NANOYOU Teachers Training Kit in Nanotechnologies

Chapter 1 – Medicine and Healthcare

MODULE 2- Applications of Nanotechnologies

Written by Luisa Filipponi and Duncan Sutherland
Interdisciplinary Nanoscience Centre (iNANO)
Aarhus University, Denmark
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Chapter 1: Applications of Nanotechnologies – Medicine & Healthcare

The application of nanotechnologies to the medical sector is referred to as Nanomedicine. Specifically, this area of application uses **nanometre scale materials and nano-enabled techniques to diagnose, monitor, treat and prevent diseases**. These include cardiovascular diseases, cancer, musculoskeletal and inflammatory conditions, neurodegenerative and psychiatric diseases, diabetes and infectious diseases (bacterial and viral infections, such as HIV), and more. The potential contribution of nanotechnologies in the medical sector is extremely broad and includes new diagnostic tools; imaging agents and methods; drug delivery systems and pharmaceuticals; therapies; implants and tissue engineered constructs.

Why nanotechnologies? Nanomaterials are defined as materials in the nanoscale regime, which in nanomedicine often goes beyond the 100nm line mark and up to about 500 nm. This is the size range of biomolecules (e.g. proteins, enzymes, DNA) and molecular complexes such as the ion pump. These natural nanomaterials are the constituents of larger hierarchical structures that regulate the function of the cell. Bacteria and viruses are larger (a few micrometers), but their functions (including toxicity to healthy cells) derive from the interactions between the biomolecules that compose them and the surrounding media (including surrounding cells). Basically nanotechnologies make it possible to create engineering materials (such as drug delivery systems, disease imaging probes, or even tissue engineered constructs) that have dimensions on the scale of biomolecules, which in turn is the scale that regulates the functions of cells. Nanotechnologies have the potential to improve the whole care process that starts for a patient once a disease is suspected, from diagnosis to therapy and follow-up monitoring. The aim is the development of new materials and methods to detect and treat diseases in a targeted, precise, effective and lasting way, with the ultimate goal of making medical practice safer, less intrusive and more personalised. The timescale from invention of a medical device or drug to release for clinical use is extremely long. In a few cases (such as drug delivery devices) nanotechnology is already in use for improving patient care, but in most of the areas discussed below the applications are still some years from being products.

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Diagnosis

Diagnosis of a suspected disease is one of the most critical steps in healthcare and medicine. We want the diagnosis to be fast, but also reliable, specific and accurate, and to minimise the risks of "false positives". Nanomedicine has the potential to greatly improve the entire diagnostic process. Instead of collecting a blood sample in a vial and sending this to a specialised laboratory for testing (which can take days), doctors will be able to use **miniaturised *in vitro* diagnostic devices** in their surgeries. These are small but highly integrated devices capable of carrying out many tests quickly at the same time using very small quantities of sample to perform the analysis. Some miniaturised *in vitro* diagnostic devices already exist such as the breathalysers that the police carry for alcohol screening or the portable glucose-test devices used by diabetics. These devices can measure ions, small molecules or proteins, or can test for specific DNA sequences that are diagnostic for a particular disease or medical condition. In the last years there has been a trend to make these devices even smaller, able to perform hundreds of tests at the same time and be easier to use. Nanotechnologies have an important role in this development: nanomaterials, such as nanoparticles or nanotubes, can be integrated into the device. Scientists can engineer nanomaterials to be very specific, so their use will make the device even more accurate and capable of carrying out even more tests simultaneously. Nanomaterials have the characteristic of exhibiting some peculiar quantum effects that can be used to **amplify the signal** arising from the detection. Thus the use of nanomaterials in miniaturised *in vitro* diagnostic devices will make it possible to improve the specificity of the analysis, its throughoutput (the number of tests that can be done simultaneously) and its readout. In the future, these types of devices will make it possible to perform "point-of-care diagnostics", which means making a diagnostic test anywhere and not only in the doctor's surgery or in a hospital. The nature of the sample to be tested will probably change, and become saliva rather than blood, which is much more convenient and safer to handle. This will allow for testing large numbers of patients, for instance in the event of an epidemic, or for testing large numbers of diseases or many parameters for one specific disease, which is needed for the diagnosis of complex medical conditions.

Miniaturised diagnostic devices include biosensors, microarrays and "lab-on-a-chip" (LOC) devices, also called miniaturised total analysis systems (µTAS). The first two are based on a parallel processing technique, whereas LOC devices are based on a serial processing technique.

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Biosensors

Generally speaking, a sensor is a device capable of recognising a specific chemical species and "signalling" the presence, activity or concentration of that species in solution through some chemical change. A "transducer" converts the chemical signal (such as a catalytic activity of a specific biomolecule) into a quantifiable signal (such as a change in colour or intensity) with a defined sensitivity. When the sensing is based on biomolecular recognition it is called a biosensor. There are various types of biosensors, such as antibody/antigen based, nucleic acid based and enzyme based. Also, depending on the technique used in signal transduction, biosensors are classified as optical-detection biosensors (as in the example above), electrochemical biosensors, mass-sensitive biosensors and thermal biosensors.

There are numerous **nanoparticles** that can be used as biosensor components. These work as probes recognising an analyte or differentiating between analytes of interest. In such applications some biological molecular species are attached to the surface of the nanoparticles to recognise the target of interest through a lock-and-key mechanism. The probes then signal the presence of the target by a change in colour, mass or other physical change. Nanoparticles used as elements for biosensors include quantum dots, metallic nanoparticles, silica nanoparticles, magnetic beads and fullerenes, which are hollow cages of carbon atoms, shaped like soccer balls.

Other biosensors use nanostructured particles as **nano-sieves** through which charged molecules are transported in an electric field. In this case particles with engineered nanopores are used.

Carbon nanotubes and **nanowires** are also employed for sensing. The latter can be fabricated out of a semiconductor material and their size tuned to have a specific conducting property. This, together with the ability to bind a specific analyte on their surface, yields a direct, label-free electrical readout. These nanowire biosensors allow the detection of a wide range of chemical and biological species, including low concentrations of protein and viruses, and their application ranges from the medical to the environmental sector. **Figures 1 & 2** illustrate a silicon nanowire biosensor based on biorecognition.

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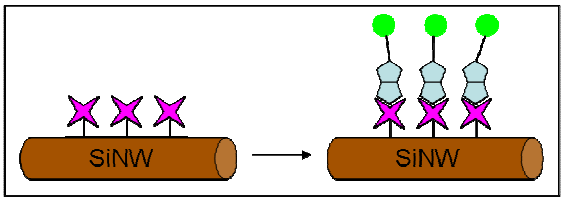


Figure 1. Biorecognition on a silicon nanowire biosensor. The surface of the nanowire is modified with avidin molecules (purple stars) which can selectively bind a streptavidin-functionalised molecule or nanoparticle. (Image credit: L. Filipponi, iNANO, Aarhus University. Creative Commons ShareAlike 3.0)

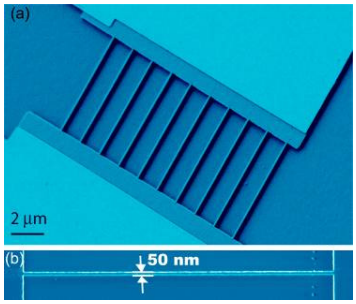
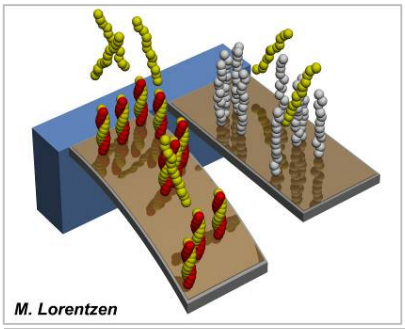


Figure 2. This scanning electron micrograph depicts the functional part of a nano-biosensor containing silicon nanowires. (Image credit: P. Mohanty, Boston University, NISE Network, www.nisenet.org, licensed under NISE network terms and conditions.)

