NANOCAP

Nanotechnology Capacity Building NGOs

Applications of Nanotechnology:

MEDICINE (Part 2)

Luisa Filipponi & Duncan Sutherland

Interdisciplinary Nanoscience Center (iNANO)

University of Aarhus, Denmark

Last update November 2007

About the Document

The present document is part of a series of papers written for the FP6 Project NANOCAP (acronym for "Nanotechnology Capacity Building NGOs"), which is a European Project that was set up to deepen the understanding of environmental, occupational health and safety risks, and ethical aspects of nanotechnology. More information about NANOCAP can be found on the project's website www.nanocap.eu.

Nanotechnology is defined and described in an introductory paper, and its application in diverse field then described in separate papers:

- Nanotechnology- A Brief Introduction
- Applications of Nanotechnology: Energy (Part 1 and Part 2)
- Applications of nanotechnology: Environment
- Applications of Nanotechnology: Medicine (part 1 and Part 2)

The aim of these documents is to provide updated, concise yet accurate information about nanotechnology in fields of high societal impact like environment, energy and medicine based on scientific literature and authoritative reports. The documents also include some visions of potential applications of nanotechnology, whilst keeping the discussion at a realistic level. These documents have been written with the purpose of supporting environmental NGOs, trade unions, as well as other groups in learning about nanotechnology and its potentials in these diverse areas of applications. The authors' intention is to help those groups forming a balanced view of nanotechnology, so to promote a constructive dialogue about this emerging technology. Other aspects of this discussion, such as potential environmental risks of nanomaterials, health and safety aspects, and ethical/societal concerns related to nanotechnology, are not covered in these papers but can be found in other sections of the NANOCAP website (www.nanocap.eu)

About the Authors

Luisa Filipponi is currently a Post Doctorate at the Interdisciplinary Nanoscience Center (iNANO) at the University of Aarhus (Denmark). She has a Bachelor degree in Chemistry (University of Bologna, Italy) and a PhD in Nano- and Microtechnology completed in 2006 at Swinburne University of Technology (Australia), through a project involving Nanotechnology Victoria (Nanovic) and the Cooperative Research Centre for Microtechnology. Her research focused on novel micro-and nanofabrication methods to develop nanoarrays for the selective detection of proteins and cells. Luisa is the author of a review for the Wiley Encyclopaedia of Biomedical Engineering (John Wiley & Sons, Inc., 2006), co-author of a chapter in the book 'Microarray Technology and its Applications' (Springer & Verlag, 2005), author and co-author of various scientific publications, as well as co-inventor of two patents. She currently focuses on science communication in particular related to nanoscience and nanotechnology.

Duncan Sutherland has worked in the area of nanotechnology for life science since 1995 publishing to date 45 scientific articles with a focus on nanostructured biomaterials and nanoscale biosensors. After completing his Ph.D in Physics at the University of Bristol he moved to Chalmers in Sweden where he became a project leader in the Chemical Physics Group of the Department of Applied Physics. During his time in Sweden he developed self-assembly based engineering approaches to nanofabrication utilising colloidal particles systems and applied them to the study of nanostructured interfaces in different life science applications. In March 2006 he moved to an Associate Professor position at the iNANO centre at the University of Aarhus, where he formed the Biointerfaces Group with research interests in biomaterials, biosensors, biofouling and nanotoxicology. In addition to his research activities, A/Prof. Sutherland is also involved in outreach activities, including communicating fundamental concepts, benefits and applications of nanotechnologies to NGOs, trade union representatives, industry members and the general public.

APPLICATIONS OF NANOTECHNOLOGY:

MEDICINE (Part 2)

Therapy

Once a disease has been identified, patient and doctor enter the challenging process of defining a therapy to fight the disease. The same disease, such as cancer, can express itself in many different forms; for instance there are at least 14 different types of breast cancer. Thus, in an ideal world, a therapy should be specific, in order to kill only the 'bad' cells, and effective, both in terms of action and time.

