MENDOS

BioMimetic Optical Sensors for Environmental Endocrine Disruptor Screening
QLK4-CT2002-02323

Project Summary

Title of the project: BioMimetic Optical Sensors for Environmental Endocrine Disruptor Screening		
Acronym of the project: MENDOS		
Type of contract **RTD**		**Total project cost** (in euro) € 2,788,248
Contract number **QLK4-CT2002-02323**	**Duration** (in months) 38 Months	**EU contribution** (in euro) € 2,004,372
Commencement date 1 January 2003		
PROJECT COORDINATOR		
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Key words (5 maximum - Please include specific keywords that best describe the project.).

endocrine disruptors, biomimetic sensors, biosensors, DNA chips,

World wide web address (the project's www address)

http://www.mendos.org

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Objectives:

Endocrine-disrupting chemicals (EDCs) are natural or synthetic chemicals that can interfere with the

hormonal system and mimic hormones or block their function. The exposure to EDCs is known to cause adverse effects in wildlife and potentially also on human health, such as abnormal sexual development of animals, increasing human breast and testicular cancer and decreasing sperm counts in men. Potentially endocrine-disrupting chemicals can be found among pesticides, plasticisers, pharmaceuticals, cosmetic ingredients, household products, industrial chemicals, but also natural products such as phytohormones. The goal of this project is the development of novel sensor and assay systems for rapid testing of environmental occurrence as well as mechanistic screening of EDCs. Main technologies employed include

- biomimetic recognition materials based on molecularly imprinted polymers (MIPs),
- highly sensitive miniaturised optical transducers,
- biosensors and assays based on EDC sensitive luminescent eukaryotic cells,
- DNA microarrays.

Results:

Biomimetic recognition materials

Molecularly imprinted polymers selectively recognising the important EDCs 17β-estradiol, benzo[a]pyrene, diethylhexylphthalate and atrazine have been developed. These materials were obtained both in bulk format as well as in the form of micro- or nanospheres useful for application in binding assays or as stationary phases in chromatography or solid phase extraction. Especially the latter application turned out to highly benefit from specific recognition properties of MIPs, allowing enrichment of trace concentrations of the target compounds from environmental matrices. Furthermore, a novel technique was developed for preparing MIP films of controlled thickness and porosity, thus opening the door to a wide variety of additional applications requiring thin and homogenous biomimetic recognition layers.

Optical transducers

An integrated optical sensor setup based on Mach-Zehnder interferometers has successfully been built up and was tested with well-established antibody reactions, demonstrating the possibility of a highly sensitive multianalyte interferometric sensor. Furthermore, the surface plasmon resonance (SPR) principle was exploited in developing two other novel optical sensing platforms, specifically: (i) a mobile SPR sensor based on prism coupling, spectral modulation and wavelength division multiplexing and (ii) a high-throughput SPR system based on angular spectroscopy of surface plasmons on an array of diffraction gratings. The SPR sensor method was combined with antibodies and MIPs for detection of selected EDCs. Antibody-based SPR sensors detected selected EDCs in sub-ng/ml concentrations.

Cell based biosensors and assays

The aim of this focus of the MENDOS project was to develop a whole-cell bioassay for pollution detection. For that purpose, on the one hand bioluminescent eukaryotic cells were genetically modified using a mammalian vector carrying a reporter gene, under the control of the Drosophila melanogaster hsp22 stress inducible promoter. Such a promoter responds to cellular stress usually induced by heat shock, but also by chemical agents like heavy metals, genotoxic agents and endocrine disruptors. The present bioassay would then give a signal dependent on both the toxicity

and the bioavailability of the tested compound.

On the other hand, a system for assessing the estrogenic activity of a sample has been established based on immobilised chemo-sensitive luminescent yeast cells. The advantages of the developed bioassay are: i) rapidity, showing a biosensor time constant of 2.5 h, ii) shelf life, allowing long term storage of the yeast cells, iii) biocompatibility, in terms of hydrogels forming a protective environment for the entrapped yeast cells protecting from contamination, thus allowing the user to work under non-sterile conditions, and finally iv) simplicity and v) cost. It could be shown that luminescent yeast based hydrogel bioassays are applicable to the analysis of environmental water samples, potentially as a primary screening tool and as the biological and chemical immobilisation parts in the construction of fibre-optic biosensors. This system thus forms a promising alternative to the currently used recombinant yeast reporter gene assays.

Finally, an aromatase activity assay was developed as a test system for the interference of xenobiotic chemicals with the steroid metabolism. This specific endpoint, in addition to the interference with hormone receptors, is of high relevance to be included in the endocrine disruption test strategy.

DNA chips

DNA chips holding oligo DNA probes for hormone responsive genes were developed for the detection of EDCs. Dedicated chip surface chemistries were developed which proved to be appropriate for the effective binding of NH2 modified 50bp long oligonucleotides. The up and down regulation of estrogen regulated genes by EDCs could be detected in ZR75 breast cancer cells using this oligo DNA chip. Furthermore, detection of antiandrogenic action of EDCs has been feasible utilizing 22RV prostate cancer cells. It could be shown that DNA chip based assays are able to reveal the presence of estrogenic compounds based on their hormonal activity even in low concentration.

Overall conclusion

Main technological developments in the field of biomimetic and biological sensor systems for EDCs could be achieved during the MENDOS project. Although one of the main goals to establish a fully functional MIP based optical sensor platform for EDC monitoring in the aqueous environment could not be reached completely, major advances in underlying MIP technology and optical sensor equipment were made, thus setting the base for successful future implementation of highly sensitive biomimetic optical sensors. In addition, MIP based solid phase extraction materials were obtained, that could significantly facilitate instrumental analysis of EDCs in environmental samples. Finally, biological sensors and assays for EDCs were established, which – after further validation – are prospectively useful tools for high-throughput laboratory based or in-the-field endeavours of screening for endocrine disrupting activity.

Benefits and Beneficiaries:

The main benefit for EU policies envisaged for the MENDOS project was the development of field deployable monitoring systems for EDCs in the aqueous environment; indeed, significant technological developments have been realised on the way to this final goal. On the one hand, biosensors and DNA chips have been established which can be used in screening for estrogenic activity of environmental samples or chemical libraries, an application relevant for EU policies, both with respect to water quality (Water Framework Directive) and chemical safety (REACH).

On the other hand, it has been possible to integrate the two basic technologies of biomimetic recognition materials based on MIPs and of highly sensitive optical transducers, opening the way to a multitude of future applications in environmental and industrial sensing. While this is clearly beneficial for EU environmental policies – whether EDC related or not – there is moreover a pronounced economic relevance in terms of a build-up of European know-how in key technologies for next generation optical chemical sensors.

In terms of a contribution to EU social objectives, the goal of the MENDOS project has been to offer tools for research into the activity mechanisms and actual risks of EDCs and thus help to protect the health and increase quality of life of European population. Especially the prototypes of cell based biosensors and bioassays as well as the DNA chips developed within MENDOS probably will be valuable tools for EDC research and risk assessment. In addition, solid phase extraction applications based on MIPs were developed that could successfully be introduced to more traditional instrumental analysis of EDCs in environmental monitoring schemes.