]; Bocquet, Bertrand[1]; Supiot, Philippe[2]

[1] *Institute of Electronic, Microelectronic and Nanotechnology (UMR-8520) y, University of Lille1, France.*
[2] *Laboratory of Process Engineering of Reactive fluids-Materials Interactions (EA 3571), University of Lille1, France*
[3] *Laboratory of Biological Process, Enzymatic and Microbial Engineering (EA 1026), University of Lille1. France*

Abdenour.abbas@iemn.univ-lille1.fr

The immobilization of biological active species is the crucial step for the fabrication of bio/chemical micro-electromechanical systems (BioMEMS). Several fields are concerned including biological analysis, chemical microreactors, environmental investigations and clinical diagnosis. The fixation of biomolecules through a covalent bound and intermediate linker is one of the most interesting methods. It allows a more stable linkage than that through adsorption phenomena, and gives best access to the active site of the molecule. This work aims to covalently biofunctionalise a microchannel using cold plasma polymerization.
First, microchannels measuring 50µm width and 150µm depth were etched in a silicon wafer. The first level supporting the microfluidic circuits was coated with a thin film to get primary amine functions on the surface. This aminofunctionalisation was performed by plasma polymerization of allylamine monomer in a home-built radio-frequency plasma reactor. The allylamine polymerization essentially involves the amine functionalities and/or the double bonds (1). We have developed a process to favour the double bonds pathway, and thus promote the retention of amine functional groups (fig.1). Infrared spectroscopy (FTIR) and UV-Visible absorption analysis show that a polymer surface with 15 NH_2 molecules/nm^2 is achievable. This could permit a multipoint attachment of molecules. Beside this, plasma polymerization enables the amino-functionalisation of any surface with integrated sensors within a few minutes. Finally, we used a glass substrate to close the microchannels. The last step of the bio-functionnalisation is the enzyme immobilization. We chose Trypsin (EC 3.4.21.4) as enzyme model. A glutaraldehyde solution was injected into the microchannel and coupled to the aminated surface. This activation was followed by trypsin immobilization and amine reduction (Fig. 2). The developed process allows us to obtain either a surface with some scattered enzymes or a surface covered with an enzyme monolayer, as shown by atomic force microscopy (AFM) (Fig. 3) and X-ray photoelectron spectroscopy. This feature is of a great importance for the analysis of enzymatic kinetics. To demonstrate the efficiency of the immobilization process, tryptic activity was monitored by fluorescent microscopy according to the hydrolysis reaction of *Nα*-Benzoyl-L-Arginine-7-Amido-4-MethylCoumarin (BA-AMC):
BA-AMC + H_2O → *Nα*-Benzoyl-L-Arginine + 7-Amino-4-MethylCoumarin (AMC).
The results obtained show that the immobilized enzyme remains active for multiple use cycles (Fig. 4). This feature is important for a best understanding of enzyme catalysis in microchannels or for further sensing applications.

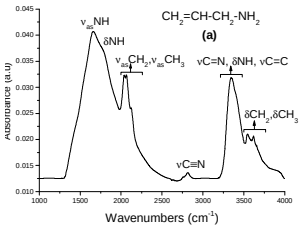

Figure 1: Infrared spectrum of allylamine plasma polymer.(a) allylamine monomer formula.

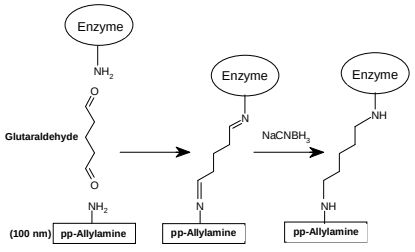

Figure 2: Biofunctionalisation mechanism of the biosensor surface .

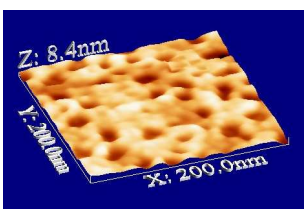

Figure 3: AFM surface topography of protein monolayer.

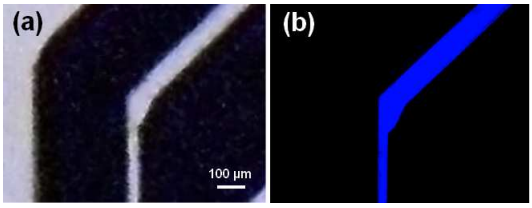

Figure 4: A microchannel observed with fluorescence microscopy before (a) and after (b) the addition of the substrate BA-AMC. In blue, the fluorescent product (AMC) of the enzyme. reaction.

(1) Choukourov, A. *et al.* Mechanistic studies of plasma polymerization of allylamine. *J. Phys. Chem. B* 2005, 109, 23086-95