Nanoscale biosensors have the potential to greatly aid in the **diagnosis** of diseases and **monitoring of therapies**. A large number of approaches have been developed in a recent years while relatively few have so far been converted into clinical diagnostic tools; therefore their wide application in patient care is foreseen in the next 5-10 years.

Cantilever biosensor



A cantilever biosensor is a biosensor made of numerous "arms" (called cantilevers) which are tens of micrometres long but very thin (a few micrometres). These devices are fabricated through lithography and etching. The surface of the cantilever is functionalised with a **nano-meter thick layer** of coating which ensures anchorage of the probe material (which can be a DNA strand or a protein, for example). Each cantilever is different and

Figure 3. Schematic diagram of a cantilever based biosensor. The yellow molecules bind specifically to the red molecules on the right hand cantilever and are detected by the bending of the cantilever. (Image credit: M. Lorenzen, iNANO, University of Aarhus. Copyright © M. Lorentzen.)

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can probe for a different target, as schematised in **Figure 3**. In this type of sensor, the adsorption of the analyte to the specific targets on a cantilever causes a surface stress and bends the cantilever. The most common read-out is optical, where the angular perturbation of a laser beam is measured following bending of the cantilever. Although common, this method suffers from the limitation that measurements are difficult in opaque liquids, such as blood, because of the absorption of the laser light. An alternative to this method is the piezoresistive read-out, where a piezoresistor is integrated into the cantilever. Upon detection of the analyte, the stress applied to the resister changes, which is reflected in a change of its resistance, which in turn is measured as an electrical signal. This approach offers the advantage of allowing the detection in opaque media, the possibility of miniaturising the sensor and incorporating it in portable devices for point-of-use sensing.

Plasmonic biosensors

The optical properties of **noble metal nanoparticles** have received significant research attention in recent years for their potential as components in many applications, including chemical/biochemical sensors. The optical properties of noble metal nanoparticles are dominated by an effect called Localised Surface Plasmon Resonance (LSPR), which was described in **Chapter 4 of Module 1 "Fundamental 'nano-effects'"**. One of the consequences of the LSPR effect in metal nanoparticles is that they have very **strong visible absorption** due to the resonant coherent oscillation of the plasmons. As a result, colloids of metal nanoparticles such as gold or silver can display colours that are not found in their bulk form, like red, purple or orange, depending on the nanoparticles' shape, size and surrounding media. The energy of LSPRs is sensitive to the **dielectric function** of the material and the surroundings and to the shape and size of the nanoparticle. This means that if a ligand such as a protein attaches to the surface of the metal nanoparticle, its LSPR energy changes. Similarly, the LSPR effect is sensitive to other variations such as the distance between the nanoparticles, which can be changed by the presence of surfactants or ions. The fact that the LSPR depends on the dielectric environment means that the refractive index can be used as the sensing parameter: changes in the local dielectric environment, induced by the sensing process, are used to detecting the binding of molecules in the particle nano-environment.

In a plasmonic biosensor the nanoparticles can be dispersed in a medium (in which case the biosensor is a **colloidal plasmonic biosensor**) or supported on a surface (**surface plasmonic biosensor**). Both types of

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sensors exploit the fact that the sensing event changes the LSPR of the metal nanoparticles, but use different read-out report strategies:

- In a **colloidal plasmonic biosensor** (for instance made of gold nanoparticles) the sensing event results in a change of aggregation among the nanoparticles that form the colloid (**Figure 4**), which can determine a colour change of the colloid. Absorption spectroscopy is used to quantify the biosensing event. In the case of gold colloid, which is normally red, the sensing event can result in the colloid becoming blue. Thus metal colloids can be used as **plasmonic colorimetric biosensors**. In nanomedicine this effect is used for instance for genetic screening, where scientist look for a specific gene sequence in a sample which can be indicative for a specific disease. How is this done? First, the sequence of bases in the target DNA is identified. Then two sets of gold particles are prepared – one has DNA attached that binds to one end of the target DNA, and the second set carries DNA that binds to the other end. The nanoparticles are dispersed in water. When the target DNA is added, it binds both types of nanoparticle together, linking them to form an aggregate. The formation of this aggregate causes a shift in the light-scattering spectrum from the solution, that is, a colour change in the solution which can easily be detected. An example is illustrated in **Figure 5**.

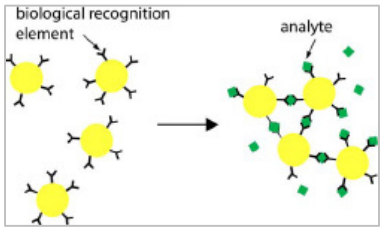

Figure 4. Schematic representation of a colloidal plasmonic biosensor.

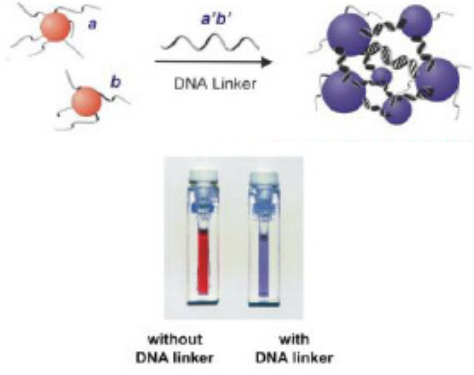

Figure 5. A plasmonic colloidal nanosensor. (Image credit: reprinted with permission from Jin et al., Journal of American Chemical Society (2003), 125 (6), 1643- . Copyright 2003 American Chemical Society.)

EXPERIMENT C in the Experiment Module deals with synthesising and testing a **gold colloidal plasmonic nano-sensor**. In the experiment, students use electrolytes to see the colour change due to the change in aggregation of the nanoparticles as the salt is added to the colloid.

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- In a **surface plasmonic sensor**, metal nanoparticles are immobilised on a surface as illustrated in **Figure 6**(a). The metal nanoparticles are attached to the surface by means of chemical linkers or prepared by nanolithography (b), and are then modified with the sensor moiety (c). The analyte (the target) attaches from solution specifically onto the recognition function adsorbed onto the particles (d), causing a change in the refractive index around the particle, resulting in an LSPR shift. The LSPR shift is measured through a technique called extinction spectroscopy (e).

