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Nanotechnology in medical applications: state-of-the-art in materials and devices

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Abstract

Nanotechnology in medical applications: state-of-the-art in materials and devices

Nanotechnology is an extremely powerful emerging technology, which is expected to have a substantial impact on medical technology now and in the future. The potential impact of novel nanomedical applications on disease diagnosis, therapy, and prevention is foreseen to change health care in a fundamental way. Furthermore, therapeutic selection can increasingly be tailored to each patient's profile. This report presents the state-of-the-art in the area of promising nanotechnology approaches for medical technology. In particular, relevant applications are reported in surgery, cancer diagnosis and therapy, biodetection of disease markers, molecular imaging, implant technology, tissue engineering, and devices for drug, protein, gene and radionuclide delivery. Many medical nanotechnology applications are still in their infancy. However, an increasing number of products is currently under clinical investigation and some products are already commercially available, such as surgical blades and suture needles, contrast-enhancing agents for magnetic resonance imaging, bone replacement materials, wound dressings, anti-microbial textiles, chips for *in vitro* molecular diagnostics, microcantilevers, and microneedles.

Keywords: nanotechnology; medical technology; biosensors; molecular imaging; implants; cancer diagnostics; cancer therapy; *in vitro* diagnostics

Rapport in het kort

Nanotechnologie in medische toepassingen: stand der wetenschap in materialen en producten

Nanotechnologie is een uitermate krachtige, opkomende technologie die op dit moment al toegepast wordt en in de toekomst een aanzienlijke invloed zal hebben op de medische technologie. Innovatieve nanomedische toepassingen kunnen de gezondheidszorg op fundamentele wijze veranderen, omdat er nieuwe mogelijkheden beschikbaar komen voor diagnose, behandeling en preventie van ziekte. Verder kunnen behandelmethodes in toenemende mate precies op maat worden gemaakt gebruikmakend van het profiel van de patiënt. Dit rapport geeft een overzicht van de "state-of-the-art" op het gebied van veelbelovende nanotechnologische ontwikkelingen in de medische technologie. Met name worden relevante toepassingen besproken in chirurgie, diagnose en behandeling van kanker, bepaling van ziekte-specifieke stoffen in het lichaam, beeldvormende technieken, implantaten, tissue engineering en toediening van geneesmiddelen, eiwitten, genen en radionucliden. Veel toepassingen van nanotechnologie in de medische technologie staan nog in de kinderschoenen. Een toenemend aantal producten wordt echter momenteel onderzocht in klinische studies en sommige zijn al commercieel verkrijgbaar, waaronder chirurgische mesjes en hechtnaalden, contrastmiddelen voor beeldvorming met magnetische resonantie, botvervangende materialen, wondbehandelingsproducten, antimicrobieel textiel, chips voor *in vitro* moleculaire diagnostiek, "microcantilevers" en micronaalden.

Trefwoorden: nanotechnologie; medische technologie; biosensoren; moleculaire beeldvorming; implantaten; kankerdiagnostiek; kankertherapie; *in vitro* diagnostiek

Summary

Nanotechnology is an emerging technology seeking to exploit distinct technological advances of controlling the structure of materials at a reduced dimensional scale approaching individual molecules and their organised aggregates or supramolecular structures. Basically, the nanometre-length scale is creating possibilities for novel materials that can be used for the construction of devices and systems. Nanotechnology must be distinguished from the nanoscience enabling such technology. Basically, nanoscience is the study of phenomena and material properties at nanoscale, while nanotechnology is applying the resulting knowledge to create novel materials and structures. Knowledge in nanoscience and nanotechnology is increasing worldwide, leading to great scientific advances. In turn, this is expected to lead to fundamental changes in the way that materials, devices, and systems are understood and created. Application in life sciences research, particularly at the cell level sets the stage for an exciting role of nanotechnology in healthcare.

In this report a general overview of the state-of-the-art in novel nanomaterials and recent advances of nanotechnology applications are presented, focussing on promising medical applications. Relevant medical areas are surgery, therapy, diagnostics, imaging, implant technology, bionics, bio-active surfaces, tissue engineering, textiles, actuators, and delivery systems. Products which are either commercially available or currently being developed at several companies are also included, illustrating the significant progress and challenges in nanotechnology.

Novel nanomaterials are envisaged to have a major impact on a number of different relevant areas. Materials with high performance and unique properties can be produced that traditional synthesis/manufacturing methods could not create. Carbon nanotubes and inorganic nanowires exhibit extraordinary mechanical, electric, electronic, thermal, and optical properties offering especially the electronic industry properties that few materials platforms could ever hope to match. Although nanotube/wire electronics has been speculated about for well over a decade, the first products are now about to reach the market or are beginning to appear in the form of field emission displays, sensors, and non-volatile memory. Quantum dots (semiconductor nanocrystals) possess remarkable optical and electronic properties that can be precisely tuned by changing their size and composition. Due to their relatively inexpensive and simple synthesis quantum dots have already entered the market for experimental biomedical imaging applications. Dendrimers (complex spherical macromolecules) have improved physical, chemical, and biological properties compared to traditional polymers. Some unique properties are related to their globular shape and the presence of internal cavities offering the possibility as medical nanovehicles. In addition to these examples of individual nanoparticles, new or enhanced materials can be constructed using structural surface modifications of macro-, micro- as well as nanomaterials. Essentially, an increase in surface area and roughness attributes to an enhancement of absorbent, adsorbent, and catalytic properties. Control of surface properties at nanolevel was shown to increase the biocompatibility of the materials.

It is difficult to accurately predict the timescale of developments, but it is anticipated that within the next few years the application of nanomaterials and nanotechnology-based manufacturing will have an established role in medical technology. Some surgical aids already benefit from nano-structured material, such as surgical blades with nanometre-thick diamond coating and surface roughness in the same order of magnitude, and suture needles incorporating nano-sized stainless steel particles. Other nanotechnological approaches might allow for nanosurgery, a minimally invasive alternative to traditional surgery, based on nanoneedles and laser technologies such as optical tweezers and “nanoscissors”.

Biomedical nanotechnology presents revolutionary opportunities in the fight against many diseases. An area with near-term potential is detecting molecules associated with diseases such as cancer, diabetes mellitus, neurodegenerative diseases, as well as detecting microorganisms and viruses associated with infections, such as pathogenic bacteria, fungi, and HIV viruses. Macroscale devices constructed from exquisitely sensitive nanoscale components, such as micro-/nanocantilevers, nanotubes, and nanowires, can detect even the rarest biomolecular signals at a very early stage of the disease. Development of these devices is in the proof-of-concept phase, though entering the market may be sooner than expected. However, a different approach of molecular sensing *in vivo* involves the use of implantable sensors which is still hampered by unwanted biofouling impairing long-term stability of continuous sensors caused by blood components and factors of the immune system. Nanotechnology might yield nano-structured surfaces preventing this non-specific protein adsorption.
Molecular imaging is providing increasing power to studies of animal models of disease and is beginning to be used in clinical investigations as a non-invasive means of monitoring disease progress and response to therapeutics. Molecular imaging agents will allow clinicians to detect diseases in its earliest, most treatable, presymptomatic stage. Combination of precise targeting using specific antibodies and imaging enhancement properties of nanoparticles are the key to greatly enhance the power of magnetic resonance imaging, optical imaging, nuclear imaging and ultrasonic imaging. One of the great achievements foreseen is the ability to identify tumours that are far smaller than those detectable with today's technology, before they are even visible with the human eye.
The above described advances in medical diagnostics are rivalled by the progress made in therapeutics enabled by nanotechnology. Especially in the field of cancer therapy promising applications are being developed. Several novel nanoparticles will respond to externally applied physical stimuli in ways that make them suitable therapeutics or therapeutic delivery systems. For example, magnetic iron oxide nanoparticles, gold-coated silica nanoshells, and carbon nanotubes can transform electro-magnetic energy into heat causing a temperature increase lethal to cancer cells merely by increasing the magnetic field or by irradiation with an external laser source of near-infra red light at the very location where these nanoparticles are bound to or internalised within tumour cells. Moreover, the delivery of chemotherapy and photosensitisers to tumours, and activating them *in situ* is possible. Also in other areas, drug delivery is one of the major application fields for nanotechnology. Nanoparticle-mediated transport across the blood-brain barrier could not only provide an effective treatment for brain tumours, but also for other central nervous system related-diseases such as Alzheimer's and Parkinson's. Furthermore, non-viral gene delivery systems for gene therapy, nanoneedles for cell surgery and delivery of molecules into the cell nucleus, nanocrystalline silver particles with antimicrobial activity or haemostatic agents on wound care products, microchip-based drug delivery systems for programmable drug release, and nanoporous drug eluting coatings on stents are examples of new nanotechnology materials and devices in drug delivery applications.
In the future, a modular approach to construct delivery systems which combine targeting, imaging and therapeutic functionalities into multifunctional nanoplatforms may allow for new refined non-invasive procedures. These nanoplatforms would localise to target cells, enable diagnostics and subsequently deliver therapeutics with great precision. Such modular approaches to nanodevice construction can potentially be more powerful than current treatment modalities, but are inherently more complex than existing small molecule or protein therapeutics.
Another important field of application for nanotechnology are biomaterials used for example in orthopaedic or dental implants or as scaffolds for tissue engineered products. If the design

of for example a hip implant is carried out at nanolevel, it might become possible to construct an implant which closely mimicks the mechanical properties of human bone, preventing stress-shielding and the subsequent loss of surrounding bone tissue. Furthermore, surface modifications at nanolevel of biomaterials or their coatings might greatly enhance the biocompatibility by favouring the interaction of living cells with the biomaterial, especially by their beneficial effect on cell adhesion and proliferation. Together with the control of nanoporosity allowing vascularisation and the growth of cells inside the biomaterial, the nano-structured surfaces of biomaterials also allow the creation of novel types of scaffolds for tissue-engineered products. A promising approach for the latter application are nanofibres produced using self-assembling peptides with engineering functionality and biodegradability.
Medical devices for *in vitro* diagnostics, such as gene-, protein- or lab-on-a-chip devices, do not have any of the safety concerns associated with nanoparticles introduced into the body. Numerous devices and systems for sequencing single molecules of DNA are feasible. Nanopores are finding use as new nanoscale technology for cancer detection enabling ultrarapid and real-time DNA sequencers. In general, developments in protein-chips and lab-on-a-chip devices are more challenging compared to gene-chips and these devices are anticipated to play an important role in medicine of the future, which will be personalised and will combine diagnostics with therapeutics into a new emerging medical area called theranostics.
Nanomedicine is now within the realm of reality, though there is some concern about the safety of nanoparticles introduced in the human body. Research is in progress to address this issue. Examples of medical devices utilising nanotechnology, which are already on the market are surgical tools with enhanced properties, nano-sized contrast agents for molecular imaging, bone replacement materials constructed from nanostructured materials, pacemakers and hearing aids of reduced size and increased power, lab-on-a-chip devices for in vitro diagnostics, wound dressings containing nanocrystalline silver particles, microcantilevers, and microneedle-based systems for minimally invasive drug administration. Over the next ten to twenty years nanotechnology may fundamentally transform science, technology, and society offering a significant opportunity to enhance human health in novel ways, especially by enabling early disease detection and diagnosis, as well as precise and effective therapy tailored to the patient.

Preface

This report describes the state-of-the-art of materials and devices in the area of nanotechnology in medical applications. The review was performed on the request of the Department of Pharmaceutical Affairs and Medical Technology of the Ministry of Health, Welfare and Sports in the Netherlands. The information gathered here is presented as basic information to staff of this department who are involved in determining Dutch policy on medical technology issues, but it may also be useful for other parties in the fields of nanotechnology and/or medical technology. Concurrently, a second report was written which evaluates possible risks for human health related to the application of nanotechnology in medical practice.[1]
For the overview presented here, scientific literature was evaluated and included when available either in journal publication or early online publication on the internet before the 1st of August 2005.

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[1] De Jong WH, Roszek B and Geertsma RE (2005). Nanotechnology in medical applications: possible risks for human health. RIVM report 265001002, Bilthoven.

Contents

1. Introduction

Technology is generally regarded as the utilization or application of science to benefit society. Nanotechnology is an emerging technology, which is no longer just a vision for the future as it was generally seen at the end of 20th century. Instead, nanotechnology is a ubiquitous technology with a potential to impact on every aspect of modern human civilization. An incredibly diverse range of areas will be affected, such as agriculture, communication, energy generation/transmission, computers, environmental monitoring, food manufacturing/processing, health care, personal care, space travel, robotics, but probably the biggest impact will be in medical technology.

In general, nanotechnology is acknowledged to represent a new frontier in science and technology of the 21st century. One may argue, however, that nanotechnology is not as contemporary as it seems to be. Although the word nanotechnology is relatively new, the "natural version" of nanotechnology was already in pole position with procreation of life itself thousands of millions of years ago (see Appendix A). All natural materials and systems establish their foundation at the nanoscale. Basically, the biological building blocks of life are nano-entities that possess unique properties determined by the size, folding, and patterns at nanoscale. The genetic material desoxyribonucleic acid (DNA) is composed of four nucleotide bases in sizes ranging in the sub-nanometre scale, and the diameter of the double-helix structure of DNA is in the nanometre range. The same is true for proteins and cell membranes which consist of lipids and proteins.

Manufacturing "non-natural" nanomaterials faces many challenges, often requiring a specific approach. Miniaturisation has been applied quite successfully for some decades now. This "top-down" approach has traditionally been used in the fabrication of electronics in the semiconductor technology industry. However, nanotechnology is more than miniaturisation alone. In order to explore the full potential of nanotechnology a new paradigm has to be set. This is accomplished by a "bottom-up" approach building structures from more basic materials. Self-assembly is a bottom-up technique that attracts much attention. In fact, this concept is not new. Biological systems are built predominantly using self-assembly. What is new is the enhanced ability to "externally" or "specifically" or "intentionally" control the structure at the nanoscale and manufacture new materials such as nanotubes, nanowires, and nanocapsules.

Nanomaterials are difficult to maintain as individual particles. One reason for this is their marked propensity to agglomerate because agglomeration reduces the enormous surface area in relation to the volume of the nanomaterial, which is energetically unfavourable. Appropriate "bottom-up" approaches and applying precision surface engineering may overcome agglomeration.

Nevertheless, nanotechnology offers very interesting possibilities of developing new innovative products for many areas of daily life, some of which have already been realised. Also the field of medical technology has already started to benefit from the progress in nanotechnology. Many revolutionary applications, such as novel sensing technologies, surface modifications, and implant technologies are currently being developed. Probably the most significant impacts of nanotechnology will be at the biomaterials/living tissue interface and the non-biological/cell interface, e.g. human-machine (for example retinal prosthesis). Whether a prosthetic implant is accepted or rejected, whether a drug is effective or whether living tissue will regenerate are all questions which could be approached at the nanometre scale. Interfacing materials with human biology/physiology is one of the exciting new

frontiers of nanotechnology in the field of medical applications. Nanotechnology will definitely be a strategic branch for science and engineering during the coming century. It will fundamentally restructure the technologies currently used for manufacturing, medicine, communication, computation, transportation and many other application areas.

1.1 Scope

This report focuses on recent advances of nanotechnology applications in medical technology. An overview of the state-of-the-art in nanomaterials and (medical) devices is given. Relevant medical areas are surgery, therapy, diagnostics, imaging, implant technology, bio-active surfaces, tissue engineering, textiles, actuators, and drug/gene delivery materials and systems. Products that are already on the market or are currently being developed illustrate the significant progress in nanotechnology.

1.2 Methodology

The state-of-the-art in nanomaterials and devices was based on literature searches, internet searches, and proceedings of conferences. Literature was identified from several sources including electronic databases and cross-checking of reference lists. Electronic databases consulted were Scopus™ (Elsevier BV) and PubMed (US National Library of Medicine). Searches were restricted to a selection of the most relevant application areas of medical technology due to the vast number of publications. Several conferences on medical applications of nanotechnology were attended and some topics discussed were included in this report. Product information was obtained using manufacturers' websites retrieved from Google (www.google.com) or Nanovip.Com, a web-searchable database focused on nanotechnology companies. It should be noted that applications of nanotechnology in drug discovery research, providing tools for a fast screening of large arrays of candidate substances, were not included in the report. Furthermore, applications on liposome-based drug delivery and viruses as delivery vehicle were excluded.
In order to be able to place the nanotechnology applications in medical technology in the right perspective, it is necessary to have a basic understanding of the origin of the unique properties of nanomaterials. Therefore this report starts with a short section explaining the definitions and features of nanoscience and nanotechnology, followed by a more elaborate overview of the novel nanomaterials and their specific properties which have opened the horizon for the applications in medical technology which are described in the main part of the report. Furthermore, in this report greater emphasis is given on highlighting promising nanotechnology-based approaches in medical technology than on consensus taxonomies of scientific/engineering disciplines.

2. Nanoscience and nanotechnology

2.1 Definitions

In a recently published report of The Royal Society & The Royal Academy of Engineering (2004) definitions were given for nanoscience and nanotechnology:

> ***Nanoscience*** *is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at larger scale.*
>
> ***Nanotechnology*** *is the design, characterisation, production and applications of structures, devices and systems by controlling shape and size at the nanometre scale.*

2.2 Nanoscale features

The prefix nano is from the Greek word nanos (νανοσ) which means dwarf. Commonly, nano is associated with the SI length unit metre and denotes one-billionth (10^{-9}). Thus, nanomaterials are characterised at the nanometre scale in one, two or three dimensions, leading to quantum wells (e.g., thin films, layers, surface coatings), quantum wires (e.g., nanotubes, nanowires) or quantum dots (qdots), respectively (Figure 1). Nanoparticles with a diameter of less <100 nm are for example fullerenes, dendrimers and semiconductor quantum dots.

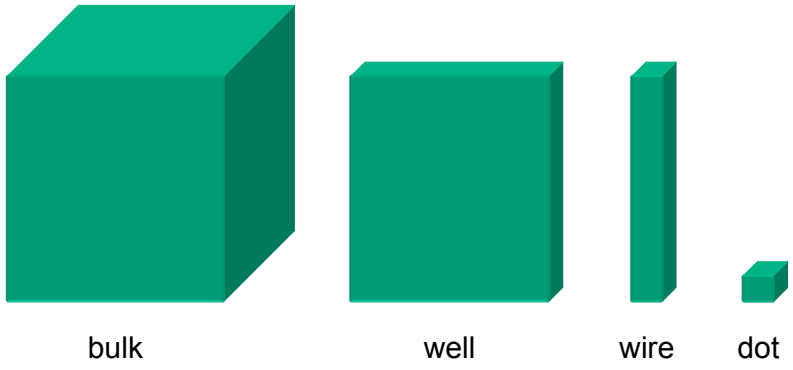

Figure 1. Progressive diminishing of size up nanometre dimensions for rectangular structures. The principle is also valid for curvilinear structures, such as spheres, discs and tubes.

The word quantum is associated with these three structures because profound changes in material properties emanate from the quantum mechanical nature of physics that rules the world in the ultra-small and where material properties no longer obey the classical macroscopic laws of physics. Materials can be scaled down many orders of magnitude from macroscopic to microscopic without any or little change in expected properties occurring. However, when the nanoworld is entered, characteristic changes are observed. For the time

being no strict dimensional limits can be defined for this phenomenon. At the nanoscale, physics, chemistry, biology, material science, and engineering converge toward the same principles and tools. As a result, progress in nanoscience will have very far-reaching impact. The nanoscale is not just another step toward miniaturisation, but a qualitatively new scale. The change in behaviour is dominated in the first place by quantum mechanics, as mentioned above and is additionally attributable to material confinement in small structures, and the increase in surface area per volume (or mass unit) (Figure 2). At the larger end of the nanometre scale other phenomena are crucial, such as surface tension and Brownian motion.

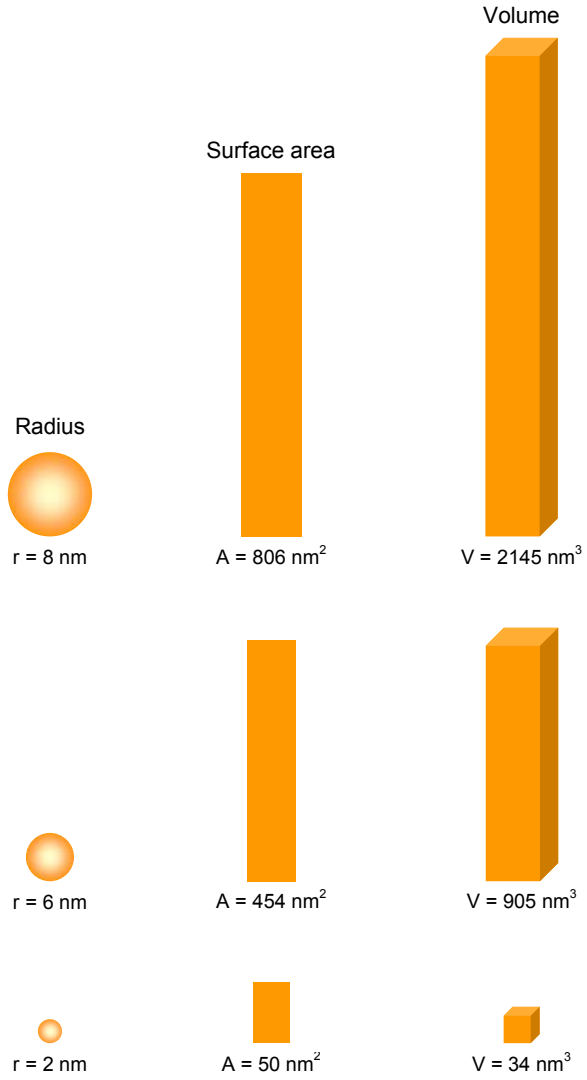

Figure 2. Interrelationships of radius, surface area, and volume of a quantum dot. Note that the volume decreases more rapidly than surface area for a given decrease in radius. Hence, surface area to volume ratio increases dramatically for a lower radius compared to the ratio for a higher radius. Drawn to scale approximately.

Nanoscience is concerned with understanding these effects and their influence on material properties. Nanotechnology aims to exploit these effects to create structures, devices, and systems with novel properties and functions due to their size (The Royal Society & The Royal Academy of Engineering, 2004). In contrast to other key technologies, such as

biotechnology, information and communication technology, nanotechnology is much less well-defined and well-structured. In fact, nanotechnology is immensely complex and covers multiple disciplines ranging from physics, chemistry, and biology to engineering disciplines. This is reflected by the enormous number of publications which accelerated from 1989 onwards (Compañó and Hullmann, 2002). In 1989 about 1000 scientific articles have been published. In 1998 the number increased to more than 12.000 publications. Data for 1999 and 2000 confirm the continuation of this development (Hullmann and Meyer, 2004). Concurrently, patents as indicators of output of applied research showed a progressive increase.

3. Nanomaterials for the 21st century

This chapter is concerned with various new or enhanced nanomaterials that are envisaged to have a major impact on technological applications in general and medical technology in particular. Nanomaterials are categorized into carbon and inorganic nanomaterials. It should be noted that not only nanoparticles are discussed, but also nano-structured bulk materials, because the physical interactions at the nano-scale interface between biological structures and materials are crucial for the biological response.

3.1 Carbon nanomaterials

3.1.1 Introduction: carbon bonds and structures

The bonds between carbon atoms are such as to follow the formation of some of the most interesting nanostructures. Solid carbon at room temperature has two classical structures or allotropes: diamond and graphite. In diamond, carbon atoms are connected each to four other carbon atoms in a tetrahedral lattice structure and these bonds form a three-dimensional network. Diamond is the hardest mineral known to man and is an excellent electrical insulator. In graphite the carbon atoms are arranged in hexagons and strongly bonded into parallel planar sheets. The sheets are held together by much weaker Van der Waals forces, which is the reason why graphite can be used as material in pencils and as the basis of some lubricants. Unlike diamond, graphite is a conductor of electricity. As illustrated by these classical examples, physical properties can vary considerably within pure carbon materials.

3.1.2 C_{60}

In 1985 the discovery of the existence of a third and new carbon allotrope containing sixty perfectly symmetrically arranged carbon atoms (C_{60}) meant a major breakthrough and opened a novel field of carbon chemistry (Kroto *et al.*, 1985). The C_{60} molecule was originally and formally called buckminsterfullerene after the American architect and inventor R. Buckminster Fuller (1895-1983), who designed geodesic domes similar to the structure of C_{60}. Scientists quickly nicknamed it a “buckyball” as the spherical structure of C_{60} resembles a football (Figure 3).

Figure 3. Representation of a C_{60} molecule. Source: ©Chris Ewels 2003 (www.ewels.info).

The geometric configuration consists of 60 vertices and 32 faces, 12 of which are pentagonal and 20 hexagonal. The faces are symmetrically arrayed to form a molecular ball with a

diameter of approximately 1.0 nm. Soon after the discovery of C_{60} many other fullerene molecules with variable shapes and forms have been synthesized, such as C_{70}, C_{76}, C_{80}, and C_{84} (Dresselhaus *et al.*, 1996). The higher fullerenes are also interesting and hold several promises in their own right.

Synthesis

Initially C_{60} was produced by vaporisation of graphite into helium using a laser beam (Kroto *et al.*, 1985). This process was not scalable and could not produce the larger quantities necessary for commercial availability. Therefore, only few experiments could be performed on the material and comprehensive fundamental research or even evaluating possible applications was restricted. However, in 1990 C_{60} molecules and other fullerenes could be produced in bulk quantities, i.e. macroscopic gram amounts, using an electric carbon arc discharge apparatus (Krätschmer *et al.*, 1990). Evaporating graphite electrodes in helium under low pressure (0.1 atm) yielded 100 mg of pure material in one day, approximately. This opened completely new possibilities for experimental research and industrial use. In 1991, a new method of fullerene production was invented, providing basic engineering knowledge critical to scale up the process (Howard *et al.*, 1991). This synthesis method involves the combustion of hydrocarbon fuel under sub-atmospheric pressure and makes economical production of higher fullerenes possible. Refining and improving the production process is still continuing. C_{60} remains the easiest to produce and cheapest ($100/gram, C purity >99.9%) with prices increasing progressively for the larger fullerenes ($500/gram for C_{78}, C purity >98%) (Bucky USA, Houston, Texas, 2004). Several companies hope to produce tens of metric tons a year in the near future, which could reduce the price down to $0.20/gram.

Properties and applications

C_{60} has appealing physical, (bio)chemical, electrical and optical properties. These properties can be modified by functionalization, i.e. attaching chemical groups to a fullerene's carbon atom. When crystalline C_{60}, normally an insulator, is doped with alkali metals, such as potassium, caesium or rubidium, it can be made metallic (Haddon *et al.*, 1991). Various functionalizations have been utilised to increase hydrophilicity of fullerenes as well as to prepare new compounds with biological and pharmacological activity (Bosi *et al.*, 2003). C_{60} derivatives have a high physical and chemical affinity for the active site of various enzymes, such as HIV-1 protease (Friedman *et al.*, 1993; Schinazi *et al.*, 1993; Sijbesma *et al.*, 1993). Introducing a C_{60} molecule into the catalytic cavity of HIV-1 protease inhibits this fundamental enzyme for virus survival. Evidence has been found for superconductivity (Hebard *et al.*, 1991) and currently C_{60} is being investigated in artificial photosynthetic applications, e.g. photovoltaic devices (Cho *et al.*, 2005). Fullerenes are powerful antioxidants, reacting readily and at a high rate with free radicals, which are often the cause of cell damage or death (Chueh *et al.*, 1999; Lin *et al.*, 1999; Straface *et al.*, 1999). Fullerenes could hold promise in health and personal care where prevention of oxidative cell damage or death is desirable.

The entrapment of (metal) atoms inside the cavity of C_{60} or other fullerenes is known as a nanomaterial family called endohedral (metallo)fullerenes. Endohedral fullerenes are described with the notation $X@C_{60}$, where X is the caged atom (or atoms) and C_{60} could be any fullerene. Reactive elements can be stabilised inside the fullerene cage. Moreover, entrapped elements can change the electrical and magnetic properties of the fullerene molecule. Because endohedral fullerenes are resistant to metabolism and highly kinetically stable, they can be used as carriers for biomedical *in vivo* imaging (Cagle *et al.*, 1999) (see Section 4.4.1).

3.1.3 Carbon nanotubes

Carbon nanotubes are among the astonishing objects that science sometimes discovers and which will likely revolutionize technological developments of the 21st century. As the fourth allotrope of carbon, carbon nanotubes are also molecules consisting solely of carbon atoms. They can be considered as elongated fullerenes. Though the expectations of fullerenes were quite high after their discovery, only few applications have actually reached the market. However, for carbon nanotubes enthusiastic predictions are being made as their physical properties, i.e. mechanical, electronic, thermal and optical, exceed those of commonly used materials.

In 1991 Sumio Iijima reported the existence of concentric multi-walled carbon nanotubes (MWCNTs) as by-products of the formation of fullerenes (Iijima, 1991). The real breakthrough occurred two years later when two research groups (Bethune *et al.*, 1993; Iijima and Ichihashi, 1993) independently discovered, again unexpectedly, single-walled carbon nanotubes (SWCNTs) consisting of a single seamless cylindrical wall of carbon atoms. MWCNTs can be considered as a collection of concentric SWCNTs with different diameters similar to cylinders within cylinders. SWCNTs were really new nano-objects having specific properties and behaviours. Recently, double-walled carbon nanotubes (DWCNTs) have been synthesized in pure form (Endo *et al.*, 2005b). These intermediate structures are likely to have superior material properties and could replace MWCNTs or SWCNTs in several applications.

Structure

Carbon nanotubes are in essence rolled-up, highly ordered hexagonal carbon honeycomb sheets, which at the end can be closed or open (Figure 4). A detailed description of the topology of carbon nanotubes is included in Appendix B.1.

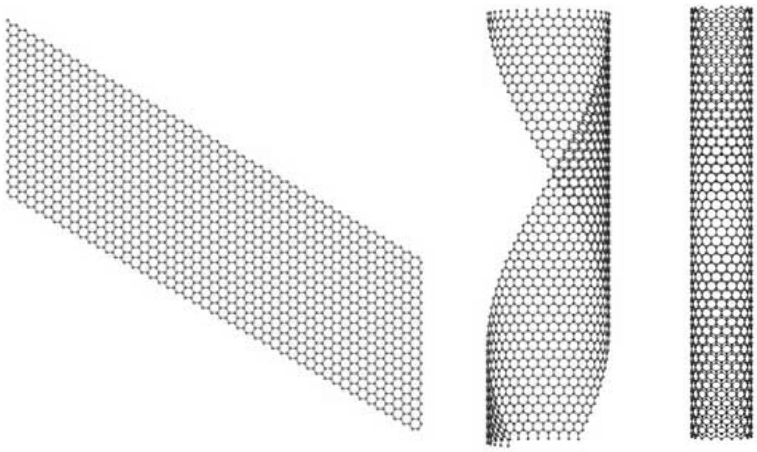

Figure 4. Illustration of a flat carbon sheet (left) rolled-up into a partially rolled-up sheet (middle) and carbon nanotube (right). Source: ©Chris Ewels 2003 (www.ewels.info).

At least one dimension of the tube should be 100 nm or less. Calculations have shown that a diameter greater than 2.5 nm is energetically not favourable to maintain a tubular morphology (Tersoff and Ruoff, 1994). Although SWCNTs with diameters of 0.4 nm have been synthesized (Wang *et al.*, 2000), a suitable energetic compromise is reached for ~1.4 nm, the most frequent diameter encountered in SWCNTs regardless of the synthesis techniques. The

smallest, stable, SWCNT grown inside a MWCNT is just 0.3 nm in diameter and has only four hexagons around its circumference (Zhao *et al.*, 2004a). The length of carbon tubes is not as restricted, depending on the synthesis conditions and varies from tens of micrometers to hundreds, or more. Recently, a research team in the USA has established a record length synthesizing four centimetres long individual SWCNTs (Zheng *et al.*, 2004b). Moreover, long strands of ordered SWCNTs with a length of ~20-30 cm, a strand diameter of 0.3-0.5 mm and carbon nanotube diameter of ~1 nm have also been accomplished (Quy *et al.*, 2005; Zhu *et al.*, 2002). Nowadays SWCNTs can be grown continuously without any apparent length limitation (Wang *et al.*, 2005b). These physical dimensions make SWCNTs unique examples of molecules with enormous aspect ratios (ratio of length to diameter) and can be considered as nearly one-dimensional structures.

Synthesis

Several technologies (solid and gaseous carbon-based production techniques) are currently used to make carbon nanotubes, including electric arc discharge (Ebbesen and Ajayan, 1992; Iijima, 1991), laser ablation (Guo *et al.*, 1995; Thess *et al.*, 1996), and chemical vapour deposition (Kong *et al.*, 1998; Li *et al.*, 1996; Ren *et al.*, 1998) (see Appendix B.2). The exact growth mechanism of carbon nanotubes is not fully understood yet.

The synthesis processes vary considerably with respect to structural quality, purity, scalability, and type of carbon nanotube synthesized, i.e. morphology and helicity. In general the synthesis yields a mix of different kinds of carbon nanotubes, some metallic and some semiconducting. The purification of SWCNTs is difficult to perform since SWCNTs can have defects and contain a lot of metal catalyst. Scalability of production processes is an essential commercial prerequisite. Some methods use equipment that simply cannot be made bigger and the only way to increase production is to make more pieces of equipment, which will not decrease costs significantly. On the long term laser-based methods are generally considered not competitive compared to the chemical vapour deposition method providing good perspectives of large-scale and low-cost processes for the mass production of carbon nanotubes.

SWCNTs were first marketed by Carbon Nanotechnologies, Inc. (Houston, Texas, USA) with prices as high as $1000/gram for raw material in 2002. Prices are steadily declining with Nanoledge S.A. (Montpellier, France) offering SWCNTs, though synthesized by the electric arc method, at a price of €65/gram (2005 data). For the time being SWCNTs synthesized using the chemical vapour deposition method yield a slightly higher price of ~$200-400/gram which is marketed for example by Nanostructured & Amorphous Materials, Inc. (Los Alamos, New Mexico, USA). Getting to the market has been hampered by high prices and production bottlenecks. However, some forty global producers have now reached a point where the combination of decreasing prices and increased availability will enable more widespread applications. In the near future prices as low as $0.03/gram of raw high concentration SWCNTs soot are expected. Other major European suppliers of carbon nanotubes are Thomas Swan & Co., Ltd. (Consett, Durham, UK) and Nanocyl S.A. (Sambreville, Belgium).

