BIO-MEDNANO (NMP4-CT-2006-017350)

Publishable Executive Summary

This BIO-MEDNANO STREP-project focuses on **improving enzymatic electron transfer reactions for application towards integrated bio-powered biosensing systems for diagnosis and healthcare**. The project aims to improve such systems by: *screening for novel enzymes; modification of enzymes; design of novel nano-structured scaffolds for enzyme immobilisation*, to provide devices with improved stability and electron transfer efficiency (sensitivity and/or power output).

The fundamental project objective is to increase understanding and overcome the present limitations of biofuel cell and biosensor devices based on biological electron transfer systems. The initial target systems will be based on development of prototype biosensors for the intermittent determination of **glucose and catecholamine neurotransmitter** levels in clinical samples, and of a biofuel cell functioning on *in-vivo* available biofuels.

The project commenced on 1st July 2006, and the first meeting of the consortium was held in Brussels in early July 2006 to plan for project research and management. Subsequent consortium meetings have been held in Rome (December 2006) and Dublin (May 2007).

Project progress during this reporting period has been good, with some slight delay in commencement of activities by partners due to delays in the recruitment of personnel. Management of the consortium has been facilitated by recruitment of a part-time assistant, Sarah Knight, by the co-ordinating partner (NUIG), and by the establishment of a dedicated project web-site for the consortium http://www.nuigalway.ie/biomednano/biomednano_home.php. The web-site, in addition to a public section, has also facilitated document sharing in the members only section.

Scientific progress during this period, whilst devolved into separate workpackages in the report, has also been strengthened by the close inter-relationship between each of the work-packages and, since commencement of the project, between the consortium partners, with exchange of both materials and personnel between several of the partners already occurring at this early stage of the project.

During this reporting period various redox enzymes available from project partners have been produced and purified, or purchased from commercial sources, and the kinetic and electrochemical characterisation of these has been initiated. Entrapment of the enzyme in a conductive polymer (PEDOT) has been tested to see whether the enzyme still retains its activity after entrapment. Novel microbial enzymes have also been screened from metagenomic libraries, and engineering of glucose oxidase has been carried out to improve enzyme immobilisation. A mathematical model to simulate the behaviour of an immobilised enzyme layer in a biosensor has been

developed, and the model has been experimentally tested for two enzyme-based biosensors.

A comprehensive survey of redox mediators suitable for the enzymes targeted in this project has been prepared and recommendations on mediators for screening of new “oxygenase” enzymes have been made. In addition, based upon the findings in the survey, a library of osmium/ruthenium-based redox mediator complexes of different redox potentials is being prepared, with each complex in the library amenable to simple immobilisation chemistry. This immobilisation can be achieved either through ligand substitution with a suitable polymer support or through covalent coupling of designed functional groups of the complex to supports.

Several methods of enzyme immobilisation were investigated during this period. These included physical entrapment, covalent coupling on self assembled mercaptocarboxylic acid modified surfaces, a novel soft landing technique and the adsorption onto carbon nanotubes. Obtained enzymatic electrodes were characterised voltammetrically in the presence and absence of mediators. For covalent immobilisation of proteins and mediators, molecular bridges were synthesised by means of electroreduction of diazonium compounds. An extensive review on the theory of the kinetics of mediated enzyme reactions was prepared.

A number of methods of template assembly for subsequent production of mesostructured gold electrodes by electrodeposition were investigated in order to select the most suitable one, which yields good quality colloidal crystals and allows their preparation in relatively short time. A vertical deposition method has, to date, proved to be the most effective, producing surfaces with only small imperfections. Template deposition under reduced and controlled pressure is to be attempted to alleviate these imperfections. In addition, enzymes have been successfully immobilised within conducting polymer (PEDOT) matrices on high-surface area carbon foams. Attempts, however, to produce porous carbon foams from polymeric precursors have not as yet been successful. Improvements in thick film technology for the mass-production of structured electrode surfaces for the project have also been achieved.

Finally, some initial investigations into the use of carbon nanotube-modified surfaces as glucose biosensors and for the production of electricity in a biofuel cell have been successfully undertaken.