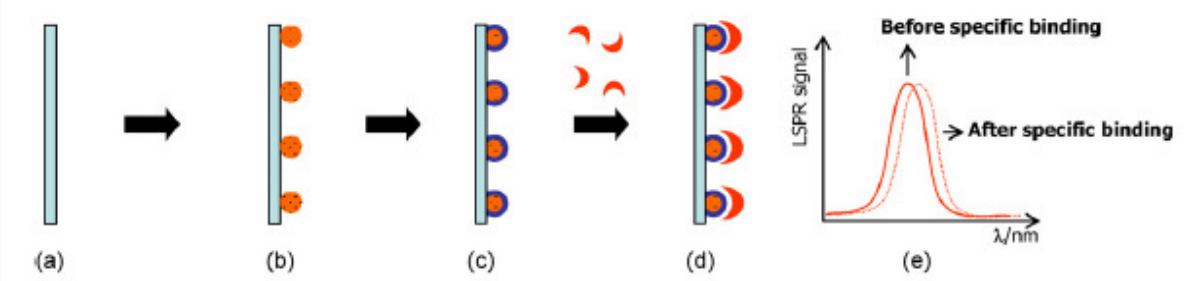

Figure 6. Schematic representation of the preparation and response of LSPR biosensors based on refractive index changes. (Image credit: Reprinted from: Borja Sepúlveda et al., "LSPR-based Nanobiosensors", Nano Today (2009), 4 (3), 244-251, with permission from Elsevier.)

Artificial nose biosensor

An artificial nose biosensor is a device that mimics the ability of some mammals, like dogs, to detect explosives and drugs through their olfactory system. Recent research has shown that this fine capacity in dogs may also be used to detect molecules which, if present, are early indicators of various diseases such as cancer. Numerous research programmes are under way around the world to create an artificial nose; one is the European project called BOND. The application of such types of biosensor ranges from medicine (early disease detection) and security (explosives detection) to the food industry (to measure whether food has gone off). This type of biosensor is an electrochemical sensor that mimics the natural mammal olfactory system. The nose-biosensor, like our own noses, is composed of three main parts, a biomolecule receptor, an electrode and a transducer. When the detector finds the target, a chemical reaction occurs between the detector and the receptor biomolecules for odour. The chemical reaction between the receptor and the substance we smell emits a chemical signal. The electrode translates this

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chemical signal into electrical signals and transports them to the brain, or, in the case of the nose biosensor, they are transported to a transducer. In mammals the equivalent of the electrodes would be the neurons. The transducer (the brain in mammals) receives the electrical signal and translates it into analytical information. In the nose biosensor the transducer would give information on a screen.

***NANOYOU VIRTUAL GAME**: Students can become "nano-scientists" and fabricate and test a nose biosensor through the NANOYOU Virtual Game. Details are found at www.nanoyou.eu/virtual-lab.html*

***NANOYOU DILEMMA**: Diagnostic nanosensors (cantilever-based, plasmonic, nanowires-based, etc.) will allow for the early detection of various diseases, like cancer, at the very onset of the symptoms, before the disease is perceived by the patient. Early detection means a higher chance of successfully treating and overcoming the disease. On the other hand, some worry that this will give doctors access to a large amount of personal information. The question is: where is this information going to be stored, and who will have access to it? Also, what if those devices are used not as a diagnostic tool but as a mean to assess a person´s medical condition by other entities, such as insurance companies or job agencies? The dilemma is: "Should nanosensors be used to diagnose medical conditions in the early stages when there are still no definite restrictions in place to protect patients´ privacy?" This dilemma is part of the **NANOYOU Role Play Card Game** (see www.nanoyou.eu/en/decide)*

Microarrays

These devices are used for diagnostic purposes such as DNA analysis (DNA microarray), protein detection (protein microarrays) as well as whole cell analysis. Microarrays are platforms made of hundreds of detection sites that have micron-size dimension and allow the specific detection of a (bio)chemical within a mixture or the simultaneous detection of many (bio)chemicals. The detection is related to the chemical functionality on the micron-sized spots in the array and it leads to a single chemical "yes/no" reaction per spot. Microarrays are used as screening tools, not only for diagnostic purposes but also for **screening new drugs**. Nanotechnology can impact microarray technology by creating densely packed, smaller, nano-sized arrays (nanoarrays) that could allow faster screening of a larger number of (bio)chemicals. There are however some problems associated with the handling of ultra-small quantities of liquid. So nanotechnologies offer the most promising advantages in sample detection on arrays. The conventional method used for detecting the "yes/no" reaction at each spot is

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fluorescence. This technique uses fluorescent probes made of organic molecules attached to the species to be detected (e.g. a protein or a fragment of DNA): when reaction occurs, this is attached to the detection spot, which becomes fluorescent in a "colour" corresponding to the emission of the fluorescent probe. Fluorescent staining suffers from some disadvantages, mainly fast bleaching of the fluorescent molecules (that is, loss of "brightness" of the colour in time during imaging); limited number of dye molecules that have distinct "colours" and that can be can be simultaneously imaged; and limited sensitivity. Nanoparticles in the form of **quantum dots** (QD) can be used as an alternative to conventional organic dyes, being more stable, sensitive and monochromatic. A substantial (ten-fold) enhancement in sensitivity compared to common fluorescent markers has been accomplished through the use of gold and silver particles of uniform dimensions in the range 40 nm to 120 nm. Signal amplification is also obtained using metal nanoparticle labels, such as DNA-modified gold nanoparticles. These nano-sized probes have molecules attached to their surface that ensure the selectivity of the detection, while the nano-properties of the probe are responsible for enhancing the signal. The overall effect is an improvement in the sensitivity and selectivity of microarray technology.

Nanobarcodes

The unique properties of nanoparticles, such as the relationship between particle size and colour, can also be used to create multiplexed detection systems in the form of nanobarcodes, for instance using QD to create different colour-based codes. Alternatively, fragments of DNA on nanospheres can be used to create a "bio-barcode", for instance for protein detection. A bio-barcode has been used to detect small levels of the cancer marker prostate-specific antigen (PSA) in serum. The results showed an increased sensitivity to the PSA protein compared to conventional protein assays, demonstrating the potential of such approaches for detecting cancers at an earlier stage.

Lab-On-A-Chip

These devices are "miniaturised integrated laboratories" that allow the separation and analysis of biological samples (e.g. blood) in a single device. They are made of microfluidic systems, including micro-pumps and micro-valves, integrated with microelectronic components. The device can also integrate one or more sensors. As with microarray technology, the impact of nanotechnologies in this area is in further miniaturisation of these devices, although the handing of ultra-small volumes of samples would pose a problem. Presently, nanotechnologies are making an impact in improving specific components and functions of lab-on-a-chip devices. For instance, analysis is commonly done by dielectrophoresis,

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where non-uniform alternating electrical fields are used to separate and guide small objects through field gradients. This manipulation requires high electrical field strengths that can be obtained using nanosized electrodes. Another example is nanopore-based separation systems that can be integrated in the membranes used in lab-on-a-chip devices. For instance, nanopore-membranes are proposed for DNA sequencing.

Imaging

The second step in the diagnosis of a disease involves *in vivo* imaging, which searches for the symptoms of the disease within the live tissue suspected of being infected without the need to perform surgery. Nanotechnologies are having a very important impact in this area, particularly by developing molecular imaging agents. The latest improvements in the area of imaging deal with the capability of tracking changes at the cellular and molecular level through the analysis of some specific biological markers (a technique called “targeted molecular imaging” or “nano-imaging”). A biomarker is an indicator of a biological process or state, such as a disease, or the response to a therapeutic intervention. This can be an altered gene, or a change in protein production, or even a physical feature of a cell. The aim is to detect biomarkers of disease and diagnose illnesses before or at the onset of the first symptoms, in this way making *in vivo* imaging a tool for the early detection of a disease. Effective early detection is crucial for planning a therapy with less severe and costly therapeutic demands, especially in diseases such as cancers, where timing is vital for the success of the treatment. Biomarkers could also be used as early indicators of the success of a treatment, thus reducing treatment time and cost. Targeted molecular imaging is important not only for diagnostic purposes, and for monitoring the progress of a therapy, but also for research in controlled drug release, in assessing the distribution of a drug within the patient’s body, and for the early detection of unexpected and potentially toxic drug accumulations. The ability to trace the distribution of a drug leads to the possibility of activating it only when and where needed, thus reducing potential drug toxicity.

