eBIOSENSE

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Electrical Bio-Sensor Arrays for Analyses of Harmful Micro Organisms and Microbial Toxins
LSHB-CT-2004-512009

Executive Summary

Objective. The eBIOSENSE project develops a platform for analysis of harmful micro organisms and their toxic products. The platform is based on electric biochip arrays, which will enable parallel and simultaneous identification and quantification of specific nucleic acids, microbial proteins and toxic microbial products. The use of DNA and antibody based analyses for identification of microorganisms, rather than traditional classification in species, offers a great advantage in the assessment of health hazards, since in many cases only certain strains of a species are pathogenic and many toxins are produced by several species. Thus, the eBIOSENSE concept is to assess the pathogenicity factors rather than the species name.

Two versions of arrays are developed: nucleic acid based chips and protein based chips. The targets are recognized and captured by DNA probes or antibodies on the silicon based electrochemical transducer surface, and labelled with an enzyme which produces an electro-active product, thus generating an electric current which correlates with the concentration of the target analyte in the sample. During the project time chips will be developed for pathogenic strains of the *Bacillus cereus* group, shiga-toxin producing *E. coli* strains (called STEC, VTEC, or EHEC), pathogenic *Staphylococcus*, *Legionella*, *Salmonella enterica* and mycotoxins.

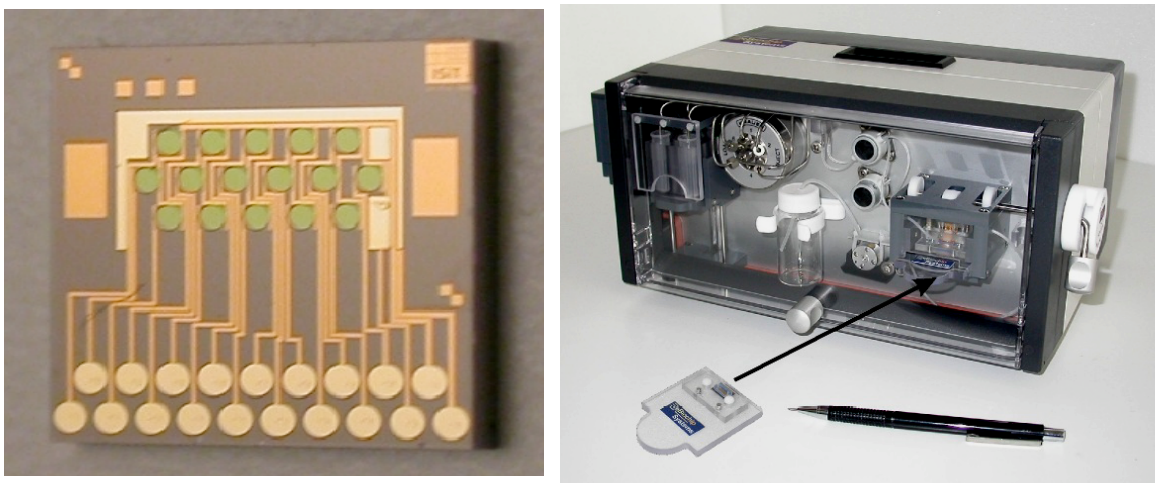

Fig 1. An electric biochip. Left: The 9x10 mm electric chip array with 16 positions. Right: The biochip reader with chip holding cassette, sample handling equipment for the assay.

When a DNA chip surface is exposed to a sample, the target DNA molecules simultaneously hybridise with the capturing DNA-probe on the electrode and with a soluble detection probe (see Fig 2). Thereafter, the detection probe is labelled with alkaline phosphatase. The signal is then generated by addition of p-aminophenyl phosphate (pAPP) which reacts with the enzyme and generates p-aminophenol (pAP) which is oxidized to quinoneimine (QI) at the anode. The two electroactive components pAP and QI are then redox recycled between the anode and cathode which generates a current from the chip, as illustrated in Fig 2.