A therapy normally involves a pharmaceutical route (drugs) to kill the disease from the inside of the body, or, when a pharmaceutical therapy is not possible or not effective, other routes to fight the disease from the 'outside' of the body, such as chemotherapy to fight cancer. In certain circumstances, surgery is required, and an organ-substitute is inserted in the body in the form of an implant or a donated organ. In all those approaches, which are often used in combination, the aim is always the same: to eliminate selectively the source of the disease in a long-lasting way. Nanotechnology is making a tremendous impact in this field, with new drugs and new type of treatments under development, some of which have already proven clinically effective and have entered the market[*].

DRUG DEVELOPMENT AND TARGETED DRUG DELIVERY

Advancement in the field of pharmacology deal with two main concepts: development of new biologically active drugs (drug discovery) and development of new drug delivery systems able to reach the specific site of the disease. Drug delivery systems (DDS) are not a new concept: research in this field started in the mid 1960s and resulted in the type of drugs we use today, that is, medicines where the active ingredient is encapsulated and released inside the body by gradual dissolution, osmotic effects, or other mechanisms. DDS are in the form of 'pills' that we frequently take and that can release gradually their active component in time (slow release drugs) or dissolve based on some physiological conditions (e.g., acidity of the environment). DDS also exist in the form of implants, inserts, or other drug-releasing systems.

One of the bottlenecks in drug discovery is the necessity of screening thousands of candidate drugs for their efficacy in fighting targeted macromolecules in a disease state. Micro- and, now, nanotechnology have enabled the development of

[*] For a recent review of nanotechnology enabled drugs and therapies that have reached the clinical or commercial stage see reference 5.

microarray platform and new detection methods (including label-free) to investigate the effects of candidate drugs against disease macromolecules with unprecedented speed.

Pharmaceutical drugs developed using conventional synthetical routes are limited by problems such as low efficacy, low solubility in water and lack of selectivity. In addition, physiological barriers often prevent the drug from reaching and acting at the target site-a phenomenon called drug resistance. The low solubility and limited bioavailability of conventional drugs is responsible for their limited effectiveness: the body often clears away the drug before its action has fully occurred. The efficacy of drugs is also dependent on the dose used, but dose-dependent side effects often limit their acceptable usage. The lack of selectivity is especially detrimental for instance in cancer therapies, since anti-cancer drugs, usually used in large volume of distribution, are toxic to both normal and cancer cells.

A recognised need exists to improve drug composition, delivery, release and action, and thus to develop new drugs that can act at the specific site of the disease, maximizing the drug's therapeutic action while minimizing side effects. For drugs to be able to do so, the delivery systems need to be miniaturized in size to become much smaller than the target, and specific in composition to elicit a certain response. With the use of nanotechnology, **targeted drugs** (in terms of composition and delivery system) are becoming a reality. In the future this will lead to targeted therapies and personalized medicine. The aim is to design and deliver drugs in such a way that they can recognize the ´bad` cells at a molecular level and penetrate the cell membrane and act inside the infected cell. This is often crucial for the efficacy of a drug, since it is across the cell membrane and inside the cell that most virus replication and other disease conditions take place. This way, the treatment will be delivered where is needed and will be specific, eliminating the problem of the drug killing healthy cells. Moreover, specific properties could be incorporated into the nano-DDS, so that the drug could be activated only upon reaching the target, and the active component released at a controlled rate. **Controlled activation** could be linked to some environment properties, such as pH, or ´lock-and-key` molecular recognition mechanisms[1]. **Figure 4** shows a schematic representation of the process of packaging of active drugs into nanoparticles for the delivery to the surface of a cell.

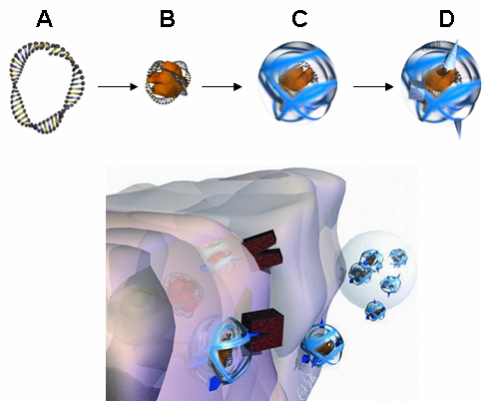

Figure 4. Design of a drug delivery system for targeted cell treatment. Plasmid DNA (A) is condensed by electrostatic interaction with a polycation to form a nanoparticle (B) in the size range 40-150nm. A hydrophilic polymer is grafted to the surface of the nanoparticle (C). Incorporation of cell specific ligands (D) onto the polymer-modified surface facilitates targeted drug delivery (bottom image). Image courtesy of K. Howard (iNANO, University of Aarhus).