Mechanical properties and applications

Several studies have been performed to characterize the extraordinary mechanical properties of carbon nanotubes. For a recent review on this topic see Dresselhaus *et al.* (2004). Young's modulus (a measure of the stiffness of a material) of carbon nanotubes is larger than that of any known material (see Box 1). Carbon nanotubes have also remarkable tensile strength that is a multiple tenfold of steel (Yu *et al.*, 2000c). Defect-free MWCNTs with tube ends well capped are expected to have very high tensile strength. Although it is difficult to perform

such measurements in a reliable manner, a tensile strength value of ~150 GPa has actually been measured for electric arc MWCNTs (see Appendix B.3).
Carbon nanotubes are exceedingly tough materials. MWCNT can be bent repeatedly without undergoing catastrophic failure, suggesting that they are remarkably flexible and resilient (Falvo *et al.*, 1997). The extraordinary flexibility and resistance to fracture distinguishes carbon nanotubes from graphitic fibres as engineering material. The ability of carbon nanotubes to elastically sustain loads at large deflection angles enables them to store or absorb considerable energy (Wong *et al.*, 1997).

Box 1. Mechanical properties of materials compared to carbon nanotubes

Specimen	Young's modulus	Reference
Human chondrocytes	0.7 kPa	(Jones *et al.*, 1999)
Human cartilage	0.7 MPa	(Mow *et al.*, 1980)
Human tendon	550-1200 MPa	(Maganaris and Paul, 1999)
Human subchondral bone (wet, micro)	1.2 GPa	(Choi *et al.*, 1990)
Human cancellous bone (dry/wet, micro)	1-20 GPa	(Rho *et al.*, 1993)
Human cortical bone (wet, micro)	5.4 GPa	(Choi *et al.*, 1990)
Human cortical bone (wet, macro)	14-20 GPa	(Reilly *et al.*, 1974)
Human cortical bone (dry, submicro)	23 GPa	(Rho *et al.*, 1997)
Titanium alloys (commercial)	110 GPa	(Lynch, 1989)
Plain carbon steel	205 GPa	(Lynch, 1989)
Diamond	1.04 TPa	(Dresselhaus *et al.*, 1996)
Graphite	1.06 TPa	(Dresselhaus *et al.*, 1996)
MWCNT	0.81 TPa	(Salvetat *et al.*, 1999)
	0.91 TPa	(Demczyk *et al.*, 2002)
	1.28 TPa	(Wong *et al.*, 1997)
	1.81 TPa	(Treacy *et al.*, 1996)
SWCNT	1.00 TPa	(Yu *et al.*, 2000a)
	1.25 TPa	(Krishnan *et al.*, 1998)

TPa = 1000 GPa

Carbon nanotubes are potentially the ultimate material for high-strength materials applications such as reinforcement of composite materials or cable components. Applications in sports engineering are emerging, such as VS Nanotube Power and VS Nanotube Drivemade tennis rackets ($200.-) made of high modulus graphite and carbon nanotubes by Babolat VS North America, Inc. (Boulder, Colorado, USA) and Synergy SL hockey sticks and bicycle components (e.g. handlebars) incorporating Zyvex's NanoSolve™ carbon nanotechnology by Easton Sports, Inc. (Van Nuys, California, USA). Mechanical applications could also be in armour, although a suitable matrix must be chosen to exploit the mechanical properties in a macroscopic product. Furthermore, the exceptional mechanical properties make carbon nanotubes ideal tips for force sensors in scanning probe microscopy, such as atomic force microscopy (Dai *et al.*, 1996; Hafner *et al.*, 2001). The carbon nanotube ends can also be specifically functionalised or coated with metals allowing improvement of imaging techniques in chemical force microscopy or magnetic force microscopy, respectively (Cheung *et al.*, 2000; Deng *et al.*, 2004; Wong *et al.*, 1998). MWCNT scanning probe tips can be bought from Seiko Instruments, Inc. (Chiba, Japan), Daiken Chemical Co., Ltd. (Osaka, Japan) and Piezomax, Inc. (Middleton, Wisconsin, USA). Theoretically, these extraordinary mechanical properties would provide unique opportunities to engineer carbon nanotube-reinforced composites for orthopaedic and dental implants when embedded into appropriate matrices (e.g., metals, ceramics, and polymers) (see Section 4.5).

Electric and electronic properties and applications

Carbon nanotubes have exceptional electric and electronic properties. They are metallic or semiconducting depending on the precise structure, i.e. the helicity and diameter of the tube (see Appendix B.4) (Hamada *et al.*, 1992; Mintmire *et al.*, 1992; Saito *et al.*, 1992). Metallic carbon nanotubes can transport very high current densities of up to 10^9 A/cm^2 without being damaged (Yao *et al.*, 2000). Normal metal wires (e.g. copper, gold) can transport current up to 10^5-10^6 A/cm^2 and higher currents would vaporise these metals because of resistive heating. Metallic carbon nanotubes conduct electricity with essentially no resistance at room temperature. This phenomenon is known as ballistic transport, where the electrons can be considered as moving freely through the structure, without any scattering from atoms or defects (Javey *et al.*, 2003; White and Todorov, 1998). In the following section highlights of some current or potential applications, made possible because of the electric and electronic properties, are described. A more elaborate description of the possibilities with the appropriate references can be found in Appendix B.4.

Field emission applications

Carbon nanotubes can be used for flat panel displays, lighting applications such as vacuum-tube elements, household light bulbs and flat panel luminescent lamps, gas discharge tubes, X-ray generators, and electron guns for the next generation scanning electron microscopes and transmission electron microscopes. In 1999 the world's first 4.5-inch flat panel device using carbon nanotubes was developed by Samsung Advanced Institute of Technology. Carbon nanotubes might be an alternative to bulky cathode-ray tubes, such as used for televisions and computer monitors, and the more recent liquid-crystal panels and plasma displays. Based on prototypes, the advantages of carbon nanotubes in flat panel displays are suggested to be low power consumption, high brightness, viewable from any angle, fast response rate, wide operating temperature range, no burn-in, lightness, and thinness. Other major companies involved are Motorola Labs and Eikos. Nanotube-based cold cathodes for compact, portable, and miniature X-ray generators are manufactured by Xintek, Inc. These X-ray tubes can be set up in a narrow space and possibly X-ray endoscopic imaging and provide improved high-resolution images in industrial, biological and medical applications.

Nanoelectronic applications

As carbon nanotubes behave like electrical conductors or semiconductors, they could be extremely useful for nanoscale electronics applications. An all-carbon-based nanoelectronic technology can be foreseen in which the electric wiring consists of metallic carbon nanotubes and the active devices are made of semiconductor carbon nanotubes. A lot of progress is yet to be achieved before routine production of carbon nanotube-based integrated circuits becomes possible, though it is currently feasible in an experimental set-up to build a nanocircuit that has wires, switches and memory elements made entirely from carbon nanotubes and other molecules. Currently, Nantero Inc. (Woburn, Massachusetts, USA) is developing NRAM™, a high density non-volatile random acess memory chip using carbon nanotubes as the active memory elements.

Chemical gas sensors

Semiconductor SWCNTs are highly sensitive to detect changes in the chemical composition of the surrounding atmosphere at room temperature. Carbon nanotube-based chemical gas sensors have great (commercial) potential in numerous areas ranging from medical applications, environmental monitoring, agricultural applications to the chemical industry and beyond. Currently, Nanomix Inc. is developing a medical capnography sensor (see Section 4.3.2).

Shielding material for electromagnetic radiation
A plastic composite of carbon nanotubes could provide shielding for electromagnetic interference which is of much concern to military applications. Nowadays command, control and communications are highly digitised and the system must be protected from weapons that emit electromagnetic pulses.

Battery technology
Carbon nanotubes can be used as additives in lithium-ion battery systems, lead-acid batteries, and electric double-layer capacitors improving their performance. The merit of electric double-layer capacitors is their high discharge rate, which make them applicable as hybrid energy source for electric vehicles and portable electric devices. Implantable active medical devices relying on battery technology for device powering could benefit from extended battery lifetime reducing the need for early replacement.

Electromechanical actuators
Carbon nanotubes are not only capable of sensing but also of actuation. Application of an external stimulus, such as an electrical charge, can change the dimensions of the materials reversibly. Termed “artificial muscles”, such actuators provide wonderful opportunities. An “artificial muscle” has been made consisting of two SWCNT-based paper strips on both sides of an insulating double-layer side tape suspended in a liquid environment. Another example is nanotweezers able to grab, manipulate, and release nano-objects. Carbon nanotube actuators could potentially be used in hostile environments such as in robotics used for planetary exploration, and in medical applications.

Thermal properties and applications
Prior to the discovery of carbon nanotubes, diamond was the best thermal conductor with the highest thermal conductivity of 2600 W/m·K for blue diamond at room temperature. Deduction of experimental values and theoretical calculations for SWCNTs yielded a thermal conductivity ranging from 2980 up to 6600 W/m·K (Berber *et al.*, 2000; Che *et al.*, 2000; Hone *et al.*, 1999; Hone *et al.*, 2001).
These thermal properties are of great importance for thermal management of nano/microelectronic devices. As over the past decades engineers have scaled down silicon-based devices to create very dense arrays of devices for information processing, rapid overheating poses serious problems. Dissipation of heat is widely accepted as a prerequisite for the next step in miniaturisation of electronic devices. Theoretically, carbon nanotubes could efficiently transport heat away from the chip and cool smaller chip circuits.

Fluorescence properties and applications
Fluorescence of carbon nanotubes can be added to the list of unique physical properties. A groundbreaking discovery in 2002 showed that dozens of varieties of semiconductor SWCNTs emitted their own unique fluorescent signature (Bachilo *et al.*, 2002; O'Connell *et al.*, 2002). Fluorescence occurs when a substance (or molecular probe) absorbs high-energy photons of a defined light source and emits a signal with different spectral characteristics (lower-energy photons) in response. The spectral characteristics can be resolved with an emission filter and captured by a high-sensitivity camera. Generally, laser sources are used because they offer power delivery at narrower and better-defined spectral windows. SWCNTs absorb and emit light in the near-infrared spectrum (700-1000 nm) where human tissue and biological fluids are particularly transparent (Wray *et al.*, 1988). In biological tissue, for instance leukocytes, SWCNTs retain their optical properties, and cell properties such as shape, rate of growth, and ability to adhere to surfaces, are not affected (Cherukuri *et al.*,

2004). Unlike most single molecules (Trautman *et al.*, 1994) or semiconductor nanoparticles (Nirmal *et al.*, 1996), the fluorescence of SWCNTs does not show any spectral or intensity fluctuations which could enable a stable infrared photon source.
Potential medical applications are envisaged in cancer therapy (see Section 4.2.1) and in fluorescence-based biosensors (see Section 4.3.2). Although long-term studies on toxicity and biodistribution must be completed before carbon nanotubes can be applied in medical tests, the fluorescence properties indicate that carbon nanotubes could be useful as imaging markers in laboratory *in vitro* studies, particularly in cases where the bleaching, toxicity and degradation of more traditional markers are problematic. Moreover, since carbon nanotubes fluoresce with a single wavelength of light, it may be possible to tailor different sizes of nanotubes and tune their electrical properties to seek specific targets, and thus diagnose multiple diseases in a single test. In addition to biomedical applications, fluorescence properties of carbon nanotubes could be useful in compact, easy-to-integrate nanophotonic devices.

3.1.4 Other carbon nanotube-based materials

Doped carbon nanotubes

Carbon atoms in all types of nanotubes can be substituted with another element such as boron and/or nitrogen. Synthesis of these B- and/or N-doped carbon nanotubes was first reported in 1994 (Stephan *et al.*, 1994). Besides partial substitution, carbon can be completely exchanged. An amazing result of such attempts is a sandwich-like structure made of multi-layered concentric nanotubes whose constituting coaxial tubes are alternatively made of carbon graphenes and boron nitride graphenes (Suenaga *et al.*, 1997). Synthesis techniques are similar to those used for the synthesis of pure carbon nanotubes, i.e. arc discharge, laser ablation, and chemical vapour deposition. For an overview see e.g., Ma *et al.* (2004). The benefits of doping are better control of electronic properties of nanotubes and for low concentrations of dopants (<0.5 %) mechanical properties do not deteriorate. For instance, B-doped MWCNTs exclusively exhibit metallic conductivity (Carroll *et al.*, 1998) in contrast to pure carbon nanotubes whose properties vary between metallic and semiconducting depending on helicity and diameter. Other benefits are enhanced field emission of B-doped (Charlier *et al.*, 2002) and N-doped MWCNTs (Golberg *et al.*, 2003) compared to undoped MWCNTs, i.e. turn-on voltages of ~1, 2, and 3 V/μm, respectively. Thus, both B- and N-doped carbon nanotubes may have great potential as building blocks for stable and intense field emission sources. Moreover, N-doped MWCNTs can be used as gas sensors exhibiting fast response (<1 s) and saturation within 2-3 s (Villalpando-Paéz *et al.*, 2004). N-doped MWCNTs are good candidates for the detection of low vapour concentrations of acetone, chloroform, and ethanol, important in the fabrication of alcoholmeters. Li-ion rechargeable batteries could also benefit from the N-doped MWCNTs (Mukhopadhyay *et al.*, 2002).

Endohedral carbon nanotubes

The inner cavity of SWCNTs or MWCNTs can be filled, partially or entirely, by other atoms, molecules, compounds, or crystals. Similar to the case of fullerenes such hybrid nanotubes are denoted as X@SWCNT or X@MWCNT where X is the atom, molecule, etc. involved. For the first time in 1998 SWCNTs were filled with C_{60} by thermally annealing C_{60} powders over SWCNTs at >600 °C under vacuum (Smith *et al.*, 1998). C_{60} molecules are arranged as a self-assembled chain within the SWCNT resembling a nanoscopic peapod. Endohedral fullerenes have also been introduced inside SWCNTs and more complex nanotube-based hybrid materials have been synthesized such as Gd@C_{82}@SWCNTs (Suenaga *et al.*, 2000). Nanotube-based hybrid materials could be applied in electronics and micro-electro-mechanical systems. In general, though, fullerenes, carbon nanotubes and peapods have yet to

find extensive (bio)medical applications, in part because of their extreme hydrophobicity, presumably poor biocompatibility, and high chemical stability. But controlled functionalization of these carbon nanomaterials may be used to modify these features (Sayes *et al.*, 2004).

Functionalised carbon nanotubes
Functionalisation of carbon nanotubes has important consequences for their properties and applications. Although covalent attachment of molecules on the nanotube sidewalls proves to be difficult, functionalisation by non-covalent adsorption of (biological) molecules is easier. Non-covalently functionalised SWCNTs preserve the sp^2 hybrid bonds (no bonds are broken) and thus the carbon nanotube electronic structure, and provide sites for selective binding (Chen *et al.*, 2001). The unique properties of carbon nanotubes when utilised in conjunction with biomolecular recognition capabilities (e.g., antibodies) could lead to miniature electronic devices, including probes and sensors. Properties and applications of functionalised carbon nanotubes have been reviewed recently (Sun *et al.*, 2002).

3.2 Inorganic nanomaterials

3.2.1 Inorganic fullerene-like molecules
Theoretical studies suggest that fully inorganic fullerene-like molecules comprising C_{60} analogs with silicon atoms and group 13/15 (e.g. boron-nitrogen) should be realisable, but experimental confirmation has been lacking. Recently, the synthesis and structural characterisation of soluble and entirely inorganic spherical fullerene-like molecules have been reported incorporating Cu, Cl, Fe, C, P, and N (Bai *et al.*, 2004). These molecules are currently only of scientific interest and no applications are yet foreseen.

3.2.2 Inorganic nanotubes
Synthesis
The synthesis of various inorganic nanotubes has been reported during the last few years (for a recent review see Tenne and Rao (2004)). The structure of inorganic nanotubes is comparable with the structure of carbon nanotubes. Some of the important inorganic nanotubes synthesized are chalcogenides, e.g. MoS_2 (Chhowalla and Amaratunga, 2000), oxides, e.g. TiO_2 (Kasuga *et al.*, 1998), nitrides, e.g. BN (Gleize *et al.*, 1994), halides, e.g. $NiCl_2$ (Hacohen *et al.*, 1998), and metals, e.g. Ni (Bao *et al.*, 2001). Synthesis techniques are similar to those of carbon nanotubes: arc discharge (Chhowalla and Amaratunga, 2000), and laser ablation (Parilla *et al.*, 2004). In addition, appropriate chemical reactions such as sol-gel chemistry are most successful techniques (Kasuga *et al.*, 1998). Sol-gel chemistry is a versatile route for synthesizing inorganic materials. This method involves hydrolysis of a molecular precursor followed by thermal treatment, typically in air.

Properties and applications
The physical properties of inorganic nanotubes are relatively less explored. For WS_2 nanotubes mechanical properties are lower compared to carbon nanotubes, i.e. Young's modulus is ~140 GPa and tensile strength ~13 GPa (Kaplan-Ashiri *et al.*, 2004). Electronic properties have been mostly studied in theory and experimental confirmations are scarce. Single-walled MoS_2 nanotubes are metallic, in contrast to bulk MoS_2 (Remskar *et al.*, 2003). Field emission currents are very reproducible and stable in bundles of iodine-doped single-walled MoS_2 nanotubes (Nemanic *et al.*, 2003). TiO_2 nanotubes may be useful as well-

adhered bioactive surface layers on titanium implant metals for orthopaedic and dental implants (Oh *et al.*, 2005), as well as for photocatalyst and other sensor applications (Park *et al.*, 2003) (see Section 4.5.1). Silica nanotubes have become atrtactive starting materials for creating multifunctional nanodevices, largely because the inside and outside of the nanotube can be modified independently from one another, for instance with a layer of magnetite (Fe_3O_4) nanoparticles (Son *et al.*, 2005). The resulting nanotubes display similar magnetic characteristics as magnetic silica nanoparticles and they can be used to facilitate and enhance biointeractions between the outer surfaces of magnetic nanotubes and a specific target surface. Potential medical applications are foreseen in multifunctional targeted drug delivery (see Section 4.10.4).

3.2.3 Inorganic nanowires

Synthesis

In contrast to nanotubes, nanowires have no inner cavity. Nanowire properties can differ distinctly from those of their corresponding crystalline bulk materials, though, some properties are similar. Nanowires can be synthesized using a large variety of materials such as metals, e.g. Ag (Braun *et al.*, 1998), semimetals, e.g. Bi (Zhang *et al.*, 1998), semiconductors, e.g. CdS (Routkevitch *et al.*, 1996), and superconductors, e.g. Zn (Li *et al.*, 2000). A plethora of articles have been published on the synthesis approaches. The most common synthesis methods are template-assisted synthesis (Martin, 1994), including vapour and electrochemical deposition, and vapour-liquid-solid growth, especially successful for semiconductor nanowires (Wu and Yang, 2000).

Properties

Various properties of nanowires, such as electrical, magnetic, thermoelectric, thermal conductivity, and optical, have been investigated. Device functionalities common in conventional semiconductor technologies have been demonstrated using nanowires, such as field-effect transistors (Cui and Lieber, 2001), p-n junction diodes and logic gates (Huang *et al.*, 2001), light-emitting diodes (Duan *et al.*, 2001; Gudiksen *et al.*, 2002), non-volatile memory and switches (Duan *et al.*, 2002), and oscillators (Friedman *et al.*, 2005) showing their promise as building blocks for the construction of complex integrated circuits. Next to the concept of construction of nanowire-based electronic devices is the development of a feasible method for integration, reliable mass production, effective assembly techniques, and quality-control methods. In order to maintain the growing rate of device density and functionality in the existing electronics industry, new kinds of complementary electronic devices will emerge from this “bottom-up” approach, different from what has been produced by the traditional “top-down” approach, i.e. lithography, as pursued by conventional electronics.

Applications

Nanowire sensors are an attractive application field enabling devices which will be smaller, more sensitive, demand less power, and react faster than macroscopic alternatives. Silicon nanowire field-effect transistor devices have been used as pH sensors (Cui *et al.*, 2001). The same approach, i.e. monitoring the nanowire conductance change due to molecule-nanowire interaction, was used for the detection of the binding of biomolecules such as streptavidin to biotin-modified Si nanowires (Cui *et al.*, 2001). The device has high sensitivity and could detect streptavidin binding down to 10 pM (pico (p) = 10^{-12}) concentration. An extension of this device concept to detect multiple analytes, could provide for fast, sensitive and *in situ* screening procedures (see Section 4.3.3 for more applications in medical technology).

Nanowires can be synthesized with controlled magnetic properties to achieve a variety of magnetic applications, including magnetic information storage as, potentially, the most interesting one (Thurn-Albrecht *et al.*, 2000). Nanowires can form stable and highly dense magnetic memory arrays.
Potential applications for nanowires are thermoelectric cooling and the conversion between thermal and electric energy (Chen *et al.*, 2003a). Although the application of nanowires to thermoelectrics seems very promising, these materials are still in the research phase of the development cycle and far from being commercialised.
Optical applications are also envisaged for nanowires. Light emission from p-n junctions is especially interesting for laser applications (Duan *et al.*, 2003). Photocurrent response to UV light irradiation suggests that ZnO nanowires could be a good candidate for optoelectronic switches (Kind *et al.*, 2002). Nanowires may also be used as barcode tags for optical read out (Nicewarner-Peña *et al.*, 2001). Nanowires have also been proposed for use in inorganic-organic solar cells (Huynh *et al.*, 2002). In addition, nanoscale light-emitting diodes with colours ranging from the ultraviolet to near-infrared region could be combined with microfluidics in lab-on-a-chip systems to produce highly integrated analytic systems that might enable applications ranging from high-throughput screening to medical diagnostics to be developed (Huang *et al.*, 2005).

3.2.4 Quantum dots

Quantum dots are spherical nano-sized crystals. They can be made of nearly every semiconductor metal (e.g., CdS, CdSe, CdTe, ZnS, PbS), but alloys and other metals (e.g. Au) can also be used (Alivisatos, 1996; Bailey and Nie, 2003; Zheng *et al.*, 2004a). The prototypical quantum dot is cadmium selenide (CdSe). Quantum dots range between 2 and 10 nm in diameter (10 to 50 atoms). Generally, quantum dots consist of a semiconductor core, overcoated by a shell (e.g., ZnS) to improve optical properties, and a cap enabling improved solubility in aqueous buffers (Figure 5).

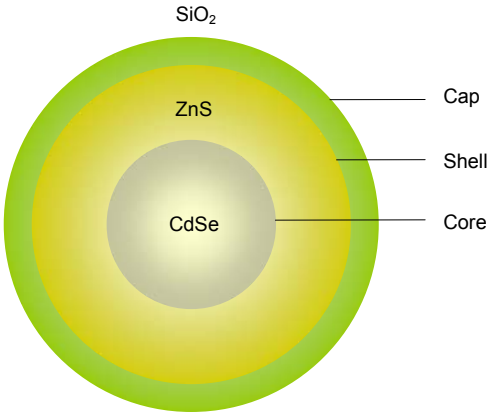

Figure 5. Schematic representation of a quantum dot. The cadmium selenide core is surrounded by a shell of zinc sulphide. Finally, a cap of silica encapsulates the binary quantum dot.The diameter of quantum dots ranges between 2-10 nm.

Synthesis

In the 1980s traditional lithography-based techniques (a combination of electron beam lithography and etching) were used to make quantum dots. However, these quantum dots are only in the nanometre scale in one dimension. The other two dimensions are limited by the

resolution of the lithography. In the early 1990s, quantum dots were mainly prepared in aqueous solution with added stabilizing agents. This procedure yielded low-quality quantum dots with poor fluorescence efficiencies and large size variations. From 1993 onwards, the high-temperature organometallic procedure was used for growing quantum dots (Murray *et al.*, 1993). This procedure yields nearly perfect crystal structures and narrow size variations, but the fluorescence is still relatively low. The deposition of a surface-capping layer such as ZnS or CdS was found to dramatically increase the fluorescence properties of CdSe nanocrystals (Hines and Guyot-Sionnest, 1996). The resulting quantum dots are highly hydrophobic and only soluble in nonpolar solvents. The art of quantum dot synthesis is evolving as alternative precursor materials, such as CdO, can be used to prepare high quality CdS, CdSe, and CdTe nanocrystals (Peng and Peng, 2001). In contrast to traditional binary quantum dots, and core/shell nanocrystals, the quantum dots synthesized show excellent quantum yields without an inorganic capping layer. The size of the quantum dot can be controlled by temperature (>300 °C) and period of time, ranging from minutes to hours depending on the desired particle size.

Properties

Quantum dots take advantage of the quantum confinement effect, giving these nanoparticles unique optical and electronic properties. A theoretical framework for these properties was already described in 1982 by two research teams in the former Soviet Union (Efros and Efros, 1982; Ekimov and Onushchenko, 1982). Fluorescence semiconductor quantum dots offer advantages in that they have a tunable absorption spectrum, which is very broad, extending from the ultraviolet to a cut-off wavelength in the visible spectrum. Emission is confined to a narrow band and can also be tuned. Absorption and emission characteristics are dictated by size for binary quantum dots or by composition/internal structure independently of size for alloyed semiconductor quantum dots, such as CdSeTe (Bailey and Nie, 2003). When illuminated, smaller binary quantum dots emit shorter wavelength, such as blue, whereas larger dots emit longer wavelength, such as red (Figure 6). Moreover, quantum dots have brighter emission and good photostability.

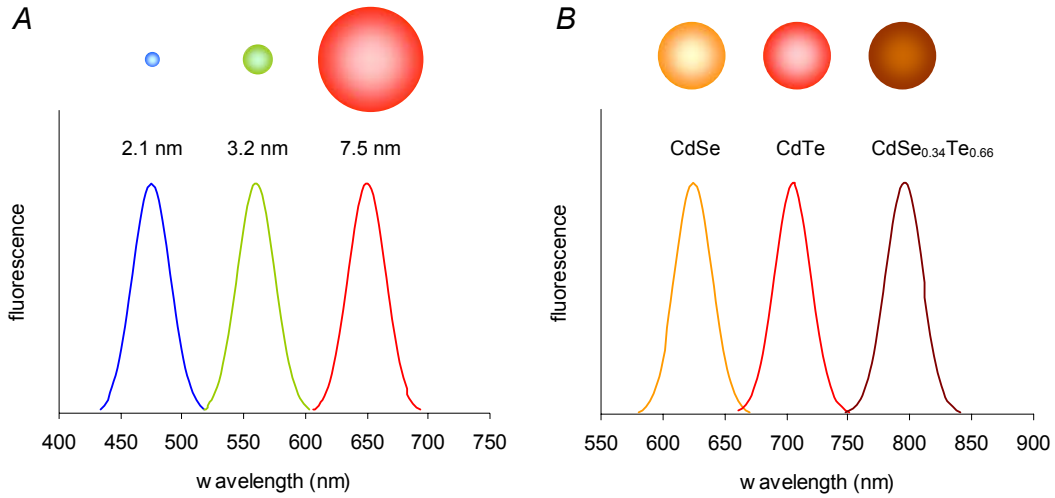

Figure 6. Optical properties of binary and alloyed quantum dots. Schematic drawings of three CdSe quantum dots with different diameters (A) and three quantum dots (mean diameter ~5 nm) with different composition (B) and their corresponding fluorescence emission spectra. Modified from Bailey and Nie (2003).

Quantum dots are rendered water-soluble using several synthesis strategies, such as water-soluble ligands (Chan and Nie, 1998), silanization (Bruchez *et al.*, 1998), organic dendrons (Wang *et al.*, 2002a), cysteines (Sukhanova *et al.*, 2002), dihydrolipoic acid (Jaiswal *et al.*, 2003), encapsulation with block-copolymer micelles (Dubertret *et al.*, 2002), with amphiphilic polymers (Wu *et al.*, 2003b), amphiphilic polymers conjugated with poly(ethylene glycol) (Ballou *et al.*, 2004), and surface coating with phytochelatin-related peptides (Pinaud *et al.*, 2004). All these synthesis strategies have effectively solubilized CdSe or CdSe/ZnS quantum dots. In addition, quantum dots can be conjugated to biological molecules such as proteins, oligonucleids, small molecules, etc. which are used to direct binding of the quantum dots to areas of interest for biolabelling and biosensing (Bruchez *et al.*, 1998; Chan and Nie, 1998). Quantum dot bioconjugates are often used as simple replacements for analogous conventional dye conjugates when superior performance is required to achieve lower limits of detection, more quantitative results, more photo-stable samples, or higher levels of multiplexability. In combination, these spectral properties, unmatched by any known organic dye fluorophore, permit the systematic generation of probes that have different biochemical specificities and can be excited and detected simultaneously. A variety of colours of quantum dots are now available commercially from Quantum Dot Corporation (Hayward, California, USA) and Evident Technology (Troy, New York, USA). Recently, Evident Technology has announced the introduction of the first commercially available non-heavy metal quantum dots for life science research. These new quantum dots, called T2-MP EviTags™, feature a ternary core consisting of indium gallium phosphide coated with a metallic plating shell and a natural coating on the outer layer. The T2-MP EviTags™ offer a potential range of benefits over traditional quantum dots, especially the possibility of lower toxicity, and a wider range of colours into the near infrared.

Applications

Biomedical monitoring applications have taken considerable advantage of using quantum dots for sensitive optical imaging in fixed cells and tissues, living cells and animal models (see Section 4.4.2). Electronic applications of quantum dots are envisaged in future high-speed electronic and photonic devices. Quantum dots provide a promising way forward for a new generation of lasers (Huang *et al.*, 2000), infrared photodetectors (Kim *et al.*, 2000), photovoltaic devices (Pan *et al.*, 2000), and optical data storage media (Son *et al.*, 2001).

3.2.5 Dendrimers

Dendrimers are synthetic, complex, and spherical molecules with very well-defined chemical structures first synthesized in the early 1980s (Newkome *et al.*, 1985; Tomalia *et al.*, 1985). The term dendrimers originates from “dendron” meaning tree in Greek. Other terms used were arborols from the Latin word “arbor” also meaning tree, or cascade molecule, but dendrimer is now the generally accepted term. From a polymer chemistry point of view, dendrimers are nearly perfect monodisperse macromolecules with a regular and highly branched three-dimensional, or fractal architecture. They consist of three major architectural components: core, branches and end groups at the periphery (Figure 7). The macromolecule constituents radiate in branching form from the central core, creating an internal cavity as well as a sphere of end groups that can be tailored according to requirements.

Synthesis

The first dendritic structures thoroughly investigated were polyamidoamine (PAMAM) dendrimers also known as starburst dendrimers. They were first synthesized by an iterative synthetic methodology (Newkome *et al.*, 1985; Tomalia *et al.*, 1985). The iterative sequence of reaction steps leads to a higher generation dendrimer after each iteration. The creation of

dendrimers, using specifically-designed chemical reactions, is one the best examples of controlled hierarchical synthesis, an approach that allows the "bottom-up" creation of complex systems. Each new layer creates a "new" generation, with double the number of end groups at the periphery and approximately doubles the molecular weight of the previous generation. The functional end groups can be modified for various purposes, including sensing, catalysis or biochemical activity. One of the most appealing aspects of technologies based on dendrimers is that it is relatively easy to control their size, composition, and chemical reactivity very precisely. As the branches growing from the core molecule become longer and more dispersed (in 4 and higher generations) dendrimers adopt a globular or spherical structure (Caminati *et al.*, 1990). Dendrimers become densely packed as they extend out to the periphery, which forms a closed membrane-like structure. When a critical branched state is reached dendrimers cannot grow anymore because of lack of space. This is called the "starburst" effect (Fischer and Vögtle, 1999). The dimensions of dendritic macromolecules are in the region of several to one hundred nanometres. The 10^{th} generation PAMAM contains 6141 monomer units and is ~12 nm in diameter (Tomalia *et al.*, 1990).

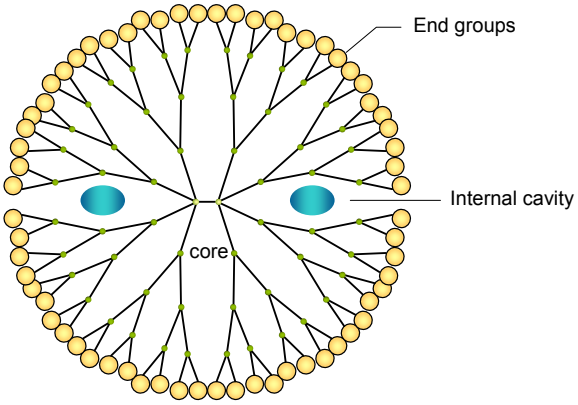

Figure 7. Schematic representation of a dendrimer. The dendrimer is built from an initiator core in sequential shells, called generations (depicted is a 4^{th} generation). The internal cavity of the "dendritic box" encapsulates quest molecules.

Properties

Dendrimers show some significantly improved physical and chemical properties compared to traditional polymers. In solution dendrimers form a tightly packed ball affecting their rheological properties, i.e. as molecular weight increases, viscosity increases to a maximum at the 4^{th} generation and subsequently decreases (Mourey *et al.*, 1992). The nature and quantity of the peripheral end groups strongly affect the solubility and reactivity of dendrimers (Fréchet, 1994). Dendrimers terminated in hydrophilic groups are soluble in polar solvents, while dendrimers having hydrophobic end groups are soluble in nonpolar solvents. Lower generation of dendrimers which are large enough to be spherical but do not form a tightly packed surface, have enormous surface areas in relation to volume, i.e. up to 1000 m^2/g (Alper, 1991). The presence of the internal cavity and globular shape enables encapsulation of guest molecules in the macromolecular interior (Jansen *et al.*, 1994). Small molecules or a probe can be trapped inside a "dendritic box". Subsequently, a shell is formed on the surface of the dendrimer by a chemical reaction of the end groups and the probe is encapsulated yielding a molecular container of nanoscopic dimensions. Opening of the box is achieved by hydrolysing, pH-triggered cleavage (Jansen *et al.*, 1995), or a photochemical reaction of the outer shell liberating the probe (Archut *et al.*, 1998). Dendrimers can act as

extremely efficient light-harvesting antennae (Gilat *et al.*, 1999). Absorbing dyes are placed at the periphery of the dendrimer and transfer the light energy to another chromophore located in the core. Biological properties are crucial for biomedical applications. Cationic dendrimers (e.g., amine-terminated PAMAM) are generally haemolytic and cytotoxic. The toxicity is generation-dependent and increases with the number of surface end groups (Roberts *et al.*, 1996). In rats anionic dendrimers, with carboxylate end groups, are not cytotoxic over a broad concentration range (Malik *et al.*, 2000).