Diagnostic imaging

Techniques such as X-ray, computer tomography (CT), ultrasound (US), magnetic resonance imaging (MRI) and nuclear medicine (NM) are well established imaging techniques, widely used in both medicine and biochemical research. Originally, imaging techniques could only detect changes in the appearance of a tissue when the symptoms of the disease were relatively advanced. Later, targeting and contrast

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agents were introduced to mark the disease site at the tissue level, increasing imaging specificity and resolution. It is in this specific area that nanotechnologies are making their greatest contribution by developing better contrast agents for nearly all imaging techniques. The physiochemical characteristics of the nanoparticles (particle size, surface charge, surface coating and stability) allow the redirection and concentration of the marker at the site of interest. An example of nanoparticles used in research for imaging is perfluorocarbon nanoparticles employed as contrast agents for **nuclear imaging**, **magnetic resonance imaging and ultrasound**, with applications in the imaging of blood clots, angiogenesis, cancer metastases and other pathogenic changes in blood vessels. Gadolium complexes have been incorporated into emulsion nanoparticles for the molecular imaging of thrombi, resulting in a dramatic enhancement of the signal compared to conventional MRI contrast agents. **Fullerenes** are also used in magnetic resonance imaging research, filled with smaller molecules that act as contrast-enhancement agents. Metals and silicon nanoparticles are also used to enhance MRI. Silicon particles fabricated into different shapes and coated with conductive layers can have enhanced magnetic resonance interactions with an imaging field.

In **X-ray imaging,** to enhance the signal an agent must deliver a detectable number of heavy atoms into targeted tissue without toxic effects. Nanoparticles of heavy metals have the highest density of surface atoms but they must be inert and stable. Nanoparticles of inert metals like silver and gold are too expensive and would make the technique not cost-effective. A solution has been proposed by General Electric in the form of **nanoparticles made of heavy metal compounds encapsulated in gold shells**. The added advantage is that organic compounds with sulphide (-S-H) groups (thiols) can easily be attached to the gold surface through the thiol end (forming an S-Au bond). The thiol molecule can be functionalised at the other end with groups that act as receptors for specific binding of antigens, antibodies or even target compounds on the surface of the cell. By targeting receptors unique to a certain type of cancer cell, gold nanoparticles can enhance an X-ray image of a suspected cancer tissue by many orders of magnitude.

Gold nanoshells are a promising material for the optical imaging of cancer. Optical technologies could provide high resolution, non-invasive functional imaging of tissue at competitive costs. However, presently these technologies are limited by the inherently weak optical signals which come from the endogenous chromophores and the small spectral differences between normal and diseased tissue. Gold nanoshells are made of a dielectric core (silica) covered by a thin metallic (gold) shield. Gold

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nanoshells possess physical properties similar to gold colloid (described before), in particular, a strong optical absorption due to the collective electronic response of the metal to light (the LSPR effect). By changing the relative dimensions of the core and shell, the optical resonance of the nanoparticles can be precisely and systematically varied over a broad region ranging from the near-UV to the mid-infrared (**Figure 7**).This range includes the near-infrared (NIR) wavelength region where tissue transmissivity is higher. Researchers are using these gold nanoshells cells as contrast agents for optical coherence tomography (OCT)[1] of cancer cells. As we will discuss later, gold nanoshells are also capable of treating cancel cells through overheating of the cells. This will be discussed in the next section.

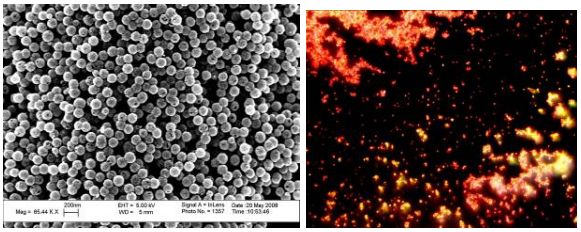

Figure 7. (left) SEM image of gold nanoshells each being about 120 nm; (right) Optical image of the same nanoshells after being dispersed in water and dried on a microscope slide. The colours are due to selective scattering of light by the nanoparticles. (Images credit: G. Koeing, University of Wisconsin-Madison, NISE Network, www.nisenet.org, licensed under NISE network terms and conditions.)

In situ diagnostic devices

In recent years *in-situ* diagnostic devices have been developed, such as wireless capsule endoscopy cameras. These devices can be swallowed by the patient and make it possible to closely monitor and locate the site of bleeding and other intestinal problems. Currently many of these devices, such as the CamPill® produced and sold by Given Imaging. Ltd, can only image the problem. In the future these devices could also incorporate sensors for the detection of specific chemicals, pH, bacteria, viruses, etc. Micro- and nanotechnologies now allow the creation of extremely small sensors; therefore research is being conducted on integrating them into swallowable capsules. This will widen their applicability and utility. In the future, they could also be drug-loaded for targeted drug delivery.

[1] Optical coherent tomography (OCT) is a state-of-the-art imaging technique which produces high resolution (typically 10-15 µm) real-time cross-sectional images through biological tissues. The method is often described as an optical analogue to ultrasound.

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Therapy

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 remove only the "bad" cells, and effective, both in terms of action and time.

A therapy normally involves a pharmaceutical route (drugs) to treat 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 radiative therapies. In some circumstances, surgery is required, and an organ-substitute is inserted into the body in the form of an implant or a donated organ. In all these 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. Nanotechnologies are making a tremendous impact in this field, with new drugs and new types of treatments under development, some of which have already proven clinically effective and have entered the market.

Drug development and targeted drug delivery

Advances in the field of pharmacology stem from 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 same form as the "pills" that we frequently take and that can release their active component gradually in time (slow release drugs) or that 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.

Drug design and screening

The structure of biological macromolecules defines a three-dimensional nano-environment that mediates specific functions in the cell. The design of new drugs requires a very detailed understanding of this nano-environment. Therefore gaining insight into the structure of macromolecules on the nanoscale through electron microscopy, nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallography is of fundamental importance for understanding biological processes and for the development of new medicines.

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

Targeted-drug delivery

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, maximising the drug's therapeutic action while minimising side effects. For drugs to be able to do so, the delivery systems need to be miniaturised to become much smaller than the target, and specific in composition to elicit a certain response. With the use of nanotechnologies, targeted drugs (in terms of composition and delivery system) are becoming a reality. In the future this could lead to targeted therapies and personalised medicine. The aim is to design and deliver drugs in such a way that they can recognise the "bad" cells at a molecular level, penetrate the cell membrane and act inside the infected cell. This is often crucial for the efficacy of a drug, since most virus replication and other disease conditions take place across the cell membrane and inside the cell. This way, the treatment will be delivered where is needed and will be specific, eliminating the problem of the drug killing healthy cells. An example of this approach is siRNA drug delivery.

