POSITIVE NEWSLETTER 3: NOVEL SURFACE FUNCTIONALISATION CHEMISTRIES – SPECIFIC BIOSENSING FOR THE FAST AND SAFE DETERMINATION OF SENSITIZATION TO MULTIPLE FOOD ALLERGENS

Six European research centres and two industry partners have joined in a new European research consortium called POSITIVE. The goal of POSITIVE is to develop new rapid and multi-assay diagnostics for determining sensitization to food allergens. The European Union supports the consortium during a three-year period with 2.9 MEuro through its Seventh Framework Programme.

Food allergies can provoke clinical reactions whose most severe is anaphylaxis, with respiratory and/or cardiovascular problems that might result in death. They are common in 1-2% of adults and up to 8% of children, corresponding to a serious public health problem that affects over **15 million people in Europe** from infants to the elderly and its prevalence is increasing.

POSITIVE will develop a diagnostic platform that can quickly and safely identify the sensitization of a patient to multiple food allergens so as to be able to proscribe a suitable diet and lifestyle. Ideally it will be a rapid system with little hands-on time, so as to be used at point of care (PoC) in an intensive care unit by paramedics.

The consortium will develop a state-of-the-art diagnostics Lab-on-a-Chip platform via an integrated microfluidic sample preparation technique capable of serum preparation from whole blood of volumes, <100µl. The detection will be based on ultrasensitive photonic biosensors that are integrated into the lab-on-chip device. A final prototype consisting of a packaged biochip and reader will be used on clinical samples in order to determine sensitization to allergens such as that for hen's eggs, cow's milk, peanuts, wheat, tree nuts, fish, sesame, and shrimp ingestion.

More information about POSITIVE and its partners can be found in the attached project flyer or on the POSITIVE website http://www.fp7positive.eu ,

ABOUT THE POSITIVE CONSORTIUM:

Positive project manager and main contact person:

Dr. Daniel Hill, UVEG – Universitat de Valencia (http://www.uv.es/umdo)

Other partners:

Centre Suisse d'Electronique et de Microtechnique (http://www.csem.ch)

Farfield Group Ltd (www.farfield-group.com)

Charite Universitaetsmedizin Berlin (http://www.charite.de)

Phylogene SA (http://www.phylogene.com)

Università degli Studi Di Trento (http://science.unitn.it/~semicon/)

Consiglio Nazionale Delle Ricerche (http://www.icrm.cnr.it)

Royal Institute of Technology - Microsystem Technology Lab (http://www.ee.kth.se/mst)

Highlights of technology developed in the second 12 months of Positive:

During the past 12 months many innovative technologies have been developed whilst we have been working towards the novel Positive platform. Through the ingenious use of a substitute membrane for biosensing the project has ended the year proceeding to plan if not slightly behind schedule which is not unusual in these types of projects. From protocols developed in the project state of art porous silicon for polarimetric biosensor purposes is being produced, independently of all other tasks for its later inclusion in the final device. We note:

- **Functionalised porous silicon membrane based sensor**
  - Protocols have been developed for high yield and reproducibility production of stable high quality free standing porous silicon membranes with tunable pore sizes (40-100 nm), controllable homogenous thickness (>3µm) and <15mm diameter.
  - Successful coating of porous membranes surfaces with a functional polymer.
  - Pores roughness has been greatly reduced increasing the optical birefringence by a factor of 50% and easing liquid flux.
  - Lowest limit of detection at 1500nm for detection of alcohols with different refractive indices was 6.25×10^{-6} RIU for porSi membranes prepared from (100) Si.
- **Polarimetric readout platform**
  - Real-time sensing volumetric and biosensing results with a throughput of one data point per second.
  - Study of the wavelength influence over the detection limit by using three different wavelengths: 808 nm, 980 nm and 1500 nm.
  - Detection limit for volumetric sensing the same order of magnitude for the three wavelengths studied but 980 nm is preferred for low cost detectors.
  - Salt injection experiments for porous alumina demonstrated a limit of detection of 2.7×10^{-6} RIU (@980 nm).
- **Biosensing**
  - Protein spotted and blocked functionalised alumina stored at 4ºC without desiccant shown to maintain protein activity for at least 4 months.
  - Protein physisorption of BSA on porous alumina demonstrated responses are proportional to concentrations.
  - Biosensing has been reproducibly seen when running a bioassay of primary IgG at different concentrations to porous alumina membranes with various surface chemistries followed by a secondary IgG.
- **Cartridge**
  - Components successfully designed and realized in Y1 (sensor chip, microfluidic flow cells, blood filter) have been implemented within a prototype semi-disposable cartridge.
  - The cartridge has been tested fluidically showing the basic functionality. Optimization of the design is ongoing.
- **Platform**
  - Components successfully designed and realized in Y1 (temperature control unit, flow control, vacuum pump, software control have been implemented within a breadboard instrument.
  - The platform has been tested fluidically with the cartridge showing the basic functionality of the platform with the cartridge. The cartridge has all fluids on-board and is actuated pneumatically through the instrument.
  - Based upon experimental results the optical instrumentation has recently undergone further optimization. Optical functionality tests have shown the general suitability for multispot phase change measurements.

