



GOODFOOD



In this Issue

- *GoodFood WP3: Rapid detection of Toxigenic fungi & Mycotoxins by Microsystem Technology*.....2
- *GoodFood WP4: Microsystems technology solutions for the rapid detection of viable Foodborne Pathogens*5
- *GoodFood Demo-Projects*.....8
- *Grenoble's Workshop*9

The GoodFood 4th Newsletter

Welcome to the GoodFood's fourth Newsletter, which is regularly disseminated in order to keep you up to date on the achievements of the project.

The GoodFood project is an Integrated Project presented within the IST thematic area of EC VI FP and aims at developing a new generation of analytical methods based on Micro and Nanotechnology (M&NT) solutions for the safety and quality assurance along the food chain in the agrofood industry.

GoodFood is now three years old. Only five months lack to the conclusion of the project and these months will be crucial for the demonstration activities and to terminate the final tasks.

The GoodFood consortium will continue to keep you updated on its activities and results. Our next Newsletter is scheduled for June 2007, with new technical results and information coming from the GoodFood research activities.

More detailed information can be found at our website: www.goodfood-project.org.

In this issue a detailed description of the objectives and results achieved within GoodFood concerning the detection of toxigenic fungi and mycotoxins and of foodborne pathogens by using microsystem technology is reported.

The results of the second GoodFood Open Day, held in Grenoble (France) on the 15th of November, 2006, is also presented. The event was devoted to the milk and dairy food sector.

Finally, GoodFood launched last year a new initiative focused on the set-up of demonstration activities to be implemented for the final period of the project (January to June 2007) and four specific technological implementations coming from the R&D activities of the project were open for the involvement of new external interested organisations. In this issue the aims of the four different demonstration activities are presented.

Project Co-ordinator:

Dr. Carles Cané

Centro Nacional de Microelectrónica - CSIC
Campus UAB E-08193 Bellaterra SPAIN
Tel: +34-935947700 Fax: +34-935801496
Email: goodfood@cnm.es



GoodFood WP3: Rapid Detection of Toxigenic Fungi & Mycotoxins by Microsystems technology

INTRODUCTION

Moulds are among the most important spoilage organisms of food. These contaminants can reduce the nutritional value, the technical quality, cause dry matter loss, heating, off-odours and form allergenic spores, harmful to animals and humans. In addition the occurrence of toxigenic fungi and relative mycotoxins in plant products, is supposed to be one of the most important social and economic concern in the European Union. The objectives of this WP are to develop new microsystems for rapid detection of toxigenic fungi and mycotoxins that can be used for food testing directly after a sample treatment. It is pursued to offer a solution for on-site testing during the manufacturing process. Many foodstuffs may suffer from fungi and toxigenic contamination, and the aim is to develop systems usable in a broad range of applications. The same technology could be applicable in other steps of the food chain, such as in crops in pre and post harvest.

MICRO-SYSTEMS TECHNOLOGY

The microsystems technology for fungal detection (ochratoxigenic *Aspergillus* species occurring on grape and *Penicillium expansum* on apple/pear) in this WP is based on the quantitative real-time PCR in a low density array format, an optical sensor and an electrochemical sensor using two immobilisation methods to observe which provides better attachment chemistry for DNA onto the surface of the device. The final steps used in the process for DNA immobilisation in both cases are the same. After DNA has been immobilised to the chip surface they are mounted onto developed PCB dipsticks via conductive glue attach. The chips are then packaged with glob top

to seal the back contact of the chips from electrolyte. This ensures the back contact is isolated from the buffer carrier solution at all times and only the surface of the chip is exposed. If the metal back contact were exposed to the solution the system would short-circuit since the silicon and SiO₂ layers of the chip would be bypassed. A photograph of a packaged chip on the developed PCB dipstick is given in figure 1.