Targeted drugs and targeted DDS could allow the creation of drug formulations with optimal loading, which deliver to the body only the necessary amount of the drugs and reduce side effects for the patients. Together with the possibility of having nano-DDS that are biodegradable inside the body, this

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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 expiry date or is no longer functioning. This will allow the improvement of drug shelf-life and better monitoring of drug safety.

siRNA drug delivery

RNA interference is a natural, fundamental mechanism in gene regulation that occurs in both plants and animals, humans included. Genes carry the genetic material of an individual, the DNA, and are contained in the nucleus of a cell. When genes are express (that is, activated), the genetic information is copied from DNA to messenger molecules, called messenger RNA (mRNA), which then orchestrate the formation of proteins outside the nucleus of the cell. In 1998 Andrew Fire and Craig Mello discovered that double stranded RNA (dsRNA) can interfere with and break down the mRNA for a specific gene, thus stopping the production of a specific protein. The gene in therefore "silenced" and the production of the protein is turned off. Fire and Mello found that this RNA interference mechanism is specific and can be obtained with a few molecules of dsRNA, and that the effect of dsRNA could spread from cell to cell and from tissue to tissue, and even passed on to offspring. The discovery won the scientists the Nobel Prize in medicine in 2006. Now researchers know that RNA interference plays an important role in switching off genes during an organism's development and controlling cellular functions. But the discovery of RNA interference not only enables scientists to better understand the fundamentals of gene regulation; it also opens new possibilities for genetic engineering in biological and medical research. In the laboratory scientists can now tailor RNA molecules – silencing RNAs – that activate the breakdown of endogenous mRNAs (that is, RNA that belongs to that specific cell). When silencing RNA (siRNA) molecules enter the cell they activate RNA interference, and endogenous mRNA molecules that bind to the added siRNA are destroyed. Researchers are now hoping to use RNA interference to treat diseases like viral infections, cardiovascular diseases, cancer and metabolic disorders. So far, many experiments with RNA interference have yielded promising results, but in order to maximise the therapeutic efficacy of the technique some fundamental difficulties have first to be overcome. These include low stability of siRNA in biological fluids and low specificity of action due to gene off-target effects caused by the similarity in behaviour of synthetic siRNA with natural microRNA produced by the cell. Therefore, there is the need to develop delivery methods capable of overcoming the extracellular and intracellular

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barriers and getting the siRNA molecules into the right type of cell (targeted delivery) whilst maintaining the stability of siRNA.

Researchers at iNANO (Aarhus University) and other institutions worldwide are developing **nanocarriers for the targeted delivery of siRNA**. For example, they are studying a novel chitosan-based siRNA nanoparticle delivery system for RNA interference *in vitro* and *in vivo*. Chitosan is a naturally occurring cationic polysaccharide that has been widely used in drug delivery systems. It contains positively charged amine groups that can interact with the negatively charged backbone of siRNA and form polyplexes in the form of nanoparticles about 200 nm in size. The protonated amine groups allow transport across cellular membranes and subsequent endocytosis into cells. It has been shown that a chitosan/siRNA nanoparticle delivery system silences genes both *in vivo* and *in vitro*. Moreover, this delivery system has been shown to be biocompatible, non-toxic and biodegradable. Another requirement in targeted siRNA delivery is the capability of the carrier to reach a specific cellular compartment and release the cargo (the siRNA) inside that cell. Synthetic vectors based on polycations such as poly-L-lysine have been widely used but have several drawbacks such as high cellular toxicity, sequestration in subcellular compartments and lack of intracellular targeting. In contrast, bioresponsive copolypeptides containing reducible disulfide bridges that respond to intracellular acidity conditions have proven advantageous in delivering nucleic acids into cells. These systems exploit the redox potential gradient existing between the extracellular and the intracellular environment so that the disulfide bridges are broken (and the cargo released) only in one compartment of the cell (in this case, the nucleus). For these reasons research is being conducted in developing nano-carriers rich in histidine groups.

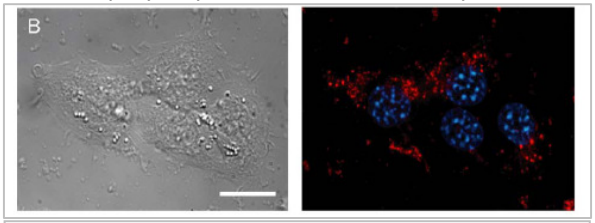

Figure 8. Live cellular uptake of chitosan/siRNA nanoparticles into NIH 3T3 cells. Fluorescence microscopy was used to visualise cellular uptake and translocation of Cy5-labeled siRNA within Chitosan nanoparticles after 4 hours of reaction. Images show fluorescent overlay of siRNA (red Cy5-labeled) and nuclei (blue Hoechst-labelled) adjacent to phase-contrast image (scale bar, 10 µm). (Image credit: reprinted by permission from Macmillan Publishers: K.A. Howard et al., Molecular Therapy (2006), 14(4), 476-484. Copyright © 2006.)

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Stimuli-activated drug delivery

In this area of research the idea is to incorporate some specific properties into the delivery system so that the drug can be **activated only upon reaching the target**, and the active component released at a controlled rate. This is called stimuli-activated drug delivery. Controlled activation could be linked to some environmental properties, such as pH, or "lock-and-key" molecular recognition mechanisms. One example is stimuli-activated gene delivery.

In **gene therapy** one of the biggest challenges is the targeted delivery of the nuclei acid load to the target (e.g. plasmid-DNA or siRNA) either to silence (RNA silencing) or to activate the expression of a protein as a way to treat a number of diseases. In the previous section we discussed how nanocarrier delivery systems formed by electrostatic interactions between cation polymers and DNA or RNA have been developed to overcome extracellular and intracellular barriers to maximise the delivery of the nucleic acids in the cell. One way to control the spatial and temporal activity of nucleic acids is to use polymers that change properties in response to stimuli such as temperature and redox potential gradients. This approach to targeted delivery is being investigated at iNANO and is schematically illustrated in **Figure 9**. The idea is to utilise a nanocarrier that passively accumulates in the diseased tissues (e.g. tumours), followed by stimuli-induced activation at the required site (inside or outside the cell). In the case of **thermoresponsive systems**, the application of heat in precise locations of the tissue can induce the deposition of the nanocarrier in the extracellular target region.

Researchers are studying for instance the use of a thermoresponsive polymer to form a polyplex with plasmid DNA and to use AFM to visualise the resulting nanoparticles. They found that they could change the size of the polyplex nanoparticles from around 50nm to

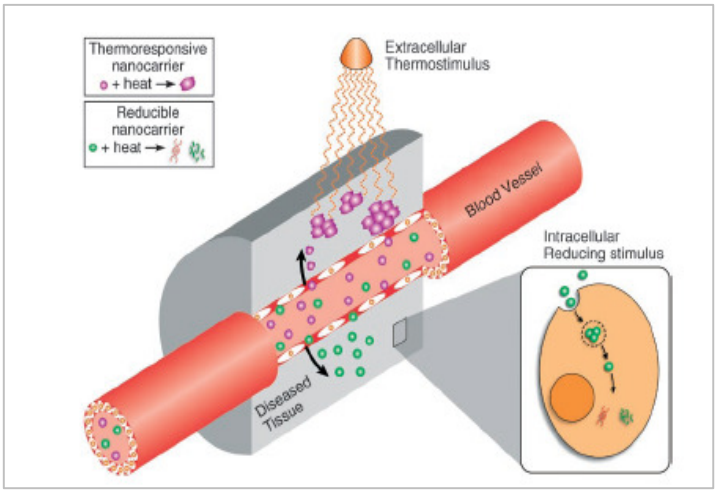

Figure 9. A schematic representation of a nanocarrier thermo-activated for gene therapy. (Image credit: Howard et al., Small (2007), Volume 3, Issue 1, pp. 54-57. Copyright © Wiley-VCH GmbH & Co. KGaA. Reproduced with permission.