Partner feature:

The Institute of Molecular Recognition Chemistry (ICRM), based in Milan, is one of the several research institutes of Italian National Research Council (CNR). ICRM employs 75 scientists and technicians. The Institute has been actively involved in research activities in Biomolecules (natural bioactive substances & synthesis of compounds of biological interest); Chemical biotechnologies (bioconversions & analytical methodologies); Mechanisms of bioregulation (molecular basis of biological regulation & experimental & theoretical studies of molecular recognition). Research facilities: CNR is equipped to carry out monomer and polymer synthesis; IR, circular dicroism, viscometer, spectrophotometer and different chromatographic systems are available to characterize and purify polymers. CNR possess the following instruments: 1) Microarray platform including: spotting station, automated hybridization station and LIF scanners 2) Various capillary electrophoresis units 3) MegaBACE Capillary Array DNA Sequencer 4) Microchip electrophoresis units.

The Analytical Microsystem group, led by Dr. Marcella Chiari, carries out research activities aimed at developing micro-analytical techniques for genomics and proteomics. It is active in different projects, organized around the following themes: 1) Chemical aspects of microarray technology 2) Automated systems for the analysis of gene and protein expression, 3) Miniaturized analytical systems for microchip electrophoresis, CNR has an internationally recognized know-how in the production of polymeric coatings for analytical devices microchip electrophoresis and microarrays on different materials including glass, silicon oxide and nitride, polydimethylsiloxane, COC, ITO and gold. The team, comprising organic, bio-organic and computational chemists, biochemists and biotechnologists, is equipped to carry out monomer and polymer synthesis; IR, circular dicroism, and different chromatographic systems are available to test and purify polymers; DPI (Dual Polarization Interferometry) is available to characterize coatings.

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Participants

Marcella Chiari (F) Principal investigator; World-wide recognized experience in development of hydrophilic linear polymers. She has developed a number of new hydrophilic acrylic monomers and polymers to be used in capillary electrophoresis as DNA sieving matrices and as capillary coatings. She is active in the area of protein and DNA microarray. Her activity is documented by over 130 publications and several patents. She has been a contractor of the EC several times and is responsible for several national research programs.

Marina CRETICH (F), Research scientist at National research council of Italy at the Institute of Chemistry of Molecular Recognition (ICRM) in Milano. Laurea degree in Biological Sciences, specialty Molecular Biology, at Università degli Studi di Milano (1998). Her research interests, documented by more than 40 JCR publications, cover the field of analytical microsystems: microarrays, lab on chip, microchip electrophoresis and microfluidics. She is actively involved in several national and international projects on the development of microsystems for diagnosis and monitoring.

Laura Sola (F), Ph.D at ICRM, has a consolidate knowledge in polymer science and associated organic chemistry. Her main activity is coating and coating chemistry. She has experience in different analytical techniques for polymer characterization (GPC, NMR, IR), as well as in micro- and nanosystems for measurement and testing of coatings (AFM, XRR).

Surface functionalization of porous membranes

The surface functionalization is one of the most important components of a sensor platform. The surface chemistry greatly affects the specificity of the target and background binding as the local environmental conditions that exist following immobilization of the probe to the sensor surface strongly influence protein interactions. Additionally, the orientation and crowding effects are critical for the functionality of bound proteins.

The widely used approach of surface functionalization through monodimensional (1-D) coatings implies modification of the support by various types of organosilanes such as aldehyde, epoxy, mercapto or amino silanes. The 1-D surface chemistries however often suffer from drawbacks, such as spot smearing, poor spot morphology and protein denaturation due to hyrophobicity. They also suffer from loss in probe functionality that results from the close proximity of the probe to the sensor surface.

