Publishable Executive Summary

Background

The SANTS project, which stands for Synthesis and Application of Nanostructured Tethered Silicates, is designed to improve knowledge in nanoscale manufacturing and create new molecular materials for both biosensors and biocatalysed synthetic chemistry via the use of techniques inspired by natural biosilication processes. The project is based on the observation that diatoms, which are small unicellular algae, possess internal silica skeletons that are laid down due to the silica precipitating activity of specialised proteins called silaffins. These proteins have a highly repeating structure in which the repeated sequences are decorated with additional amine and phosphate groups. Whilst silaffins themselves are potent silica precipitants, synthetic peptides corresponding to these repeats will also act as silicating agents and will allow silica nanoparticles to be generated, provided phosphate is also supplied. Moreover, several polyamines, which can be viewed as peptide mimetics also act as silica precipitants. The biotechnological facility of these observations is that the biosilicates can entrap and immobilise a wide range of enzymes with dramatically higher efficiency than conventional immobilisation procedures, and that biosilica entrapment also serves to stabilise and protect the entrapped enzymes.

The primary objective of the NMP work programme is to promote real breakthroughs, based on scientific and technical excellence. Within the SANTS project two main areas are targeted:

- Biosensors - reagentless analytical devices employing intimately entrapped enzymes.
- Biocatalysis - functionalised supports with entrapped enzymes for green chemistry.

To fulfil these objectives a number of University partners have been chosen alongside two small to medium-sized enterprise (SME) companies. The University partners consist of the Universities of Leeds (coordinator), Alcalá, Warwick, Hull, Ghent, Crete and INSA (Toulouse). The two SME companies are C-Tech Innovation Ltd and Sarissa Biomedical Ltd. The project coordinator can be contacted using the following details:

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Project Progress: First 12 Months

Initially, a project logo was designed (shown below) and a website was constructed (www.sants-nanosilicates.com). The website has been used by all of the partners to share information and by the coordinators for posting new information and technical documents that can be accessed by all of the project partners. There is also an area of the website that can be accessed by the general public and any other outside entity which may be interested in the technologies that the SANTS project uses and is developing.

The project SANTS logo designed by Dr F. Neville, University of Leeds (Partner 1)

The production and characterisation of nanosilicate particles catalysed by R5, PEI and poly-L-lysine has successfully been achieved. As a result a number of common protocols are used by the project partners in the production of nanoparticles. The project has progressed well from the generation of silica particles to the point where a number of enzymes (glucose oxidase, acetylcholinesterase, lipases and mannitol dehydrogenase) can be entrapped using both a batch wise method and one involving the use of a microfluidic reactor. Many potential sources of R5 peptide has been scrutinised and a reliable supplier chosen so that a high quality peptide can be supplied to all of the necessary partners. R5 peptide with an N-terminal thiol has also been sourced and supplied as well as R5 multimers. The future use of R5 peptide will be much cheaper and much more viable through the design of a recombinant R5 expression vector that will enable the production of single R5 peptide as well as a variety of multimers.

A microfluidic reactor for the production of R5 and PEI catalysed silica nanoparticles has been developed that allows the rapid production of nanoparticles that have been functionalised with enzyme. R5, PEI and poly-L-lysine have been investigated in the preparation of ultra-microbiosensors via the use of cross-linking agents. Current work is centred upon the optimisation of these devices. R5 peptide has also been used to successfully silicate the surface of gold electrodes where the R5 peptide attaches directly with the electrode via an N-terminal thiol group. Preliminary studies indicate that this method can also be used to functionalise the electrode surfaces with enzyme and electrochemical characterisation has been utilised to monitor improvements in the enzyme entrapment process.

A significant portion of work has also centred upon the production and characterisation of expression vectors that will allow the preparation of Cal B and BCL lipases as well as mannitol dehydrogenase. These enzymes have significant industrial importance and the ability of the SANTS project partners to produce their own enzymes for entrapment studies will be greatly advantageous. Currently work is focusing upon the optimisation of the expression systems and the subsequent purification of these enzymes so that they can be compared to commercially available products.

Finally, FTIR-Attenuated Total Reflectance and Raman spectroscopy have been used to investigate the occlusion of poly-L-lysine and enzyme within silica nanoparticles. This work will be extended to include particles that have been generated using R5 peptide and have had many more enzyme types entrapped. Preliminary work has also begun to investigate the internal structure of silica nanoparticles using high resolution transmission electron microscopy, physical and chemical gas adsorption as well as mercury intrusion porosimetry.

Adaptation of the natural silication process through the use of synthetic peptides and peptide mimics to catalyse nanosilica production will be explored by the Project SANTS partners. The materials produced will be of use in the production of biocatalytic matrices, ultramicro-biosensors and nanoparticulate optical biosensors. Manufacturing protocols will be developed that allow the reproducible deposition of nanostructured surfaces for sensing and synthetic applications and also, the potential exists for the production of self-assembling and self-organising structures. This could lead to the development of a range of materials that will be of significant use in a range of industrially important areas.