Applications
Numerous potential medical applications are foreseen. Dendrimers have been applied in *in vitro* diagnostics for heart muscle damage, tested as contrast agents for medical resonance imaging (Wiener *et al.*, 1994), have been used as photocross-linkable "glue" to seal large corneal lacerations in ophthalmic surgery (Grinstaff, 2002), topical microbicide with activity against herpes simplex virus infection (Bourne *et al.*, 2000) and HIV-1 (Witvrouw *et al.*, 2000), used in drug delivery (e.g., Zanini & Roy (1998), Zhou *et al.* (1999), Twyman *et al.* (1999), Liu *et al.* (2000)), in targeting tumour cells (Wiener *et al.*, 1997), in gene therapy (Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 2000), and in boron neutron capture therapy for cancer treatment (Barth *et al.*, 1994) (see also Section 4.2 and 4.4.1).

3.2.6 Nanoporous material
Nanoporous materials are materials with holes less than 100 nm, although some interesting microporous materials in the submicron (>100 nm) scale exist. Therefore, the arbitrary "nano" limit of 100 nm should not be too strict. Nanoporous materials can be made with a variety of pore sizes, shapes and densities by varying the conditions of pore formation. A crucial feature of nanoporous materials is the increase of surface area of the material. In general, surface area increase of nanoporous materials improves catalytic, absorbent and adsorbent properties. Adsorbing is like absorbing but adsorbed material is concentrated on the surface rather than inside. Nanoporous materials can be made out of many substances such as carbon, silicon, silicates, ceramics, polymers, and minerals. Potential applications could be in environmental remediation and implant technology. For instance nanoporous silicon is instable and could be used as biodegradable material for medical implants including tissue engineered products, whether for structural support or drug delivery (see Section 4.10).

4. Novel medical applications

In the previous chapter an overview was provided of the state-of-the-art of nanomaterials in general and the impact it is expected to have on technological applications. In this chapter, those applications which are in the field of medical technology will be elaborated on. It will become clear from the diversity of subjects that nanotechnology is potentially influencing a very broad range of medical applications, ranging from traditional surgical aids such as blades or needles to advanced diagnostic tools such as biosensors or molecular imaging techniques and to implantable materials for orthopaedic, dental, and cardiovascular interventions.

4.1 Surgery

4.1.1 Conventional surgical tools

Surgical blades

The performance of surgical blades can be enhanced significantly when microstructured hard metal is coated with diamond and processed. Major advantages of the diamond nano-layers in this application are low physical adhesion to materials or tissues and chemical/biological inertness. In addition, diamond has a low friction coefficient decreasing the penetration force necessary. Medical specialities that could benefit most from this type of product enhancement are in the field of ophthalmic surgery and neurosurgery. Manufacturer GFD Gesellschaft für Diamantprodukte mbH (Ulm, Germany) has developed Diamaze PSD (Plasma Sharpened Diamond) which is on the market. This blade is plasma-polished which decreases the thickness of the coating from 5-25 µm to 0.5 µm and concurrently diminishes the surface roughness to 20-40 nm, approximately.

Suture needles

New suture needles for ophthalmic and plastic surgery are made of stainless steel incorporating nano-size particles (1-10 nm quasi crystals) by using thermal ageing techniques (Wilkinson, 2004). Such needles have good ductility, exceptional strength, and corrosion resistance. Currently, AB Sandvik Materials Technology (Sandvik, Sweden) is commercially distributing Sandvik Bioline 1RK91™ needles.

4.1.2 Minimally invasive surgery

Catheters for minimally invasive surgery

The development and use of catheters has greatly contributed to the advancement of minimally invasive surgery. For instance, intravascular devices are nowadays prominent in interventional cardiology. However, blood components give rise to thrombus formation on the surface of catheters and new materials are being tested to enhance the performance. Recently, MWCNTs have been incorporated as a filler in nylon-12 (matrix) for the fabrication of a nanotube-based polymer-reinforced catheter (Endo *et al.*, 2005a). The device was tested *in vitro* (coagulation test) and *in vivo* in dogs (thrombogenicity) and demonstrated reduced thrombogenicity and improved mechanical properties, i.e. better recovery to original shape, very good handling characteristics, and high resistance to fracture. Improved

electrostatic properties and the dense surface topology caused by the nucleation function of the carbon nanotubes has probably contributed to the antithrombotic property.

4.1.3 Optical nanosurgery

Optical tweezers

Single-gradient optical trap, often referred to as optical tweezers, is a powerful technique for non-invasive manipulation of micron-sized objects, including single living cells, organelles within cells, and viruses (Ashkin *et al.*, 1987; Ashkin and Dziedzic, 1987) or for nanomanipulation of biological molecules, e.g. DNA (Bennink *et al.*, 2001). Rather than using mechanical tools such as forceps or tweezers, light is applied to control the dynamics of objects or particles. When a continuous wave laser beam strikes an object, forces arising from the momentum of the light itself can precisely reposition objects by steering the laser beam. Although the technique has often been claimed to be non-intrusive, evidence suggests that certain wavelengths of near-infrared continuous-wave laser light from optical tweezers can produce stress in nematodes (*Caenorhabditis elegans)* by expressing an integrated heat-shock-responsive reporter gene (*Escherichia coli lacZ*) (Leitz *et al.*, 2002).

Femtosecond laser neurosurgery

Ultra-short pulse laser can perform extremely precise surgery and cut nano-sized cell structures as has been shown in nerve cells (Yanik *et al.*, 2004). Usually, conventional lasers first heat the target area, and then cut it, but this increases the risk for tissue damage. The advantage of "nanoscissors" is its ability to cut cell organelles without harming surrounding tissue. The technique uses a series of low-energy femtosecond (femto = 10^{-15}) near-infrared laser pulses and was investigated in anaesthetized *C. elegans.* Axons were cut by vaporization of 0.2-0.3 femtolitres, assuming an axon diameter of 300 nm. The low energy and short laser pulse should significantly reduce mechanical effects, i.e. plasma extension and shock waves, heat accumulation and extended thermal damage to surrounding tissue with respect to other laser-surgery techniques (Colombelli *et al.*, 2004). A remarkable finding was that nearly 50% of cut axons exhibited regrowth within 24 hours. The femtosecond laser axotomy technique could be used to investigate mechanisms affecting nerve cell regeneration and development. Other applications of femtosecond laser systems are in ophthalmology for corneal refractive surgery (Juhasz *et al.*, 1999) and dermatology (Kumru *et al.*, 2005).

4.1.4 Future directions

Nanotechnological tools such as optical tweezers used for cell manipulation and immobilisation, "nanoscissors" for cell compartment restructuring, and nanoneedles used as external communicators to deliver/withdraw substances (see Section 4.10.1) have been proposed to enable a novel "lab-in-a-cell" concept (Andersson and van den Berg, 2004) that could eventually lead to cell surgery.

4.2 Nanoparticles / platforms for cancer therapy

Well-established therapies commonly employed in cancer treatment include surgery, chemotherapy, immunotherapy, and radiotherapy. Nanotechnology offers tremendous opportunities to aid and improve these conventional therapies and could eventually offer new approaches, for example localised heating (see Section 4.2.1) or reactive oxygen generation (see Section 4.2.2). The power of nanotechnology is envisaged by the US National Cancer

Institute that is engaged in concerted funding efforts. The National Cancer Institute proclaims the elimination of suffering and death from cancer by 2015, definitely an ambitious goal.

4.2.1 Thermotherapy

In general, thermotherapy refers to hyperthermia as well as thermal ablation therapy. Hyperthermia therapy is based on the fact that tumour cells are more sensitive to temperature increase than normal tissue cells (Cavaliere *et al.*, 1967). It involves tumour heating to temperatures between 41-45 °C inducing almost reversible damage to cells and tissues. For thermal ablation therapy higher temperatures are applied, i.e. ranging from 50 °C to 70 °C, leading to the destruction of pathologically degenerated cells. In case of successful treatment, either the tumour disappears, diminishes, or at least stops growing. Thermotherapy can be applied as stand-alone therapy, i.e. thermal ablation, or as adjuvant therapy enhancing the efficacy of chemotherapy and/or irradiation (for a review see e.g. Wust *et al*. (2002)). Hyperthermia therapy in combination with immunotherapy could also offer feasible treatment of advanced malignancies (Ito *et al.*, 2003). Heating methods can use energy sources based on radiofrequency electric fields, magnetic fields, microwave, ultrasound, and optical applicators. In clinical settings radiofrequency electric field-induced heating is most commonly used, although application is still hampered due to factors of such as heterogeneity of tissue electrical conductivities, position of electrodes, adhesion of electrodes at uneven sites, and tumour size which make selective heating very difficult (Abe *et al.*, 1986).

Photo-thermal ablation therapy using silica nanoshells

Nanoshells can be used for photo-thermal ablation of tumour tissue as demonstrated both human breast carcinoma cells *in vitro* and in a murine model *in vivo* (Hirsch *et al.*, 2003; Loo *et al.*, 2004; O'Neal *et al.*, 2004). Nanoshells are layered, spherical nanoparticles consisting of a dielectric silica (SiO_2) core coated with a thin metal shell (Figure 8). These ceramic-based nanoparticles have a diameter of 100-200 nm and the coating is 10 nm thick. By manipulating the size of the silica core and the thickness of the gold shell, the plasmon resonance response can be tuned (Averitt *et al.*, 1997). This is a phenomenon whereby light induces collective oscillations of conductive metal electrons at the nanoshell surface. The nanoshell's plasmon resonance, in turn, determines the absorbing and scattering properties of the particle, so that the nanoshell can be selectively activated. Gold usually absorbs light from the visible to ultraviolet part of the electromagnetic spectrum, which can burn tissue. However, electromagnetic interactions between the gold nanoparticles change the property of the metal, making it absorb light from the near-infrared, which can easily penetrate several centimetres of (human) tissue without harming it. In the murine model poly(ethylene glycol)-passivated gold-coated silica nanoshells were injected interstitially ~5 mm into the tumour volume (tumour size 3-5.5 mm). Poly(ethylene glycol) is used to increase circulation time (Gref *et al.*, 1994) as well as to reduce non-specific attachment or uptake (Åkerman *et al.*, 2002). The blood vessels inside tumours develop poorly, allowing the nanoshells to leak out and accumulate inside tumours. Six hours after the injections an external laser source of near-infrared light (power density ~4-35 W/cm^2) is applied through the skin for 3-7 minutes. The gold nanoparticles readily absorb the energy and turn it into heat resulting in an average temperature increase of ~37 °C which induces irreversible cancerous tissue damage. The heating is localised and does not affect healthy tissue adjacent to the tumour. The mice remain cancer-free after the treatment, whereas growth of tumours in the control group, i.e. no treatment and sham group, continues rapidly (O'Neal *et al.*, 2004). It is also possible to attach biological markers, such as antibodies and proteins, to the nanoshells, in order to direct them to their target tissues (Loo *et al.*, 2005). This non-invasive localised thermal ablation

technology can be used to replace or supplement chemotherapy and surgery and is licensed to the company Nanospectra Biosciences, Inc. (Houston, Texas, USA).

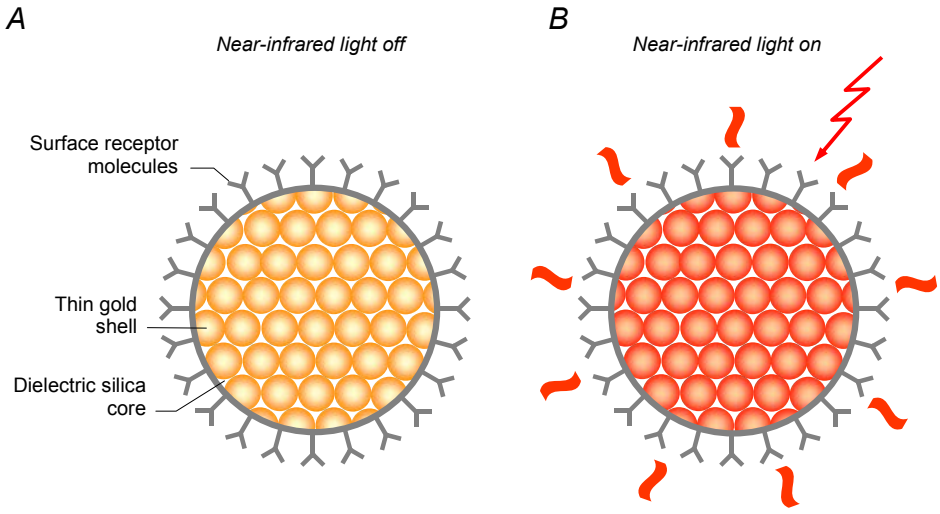

Figure 8. Photo-thermal ablation therapy using gold-coated silica nanoshells. Surface receptor molecules, e.g. antibodies, are used for targeting (A). Once accumulated inside a tumour, near-infrared light is used to activate the gold nanoparticles. The gold nanoparticles absorb near-infrared light turning it into heat which is lethal to cancer cells (B).

The optical properties of smaller silica shells (2-3 nm) have also been tested in theory (Sun *et al.*, 2004). The advantage of using smaller particles is that they can be inserted into any part of the human body to treat cancer cells in their infancy. The biocompatibility of silica and gold at the nanoscale has yet to be investigated, as gold particles become very reactive when they are reduced to a very small size, see also De Jong *et al*. (2005).

Photo-thermal ablation therapy using carbon nanotubes

Functionalised SWCNTs can achieve near-infrared light-triggered selective tumour cell destruction without harming normal cells *in vitro* (Kam *et al.*, 2005). Folate-functionalised SWCNTs are internalised inside HeLa tumour cells as the surface of these cancer cells is covered with abundant folate receptors. Continuous near-infrared light radiation by a laser (power density 1.4 W/cm^2) for 2 min causes excessive local heating and triggers cel death. Compared to the optical properties of other nanomaterials, such as gold-coated silica nanoshells (e.g., Hirsch *et al.*, (2003), SWCNTs are favourable with lower laser power and shorter radiation times necessary for effective tumour cell destruction. It should be noted that pulsed (six 10-s on-and-off pulses) laser radiation (1.4 W/cm^2) causes delivery of DNA-SWCNT conjugates without destroying cells. Hence, the optical and transporting properties of SWCNTs could lead to new classes of novel nanomaterials for drug delivery and cancer therapy.

Magnetic field-induced thermotherapy using magnetic nanoparticles

Another approach uses magnetic fields in conjunction with magnetic nanoparticles, such as superparamagnetic iron oxide nanoparticles (~15 nm in diameter) (Jordan *et al.*, 1999; Jordan *et al.*, 2001), paramagnetic copper-nickel alloy nanoparticles (~400 nm in diameter) (Bettge *et al.*, 2004), or magnetite (Fe_3O_4) cationic liposomes (~10-40 nm in diameter) (Shinkai *et al.*, 1996; Yanase *et al.*, 1997; Yanase *et al.*, 1998). These nanoparticles remain silent until

activated in the treatment zone by the application of a localised magnetic field. Once an alternating magnetic field is applied heat is generated within the nanoparticles providing selective heating to (cancerous) tissues loaded with the thermal agent only. The amount of heat generated depends on the type of particle, as well as the frequency and strength of the applied magnetic field.
MagForce Nanotechnologies GmbH (Berlin, Germany) has developed an AC magnetic field applicator (MFH® 300F) in conjunction with MagForce® nanoparticles for hyper-thermia treatment of (even deep-seated) brain tumours, also known as "magnetic fluid hyperthermia". The magnetic fluid consists of superparamagnetic iron oxide nanoparticles in aqueous solution administered by stereotactic navigation-based injection into brain tumour tissue. The iron oxide is covered by an aminosilane type shell. Due to the universal design of the magnetic applicator, it can be used for hyperthermia as well as thermal ablation treatment of malignancies in any part of the human body (Gneveckow *et al.*, 2004). Several clinical investigations, i.e. feasibility and efficacy studies, are being performed at the Charité University Hospital in Berlin including patients with tumours in different body parts, i.e. brain and prostate. The entire treatment takes approximately 2 hours, including the therapy with 60 minutes, heating periods and time for preparing the patient.
A more advanced approach is currently being developed by Triton BioSystems, Inc. (Chelmsford, Massachusetts, USA). A non-invasive targeted therapy for the treatment of solid breast tumours, referred to as the Targeted Nano-Therapeutics™ System, consists of polymer-coated superparamagnetic iron oxide nanoparticles bound to monoclonal antibodies about 40 nm long. This "bioprobe" is injected into the blood circulation, where the antibodies detect the unique chemical signature of cancer cells and bind to their membrane receptors. Triton BioSystems anticipates that it will start clinical investigations in 2006. With the product development still largely in the research phase, commercialisation is several years away.

4.2.2 Photodynamic therapy

Photodynamic therapy is an emerging treatment modality where a light-sensitive molecule or photosensitiser exposed to visible or near-infrared light induces cytotoxic effects in the presence of oxygen. When photosensitisers are irradiated, the excited molecules can transfer their energy to molecular oxygen. Two types of photodynamic reactions are observed. First, reactions in which electron of hydrogen-transfer occurs producing reactive oxygen species (ROS) or free radicals, such as superoxide (O_2^-), hydrogen peroxide, hydroxyl and hydroperoxyl radicals. Second, reactions in which an electron spin exchange occurs between the photosensitiser and triplet oxygen ($^3O_2$), resulting in the production of cytotoxic singlet oxygen ($^1O_2$). Singlet oxygen is accepted as the main mediator of photocytotoxicity in photodynamic therapy, causing irreversible cell damage by oxidation and degradation of (intra)cellular biomembrane structures, but with minimal systemic toxicity (e.g., Henderson and Dougherty (1992)).
Photodynamic therapy can be used to treat a variety of oncological, cardiovascular, dermatological, ophthalmic, and immunological disorders (Boehncke *et al.*, 1994; Donati *et al.*, 1999; Ortu *et al.*, 1992; Pass, 1993; Trauner and Hasan, 1996). Compared with conventional surgery, the approach is non-invasive, enables accurate targeting, repeated administration without total-dose limitations associated with radiotherapy, and results in little or no scarring after healing.
A common problem among many first and second-generation photosensitisers, e.g. Photofrin® I, Photofrin® II (Axcan Pharma Inc., Mont-Saint-Hilaire, Quebec, Canada), methylene blue, porphyrin, phthalocyanine, is the difficulty in preparing appropriate pharmaceutical formulations. The most potent photosensitisers are hydrophobic and poorly

water-soluble and therefore difficult to administer as such especially when intravenous injection is needed. This issue calls for the use of advanced delivery systems and different strategies have been investigated, which mainly include polymer-photosensitiser conjugation as well as encapsulation of the photosensitiser in colloidal carriers such as liposomes, oil-dispersions, and polymeric particles (Konan *et al.*, 2002). Recently, nanoparticles have received increasing attention as potential delivery systems for photodynamic therapy agents.

Quantum dots as photosensitisers and carriers

Quantum dots offer several advantages as potential delivery systems for photosensitisers. The optical properties of this nanomaterial can be tuned to absorb and emit in the near-infrared region of the spectrum by changing their size and composition. Light of low intensity can be used to penetrate tissue several centimetres allowing access to deep-seated tumours. Importantly, the surface coating of quantum dots can be functionalised to make them more water soluble and biocompatible (Bruchez *et al.*, 1998; Chan and Nie, 1998; Dubertret *et al.*, 2002), which facilitates systemic delivery.

Quantum dots can act as photosensitiser alone generating reactive singlet oxygen as well as promote the effect of classical photosensitisers linked to quantum dots (Figure 9). Close steric proximity between quantum dot and photosensitisers ensures a highly efficient fluorescence resonance energy transfer (FRET) and increased photosensitising power. The proof-of-concept of cadmium selenide (CdSe) quantum dot-based FRET to facilitate excitation of photosensitisers, such as phtalocyanines, has been demonstrated in oxygen-saturated toluene solution (Samia *et al.*, 2003). However, CdSe-generated singlet oxygen is rather low, i.e. 5% versus 43% reported for classical photosensitisers (He *et al.*, 1997). But the photobleaching of classical photosensitisers is rapid compared with that of quantum dots. Nevertheless, the prolonged and repetitive exposure of quantum dot-treated cells to irradiation may have the potential to mediate a high steady-state level of singlet oxygen, enough perhaps to induce apoptotic and/or necrotic cell death in the target tissue.

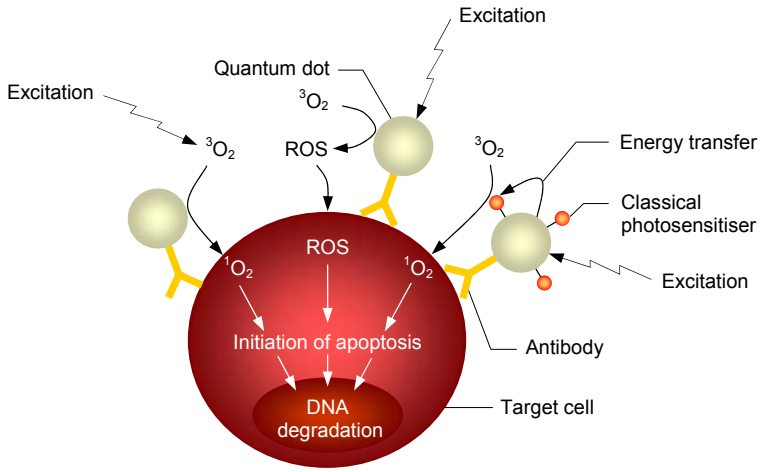

Figure 9. Schematic illustration of possible mechanisms for quantum dot-induced photodynamic therapy. Visible or near infrared light excites quantum dots conjugated with antibodies to provide targeting to specific cell types. Energy is transferred to triplet oxygen ($^3O_2$) generating radical oxygen species (ROS) or to a photosensitiser via fluorescence resonance energy transfer (FRET) to triplet oxygen producing singlet oxygen ($^1O_2$). ROS and singlet oxygen cause cytotoxic reactions in cells via apoptosis, a self-destructing mechanism of the cell itself. Reprinted with permission from Nature Publishing Group, modified from Bakalova et al. (2004).

Ceramic-based nanoparticles as carriers

Ceramic-based nanoparticles have the potential to act as delivery system for photosensitiser agents (Roy *et al.*, 2003; Yan and Kopelman, 2003). For example, silica-based nanospheres (~30 nm in diameter) doped with water-insoluble photosensitisers are efficiently taken up into the cytosol of tumour cells and generate singlet oxygen *in vitro* (Roy *et al.*, 2003). The size of the nanosphere is important because the lifetime of $^1O_2$ in aqueous media is in the microsecond domain, during which interval it can diffuse over a radial distance of at least 100 nm (Lindig *et al.*, 1980). Silica-based nanospheres are highly stable and are unlikely to release any embedded substances, although their porous matrix is permeable to triplet as well as singlet oxygen and subsequent light irradiation results in significant cell death. Experiments using silica-based nanospheres in tumour-model animals are in progress.

Other nanomaterials

Other approaches involve the incorporation/encapsulation of hydrophobic photosensitisers into sub-200 nm nanoparticles composed of biodegradable polymers (Konan *et al.*, 2003b), and polyacrylamide (Tang *et al.*, 2005), or photosensitiser-stabilised gold nanoparticles (Hone *et al.*, 2002). The photocytotoxicity of the polymeric nanoparticles has been evaluated on mouse mammary tumour cells *in vitro* (Konan *et al.*, 2003a), rat glioma tumour cells *in vitro* (Tang *et al.*, 2005), as well as on an *in vivo* chick embryo model (Vargas *et al.*, 2004) showing inhibition of tumour cell growth more effectively than free photosensitisers and selective destruction of chick embryo vasculature of the chorioallantoic membrane while protecting surrounding tissues. In addition, the biodegradable nanoparticles show an increased residence time in blood vessels that could diminish the doses of photosensitiser agents and might be useful to overcome the post-treatment accumulation of the free drug in the skin and the eye which may last for at least one month (Dillon *et al.*, 1988), and the adverse effects seen during photodynamic treatment of choroidal neovascularisation (Donati *et al.*, 1999) associated with age-related macular degeneration, one of the leading causes of blindness in elderly people in Western countries (Klein *et al.*, 1992). Furthermore, sterilisation of the polymeric nanoparticles by membrane filtration is feasible offering great advantages because many photosensitising agents may be denatured by heat or gamma sterilisation.

Nanoplatforms based on nanocomposite particles

A magnetic core of γ-Fe_2O_3 can be embedded within silica-based nanospheres functionalised with a targeting agent (Levy *et al.*, 2002). Applying a DC magnetic field results in a selective magnetocytolysis of targeted cells only (Bergey *et al.*, 2002). DC magnetic fields can be generated by medical magnetic resonance imaging devices and require less power compared to devices generating AC magnetic fields used for thermotherapy. Experiments involving the synthesis of functionalised magnetic nanoparticles as carriers of photosensitisers to develop a nanoplatform with “dual lethality” combining the photocytotoxic effect of the photosensitiser with the magnetocytolytic property are also in progress.

Recently, the synthesis of magnetic nanoparticles (γ-Fe_2O_3 crystallites, ~12 nm) and CdSe-based quantum dots (~3.5 nm) encapsulated in a silica shell (~50 nm in diameter) has been reported (Yi *et al.*, 2005). These materials have potential in combining targeting, bioimaging, biolabelling, and biosensing applications enabling novel platforms that are aimed at deployment for clinical applications in cancer research.

4.2.3 Chemotherapy

Nano-structured polymer capsules

Nano-structured polymer capsules could be used to deliver chemotherapy directly to tumours, leaving adjacent tissue intact (Radt *et al.*, 2004). The concept of the capsule is basically a templating core (~1 µm in diameter), which contains drug particles, surrounded by multi-layered polymer spheres with embedded light-absorbing gold nanoparticles (~6 nm in diameter) (Figure 10). A lipid bilayer and tumour-specific antibodies form an outer layer. When injected into the bloodstream, the nano-structured capsules will concentrate inside tumours. When a sufficient number of capsules have gathered in malignant cells, a low-energy pulse from a near-infrared laser is applied. A 10-nanosecond laser pulse is brief enough to heat the gold nanoparticles which swell up to 50 nm in diameter. The pulse is too short to damage the contents of the nano-structured capsules, but will melt the gold, rupture the polymer spheres and the nano-structured capsules will subsequently release their contents. In clinical use the laser could be targeted through the skin, or be beamed inside the body via an endoscope. The infrared energy (100 mJ/cm²) needed for the rupture of the nano-structured capsules is well within safety limits. The next refinement will be the down-sizing of the capsules from around 1 µm to a couple of hundred nanometres by using smaller drug particles. Clinical use and even animal tests are not yet within reach. However, photo-activating the capsules without damaging surrounding tissue is particularly interesting therapeutically.

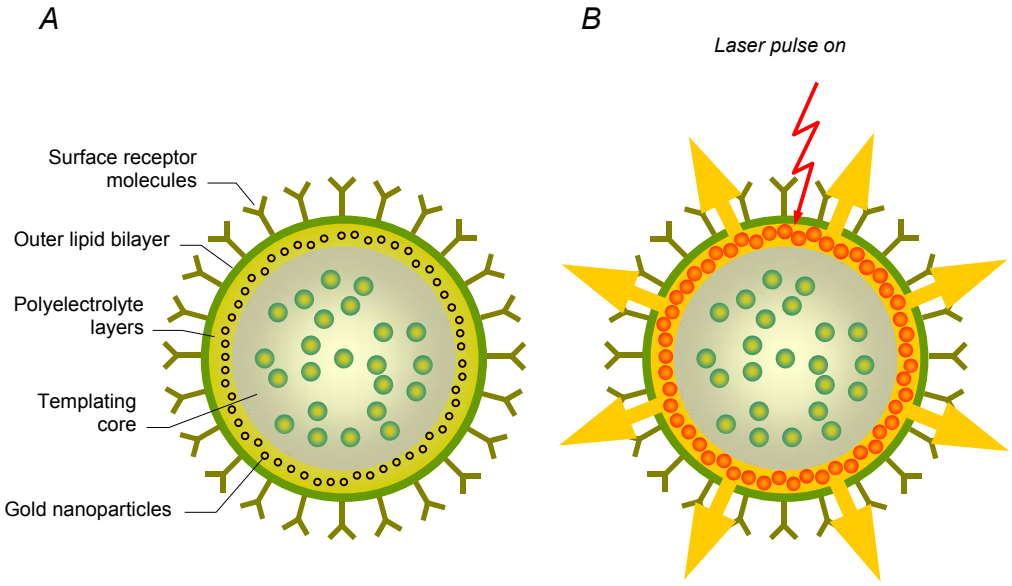

Figure 10. Schematic representation of an optically addressable nano-structured capsule for controlled delivery. A templating core with content is covered with a seven layer polyelectrolyte shell (A). The outer part of the capsule contains additional lipid bilayers with surface receptor molecules. Light-absorbing gold nanoparticles are embedded in the polyelectrolyte layers. A short laser pulse heats the gold nanoparticles which swell in diameter causing the rupture of the polymer capsule (B). Not drawn to scale.

Dendrimers

Therapeutic agents can be attached to or embedded in a dendrimer to direct the delivery. A more advanced concept of drug delivery has been explored independently by three research teams (Amir *et al.*, 2003; de Groot *et al.*, 2003; Li *et al.*, 2003c). Triggered by a specific

chemical signal, the dendrimer skeleton falls apart in a chain reaction of several steps, releasing the constituent molecules. Released drugs, e.g. paclitaxel, kill cancerous cells whereas the degradation products of the dendrimer skeleton are not cytotoxic (de Groot *et al.*, 2003). A possible risk is if the trigger is activated at the wrong time or place, in which case the result could be dangerous.

Nanocells

The fundamental challenges in cancer chemotherapy are its toxicity to healthy cells and drug resistance by cancer cells. In cancer therapy anti-angiogenesis therapy is an elegant concept based on the starvation of tumour cell by impairment of blood supply. However, lack of oxygen prompt tumour cells to release a cell signaling molecule known as hypoxia-inducible factor-1α (Semenza, 2000), which triggers metastasis (Pennacchietti *et al.*, 2003; Rofstad *et al.*, 2002) and the development of resistance to further chemotherapy (Blagosklonny, 2004; Yu *et al.*, 2002). An obvious solution would be combining chemotherapy and anti-angiogenesis. But this combination therapy confronts an inherent engineering problem. Long-term shutdown of tumour blood vessels by an anti-angiogenesis agent can prevent the tumour from receiving a chemotherapy agent. Also, the two drugs behave differently and are delivered on different schedules: anti-angiogenics over a prolonged period and chemotherapy in cycles (Kerbel and Folkman, 2002).

Recently, a multifunctional nanoparticle has been designed enabling this combination. The dual-chamber, double-acting, drug-packing "nanocell" (180-200 nm in diameter) proved effective and safe, with prolonged survival, against two distinct forms of cancers, i.e. melanoma and Lewis lung cancer, in mice (Sengupta *et al.*, 2005). Using two different widely studied biocompatible polymers, a balloon within a balloon was created, resembling an actual cell. The outer membrane of the nanocell, made of pegylated-phospholipid block-copolymer, was loaded with the anti-angiogenic drug combrestastatin. The inner balloon, composed of the biodegradable and nonbioactive poly-lactic-*co*-glycolic acid, was loaded with the chemotherapy agent doxorubicin. Pegylation of the outer membrane creates a "stealth" surface chemistry that allows the nanocells to evade the immune system. The size of the nanocells allows tumour cells to take them up preferentially compared to other (healthy) cells. Once the nanocell is inside the tumour, its outer membrane disintegrates, rapidly deploying the anti-angiogenic drug. The blood vessels feeding the tumour then collapse, trapping the loaded nanoparticle in the tumour, where it slowly releases the chemotherapeutic agent. In an *in vivo* mouse model the double-loaded nanocell demonstrates tumour inhibition, stops angiogenesis and avoids systemic toxicity much better than other treatment and delivery variations. Moreover, the nanocell works better against melanoma than lung cancer, indicating the need to systematically evaluate drug combinations and loading mechanisms for different cancers.

4.2.4 Radiotherapy

Dendrimers for boron neutron capture therapy

Dendrimers can be used in boron neutron capture therapy, which is an experimental approach to cancer treatment using a two-step process (Barth *et al.*, 1994). First, a patient is injected with a non-radioactive pharmaceutical which selectively migrates to cancer cells. This component contains a stable isotope of boron (^{10}B). Next, the patient is irradiated by a neutron beam of low-energy or thermal neutrons. The neutrons in the beam interact with the boron in the tumour causing the boron atom to split into an alpha particle (high-energy helium-4 nucleus) and a lithium-7 ion. Both of these particles have a very short range and destroy tumour cells in which it is contained. In order to sustain a lethal reaction a large number of boron atoms must be delivered to each cancer cell requiring selective delivery.

PAMAM dendrimers containing folic acid conjugates of boronated poly(ethylene glycol) units have been prepared to target folate receptors abundantly expressed in a variety of human tumours (Shukla *et al.*, 2003). These i*n vitro* studies showed receptor-dependent uptake of the dendrimer conjugates.

Carbon nanotubes for boron neutron capture therapy
Recently, water-soluble SWCNTs with appended C_2B_9 units have been shown to be promising nanovehicles for boron delivery to tumour cells *in vitro* (Yinghuai *et al.*, 2005). Tumour tissue shows enhanced accululation and retention of these modified SWCNTs. The actual mechanism of acculumation has not yet been determined.

Gold nanoparticles
Intravenous injection of gold nanoparticles (~2 nm in diameter) can enhance radiotherapy (X-rays) and results in eradication of subcutaneous mammary tumours in mice (Hainfeld *et al.*, 2004). One-year survival is 86% versus 20% with X-rays alone. Apparently, gold nanoparticles are non-toxic to mice and are cleared from the body through the kidneys.

