Project Number: 257401

A highly integrated and sensitive PORous SIlicon based lab on a chip for multiple quantitaTIVE monitoring of Food allergies at point of care.

Specific Targeted Research Project
Information Society Technologies

D6.1A: Report on the receptor immobilization methods on porSi structures and conditioning requirements for immobilised receptors.

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

1 About this addendum

1.1 Introduction

The set-up of a functionalization protocol for porSi structure and the optimization of binding conditions for food allergens have previously been reported in D6.1 and D6.2.

The reviewers recommendations following the consortiums report on the first reporting period included that D6.1 would be accepted on the condition that receptor immobilisation in a porous silicon device was demonstrated. Specifically:

"D6.1 Report on the receptor immobilisation methods on porSi structures and conditioning requirements for immobilised receptors

Conditionally Approved *Receptor immobilisation should be demonstrated in the final selected porous Si device"*

This report, an addendum to D6.1, aims to meet this condition by reporting on recent experiments performed with the second generation of porSi membranes where surface functionalisation by the copolymer has finally been demonstrated throughout the entire thickness. This combined with the already demonstrated ability of the copolymer to act as an effective surface chemistry for silicon oxide (the same surface chemistry as that of porSi) in allergen specific IgE detection would strongly suggest that receptor immobilisation can now be done in a final selected porous Si device. It is quite possible that this can be shown in the upcoming intermediate review meeting.

Structure of this deliverable

The report is organized according to the following sections

2 Description of work performed

2.1 Introduction

The activity of Workpackage 6 is aimed at modifying the porSi structures in order to obtain a surface for receptors (allergens) immobilization with high binding capacity, preservation of native conformation of proteins and low background noise.

Due to the temporary lack of suitable porous silicon membranes from UNITN flat silicon oxide substrates were substituted to develop the surface chemistry. Thanks to work performed on flat silicon oxide slides and LIF detection, allergen microarray for specif IgE analysis were developed

(see D6.1, D6.2 and D6.3) based on coating with copoly(DMA-NAS-MAPS) [1,2]. This copolymer is a terpolymer made of *N,N*-dimethylacrylamide (DMA, 97% of moles), *N,N*-acryloyloxysuccinimide (NAS, 2%), and 3-(trimethoxysilyl)propyl methacrylate (MAPS, 1%), synthesized by free radical copolymerization (Figure 1).

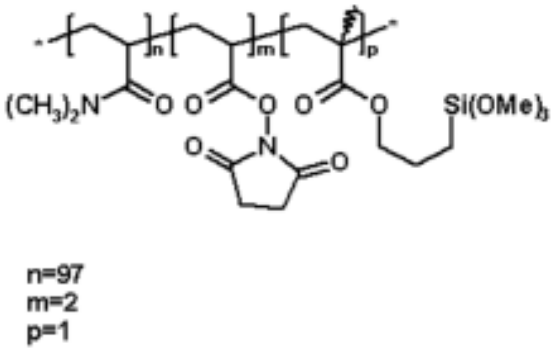

Figura 1: copoly(DMA-NAS-MAPS)

The polymer self-adsorbs onto the surface, the silanating moiety stabilizes the film, whereas the *N,N*-acryloyloxysuccinimide ester covalently binds the bioprobes.

2.2 Coating of first generation of porSi structures

A coating procedure was specifically devised for the porSi membranes in order to assure an efficient infiltration of the polymer solution into their nanopores and to preserve their physical properties. The procedure is based on the following steps:

1) Oxygen plasma treatment for 10 minute. (Due to oxidation, this step dramatically enhances the wettability of the samples and reproducibility of functionalization)

2) Immersion of porSi structure into a 1% w/v polymer into water solution of ammonium sulphate at 20% saturation level for 30 minutes applying a controlled (500 mbar) vacuum. Applying a vacuum is essential to let the air out from the nanopores and the polymer solution to infiltrate.

3) Rinsing in water under a mild vacuum for 15 minutes

4) Drying at room temperature under a high vacuum (50 mbar) for 15 minutes.

Problems of poor mechanical stability for the porSi emerged however with the fragility of the self-standing membranes hindering the development of flow-through coating procedures. When membranes of the first generation were glued to supports or embedded into chips, a poor reproducibility of membrane permeability was observed leading to some inconsistencies in protein functionalization.

2.3 Coating of second generation of porSi membranes

Very recently a second generation of porSi membranes became available from partner UNITN (D3.4R). As mentioned in the aforementioned report, for speed the porSi samples were glued onto solid plastic and aluminium supports and deposited onto a filter to which a controlled vacuum of 300-400 mBar was applied. Under these conditions, *a droplet of 2μL of solution was completely sucked through the membranes within a few minutes.* The consistency of permeability of the second generation of samples allowed us to perform a coating procedure in a controlled way (as described in D3.4R):

1) Oxygen plasma treatment

2) flow of 10 ul of polymer solution (copoly(DMA-NAS-MAPS) 1% w/v in ammonium sulphate solution at 40% saturation level)

3) rinse with 10 ul of water

4) drying at 80 °C, under vacuum for 15 minutes.

2.4 Immobilization of protein on the second generation of porSi membranes

PorSi membranes coated as described above were incubated overnight with 2 µl of Cy5 labeled Rabbit ImmunoglobulinG (10 ug/ml in PBS buffer).

Membranes were then characterized by UNITN using a micro-photoluminescence (uPL) set up either before or after washing. Results of this experiment are described in D3.4R. Briefly, *the uPL spectra clearly showed the presence of the fluorescent protein before and after membrane washing.* The data displays a large dispersion of fluorescent intensities probably due to the membrane bending and to a non optimal washing procedure. A systematic decrease in fluorescent signal is recorded on the sample after washing but *fluorescence is detectable along the entire pore depth confirming the permeability of the second generation porSi samples*.

3 Conclusions

The work performed in WP6 has led to a protocol specifically devised and demonstrated for porSi coating using copoly(DMA-NAS-MAPS) and to the optimization of immobilization conditions on flat coated silicon for the entire set of POSITIVE food allergens. Since the regular supply of permeable (second generation) porSi samples is now assured, the functionalisation protocols are undergoing further optimisation and a real bioassay will be shown in a later report, and possibly in the upcoming intermediate review meeting.

4 Bibliography

[1] Cretich M.; Pirri G.; Damin F.; Solinas I.; Chiari M.; A new polymeric coating for protein microarrays. *Anal Biochem* **2004,** 332, 67-74

[2] Cretich M.; Di Carlo G.; Longhi R.; Gotti C.; Spinella N.; Coffa S.; Galati C.; Renna L.; Chiari M. High sensitivity protein assays on microarray silicon slides. *Anal Chem* **2009**, 81, 5197-5203