The proximity of probes to a solid surface, generally leads to reduced specificity of target binding and loss of probe functionality. One of the main sources of protein denaturation is the strong interaction between the proteins and the surface. The surface proximity can also result in interactions between the target and the surface, which may further hinder specific probe-target binding. A related effect is the orientation of immobilized probes; for randomly oriented probe proteins a large portion of the active sites may be inaccessible to targets in solution. This is especially likely for probe proteins that are randomly oriented on a solid, as the active binding site will be obstructed by the solid surface for a majority of the probes. These effects are significantly reduced for the so called 2D surface coatings, which describe surface chemistries consisting of functionalized polymers that are bound to solid surfaces in a brush type configuration. For probes immobilized on a polymeric scaffold, the effects of orientation are mitigated because binding sites can remain accessible if the polymer permits diffusion of the target protein to the probe binding site.

The focus of the activity at CNR in Positive is the design of 2D polymeric coatings with optimal features (thickness and probe density) specifically tailored to the characteristics of the sensor.

CNR has experience in the synthesis of functional polymers and in the development of coating procedures that are characterized by i) robustness, ii) high compatibility with sensor structure iii) precise control of coating thickness. The unit has completed the development and characterization of a three components polymer: DMA (dimethylacrylamide), MAPS (3-(trimethoxysilyl)propyl methacrylate), and NAS (acryloyloxysuccinimide) (Figure 1). [1]

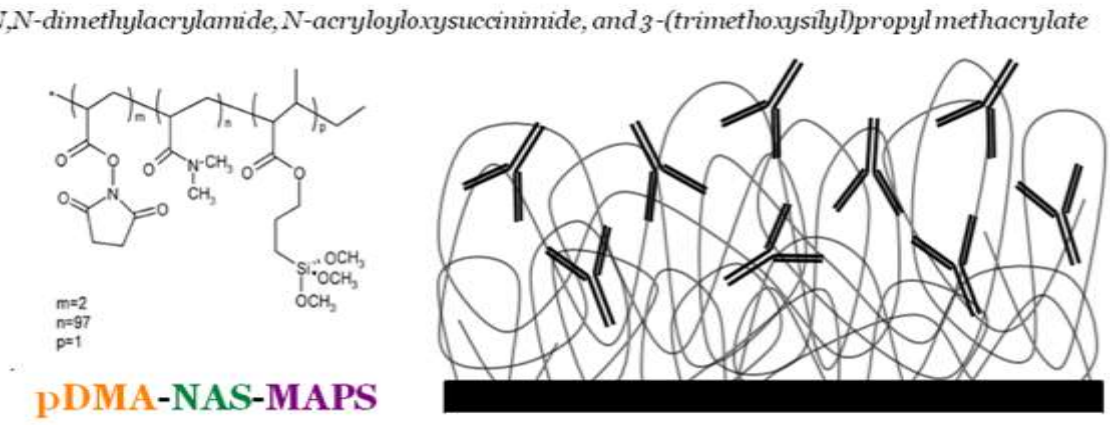

Figure 1 (a) The three major components of the polymer are DMA, NAS and MAPS in ratio 97:2:1. NAS provides the active groups, which specifically bind amine functional groups on the probe, the MAPS covalently binds to the oxide surface and DMA adsorbs to the oxide surface and provides the backbone of the polymer. (b) The polymer swells upon hydration to provide a near liquid environment for the probes. [2]

This polymer forms a film on the surface of various materials by dip and rinse coating. The coating procedure is fast and highly compatible with the characteristics of different sensors. DMA provides the majority of the polymeric structure and also adsorbs to the oxide surface, MAPS covalently binds to the oxide and NAS is the active groups that covalently binds the functional amine groups on the DNA probes.

This polymer has been used by the CNR unit to functionalize the surface of porous silicon. The coating process devised for porous silicon was not applicable to alumina, the alternative porous material considered in Positive. With this latter material a two-step procedure was devised. In the first step, a monolayer of MAPS was grafted to the surface followed by a radical polymerization of DMA and NAS monomers. The process was carried out in dimethylformamide and the polymer was covalently grafted to the surface through the incorporation of MAPS allyl moieties. Alternatively 1-D coatings have also been considered. Research is ongoing to determine whether polymer swelling is an obstacle to liquid flow in nanochannels.

1. G. Pirri, F. Damin, M. Chiari, E. Bontempi, and L.E. Depero, "Characterization of a polymeric adsorbed coating for DNA microarray glass slides," Analytical Chemistry, vol. 76, 2004, pp. 1352–1358.

2. A. Yalçın, F. Damin, E. Özkumur, G. di Carlo, B.B. Goldberg, M. Chiari, and M.S. Ünlü, "Direct observation of conformation of a polymeric coating with implications in microarray applications," Analytical Chemistry, vol. 81, 2008, pp. 625–630.

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