

# NUSNNI FOCUS GROUPS

**FOCUS GROUP:** Nanobiotechnology

**FOCUS GROUP CHAIRS:** A/Prof Feng Si-Shen (Chemical & Biomolecular Engrg, FOE)

A/Prof Sheu Fwu-Shan (Biological Sciences, FOS)

## Focus Group Information

### 1. Objectives & Planned Deliverables

Nanobiotechnology is to apply and further develop nanotechnology to solve problems in biology and medicine, which describes and explains biological structures/functions/mechanisms and human physiology/pathology/treatment at the molecular and atomic scale. Nanobiotechnology will provide in-depth knowledge to understand the mechanisms of mysterious life phenomena and currently incurable diseases and thus to revolutionize the current practice in biology and medicine. The overall objective of this Focused Group is to build a world-class nanobiotechnology team at NUS, which include high quality team members of leadership in their areas at the cutting edge of nanobiotechnology, world-class laboratories with state-of-the-art facilities, well deigned educational program, and wide network for international interactions.

The focused areas in which we pursue the international leadership include (1) Chemotherapeutic engineering, (2) Biosensors, (3) Cellular and molecular biomechanics, (4) Molecular imaging, (5) Lab-on-a-chip, (6) Molecular diagnosis, and (7) Gene therapy.

Deliverables include (1) scientific papers in internationally refereed journals of high impact, (2) keynote/invited presentations in international conferences, (3) external funding and investment, (4) world-class PhD and MEng/MS students, (5) nanobiotechnology patents, (6) spin-off companies, (7) nanobiotechnology and human health care products.

### 2. Research Plan & Focus

There are 7 research areas in the NUSNNI nanobiotechnology focused group:

(1) Nanoparticle Technology for Biomedical Applications. Nanoparticle technology could be one of the most promising and most prospective areas of the widely defined nanobiotechnology. With the effort in the past few years, our Nanobiotechnology Focus Group has begun to play a leading role and has won certain international reputation in the world in nanoparticle technology for biomedical application. A few invited reviews have been published in internationally prestigious journals such as *Chemical Engineering Sciences*, *Current Medicinal Chemistry*, *Expert Review of Medical devices*. External fund application has been successful. A few BMRC grants (including a SCS fund) have been successfully applied. Nanoparticles developed include those of biodegradable polymers such as PLA, PLGA, PELA, etc and other bioadhesive materials such as medical clay, lipid bilayer vesicles (liposomes), solid lipid nanoparticles, micelles, liposomes-in-microsphere (LIM), colloidosomes, etc. Applications include cancer chemotherapy, cardiovascular tissue repair, delivery of therapeutic agents and diagnostic materials across the blood-brain barrier (BBB), gene therapy, etc. Paclitaxel has been used as a prototype drug due to its excellent therapeutic effects against a wide spectrum of cancers, cardiovascular restenosis, Parkinson's disease, AIDS, etc and its great commercial value as the best seller in the world market. Attention has been given to (i) nanoparticle formulation techniques, (ii) emulsification process and recognition of natural emulsifiers such as phospholipids, cholesterol and PEGylated vitamin E, etc, (iii) other coating techniques, (iv) characterization techniques, in vitro measurement techniques (HPLC, MS, etc), (v) cell line techniques, (vi) animal test techniques, (vii) molecular imaging, (viii) development of techniques and theories to measure and analyze drug-cell interactions and nanoparticle-cell interactions, (ix) development of new nanomachines and nanostructure for detection and treatment of diseases, e.g.

nanobiosensors and nanoparticles for imaging, etc, (x) nanoparticles to cross various physiological barriers for drug delivery such as the blood-brain barrier (BBB), the gastrointestinal barrier (GI barrier), the mucosal barrier, the microcirculatory barrier, etc, (xi) mathematical modeling and computer simulation of chemotherapy for cancers and other diseases.