4.2.5 Future directions in cancer therapy
Future efforts in cancer therapy are envisaged to be driven by multi-functionality and modularity, i.e. creating functional modalities that can be assembled into nanoplatforms and can be modified to meet the particular demands of a given clinical situation. Proof-of-principle has already been demonstrated for dendrimer-based nanoplatforms (Choi *et al.*, 2005; Choi and Baker, 2005; Patri *et al.*, 2004; Quintana *et al.*, 2002; Thomas *et al.*, 2004; Thomas *et al.*, 2005) and nanoshell-based platforms (Loo *et al.*, 2005) in cell studies *in vitro*. In addition, promising results have been obtained using dendrimer-based nanoplatforms as effective treatment in a murine model *in vivo* (Kukowska-Latallo *et al.*, 2005).
These nanoplatforms are independently coupled to targeting, and imaging/monitoring modalities, and can deliver selectively therapeutics intracellularly for growth suppression. Targeting modalities can be based on the recognition properties of cell-surface receptor ligands, monoclonal antibodies, nanobodies, or aptamers. Nanobodies are the smallest fragments of naturally occurring heavy-chain antibodies that have evolved to be fully functional in the absence of a light chain (Hamers-Casterman *et al.*, 1993) and have shown effective targeting in adenocarcinoma tumour-bearing mice (Cortez-Retamozo *et al.*, 2004) and have attracted attention as nanoparticles crossing the blood-brain barrier. Aptamers are DNA or RNA oligonucleotides that fold by intramolecular interaction into unique three-dimensional conformations capable of binding to target antigens with high affinity and specificity (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Aptamers are quickly emerging as a new powerful class of ligands that rival with antibodies in their potential for diagnostic and therapeutic applications (Brody and Gold, 2000). Recently, nanoparticle-aptamer bioconjugates have been developed and demonstrated proof-of-concept for targeted delivery in prostate cancer cells (Farokhzad *et al.*, 2004). The imaging/monitoring modalities can be based on traditional fluorophores, or quantum dots and superparamagnetic iron oxide nanoparticles. These constructed nanoplatforms would localise to target cells and could deliver their therapeutic payload with great precision where they are likely to be most effective, that is within the cell or even within specific organelles. Not only anticancer drugs, but also physically triggered chemical substances, such as photosensitisers, superparamagnetic iron oxide nanoparticles, or isotopes, can be applied as therapeutic agent. In particular, photodynamic therapy would benefit from the embodiment of a nanoscale light-generating system, such as the luceferin-luciferase pair, in such a way as to trigger light production only after take up by targeted cells (Theodossiou *et al.*, 2003). If successful, such

an approach would greatly extend the usefulness of photodynamic therapy to include treatment of tumours deep within the body.
Currently, NanoCure™ Corporation (Ann Arbor, Michigan, USA) is developing dendrimer-based nanoplatforms capable of delivering therapeutics (drugs and genes) to specific targeted cells coupled with imaging/monitoring modality. The initial target applications for this technology are cancers of the neck, breast and prostate. The proof-of-principle for treatment of cancer tumours of the neck has been demonstrated in animal models. Availability for clinical investigations is anticipated within three years. Additional research is ongoing to complete the proof-of-principles for breast and prostate cancer.
Thus, the modular approach to nanodevice constructions may allow for "smart" nanomolecular therapeutics that would facilitate the concept of non-intrusive sensing, signalling, and intervention for cancer. In addition, the approach may allow for interchangeable therapeutic nanoplatforms enabling new refined non-invasive procedures that can potentially be more powerful than current treatment modalities, but is inherently more complex than existing small molecule or protein therapeutics.
It is anticipated that most efforts will generate products in clinical investigations or even in clinical use within five to ten years. More difficult technological and biological problems or the integration of multiple technological components will require at least five years but have the potential of making paradigm-changing impacts on detection, treatment and prevention of cancer. Clinical investigations with targeted sensitisers (heat, magnetic field, light, radiation) are expected to start within one to three years and preliminary clinical assessments could be available within at least three to five years.

4.3 Biosensors / biodetection

A biosensor is generally defined as a measurement system that consists of a probe with a sensitive biological recognition element, or bioreceptor, a physicochemical detector component, and a transducer in between (see Box 2). A nanobiosensor or nanosensor is a biosensor that has dimensions on the nanometre size scale. Nanosensors could provide the tools to investigate important biological processes at the cellular level *in vivo*. Two types of nanosensors with medical application possibilities are cantilever array sensors and nanotube/nanowire sensors.

4.3.1 Cantilever array sensors

Microfabricated cantilever array sensors are used as ultra-sensitive mechanical sensors converting (bio)chemical or physical processes into a recordable (electrical) signal in microelectromechanical systems (MEMS) or nanoelectromechanical systems (NEMS) (see Appendix C). Cantilevers are typically rectangular-shaped silicon bars. The unique feature of microcantilevers is their ability to undergo bending due to molecular adsorption or binding-induced changes in surface tension. The major advantages of such miniaturised sensors are their small size, fast response times, high sensitivity, and direct transduction without the need for any labels.

Cantilever sensors for diagnosis of diabetes mellitus

Medical applications of cantilever-based sensors have been proposed for early diagnosis of diabetes mellitus (Lang *et al.*, 2002) and can improve blood glucose monitoring using small and ultra-sensitive analytical platforms (Pei *et al.*, 2004; Yan *et al.*, 2004). In patients with diabetes mellitus, ketones are produced due to the deterioration of blood insulin concentrations. Acetone is one of these ketones which is excreted in urine or expired as

vapour in exhaled air. Disposable test kits are used to detect acetone in urine. Acetone in exhaled air can only be detected by the physician as a putrid smell without any quantification. Small amounts of acetone in a patient's breath can be detected by cantilever array sensor technique which may attribute to early diagnosis of diabetes mellitus.

Box 2. Principle of a biosensor

A biosensor consists of three parts: the sensitive biological element (bioreceptor), the transducer, and the detector element. The interaction of an analyte, e.g. a particular chemical component, virus or micro-organism, with the bioreceptor is designed to generate an effect picked up by a transducer, which converts the information into a measurable effect by the detector, for instance an electric signal. Bioreceptors are used because of their specificity. They enable measurement with minimum interference from other components in complex mixtures. The bioreceptor is a biological molecule (e.g., an antibody/antigen, DNA, protein, or enzyme), or a living biological system (e.g., cells, tissues, or whole organisms) that utilises a biochemical mechanism of recognition. The sampling component of a biosensor contains a bio-sensitive layer that can either contain bioreceptors or be made of bioreceptors covalently attached to the transducer. Transduction can be accomplished by optical, electrochemical, and mass detection methods.

Cantilever sensors for bacteria, fungi, viruses

Devices have also been developed to detect bacteria (Ilic *et al.*, 2001), fungal spores (Nugaeva *et al.*, 2005), and viruses (Gupta *et al.*, 2004). The interaction between specific antibodies, for instance antibodies to *Escherichia coli*, immobilised on the surface of the cantilever, and antigens on cell membrane surface results in additional mass loading detected by the device. The detection sensitivity is in the order of a single bacterium corresponding to a mass of ~1 pg (pico (p) = 10^{-12}), single fungal spore, and single vaccinia virus particles corresponding to a mass of ~10 fg (femto (f) = 10^{-15}). Cantilever arrays allow detection of vital functionalised fungal spores *in situ* within ~4 hours, which is more than ten times faster than current applied procedures for fungal detection (Nugaeva *et al.*, 2005).
Recently, a NEMS device with molecular recognition for virus particle detection has been developed, allowing improvement of the detection sensitivity up to 6 bound baculovirus particles (Ilic *et al.*, 2004b). Once these devices with on-chip antibody-based recognition are integrated with sample concentrators, nanomechanical oscillators may prove to present a viable strategy for ultra-sensitive detection of airborne bacteria, fungi, and virus particles.

Cantilever sensors for cancer diagnosis

Cantilever arrays can aid cancer diagnosis and can be engineered to bind to molecules associated with cancer, such as DNA sequences (Fritz *et al.*, 2000; Su *et al.*, 2003), single-nucleotide polymorphisms (Fritz *et al.*, 2000; Hansen *et al.*, 2001), and proteins (Wu *et al.*, 2001a; Wu *et al.*, 2001b). When the cancer-associated molecules bind to the cantilevers, changes in surface tension cause the cantilever to bend (Figure 11). By monitoring whether or not the cantilevers are bending, the presence of cancer-associated molecules can be demonstrated. Significant bending should be evident when the molecules are present in very low DNA concentrations (femtomoles detection). Recently, the mass detection limitation of NEMS cantilevers has been improved to the enumeration of a single DNA molecule consisting of ~1600 base pairs and weighing ~1000 kD, which is ~1 ag (atto (a) = 10^{-18}) (see Box 3) (Ilic *et al.*, 2005). The cantilever technology could be useful in high-throughput nanomechanical genomic analysis and proteomics detecting early molecular events in the development of cancer. Microcantilever-based, multiplexed DNA assays to detect mutations have recently been introduced (Chen *et al.*, 2004). The specificity and sensitivity of these arrays do not yet offer substantial advantages over conventional detection methods, although the use of nanoparticle probes might allow for individual single-pair mismatch

discrimination. Rather, the breakthrough potential of micro- and nanomechanical cantilevers resides in their extraordinary multiplexing capabilities. It is realistic to envision arrays of thousands of cantilevers constructed on individual centimetre-sized chips, enabling the simultaneous reading of proteomic profiles or, ultimately, the entire proteome.
Currently, Protiveris Inc. (Rockville, Maryland, USA) is developing the VeriScan™ 3000 System, a microfluidic optical reader that utilizes arrays of microcantilevers with customizable surfaces to measure distinct biomolecular interactions, including interactions between proteins, antibodies, antigens, or DNA. In Europe, the Optonanogen project is aiming to develop a protable device using an array of 20 cantilevers and a microfluid header, which are due to be low-cost components that would be disposable. Optonanogen is a consortium of research institutes (Spain, France and UK) and a spin-off company (Senia SL, Madrid, Spain).

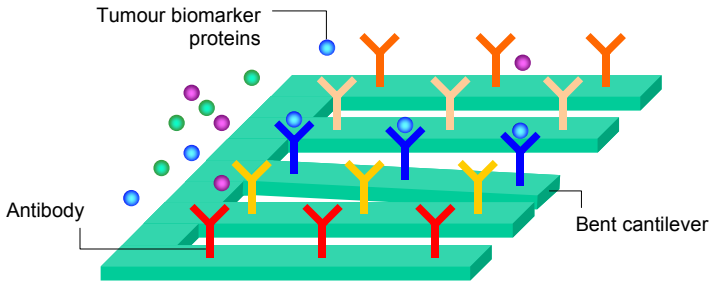

Figure 11. Cantilever array sensor. The biomarker are affinity-bound to the cantilevers and cause them to bend. The deflections of the cantilever beams can be directly observed with lasers. Alternatively, the shift in resonant frequencies caused by the binding can be electronically detected. The breakthrough potential in cantilever technology is the multiplexing modality, i.e. the ability to sense a large number of different proteins at the same time, in real time. Reprinted with permission from Nature Publishing Group, modified from Ferrari (2005).

Box 3. Mass unit of DNA, protein, and other organic molecules

The mass of DNA, proteins and other organic molecules is usually expressed in Daltons (D). A Dalton, also known as an atomic mass unit, is roughly the mass of a single proton or neutron. In relation to other units of mass, a Dalton is one-thousandth of a zeptogram, which is one-thousandth of a attogram, which is one-thousandth of a femtogram.

4.3.2 Nanotube-based sensors

Nanotube-based sensors for blood glucose monitoring

Carbon nanotubes are promising sensing candidates to monitor glucose in blood and urine. MWCNTs as well as SWCNTs have been used to develop enzymatic amperometric biosensors (Joshi *et al.*, 2005; Sotiropoulou and Chaniotakis, 2003; Wang *et al.*, 2003) or fluorimetric biosensors (Barone *et al.*, 2004). The enzyme glucose oxidase is either immobilised inside MWCNTs or non-covalently attached to the surface of SWCNTs enabling the catalysis of glucose with hydrogen peroxide as co-product. For the amperometric biosensor the enzyme immobilisation allows for the direct electron transfer from the enzyme to a gold or platinum transducer producing the response current. The fluorescence biosensor could be used in a new type of implantable biological sensor such as near-infrared nanoscale

sensor. This sensor could be inserted into tissue, excited with a laser pointer, and provide real-time, continuous monitoring of blood glucose levels. It consists of protein-encapsulated SWCNTs functionalised with potassium ferrocyanide, a substance that is sensitive to hydrogen peroxide. The ferrocyanide ion adsorbs on the surface through the porous monolayer. When present, hydrogen peroxide will form a complex with the ion, which changes the electron density of the carbon nanotube and consequently its optical properties. The more glucose that is present, the brighter the carbon nanotube will fluoresce. The sensor can be loaded into a porous capillary and inserted into tissue. As carbon nanotubes do not degrade like organic molecules that fluoresce, these nanoparticle optical sensors would be suitable for long-term monitoring applications. Proof-of-concept studies to detect glucose levels have been performed *in vitro*, i.e. in blood samples. Practical use is five to ten years ahead, according to the researchers (Barone *et al.*, 2004).
Self-assembled peptide nanotubes can be used in an electrochemical biosensor (Yemini *et al.*, 2005). The presence of the peptide nanotubes improves the sensitivity of the device several fold. Peptide nanotubes offer several advantages over carbon nanotubes, since they are biocompatible, water-soluble, inexpensive, easy to manufacture, and can be chemically modified by targeting their amino or carboxyl groups. The sensing technique can be used as a platform for ultra-sensitive detection of biological and chemical agents.

Nanotube-based sensors for DNA detection
MWCNT-based nanoelectrode arrays embedded in SiO_2 matrix have been integrated into a electrochemical system for ultra-sensitive and rapid DNA detection (Cai *et al.*, 2003; Koehne *et al.*, 2004; Li *et al.*, 2003b). A bottom-up approach is used for the fabrication of individually addressed nanoelectrode arrays, that results in precisely positioned and well aligned MWCNT arrays on a silicon wafer. Subsequently, the open ends of MWCNTs are functionalised with oligonucleotide probes. Combining the nanoelectrode arrays with redox-active molecule-mediated (e.g., $Ru(bpy)_3^{2+}$) guanine oxidation, the hybridisation of less than a few attomoles of oligonucleotide targets ($\sim 3.5 \times 10^6$ DNA molecules) can be easily detected by voltametric measurement. The proof-of-concept has been demonstrated for clinical relevant DNA molecules related to wild-type alleles associated with cancer genes (Li *et al.*, 2003b). Furher optimisation of the system could yield detections below one attomole.

Nanotube-based sensors for capnography
Carbon nanotube-based chemical gas sensors have great potential in medical applications. Currently, Nanomix Inc. (Emeryville, California, USA) is developing a medical capnography sensor using polyethylene-imine-coated carbon nanotubes. Capnography is the measurement of carbon dioxide concentration in human respiration and is a indicator of patient status during administration of anaesthesia. The tiny, low-power sensor will be the first disposable electronic capnography sensor and has the potential to extend the reach of quantitative respiratory monitoring beyond the operating room and into ambulatory and emergency settings as well as doctors' offices.

Other carbon nanotube-based biosensors
Various applications have been reported illustrating the broad potential of carbon nanotube-based biosensors, such as biosensing platforms for the simultaneous detection of dopamine and ascorbic acid for the diagnosis of Parkinson's disease (Jiang *et al.*, 2004; Wang *et al.*, 2002b), and dopamine and serotonin (Wu *et al.*, 2003a), and a nitric oxide radical biosensor (Wang *et al.*, 2005c). Recently, a more generalized approach for enzyme-based biosensors has been demonstrated by immobilising enzymes in redox hydrogels incorporating SWCNTs (Joshi *et al.*, 2005).

4.3.3 Nanowire-based sensors

Nanowire-based electrical detection of single viruses

Semiconducting silicon nanowires can be configured as field-effect transistors for the electrical detection of viruses in solutions (Patolsky *et al.*, 2004) (see Section 3.2.3). When a single charged virus binds to receptors (e.g., antibodies) linked to the nanodevice the conductance of a semiconducting nanowire changes from the baseline value, and when the virus unbinds, the conductance returns to the baseline value (Figure 12). The conductance of a second nanowire device without receptors should show no change during the same time period and can serve as an internal control. Nanowires are confined to a central region that is coupled to a microfluidic channel for sample delivery and the conductance response can be recorded while solutions with viruses flow at a constant rate. Modification of different nanowires within an array with receptors specific for different viruses provides a means for simultaneous detection of multiple viruses at the single particle level. The potential of nanowire-based electrical detection of viruses exceeds the capabilities of other methods such as polymerase chain reaction-based assays (Gardner *et al.*, 2003) and micromechanical devices (Gupta *et al.*, 2004).

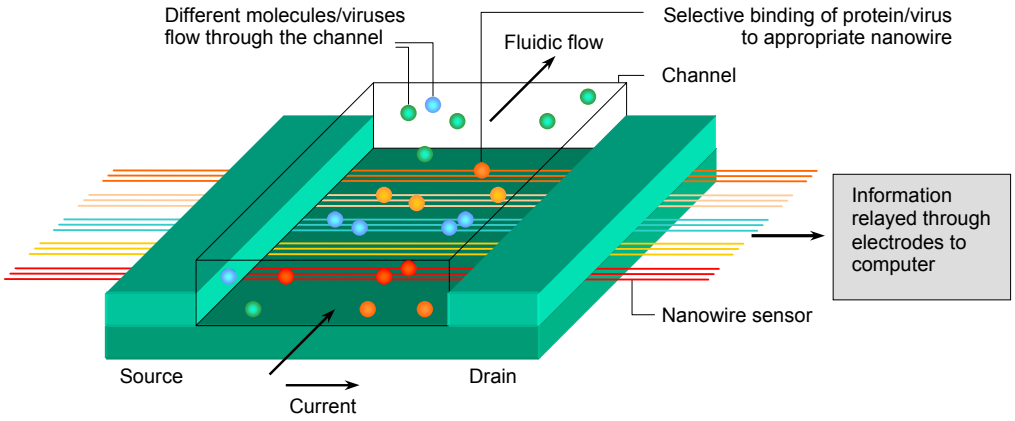

Figure 12. Nanowire-based sensors deployed within a microfluidic system. Different colours indicate that different molecules/viruses adsorb or affinity-bind to different nanowire sensors. The binding causes a change in conductance of the wires, which can be electronically and quantitatively detected in real time. The working principle is that of a (biologically) gated transistor. The nanosize of the wire is required to attain high signal-to-noise ratios. Reprinted with permission from Nature Publishing Group, modified from Ferrari (2005).

Nanowire-based electrical detection of biomolecules

Silicon nanowire field-effect transistor devices have been used for detection of small-molecule inhibitors of ATP binding to AbI, which is a protein kinase whose activity is responsible for chronic myelogenous leukaemia (Wang *et al.*, 2005a). In addition, real-time, labelfree detection of DNA and DNA mismatches is also feasible (Hahm and Lieber, 2004). Silicon nanowire sensors functionalised with peptide nucleic acid receptors can distinguish wild-type from the mutation type in the cystic fibrosis transmembrane receptor. Cystic fibrosis is one of the most common fatal genetic diseases among populations of European origin.

4.3.4 Other optical-based sensors

Normal Raman spectrometry detects physiological concentrations of glucose *in vitro* from a simulated aqueous humour solution, in serum and in blood, though high laser power and long acquisition time render normal Raman spectrometry clinically not practicable. However, surface-enhanced Raman spectrometry possesses many advantages allowing chemical analysis of in vivo molecular substances including high specificity, micromolar to picomolar concentration sensitivity, and interfacial generality. For the first time the concept-of-proof toward the development of a glucose sensor using surface-enhanced Raman spectroscopy has recently been demonstrated (Shafer-Peltier *et al.*, 2003). Glucose is partitioned into an alkanethiol monolayer (~2 nm thick) adsorbed on a silver film (200 nm thick) over nanosphere surface (polystyrene latex spheres ~390 nm in diameter). Spectra are measured from backscattered laser light indicating the glucose concentration. On the long term, the surface-enhanced Raman spectrometry substrate can be miniaturised to a microscale of even nanoscale device that can be implanted subcutaneously or can be incorporated as a component of a prosthetic lens in the eye with little or no discomfort to diabetic individuals.

4.3.5 Nanoarray-based biodetection

Ultra-sensitive virus detection

Viruses in human blood samples, such as HIV-1, can be detected using nanoscale antibody array-based devices (Lee *et al.*, 2004). Dip-pen nanolithography was used to pattern 16-mercaptohexadecanoic acid into an array of 60 nm dots on a gold thin film. Monoclonal antibodies to the HIV-1 p24 antigen were immobilised on the dots. The analysis consists of immersing the array for one hour in a blood plasma sample. Subsequently, the signal from the antigen-array binding was amplified using gold nanoparticles probes functionalised with polyclonal antibodies in a solution for one more hour. A measurable amount of HIV-1 p24 antigen in blood plasma from humans with less than 50 copies of RNA/ml is feasible demonstrating that nano-based assays can far exceed the 5 pg/ml (pico (p) = 10^{-12}) detection limit of conventional enzyme-linked immunosorbent assays and provide sensitivity comparable to a polymerase chain reaction-based assay, without target amplification. Nano-based array biodetection could enable HIV-1 diagnosis in mother-to-child transmission.

4.3.6 Nanoparticle-based biodetection

Ultra-sensitive detection of pathogenic biomarkers

One of the major drawbacks of conventional protein or antigen detection methods (e.g., enzyme-linked immunoassays, blotting assays) is the relative insensitivity for the target. Ultra-sensitive tests are needed for patient screening and diagnosis in the early stage of diseases enabling detection of very low concentrations of pathogenic biomarkers and conclusive confirmation of the disease in living patients.

Recently, an ultra-sensitive bio-bar code assay has been developed for the detection of protein/antigen analytes at clinically relevant attomolar (atto = 10^{-18}) concentrations which is five to six orders of magnitude less compared to conventional clinical assays (Nam *et al.*, 2003). The bio-bar code assay uses two types of probes: gold nanoparticle (13-30 nm in diameter) probes heavily functionalised with hundreds of identical hybridized oligonucleotides (DNA strands or “bar-code DNA” acting as an identification label) and polyclonal antibodies, and magnetic microparticle (1-µm diameter polyamine particle with magnetic iron oxide core) probes functionalised with monoclonal antibodies. The polyclonal and monoclonal antibodies recognize and bind to the same target protein, sandwiching the protein between the nano- and microparticle (Figure 13). After the “sandwich” is removed magnetically from the solution, the bar-code DNA strands are released and read using

standard DNA detection methodologies. The increased sensitivity of the assay derives mainly from the very effective sequestration of the protein/antigen and the amplification process that occurs as a result of the large number of barcode DNA strands (for 13 nm nanoparticles, each nanoparticle can support up to 100 strands of DNA) released for each recognition and binding event.

The bio-bar code assay technology has been tested to detect very low concentrations free of prostate-specific antigens (Nam *et al.*, 2003). Prostate-specific antigens are associated with prostate and breast cancer. In women with breast cancer, free prostate-specific antigen is found in serum at much lower concentration than in men and it is being explored as a breast cancer screening target (Black *et al.*, 2000). Recently, the bio-bar code assay technology has successfully been applied for the first time to detect amyloid-β-derived diffusible ligands in cerebrospinal fluid of living patients with Alzheimer's disease (Georganopoulou *et al.*, 2005). Amyloid-β-derived diffusible ligands are found in brain tissue of individuals with Alzheimer's disease where they cause neurological damage (Gong *et al.*, 2003), but ligand concentrations in blood were too low to be detected until now. The bio-bar code assay technology can be used to identify these markers before symptoms develop and the disease may be treated in its nascent form when treatments may be most effective. In fact, the assay could be extended to potential applications such as blood screening concerning HIV, prions (Creuzfeldt-Jakob disease (CJD) and variant CJD agents), many forms of cancer, and certain cardiac and pulmonary markers.

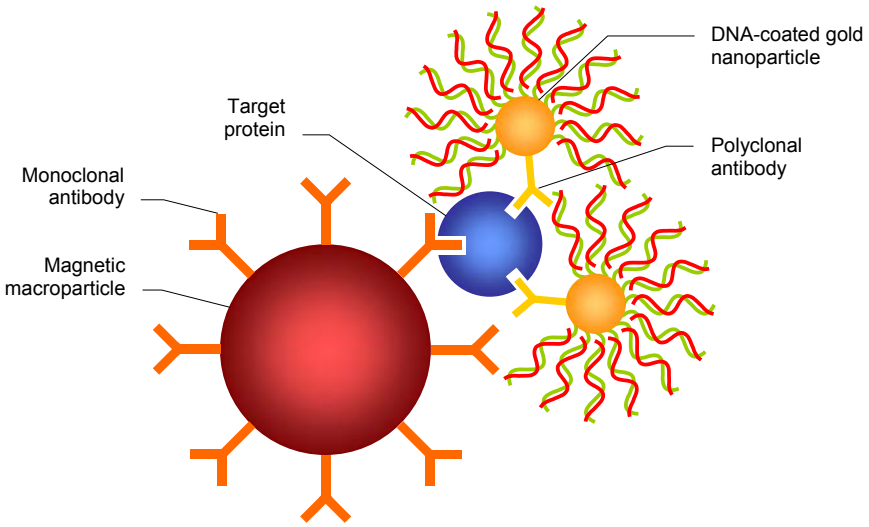

Figure 13. Sandwiched target protein for bio-bar code assay. DNA-coated (oligonucleotides) gold nanoparticles form the basis of the bio-bar code assay using larger magnetic macroparticles to detect attomolar concentrations of serum proteins. In this case a monoclonal antibody to prostate specific antigen (PSA) is attached to the magnetic macroparticle capturing free PSA. A second polyclonal antibody to PSA, attached to the nanoparticle, creates a sandwich of the captured protein and the two particles that is easily separated using a magnetic field. Modified from Nam et al. (2003).

These scientific breakthroughs could have profound clinical implications for research, therapeutic cerebrospinal fluid screening as well as wide scale blood screening. The molecular detection method has the potential for massive multiplexing and simultaneous detection of many analytes in one solution. However, before this assay can be used clinically, statistical validation with a large patient pool is needed. The bio-bar code assay technology

has been licensed to Nanosphere, Inc. (Northbrook, Illinois, USA). Currently, Nanosphere is developing an easy-to-use platform, the Verigene™ System, that automates the analysis and ultrasensitive detection of proteins and nucleic acids using a modular microfluidic lab-on-a-chip (Shaikh *et al.*, 2005).

Ultra-sensitive detection of single bacteria

Recently, dye-doped silica nanoparticles have been used to develop an assay tool for *in situ* pathogen quantification in water samples enabling the detection of one bacterium cell (Zhao *et al.*, 2004b). This ultra-sensitive detection method uses fluorescent-bioconjugated silica nanoparticles (~60 nm in diameter). Within each silica nanoparticle thousands of fluorescent dye molecules are trapped. The silica matrix not only provides high photostability of the dye molecules inside the nanoparticle, but it also enables easy modification of the surface by conjugation of various biomolecules to the nanoparticles. Monoclonal antibodies against antigens of bacteria are covalently immobilised onto the nanoparticles, which are then used in an immunoassay. High fluorescent signal amplification is achieved when the antibody-bioconjugated nanoparticles bind to antigens on the surface of the bacteria enabling detection of bacteria using a spectrofluorometer. The single-bacterium assay can be adapted for multiple-sample determination (>300 samples at one time) and is rapid, taking <20 minutes to complete sample preparation, instrumentation preparation, and sample determination. In addition, the bioassay can be used for multiple-pathogen quantification *in situ* with high specificity.

4.3.7 Future challenges

Current developments in cantilever array sensors are towards improvement of medical diagnostics tools, e.g. new ways to characterise complex solutions such as small amounts of blood or body-fluid samples. On the other hand, from a scientific point of view, the challenge lies in optimising cantilever sensors to improve the sensitivity until the ultimate limit is reached, which may be the nanomechanical detection of individual molecules. Further refinement of *in vitro* nanotechnology systems (cantilevers, nanowires) for rapid, sensitive analysis of disease biomarkers might take place within the next five years. Such systems could be easily expanded as new biomarkers are identified.

Current implantable biosensors, equipped with technology to relay sensed information extracorporeally, are facing serious problems such as unwanted biofouling, i.e. non-specific adsorption of blood components and factors of the immune system on the sensing surfaces resulting in rapid loss of the ability of the sensor to detect the particular protein over the background signal (Desai *et al.*, 2000). Developing surface nanostrucures for implantable molecular sensors might tackle this still unsolved problem of biofouling. More realistically, nanotechnology might be expected to yield novel, biofouling-indifferent sensing strategies, based for instance on the measurement of physical properties.

4.4 In vivo diagnostics using molecular imaging

Molecular imaging is the science of visually representing, characterizing, and quantifying (sub)cellular biological processes in intact organisms. These processes include gene expression, protein-protein interaction, signal transduction, cellular metabolism, and both intracellular and intercellular trafficking. Molecular imaging has the potential to quantify these events in three spatial dimensions, and to monitor these events serially in time, i.e. in a temporal dimension. Thus, biological processes can be identified in space and time. The emergence of molecular imaging has coincided with and has been made possible by the

enormous advances in molecular biology, cell biology, transgenic animals, as well as the development of imaging probes that are specific, reproducible, and quantifiable.
Molecular imaging was once the sole domain of nuclear medicine, with tracers developed for positron emission tomography (PET) and single photon emission computed tomography (SPECT). However, tools involving magnetic resonance imaging (MRI), optical imaging, and ultrasound have evolved steadily in recent years as the prerequisite of an efficient agent for imaging targets at the level of molecular structures, such as enzymes, receptors, and genes. Targeted images are produced by identifying an antibody or peptide with affinity to the target, which is then attached to magnetic compounds for MRI (Lanza *et al.*, 2004), acoustic microbubbles or liposomes for ultrasound (Hamilton *et al.*, 2004; Lanza *et al.*, 1996), isotopes for nuclear imaging (Kolodgie *et al.*, 2003), or fluorescence/bioluminescent probes for optical imaging (Weissleder and Ntziachristos, 2003). In fluorescence imaging, the energy from an external source of light is absorbed and almost immediately re-emitted at a longer, lower-energy wavelength. The depth penetration of fluorescence imaging ranges from micrometres to centimetres. In luminescence imaging light is produced from a chemical reaction without an excitation light. Bioluminescence is a subset of chemiluminescence, in which the light-producing chemical reaction occurs inside an organism.

4.4.1 Magnetic resonance imaging

MRI has evolved as a major important diagnostic technique in clinical radiology. The advent of high magnetic fields, improved gradient coils and pulse sequences has provided the means to obtain three-dimensional images of humans at near cellular resolution. Signal intensity in tissue is manipulated by administration of exogenous contrast agents, which sharpen the contrast by affecting the magnetic spin of protons in water molecules in their proximity. Traditional MRI contrast agents are classified into paramagnetic, and superparamagnetic materials. Metal ion toxicity is an unfortunate consequence of physiologic administration of contrast agents but can be mitigated somewhat by complexation of the metals with organic molecules.

Paramagnetic nanoparticles-based MRI

Paramagnetic metals used for enhanced MRI contrast, such as gadolinium, iron, chromium and manganese, have permanent magnetic fields, though the magnetic moments are unaligned (Thunus and Lejeune, 1999). Upon exposure to an external magnetic field, moments become aligned generating a strong local magnetic field. Paramagnetic metal ions cause an enhancement of the proton signal resulting in “bright” image contrast. Commonly used MRI contrast agents are gadolinium chelates, which tend to be non-specific with rapid accumulation in the liver allowing only a short time imaging window (Kubaska *et al.*, 2001).
Superparamagnetic materials consist of an inorganic core of iron oxide, such as magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) or other insoluble ferrites, and generate greater magnetic moments than individual iron ions. In most cases superparamagnetic materials are of interest for *in vivo* applications, as they do not lose their magnetic moment after removing the magnetic field and differentiate between healthy and pathological tissues. Superparamagnetic substances minimize the proton signal resulting in negative contrast, i.e. darkening of the image (Kehagias *et al.*, 2001). In general, these particles are categorised based on nominal diameter into superparamagnetic iron oxides (50-500 nm) and ultra-small superparamagnetic iron particles (<50 nm), which dictates their physiochemical and pharmacokinetic properties. The first ultra-small superparamagnetic iron oxide particles were developed in the late 1980s (Weissleder *et al.*, 1990). Since than a wide variety of particles have been produced differing in size, type of coating (e.g., dextran, silicones, poly(ethylene glycol), and albumin), and encapsulation used (e.g., liposomes) (Martina *et al.*, 2005). These magnetic nanoparticles

have proved useful in several applications including imaging of the gastrointestinal tract, liver (Mergo *et al.*, 1998; Saini *et al.*, 2000), spleen (Harisinghani *et al.*, 2001), lymph nodes (Harisinghani *et al.*, 1997), intracranial tumours (Varallyay *et al.*, 2002), and abscesses in peripheral soft tissues (Gellissen *et al.*, 1999). Ultra-small paramagnetic iron oxide contrast agents are also useful as blood-pool agents for MR angiography (Anzai *et al.*, 1997), coronary angiography (Taylor *et al.*, 1999), and lymphangiography (Harisinghani *et al.*, 1999). However, conventional synthesis methods of most of these iron particles yield less well-defined nanocrystalline size, stoichiometry, and magnetism resulting in a non-optimal magnetic resonance contrast-enhancing effect. Recently, synthetically controlled magnetite nanocrystals with tuneable size-dependent magnetism and magnetic resonance properties have been developed and could be used as probe systems for the diagnosis of breast cancer (Jun *et al.*, 2005).

Nowadays superparamagnetic materials can be purchased commercially. Two examples of superparamagnetic iron particles on the market are Lumirem®, silicon-coated magnetic particles 300 nm in diameter, and Endorem™, dextran-coated magnetic particles 150 nm in diameter, both manufactured by Advanced Magnetics, Inc. (Cambridge, Massachusetts, USA). Lumirem® is used to distinguish the loops of the bowel from other abdominal structures and physiology. Endorem™ is used for the detection of liver lesions. Currently, ultra-small superparamagnetic iron oxide particles are under development. Two examples are Supravist™ manufactured by Schering AG (Berlin, Germany), and Sinerem® from Guerbet SA (Roissy, France) for the European market, which is the same as ferumoxtran-10 or Combidex® from Advanced Magnetics for the US market. Supravist™ is used as positive enhancing blood pool agent and Sinerem® is used to detect metastatic disease in lymph nodes. Recently, the advisory panel of the US Food and Drug Administration voted against recommending approval of Advanced Magnetics' Combidex®. Safety and efficacy of this imaging agent is questioned.