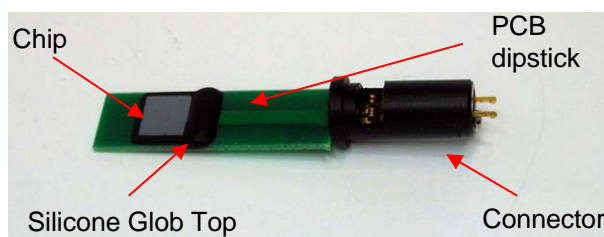


Figure 1: Photograph of a packaged chip

Ability of target DNA recognition by the developed microsystem is proved by figure 2 where Nyquist plots for two silicon sensors before (b) and after (a) DNA hybridisation are presented.



GoodFood WP3: Rapid Detection of Toxicogenic Fungi & Mycotoxins by Microsystems technology

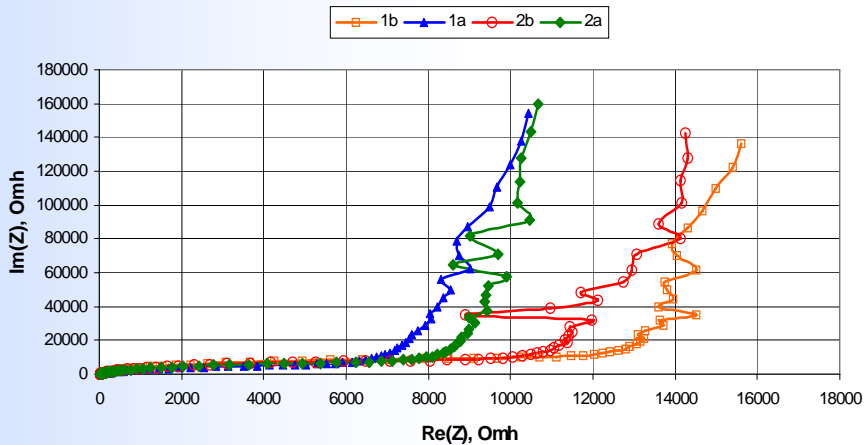


Figure 2: Nyquist plots for two DNA chips before (b) and after (a) DNA hybridisation

The microsystems technology for the detection of mycotoxins in food and milk (and their processed products) is based on nanoelectrode array transducers. Progress in this period has resulted in the successful fabrication of the final nanoelectrode sensor design by e-beam lithography, based on a cell-on-a-chip format. The e-beam lithographic technique has been used for the fabrication of nanoelectrodes, and it is based on the same principle as the FIB (Focused Ion Beam) approach, by creating

nanopore openings in the silicon nitride top layer, however there are more processing steps involved (Figure 3). The e-beam technique is designed for the mass-production of nanoelectrode arrays in which full wafers at a time are processed. The smaller size of the electron beam can facilitate the creation of smaller nanopore dimensions than the focused ion beam, and can also be used to produce high-density nanoelectrode arrays (Figure 4), thus greatly enhancing the sensor signal.

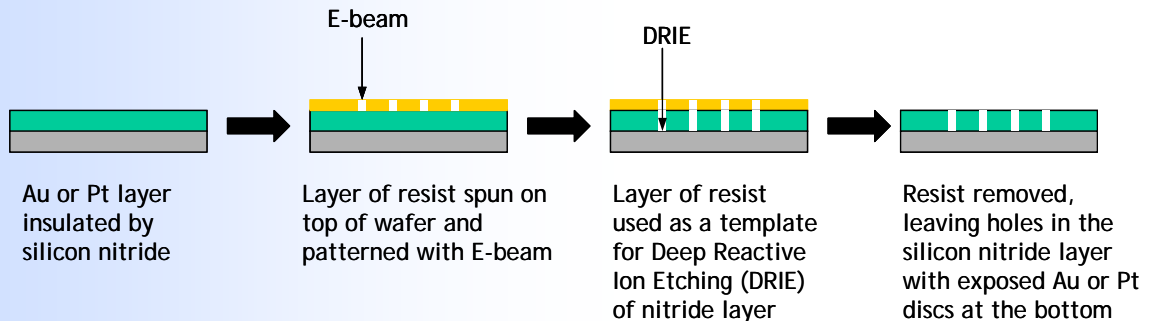


Figure 3: Fabrication approach of large-scale nanoelectrode array production using silicon-based wafers pre-patterned by photolithography. A combination of e-beam lithography and Deep Reactive Ion Etching (DRIE) is used to create nanopore openings in the top insulating layer, creating recessed disc nanoelectrodes.