Nano-sized drug carriers that are currently under development include either materials that self-assemble, or conjugated multicomponent systems, for instance a drug linked to a protein and a polymer (called polymer-drug conjugate). Numerous nanosystems are now investigated, and include micelles, nanoemulsions, nanotubes, nanofibres, liposomes, dendrimers, polymer therapeutics, nanoparticles, nanocapsules, nanospheres and hydrogels[2]. Some of these nano-sized drug carriers are established in the field of drug delivery, such as liposomes, others have made their way to the market in recent years, such as polymer-protein conjugates (polymer pharmaceutics). Many are now used for treating some forms of cancer, hepatitis, and leukaemia[3-5].

Targeted drugs and targeted DDS could allow the creation of drug formulations with **optimal loading**, therefore delivering to the body only the necessary amount of the drugs and reducing side effects for the patients. Together with the possibility of having nano-DDS that are **biodegradable** inside the body, this will help to reduce drug toxicity. **Drug safety** can be further enhanced by the possibility of introducing inside the drug formulation a label that changes colour when the drug reaches its expiring date or is no longer functioning. This will allow the improvement of drug **shelf-life** and better monitoring of drug safety.

Finally, the future of nano-DDS enabled by nanotechnology could be **miniaturised implantable chips** loaded with different drugs that can be released upon external stimuli. This could free patients, like diabetics, from having to administer drugs repeatedly during the day.

NEW EXTERNALLY ACTIVATED THERAPIES THAT USE NANOPARTICLES

One of the distinguishing properties of nano-sized drug carriers is their ability to accumulate passively in cancerous solid tumour tissue due to an effect called **´enhanced permeability and retention` (EPR).** This passive mechanism has been attributed to the ´leaky` nature of tumour vessels. The blood vessels that supply tumours with nutrients have tiny gaps in them that allow nano-DDS (60-400nm in size) to get in the tumour region and accumulate in. This further enhances the targeted approach to treating infected cells. Moreover, this allows the accumulation of therapeutic agents inside the tumour region and activating it using an external source. Based on this concept some **new anti-cancer therapies** have been developed and have entered advanced clinical trial stages. In these therapies, nanoparticles are delivered to the tumour site where they accumulate. An external source is then used to specifically activate the nanoparticles and overheat the tumour region (the therapy is called hyperthermia). Thanks to the EPR effect, the nanoparticles accumulate *only* in the tumour region so the treatment is extremely localised and healthy tissues are not affected. Overheating the tumour site can be done for instance by activating magnetic nanoparticles with an alternating magnetic field, where they start vibrating and generate heat. This is the principle based on which a new anti-cancer therapy has been developed, called MagForce®, which has entered Phase II clinical trial in 2007 for the treatment of prostate cancer[6]. Another approach uses silica nanoparticles covered with a shell of gold (and for this called **nanoshells**) designed to absorb light in the near infrared (NIR) region. This is the region where light penetration through tissue is optimal. The nanoshells absorb NIR light, delivered with a laser, converting light into heat. In animal model studies, the nanoshell treatment induced complete resorption of a tumour in 10 days and all animals remained healthy and tumour-free for more then 3 months after treatment[7]. These examples show the innovative approach to tumour treatment enabled by nanoparticles.

THERANOSTIC

One of the most exciting opportunities that nanotechnology has brought to the therapeutic field is the possibility of integrating the diagnosis, therapy and follow-up of a disease. This is referred to as theranostic, and could be enabled by nanoparticles incorporated inside a drug that can change some property-such as colour- once the drug has reached the target (for instance, quantum dots). Drugs could therefore have a **feed-back action**. Together with a slow, targeted release system, the nanoparticles could gradually change colour during the drug action, therefore informing doctors of the progress of a therapy. This approach is called **´find, fight and follow`** and could become a reality thanks to the parallel progress of the field of molecular imaging. One vision is that, one day, the entire process of diagnosis, pre-imaging, therapy and post-imaging of a specific disease will be integrated. Moreover, being this process specific for a certain disease thanks to a targeted approach, more then one disease could be individually tracked, treated and monitored, in what is conceived as a **multifunctional therapy**.