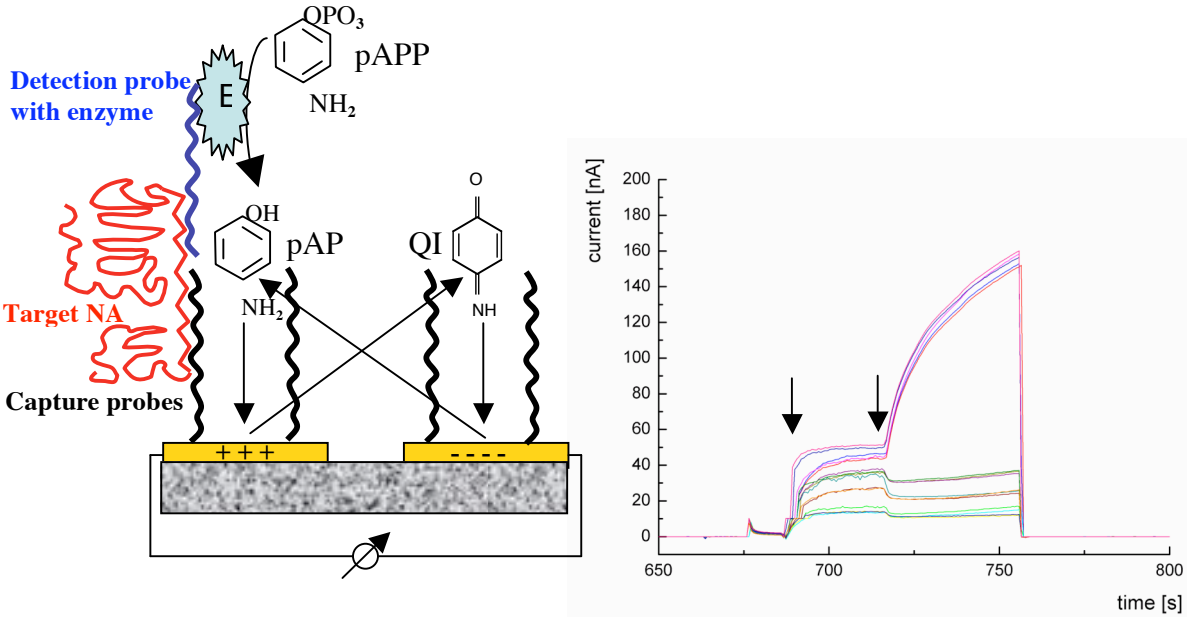

Fig 2. Left: Principle of signal generation with an electric DNA chip. Right: responses from 16 electrodes of a chip. The left arrow shows time of pAPP application. The second arrow the subsequent stop-flow. The initial slope (nA/sec) generated during the stop-flow is used as signal.

In an antibody based biochip, the DNA capture probe is replaced by a specific antibody that recognises and binds the target analyte where-after an enzyme-antibody conjugate is used to label the bound target molecules. Enzyme reaction and signal generation are the same as for the DNA chip.

Results. During the first project year the main activities were put on developing the chip arrays and instrumentation in which all the steps of the assay are automatised. Large inputs were also made on design and evaluation of suitable DNA probes and antibodies, and on the immobilisation of the capturing molecules on the chip surface.

During the second project year silicon chip arrays with 16 positions have been designed and manufactured for analysis of several pathogens and toxins: 1) A DNA chip for simultaneous detection of the genes involved in synthesis of the four known toxins produced by many strains of the common food pathogens of the *Bacillus cereus* group. 2) A DNA chip for analysis of the pathogenic *E. coli* strains called EHEC. 3) An RNA-chip for analysis of *Legionella*. 4) A DNA chip for analysis of *Salmonella* and fumonisin producing *Fusarium*. 5) An antibody based chips for analysis of several staphylococcal toxins and for the O157

antigen of EHEC bacteria. For DNA analysis different primary enrichment methods are used depending on the type of sample to be analysed. Analysis of a single colony of suspected pathogenic *Bacillus cereus* provides in 45 min the full picture of which of the 8 known toxin-encoding genes that are present in the bacterial strain. The chip for quantitative analysis of staphylococcal enterotoxin B, currently detects 15 ng toxin in a 250 μL sample.

Exploitation and dissemination. The electric biochips will improve the risk assessment of food and feed products in several ways. The quality of the risk assessment will be improved by the introduction of DNA-analyses. They will give information of the real potential for toxin production and other pathogenicity factors, rather than the taxonomic information provided by traditional tests. This taxonomic information is often poorly related to the real pathogenicity of a micro-organism. The EHEC and the *Bacillus* chips can illustrate this: EHEC is traditionally identified with the serological analysis for presence of the antigen O157:H7, but there are many other serological types of *E. coli* that produce the shiga-like toxin that cause the serious disease of EHEC. Many but not all *Bacillus cereus* strains produce haemolysins, and other toxins but some of these toxins are also produced by other *Bacillus* species. Thus, only a DNA based analysis gives the information of the health hazard related to these organisms. DNA based analysis can also be made with PCR that is rapidly gaining use in food analysis. Compared to the PCR technology, the silicon based chips offer the potential of faster and simultaneous analysis of a large number of genes in a sample with disposable chips operated by a portable alarm-type detector. The advantage offered by the hybridization-based form of analysis with these chips include also full automation of the detection processes once the sample is provided. The electric chips can also be used as rapid and sensitive detectors of the PCR products.

The third project year will include an evaluation of the performance of an antibody based chip and a DNA-chip in comparison with conventional analyses.

The organisms/toxins employed in the eBIOSENSE project are specifically associated with food and water born health hazards. However, the technology is generic and a wide range of analytical applications, e.g. rapid determination of antibiotics resistance of bacteria, are expected based on the results of the project.

Contractors: The Fraunhofer Institute for Silicon Technology develops and produces the silicon chip arrays. The instrumentation is developed by eBiochip Systems GmbH. Specific chips for analytical applications and analytical protocols are developed by Ernst-Moriz-Arndt University, Greifswald, Kungl. Tekniska Högskolan, Stockholm, Plant Research International, Wageningen, Scanbec Oy, Oulu, University of Gdansk, and University of Oulu. Raisio Benecol Ltd is a manufacturer of food and feed diagnostics and together with CREA, Paris, they contribute to the project as experienced end-user of analytical techniques, with focus in the food and feed area.

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