GoodFood WP3: Rapid Detection of Toxicogenic Fungi & Mycotoxins by Microsystem Technology

In addition, progress on the coupling of the mycotoxin ELISA scheme with surface-modified cell-on-a-chip microelectrodes has resulted in a greater sensitivity for Aflatoxin M1 detection than screen-printed electrodes. The use of an ELISA scheme is necessary for recognizing the mycotoxins and for improving the detection sensitivity and selectivity. Figure 5 shows a schematic representation of the coupling of the cross-linker end terminal group with the amine group of the primary BSA-antibody conjugate, and all further steps of the immunoassay. Detection of the electroactive enzyme product, 1-naphthol, is made by the microelectrodes using differential pulse voltammetry.

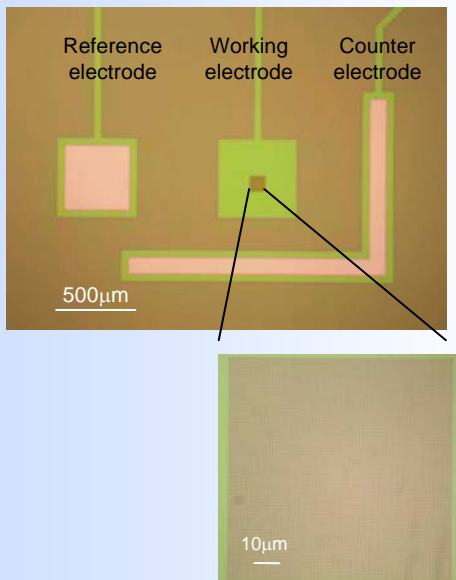


Figure 4: Electrochemical cell-on-a-chip devised for mycotoxin detection and magnification of the $100 \times 100 \mu\text{m}^2$ working electrode area showing a nanoelectrode array. Nanoelectrodes diameter is 250nm.

The Team of Scientists working on this project comprises of experienced biologists, chemists and engineers from

CNR-ISPA- Bari, Italy

CNR- IMM, Lecce, Italy

Cranfield University, Bedford, UK

Tyndall National Institute, Cork, Ireland

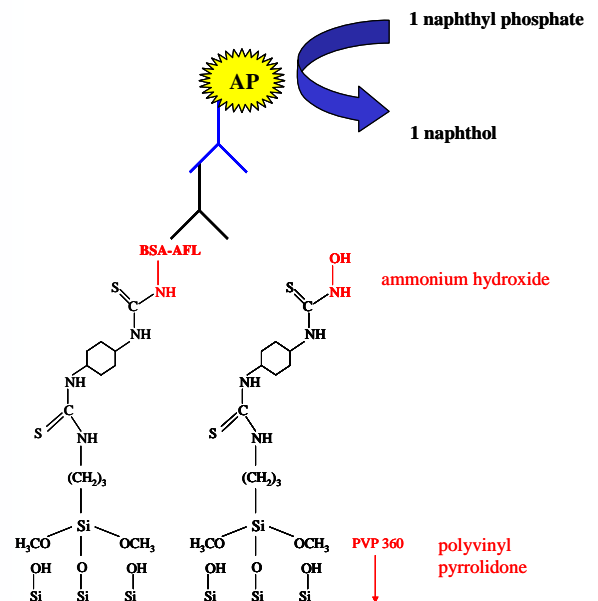


Figure 5: Schematic representation of the coupling of the cross-linker end terminal group with the amine group of the primary BSA-antibody conjugate, and all further steps of the immunoassay.



GoodFood WP4: Microsystems Technology Solutions for the Rapid Detection of Viable Foodborne Pathogens

INTRODUCTION

Food-borne illness is a threat to the health and well-being of the public and represents a major economic loss to the European economy in terms of lost working days and health costs. Although no figures are currently available, the annual cost of food-borne illness in the United States is estimated to be \$6.7 billion, up to \$100 million in New Zealand and approximately \$123 million in Sweden. The major bacterial food poisoning agents include *Salmonella*, *Listeria monocytogenes* and *Campylobacter*.