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more than 200nm by heating the particles. The AFM images revealed that smaller particles merged into larger ones during the temperature treatment. Since the ability of polyplexes to cross vasculature endothelial barriers and enter/exit tissues depends on particle size, a thermostimulus could be used to control migration inside tissues. The idea is to apply a thermostimulus in the diseased tissue so as to induce a size increase of the nanoparticles and prevent these from re-entry into the bloodstream (see Figure 9). This general approach could be used for nanocarriers containing drugs or imaging agents for therapeutic and diagnostic applications.

Current and future nano-drug carriers

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 being investigated, and include micelles, nanoemulsions, nanotubes, nanofibres, liposomes, dendrimers, polymer therapeutics, nanoparticles, nanocapsules, nanospheres and hydrogels. 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. An example is an anti-cancer drug called DOXIL (from Sequus Pharmaceuticals).

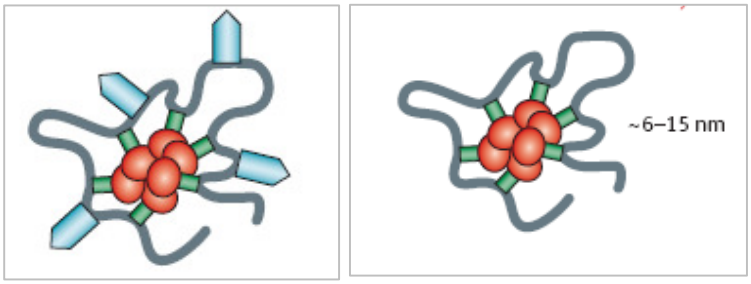

Figure 10. (left) Schematic representation of a polymer-drug conjugate, and of a targeted polymer-drug conjugate (right). (Image credit: Reprinted by permission from Macmillan Publishers: Duncan R., Nature Reviews Cancer (2006), 6(9), pp. 688-701. Copyright © 2006.)

The future of nano-DDS enabled by nanotechnologies could be miniaturised implantable chips loaded with different drugs that can be released upon external stimuli. This could free patients such as diabetics from having to administer drugs repeatedly during the day. Research in this area is very active but still requires years for its commercial realisation.

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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 enter the tumour region and accumulate there. 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. Another approach uses gold nanoshells (described before) designed to absorb light in the near infrared (NIR) region. This is the region where light penetration through tissue is optimal (800-1300 nm). The **nanoshells absorb NIR light**, delivered with a laser, converting light into heat. In animal model studies, the nanoshell treatment has shown to induce complete resorption of a tumour in 10 days and all animals remained healthy and tumour-free for more than 3 months after treatment. These examples show the innovative approach to tumour treatment enabled by nanoparticles.

Theranostics

One of the most exciting opportunities that nanotechnologies have brought to the therapeutic field is the possibility of integrating the diagnosis, therapy and follow-up of a disease. This is referred to as theranostics, 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 feedback action. Together with a slow, targeted release system, the nanoparticles could gradually change colour during the drug action, thereby informing doctors of the progress of a therapy. This approach is called "find, fight and follow" and could become a reality thanks

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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. An example of theranostics is the use of gold nanoshells for imaging and treating cancer cells at the same time.

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 and biomaterial engineering

Tissue engineering 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 extracellular 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 three-dimensional nano 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

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cellular growth. For instance, one of the pioneering works in this field was published in the late 1970s. 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 nanotopography 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 now have access to techniques to produce macro-scale structures with nanometre details. Conventional polymer chemistry combined with new nanofabrication methods is 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 nanocomposites.

Close to the field of tissue engineering, and in many cases an integral part of it, is **biomaterial engineering**. Materials used in regenerative medicine are called biomaterials in the sense of being able to trigger and support a biological response. One of the distinguishing features of nanotechnologies is their 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. Nanotechnologies can also be used to fabricate implants made of materials that are more resorbable, to increase functionality and durability. For instance, nano-coatings are being studied to better integrate synthetic implants with the biological tissue, in order to prevent tissue inflammation and the onset of rejection.

Nanotechnologies are also 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) and for this reason are called “smart biomaterials”. Moreover, research is being conducted to include nanoscale patterns 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 restore 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

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(e.g. cancer cells) and viruses. This could be used to collect information on the implant status and activity. This feedback information could be used to maximise the implant efficacy and safety.

Tissue and biomaterial engineering have application in basically all aspects of regenerative medicine, i.e. neuroprosthetics and neuron regeneration (e.g. spinal cord repair), bone restoration, hearing and vision restoration, motor restoration, etc.

Nanoengineering bone regeneration

Bones and teeth are material that have to bear complex loads of moving bodies, provide a protective cage for vital organs, anchor tendons and muscles, and act as joints and levers. This functional complexity is reflected in a structural complexity. Bones have a complex hierarchical structure at the nano-, micro- and macro-levels, which determines their amazing properties.

The "classic" way to promote the re-growth of bone after injury is to provide a **scaffold** into which bone-forming cells can migrate and grow attached (fused) to the scaffold. In the past numerous scaffolds have been used, mainly coated with hydroxylapatite which is a natural bone component. More recently, research has focused on trying to mimic the nanostructure of the scaffold. This involves working on the topography of the scaffold (surface nano-roughness) but also at anchoring specific biomolecules to the nano-surface of the scaffold. The idea is to mimic the natural fine organisation of natural bone, which is a combination of nano-topography and (bio)chemistry.

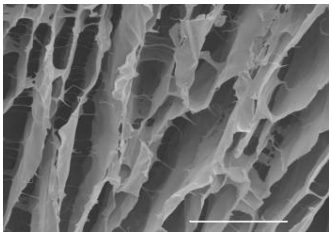

Figure 11. This scanning electron microscope image shows a hydrogel scaffold grown for studying brain tissue engineering and nerve regeneration. The image is 100 µm wide (Image credit: D Nisbet, Monash University, NISE network, www.nisenet.org, licensed under NISE network terms and conditions.)

A novel approach is the development of **"artificial bone"**, which means using macromolecules that self-assemble into large structures that mimic the natural structure of bone. This is a bottom-up approach to bone engineering which leads to materials with nano-scale level control. For instance some researchers have developed a bone scaffold by biomimetic synthesis of nano-hydrohylapatite and collagen. Collagen is the most plentiful protein in the human body. It is found in most human tissues including bone, cartilage, the heart, eye and skin and gives these tissues their structural strength. These biomaterials

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assemble into 3D mineralised fibrils that mimic key features of human bone. This material shows some similarities with natural bone in terms of hierarchical micro- and nanostructure, and three-dimensional porosity. Cells seeded *in vitro* over this scaffold grew and proliferated well. The advantage of this approach is that the "building blocks" are biomimetic macromolecules: once assembled, the final "macro" material can integrate with natural tissues, opening the way to new clinical approaches to bone regeneration.

When these synthetic nanofibres form, they make a gel that could be used as a sort of glue in bone fractures or to create a scaffold for other tissues to regenerate onto. As a result of its chemical structure the nanofibre gel would encourage attachment of natural bone cells which would help to patch up the fracture. The gel could also be used to improve implants or hip and other joint replacements.