Using superparamagnetic nanoparticles such as ferumoxtran-10 may improve the ability to detect cancer that has spread to the lymph nodes and may help plan effective cancer diagnosis as was demonstrated in patients with different types of cancer (Deserno *et al.*, 2004; Harisinghani and Weissleder, 2004). A computer program linked to an MRI scan tracked the nanoparticles and recognised abnormal patterns indicating the presence of cancer. The results showed a specificity of 92%, and a sensitivity of 98%, i.e. percentage of the cases in which cancer had spread to the lymph nodes was correctly predicted. The data can be used to reconstruct a three-dimensional (virtual) picture of the patient's lymph nodes. Although the technique has to be validated in a larger group of patients, it offers the possibility of a more precise intervention with less chance of error whether lymph nodes are involved in cancer. If surgery is needed to remove nodes for analysis, then this technique could ensure that surgery is as minimal as possible.

Endohedral metallofullerenes-based MRI

Endohedral metallofullerenes are fullerenes that encapsulate metal atom(s) inside the fullerene cavity. Having lanthanoid metal atoms inside, especially gadolinium, render endohereal fullerenes suitable as (paramagnetic) contrast agents for diagnostic nuclear medicine, although low solubility of $Gd@C_{60}$ hampered their potential use in medical applications. However, procedures were developed to improve the solubility of $Gd@C_{60}$ (Bolskar *et al.*, 2003) and other endohedral metallofullerenes (Kato *et al.*, 2003). Functionalization of these fullerenes through derivatization chemistry attributes to the development of the next generation of MRI contrast agents. Physicochemical characterization (proton relaxivities as function of temperature and magnetic field) of water-soluble malonate derivatives, such as $Gd@C_{60}[C(COOH)_2]_{10}$, and polyhydroxy derivatives, $Gd@C_{60}(OH)_x$,

have shown promising results with regard to their efficacy (Tóth *et al.*, 2005). In addition, strong pH dependency of the proton relaxivities makes these gadofullerene derivatives good candidates for pH-responsive MRI contrast agent applications. Moreover, an *in vivo* biodistribution study demonstrated that $Gd@C_{60}[C(COOH)_2]_{10}$ is the first water-soluble endohedral metallofullerene with decreased uptake of the reticulo-endothelial system (see Box 4) and facile excretion via the urinary track opening new opportunities for medical applications (Bolskar *et al.*, 2003).

Box 4. Reticulo-endothelial system

The reticulo-endothelial system is composed of monocytes and macrophages and located in reticular connective tissue, for example in the spleen. These cells are responsible for phagocytosing and removing cellular debris, pathogens, and foreign substances from the bloodstream. In tumorous lymp nodes macrophages have been replaced by malignant cells, which lack reticulo-endothelial activity and shown no uptake of ultrasmall superparamagnetic iron particles (Bellin *et al.*, 1998; Guimaraes *et al.*, 1994). The reticulo-endothelial system is designed to sequester macromolecules, both relatively small (<70 nm) and large (>300 nm) (Moghimi *et al.*, 2001).

Currently, Luna Innovations Inc. (Blacksburg, Virginia, USA) is developing Trimetaspheres™, endohedral fullerenes enclosing three metal atoms. Trimetaspheres™ are expected to have a major impact as a contrast agent for MRI providing enhanced images at least 25 times better than currently marketed contrast agents. They can also be modified chemically to make them soluble and to attach specific molecules that detect cancer cells, or other targeted cells.

Perfluorocarbon-based MRI

Early detection of vascular microthrombi along the intimal surface of unstable atherosclerotic plaques has been demonstrated using paramagnetic gadolinium nanoparticles such as gadolinium diethylene-triamine-pentaacetic (Gd-DTPA) acid-bi-oleate or Gd-DTPA phosphatidylethanolamine (Winter *et al.*, 2003b). Bristol-Myers Squibb Medical Imaging, Inc. (N. Billerica, Massachusetts, USA) is working to test and commercialise the molecular imaging agent created by Kereos, Inc. (St. Louis, Missouri, USA) to visualize unstable plaques in coronary arteries, which play an important role in heart attacks, in a MRI scan. Conventional imaging techniques can determine whether plaques have narrowed the arteries, but information about the quality of the plaque is restricted. The new agent will selectively stick to the plaque and shine brightly in an MRI scan. It consists of a droplet (~250 nm in diameter) of perfluorocarbon, an inert liquid, surrounded by a layer of lipid. The lipid is hydrophobic, so the water in blood plasma repels it and causes it to stick to the perfluorocarbon. The lipid in turn holds a layer of chelated gadolinium, a metal chemically bonded to organic molecules to render it non-toxic. The gadolinium will stand out strongly in an MRI image. Scattered across the surface of the droplet are a series of targeting ligands that will stick to fibrin on the surface of the plaque. Because the droplets are not chemically bonded, one droplet holding only 100 ligands can carry 100.000 gadolinium molecules, increasing by 1000-fold the brightness at each binding site.

Dendrimers-based MRI

PAMAM dendrimer gadolinium conjugates have already been used experimentally to deliver MRI contrast agents more than a decade ago (Wiener *et al.*, 1994). In murine models, dendrimers conjugated with either folate or monoclonal antibodies have been used as gadolinium nanocarriers to evaluate the biodistribution in ovarian cancer xenografts (Konda

et al., 2002) and to image the lymphatic drainage of breast cancer (Kobayashi and Brechbiel, 2004). These dendrimer-based contrast agents might be used to non-invasively detect cancer cells in the lymph nodes of patients, to provide early signals of the disease, or information about the patterns of metastatic spread. This procedure could be used clinically instead of sentinel lymph node biopsy, which is a surgical approach for the assessment of metastatic involvement of lymph nodes. It is based on the hypothesis that if the node that is nearest to a tumour is negative, the others along the same pattern of spread will also be negative. In addition to the function as tumor-specific contrast agents, dendrimers might also be employed as therapeutic drugs for either neutron capture therapy or in conjunction with radioimmunotherapy.
Gadomer-17 is a dendrimer with 24 peripheral gadolinium complexes that has been designed for contrast-rich magnetic resonance angiography by Schering AG (Berlin, Germany). In animal studies gadomer-17 has demonstrated high relaxivity and long intravascular retention and provides an effective contrast agent for the imaging of blood vessels, such as abdominal blood vessels (Dong *et al.*, 1998), coronary arteries (Gerber *et al.*, 2002), and pulmonary arteries (Abolmaali *et al.*, 2002).

4.4.2 Quantum dots-based optical imaging

Fixed cells and tissue imaging

The feasibility of using quantum dots for antigen detection in fixed cellular monolayers was first demonstrated in 1998 (Bruchez *et al.*, 1998). By labelling nuclear antigens with green silica-coated CdSe/ZnS quantum dots and F-actin filaments with red quantum dots in fixed mouse fibroblasts, these two spatially distinct intracellular antigens were simultaneously detected. For cellular labelling quantum dots are ~20 times brighter and dramatically more photostable over many weeks after injection than organic fluorophores (Chan and Nie, 1998). Recently, specific genomic sequences and antigens in tissue sections have been labelled (Sukhanova *et al.*, 2002; Sukhanova *et al.*, 2004; Xiao and Barker, 2004).

Live cell imaging

Live cell imaging is a more difficult task compared to fixed cells and tissues due to the care that must be taken to keep cells alive and due to the challenge of delivering probes across the plasma membrane for studying intracellular targets. In vivo applications of quantum dots have been demonstrated for labelling cellular surface antigens (Chan and Nie, 1998). By covalently conjugating mercaptoacetic acid-coated CdSe/ZnS quantum dots to the transferrin protein, quantum dots were spontaneously endocytosed by cancer cells and retained their bright fluorescence, indicating that quantum dots can be used as intracellular labels. For intracellular staining of cells poly(ethylene glycol)-coated CdSe/ZnS quantum dots with green emission were injected into single cells of a *Xenopus laevis* embryo (Dubertret *et al.*, 2002). Microscopic fluorescence imaging allowed real-time monitoring of cell lineage and differentiation. Remarkably, most of the embryos exhibited normal development, and there was no evidence of toxicity, even with the injection of over one thousand million quantum dot particles per cell.
Recently, the true advantages of quantum dots for live cell imaging have been demonstrated by labelling plasma membrane receptors, such as glycine receptors (Dahan *et al.*, 2003) and erbB/HER receptors (Lidke *et al.*, 2004) enabling real-time tracking of biomarkers and imaging single molecules. The data provide new insights into the mechanism of ligand-receptor interactions.
Targeting of quantum dots to specific cytoplasmic or nuclear locations for monitoring biological events is a more difficult task as the plasma membrane barrier and the entrapment of quantum dots in the endocytic pathway has to be circumvented. Different mechanisms

have been used to deliver quantum dots into the cells, such as microinjection (Dubertret *et al.*, 2002), non-specific uptake of quantum dots through endocytosis (Jaiswal *et al.*, 2003), conjugation of quantum dots to translocating proteins (Chan and Nie, 1998) or cationic peptides (Pinaud *et al.*, 2004), or specific membrane receptors (Lidke *et al.*, 2004). All these techniques have successfully delivered quantum dots into cells, although it seems that the peptide-mechanism may be the most efficient.

In vivo imaging

In order to benefit from the advantageous optical properties of quantum dots as *in vivo* labels, a number of issues must be addressed. First, the relatively large size and surface area of quantum dots allow the attachment of multiple targeting probes to each label of enhanced binding specificity. However, this size (~4-20 nm in diameter following bioconjugation) has the disadvantage of being too large to penetrate through the vascular endothelium, and too large to be excreted in the urine. The accessible targets for systemically administrated quantum dot probes could be limited to those of vascular exposure, such as endothelial receptors. Also, nanoparticles are non-specifically taken up by phagocytic cells in the organs of the reticulo-endothelial system (most notably by the liver and spleen). This non-specific targeting can be reduced by coating nanoparticles with hydrophilic polymers such as poly(ethylene glycol) to allow greater vascular circulation time, but non-specific uptake cannot be eliminated completely (Åkerman *et al.*, 2002; Ballou *et al.*, 2004).

Quantum dots were first used to target tissue-specific vascular markers by intravenous injection in live mice (Åkerman *et al.*, 2002). CdSe/ZnS quantum dots with either green or red emission were conjugated with tissue-specific peptides targeting lung blood vessels, tumour blood vessels, or tumour lymphatic vessels. Fluorescence visualization of mouse tissue showed uptake in the target tissue, but non-specific uptake by the reticulo-endothelial system was also observed. As long as the target organ is part of the reticulo-endothelial system, such as a lymph node, this is not a problem. Targeting via transdermal injection has been shown in porcine sentinel lymph nodes using a near-infrared fluorescence imaging system (Kim *et al.*, 2004). In this study near-infrared CdTe/CdSe quantum dots were used. Near-infrared light has the advantage to be attenuated less by biological tissue and many types of quantum dots have recently been developed with emission within this range (Bailey and Nie, 2003; Hines and Scholes, 2003; Kim *et al.*, 2003; Yu *et al.*, 2003). The injected quantum dots were phagocytosed non-specifically by dendritic cells which migrated to sentinel lymph nodes. The quantum dots could be followed visually to the lymph system even 1 cm under the skin surface of the animals. This new imaging technique allows surgeons to see clearly the target lymph nodes without cutting the animals' skin and is a significant improvement over the dye/radioactivity method currently used for several reasons. Throughout the procedure, the quantum dots are clearly visible allowing the surgeon to see not only the lymph nodes, but also the underlying anatomy. The pathologist/surgeon can focus on specific parts that would be most likely to contain malignant cells, if cancer were present. The imaging technique minimized inaccuracies and permitted real-time confirmation of the total removal of the target lymph nodes, drastically reducing the potential for repeated procedures and unwanted trauma.

The technique has not been applied in humans yet. Before quantum dot clinical applications become possible, the biocompatibility of these nanoparticles must be thoroughly investigated. So far, nearly all of the publications on the in vivo use of quantum dots have reported normal organism development and no detectable toxicity (Ballou *et al.*, 2004; Dubertret *et al.*, 2002; Jaiswal *et al.*, 2003; Larson *et al.*, 2003). However, long term stability has not been investigated, and it is unlikely that systemically administered quantum dots will be completely excreted from the body prior to degradation. Recently, the cytotoxicity of

CdSe/ZnS quantum dots with various coatings has been determined in cultured liver cells (Derfus *et al.*, 2004). The results showed that surface coatings must be sufficiently stable to prevent oxidation of the quantum dot surface, which results in the release of toxic and carcinogenic cadmium ions. For stability *in vivo*, the amphiphilic polymer coating results in a robust layer.

In vivo tumour targeting and imaging

Targeted molecular imaging of tumours was first demonstrated in nude mice using quantum dots (Gao *et al.*, 2004). Nude mice lack a thymus and a functional immune system. Therefore, a human xenograft of tumour cells will be accepted and grow in nude mice. This xenograft tumour model is therefore an excellent model to study in vivo targeting of therapeutics to human cancer cells. Subdermal tumours require only a shallow penetration depth for imaging. Moreover, the vasculature of most cancer tissue is highly disordered, causing exposed interstitial tissue, so that tumour antigens are in direct contact with blood. Nude mice with human prostate tumours were injected intravenously with poly(ethylene glycol)-conjugated quantum dots functionalised with anti-bodies against the prostate-specific membrane antigen. Quantum dot accumulation in the tumour was primarily due to antibody-antigen binding, but was also aided by the enhanced permeability and retention effect characteristic for tumour vasculature. The permeability and retention effect is due to the inherent vasculature permeability of the microenvironment of cancerous tissue, combined with the lack of lymphatic drainage. Due to the permeability and retention effect alone, it was found that nonconjugated poly(ethylene glycol) quantum dots accumulated in induced mouse tumours, demonstrating tumour contrast, but much less efficiently than actively targeted probes.

Recently, an intraoperative highly sensitive technique for pulmonary sentinel lymph node mapping using near-infrared fluorescent quantum dots has been developed (Soltesz *et al.*, 2005). The study showed the feasibility of the technique for mapping pulmonary lymphatic drainage and guiding excision of the sentinel lymph node in a porcine model. In addition, the application of quantum dots in multiphoton intravital microscopy shows great versatility for studying tumour pathophysiology (Stroh *et al.*, 2005). Intravital microscopy is a powerful imaging technique that allows continuous non-invasive monitoring of molecular and cellular processes in intact living tissue with 1-10 μm resolution (Jain *et al.*, 2002). Quantum dots can be customized to concurrently image and differentiate tumour vessels from both perivascular cells and matrix and to monitor the trafficking of bone marrow-derived precursor cells to the tumour vasculature allowing to investigate the degree to which the vascular and perivascular structures are formed or remodelled in response to cell homing.

4.4.3 Emissive polymersomes-based optical imaging

Emissive porphyrin fluorophores within a polymersome membrane can be used to optically image and target tissue structures more than one centimetre below the skin surface using near-infrared light (Ghoroghchian *et al.*, 2005). Developed in the mid-1990s a polymersome consists of two layers of synthetic co-polymers with a hydrophobic core (Discher *et al.*, 1999; Discher and Eisenberg, 2002). The nano-sized particles are featured by self-assembly: simply mixing all components together creates functional nanometre-sized, cell-like vesicles with the fluorophores evenly dispersed within the core. Injected in rodents the polymersomes can be used to target markers on the surface of a specific type of tumour cells. When exposed to near-infrared light, the fluorosphores within the polymersome emit near-infrared light that can be detected. Emissive polymersomes have the potential to visualize deeper tissue structures and to transport therapeutics directly to a tumour. Emissive polymersomes were suggested to define the first nanotechnological optical imaging platform based on non-aggregating “soft matter” (polymers and porphyrins) unlike the use of semiconductor-based

quantum dots which are considered “hard matter”. The technology will enable optical imaging of cancer tissue that will be less costly and more accessible than MRI-based methods and free of harmful side effects associated with radioactivity.

4.4.4 Ultrasonic imaging

Microbubbles

Currently known gas-filled (perfluorocarbon or sulphur hexafluoride) echogenic contrast agents are microspheres coated with phospholipids, surfactant, denatured human serum albumin, or synthetic polymer with an average diameter of ~1-2 μm. Thus, they can be regarded on the upper boundary of nanodimensions (Unger *et al.*, 2004). Acoustic studies have shown that microbubble suspensions contain a substantial population of nanobubbles (Chin and Burns, 2000). Ultrasound energy is efficiently reflected by microbubbles, hence their use as contrast enhancer in medical ultrasonic imaging. Microbubbles are injected intravenously as a bolus injection enabling real time visualization of blood flow as a way to differentiate pathologic from normal tissue. In addition to using microbubbles for microcirculation, it is also possible to use microbubbles as targeted contrast agents that bind to selected cells providing a more precise method of molecular imaging. Important identified targets for gas-filled microbubbles are intravascular vulnerable plaques as shown in human endothelial cell cultures (Villanueva *et al.*, 1998), in a murine model activated leukocytes (Lindner *et al.*, 2000a; Lindner *et al.*, 2000b), endothelial cell adhesion molecules expressed during inflammatory responses (Lindner *et al.*, 2001), endothelial markers expressed in early tumour angiogenesis (Ellegala *et al.*, 2003; Weller *et al.*, 2005), and acute cardiac allograft rejection (Weller *et al.*, 2003), and in a canine model cardiac thrombosis in a canine model (Takeuchi *et al.*, 1999).

Currently, two perfluoropropane-based microbubble products are commercially available for diagnostic imaging. Definity® was developed by ImaRex Therapeutics, Inc. (Tucson, Arizona, USA) and is currently marketed by Bristol-Meyer Squibb Medical Imaging, Inc. (N. Billerica, Massachusetts, USA). The second product is Optison™ marketed by Amersham Biosciences UK, Ltd. (Little Chalfont, UK). Optison™ comprises of microbubbles stabilized by human serum albumin. Another marketed product developed by Astra Tech AB (Mölndal, Sweden) is SonoVue® consisting of sulphur hexafluoride.

Liquid perfluorocarbon nanoemulsions

Newer types of ultrasonic imaging enhancers are emerging, such as liquid perfluorocarbon nanoemulsions. Compared with microbubbles which have an average diameter of one micron, the perfluorocarbon nanoemulsions can be ~200 nm in diameter. Perfluorocarbon materials have a range of different boiling points and nanoemulsions can be designed to undergo the phase transition from liquid to gaseous state at a range of different temperatures. In addition, insonation with ultrasound energy can also be used to stimulate the transition from liquid to gas. As the particle becomes a gas bubble, the acoustic properties change (Lanza and Wickline, 2001).

ImaRex Therapeutics, Inc. (Tucson, Arizona, USA) is developing NanoInvasive™ therapies for the treatment of cardiovascular diseases, central nervous system diseases, and cancers using ultrasound combined with nanobubbles.

4.4.5 Nuclear imaging

Contrast agents for nuclear imaging are being developed by researchers at the University of Washington collaborating with Dow Chemical Company (Midland, Michigan, USA) and Philips Medical BV (Best, The Netherlands). The contrast agents comprise of perfluoro-

carbon nanoparticles suspended in emulsion. Radionuclides such as technetium-99m are attached to the nanoparticles to provide contrast that allows for SPECT imaging. Moreover, perfluorocarbon nanoparticles are labelled with a specific ligand that causes the agent to target newly developing blood vessels. When injected into the body, the resulting agent will find and illuminate these vessels. The university research group will develop target-specific nanoparticles. Dow Chemical will develop and provide proprietary technology to attach radionuclides to nanoparticles for diagnostic applications. Philips Medical will develop the imaging technology enabling visualization of these particles and provide quantitative measurements of the disease process. Kereos, Inc. (St. Louis, Missouri, USA) will evaluate the contrast agents evolving from research and bring the most promising to development.

4.4.6 Future directions in molecular imaging

One of the most pressing needs in oncology is for imaging contrast agents than can identify tumours that are by far more smaller than those detectable with today's technology, at a scale of 100.000 cells rather than 1.000.000.000 cells. Achieving this level of sensitivity requires better targeting of imaging agents and generation of a bigger imaging signal, both of which nanoscale devices are capable. First-generation nanoscale imaging contrast agents are already pointing the way to new methods for tracing tumours and metastatic lesions much earlier in their development, before they are even visible to the eye.

Imaging agents should also be targeted to changes that occur in the tumour micro-environment, such as angiogenesis, that are currently beyond the capability to detect in the human body. Sustained angiogenesis is an important marker for use in early detection of cancer, as it is found in pre-malignant lesions (Hanahan and Folkman, 1996) and might be expected to be an early-to-midstage event in human cancers (Hanahan and Weinberg, 2000). Several research groups have successfully imaged angiogenesis with MRI in animal models by various formulations of derivatized nanoparticles targeted by $\alpha_v\beta_3$-integrins expressed by growing capillaries (Anderson *et al.*, 2000; Schmieder *et al.*, 2005; Sipkins *et al.*, 1998; Winter *et al.*, 2003c; Winter *et al.*, 2003a). Given that angiogenesis occurs in distinct stages and that angiogenic therapies will need to be specific for a given angiogenic state, imaging agents that can distinguish among these stages will be invaluable for obtaining optimal benefit from therapeutics that target angiogenesis.

Other challenges are envisaged in developing so-called reporters of efficacy: highly sensitive imaging agents and *ex vivo* diagnostics that can determine whether a therapeutic agent is reaching its intended target and whether that agent is killing malignant or support cells, such as growing blood vessels. The greatest potential for immediate results would focus on detecting apoptosis following cancer therapy. Such systems could be constructed using nanoparticles containing an imaging contrast agent and a targeting molecule that recognizes a biochemical signal seen only when cells undergo apoptosis. Using superparamagnetic iron oxide nanoparticles, which act as powerful imaging contrast agent, conjugated with the molecule annexin-V, which recognises the phosphatidylserine that is expressed on the membrane surface of apoptic cells, detection of therapeutic-induced apoptosis is feasible in isolated cells (Schellenberger *et al.*, 2002) and in tumour-bearing mice (Jung *et al.*, 2004). Further development of this type of system could provide clinicians with a way of determining therapeutic efficacy in a matter of days after treatment. Targeted nanoscale devices may also enable surgeons to perform preoperative, contour-defining imaging of tumours and intraoperative visualisation of the lesion (Kircher *et al.*, 2003) or to detect micrometastases in lymph nodes (Harisinghani *et al.*, 2003) or tissues distant from the primary tumour.

4.5 Implantable materials for orthopaedics and dentistry

Conventional orthopaedic/dental implants suffer from a restricted lifetime caused by implant failure. Artificial joint replacements such as for hip joint, need revisions within 10-15 years (Emery *et al.*, 1997). Obviously, this implant lifetime is not long enough, especially for young patients who are faced with frequent, complicated and expensive revision surgery. Therefore, extending the lifetime up to several decades would eliminate considerable patient suffering and save health care costs. Failure rate can be decreased if implant material stimulates rapid formation of new bone or if an implant is firmly fixed within adjacent bone (osseointegration). Initially, metallic implants preferred for joint replacement were stainless steel and cobalt chrome alloys primarily used for their good mechanical properties. However, the high Young's modulus of these materials resulted in stress-shielding and bone resorption. Stress-shielding should be avoided, since living bone must be under some tensile load to remain healthy. Osseointegration minimises stress and strain imbalances at the tissue-implant interface. Therefore, the implantable material properties should match the mechanical characteristics of the surrounding bone tissue.
Bone is a biocomposite material of which the major constituents are a complex mineral mixture (60-70% by weight) of calcium and phosphate in the form of hydroxyapatite, proteins (20-30%) with predominately type I collagen fibrils, and water (10%). The dimensions of the mineral and organic constituents are on the nanometre scale (Rho *et al.*, 1998). Hydroxyapatite is between 2-5 nm in diameter and ~50 nm in length, and the estimated Young's modulus is 110 GPa. X-ray diffraction analysis showed an identical structure to synthetic hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Germine and Parsons, 1988), which is often used as bioactive ceramic coating material on titanium and cobalt-chromium implants. However, chemical analysis indicates presence of impurities such as carbonate, citrate, sodium, magnesium, fluoride and other ions (Rey, 1990). Collagen fibrils are composed of three identical collagen fibres that are woven in a triple-helix to form a cylinder of ~80-300 nm×1.5 nm. Obviously, bone cells are accustomed to interacting with nano-structured materials, i.e. materials with grain size of <100 nm in at least one dimension. It is assumed that nano-structured materials are very biocompatible.
Engineers and scientists are continuously investigating novel materials and designs for orthopaedic/dental implants often inspired on micron-structured materials. However, current research on nanomaterials in orthopaedics is intriguing because nanomaterials can be manufactured to sizes similar to bone constituents, and nanomaterials can emulate the fibrous nature of bone constituents. Moreover, material surface modification is a primary tool to enhance the performance of orthopaedic implants.

4.5.1 Implant coatings

Nano-structured implant coatings

Several investigations have been performed exploring the properties of different coating materials such as nano-structured diamond, hydroxyapatite, and metallo-ceramic coatings. Chemical vapour deposition has been the most successful method of producing nano-structured diamond coatings achieving a surface roughness of ~15 nm (Toprani *et al.*, 2000). Although structure and hardness is strongly affected by processing parameters, it has been shown that nano-structured diamond coatings have good adhesion to titanium alloys (Ti-6Al-4V) and poor adhesion to cobalt-chrome and steel substrates (Catledge *et al.*, 2002a). Ultrahigh hardness, improved toughness, low friction, good adhesion to titanium alloys, and biocompatibility characteristics are promising. Life time of orthopaedic implants could increase to upwards of 40 years (Catledge *et al.*, 2002b).

Nano-structured hydroxyapatite coatings for biomedical implants have attracted attention since the adoption of thin-film depositing processes from the electronic industry during the last decade. In general, hydroxyapatite coatings are more accepted in dentistry than in orthopaedics, but the potential in both fields is high. Hydroxyapatite promotes bone formation around the implant, increases osteoblast (bone-forming cell) functions such as adhesion, proliferation, and mineralisation. However, it is unlikely that bulk synthetic hydroxyapatite will be used as load-bearing implant since fracture toughness (~1.0 $MPa \cdot m^{1/2}$) and flexural strength (<140 MPa) are low (Ogiso *et al.*, 1996). Further research is necessary for long-term *in vivo* performance.
Currently, Inframat, Inc. (Farmington, Connecticut, USA) and Spire Biomedical, Inc. (Bedford, Massachusetts, USA) are developing orthopaedic and dental implants using nano-structured hydroxyapatite coatings. Inframat is developing the next generation nano-structured hydroxyapatite coatings for hip, knee, and dental prostheses using a room temperature electrophoretic deposition technique. Knee and dental implants require high bond strength coatings to accommodate the greater impact loads and related higher stresses than hip prostheses. Spire Biomedical is developing a new family of "smart" nanophase (i.e., grain sizes less than 100 nm in at least one direction) coatings that will enhance bone integration and promote better device fixation. Although hydroxyapatite coatings are now widely used to encourage device fixation and stability, they can lead to undesirable soft tissue, as well as growth of desirable hard tissue. Spire's nanophase hydroxyapatite coatings are modified to selectively encourage hard tissue growth on implants while discouraging the formation of soft tissue growth that can result in non-optimal performance.
Nano-structured metalloceramic coatings are still in the early stage of development and *in vitro* testing. A nanocrystalline multilayer (Cr/CrTi/CrTiN) coating was deposited on a Co-Cr-Mo substrate (Catledge *et al.*, 2002b). Using ion-beam assisted deposition yields a grain size of the nanocrystalline structure of ~10 nm. The Cr/CrTi metallic layer at the interface increases the adhesion to the Co-Cr substrate, whereas the CrTiN surface layer is covalent in nature and will enhance scratch and wear resistance of the coating. Advantages are high hardness, very low surface roughness, good adhesion and corrosion resistance. Currently, Spire Biomedical is developing super hard ceramic-like coatings on metal substrates to improve performance of orthopaedic implants.

Nanoporous ceramic implant coatings
A different approach to improve implant properties is anodisation of aluminium. Anodisation is widely used for producing corrosion resistant aluminium parts (Thompson, 1997). It has the effect of covering metallic aluminium with a strongly adherent surface layer principally composed of ceramic alumina (Al_2O_3). This technique was used to create a nanoporous alumina layer on top of a titanium alloy implant (Briggs *et al.*, 2004; Karlsson *et al.*, 2003). The pores in the layer are roughly cylindrical and parallel to each other running perpendicular to the layer surface. At the base of each nanopore is a thin barrier layer of oxide separating the pore from the metallic aluminium underneath. The anodisation process produces a ceramic alumina layer containing ~$5 \cdot 10^8$ pores /cm^2 with nanopores ~160-200 nm in diameter and 1-5 μm in height. Mechanical testing showed a highly adherent nanoporous alumina coating on titanium alloys likely to withstand stresses in excess of 20 MPa in shear and 10 MPa in tension, i.e. similar to that of the bone in which it might be implanted. For comparison, the shear strength of bovine cortical bone has been measured to be 34 MPa and shear strength of hydroxyapatite deposited on polished Ti-6Al-4V surfaces was found to be ~12 MPa (Wei *et al.*, 1999). Human osteoblast cell response *in vitro* showed normal osteoblast adhesion, morphology, and proliferation indicating that nanoporous alumina coatings could improve implant designs. It should be noted that nanoporous alumina has the

potential of being rendered bioactive by loading the porous structure with appropriate bioactive agents improving cell response and facilitate osseoinductive activity.

Novel nanomaterials for implant coatings

Titanium and titanium alloys have been successfully used as orthopaedic and dental implants because these materials osseointegrate, i.e. direct chemical or physical bonding with adjacent bone surface without forming a fibrous tissue interface. For the optimisation of bone growth surface treatments have been applied, such as surface roughening by sandblasting, hydroxyapatite coating (Ducheyne *et al.*, 1986), formation of titanium dioxide or titania (Uchida *et al.*, 2003), and recently novel nanomaterials such as helical rosette nanotubes (Chun *et al.*, 2004) and titania nanotubes (Oh *et al.*, 2005).

Helical rosette nanotubes are a new class of organic nanomaterial featuring two basic DNA components, i.e. guanine and cytosine (Fenniri *et al.*, 2001). In water guanine and cytosine self-assemble spontaneously to form rosettes which then stack up to form a nanotube with a hollow core of 11 Å, an outer diameter of ~4.0 nm and up to several millimetres long resembling biologically-occurring nano-structured constituents in bone. In a study *in vitro* titanium was coated with lysine and arginine-functionalised helical rosette nanotubes to determine osteoblast adhesion (Chun *et al.*, 2004). The results of this study showed that functionalised helical rosette nanotubes enhanced osteoblast function and could improve the next generation of orthopaedic implants.

Titania nanotubes can be fabricated on the surface of a titanium substrate using anodisation. The dimensions of titania nanotubes are ~100 nm outer diameter, ~70 nm inner diameter, ~15 nm wall thickness, and ~250 nm in height. The vertically aligned arrays of titania nanotubes are made bioactive by chemical treatment (immersion in NaOH) followed by heat treatment (600°C). On a bare metallic titanium surface this treatment has already been shown to accelerate hydroxyapatite formation due to the formation of a sodium titanate layer (Kim *et al.*, 1996). The subsequent heat treatment crystallises the amorphous titania nanotubes into the anatase structure which is more efficient in nucleation and growth of hydroxyapatite (Uchida *et al.*, 2003). However, the chemical treatment is of greater significance for the bioactivity improvement. On the very top of the titania nanotube walls an additional, extremely fine, and predominantly nanofibre-like structure and in some cases a nanoribbon-like structure is deposited (Oh *et al.*, 2005). This “nano-inspired nanostructure” is likely to be sodium titanate which has the composition of $Na_2Ti_5O_{11}$ or $Na_2Ti_6O_{13}$. The nanofibre structure is ~8 nm in diameter and ~50-100 nm long. It should be noted that the concept of growing even finer-scale structures from a given nanostructure could be of significant interest for basic materials development for nanotechnology. For the evaluation of hydroxyapatite formation specimens, i.e. titania nanotubes covered with or without sodium titanate nanofibres on top of the titanium substrate, were soaked in a simulated body fluid solution, subsequently dried, and examined by scanning electron microscopy. The results showed that the kinetics of hydroxyapatite formation by the presence of the nanostructure. In the specimen without sodium titanate nanofibres hydroxyapatite in detectable quantity was formed after seven days, whereas one day was sufficient in the specimen with nanofibres. Interestingly, hydroxyapatite was structured as a nanofibre with a diameter of ~25 nm. Although nanofibre hydroxyapatite is coarser than its precursor sodium titanate (~8 nm), it is the smallest feature hydroxyapatite reported so far. The next step would be to investigate the effect of this composite structure on the osteoblast response *in vitro*.

4.5.2 Surface modifications

Nano-structured surfaces using novel nanofibres

Biomedical investigations dealing with the potential use of organic nanomaterials, such as carbon nanofibres, in orthopaedic applications are scarce (Elias *et al.*, 2002; Price *et al.*, 2003a; Price *et al.*, 2003b). Carbon nanofibres do not possess as regular a helical carbon structure as carbon nanotubes. Therefore, mechanical properties of carbon nanofibres are less impressive, e.g. Pyrograf® III manufactured by Applied Sciences, Inc. (Cedarville, Ohio, USA) has a Young's modulus of 400 GPa, which is, nevertheless, still higher compared to human bone. Importantly, the aspect ratio and physical shape of carbon nanofibres mimic the crystalline dimensions of hydroxyapatite found in bone. Additionally, the dimensions of carbon nanofibres are similar to type I collagen fibres. It is hypothesized that another key parameter to emulate in nanostructures for bone implants is the constituent fibrous nature of bone (Price *et al.*, 2003b). Nanofibre rather than nanospherical material simulates more closely the nanometre dimensions of hydroxyapatite crystals and collagen fibres in bone that osteoblasts are accustomed to interacting with (Kaplan *et al.*, 1994).

Recently, a study *in vitro* has been performed to determine osteoblast function using a carbon nanofibre-reinforced polycarbonate urethane composite (Webster *et al.*, 2004). Carbon nanofibres embedded in the polymer matrix were tested with either nanophase (i.e., at least one dimensions <100 nm) or submicron (>100 nm) dimensions in human osteoblast and mouse fibroblast cell cultures. The experiments showed for the first time increased osteoblast functions with increasing amounts of carbon nanofibres in the nanophase composite. In contrast, at the same time fibrous-tissue encapsulation decreased on the reinforced composite. Fibrous encapsulation at the tissue-implant interface decreases the effectiveness of the necessary osseointegration of the implant and often results in clinical failures (Kaplan *et al.*, 1994).

