Immobilization of enzyme by entrapment in polymer thin film synthesized by RPECVD

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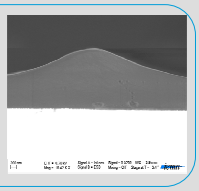
Context and purpose

Following the fast evolution of microfluidics and nanotechnology, the elaboration of efficient enzyme immobilization processes is becoming of great interest for the development of new and original analytical tools or microreactors Recently, cold plasma polymerization of 1,1,3,3,tetramethyldisiloxane (TMDSO) has been successfully used for the simple fabrication of microchannels [1]. In the context of BioMEMS manufacturing, we present a fast, innovative, and biocompatible method for the rapid fabrication of bioactive coatings using this plasma polymerized 1,1,3,3,tetramethyldisiloxane (ppTMDS) as carrier matrix. Using β-galactosidase and pepsine as enzymes, we aim to develop a one- or two-steps immobilization procedure in order to fabricate a bio-functionnal layer where the enzymes are expected to be entrapped into the polymer matrix while preserving their native structure and their activity.



Experimental procedure

Two distinct enzyme physical immobilization methodologies

- a one-step procedure for which enzyme is in solution with the TMDSO monomer

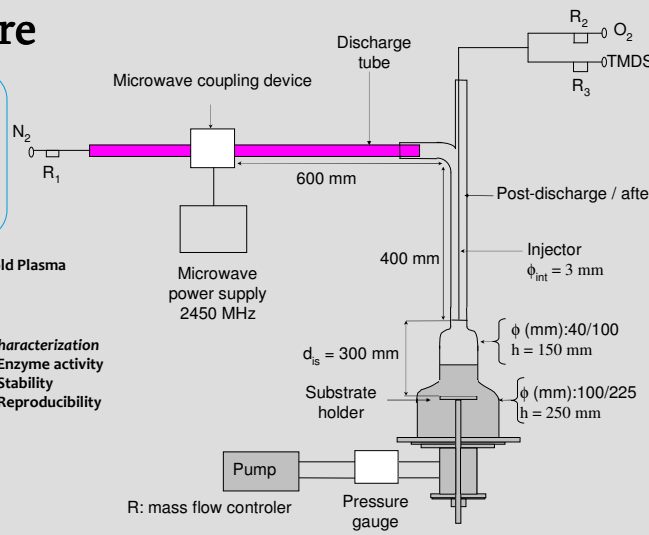
- a two-step procedure for which the enzyme is adsorbed on the surface (aluminium or silicium) before its exposition to the flux

TMDSO Ethanol β-galactosidase Cold Plasma

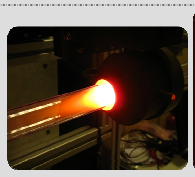
Variable
- Deposition order of constituents
- Deposition duration
⇒ Polymer thickness

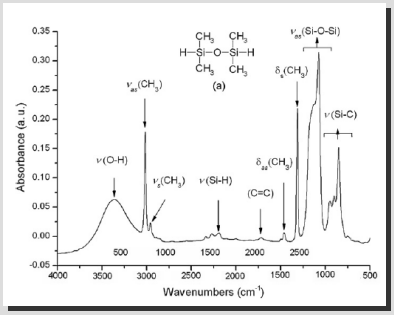
Characterization
- Enzyme activity
- Stability
- Reproducibility

Techniques used
- FTIR (Chemical surface state)
- GBX Digidrop (Contact angle)
- Microscopie MEB (Thickness)
- Nanoscope/AFM (Surface topography)



Experimental RPECVD* set-up
* Remote Plasma Enhanced Chemical Vapor Deposition

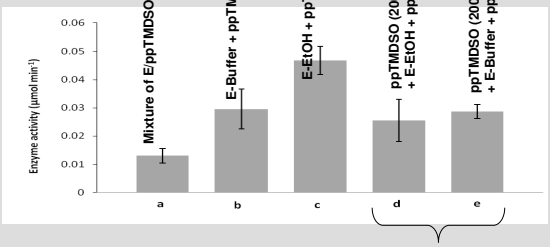




Infrared spectrum (FTIR) of plasma polymerized TMDS (ppTMDS) and semi-developed formula of the monomer

Results

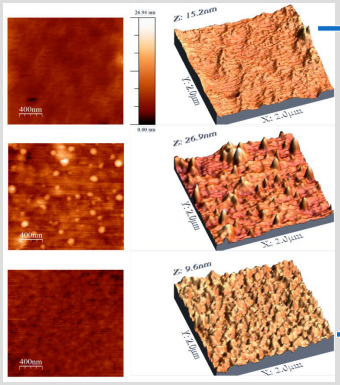
Influence of deposited enzyme support nature



Direct influence on the nature of the support on the deposit and the activity of the enzyme

ppTMDSO film hydrophobicity >> Silicon hydrophobicity

Surface Topography by AFM



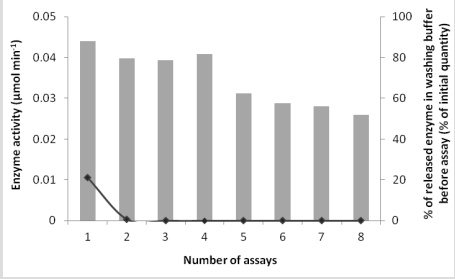
Cold plasma treatment

Polymerization

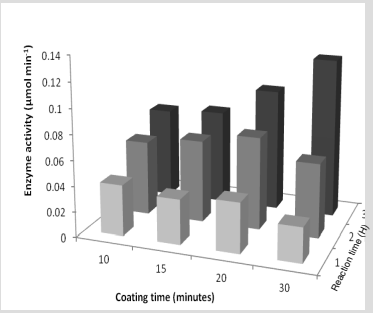
Agregates of β-Gal

Polymer re-organization during the contact with β-Gal

Influence of deposited enzyme support nature



Activity loss of only 23% after 8 washes (thickness=200nm)



- Polymer thickness ↑ ⇔ Better retention of β-Gal
- Diffusional limitations
- No uniformity of polymer thickness
- Compromise between Thickness / Quantity of adsorbed enzyme / Enzyme activity

	Film thickness (nm)		
	~200	~500	~650
Released enzyme quantity (µg)	14.06 ± 1.44	8.33 ± 0.23	5.53 ± 1.49

	Km^{app} (mM)	$Vmax^{app}$ (µmol.min⁻¹.mg enzyme⁻¹)
« free » β-galactosidase	2.23	9.83
Immobilized β-galactosidase	0.74	1.52

Conclusion and perspectives

Cold plasma technology allows fast immobilization of enzyme while retaining their bioactivity after several assays. The results reveal the feasibility of this physical non-conventional immobilization process. Further investigations and optimizations of the technological process will certainly enable the development of new biofunctional coatings for specific applications . Integration of this technology in microsystems fits into this context [1-3].

[1] Abbas A., Supiot P., Mille V., Guillochon D., Bocquet B., *Journal of Michromechanics and microengineering* (2009), **19**, 045022, 1–8.
[2] Bocquet B., Bourzgui NE., Guhel Y., Mille V., Vivien C., Supiot P., *Proc. Of SPIE, vol. 5345, 118-129.*
[3] A. Elagli, K. Belhacene, C. Vivien, P. Dhulster, R. Froidevaux and P. Supiot. J. Mol. Catal. B: Enzym. Online 30/09/2014.