NANOYOU DILEMMA: The example of artificial bone is one of the NANOYOU dilemmas, part of the **NANOYOU Role Play Card Game** (see www.nanoyou.eu/en/decide).The dilemma arises from the fact that if such technology is developed to heal bones, the natural progression might be that it is then used to strengthen otherwise healthy bones to make them almost unbreakable. This solution might not be available for some people (due to cost or accessibility restriction), so it could become a human-enhancement tool only for a few. The dilemma is: "Is it acceptable to use processes developed for medical treatment to enhance the human body?"

Nanoengineering neuron regeneration

The loss of neuron functions is one of the most dramatic medical conditions in terms of consequences for the patient: it can interfere with basic functions (like movement) and cognitive capabilities. There are numerous neurodegenerative diseases (Parkinson´s, Alzheimer's, etc.) that are connected with neurological damage (**neurodegenerative diseases**). Neuron function loss can also derive from a severe accident (**spinal cord injury**) or a minor one (**peripheral neuron damage**). The loss or impairment of a person's neurological function has detrimental effects on his/her life. The research in this field is therefore massive, and covers a very wide range of disciplines and sub-disciplines. There are basically

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two main approaches to neuron regeneration: tissue engineering and neuron prosthetics. Until recently these two approaches were fairly separate, mainly due to the type of materials employed in the two approaches: "soft" biomaterials in the first case (biopolymers, proteins, peptides, etc.), and microchip-type materials in the second case (semiconductors, metals, etc). With the advent of nanotechnologies these two types of materials are starting to be integrated not just in terms of physical attachment (e.g. protein coatings) but in terms of *function*: for instance, in nanotechnologies biomolecules are used as nano-scale motors, or as energy-harvesting materials. Therefore in the future the approach to neuron regeneration will be in the form of hybrid nano-scale devices. Below is a short review of neuron tissue engineering; neuroprosthetics is discussed in the next section.

Neuron tissue engineering

The use of scaffolds to encourage neuron re-growth after injury is an established method. At first simple bio-compatible polymers were used; nowadays the need is recognised to engineer the scaffold at the nano-scale level in two ways: physical, by inserting nano-scale "paths" to encourage directional growth, and biochemical, by adding "cues" in the form of neuron growth factors and other essential biomolecules to encourage re-growth. These two elements must be engineered so that their coordinated action results in neuron re-growth. In the last few years the research in this area has been impressive and nanotechnologies have been the enabling tool. For instance researchers at the Stupp Laboratory (Northwestern University, USA) 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 work is to engineer nano-scaffolds that can support the re-growth of neurons to heal patients affected by neurodegenerative disease or severe neuronal losses, as in the case of spinal cord injury.

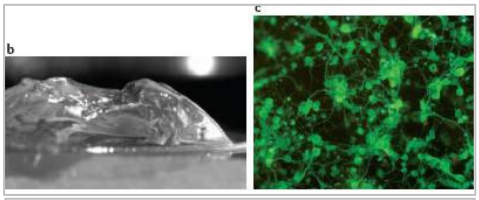

Figure 12. An engineered nanomaterial that supports specific cellular growth and can promote desired neurobiological effects. The material is formed of small bioactive molecules that resemble parts of natural proteins which spontaneously assemble in nanofibres (A) making a macro-gel (B). The gel supports and steers the growth of stem cells (C). (Image credit: Reprinted by permission from Macmillan Publishers Ltd: G.A. Silva, Nature Reviews (2006), 7 (1), 65-74. Copyright © 2006).

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Neuroprosthetics

A “neuron prosthesis” is a device implanted to restore a lost or damaged neuron function. There are two main kinds of neuron prosthesis: motor and sensory. Much progress in the last decade has been enabled by miniaturisation. Nanotechnologies offer opportunities to continue this miniaturisation trend but also to introduce new features, like electrodes that actively interface with the nerves, smaller and more powerful sensors, actuators, and control systems throughout the prosthesis to render it more natural and effective.

A **motor neuroprosthetic device** takes the signals from the brain or motor pathway and converts that information into control of an actuator device to execute the patient’s intentions. Examples are artificial limbs or hands. The task is extremely complicated, since the motor neuroprosthesis must also be integrated with the owner’s nervous system. Therefore prostheses have a sophisticated distributed network of control, actuation and feedback. Limb and hand prosthesis also need to be mechanically similar to natural limbs, otherwise the task of learning to use them will be difficult. Therefore to be effective the device must be designed with nanoactuators and nanosensors fully integrated with the control system from design to implementation, including the very special ergonomic interface between patient and device.

What can nanotechnologies do? Progress in current motor neuroprosthetic devices has already been made possible by nanotechnologies, which are enabling more natural prosthesis by providing:

- Smaller and more affordable **sensors**, processor elements, and the wiring and interconnectors needed to network them in a distributed control system;
- Smaller, more powerful, efficient and responsive **actuators** that move smoothly, resembling natural movement. This is possible because these actuators are based on molecular forces.
- Engineering **materials** that match the strength/weight ratios, elasticity/rigidity, and mechanical energy storage characteristics of key components of natural limbs.

A key contribution of nanoscience is in the form of novel materials (e.g. carbon nanotubes) that can become elements of new sensors, computing elements and even artificial muscles. Nanoscale magnetometers, accelerometers, pressure sensors and gyroscopic devices will be able to more precisely

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detect even minute movements and angle changes. These nanomaterials will support the design of internal movement devices to render the prosthetic movement more natural, and to ensure the accurate transmission of control and feedback information between device and patient. The impact of nanotechnologies will not be in the form of miniaturised robots but rather assemblies of cooperating interconnected networks that compute, communicate, sensing, actuate, and so on, to make up a prosthesis that resembles as much as possible the natural lost limb.

Motor neuroprosthetics commonly integrate **sensory neuroprosthetics** as well, since the reconstruction of motor functions needs to be associated with the reconstruction of haptics – the sense of touch (or more specifically the sense of pressure and force feedback from the body to the brain). Haptics, along with vision, hearing, and balance, is an essential component of the stream of feedback information that the nervous system sends to the brain. So-called "**smart materials"** (or dynamic materials, meaning materials that change their shape or function in response to an external stimulation) and nanosensors are expected to provide many options to implement sensory neuroprosthetics.

Research in the area of motor and sensory prosthetics is very active and is not only directed to the fabrication of human motor prosthesis but also **robotics** for computer assisted-surgery, deep-marine investigation, astronomy, etc.

In addition to neuroprosthetics that restore motion functions, **vision and auditory neuroprosthetics** are also extremely important. Both technologies have made enormous progress in the last decade and much has been enabled by micro and nano-technologies. One such example is **cochlear implants**, which are a type of electronic neuroprosthesis implanted in the middle ear, where they stimulate the ossicles electromechanically, rather than acoustically, through either electromagnetic or piezoelectric transducers. Nanotechnologies have enhanced microelectronics, batteries and micromechanical transducers in cochlear implant devices. Problems in those devices still exist, in particular refinement of the signal processing (for instance to recognise voice pitch in music), improvement in their long-term biocompatibility (build-up of biofilms and plaques) and prevention of bacterial fungal infections. Nanomaterials are expected to have an important impact in all these areas.