Osteoblast functions, i.e. cell adhesion, cell activity, and calcium deposition, were also tested using crystal-structured nanofibre alumina *in vitro* (Webster *et al.*, 2005). Alumina nanofibre (2 nm in diameter and >50 nm in length) powders were compressed and sintered in air at five different temperatures ranging from 400 °C to 1200 °C yielding five compacts with differences in crystalline phase, chemical composition, and surface roughness (topography). Improved osteoblast functions were shown for nanofibre alumina with delta and theta crystalline phase which was obtained using a sintering temperature of 1000 °C. In fact, osteoblast cell activity was increased as well as calcium deposition. However, osteoblast adhesion showed no difference among the various nanofibre alumina compacts. Researchers concluded that nanofibre alumina could be used for future design of orthopaedic materials with increased cytocompatibility properties.

Implant surface roughness modification

Another way to improve the performance of orthopaedic/dental implants can be achieved by modification of the surface roughness, specifically by creating nanometre-scale roughness. Cell responses might be triggered by changes in surface roughness, i.e. in horizontal as well as vertical direction, in the nanometre domain (<100 nm) rather than on submicron scale (>100 nm). Increased *in vitro* osteoblast function and osteoclastic (bone-resorbing cells) response was correlated with nanometre surface roughness (ranging from ~20-300 nm) for nanophase alumina (Webster *et al.*, 2001), and poly-lactic-*co*-glycolic acid (PLGA) cast of carbon nanofibres (Price *et al.*, 2004). In addition, inhibiting *in vitro* fibroblast proliferation was also correlated for nanophase alumina (Webster *et al.*, 2001), copolymer mixtures of polystyrene and polybromostyrene (Dalby *et al.*, 2002; Dalby *et al.*, 2003), PLGA (Vance *et al.*, 2004), and ceramics (Mustafa *et al.*, 2005). More research is needed to determine optimal topographical parameters affecting a wide range of cell functions.

4.5.3 Bone replacement materials

Hydroxyapatite nanoparticles used as bone replacement material, i.e. bone cement with improved mechanical properties, is commercially available. Indications for bone replacement materials are bone fractures, periprosthetic fractures during hip prosthesis revision surgery, acetabulum reconstruction, osteotomies, filling cages in spinal column surgery, and filling in defects in children. Ostim® is an injectable bone matrix in paste form manufactured by Osartis GmbH & Co. KG (Obernburg, Germany) and received CE marking in 2002. Ostim® is 100% synthetic nanoparticular hydroxyapatite and is fully absorbed after a few months. In May 2005, Angstrom Medica, Inc. (Woburn, Massachusetts, USA) obtained FDA approval to commercialise their engineered synthetic bone product NanOss™. The material is composed of hydroxyapatite nanocrystals, sized and shaped like native bone crystals with the strength of stainless steel. Another synthetic replacement material for cancellous bone grafts is VITOSS® produced by Orthovita, Inc. (Malvern, Pennsylvania, USA). VITOSS® contains β-tricalcium phosphate nanoparticles with a diameter of ~100 nm. It is engineered to resemble human cancellous bone in porosity and structure. Higher porosity and larger surface area, compared to conventional tri-calcium phosphate, facilitates faster and increased bioresorption and vascular invasion (Szpalski and Gunzburg, 2002). VITOSS® received CE marking in 2000. Though the next product is in the strictest sense not nano-structured, it is worthwhile to mention OsSatura™ manufactured by IsoTis International (Lausanne, Switzerland). OsSatura™ is composed of approximately 80% hydroxyapatite and 20% β-tricalcium phosphate. It is a porous biomaterial featuring interconnected macropores and micropores with an approximate total porosity of 75%. The macropores are responsible for the osteoconduction, whilst the proprietary microporous structure is responsible for the osteoinduction. OsSatura™ has been launched in the EU in early 2003.

4.5.4 Tissue Engineering

Tissue engineering has been defined as “the application of principles and methods of engineering and life sciences towards fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function” (Skalak and Fox, 1988). The products that arise from these techniques may provide an alternative to available therapies to replace damaged, injured or missing body tissues. Tissue-engineered products (TEPs) typically are a combination of three components, i.e. isolated cells, an extracellular matrix and signal molecules, such as growth factors. Nanotechnology provides new possibilities for the extracellular matrix, often referred to as the scaffold.

The extracellular matrix serves three primary roles. First, it facilitates the localisation and delivery of cells in the body. Second, it defines and maintains a three-dimensional space for the formation of new tissues with an appropriate structure. Third, it guides the development of new tissues with appropriate function. The interaction of the cells and the extracellular matrix is of great importance for the intended function of the final product.

Thus, several of the principles described in the section above for implantable materials in orthopaedics and dentistry, providing greater biocompatibility and/or stimulating the ingrowth of cells into the material *in situ* are at least equally valid for tissue engineering scaffolds. Micro- and nano-structured surfaces of scaffold materials have important beneficial effects on cell adhesion and proliferation. Control of (nano)porosity is essential to obtain 3-dimensional constructs with the appropriate desired properties. Also the possibility to tailor mechanical characteristics, matching the target tissue as closely as possible, leads to increased possibilities for successfully functioning TEPs.

Particularly interesting are structures prepared from nanofibres. The most efficient technique to produce polymer nanofibres, suitable for application in TEPs, and with a potential for mass

production, is electrospinning (Jayaraman *et al.*, 2004). The dimension and structure of electrospun polymeric nanofibre mats resembles closely the collagen phase of the natural extracellular matrix. This, combined with excellent physical properties such as high surface area, high porosity, interconnective pores of the nanofibre matrices and appropriate mechanical properties, well-controlled bio-degradation rates and biocompatibility, make (synthetic) biodegradable polymeric nanofibre matrices ideal candidates for developing scaffolds for TEPs, as reviewed by Nair *et al.* (2004).
The application of carbon nanotubes shows also promising capabilities for directing and guiding live biological cells. Functionalised MWCNTs (Mattson *et al.*, 2000; Zhang *et al.*, 2005) as well as functionalised SWCNTs (Hu *et al.*, 2005) promote neurite outgrowth, branching, and the formation of synpatically communicative neural networks *in vitro* which are essential for the repair or regeneration of the central nervous system after brain or spinal cord injury. Besides these potential capabilities which are long awaited in clinical neuroscience research, functionalised SWCNTs can also present ideal materials for artificial bone. Functionalised SWCNTs mimic the role of collagen as the scaffold for the nucleation and growth of hydroxyapatite in bone and can be applied as supporting scaffold for bone therapy (Zhao *et al.*, 2005). Functionalised SWCNTs could be implanted as solutions or substratum in fractures to promote growth of bone.
Another promising approach by which novel biocompatible materials can be fabricated is molecular self-assembly. Using natural processes as a guide, substantial advances have been achieved at the interface of nanomaterials, including the fabrication of 3-dimensional scaffolds for tissue engineering. A review on the possibilities of nanofibres constructed from peptides and proteins undergoing self-assembly, and leading to very well ordered structures with remarkable regularity was published by Zhang (2003). Scaffolds produced using tailor-made self-assembling peptides, with engineering functionality and biodegradability are foreseen to contribute to the development of new biomedical applications such as TEPs.

4.6 Implantable materials for vascular interventions

Endovascular implants such as coronary and peripheral stents are nowadays frequently used in cardiovascular and non-cardiovascular interventions. Endovascular stents are, in general, metallic devices employed in stenosed arteries to support the blood vessel wall following balloon angioplasty. Metallic coronary stents were clinically introduced in 1987 (Puel *et al.*, 1987; Sigwart *et al.*, 1987) and biodegradable coronary stents made of poly(L-lactic acid) in 2000 (Tamai *et al.*, 2000). However, stent implantation often leads within several months after the intervention to re-narrowing of the coronary artery at the site of stent deployment known as in-stent restenosis (Ellis *et al.*, 1992; Savage *et al.*, 1994). This is caused by proliferation of smooth muscle cells in the blood vessel wall, i.e. neointimal hyperplasia (Hoffmann *et al.*, 1996; Schwartz *et al.*, 1992). Consequently, research is focused to explore more efficient and longer-lasting stenting solutions. One of the more effective methods involves employing stents covered with an appropriate quantity of drugs that can be released when placed in an endovascular situation. These drug-eluting stents were clinically introduced in coronary arteries in 2000 (Sousa *et al.*, 2001) and contain agents that inhibit inflammation, e.g. dexamethasone (Liu *et al.*, 2003b), or cellular proliferation, e.g. sirolimus (Sousa *et al.*, 2001), paclitaxel (Grube *et al.*, 2003) and tacrolimus (Grube *et al.*, 2004). In general, a coating matrix is required for most drugs, but some drugs can be loaded directly onto metallic surfaces, e.g. paclitaxel. The coating ensures drug retention during deployment and modulates drug-eluting kinetics. Compared to bare metal stents drug-eluting stents reduce considerably the incidence of restenosis (for a recent meta-analysis on clinical trials

see e.g. Babapulle *et al*. (2004)). Drug-eluting stents are being evaluated in animal models, clinical trials, implanted into patients and have become the most widely used modality for coronary revascularisation. Several drug-eluting stents have already received CE-marking, including the CYPHER™ sirolimus-eluting coronary stent (Cordis Corporation, Miami Lakes, Florida, USA) in April 2002, the TAXUS® Express™ pactitaxel-eluting coronary stent (Boston Scientific Corporation, Natick, Massachusetts, USA) in April 2003, and the Endeavor™ ABT-578 (sirolimus-analogue drug) eluting stent (Medtronic Inc., Minneapolis, Minnesota, USA) in July 2005.

4.6.1 Stent coatings

Bare metal coronary stents can induce platelet activation by shear stress, release of metal ions, and contact to the blood vessel wall triggering thrombosis. Traditionally, surface coatings on stents are applied to reduce thrombogenicity by lowering blood protein adsorption and thrombocyte aggregation. Applying nanoporous ceramic coatings may even further improve efficient stenting. Currently, several nanoporous ceramic coatings, e.g. aluminium oxide and hydroxyapatite, are being developed at companies such as AlCove Surface GmbH (Gladbeck, Germany), Debiotech SA (Lausanne, Switzerland), and MIV Therapeutics, Inc. (Vancouver, British-Colombia, Canada). MIV Therapeutics is conducting a full range of biocompatibility and histopathology tests on nanoporous hydroxyapatite coated stents (Rajtar *et al.*, 2004) and could initiate porcine trials of this product with the potential to reach human clinical trials by the end of 2005.

4.6.2 Stent coatings with drug-eluting capacity

Nanoporous technology could be used for the development of next generations of coronary stents with the capacity to carry drugs or acting as a semi-permeable barrier. Recently, a beneficial effect was suggested in a rabbit model of restenosis using a tacrolimus-eluting stent from a nanoporous ceramic aluminium oxide coating (Wieneke *et al.*, 2003). However, a porcine model of restenosis could not confirm this effect (Kollum *et al.*, 2005). Particle debris shed from a drug-eluting nanoporous aluminium oxide coating of a stainless steel stent counteracted potential antiproliferative effects of stent-based tacrolimus delivery. Researchers recommended drug-eluting stent coatings to be routinely tested for being tightly anchored into the stent surface. Besides, nanoporous silicon carbide (SiC) membranes have low protein adsorption *in vitro* compared to the best commercially available polymeric membranes specifically designed for low protein adsorption (Rosenbloom *et al.*, 2004). This resistance to protein fouling is desirable property of nanoporous SiC. Furthermore, bulk SiC has been shown to have good biocompatibility (Kotzar *et al.*, 2002) and hemocompatibility (Bolz *et al.*, 1996; Carrié *et al.*, 2001; Monnink *et al.*, 1999). MIV Therapeutics, Inc. is evaluating a new ultra-thin multilayer drug-eluting hydroxyapatite coating in a porcine model. Preliminary results indicate that the addition of paclitaxel does not adversely affect the coating properties.

4.7 Active implantable devices and bionics

An implantable device, or endoprosthesis, is an artificial structure or system in which the remaining functional parts of a previously fully developed physiological system are structurally supported or stimulated to restore (some) function. Prostheses are typically used to replace parts or restore functions lost by injury (traumatic), disease, or missing from birth (congenital). In the next section active implantable devices are categorised as implantable

devices for vision rehabilitation, cardiac arrhythmia, hearing impairment, motor control, and drug delivery.

4.7.1 Vision rehabilitation

Current developments in vision rehabilitation are essentially limited to retinitis pigmentosa-induced blindness, which for the time being is untreatable. Retinitis pigmentosa is a hereditary retinal dystrophy resulting in a progressive degeneration of photoreceptors in the outer retina and eventually leading to complete blindness. Clinically, the illness starts with loss of night-vision and concentrically decreasing visual field. Ultimately, some central vision is left, if any at all, leading to tunnel-vision. For developed countries the prevalence figures suggest that retinitis pigmentosa affects one in 5000 resulting in ~100.000 sufferers in Europe alone and 1.5 million worldwide (Easty and Sparrow, 1999). In contrast to retinitis pigmentosa, where the peripheral vision is affected, in age-related macular degeneration central vision is most afflicted. The task to restore loss of central vision is much more demanding.

Restoring vision with electrical devices is very difficult due to the very complex form of information processing. Electrical stimulation of the retina, optic nerve, or occipital cortex elicits cortical sensations called phosphenes, i.e. the brain interprets the signals as lights and visual patterns. Phosphenes are examples of entoptic phenomena, i.e. visual images which arise within the human visual system, and not as a reflection of the surrounding world. Two kinds of retinal implants will be shortly reviewed below.

Although current retina implants should be regarded as microdevices, nanotechnology can be used for significant improvement in case of device materials and manufacturing (e.g., nanoporous and nanoscale electrodes), cell adhesion and biocompatibility (e.g., diamond-like coatings), and signal transmission.

Subretinal implant

The subretinal device is located between the pigment layer epithelial layer and the outer layer of the retina, which contains the photoreceptor cells. Microphotodiode arrays are manufactured on a silicon wafer (~25-100 μm thick and 2-3 mm in diameter) using CMOS process technology. In the subretinal device, several thousands of independently functioning light-sensitive microphotodiodes equipped with microelectrode of gold or titanium nitride are arranged on the wafer and placed in the subretinal space. Light emanating from visible objects falls on the device and activates the microphotodiodes, which in turn activate the corresponding microelectrodes that are supposed to stimulate the surviving retinal neurons postsynaptic to the photoreceptor layer. Thus, the subretinal concept is replacing damaged photoreceptors by artificial ones. Subretinal prostheses have several advantages: the remaining intact neural network of the retina is still capable of processing electrical signals, positioning and fixing of the implant in the subretinal space is relatively easy, no camera or image processing is required, and eye movement can still be used to locate objects. Feasibilities studies were conducted on isolated retina (Stett *et al.*, 2000; Zrenner *et al.*, 1999), in rats (Zrenner *et al.*, 1999), rabbits (Chow and Chow, 1997; Schwahn *et al.*, 2001), cats (Chow *et al.*, 2001; Chow *et al.*, 2002), and the first silicon-based vision prosthesis was implanted in six blind patients in 2000-2001 (Chow *et al.*, 2004). During follow-up, ranging from 6-18 months, of this pilot clinical investigation visual function improvements have been demonstrated and no significant side-effects were noticed. Optobionics, Corp. (Naperville, Illinois, USA) developed the ASR® (artificial silicon retina) device and has implanted recently additional 20 patients as part of an expanded safety and feasibility study. In Europe Retina Implant AG (Reutlingen, Germany) is developing subretinal implants.

Epiretinal implant

In contrast, the epiretinal device is implanted onto the innermost layer of the retina (i.e., vitreal side) that contains the retinal ganglion cells. In the epiretinal approach, microelectrode arrays are controlled by electronic circuitry connected via a radio frequency telemetry link to an image acquisition and processing unit (Liu *et al.*, 2003a). For image acquisition a very small field sensor like a highly-miniaturised CMOS camera is placed outside the eye, for instance in spectacles. For information processing a retina encoder with potential learning capabilities can be a wearable module for instance around the waist (Eckmiller, 1997). Another approach is to implant an epiretinal sensor within an intraocular plastic lens that replaces the natural lens (Rizzo *et al.*, 1999). The epiretinal implant is connected to retinal ganglion cells and their axons. Electrical stimulation generated by the implant is supposed to activate the retinal ganglion cells or bipolar cells. Feasibility studies were conducted on isolated retina (Grumet *et al.*, 2000), in cats (Schanze *et al.*, 2002; Wilms *et al.*, 2003), and dogs (Majji *et al.*, 1999), and in acute (Rizzo *et al.*, 2003a; Rizzo *et al.*, 2003b) and chronic conditions (Humayun *et al.*, 2003) in humans. After 10 weeks, no side effects were reported, and the electrical stimulation of the electrodes resulted in consistently reproducible perception of phosphenes. In Europe, IIP Technologies GmbH (Bonn, Germany) is performing clinical investigations using learning epiretinal visual prostheses in patients with retinitis pigmentosa (Feucht *et al.*, 2005) and has started a feasibility study in patients with age-related macular degenerative disease in November 2004. In the USA, Second Sight Medical Products, Inc. (Sylman, California) is currently in the first phase of development of a retinal prosthesis and is conducting two clinical investigations approved by the FDA.

Artificial synapse chip

A different approach for vision rehabilitation could be envisaged with the concept of an artificial synapse chip. Recently, this chip was built mimicking the connection between neurons by controlled repeatable release of neurotransmitters (Peterman *et al.*, 2003b). Though this prototype neural interface is still in its infancy, it could be a powerful application of a neurotransmitter-based flexible retinal prosthesis (Peterman *et al.*, 2003a). Essentially, the device (8×8 mm) consists of four apertures, each connected to a microfluidic channel. Using standard microfabrication techniques a thin layer (1.6 μm thick) of silicon nitride is deposited on a silicon wafer. Reactive ion etching is used to etch four circular apertures in a 2×2 array (5 μm in diameter, 125 μm centre to centre) and gold electrodes (150 nm) for controlling the electroosmotic flow were deposited. The microfluidic channels are patterned using lithography. Fluid injection as well as fluid withdrawal of minute quantities of neurostimulant solution in the order of picolitres (pico = 10^{-12}) at well defined locations is electric field-driven. The feasibility of the device for chemical neurotransmission in biological systems *in vitro* was demonstrated using cell cultures on the chip (Peterman *et al.*, 2004). Small amounts of released bradykinin changed intracellular calcium visualized by a fluorescence dye. For use as a retinal prosthesis the conceptual device needs improvement regarding an increase of the number of apertures required for better fluidic interfacing and fluidic interconnects, and reduction of the dimensions of device making it suitable for implantation. In addition, the conceptual device could be composed of soft material less damaging for the retina than electrode arrays. The stimulation of a functional retinal network using chemical instead of electrical activation provides a new approach in vision rehabilitation.

4.7.2 Pacemakers and hearing aids

New pacemakers and hearing aids will be equipped with nanosensors that not only use nanomaterials to sense, but which also employ nanoelectronics technology, i.e. spintronics.

Application of spintronics in pacemakers will enable non-invasive high-speed communication. For hearing aids these tiny sensors will automatically adjust to accommodate the source of sounds. For example, if a phone is held to the wearer's ear, the hearing aid will automatically switch modes without the user's intervention. The nanosensor uses giant magnetoresistive elements embedded in conventional integrated circuits. Giant magnetoresistance is a quantum mechanical effect observed in layered magnetic thin-film structures that are composed of alternating layers (~3 nm thick) of ferromagnetic and nonmagnetic materials (Prinz, 1998; Wolf *et al.*, 2001). The sensor element is combined with conventional electronics on a single chip measuring 1.2×1.3×0.2 mm. When subjected to relatively small magnetic fields giant magnetoresistors provide a very large (i.e. "giant") signal. Spintronic technology focuses on the spin of electrons rather than their charge and enables size reduction and power enhancement of sensor-based components. Currently, NVE Corp. (Eden Prairie, Minnesota, USA) manufactures spintronic-based sensors for the pacemaker market (St. Jude Medical Inc., St. Paul, Minnesota, USA) and hearing aid market (Starkey Laboratories Inc., Minneapolis, Minnesota, USA).

4.7.3 Neuro-engineered systems for motor control

Applications of new engineering technologies in neuroscience can be used to remedy severe symptoms in neurodegenerative diseases (e.g., rigidity and tremors in Parkinson's disease) (Vesper *et al.*, 2002), restore motor/blader/bowel function after traumatic lesion of the central nervous system (Creasey *et al.*, 2001; Davis *et al.*, 2001), or learn subjects with motor disabilities to utilise their brain activity for different purpose (Leuthardt *et al.*, 2004). The development of neuroengineered systems, such as neuroprostheses and brain-computer interfaces, requires neural-microelectronic interfaces with neural sensing and stimulation devices and has been pushed by new technologies, including micromachining, MEMS, and nanotechnology. Recently, a novel micromachined stimulating probe has been tested in a rat model *in vivo* for deep-brain stimulation which can improve symptoms of Parkinson's disease (Motta and Judy, 2005). The stimulating probe has geometric and mechanical properties that allow accurate positioning in the brain, while minimizing tissue damage, and stimulation of the subthalamic nucleus. The probe shank is coated with gold and the electrode interconnects are insulated with silicon nitride for biocompatibility.

4.7.4 Microchip-based drug delivery systems

Microchip-based drug delivery systems are devices incorporating micrometer-scale pumps, valves, and flow channels and allow controlled release of single or multiple drugs on demand. Micro- and nanotechnology-based methods (e.g., UV-photolithography, reactive ion etching, chemical vapour deposition, electron beam evaporation) can be used for the fabrication of these silicon-based chips. These delivery systems allow high system dynamics, i.e. fast actuation and detection. The devices are particular useful for long-term treatment of conditions requiring intermittent or continuous drug release after implantation in a patient. Some devices can be refilled during use and others are not designed to be refilled. The release mechanism can be based on the electrochemical dissolution of thin anode membranes covering microcompartments, which are filled with drugs. Controlled delivery can be designed to release pulses of different drugs by using different materials for the compartment membrane.

The feasibility of solid-state silicon microchip for controlled release has been demonstrated in proof-of-principle release studies (Santini *et al.*, 1999). The release mechanism of this prototype is based irreversible metallic valves dissolved by a electrochemical reaction of thin (300 nm) gold anode membranes covering microcompartments filled with substances.

Alternatively, microchips can use reversible polymer valves or microactuators consisting of a blend of hydrogel and an electronically conducting redox polymer (Madou and Forkley, 2000). By electropolymerising these polymers onto electrodes, compartments can be opened or closed via the swelling and shrinking processes of the polymer system in response to electrochemical actuation (Low *et al.*, 2000).
DebioSTAR™ is an innovative implantable drug delivery technology currently being developed by Debiotech SA (Lausanne, Switzerland). Using a nanoporous membrane (pore size up to 250 nm) the drug release can be precisely controlled over a prolonged period of time. The membrane is fully biocompatible and can be used in single use or refillable delivery systems. DebioSTAR™ can be used as a passive device as well as an active-pressurised system. Moreover, Debiotech is developing implantable piezo-actuated silicon micropumps, known as MIP (dimensions 16×12×1.86 mm), for programmable drug infusion systems using a MEMS approach to miniaturise pump, actuators, connectors, and channels. Other medical devices are being developed by ChipRx, Inc. (Lexington, Kentucky, USA), and MicroCHIPS, Inc. (Bedford, Massachusetts, USA).

4.7.5 Prosthetic knee system

Conventional leg prosthetics for transfemoral amputees is frequently hampered by stumbling, falling, or walking with an unsual gait of the user. Combining microelectronics and nanotechnology to develop a microprocessor-controlled artificial knee with learning capacity to move more naturally can protect the individual from a potential strumble and fall, reducing fear and increasing confidence. A synergy of artificial intelligence, advanced sensor and magnetorheological actuator technologies has resulted in the development of the Rheo Knee™ (Össur, Reykjavik, Iceland). A microprocessor sends signals to the joint filled with a magnetorheologic fluid actuator made of oil mixed with small iron particles (~100-1000 nm). The magnetorheologic fluid actuator uses magnetic fields to vary instantaneously the knee resistance. Application of a magnetic field polarizes the particles causing a dramatic and smooth change in the suspension's rheological properties. The change is reversible and the particles loose their polarization upon removing the magnetic field. Magnetorheological fluids are mostly used for shock absorbing systems. The resistance of the fluid is activated only when the user needs it most, allowing more natural and effortless motion. Clinical evaluations showed that magnetorheological-based systems offer advantages over mechanically passive designs and hydraulic-based designs (Herr and Wilkenfeld, 2003; Johansson *et al.*, 2005).

4.7.6 Current and future developments

The assessment of clinical studies involving sub- and epiretinal implants should be available over the next five years. The results would allow conclusions to be drawn regarding their efficacy and acceptance to the blind community. According to these conclusions, therapeutic implants and associated training programs might become available. Preliminary assessments of devices which artificially release neurotransmitters, as well as light-controlled chemical stimulation techniques should become available. In five years time this area of research will still be in an active stage of development. However, at this time, an appreciation of the relative usefulness of each approach might become apparent.
Current developments on microchip systems are involving the fabrication of "smart" devices allowing real-time control of drug dosage according to the patient's therapeutic requirements or biological/physical stimulus. Nano-structured "smart" membranes/surfaces are likely to advance the development of programmable, or feedback-controlled, *in vivo* drug delivery devices. Combining a "smart" surface or membrane with an otherwise diffusion-controlled

delivery device permits the release rate to be regulated by changing the permeability of the membrane. Switchable surfaces and membranes can be controlled by light (Ichimura *et al.*, 2000), heat (Lin *et al.*, 1995), pH (Wilson and Whitesides, 1988), and redox and amperometric reactions (Willner and Katz, 2000). An electronically controllable surface would enable direct switching of release rates.
Another extension of the release-controlled microchip would be the development of a passive, polymer microchip without electronics, power supply, or microprocessors made of biodegradable material. Once implanted, such a device would not need to be removed. Recently, microchip devices (1.2 cm in diameter, and ~500 μm thick) with 36 drug compartments have been fabricated from biodegradable poly(L-lactic acid) (Grayson *et al.*, 2003). The drug reservoirs are covered with poly(D,L-lactic-co-glycolic acid) membranes of different molecular masses allowing controlled timing of drug release. On the long-term developments on microchip systems consist of strategies to couple "smart" devices to other implants, such as biosensors, pacemakers, and stents. Many efforts are focused on the treatment of diabetes, with systems to sense blood glucose levels and release insulin in response. This integration has been hampered by difficulties to develop stable biosensors and *in vivo* glucose sensors for long-term implantation are not yet available (Kerner, 2001). A major part of the contribution of nanotechnology resides in some functional components of these devices. In addition, nano-structured materials or surface coatings are expected to improve the biocompatibility of active implantable devices.
It should be noted that other neuroprostheses, e.g. implantable neuromuscular stimulators for bladder/bowel emptying and motor control, will also benefit from enhanced surface modifications. Furthermore, nanotechnology is being applied to improve the signal transmission of stimulation electrodes. It may be argued that the classification of these intelligent devices do not enter the domain of nanotechnology in the strict sense of size dimensions, but instead can be regarded as microtechnology-based devices. It has already been proposed that the definition of nanotechnology should have less stringent limitations on the exact dimensions (Whitesides, 2003). Instead the "right" size should be defined in an operational manner addressing the biological needs. Although the contribution of nanotechnology in the development of these implantable devices may be limited, some specific functional components can only be created if advanced nanotechnologies are applied.
An area of future research of considerable importance is biomechatronics. Biomechatronics focuses on the interactivity of biological organs, including the brain and the neuromuscular-skeletal system, with electromechanical devices and systems (Veltink *et al.*, 2001). A new generation of artificial limbs for amputees are anticipated that can communicate with the users' nervous system and for stroke patients mentally-controlled electrical muscle stimulators. Although human studies demonstrate the feasibility of using brain signals to command and control external devices, many years of development and clinical testing will be required.

4.8 Textiles and wound care products

4.8.1 Antimicrobial textile surfaces

Neither natural nor synthetic textile fibres are resistant to bacterial or pathogenic fungi. Therefore, antibacterial disinfection and finishing techniques have been developed for many types of textiles including treatment of textile fibres by padding cotton and polyester fabrics with nano-sized silver colloidal solutions (25-50 ppm) (Lee *et al.*, 2003) or by melt-spinning of polypropylene and silver nanoparticles with a average diameter of 15 nm (Yeo *et al.*, 2003). Polypropylene is widely used for sanitary applications such as surgical masks, diapers,

filters, hygienic bands, etc. The fabrics showed excellent antibacterial efficacy which was mainly attributable to silver nanoparticles placed on the outside of the fibre and not to nanoparticles located inside the fibre having a negligible antibacterial effect. Although concentration of silver nanoparticles decreased after 20 wash cycles the antibacterial efficacy had a good laundering durability. The Textile Performance Group of company Hyosung (Seoul, South Korea) has developed a nanotechnology fibre with permanent and powerful antibiotic effect known as BIOSILVER/Mipan Nano-Magic Silver®. In Europe JR Nanotech plc (London, UK) introduced various Nano-silver polymers and cotton yarns. Nano-silver can be used to control infection in operation rooms (gown, linen, masks, uniforms) and personal hygiene (incontinent pads, nappies).

4.8.2 Biomedical "smart" textiles

The term "smart" textiles is derived from intelligent or smart materials. Smart textiles are context-aware textiles which are able to react and adapt to stimulus from the environment. Smart textiles can be divided in passive smart textiles, active smart textiles, and very smart textiles (Van Langenhove *et al.*, 2004). Passive smart textiles can only sense the environment and they are sensors. Active smart textiles have a sensing function and they act also as actuators. Very smart textiles take a step further, allowing to adapt their behaviour to the circumstances.

Smart textiles play a key role in the development of biocommunicative clothes for ambulatory measurement and monitoring of vital physiological, kinematic, and behavioural human parameters (Edmison *et al.*, 2004; Grossman, 2004; Guilemaud *et al.*, 2004). Integration of sensors, actuators, and communication systems into woven or knitted textiles is now feasible providing light and wearable user-friendly electronic systems capable of exchanging information with other health-related information systems (Lukowicz *et al.*, 2004). Examples of wearable prototypes are Georgia Tech Wearable Motherboard™ (Park and Jayaraman, 2003), LifeShirt® (Grossman, 2004), Mamagoose pyjama, VTAMN system (Vêtement de Télé-Assistance Médicale Nomade) (Weber *et al.*, 2004), and WEALTHY system (Paradiso *et al.*, 2004). The Georgia Tech Wearable Motherboard™ is currently being manufactured for commercial use under the name Smart Shirt by Sensatex™, Inc. (Bethesda, Maryland, USA). LifeShirt® is commercially distributed by Vivometrics®, Inc. (Ventura, California, USA). Mamagoose pyjama is manufactured by Verhaert Design & Development NV (Kruibeke, Belgium). VTAMN and WEALTHY systems are being developed in research projects financed by the French Ministry of Research and the European Commission, respectively. Foreseen applications in healthcare are medical monitoring in obstetrics, pharmaceutical trials, geriatric care, (post-surgery) rehabilitation, detection of sudden infant death syndrome, mental health and drug delivery. The next step could be the development and integration of new nanotechnology-based functional textiles with new mechanical, electrical, and/or optical properties (Bayindir *et al.*, 2004; Devaux, 2003; Hyde *et al.*, 2005; Lemieux *et al.*, 2003; Subbiah *et al.*, 2005).

Woven fabrics and textiles using carbon nanotubes

Carbon nanotubes yarns have been made by adapting traditional textile spinning techniques using wet spinning techniques for SWCNTs (Dalton *et al.*, 2003; Ericson *et al.*, 2004; Vigolo *et al.*, 2000) and dry spinning techniques for MWCNTs and SWCNTs (Jiang *et al.*, 2002; Li *et al.*, 2004). MWCNTs can be drawn from a "forest" of similar length tubes deposited on a substrate (Baughman, 2000). Applying a twist at the same time results in various forms of yarns, including two-ply and four-ply yarns as well as knitted and knotted yarns (Zhang *et al.*, 2004a). Currently, the length of the yarn is limited to about one metre. However, the spinning process is amenable to automation, which would enable the production of continuous yarns.

Applications are envisaged in wearable electronic textiles, protective clothing, and artificial muscles.

4.8.3 Wound care products

Wound care delivery platforms

Recent advancements in electrospinning enable the production of nanoscale (biodegradable) polymer fibres which have potential applications in wound care (Katti *et al.*, 2004). Alltracel Pharmaceuticals plc (Dublin, Ireland) is developing first-aid wound care products in collaboration with nanofibre technology company ELMARCO s.r.o. (Liberec, Czech Republic). The collaboration focuses on the development of a new delivery platform for Alltracel's m•doc™ (micro-dispersed oxidized cellulose) technology, which is used as effective and efficient haemostatic agent in wound care. ELMARCO has developed a novel process for spinning polymers into nanofibrous non-woven materials for a range of biomedical, chemical, industrial, microelectronic applications.

Nanoparticles and nanofibres for wound dressings

Wound dressings have also been developed and evaluated. The formulation of these dressings can be based on a bilayer of silver-coated, high-density polyethylene mesh with a rayon adsorptive polyester core. The dressing delivers nanocrystalline silver from a nonadherent, nonabrasive surface. *In vitro* studies have shown that the sustained release of this ionized nanocrystalline silver maintains an effective anti-bacterial and fungicidal activity (Wright *et al.*, 1999; Yin *et al.*, 1999). In addition, nanocrystalline silver dressings have been clinically tested in a variety of patients with burn wounds (Tredget *et al.*, 1998), ulcers and other non-healing wounds (Sibbald *et al.*, 2001) facilitating wound care by adequate debridement, and bacterial and moisture balance. Nanocrystalline silver wound dressings are commercially available, such as Acticoat™ manufactured by Smith & Nephew plc (London, UK).

Furthermore, wound dressings composed of an electrospun polyurethane nanofibrous membrane and silk fibroin nanofibres have been developed (Khil *et al.*, 2003; Min *et al.*, 2004). These electrospun materials are characterized by a wide range of pore size distribution, high porosity, and high surface area-to-volume ratio, which are favourable parameters for cell attachment, growth and proliferation. *In vitro* studies have shown enhanced cell adhesion and spreading of type I collagen (Min *et al.*, 2004). The porous structure is particularly important for fluid exudation from the wound, avoiding wound desiccation, and impairing exogenous microorganism infection.