REGENERATIVE MEDICINE

At times, the only way to treat a disease is the removal of the infected organ or tissue. The loss can also derive from an injury or a congenital condition (e.g., vision or hearing impairment). To compensate for the lost or impaired body function, an artificial construct is implanted in the body. Depending on the type, site and extent of the loss, this construct can be in the form of an engineered tissue or an implant.

Tissue engineering

This discipline deals with the fabrication of **artificial scaffolds** to support the growth of donor cells, which differentiate and grow into a tissue that mimics the lost, natural one. This tissue-engineered construct is then implanted in the patient and, in time, resorbed by the body and fully integrated by the host tissue. Current applications of tissue engineered constructs include engineering of the skin, cartilage and bone for autologous implantation (i.e., implantation of a tissue regenerated by seeding cells of the patient).

The 'scaffold' that supports cell growth is the core of this technique. In the body, cells are supported in their growth and function by a natural scaffold, called the extra cellular matrix (ECM). This is a very complex and intricate 'web' of nanofibres that provide the mechanical architecture for cellular growth. Moreover, the ECM is filled with small molecules (e.g., growth factors) and proteins that direct many cell processes, such as adhesion, migration, growth, differentiation, secretion and gene expression. The three-dimensional spatial organisation of these 'cues' is critical for controlling the entire cell life cycle. Ultimately, this complex nano, three-dimensional architecture guides cells to form tissues as complex as bone, liver, kidney and heart. The biggest challenge in regenerative medicine is the artificial replication of this 'perfect nano-scaffold'. The ability to engineer materials to have a similar level of complexity is now becoming a reality thanks to nanotechnology.

Microfabrication techniques derived from the semiconductor industry (such as photolithography or ion beam lithography) have long been used for the fabrication of microstructures to support and control cellular growth. For instance, one of the pioneering works in this field was published in the late 1970s[8]. In recent years new nanotechnology techniques have enabled studies at higher and higher resolution revealing the nanoscale detail of the ECM. Analytical tools like the AFM and nanofabrication tools have allowed scientists to fabricate scaffolds with nanoscale features. A great deal of research is now dedicated to engineering scaffolds with **tailored material composition** and properties, including topography and controlled alignment, to study how these can support and direct cellular growth. The aim is the fabrication of scaffolds that most closely resemble natural ECM. Researchers have now access to techniques to produce macro-scale structures with **nanometre details**. Conventional polymer chemistry combined

with new nanofabrication methods are now used to manufacture a wide range of structures, such as nanofibres of different and well defined diameters and surface properties; nanofibrous and porous scaffolds; nanowires, nanotubes, nanospheres and nano-composites. For instance, researchers from the Stupp Laboratory (Northwester University, USA)[9] have fabricated a nanogel of elongated micelles arranged in a nanofibre matrix and demonstrated that this can support the directional growth of neurons. The aim of this and other works is to engineer nano-scaffolds that can support the re-growth of neurons for healing patients affected of neurodegenerative disease or severe neuronal losses, like in the case of spinal cord injury.