(2) Biosensors. Biosensors represent a most plausible and exciting application area for nanobiotechnology. Nanosensors based on advanced nanomaterials are expected to emerge in the marketplace in significant volumes over circa the next ten years. Sensors constructed at the molecular scale are promising and have achieved to be extremely sensitive, selective, and responsive. For example, the U.S. Defense Department has been interested in such sensors for rapidly and accurately detecting small amounts of chemical or biological agents to allow soldiers to defend against chemical or biological attacks. In the medical diagnostics arena, nanotechnology-based biosensors could be used, for example, to replace more costly and tedious laboratory methods for monitoring a patient's blood for proteins, chemicals, and pathogens. Our goal is to build an interdisciplinary team based on the expertise developed on carbon nanotubes, to develop novel, rapid-response biochemical sensors selective for targeted chemical and biological molecules.

We have utilized high-density well-aligned carbon nanotubes, which are multi-walled and vertically aligned on a large area of substrates, such as Ta, that can be readily synthesized. In particular, Ta plate was used as a substrate and a thin cobalt (Co) layer of 8 to 50 nm was coated onto the substrate as catalyst by magnetron sputtering for the synthesis of MWNTs. The nanotubes prepared by this method have diameters of 200 nm to 400 nm and a length of about 10  $\mu\text{m}$  depending on the Co layer thickness and growth time. MWNTs at Ta substrate can be easily attached to the surface of a planar electrode using conductive silver paint as biosensing electrode. Firstly, these MWNTs have high electrochemically accessible surface area, high electrical conductivity, and useful mechanical properties for developing electrochemical sensors in selectively detection of uric acid (UA) in the presence of L-ascorbic acid (L-AA). Secondly, MWNTs can be used as a nonenzymatic sensor to detect glucose with high sensitivity and stability in alkaline medium. Thirdly, we have successfully constructed a hemin-modified MWNTs electrode in the development of novel oxygen sensor for working at a relatively low potential.

In sum, we have developed a unique expertise in electrochemical bio-sensing using multi-walled nanotubes as electrodes. We start to collaborate with MIT researchers who have developed ordered nanotubes arrays, chemically functionalized nanotubes, and sensors based on conjugated polymers. Hence, we will show how the combination of this expertise and capabilities will make it possible to produce novel state-of-the-art biochemical and contaminant sensors, based on an original concept able to selectively detect in real time a single chemical species down to nanomole concentrations.

(3) Nanomechanics of Cell and Biomolecules. As physical entities, biological structures in the human body are constantly subjected to physical interactions throughout life. The length/force scale of these interactions can range from the micro down to the picoscale. These biological structures can include as large as a single cell to as small as a single biomolecule such as a DNA or protein. Here, we seek to correlate the structures of these biological entities to their physical properties and physiological functions by investigating the mechanics governing these biophysical interactions. It is hoped that through this study, one can obtain important information on their natural structure-property-function relationship, gain further insight into important physiological functions such as wound healing and tissue regeneration and establish possible connections to human diseases such as malaria and cancer. Research areas include cellular and molecular biomechanics, nanomechanical characterization of biomaterials.

(4) Quantum Dots for Biomedical Applications. Existing ways of labeling and visualizing DNA and protein molecules rely on the light-emitting properties of a limited group of radioactive elements, chemical dyes, and protein molecules. These labeling techniques have several drawbacks: radioactive markers have short life spans while organic dyes come with a limited number of colors and may quickly lose their glow. There have been great demands for more reliable and more robust labeling fluorophores in biomedical research and applications so as to enable real-time imaging and quantitative determination of multiple-molecule types present in cells or tissues. Highly luminescent quantum dots potentially can overcome the functional limitations encountered with chemical and organic dyes. They are highly stable against photobleaching and have narrow, symmetric emission spectra. In particular, the emission wavelength of quantum dots can be continuously tuned by changing the particle size or composition, and a single light source can be used for simultaneous excitation of all different-colored dots. These novel optical properties can render quantum dots ideal fluorophores for sensitive, multicolor, and multiplexing applications in molecular biology and bioengineering. In order to understand the complexity and dynamic interactions of biological molecules, it is