4.9 Chips for molecular diagnostics

A myriad of studies is available for applications of micro- and nanotechnologies in chips for medical molecular diagnostics. Key words are for example DNA microarrays (gene chips), protein microarrays (protein chips), lab-on-a-chip devices, and cell chips. Basically, these devices or systems are constructed using techniques inspired from micro/nanoscale fabrication methods, that are used for processing, manipulation, delivery, analysis or construction of biological and chemical entities. For instance, lab-on-a-chip devices require micro-engineered surface topographies or a chemical wetting contrast in combination with electronics, which are commonly referred to as microelectromechanical systems (MEMS). Further reducing of size leads to nanoelectromechanical systems (NEMS). MEMS for diagnostic applications are sometimes referred to as biochips. These devices are used to detect cells, microorganisms, viruses, DNA and related nucleic acids, proteins, and small molecules. In general, the use of micro- and nanoscale detection technologies is justified by

(1) reducing the sensor element to the scale of target species providing higher sensitivity, (2) reducing reagent volumes and associated costs, (3) reducing time to result due to small volumes resulting in higher effective concentrations, (4) amenability of portability and miniaturisation of the entire system. Microarray devices and lab-on-a-chip devices utilise optical as well as electronic detection schemes.

4.9.1 DNA microarrays

DNA microarrays have become the most successful example of the merger between microelectronics technologies, molecular biology, and chemistry. The techniques used to define the patterns on semiconductor surfaces are utilised to construct arrays of oligonucleotides strands (sequences of nucleic acids generally fewer than 100 bases) or single-stranded cDNA or RNA molecules. Once single strands of known sequences (capture probes) are placed at specific known sites on a chip surface, hybridisation with molecules of unknown sequence (target probes) can reveal the sequence and identify biomarker genes in cancer and other diseases. This approach can be used not only to screen a whole genome, but also to distinguish between gene expression, for example different types of breast cancer (Hedenfalk *et al.*, 2001), or to predict treatment response and cancer prognosis on the basis of gene expression profiles (van 't Veer *et al.*, 2002). Screening a whole genome is usually associated with genomics, the field of study that uses powerful computer technology to understand the structure and function of all genes in an organism based on knowledge of the organism's entire DNA sequence.

Two basic approaches can be used to fabricate DNA arrays, namely optical and electrical. The optical approach uses a photolithographic mask to selectively de-protect sites where chemical reactions can be performed to build the molecule, one DNA base at a time and eventually up to 25 bases (Fodor *et al.*, 1991). This technique requires a large number of masking steps, but it can potentially lead to a higher density of molecules with a certain number of masking steps. In a similar but less costly approach, inkjet printers are used to spray single DNA bases onto lithographically-preshaped wells (little holes on a chip). The other approach takes advantage of the fact that oligonucleotides and DNA have a negative charge, due to the phosphate back-bone and can be electrophoretically transported to specified locations on chip surfaces (Heller, 1996). This can also result in higher local concentration and accelerated DNA hybridisation and electronic stringency (Sosnowski *et al.*, 1997). Both approaches are now being commercialised for single nucleotide polymorphism, short tandem repeats, insertions, deletions, and other genetic mutation analyses.

Several companies are involved in the commercialisation of DNA microarrays for research and medical diagnostic applications. For instance, Affymetrix's, Inc. (Santa Clara, California, USA) GeneChip® uses photolithographic *in situ* synthesis to immobilise oligonucleotides at each spot (Figure 14). Affymetrix's microarray platform can detect subnanogram quantities of genetic material and is one of most commonly used DNA microarray. Recently, the AmpliChip™ CYP450 Test of Roche Diagnostics (Schweiz) AG (Rotkreuz, Switzerland) has been launched in Europe and received CE marking in the fall of 2004. The test combines Roche's patented polymerase chain reaction amplification technology, which replicates even minute amounts of genetic material to detectable quantities, and Affymetrix's high-density microarray technology. The test can determine genetic differences in the cytochrome-P450-iso-enzymes 2D6 and 2C19, thus enabling clinical diagnostic laboratories to identify polymorphisms. The presence of a specific iso-enzyme determines whether an individual will metabolise a drug fast, slow, or normal. Slow metabolism will contribute to a rapid increase of drug concentration, whereas fast metabolism will lead to an early decline.

Agilent Technologies, Inc. (Palo Alto, California, USA) has developed a whole series of DNA microarrays for human genomics and uses an inkjet printing method to deposit cDNA molecules onto glass chips (length 60 nucleotides, spot size 150-200 μm).
Nanogen, Inc. (San Diego, California, USA) has developed a novel platform for electronic detection of nucleic acids on microarrays, the NanoChip® Electronic Microarray. Nanogen's first commercial product, the NanoChip® Molecular Biology Workstation, is an automated multi-purpose instrument that facilitates the detection of known DNA sequences, such as in the analysis of single nucleotide polymorphisms and short tandem repeats using the NanoChip® Electronic Microarray. The unique, open-architecture design permits researchers to define, select and build their own test panels. The accuracy of the NanoChip® Electronic Microarray has been verified recently (Børsting *et al.*, 2004). Another electronic-based microarray has been developed by Motorola Life Sciences (Pasadena, California, USA), the CodeLink eSensor™ Biochip. Microelectronic-based DNA chips appears to best fulfil the requirements of molecular diagnostics (Ferrari *et al.*, 2003). The versatility of the electronic detection platform makes it suitable for multiple applications in pharmacogenetics.

Figure 14. Affymetrix GeneChip® microarray. The actual size of the microarray is 1.28×1.28 cm. It contains 500.000 locations, each containing millions of DNA strands. Image courtesy of Affymetrix, Inc. (Palo Alto, California, USA).

Tm Bioscience Corporation (Toronto, Ontario, Canada) has developed the Tag-It™ Cystic Fibrosis Kit, a multiplexed human disease genotyping *in vitro* diagnostic test. The device simultaneously screens for the 43 mutations and variants, i.e. 23 cystic fibrosis transmembrane conductance regulator gene mutations, 4 variants (polymorphisms), and 16 additional mutations prevalent on the Northern hemisphere.

4.9.2 Protein microarrays

Protein microarrays or protein chips are also proving to be useful for molecular diagnostics. Protein analytes can be recognised by antibodies, enzymes, or aptamers (Stadtherr *et al.*, 2005) immobilised on the chip using inkjet printing methods similar to those applied for DNA microarray fabrication. For the subsequent readout detection either fluorescence- or radionuclide-based markers, or surface plasmon resonance spectroscopy can be applied (Lee and Mrksich, 2002; Mitchell, 2002).
Proteomics is providing a better understanding of pathophysiological mechanisms of human diseases. Profiling proteins on biochips will be of use for example in distinguishing the

proteins of normal cells from early-stage cancer cells and malignant metastatic cancer cells. In comparison with DNA microarrays, protein microarrays offer the possibility of developing a rapid global analysis of the entire proteome (set of all expressed proteins for a given organism), leading to protein-based diagnostics and therapeutics. Analysis of different levels of gene expression in healthy and diseased tissues by proteomic approaches is as important as the detection of mutations and polymorphisms at the genomic level and may be of more value in designing a rational therapy. Proteomic technologies are now being integrated into the drug discovery process as complimentary to genomic approaches and would fit into the emerging trend of individualised clinical treatment combining diagnostics and therapeutics (see also Section 4.9.4).
Ciphergen Biosystems, Inc. (Fremont, California, USA) has developed the ProteinChip® System for protein molecular diagnostics. The ProteinChip® System has a role on proteomics comparable to that of GeneChip® in genomics and is based on a surface-enhanced laser desorption/ionisation process. The ProteinChip® System is the first complete tool to be commercially introduced for disease-focused protein biology. Ciphergen's newer technology, the ProteinChip® Biomarker System, enables clinical researchers to rapidly discover, characterise, and validate predictive biomarker patterns in their own laboratories. ProteinChip® can be used for the profiling of serum to accurately differentiate patients with pancreatic cancer from those with other pancreatic diseases and from healthy control (Koopmann *et al.*, 2004), and for the detection of early stage ovarian cancer (Zhang *et al.*, 2004b).

4.9.3 Lab-on-a-chip

Lab-on-a-chip is another term used for micro-total analysis systems (μTAS). These devices are fabricated in glass or plastic chips and integrate different functions and functionalities. More sophisticated versions can perform sample introduction and handling, preprocessing (e.g., cell lysis, dilution, debris removal), separation (e.g., electrophoresis, chromatography), and detection, all conducted on the chip. The entire chip, including integrated electronics or optics, can be the size of a typical microscope slide or may be in a compact disk format (Duffy *et al.*, 1999; Lai *et al.*, 2004). Thus, essential features of lab-on-a-chip devices, such as small channel diameters, miniaturised pumps, mixers, heaters and valves, etc., enable the use of small sample and reagent volumes (Burns *et al.*, 1998; Figeys and Pinto, 2000). They are used to process and detect cells, proteins, DNA, and small molecules. Thus, besides genomics and proteomics, clinical diagnostics applications also include monitoring of regular metabolic parameters such as glucose, lactate, creatinine, bilirubin, urea, cholesterol and iron.
The construction of a miniaturised "total chemical analysis system" has already been proposed more than a decade ago (Manz *et al.*, 1990b; Manz *et al.*, 1990a). In 1990 the use of diagnostic microchips was unknown to the majority of (clinical) chemists and a trend towards miniaturisation of total analysis systems, having fluidic channel diameters on the micrometre scale to increase the separation performance, and proposed modular construction was identified. Fifteen years later, through the collaborative efforts of chemists, engineers, physicists, and biomedical researchers lab-on-a-chip devices are making a growing mark in biomedical research.
Caliper Life Sciences (Hopkinton, Massachusetts, USA) has developed LabChip® 3000, a capillary electrophoresis chip, that can simultaneously assay the activity of 500 different kinases, enzymes known to play a role in many human diseases, including cancer. This chip, which can analyse 12 chemicals every minute approximately is useful for finding drug candidates that bind to specific kinase while avoiding all others.
Tecan Group Ltd. (Männedorf, Switzerland) has developed the LabCD®, which is a consumable compact disc with micro-scale fluid paths, reaction chambers, and valves. Fluid

is moved along these pathways by capillary action and centrifugal forces generated by disc rotation, allowing the processing of many different assay types. It is the combination of informatics, bioassays, and miniaturisation that make this "lab-on-a-disc" truly innovative. The LabCD® system is designed to meet the needs of the rapidly growing point-of-care testing market (see Section 4.9.4). Because of its distinct ability to combine assays utilising different measurements methods, the LabCD® can be used to conduct disease specific panels from a single patient sample. The most important application of the LabCD® system is in automated DNA screening for infectious diseases. The LabCD® also has a unique ability test concurrently for different strains of the same virus from a single sample, which could have profound implications for individualised clinical therapy. For example, physicians should be able to run tests for multiple strains of hepatitis simultaneously, instead of ordering them separately.

Nanopores

Due to the versatility of lab-on-a-chip systems, these devices can be equipped with new functionalities based on emerging nanotechnologies, such as nanopores, nanoscale actuators, nanotube/wire-based detection systems, or surface structuring. In particular, nanopores (~1-2 nm in diameter) offer the potential for ultra-rapid real-time DNA sequencers (Deamer and Akeson, 2000; Howorka *et al.*, 2001; Meller *et al.*, 2000). Charged strands of DNA are driven by applied electric fields (electrophoresis) through a nanopore of a protein channel, such as an α-hemolysin protein complex, which is inserted into a lipid bilayer separating two conductive compartments, or through a solid state nanopore (Li *et al.*, 2003a; Storm *et al.*, 2003). Continued research in the field of nanopore sequencing has focused on the development of solid-state nanopores that may bypass some of the inherent limitations of protein pores. The translocation duration and current flow during transversal of individual polynucleotides are recorded. These parameters are converted into electronic signatures enabling nanopores to distinguish between polynucleotides of similar length and composition that differ only in sequence. Because nanopores can rapidly discriminate and characterise unlabeled DNA molecules at low copy number, they could eventually provide a low-cost high-throughput method of analysing DNA polynucleotides. This method can sequence more than 1000 base per second and has much potential for the detection of single polymorphisms and for gene diagnosis of pathogens.

4.9.4 Cell chips

Cell chips are comparatively new types of biochips, where entire living cells are immobilised on an array. On cell chips protein interactions can be studied without the problem of protein denaturation, which can occur after processing proteins for the use on protein arrays. Therefore, cell chips can be considered as an alternative to protein arrays, which allow the study of the sensitive membrane proteins. On the other hand, cell chips require an enormous effort in their production due to the fact that cells cannot be spotted or printed on the target surface. One approach to cell chip production is based on the functionalisation of discrete spots, providing optimal conditions for cell growth. Cell chips can be used for a number of studies including antibody screening (Schwenk *et al.*, 2002), drug discovery (Bailey *et al.*, 2002), drug target identification (Ziauddin and Sabatini, 2001), cell membrane ion channels (Fertig *et al.*, 2002), and electrical signals in neurones (Zeck and Fromherz, 2001) and heart muscle cells (Offenhäusser and Knoll, 2001).

Nanotechnology is not impacting on cell chips at the present time. Biological cells have a diameter of at least 10 μm and it is impractical to miniaturise the array to these dimensions. Nevertheless, the functionality of the cell membrane is based on nanoscaled features. For example studies of ion channels in the cell membrane involve tapered glass micropipettes

with apertures of between several 10 nm and 1.5 µm and a resolution in the same range (Fertig *et al.*, 2002). The patch clamp technique is used to measure the ion current through the membrane pore and enables an accelerating screening process for new ion channel-related drugs.
Cell chips are at a very early stage of research making it difficult to forecast their future development. There are a number of companies developing and marketing cell chips, including Cellomics, Inc. (Pittsburgh, Pensylvannia, USA), which is developing chips with a fluorescent detection system suitable for drug discovery and screening experiments, Molecular Devices, Corp. (Sunnyvale, California, USA), which is marketing patch clamp systems, such as PatchXpress® 7000A and OpusXpress® 6000A. In Europe, NanIon Technologies GmbH (München, Germany) and Sophion Bioscience A/B (Ballerup, Denmark) are two examples of companies involved. NanIon Technologies is marketing NPC©-Technology-based micro-structured cell chips replacing conventional glass micropipettes for ion channel analysis, which simplifies the patch clamp procedure (Brueggemann *et al.*, 2004). NanIon's NPC©-Technology is suitable for screening drugs for cardiac arrythmia, epilepsy, and migraine. Sophion Bioscience has developed the QPatch System.

4.9.5 Future directions in novel chip systems and molecular diagnostics

Significant progress has been made in developing highly sensitive and integrated devices for molecular diagnostics and sensing. Challenges and opportunities still exist in the era of continuous monitoring and early detection of clinically significant molecules directly from blood and other body fluids. Rapid detection technologies will be a prerequisite for emerging personalised medicine that will provide the health care providers with genetic differences and variations between individuals to be able to personalise the health care.

Novel chip systems

Novel micro- and nanotechnology-based chip systems can be equipped with electrical, magnetic or acoustic detection schemes omitting optical readout methods. Fluorescence-based readout involves not only highly precise and expensive instrumentation but also sophisticated numerical algorithms to interpret the data. Electrical detection can be based on micrometre-scale silicon-based field-effect sensors (Fritz *et al.*, 2002), electrical conductance (Burmeister *et al.*, 2004), or electrochemical methods (Albers *et al.*, 2003; Gabig-Ciminska *et al.*, 2004b). The advantage of the novel electrical chips is that the analyte DNA does not have to be amplified before used for hybridisation. Moreover, distinguishment of label-free 12-mer oligonucleotides with a single base mismatch can be performed within minutes, i.e. chips can differentiate between a specific sequence and a sequence that differs only by one base (Fritz *et al.*, 2002). This is particular important, because a potential application of DNA chips to detect point mutation associated with disease. In addition, electrochemical detection of pathogenic microorganisms on a biochip is also feasible, enabling rapid identification, i.e. in less than 4 hours, of enterotoxic *Bacillus* strains (Gabig-Ciminska *et al.*, 2004a).
Recently, eBiochip Systems GmbH (Itzehoe, Germany) has placed electrical chips on the market. Currently, FRIZ Biochem (München, Germany) is developing the LADER (Light Addressable Direct Electrical Readout) Chip Platform exploiting the difference in conductance of single (non-conducting) and double (conducting) stranded oligonucleotides. For the magnetic approach giant magnetoresistive multi-layers can be used in biosensors (Schotter *et al.*, 2002; Schotter *et al.*, 2004). Probe DNA is assembled on top of the sensor elements and complementary biotin- or superparamagnetic nanoparticles-labelled analyte DNA is hybridised to the probe DNA. The acoustic approach is under development at Akubio, Ltd. (Cambridge, UK). The acoustic chip is based on the resonant acoustic profiling

technology and is anticipated to be launched in 2005. Resonant acoustic profiling utilizes the piezoelectric properties of quartz crystals to enable real time, label-free analysis of molecular binding events.

Theranostics and point-of-care

Much progress has also been made in therapeutic micro- and nanotechnology. The possible integration of these technologies with diagnostics devices for intelligent and integrated sensing, and the ability to deliver known types and quantities of drugs, chemicals, or physical stimuli would be highly beneficial. This merger between therapy and diagnostics is also known as theranostics, which goal it is to perform a diagnostic test that aids in the selection of patients and has the ability to monitor the biological effects of a (drug) therapy rapidly, at the point-of-care.

Lab-on-a-chip devices are beginning to be applied in point-of-care testing enabling clinicians to use compact and flexible clinical chemistry testing devices suited to testing close to the patient. These analytical devices are designed to move diagnostic testing out of central laboratories into sites where health care matters, i.e. in areas where fast diagnostic monitoring can improve medical decision-making, such as intensive units and operating theatres, or where the frequency necessitates measurements physically close to the patient, for example at the physician's office or the patients' home. In the near term, lab-on-a-chip devices are envisaged to enable automate routine molecular separation and analyses, and in the process, will vastly reduce the amount of sample and expensive reagents needed for any given experiments that a lab can perform at any time. Polymerase chain reaction, mRNA profiling, mutational analysis, and protein expression profiling are just a few important laboratory tools that may soon be available as fully integrated lab-on-a-chip systems, which could enable for instance cancer researchers to rapidly characterise tumour cells based on molecular characteristics. Rapid and affordable point-of-care testing will also require disposable (smart) lab-on a-chip devices (Ahn *et al.*, 2004). Instead of silicon or glass as substrates, polymers can be used for the fabrication of these disposables devices offering several advantages like low cost, rugged construction, ease of fabrication, and rapid prototyping.

4.10 Delivery devices / tools

In recent years a plethora of studies have been published on new drug, gene, protein, and radionuclide delivery devices. Some examples are provided in the previous paragraphs on nanodevices for cancer therapy (see Section 4.2), drug eluting stents (see Section 4.6), microchip based drug delivery systems (see Section 4.7.3 and 4.7.4), smart textiles (see Section 4.8.1) and wound dressings (see Section 4.8.3). Several reviews on the subject have been published (Kipp, 2004; LaVan *et al.*, 2003; Sahoo and Labhasetwar, 2003), including a theme issue of Advanced Drug Delivery Reviews, edited by Peppas (2004). It is, however, beyond the scope of this report to elaborate on this application. Instead, some examples are given illustrating recent developments.

4.10.1 Needles for administration and monitoring

Microneedles

Advances in materials processing, such as micromachining and nanoprocessing, contribute to the development of innovative minimally invasive drug delivery devices. Traditional production techniques make it unfeasible to manufacturer needles with a diameter less than 300 μm. Microneedles are designed to be as small as possible, extremely sharp, with sub-micron tip radii, inserted into the skin, and to be high performance devices, through which

solutions (drugs, proteins, DNA, or vaccines) can be administered to the body (Henry *et al.*, 1998; Martanto *et al.*, 2004) and can be functionally combined with a MEMS device for pumping, liquid storage and liquid dosing (Griss and Stemme, 2003). Due to the small size of the needles tissue damage is limited and pain sensation is reduced or even completely avoided since microneedles do not reach the nerve endings found in deeper tissue (Kaushik *et al.*, 2001). Microneedles can be solid or hollow at almost any size and geometry because they are defined lithographically (Gardeniers *et al.*, 2003; McAllister *et al.*, 2003). They can be made of metal or silicon (e.g., Cormier *et al.* (2004), Zahn *et al.* (2000)). A novel approach is the fabrication of biodegradable polymeric microneedles with an appropriate geometry and sufficient strength to insert into skin (Park *et al.*, 2005). Microneedles can also be used for diagnostic purposes, such as sampling blood for glucose monitoring. Recently, a microfabricated dialysis microneedle has been developed which is permeable to small molecular weight molecules, yet excludes larger compounds such as proteins (Zahn *et al.*, 2005). The microdialysis microneedle can ealisy be integrated with electrochemical sensors which are prone to protein adsorption affecting the sensor signal. Microdialysis microneedles offer the potential in maximising the efficacy of portable medical monitors by decreasing size, patient discomfort, fluid volumes, and energy consumed by the device.
Microneedles are commercially available medical devices, for instance Debiotech SA (Lausanne, Switzerland) manufactures MicroJect, a microneedle array for hypodermic drug delivery and/or interstitial fluid diagnostics developed in collaboration with the Royal Institute of Technology in Stockholm (Figure 15). MicroJect can be used in combination with a MEMS micropump for insulin therapy. BioValve Technologies, Inc. (Westborough, Massachusetts, USA) is distributing MicroTrans™ with similar functionality. Micronit Microfluidics BV (Enschede, The Netherlands) has developed an array of hollow silicon-based microneedles for painless blood sampling and drug delivery. Currently, Kumetrix, Inc. (Union City, California, USA) is developing a device that contains a cartridge of disposable silicon microneedles and a handheld meter producing readout of the blood glucose level displayed on a monitor.

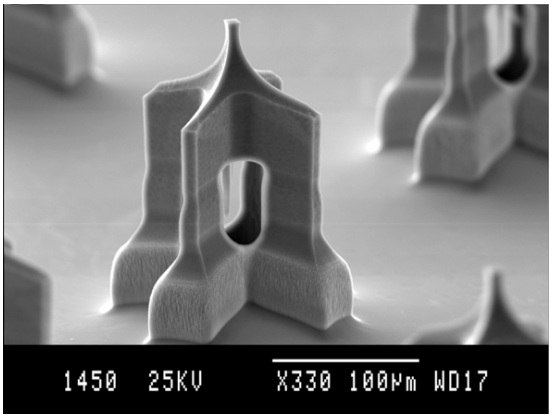

Figure 15. Microneedle for minimally invasive transdermal drug delivery.
Source: KTH, Royal Institute of Technology, Stockholm, Sweden.

Nanoneedles
Nanoneedles prepared from silicon and attached to an atomic force microscope can be used to penetrate the nucleus of living cells to deliver molecules and may be even used to carry out cell surgery (Obataya *et al.*, 2005). Typical features sizes of these nanoneedles are 200- 300

nm in diameter, and 6-8 μm in length. It was demonstrated that nanoneedles do not indent the plasma membrane and nucleus, but penetrate through the membrane. Minimal deformation of cells is essential for cell manipulation because undesired mechanical responses may interfere with the result of manipulation (Charras and Horton, 2002). By modifying the surface of a nanoneedle, various molecules such as DNA, proteins or chemicals can be loaded by standard immobilisation techniques.

4.10.2 Nanomaterials for brachytherapy and nanocapsules

BrachySil™ a silicon-based nanoscale system that takes substances direct to the tumour site based on the material known as BioSilicon™ incorporating radionuclide ^{32}P. It is biodegradable, safe to administer, cheap and effective in tumour regression. BrachySil™ is manufactured by pSivida, Ltd. (Perth, Australia). Recently, a phase IIa clinical investigation started with cancer patients treated with at Singapore General Hospital (June 2004). BrachySil™ is classed as a medical device and product launch is scheduled for 2007, following the phase IIb clinical investigation in 2005-2006.

Recently, a new protein from the poplar tree has been discovered with a remarkable structure and properties (Dgany *et al.*, 2004). The protein, called SP-1, has a nanometric, "bagel-shaped", circular form and is extremely stable. SP-1 is capable of surviving exposure to boiling, excessive acidity, salinity, organic solvents, detergents solutions and contact with proteases. It has also the ability to assemble itself into a structure composed of twelve identical units, creating a nanocapsule capable of delivering drugs to certain types of solid cancer tumours.

4.10.3 Nanoparticles for drug delivery across blood-brain barrier

The blood-brain barrier is a biological barrier in the form of tight junctions between epithelial cells, which impedes the extravasation of a large number of vascular agents. Physicochemical properties of compounds, such as lipophilicity and molecular weigth, determine to what extent the compounds can cross the blood-brain barrier. Compounds or drugs that are not ionized at physiological pH, lipophilic, and of low molecular weight can cross the barrier by diffusion mechanisms. Other essential compounds, such as amino acids, neuropeptides, and hexoses, normally need specific carriers to permeate the brain. Nanotechnology-based delivery systems have shown efficacy in *in vivo* murine models (Alyautdin *et al.*, 1997; Kreuter *et al.*, 2003; Kreuter, 2004; Schroeder *et al.*, 1998) and *in vitro* bovine and human brain capillary endothelial cell models (Kreuter *et al.*, 2003) by virtue of the properties of their constituent (surfactant) material. Polymeric nanoparticles such as poly(butylcyanoacrylate) and poly(akylcyanoacrylate) coated with suitable surfactants such as polysorbate (Tween) 80 or poloxamer (Pluronic F68) have shown to enable transport of drugs across the blood-brain barrier. The average diameter of these nanoparticles is about 200-400 nm. Similar results have been obtained with emulsifying wax/Brij® 78 nanoparticles *in vitro* and *in vivo* studies (Lockman *et al.*, 2003), and, recently, with poly(ethylene glycol)-poly(lactide) nanoparticles conjugated with cationic albumin *in vitro* (Lu *et al.*, 2005).

At present, the mechanism of nanoparticle-mediated transport of drugs across the blood-brain barrier is not fully elucidated. The most likely mechanism is endocytosis by the endothelial cells lining the brain blood capillaries. Nanoparticle-mediated drug transport to the brain depends on the overcoating of the particles with polysorbates, especially polysorbate 80. Overcoating with these materials seems to lead to the sufficient adsorption of apolipoproteins B and E from blood plasma onto the nanoparticle surface (Kreuter *et al.*, 2002). These particles then seem to mimic low density lipoprotein particles and could interact with the low density lipoprotein receptor leading to their uptake by the endothelial cells. After this the

drug may be released in the endothelial cells and diffuse into the brain interior or the particles may be transcytosed (Kreuter, 2001).
The development of nanoparticles as drug delivery system to the central nervous system could allow for more effective therapies for brain tumours in humans as has been shown in glioblastoma-bearing rats treated with doxorubicin bound to polysorbate-coated nanoparticles (Steiniger *et al.*, 2004). In addition, nanotechnology-based drug delivery could enable significant advances in the treatment of other central nervous system diseases. For example, neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease are associated with metal ion-induced protein precipitation in senile plaques located in the brain (Lovell *et al.*, 1998). Recently, chelator-conjugated nanoparticles have been shown to effectively resolubilize copper-β-amyloid aggregates *in vitro* (Cui *et al.*, 2005).
NanoDel Technologies GmbH (Magdeburg, Germany) is developing nanoparticles as a drug delivery formulation especially for diseases of the central nervous system, the NanoDel™ technology. Depending on the method of polymerisation, drugs are either attached to the surface and/or are incorporated into poly(butylcyanoacrylate) nanoparticles. NanoDel Technologies is developing a patent-protected nanoparticle/doxorubicin formulation for the treatment of brain tumours. NanoMed Pharmaceuticals™, Inc. (Kalmazzo, Michigan, USA) is developing nanoparticle-based advanced drug delivery systems using the Nanotemplate Engineering™ platform technology, which is a scaleable nanoparticle manufacturing technology that enables the production of nanoparticles <100 nm in diameter (Lockman *et al.*, 2003).

4.10.4 Nanotube-based delivery applications

Carbon nanotubes-based protein transporters

Many therapeutic agents may turn out to be proteins, but proteins can be difficult to get across the cell membrane and into the cytoplasm while still retaining their biological function. SWCNTs could become a new class of generic tool for delivering small peptides (Pantarotto *et al.*, 2004a) and proteins (Kam *et al.*, 2004; Kam and Dai, 2005) into cells *in vitro* as well as *in vivo*. Acid-oxidised SWCNTs bind various types of proteins (≤80 kD) and transport them through the cell membrane. These acid-treated carbon nanotubes are stable in water and do not aggregate, as do untreated carbon nanotubes. The uptake mechanism is not fully understood. Proposed mechanisms are endocytosis (Kam *et al.*, 2004), phagocytosis (Cherukuri *et al.*, 2004), and insertion and diffusion through the lipid bilayer of the cell membrane (Bianco *et al.*, 2005; Pantarotto *et al.*, 2004a). In many instances, a cell breaks down proteins transported via endocytosis, but the SWCNT-bound proteins avoid this fate if concurrently a small amount of the antimalarial drug chloroquine is delivered leading to swelling of the endosomal compartments and eventual rupture (Ogris *et al.*, 1998). For reasons that are still unclear, acid-treated carbon nanotubes are able to bind a large protein, human immunoglobulin (~150 kD), but are not able to transport that protein across the cell membrane. To test if carbon nanotubes can deliver small proteins that then retain their biological activity once inside the cell, SWCNTs were used to deliver the protein cytochrome *c* (cyt-*c*) which triggers apoptosis (Cai *et al.*, 1998; Zhivotovsky *et al.*, 1998). Cell line experiments showed that SWCNT-bound cyt-*c* retained its biological activity and did cause significantly higher rates of apoptosis than did either cyt-*c* or the nanotubes alone. Whether cyt-*c* remains attached to the nanotubes or whether it is released into the cytoplasm, has to be elucidated.

Magnetic silica nanotubes

Over the past several years, silicon nanotubes have become attractive starting materials for creating multifunctional nanodevices, largely because the inside and outside of the nanotube

can be modified independently of one another. This characteristic has been used to create multifunctional magnetic nanotubes (60 nm diameter, 3 μm length) which can be used as drug delivery vehicles and in magnetic-field-assisted bioseparation (Son *et al.*, 2005). Coating the inside surface of silicon nanotubes with a layer of magnetite (Fe_3O_4) nanoparticles exihibits similar superparamagnetic characteristics compared to nano-structured silica-coated magnetite particle (Yang *et al.*, 2004). Various drugs can be loaded into the pores of the magnetic nanotubes, including the anticancer agent 5-fluorouracil. Drug release rates are determined by the chemical composition of the nanotube's inner surface.

4.10.5 Nanovectors for gene therapy

Non-viral gene delivery systems can be used to deliver foreign genes intro somatic cells to supplement defective genes or provided additional biological functions. Several nanomaterial-based vectors have been developed using functionalised MWCNTs and SWCNTs (Bianco *et al.*, 2005; Pantarotto *et al.*, 2004b; Singh *et al.*, 2005), bimetallic gold/nickel nanorods (Salem *et al.*, 2003), TiO_2 nanoparticles (Paunesku *et al.*, 2003), silica nanoparticles (Roy *et al.*, 2005), calcium phosphate nanoparticles (Bisht *et al.*, 2005), magnesium phosphate and manganous phosphate (Bhakta *et al.*, 2005), and dendrimers (Kukowska-Latallo *et al.*, 2000; Luo *et al.*, 2002; Santhakumaran *et al.*, 2004). In addition, magnetic nanoparticles associated with vector DNA are used to enhance the efficiency of transfection into cells by the influence on an external magnetic field (Krötz *et al.*, 2003; Plank *et al.*, 2003; Scherer *et al.*, 2002). Recently, the succesful *in vivo* DNA transfection and modulation of the activity of neuronal cells in brains of mice has been reported using amino-functionalised organically modified silica (ORMOSIL) nanoparticles (Bharali *et al.*, 2005).

5. Conclusions

Nanotechnology offers important new tools expected to have a great impact on many areas in medical technology. It provides extraordinary opportunities not only to improve materials and medical devices but also to create new "smart" devices and technologies where existing and more conventional technologies may be reaching their limits. It is expected to accelerate scientific as well as economic activities in medical research and development.
Nanotechnology has the potential to make significant contributions to disease detection, diagnosis, therapy, and prevention. Tools are important and integral parts for early detection. Novel tools and tools complementing existing ones are envisaged. It offers opportunities in multiple platforms for parallel applications, miniaturisation, integration, and automation.
Nanotechnology could have a profound influence on disease prevention efforts because it offers innovative tools for understanding the cell as well as the differences between normal and abnormal cells. It could provide insights into the mechanism of transformation, which is fundamental in designing preventive strategies. Further, it provides novel non-invasive observation modalities into the cellular machinery. It allows for the analysis of such parameters as cellular mechanics, morphology, and cytoskeleton, which have been difficult to achieve using conventional technologies.
It could be argued that the classification of some devices do not enter the domain of nanotechnology in the strict sense of size dimensions. However, some specific functional components in electric/electronic devices that some would classify as microtechnology can only be created if advanced nanotechnologies are applied. Furthermore, nanostructuring of materials and their surfaces can play an essential role in the interaction of materials and devices with the human body.

Several medical devices have already benefited from recent developments in nanotechnology. These medical devices are in use or are currently being commercialised. In particular, the following medical devices are on the market:

- surgical tools with enhanced material properties enabling better handling,
- microcantilevers for label-free assays used in molecular *in vitro* diagnostics,
- novel nano-sized contrast agents for molecular imaging improving the quality of *in vivo* diagnostics,
- bone replacement materials obtained by nano-structured materials allowing better implant integration and bio-compatibility,
- pacemakers and hearing aids based on spintronic technology enabling size reduction and power enhancement of these medical devices,
- DNA/protein microarrays and lab-on-a-chip devices for molecular *in vitro* diagnostics
- wound dressings and textiles incorporating nanocrystalline particles with antibacterial and fungicidal activity,
- microneedle-based systems for minimally invasive drug administration or for blood substance monitoring limiting tissue damage and pain sensation.