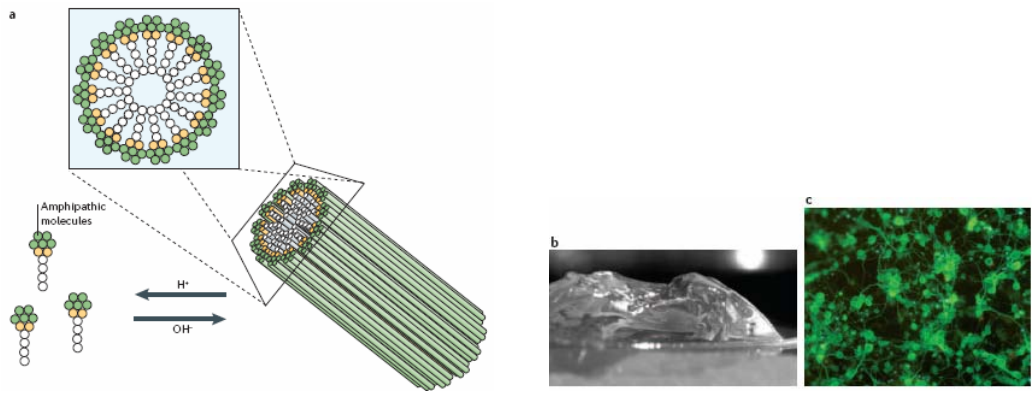

Figure 5. An engineered nanomaterial that supports specific cellular growth and can promote desired neurobiological effects. The material is formed of small bioactive molecules that resemble parts of natural proteins which spontaneously assemble in nanofibres (A) making a macro-gel (B). The gel supports and steers the growth of stem cells (C). Reprinted by permission from Macmillan Publishers Ltd: G.A. Silva, Nature Reviews 2006, 7, 65-74, Copyright 2006.

Biomaterials and Implants

Materials used in regenerative medicine are called **biomaterials** in the sense of being able to trigger and support a biological response[10-11]. One of the distinguishing features of nanotechnology is its ability to create new, functional materials. This can be exploited in the fabrication of new biomaterials that have better mechanical properties to increase the implant stability and reduce fatigue failure, for instance for orthopaedic **implants**, and materials that have enhanced electrical properties, needed for instance in neural prostheses. Nanotechnology could also be used for fabricating implants made of materials that are more resorbable, to increase functionality and durability. For instance, nano-coatings could be used to better integrate synthetic implants with the biological tissue, in order to prevent tissue inflammation and the onset of rejection.

Nanotechnology could also be employed for the fabrication of biomaterials that are **responsive** to the environment (for instance, responsive to the pH or to the

presence of specific biomolecules), for this reason called ´smart biomaterials`. Moreover, nanoscale patterns could be included in the biomaterial, to simulate the natural 'cues' and mimic molecular signalling mechanisms, in order to trigger desired biological events, like cell adhesion, differentiation and spreading. This could enable the fabrication of **dynamic implants** that are not limited to replacing a lost organ but truly restoring the loss.

Finally, **nano-sized sensors** could be inserted inside the biomaterial (for instance, nanowire biosensors) functionalised with receptors that can monitor the presence of small organic molecules, proteins, cells (e.g., cancer cells) and viruses. This could be used to collect information on the implant status and activity. This **feed-back** information could be used to maximize the implant efficacy and safety.

References

1. M. Staples, K. Daniel, M.J. Cima, R. Langer, Application of micro- and nano-electromechanical devices to drug delivery, *Pharmaceutical Research* 2006, 23(5), 847-863.

2. Nanomedicine, an European Science Foundation (ESF)-European Medical Research Councils (EMRC) Forward Look Report, 2005, available for download from the ESF website http://www.esf.org/research-areas/medical-sciences/publications.html

3. R. Duncan, The dawning era of polymer therapeutics, *Nature Reviews* 2003, 2, 347-360

4. R. Duncan, Polymer conjugates as anticancer nanomedicines, *Nature Reviews*, 2006, 6, 688-701

5. V. Wagner, A. Dullaart, A-K Bock, A. Zweck, The emerging nanomedicine landscape, *Nature Biotechnology* 2006, 24 (10), 1211-1217.

6. www.magforce.com

7. D. P. O'Neal, L.R. Hirsch, N.J. Halas, J.D. Payne, J.L. West, Photo-thermal tumor ablation in mice using near infrared-adsorbing nanoparticles, *Cancer Letters* 2004, 209, 171-176.

8. For a review see: L. Filipponi, D.V. Nicolau, "Cell Patterning" In: Wiley Encyclopedia of Biomedical Engineering, John Wiley & Sons, Inc., 2006.

9. G.A. Silva, Neuroscience nanotechnology: progress, opportunities and challenges, *Nature Reviews* 2006, 7, 65-74.

10. B. D. Ratner, S.J. Bryant, Biomaterials: where we have been and where we are going, Annual *Reviews in Biomedical Engineering* 2004, 6, 41-75.

11. S.E. Sakiyama-Elbert, J.A. Hubbell, Functional biomaterials: design of novel biomaterials, *Annual Reviews in Materials Research* 2001, 31, 183-201