desirable to monitor and visualize the interactions of multiple proteins or DNA sequences present in cells or tissues. A new strategy will be developed for quantitatively imaging the biochemical contents in organisms. For example, diverse receptors or ligands on cells can be simultaneously screened and quantitatively analyzed by using confocal micro-spectrophotometer. This could provide a direct approach to identify sets of genes and proteins that correlate with certain diseases.

(5) Laboratory-on-a-chip (LOC). LOC, an integrative platform for biomolecule detection, is an emerging technology platform to simultaneously detect multiple biomolecules (e.g., DNA, RNA, and protein) in various biological applications in a miniaturized setting. It integrates multiple processes from sample collection, DNA/RNA extraction and amplification to detection on a microfluidic platform less than a palm size. To do so, it employs the technological development in micro- and nano-fabrication and microfluidics to produce micro/nano-devices consisting of interconnected fluid reservoirs and pathways, and allows automated transport of samples through selected pathways for example by the electrokinetic forces. It is further possible to create the functional equivalent of valves and pumps capable of performing manipulations such as reagent dispensing and mixing, incubation/reaction, and sample partition and analyte detection through various biosensing and nanoparticle-detection systems. One simple example is to develop LOC for whole-cell immunological assay. It consists of micro-chambers, micro-channels and filter weirs, and was demonstrated to effectively trap, concentrate, and immuno-fluorescently detect microbial cells, which were larger in size than the weir gap. The other example is to identify hundreds of single-nucleotide polymorphism sites in biomedical researches by simultaneously examining the dissociation kinetics of all the targeted DNA (T)-probe (P) hybrids within minutes in a LOC.

(6) Luminescent Nanocomposites for Biological Labeling and Diagnostics. There have been tremendous advances in the development of in-situ labeling and screening of different biological entities, ranging from cells to DNAs. Many approaches have been developed for this purpose, such as the chemical encoding with molecular tags, organic fluorophores, fluorescent colloids, and Raman fingerprints. The development of labeling materials has been critically important. Some materials such as quantum nanodots, organic dyes and metal nanoparticles have been extensively used for biological labeling. However, their poor water solubility, lack of surface functionality, poor photochemical stability and biocompatibility, are still the main concerns. They have to be surface modified to better suit their integration with biological systems. Recently, synthesis of monodisperse polymer nanospheres has stimulated great interest and incorporation of fluorophores in these nanospheres is particularly attractive. In these nanocomposites, not only can the organic polymer stabilize the nanoparticles in a solid matrix, but also effectively combine the peculiar features of organic and inorganic components, thus resulting in novel properties. These materials can bring new and unique capabilities to a variety of biomedical applications ranging from diagnosis of diseases to novel therapies.

(7) DNA Nanoparticles: A New Means for Molecular Medicine in the Nervous System. Molecular medicine uses genetic materials that are transported into cells with the assistance of specific delivery vectors to treat those diseases that do not respond to conventional therapies well. Nonviral DNA vectors have been extensively studied as such delivery vehicles, because of their desirable properties such as the ability to induce relatively low toxicity and generate hardly any immune responses. Other potential advantages of the nonviral gene delivery systems include their capability to deal with large DNA plasmids, their simple preparation method, and flexibility in their use, as well as cell-type specificity after chemical conjugation of a targeting ligand. Our lab has been working with the development of synthetic polymer-, peptide-, and recombinant protein-based gene delivery vectors that have been tuned to fit into the application in the central nervous system (CNS). The PEGylation approach was adopted to modify polyethylenimine (PEI), a polycation that is potent in mediating gene transfer in vitro and in vivo, to improve its biocompatibility in the CNS without sacrificing its gene delivery efficiency. Of particular interest, PEGylated PEI allows repeated intrathecal administration of DNA/polymer nanoparticles, leading to prolonged transgene expression in the spinal cord. The use of cyclodextrin (CD) to link low molecular weight PEI is also another method of improving the biocompatibility of PEI. The copolymers comprise ester bonds for hydrolysis and are capable of mediating gene transfection in various types of cells as efficiently as high molecular weight PEI. To explore the feasibility of targeting entry of DNA nanoparticles into neurons through specific ligand-receptor interaction and receptor-mediated endocytosis, recombinant DNA technology has been used to produce chimeric polypeptides containing a nucleic acid binding domain linked to a receptor-binding domain of neurotrophins. The polypeptides form complexes with DNA, and target the complexes to the neurons expressing neurotrophin receptors. Receptor binding of the polypeptides triggers receptor-mediated endocytosis, delivering DNA into the neurons selectively.