The aim of this research is to develop a new and innovative approach to achieve safer food products by creating user-friendly interrogation devices for the detection of foodborne pathogens in dairy products. The technology developed is based on DNA detection and is a generic technology, which in the agrofood sector may be applied for the detection of a variety of spoilage or pathogenic organisms, GM traceability, product authenticity etc. The major advantages underpinning this approach are the portability, ease-of-use and rapidity of the proposed systems. They can be used to rapidly generate data, in an at-line situation by personnel with minimal training.

KEY AREAS

In order to fulfill the aim set out in this project, it was sub divided in to number of key areas and each area in turn assigned to a researcher with expertise in that area. To this end Microzone developed a procedure for optimizing the DNA extraction. Bionostra through sequencing identified specific primers to be used in the prototype system.

AZTI, in conjunction with these activities setup, managed and tested both the primers and isolation protocols developed by the other partners. In conjunction with this activity AZTI investigated different liquid media for optimal growth of the sought after pathogens from real food samples including milk, cheese and smoked salmon.

The second part of the project concerned sensor development and instrumentation. Three project partners worked independently on this activity namely, EPFL, Tyndall National Institute and Biomerieux. Here, different sensor types utilizing a simple, direct method for the detection of DNA–DNA interaction were developed throughout the course of this project. All systems investigated centre round electrochemical based analysis methods. Prototypes of some of the sensor platforms developed are given in Figure 6.

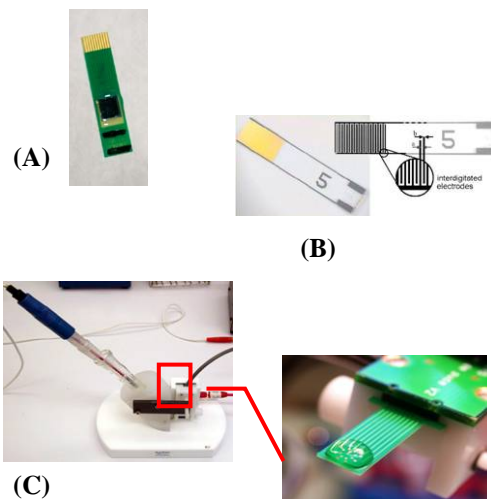


Figure 6: A) One of two Tyndall chips for dual analysis. (B) EPFL sensor based on gold interdigitated electrodes for multianalyte detection (C) ApiT chips consisting of eight working electrodes, an auxiliary electrode and a reference electrode.



GoodFood WP4: Microsystems Technology Solutions for the Rapid Detection of Viable Foodborne Pathogens

One of the main challenges to achieving highly reproducible and more sensitive sensor devices is the optimisation of the probe layer immobilisation procedures, to achieve probe layer uniformity, and the nature of the probe layer itself. Probe layer immobilisation strategies varied depending on sensor type chosen among the three partners. For example, Biomerieux developed a polypyrrole based chemical attachment method. Here, different parameters were adapted to achieve good reproducibility of electropolymerization. The parameters examined included the fixed monomer-oxidation potential, charge quantity and concentration of monomers in each step. See Figure 7 for a schematic of the immobilisation chemistry used.

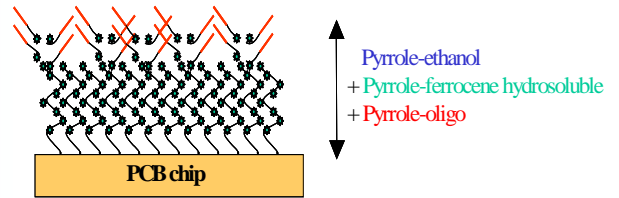
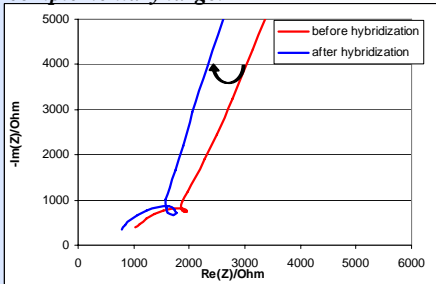