A number of other medical applications are currently being evaluated in clinical investigations or are expected to enter clinical research in the near future:

- superparamagnetic iron oxide nanoparticles administered by stereotactic navigation-based injection for hyperthermia treatment of brain/prostate tumours (now)
- retinal prostheses, to restore vision in blind patients (now)

- silicon-based nanocarrier system incorporating a radionuclide to treat tumours via brachytherapy (now)
- bio-bar code assay to detect specific ligands in the cerebrospinal fluid of patients with Alzheimer's disease (now)
- stents coated with nanoporous hydroxyapatite (within 1 year)
- superparamagnetic iron oxide nanoparticles conjugated with monoclonal antibodies, injected intravenously for selective, targeted thermotherapy to treat tumours (within 2 years)
- other targeted sensitiser nanoparticles physically triggered using heat, magnetic field, light, or radiation for tumour treatment (within 3 years)
- dendrimer-based nanoplatforms capable of delivering drugs and genes to specific targeted cells with imaging/monitoring modality (within 3 years)

A substantial part of scientific research is still in its infancy. Although much research presents only proof-of-concept results, substantial advances have been accomplished. These "prototypes" are awaiting more laboratory and preclinical investigations. Future research will undoubtedly make progress. Whether the proof-of-concepts will be transferred into realistic medical devices and eventually will be commercialised is still to be seen. Definitely, for some applications this will be several years ahead. Nevertheless, great expectations are foreseen regarding the impact of emerging nanotechnology-based devices and systems on disease detection, diagnosis, treatment and prevention.
Nanotechnology could be useful in the area of biomarker research. It could provide successful strategies for real-time and direct readout of genomic and proteomic information at the single molecule and single cell level. In addition, it would allow for multiparametric analysis using relatively small sample volumes. Nanotechnology could help provide additional sensitivity in assays through analysis of single cells and extremely limiting amounts of samples. For example biosensors or biodetectors, irrespective whether they are based on nanotubes, nanowires, nanoarrays, or nanoelectromechanical systems, will probably enter the market sooner than expected. Inexpensive hand-held biosensors may allow simple detection of diseases, within minutes, from blood samples or saliva. Early detection of illness and deviation from normal functioning will be accomplished through inexpensive biomonitoring. Biosensors could eliminate the need for maintaining large laboratories, transporting samples within facilities, and sending samples out for external analysis. Forecasts are being made that biosensors will proliferate rapidly in all health care segments.
Also in the field of molecular imaging, where the first applications are already on the market, there is much more on the horizon. Especially in the battle against cancer, valuable time is expected to be gained by the enabling of very early stage diagnoses.
Nano-structured materials or surface coatings will continue to improve the biocompatibility of a growing range of devices and scaffolds for tissue engineered products Especially, the work on nanofibrillar networks produced by self-assembly is expected to converge with advances made in cell biology to provide functional scaffolds for tissue engineering applications in the following decade.
New challenges are ahead for the testing at the point-of-care. Point-of-care testing devices enable more widespread monitoring of health parameters in disease prevention, but even more importantly, daily screening of vital health parameters in order to extend and improve quality of life. Household analysers, miniaturised "personal laboratories" can report on the daily health status. Medical decisions can be based on the measurement results, immediately available at the general practitioner's office. It is envisaged that point-of-care devices will form an integral part of human life sometime in the future.

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Appendix A – Historical perspective

Historical perspective of a few nano (scientific) milestones, adapted from Stix (2001).

3800 million years ago The first living cells emerge.

5th century BC The Greek philosopher Leucippus postulates the theory of Atomism. Leucippus' initial theory is a concept of the nature of the universe: the universe is composed of invisible, indestructible material particles. In Greek the prefix "a" means "not" and the word "tomos" means "cut". Thus, "atomos" or atom means uncuttable or undividable.

4th century BC Greek philosopher Democritus (Leucippus' disciple) expands the atomic theory. Matter consists of atoms that remain the same but form different combinations with each other in number and arrangement, eventually accomplishing the differences between things.

4th century AD Roman glassmakers use nano-sized metals, maybe not deliberately, to make decorative glasses. An archaeological curiosity is the Lycurgus cup in the British Museum: the oldest known object incorporating gold nanoparticles. An unusual feature of this goblet is its colour. When viewed in daylight it appears green. However, when light shines into the cup and transmitted through the glass, it appears red (Wagner *et al.*, 2000).

Middle Ages Gold and silver nanoparticles are used as coloured pigments in stained glass of the windows in medieval cathedrals (Erhardt, 2003).

1802 Scientist Thomas Wedgewood publishes his experiments on creating images using silver nitrate and chloride. His initial experiments are elaborated in the 19th century and the evolution of photography emerges. The process of image formation in photography depends on small colloidal particles of silver.

1861 Scientist James Clark Maxwell, who contributed to the electromagnetic theory, produces the first colour photograph.

1883 Inventor George Eastman (founder of Kodak Corp.) produces a film consisting of a long paper strip coated with an emulsion containing silver halides. Later he develops the rolled-op film.

1908 Gustav Mie provides an explanation of the dependence of the colour of glasses on metal size and kind (Mie, 1908).

1931 Max Knoll and Ernst Ruska (Nobel Prize in Physics, 1986) develop the electron microscope, which enables sub-nanometre imaging.

1959 Richard P. Feynman (Nobel Prize in Physics, 1965) proclaims his original thoughts in his visionary lecture entitled "There's Plenty of Room at the Bottom" at the California Institute of Technology (Feynman, 1959). He suggests that it would be possible to put the entire 24 volume Encyclopaedia Britannica on the head of a pin and speculates on the possibility and potential of nano-sized materials. Feynman foresees a tremendous performance increase of computing machines by miniaturisation. Ultimately he proposes that mankind can manipulate individual atoms to make structures with very unique properties.

1968 Alfred Y. Cho, John Arthur of Bell Laboratories and their colleagues invent molecular-beam epitaxy, a technique that can deposit single atomic layers on a surface.

1974 Norio Taniguchi of the University of Tokyo conceives the word "nanotechnology" to describe the production technology of materials with nanometre precision.

1981 Gerd Binnig and Heinrich Rohrer of IBM Zürich Research Laboratory (Nobel Prize in Physics, 1986) create the scanning tunnelling microscope, which can image individual atoms.

1985 Harold W. Kroto of the University of Sussex, Robert F. Curl and Richard E. Smalley of Rice University (Nobel Prize in Chemistry, 1996) discover buckminsterfullerenes, also known as buckyballs, which measure less than one nanometre in diameter.

1989 Donald M. Eigler of IBM Almaden Research Center demonstrates the ability to build structures at the atomic level using the scanning tunnelling microscope. He writes the letters of his company's name using individual xenon atoms.

1991 Sumio Iijima of NEC Fundamental Research Laboratories discovers multi-walled carbon nanotubes. Iijima synthesises single-walled carbon nanotubes in 1993.

1993 Warren Robinett of the University of North Carolina and R. Stanley Williams of the University of California at Los Angeles devise a virtual-reality system connected to a scanning tunnelling microscope that enables the user to see and touch atoms.

1998 Cees Dekker *et al*. at Delft University of Technology create a transistor from a carbon nanotube.

1999 M. Tour and Mark A. Reed of Yale University demonstrate that single molecules can act as molecular switches.

Appendix B – Carbon nanotubes

B.1 Topology of carbon nanotubes

The structure of SWCNTs can be conceptualised by wrapping a one atom-thick layer of graphite, called graphene, of a certain size into a seamless cylinder. Although carbon nanotubes are not actually made by rolling a graphene sheet, there are many ways to form a SWCNT. The various ways can be mathematically defined by the chiral vector or vector of helicity $\underline{\boldsymbol{C}}_h$, and the angle of helicity θ as follows (Dresselhaus *et al.*, 1996):

$$OA = \underline{\boldsymbol{C}}_h = n\underline{\boldsymbol{a}}_1 + m\underline{\boldsymbol{a}}_2$$

and $\cos\theta = (2n + m) / (2\sqrt{(n^2 + m^2 + nm)})$

where $\underline{\boldsymbol{a}}_1$ and $\underline{\boldsymbol{a}}_2$ are unitary vectors of the hexagonal honeycomb lattice.

The vector of helicity $\underline{\boldsymbol{C}}_h$ is perpendicular to the tube axis, while the angle of helicity θ is taken with respect to the so-called zigzag axis (Figure B.1). A pair of indices (n,m) represents the way the graphene sheet is wrapped. The integers n and m denote the number of unit vectors along two directions in the honeycomb lattice of graphene. This is often thought of as representing the number of carbon atoms around the circumference of the tubes, and the number of atoms down the tube axis.

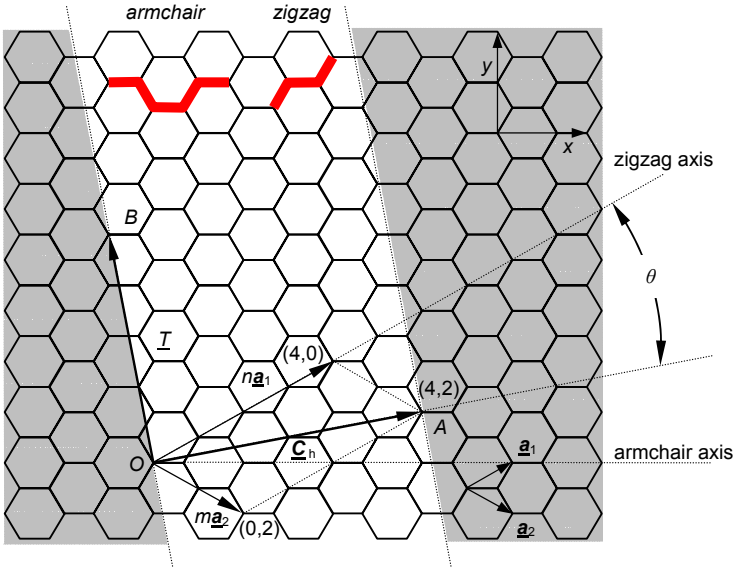

Figure B.1. Schematic diagram showing how a 2D hexagonal sheet of graphene is wrapped into a carbon nanotube (adapted from Dresselhaus et al. (1996)). In this diagram, a (4,2) nanotube is constructed.

The diameter D of the corresponding nanotube is related to $\underline{\boldsymbol{C}}_h$ as follows:

$$D = 1/\pi \cdot |\underline{\boldsymbol{C}}_h| = a_{CC}/\pi \cdot \sqrt{(3(n^2 + m^2 + nm))}$$

where 1.41 Å (graphite) $\leq a_{CC} \leq$ 1.44 Å (C_{60})

The carbon-carbon bond length (a_{CC}) is actually elongated by the curvature imposed, taking the average value of the C_{60} molecule as a reasonable upper limit, and the value of flat graphenes in genuine graphite as the lower limit. Since $\underline{\boldsymbol{C}}_h$, θ, and D are all expressed as a function of integers n and m, they are sufficient to define any SWCNT specifically, by noting them (n,m). In particular, three typical chiral structures of SWCNTs have been proposed defined by the relation between n and m:

Armchair tube	(n,n)
Zigzag tube	$(n,0)$
Helical tube	(n,m)

These structures have been confirmed using scanning tunnelling microscopy and electron diffraction studies (Dresselhaus *et al.*, 1996; Wildöer *et al.*, 1998).

B.2 Synthesis of carbon nanotubes

Using the electric carbon arc discharge method, initially used for producing C_{60} molecules (Krätschmer *et al.*, 1990), a voltage is applied between two solid carbon electrodes (electrode diameter 5-20 μm, distance between electrodes 1 mm) at reduced atmosphere of flowing inert helium or argon gas (Iijima, 1991). In this plasma-based process the applied voltage (20-25 V) creates a high temperature (800-5000 °C) discharge between the two graphite electrodes. Carbon atoms are ejected from the anode (positive electrode) and are deposited at the cathode forming a range of nanotube types, i.e. for the greater part MWCNTs. The uniformity of the plasma arc and the temperature of the deposit are crucial for yielding sufficient quantitative amounts of carbon nanotubes (Ebbesen and Ajayan, 1992). Incorporating a metal catalyst, e.g. cobalt, iron or nickel, in the central region of the anode enables the synthesis of SWCNTs (Bethune *et al.*, 1993; Iijima and Ichihashi, 1993). SWCNTs are deposited in different regions of the reactor such as around and above the cathode, and the soot is deposited all around the reactor walls and the bottom.
While the technique of laser ablation was used successfully to synthesize fullerenes for the first time in 1985 (Kroto *et al.*, 1985), it improved over the years and became apparent for the synthesis of carbon nanotubes ten years later (Guo *et al.*, 1995). Laser ablation which only makes SWCNTs, uses a high-power, pulsed Nd:YAG laser aimed at powdered graphite loaded with metal catalyst. The graphite is placed in the middle of an inert gas-filled quartz tube and subsequently in an oven maintaining a temperature of 1200 °C for the best quality. Lower temperatures affect the structural quality of carbon nanotubes. The laser beam focused on the graphite enables it to vaporize and the soot is deposited in different regions. Various improvements have been made in order to increase the production efficiency, including using a second laser (Thess *et al.*, 1996), a continuous high-energy CO_2 laser (de la Chapelle *et al.*, 1999), or a second quartz tube (Rinzler *et al.*, 1998) or pellet (Yudasaka *et al.*, 1997).
Both the arc discharge and the laser ablation techniques are limited in the volume of sample they can produce. In addition, subsequent purification steps are necessary to separate the nanotubes from undesirable by-products. These limitations have motivated the development of other techniques such as catalytic chemical vapour deposition. This technique can be used to make either SWCNTs or MWCNTs, and involves decomposing a solid carbon source or a hydrocarbon gas, such as methane, on small metallic particles (Kong *et al.*, 1998; Li *et al.*, 1996; Ren *et al.*, 1998). The temperature is lower compared to the arc discharge and laser ablation techniques, usually between 600 °C and 1000 °C. Typically, a nickel, iron, molybdenum, or cobalt catalyst is used. In general, carbon nanotubes are longer (up to hundreds of micrometers) compared to those prepared by the carbon arc method (few micrometers). This method produces carbon nanotubes with open ends.
Finally, in several gas-phase processes, such as a high-pressure carbon monoxide process, both SWCNTs and MWCNTs can be produced using a reaction that takes place on a catalyst flowing in a stream, rather than bonded to a surface. SWNCTs can be synthesized in a continuous-flow gas phase using carbon monoxide mixed with $Fe(CO)_5$ as the iron-containing catalyst precursor (Nikolaev *et al.*, 1999). This method is promising to produce bulk SWCNTs of high purity and claims to be relatively free of by-products. The temperature and pressure conditions (1200 °C, 10 atm) required are applicable to industrial plants.

B.3 Mechanical properties of carbon nanotubes

The general mechanical behaviour of solid materials is considered in terms of strain, stress, strength. Strain (ε) is a mathematical term to express the trend of the deformation change of a material. Tensile stress (σ) is a loading that tends to produce stretching on a material by the application of axially directed pulling forces. Tensile strength (σ_t) is a limit state of tensile stress. It is a measure of the amount of stress needed to pull material apart. The Young's modulus or tensile modulus (E) is a measure of the stiffness of a material and is well defined. It can be experimentally determined by the slope of the stress-strain curve during tensile tests. The Young's modulus allows engineers and scientists to calculate the behaviour of material under load. For instance, it can be used to predict the amount a material will extend under tension, or to predict the load at which a material will buckle under compression.
The quantification of mechanical properties of carbon nanotubes is not as straightforward as with (macroscopic) bulk material. Generally, using standard techniques of mechanical characterization two ends of a single specimen are mounted in an apparatus (extensiometer) that bends or extends the specimen with a controllable force. The Young's modulus of any material can be measured provided that a large enough quantity of material

is present and that the material can be shaped into a rod. The difficulties of mounting materials with nanometer diameter, or even micrometer urges for new approaches to probe the mechanics of these materials. During the last decade several new techniques have been published to analyse the mechanical properties of nanotubes by:

1. Measuring the temperature dependence of the vibration amplitude using transmission electron microscopy (TEM) which gives an estimate of the Young's modulus (Osakabe *et al.*, 1997; Treacy *et al.*, 1996). However, this technique has limitations. First, the size (diameter and length) of samples is restricted. Second, this approach cannot evaluate the strength and toughness of carbon nanotubes.
2. Observing electric field-induced vibrations using TEM (Krishnan *et al.*, 1998).
3. Examining the pattern of mechanically deformed MWCNTs in polymer composites with a TEM (Lourie *et al.*, 1998).
4. Observing thermally-induced vibrations using atomic force microscopy (AFM) (Poncharal *et al.*, 1999).
5. Measuring lateral bending and force exerted of suspended MWCNTs (Wong *et al.*, 1997) or SWCNTs ropes (Walters *et al.*, 1999) using AFM.
6. Measuring axial compression with tapping-mode AFM (Yu *et al.*, 2000b).
7. Measuring stress-strain relationship and strength-at-failure of MWCNTs (Yu *et al.*, 2000c) and SWCNTs (Yu *et al.*, 2000a) under tensile loading inside a scanning electron microscope (SEM).

Theoretical calculations of mechanical properties showed that Young's modulus is ~1 TPa and ~1.11 TPa for MWCNTs and SWCNTs, respectively (Lu, 1997). The dependence of elastic properties on the nanotube diameter and helicity angle is still controversial. It was found to be different for the theoretical models applied: either largely independent of diameter and helicity, except for very narrow tubes with diameters below ~0.6 nm (Cornwell and Wille, 1997; Lu, 1997; Robertson *et al.*, 1992), only affected by helicity (Sun and Zhao, 2005), only affected by diameter (Hernández *et al.*, 1998), or diameter and helicity angle (Yao and Lordi, 1998). Measuring the effective bending modulus for a series of MWCNT of different diameters showed a dramatic decrease from ~1 TPa to ~100 GPa with diameter increasing from 8 to 40 nm (Poncharal *et al.*, 1999). The effective bending modulus corresponds to the standard Young's modulus if the nanotube bends by stretching in the outer arc and by compression in the inner arc of the tube. The large variation in Young's modulus has also been demonstrated for tensile strength measurements (Table B.1).

Table B.1. Mechanical properties of carbon nanotubes.

		Young's modulus (TPa)		Tensile strength (GPa)		
Type	Synthesis					References
MWCNT	Arc discharge	1.81±1.37	0.40-4.15	-	-	(Treacy *et al.*, 1996)
	Arc discharge	1.28±0.59	-	14.2±8.0	?-28	(Wong *et al.*, 1997)
	Arc discharge	0.81-0.16/+0.41	-	-	-	(Salvetat *et al.*, 1999)
	CCVD	-	0.01-0.05	-	-	(Salvetat *et al.*, 1999)
	Arc discharge	-	0.27-0.95	-	11-63	(Yu *et al.*, 2000c)
	Arc discharge	0.91	-	150	-	(Demczyk *et al.*, 2002)
SWCNT	Laser ablation	1.25-0.35/+0.45	-	-	-	(Krishnan *et al.*, 1998)
	Laser ablation	-	-	45±7	-	(Walters *et al.*, 1999)
	Laser ablation	1.00	0.32-1.47	30	13-52	(Yu *et al.*, 2000a).

Values are expressed as average±SD or range.
MWCNT and SWCNT denote multi-walled and single-walled carbon nanotube, respectively.
CCVD denotes catalytic chemical vapour deposition.

B.4 Electric and electronic properties of carbon nanotubes

The structure strongly affects the electronic properties of SWCNTs which were theoretically predicted in 1992 (Dresselhaus *et al.*, 1996). The general rules for the metallicity of SWCNTs are as follows: for a given nanotube (*n,m*) (see A.1), if (*n*-*m*)/3=*i* (where *i* is an integer), then the SWCNT is metallic, otherwise the nanotube is a semiconductor. Thus, all SWCNTs with an armchair configuration are metallic, whereas zigzag nanotubes could be semiconductors except for cases in which (*n*-*m*) is a multiple of three. These results astonished the scientific community because bulk graphite behaves only as a semi-metal. The unique electronic properties of carbon nanotubes are due to the quantum confinement of electrons normal to the nanotube axis. In the radial direction the electrons are restricted. Consequently, the electrons can only propagate along the nanotube axis.

In the following sections some current or potential applications, made possible because of the electric and electronic properties, are described.

Field emission applications

Field emission is the extraction of electrons (emission) from a solid under an intense electric field. This phenomenon is a form of quantum tunnelling and is dependent on the properties as well as the shape of the material. Carbon nanotubes emit electrons from their tips into a vacuum when a potential is applied between the carbon nanotube-coated surface and an anode (de Heer *et al.*, 1995; Rinzler *et al.*, 1995). The electric field directs and accelerates the electrons towards the anode, i.e. a coated glass substrate with a low-voltage phosphor layer. The emitted electrons have sufficient energy to activate phosphors giving rise to visible light illumination. Because carbon nanotubes are so sharp, they emit electrons at lower voltages than electrodes made of most other materials and their strong carbon bonds allow carbon nanotubes to operate for longer periods without damage. In addition, no heating is required compared to other thermionic electron sources such as the heating element in cathode-ray tubes which reduces the lifetime of the tube.

Carbon nanotubes can be used as "cold" electron sources (i.e. electrons are emitted at room temperature) for flat panel displays (Choi *et al.*, 1999; Lee *et al.*, 2001; Sohn *et al.*, 2001; Wang *et al.*, 2001), lighting applications such as vacuum-tube elements (Saito *et al.*, 1998; Saito and Uemura, 2000), household light bulbs (Wei *et al.*, 2004) and flat panel luminescent lamps (Chen *et al.*, 2003b), gas discharge tubes (Rosen *et al.*, 2000), X-ray generators (Sugie *et al.*, 2001; Yue *et al.*, 2002), and electron guns for the next generation scanning electron microscopes and transmission electron microscopes (de Jonge *et al.*, 2002). In 1999 world's first 4.5-inch flat panel device using carbon nanotubes was developed by Samsung Advanced Institute of Technology (Suwon, South Korea). The luminance performance was limited to red, green, and blue light columns. This prototype was followed by a 9-inch flat panel display demonstrating moving colour images. Recently, Motorola Labs (Tempe, Arizona, USA) unveiled a working 5-inch colour video display prototype with a panel thickness of 3.3 mm using nano emissive display technology, a scalable method of growing carbon nanotubes directly on glass to enable an energy efficient design that excels at emitting electrons. Carbon nanotubes-based displays could enable the next generation of large size flat panels and may be an alternative to bulky cathode-ray tubes, such as used for televisions and computer monitors, and the more recent liquid-crystal panels and plasma displays. Based on the production of prototypes advantages of carbon nanotubes in flat panel displays are suggested to be low power consumption, high brightness, viewable from any angle, fast response rate, wide operating temperature range, no burn-in, lightness, and thinness. However, serious technical problems remain to be overcome such as addressing electronic circuits, the development of low-voltage phosphors, methods for maintaining the required vacuum, spacers withstanding the high electric fields, and the elimination of faulty pixels. Motorola and Samsung are developing commercially available solutions and Samsung anticipates the development of 38-inch displays by the end of 2006. Another major company involved in nanotube-based flat panel displays is Eikos (Franklin, Massachusetts, USA). Vacuum-tube lighting elements are marketed by Ise Electronics, Corp. (Ise, Japan). These tubes form the pixel elements in giant screen displays. Nanotube-based cold cathodes for compact, portable, and miniature X-ray generators are manufactured by Xintek, Inc. (Research Triangle Park, North Carolina, USA). These X-ray tubes can be set up in a narrow space and possibly X-ray endoscopic imaging and provide improved high-resolution images in industrial, biological and medical applications.

Nanoelectronic applications

As carbon nanotubes behave like electrical conductors or semiconductors, they could be extremely useful for nanoscale electronics applications. An all-carbon-based nanoelectronic technology can be foreseen in which the electric wiring consists of metallic carbon nanotubes and the active devices are made of semiconductor carbon nanotubes. Various basic electronic components have been demonstrated, including field-effect transistors (Martel *et al.*, 1998; Tans *et al.*, 1998), single-electron transistors (Postma *et al.*, 2001), rectifying diodes (Yao *et al.*, 1999), small logic circuits (Bachtold *et al.*, 2001; Derycke *et al.*, 2001), inverters (Liu *et al.*, 2001), and memory cells (Cui *et al.*, 2002). A field-effect nanotransistor is a nanodevice representing an important step towards implementing molecular electronics. It can be built by attachment of a semiconductor carbon nanotube across two metal electrodes (source and drain) on an insulating substrate that serves as a gate electrode. By applying a voltage to the gate electrode, the carbon nanotube can be switched from a conducting to an insulating state at room temperature.

It should be emphasized that all circuits have been fabricated experimentally one at a time and with great effort. It is obviously a long way from massively parallel, complex and automated production of silicon microchips on which the semiconductor industry is built where scaling down CMOS devices has become one of the major objectives (see Box B.1). However, possible performance of basic nanoelectronic components in very large circuits should be considered. Obviously, progress is needed before making routinely carbon nanotube-based integrated circuits, though it is currently feasible to build a nanocircuit that has wires, switches and memory

elements made entirely from carbon nanotubes and other molecules. Currently, Nantero Inc. (Woburn, Massachusetts, USA) uses carbon nanotubes for the development of next-generation semiconductor devices, such as NRAM™, a high-density non-volatile randon access memory. This chip will provide permament data storage even without power, lower power consumption, and it will be portable and highly resistant to environmental conditions, e.g. heat, cold and magnetism. Nevertheless several hurdles have to be overcome such as the synthesis of defect free nanotubes, selective preparation of either metallic or semiconductor carbon nanotubes, new ways to construct complex circuits without the aid of photolithography, for instance assembly strategies based on DNA (Keren *et al.*, 2003), and steering electronic impulses from large-scale wires down to nanoscale devices.

Box B.1. Scaling down integrated silicon technology

One of the important ingredients of silicon technology is the metal oxide semiconductor field-effect transistor (MOSFET) because of its scalability. Decreasing the dimensions of the device improves its speed and power efficiency. MOSFETs are used in complementary metallic oxide semicoductor (CMOS) integrated circuits (ICs) which are the basis of most digital electronic devices. The most representative parameter of the lithographic process capability enabling high-density ICs is the minimum half-pitch of custom lay-out metal interconnect (International Roadmap Committee, 2003). This defining metal half-pitch is taken from whatever product has the minimum value. Historically, DRAM (dynamic random access memory) has taken the leadership on metal pitch. Other parameters are also important for characterizing IC technology. For example, in the case of microprocessor units (MPUs) physical bottom gate length is most representative. Currently, MOSFETs with critical dimensions of about 90 nm are being fabricated. It is anticipated that half-pitch will decrease to 65 nm in 2007, 45 nm in 2010, 32 nm in 2013, and 22 nm in 2016. Producing such small MOSFETs in the long term will impose a tremendous challenge in IC technology. It is widely believed that current lithographic technology will reach its limit during the next decade. Optical lithography will face tremendous problems as it approaches 50 nm critical dimensions. Further miniaturisation of electronic circuits is in need for alternatives.

Chemical gas sensors

Semiconductor SWCNTs are highly sensitive to detect changes in the chemical composition of the surrounding atmosphere at room temperature. A significant conductance change of SWCNTs in response to adsorption of ammonia and nitrogen dioxide (Kong *et al.*, 2000) and oxygen (Collins *et al.*, 2000) demonstrate their ability to act as extremely sensitive gas-phase chemosensors. Electron charge transfer between nanotubes and molecules from gases adsorbed onto the nanotube surface is found to be a major mechanism determining the conductivity change upon exposure (Chang *et al.*, 2001). The determination of oxygen and carbon dioxide has been measured on a gas-sensitive MWCNT-SiO_2 composite using a platform for wireless long-term monitoring without battery lifetime issues (Ong *et al.*, 2002). High sensitivity towards water or ammonia vapours has also been reported on a MWCNT-SiO_2 composite (Varghese *et al.*, 2001).

Although chemosensors based on the electrical conductance changes of semiconductor SWCNTs are very sensitive, the method is limited by several factors, such as the inability to identify gases with poor adsorption energies, charge transfer, and diffusion kinetics. In addition, the distinction between gases or gas mixtures is challenging. However, MWCNT-based gas ionisation sensors have been shown good practical alternatives (Modi *et al.*, 2003). These sensors are supplied with a vertically aligned MWCNT film deposited on a SiO_2 substrate. The nanotubes in the film are ~25-30 nm in diameter, ~30 μm long and separated ~50 nm apart. The nanotube film is used as anode and separated by a glass insulator from the cathode, i.e. an aluminium sheet. When a voltage is applied between the anode and cathode electrons are emitted creating an electric field (field emission) which enables the formation of highly ionised gas (corona) around the nanotube tips. The corona promotes the formation of a powerful electron avalanche. This discharge can be created at relatively low voltages and each gas has a unique “breakdown” voltage. Portable nanosensors could be made enabling the determination of low-range gas concentrations (~25 ppm), fast response (20 μs), and identification of gases and gas mixtures.

Carbon nanotube-based chemical gas sensors have great (commercial) potential in numerous areas ranging from medical applications, environmental monitoring, agricultural applications to chemical industry and beyond. Currently, Nanomix Inc. (Emeryville, California, USA) is developing of a medical capnography sensor.

Shielding material for electromagnetic radiation

Due to the high electrical conductivity carbon nanotubes can be used as shielding material for electromagnetic radiation since carbon nanotubes are poor transmitters of electromagnetic energy. A plastic composite of carbon nanotubes could provide this shielding which is of much concern to military applications. Nowadays command,

control and communications are highly digitised and the system must be protected from weapons that emit electromagnetic pulses.

Battery technology

Due to the high surface-to-volume ratio, carbon nanotubes can be used as additives in lithium-ion battery systems, lead-acid batteries, and electric double-layer capacitors improving their performance (Endo *et al.*, 2001; Endo *et al.*, 2004). The merit of electric double-layer capacitors is their high discharge rate, which make them applicable as hybrid energy source for electric vehicles and portable electric devices. Implantable active medical devices relying on battery technology for device powering could benefit from extended battery lifetime reducing the need for early replacement.

Electromechanical actuators

Application of an external stimulus, such as an electrical charge, can change the dimensions of the materials reversibly. When a voltage is applied to carbon nanotubes they act conveniently as actuators. An “artificial muscle” has been made consisting of two SWCNT-based paper strips on both sides of an insulating double-layer side tape suspended in a liquid environment (Baughman *et al.*, 1999). The two strips were previously loaded with solutions containing sodium and chloride ions, respectively. When 1 V was applied between the two strips, both expand, but the strip loaded with sodium ions expands slightly more, forcing the system to bend. Another example are nanotweezers able to grab, manipulate, and release nano-objects (Kim and Lieber, 1999). This was made possible by depositing two non-interconnected gold coatings onto a glass micropipette. Subsequently, two carbon nanotube bundles (~20-50 nm in diameter) are attached to each of the gold electrodes. Applying a voltage (<8.5 V) between the two gold electrodes then opens and closes the tube tips reversibly in a controlled manner. Carbon nanotube actuators could potentially be used in hostile environments such as for robots used for planetary exploration (Baughman, 2003).

Appendix C – Biosensors

C.1 Cantilever array sensors

Originally, microfabricated cantilevers were used as force sensors to image the topography of a surface by means of techniques such as scanning force microscopy or atomic force microscopy (Binnig *et al.*, 1986). MEMS cantilevers are typically rectangular-shaped bars of silicon less than 1 μm thick and are usually assembled in arrays. NEMS cantilevers are smaller with feature sizes from hundreds to a few nanometres. New physical properties, due to the small dimensions, may dominate the operation of these devices. Cantilevers can operate in two fundamentally different modes. The first method is the static mode in which measurement of cantilever deflection is based on stress changes induced by molecular interaction on the cantilever surface (Fritz *et al.*, 2000). The upper surface of the cantilevers is usually coated with layers of titanium and/or gold of several nanometres thick to provide a reflective surface and an interface for attaching functional groups of probe molecules. The cantilever deflection is determined via a laser. For cantilever arrays a time-multiplexing procedure switches the multiple lasers on and off sequentially. The laser light is reflected by the metal-coated surface of the cantilever and impinges on the surface of a position-sensitive detector (photopotentiometer-like device) allowing determination of the position of the laser beam with micrometer precision. The second operation method is the dynamic mode in which the cantilever undergoes excitation in its fundamental vibration mode and the change in resonance frequency upon mass loading is measured (Battiston *et al.*, 2001). Coating of a specific sensor layer is performed for molecular recognition. The sensitive layer can be highly specific for molecular recognition or only partially specific to produce response patterns for various analytes, provided that each of the cantilevers is coated with a different partially specific sensor layer.

Artificial nose

In a gaseous environment, MEMS devices may be used as a so-called nanotechnology olfactory sensor (NOSE) system or artificial nose to characterize volatile vapours and odours (Baller *et al.*, 2000). Silicon cantilever sensor arrays are microfabricated using a dry-etching silicon-on insulator fabrication technique. A chip comprises of eight cantilevers, each 500 μm long, 100 μm wide, and 0.5 μm thick, arranged in a pitch of 250 μm. The upper surface of the cantilevers is coated with 2 nm of titanium and 20 nm of gold. The cantilever deflection is determined via an array of eight lasers arranged at a linear pitch of 250 μm. On top of the metal coating, the cantilever surface is functionalised with a polymer. Detection of vapours proceeds via diffusion of the vapour molecules into the polymer, resulting in a swelling of the polymer and static bending of the cantilever. The bending is specific to the interaction between the solvent vapour and the polymer in terms of time and magnitude. The recognition system uses the magnitudes of deflection of all eight cantilevers at three points in time yielding a “fingerprint” of the vapour. The device is capable to recognize simple or complex odours. The main advantages of an artificial nose are that the device does not fatigue, is reproducible, and can be placed in environments harmful to humans. It should be noted that the artificial nose can only recognize sample vapours that have been measured before. Therefore, it is a characterization tool rather than a chemical analysis tool.

Micro- and nanomechanical oscillators

Cantilever-based sensors are also used as mechanical oscillators for highly sensitive detection of adsorbed mass. A shift in resonant frequency of the cantilever allows detection of mass changes. Advanced fabrication processes can be used to further miniaturise these electromechanical systems. Smaller mechanical systems allow measurement of smaller forces, and similarly, NEMS devices can be actuated by small forces. Typical feature sizes of NEMS cantilevers are 4 μm in length, 0.5 μm in width, and 165 nm thick enabling detection of mass changes in the order of attograms (atto = 10^{-18}) (Ilic *et al.*, 2004a).

Biodiagnostics

Operating a cantilever array in liquid opens up a variety of new applications, for example in biochemistry, allowing rapid, quantitative, and qualitative detection of biomolecules. The surface of the cantilever can be functionalised via thiol chemistry with biomolecules to observe very specific biochemical reactions, such as DNA hybridization and antibody-antigen interactions (Fritz *et al.*, 2000; McKendry *et al.*, 2002).