#### **Grand Challenge Project:**

In order to focus our strength in developing a world class research program as well as promote close multidisciplinary collaboration among the members of our Nanobiotechnology Focused Group, we will develop a synergistic program in which all members are able to effectively contribute using their own expertise. We aim to become one of the world leaders in this area as well as to contribute towards the knowledge-based economy in Singapore:

### **Nanobiotechnology for Cancer Diagnosis and Treatment**

Cancer is a leading cause of deaths. More than 10 million people are diagnosed with cancer every year. It is estimated that there will be 15 million new cases every year by 2020. Cancer causes 6 million deaths every year or 12% of deaths worldwide. In the United States, about 16 million new cancer cases have been diagnosed since 1990, where there were 553,768 cancer deaths and the overall costs for cancer treatment were estimated as high as \$156.7 billion in 2001, and about 1.3 million new cases were diagnosed and more than half a million deaths were caused by cancer, i.e., one in every four deaths, in 2002. Although we have been facing such a serious situation, there has been no substantial progress in the past 50 years in fighting against cancer. The cancer death rate in US was 1.939‰ in 1950 and still 1.940‰ in 2001. It is clear that the progress in cancer treatment has been slow and inefficient and we are in crisis in fighting against cancer. Significant increment in cure rate would be unlikely achieved unless more profound knowledge of cancer pathophysiology can be pursued, new anticancer agents can be discovered and new biomedical technologies can be developed. The emerging nanotechnology brings new hope for significant breakthroughs to be achieved in the near future.

Although there is no thorough cure for cancer at late stage, early stage cancer is treatable in general and the prognosis could be great. Cancer diagnosis is thus an important and practical way to improve the cure rate. The current diagnosis techniques are usually in tissue level, which has low efficiency and fails to detect invisible cancer cells. Molecular imaging has become a high area in cancer diagnosis. Molecular labeling is a key. The existing ways of labeling and visualizing DNA and protein molecules rely on the light-emitting properties of a limited group of radioactive elements, chemical dyes, and protein molecules. These labeling techniques have several drawbacks: radioactive markers have short life spans while organic dyes come with a limited number of colors and may quickly lose their glow. There have been great demands for more reliable and more robust labeling fluorophores to enable real-time imaging and quantitative determination of multiple-molecule types present in cells. Highly luminescent quantum dots can overcome the functional limitations encountered with chemical and organic dyes. They are highly stable against photobleaching and have narrow, symmetric emission spectra. In particular, the emission wavelength of quantum dots can be continuously tuned by changing the particle size or composition, and a single light source can be used for simultaneous excitation of all different-colored dots. This synergistic program will develop robust biological nanoprobe using optically active quantum dots for multi-parameter and quantitative analysis of genes and proteins in tissues. This research will include synthesis of high quality luminescent quantum dots, a reasonable understanding of the surface chemistry for bioconjugation, and the preparation of water-soluble and biocompatible nanocrystals for real-time monitoring, simultaneous screening and quantitative bio-imaging of multiple biochemical contents in cancerous organisms by using confocal micro-spectrophotometer. The research will be focused more on cancer diagnosis (Coordinators: Dr Han Ming-Yong and Dr Zhang Yong).