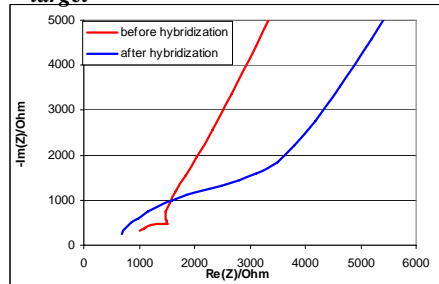
Figure 7: Mono-layer molecular architecture developed by Biomerieux

Impedance spectroscopy and differential pulse voltammetry (DPV) distinguished the interaction between the DNA probes and amplified PCR products from the test samples. Impedance spectra obtained before and after the interaction of DNA probes with the complementary PCR products clearly showed the sequence-specific hybridization (Figure 8).

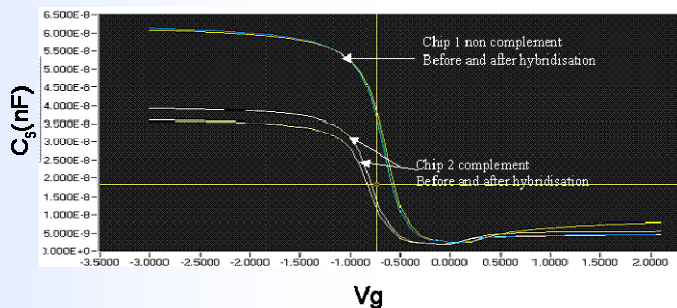
Modified electrode with non-complementary target



Modified electrode with complementary target



(a)



(b)

Figure 8: Impedance measurements taken before and after hybridisation using (a) the Biomerieux gold multianalyte based sensor, and (b) the Tyndall silicon based dual sensor.



GoodFood WP4: Microsystems Technology Solutions for the Rapid Detection of Viable Foodborne Pathogens

Results to date have been promising with as low as 50nM concentrations of DNA being detected using the Biomerieux sensor. The DNA detected in these experiments was prepared using optimised protocols. WP4 has focussed on the detection of *Salmonella* spp. This microorganism is currently implicated in most food-borne diseases. The second pathogen chosen, *Listeria monocytogenes* when detected in contaminated food has very high rates of morbidity and mortality associated with it. The target detection limits were defined by the EU Council directive 92/46/EC where a detection level of 1 cfu/25 ml or 25g of foodstuff is required. Once the required detection limits were reached for dairy products i.e. milk in this case, the prototype systems developed were subsequently used for the detecting salmonella and listeria in smoked salmon. The final phase of the Goodfood project will concentrate on validating the three sensors developed and evaluating their performance with respect to their commercial viability.

The Team of Scientists working on this project comprises of experienced biologists, chemists and engineers from

AZTI, Sukarrieta, Spain

Biomerieux, France

Bionostra, Madrid, Spain

EPFL, Lausanne, Switzerland

Microzone, West Sussex, United Kingdom

Tyndall National Institute, Cork, Ireland

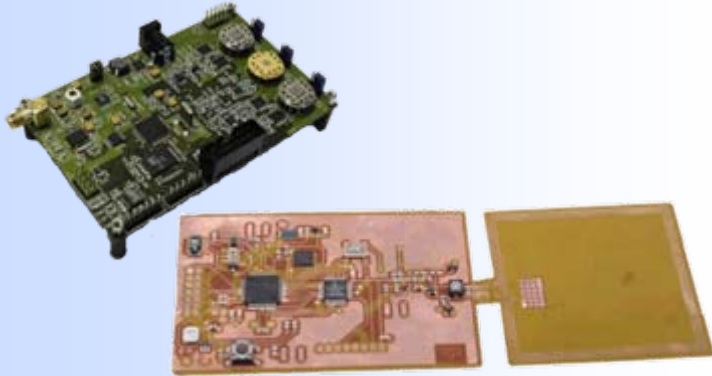


GoodFood Demo Projects

The GoodFood project has entered in a very important phase: the demonstration of the results obtained during the first three years of the project. A call was launched from October 20th to November 30th 2006 focused on the set-up of demonstration activities to be implemented for the final period of the project (January to June 2007). Different specific technological implementations coming from the R&D activities of the project were open for the involvement of new external interested organisations:

-GF-DEMO-A: *“Monitoring for the chilled/frozen fish logistic chain based on Flexible Tag Microlab (FTM) system”.*

A Demonstrator of Flexible Tag Microlab developed within the Workpackage 6, based on RFID communication with a temperature sensor and time inlay monitoring.



-GF-DEMO-B: *“Flexible tag datalogger for wine quality control”.*

A Demonstrator of Flexible Tag Microlab based on Infra-Red communication for the traceability of wine bottles, including information of environmental conditions (temperature, light and humidity) during storage and transport.



-GF-DEMO-04: *“Ambient Intelligent Site for the vineyard”.*

A Demonstrator of an Ambient Intelligence Platform developed within the Workpackage 7 with data analysis models for quality & safety monitoring during wine production.



At this moment, GoodFood has analysed several expression of interests and selected the most suitable for demonstrating the systems real environments. Selected partners will become full partners of the GoodFood project for the period of the demonstration activities. This process will allow the new partners add on the innovative R&D results coming from GoodFood and to validate the functionalities of the developed prototypes.



Grenoble Workshop: Micro&Nano Technologies for Milk and Dairy

On November 15th 2007, an open day Workshop was held in Grenoble with the aim of opening the project to the industrial and research communities from outside of the consortium. The workshop was planned for contributing both to the dissemination of the aims and achieved results of the project and also to the training of researchers and industrials through tutorials given by key persons from different countries.

The second edition of the Open GoodFood Day was devoted to the presentation of the results achieved in the project, especially for the milk and dairy food chain. External key persons on the field also presented other relevant initiatives, projects and future European research related topics.

The attendees to the Open Day in Grenoble were 80, including 50 from GoodFood and 30 external to the project.

The presentations given during the Workshop by the different speakers are available on the GoodFood web site, in the "Training activities" section.



09.15 - 09.30	<i>Welcome</i>	<i>David Holden (CEA_LETI) Augusto de Albuquerque Head of G2 Unit (Micro and Nanosystems)</i>
09.30 - 09.45	Opening remarks	<i>Carles Cané (CNM, ES)</i>
09.45 - 10.15	M&NT for food and other life sciences. Perspectives for FP7 in ICT priority	Griet Van Caenegem (DGINFSO C2,EC)
10.15 - 11.00	Contamination Risks and Assurance Schemes for Fresh Milk	Manfred Noll (Nestlé, CH)
11.00 - 11.30	<i>Coffee break</i>	
11.30 - 12.15	Commercial solutions for antibiotic detection	Benoit Granier (Unisensor, BE)
12.15 - 13.00	The Biocop project strategies for detecting a range of chemical contaminants in foods using optical biosensor technology	Chris Elliot (Institute of Agri- Food and Land Use, UK.)
13.00 - 14.30	<i>Lunch</i>	
14.30 - 14.45	FOOD FOR LIFE: Presentation of the European Technological Platform	Domenico de Martinis (ENEA BIOTEC-GEN, IT)
15.45 - 15.00	TRUEFOOD: Presentation of the Integrated Project	Domenico de Martinis (ENEA BIOTEC-GEN, IT)
15.00 - 15.30	GOODFOOD: Microsystems for antibiotic detection in milk	Guy Voirin (CSEM, CH)
15.30 - 16.00	GOODFOOD: Microsystems for pathogens in milk	Mary Manning (Tyndall National Institute, IE)
16.00- 16.30	GOODFOOD: Market issues in milk and dairy safety and quality	Jo Goossens (Bio-Sense, BE)
16.30- 16.45	Closing	Carles Cané (CNM, ES)