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Neuronal stimulation, monitoring and pain management

The cardiac pacemaker is one of the best-known and most widely used neuroprosthetics. Other types of **electronic stimulators** are cardiac defibrillators, cochlear implants, bone-growth stimulators, neural stimulators for the deep brain for controlling tremors in Parkinson's disease, neuronal stimulators for spinal cord restoration, sacral and other nerve stimulators. Nanotechnologies are impacting these electronic stimulators through improved battery technologies, biocompatible materials and surface treatments for enclosures and leads, electrode miniaturisation and efficiency improvements, and smaller-sized integrated circuits for control and power with increased speed and processing capabilities.

Electrical stimulation of neural tissue by surgically implanted neuroelectronic devices is already an established modern therapy. Integrated micro-and nanoscale devices allow many more electrodes to be applied to the target site with fine resolution and in a coordinated, dynamic way. **Recording of neural activity** is also implemented by using nano-scale electrodes, since a much larger number of recording sites is possible. These implanted probes must be resistant to challenging environments, so the nano-scale surface engineering of these probes is essential. In addition to protection of the probes, nanotechnologies are contributing in developing **improved active interfaces** between neurons and electrostimulation devices.

An example of an electronic stimulator is a deep brain stimulator. This device will make it possible to treat patients with severe Parkinson's disease – a disorder of the central nervous system that often impairs the sufferer's motor skills, speech and other functions. It has been found that the uncontrollable tremor of patients can be removed if a fine nano-size electrode is inserted into the brain to delivers a continuous electrical stimulus. This precise electrical stimulus has the effect of removing the tremors.

Reduction of size and power requirements with the integration of microelectronic devices makes it feasible in many cases to energise an implanted device by RF electromagnetic transmission of power, thus eliminating wires and batteries. This is already the case for implanted pacemakers. Improvements in energy storage through nanoengineered energy materials, such as supercapacitors and conductive polymers, coupled with low power requirements for nanoengineered electronics, will allow great improvements in terms of size and capabilities of such devices. These devices make it possible to perform electrical stimulation in selected points of nervous, sensory and neuromuscular systems. Such

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improvements might make it possible eventually to use implanted electrical stimulation for bone and tissue grafts, and to stimulate functions in the endocrine system and other organs.

NANOYOU DILEMMA: The example of deep brain stimulation is part of the **NANOYOU Role Play Card Game** (see www.nanoyou.eu/en/decide).The dilemma arises from considering a deep brain stimulator designed to treat Parkinson's disease. Now, this device could also be used to treat other medical and health conditions such as intractable epilepsy, as well as mood and eating disorders. Could this nanotechnology be used to increase the capability of the brain in areas for which it was not originally developed? An example of this might be students enhancing their concentration during exams. The dilemma is: "Is it ethically acceptable to use technologies developed for specific medical treatments for others purposes like improving human capabilities?"

Noninvasive brain-machine interfaces

The control of physical objects by the power of thought alone has always captured the imagination of humans. Until recently this possibility was only envisaged in science fiction. Now, with the aid of new technologies, and thanks to decades of studies on neuron activity in the brain, the control of machines and computers by the brain is becoming a reality. Systems are being developed where the patterns of neuronal firing in the brain are translated into electronic controls to support communication, mobility and independence of paralysed people. This is possible because the firings of neurons and the travel of ion currents along axon membranes generate an electrical current, which in turn generates a magnetic field. A steady electrical current generates a static magnetic field, but if the electrical current changes (due to neuronal activity), so does the magnetic field. Conversely, a change in external magnetic field can induce a change in electrical (neuronal) activity. Therefore magnetism can become a non-invasive communication tool with nerves without implanted electrodes and painful transcutaneous shocks.

The technique is called **magnetic monitoring** and it requires extremely sensitive **magnetometers** because the magnetic fields produced by brain activity are very small. Today **magnetoencephalography (MEG)** can map brain activity on a one-millimetre grid or less. The first generation of MEG equipment was very bulky, requiring shielded rooms, high power consumption, cryogenic cooling of detectors and significant processing time. The technique has so far been limited to research labs or extremely

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specialised medical investigations. Nanofabrication is enabling reduction in size of most components of MEG equipment (sensors, magnets, etc.) and new concepts are being developed; in prototypes this progress has already led to 1000-fold improvements in sensitivity and **reductions in size and power requirements** by factors of 10 to 100.

Magnetism can also be used **to induce electrical currents** in the neuron cell membrane like those induced by implanted electrodes, but without physical contact. **Magnetic stimulation** is a new medical technique which requires strong magnetic fields that must vary or pulse in order to generate an electric field. The impacts of nanotechnologies in this case are: nanofabrication of compounds and alloys that produce better high-temperature conducting materials. This will make it possible to reduce the size of the device and the cryogenic environment needed for the performance of superconducting magnets. Nanoparticle thin films are also being developed as shielding materials.

Numerous new **nanoscale magnetometer designs** are being developed, one of which is the **optical atomic magnetometer**. This instrument is based on the interaction of laser light with atoms oriented in a magnetic field in a gas phase. The instrument measures the change in alignment when atoms with a magnetic spin moment interact with a beam of a laser. In the absence of a magnetic field the atoms will align with the electrical and magnetic field of the laser beam crossing the atoms. Any perturbation by a magnetic field will disorient the alignment with the beam, reducing the amount of light transmitted through the gas. A prototype of such a system has been developed by NIST (US) containing about 100 billion atoms of rubidium gas in a vial the size of a grain of rice. The change of spin was easily detectable and scalable to much smaller sizes. The NIST prototype was able to detect the heartbeat of a rat. Researchers predict that with their small size and high performance such sensors could lead to **magnetocardiograms** that provide similar information to an electrocardiogram (ECG) without requiring electrodes on the patient's body, even outside clothing. This technique could become a realistic alternative to MRI and PET imaging, without injection of contrast enhancement agents or tracers. What is really exciting is that even with the laser and heating components, this new device uses relatively **low power** and can be extremely small compared to any current magnetic stimulation device. It might one day be possible to use sensors to make portable MEG helmets for brain-machine interfaces.

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Although much progress has been made and much research is underway, there are some **major obstacles** to overcome before brain-machine interfaces become a reality. Wireless signal transmission from brain implants is still futuristic, along with wearable magnetic brain-machine interfaces. Another challenge is optimisation of the microelectrodes that record neuronal activity, which tend to degrade in time due to biofilm formation. **Risk of infection** is also a major problem. Better engineering of interfaces using **nanoengineered materials** is needed to improve biocompatibility and durability and to allow lower stimulation potentials.

ELSA TOPIC*:* Neuroprosthetics raises a number of ELSA issues. Normally these types of devices are only considered when other pharmacological and neurosurgical options have been exhausted. Bioengineers and medical engineers say that their role should be to compensate for a body's deficit (as result of an accident or a disease), *not to replacing any existing function*. It should not lead to enhancement of human capabilities. Nevertheless nanotechnologies are making these developments more feasible and affordable, obliging researchers in the field, as well as regulators, ethicists and sociologists to reflect on the social, medical and ethical consequences of these devices. The same applies to the concept of cognitive prosthesis, a system developed to support and augment the cognitive abilities of its user. Of course, such devices could be extremely useful to people with impaired communication. However, the concept raises the possibility of enhancement of cognitive capabilities for other uses, which brings up many social, ethical and regulatory questions. One is access to these types of devices. Will these be affordable for everybody, covered by the social health systems, or available only to wealthy people? Is it ethical to have a technology that can enhance a person's mental ability, but only if she/he can afford it? How will this technology be regulated in situations where the mental/cognitive ability of numerous individuals is assessed (job interviews, competitions, etc.)?

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