Another technology which will be developed in this synergistic program is nanosensors. Sensors constructed at the molecular scale are promising and have achieved to be extremely sensitive, selective, and responsive, which can be used to replace more costly and tedious laboratory methods for monitoring a patient's blood for proteins, chemicals, and pathogens. This FG has utilized high-density well-aligned carbon nanotubes, which are multi-walled and vertically aligned on a large area of substrates. We have a unique expertise in developing electrochemical multi-walled nanotubes, ordered nanotubes arrays, chemically functionalized nanotubes, and conjugated polymers as nanosensors. We will build a team for further development of the nanosensor technology for cancer diagnosis (Coordinators: Prof Sheu Fwu-Shan and A/P Lim Chwee Teck).

Finally this synergistic program will develop two fully integrated laboratory-on-chip devices for cancer diagnosis. The first contains numerous functional features such as indicators for physical parameters and reaction chambers for cell growth and separation at micro- and nano-scale to rapidly identify cancer cells. Potential cancer cells can be delivered into the microfluidic device and possibly cultivated *in-vitro* followed by detection using various optical-based detection methods. The second is a beads-based lab-on-chip device to rapidly identify metabolites, genes and single-nucleotide-polymorphism (SNP) sites associated with specific cancers. The two lab-on-chip devices have several

distinct advantages over the current cell culturing and detection methods, which include ease of use for cell culture and reaction, rapid hybridization and sensitive detection, and low cost for commercial interest (Coordinator: Prof Liu Wen-Tso and A/P Sheu Fwu-Shan).

The standard treatment for cancer has been surgery plus radiotherapy in the past decades. If cancer patients fail such a treatment, he/she has only less than 10% to be cured by other treatment such as chemotherapy, immunotherapy, and molecular therapy. Chemotherapy is one of the most effective treatments available for cancer and other diseases such as cardiovascular diseases and AIDS. The present status of chemotherapy, however, is far from being satisfactory. Its efficacy is limited and patients have to suffer from severe side effects. Nanobiotechnology may provide an ideal solution for the problems in the current regime of chemotherapy and promote a new concept of chemotherapy, which may include sustained, controlled and targeted chemotherapy; personalized chemotherapy; chemotherapy across various physiological drug barriers such the gastrointestinal (GI) barrier for oral chemotherapy and the blood-brain barrier (BBB) for treatment of brain tumors and other central nerve system (CNS) diseases; and eventually, chemotherapy at home. Indeed nanobiotechnology, especially nanoparticle technology, will change the way we make drug and the way we take drug. Paclitaxel, one of the best antineoplastic drugs found from nature in the past decades, will be used as a prototype drug in this synergistic program due to its excellent therapeutic efficacy against a wide spectrum of cancers and its great commercial success as one of the best sellers among various antineoplastic agents. Our preliminary results have shown by fluorescence microscopy and cell mortality experiment with HT-29 cells that paclitaxel formulated by Vitamin E TPGS-emulsified PLGA nanoparticles can be at least 18 times more effective than the free drug Taxol<sup>®</sup> after 24 hours of cell culture, and oral chemotherapy by nanoparticles seems feasible. We shall continue to be focused on our established strength which includes synthesis of copolymers more friendly to anticancer drugs and molecular drugs (proteins and peptides), characterization and application of natural emulsifiers such as phospholipids, cholesterol and molecularly modified vitamins, surface medication techniques, enhanced molecular conjugation techniques and nanoparticle techniques. Our research will speed up to animal test and clinical trials (Coordinators: A/P Feng Si-Shen and Dr Zhang Yong).

Gene therapy will also be a focus of this synergistic program. Targeted gene delivery to selected cell types provides a means for highly specific gene expression. Improved efficiency of gene transfer could be achieved through enhancing the entry of gene vectors into the desired cells and reducing the uptake of the vectors by non-target cells. We are developing chimeric peptides containing a nucleic acid binding domain linked to a receptor-binding domain of neurotrophins to target gene delivery vectors to tumors cells. These and other functional peptides are also tried to be conjugated to cationic polymer-based DNA vectors. The developed materials would hopefully form stable nanoparticles with DNA that are suitable for *in vivo* targeting of cancer gene therapy (Coordinators: A/P Wang Shu and A/P Feng Si-Shen).

The second approach is to use a cell-specific promoter in a viral vector, which allows the control of specific gene expression in a selected cell type. Because of their cellular authentic sequences, the cell-specific promoter may reduce the chance of activating host cell defense machinery and usually are less sensitive to cytokine-induced promoter inactivation than viral promoters, thus improving the stability of gene expression. The third approach involves small interfering RNA (siRNA) technology, which is currently emerging as potentially useful method to develop highly specific double stranded RNA based gene silencing therapeutic. The aim is to develop efficient and effective siRNA delivery systems for tumors in the central nervous system (Coordinator: A/P Wang Shu and A/P Sheu Fwu-Shan).

Cellular and molecular biomechanics of cancer cells can be a fascinating area, which characterizes the rheological properties of mutant cancer cells and relates the measurable mechanical properties to their molecular basis. Changes in the rheological properties may provide useful information for cancer diagnosis and physical evidence to understand therapeutic mechanisms of various anticancer agents. Recent advances in experimental biomechanics have enabled direct and real-time mechanical probing and manipulation of single cells and molecules. Such methods are now capable of imposing and sensing forces and displacements with nano and picoscale resolutions. Our experimental techniques to probe single cells include micropipette aspiration, optical/laser traps and atomic force microscopy. Focus will be made on the original biochemical conditions of the cancerous cells as well as drug treatment for which the mechanical properties of these cells can either increase or decrease as compared to the healthy cell. Such changes in mechanical response with the underlying changes in molecular architecture as a consequence of disease development and how that changes cell shape and mobility can also be observed. Our Nano Biomechanics Laboratory has been well established in the area of nanomechanics of biological cells and molecules. It will focus its effort on investigating biomechanical responses to the progression of the disease state of cancer cells as well as the

effects arising from treatment using anticancer agents/molecular drugs. (Coordinators: A/P Lim Chwee Teck and Dr Han Min-Yong)

We have successfully obtained a few research grants from the Singapore Cancer Syndicate (SCS) and BMRC, A\*STAR. We shall file more grant applications based on this synergistic program. We shall also contact pharmaceutical companies for industrial investment on this synergistic program. International collaboration with top universities and institutions in the world will be widened and strengthened.

### **3. Websites of Affiliated Focus Group Members & Laboratories**

A/P Feng Si-Shen: <http://www.bioeng.nus.edu.sg/people/fengss/FengSS-BioE-CV2004.doc>  
<http://www.bioeng.nus.edu.sg/research/chemotherapeutic/ChemoTheraEngPoster.pdf>

A/P Sheu Fwu-Shan: <http://www.dbs.nus.edu.sg/Staff/sheu.htm>

A/P Lim Chwee Teck: [http://me.nus.edu.sg/people/staff.asp?staff\\_id=60](http://me.nus.edu.sg/people/staff.asp?staff_id=60)  
<http://www.bioeng.nus.edu.sg/nanolab/nanolab.html>

A/P Liu Wen-Tso: <http://www.eng.nus.edu.sg/civil/aboutus/facultystaff/cveliuwt/liuwt.html>

Dr Han Ming-Yong: <http://www.imre.a-star.edu.sg>

Dr Zhang Yong: <http://www.bioeng.nus.edu.sg/people/default.htm>

A/P Wang Shu: <http://www.dbs.nus.edu.sg/Staff/wangshu.htm>