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# Nanotechnology and its Implications for the Health of the EU Citizen.

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## About Nanoforum

This European Union sponsored (under FP5) Thematic Network provides a comprehensive source of information on all areas of nanotechnology to the business, scientific and social communities. The main vehicle for the thematic network is the dedicated website www.nanoforum.org. Nanoforum encompasses partners from different disciplines, brings together existing national and regional networks, shares best practice on dissemination of national, EU-wide and Venture Capital funding to boost SME creation, provides a means for the EU to interface with networks, stimulates nanotechnology in underdeveloped countries, stimulates young scientists, publicises good research and forms a network of knowledge and expertise.

Nanoforum aims to provide a linking framework for all nanotechnology activity within the European Community. It serves as a central location from which to gain access to and information about research programmes, technological developments, funding opportunities and future activities in nanotechnology within the community.

The Nanoforum consortium consists of:

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http://www.nano.org.uk

Cea-Leti (France)
http://www.minatec.com/minatec_uk/minatec/recherche.htm

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Nordic Nanotech (Denmark)
http://www.nanotech.dk/

MalschTechnoValuation (Netherlands)
http://www.malsch.demon.nl/

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# Summary

Healthcare is affected by a large number of social and economic factors. The global healthcare markets are worth several hundred billion euros per year, with pharmaceuticals accounting for the majority of this (approximately $400 billion US in 2002). This report focuses on those areas in which nanotechnology is having a direct impact and includes diagnostics, drug discovery and delivery, surgery, tissue engineering and implants.

Nanotechnology investment is increasing world-wide and the market for products utilising nanotechnology is forecast to be worth over a trillion euros within the next 10 to 15 years. Nanotechnology is already featuring within the healthcare market as the following examples illustrate:

1. Atomic force microscope (AFM) technology (which can move single atoms about) is being used to create smaller and more sensitive microarrays for use in diagnostics and drug discovery. AFMs can also be used to nanostructure surfaces, and for example make them more biocompatible.
2. Nanoparticles such as fullerenes (molecules based on a 60 carbon atom cage) and quantum dots (complexes of semi-conductor material that have unique fluorescent properties) are being exploited in many areas including imaging (e.g. enhancement of magnetic resonance imaging [MRI] and ultrasound) and drug delivery (e.g. a modified fullerene is entering clinical trials as an anti-HIV agent). Formulating drugs with nanoparticles can also improve their solubility (many drugs are not marketed because they are not very water-soluble), increase their resistance to stomach acid and enzymes (allowing better uptake from the small intestine), and allow controlled release (e.g. over days rather than minutes and hours). Nanotubes represent another mechanism for drug delivery, both as a "container" and potentially a system for "nano-injection" into cells.
3. Nanocomposites of titanium alloys, for example, can be used to improve the biocompatibility and longevity of surgical devices and implants.

4. Nanostructuring surfaces can improve cellular attachment (e.g. etching surfaces with nanoscale grooves or using instruments such as an AFM to imprint surfaces with cell attachment molecules), and direct cells to grow into defined structures. By incorporating biodegradable polymers to act as a scaffolding, these structures can be assembled into 3-dimensional "tissues". Nanostructuring can also be used to provide an anti-microbial coating on implants.

What does the future hold? Nanotechnologies will allow us to rapidly sequence an individual's DNA (nanosequencing) and thereby determine genetic susceptibility to disease, drug intolerances and drug metabolism rates (all of which comes under the area of pharmacogenomics). We will be able to target molecules to individual cells within the body for drug delivery or imaging purposes. Patient illnesses will be diagnosed more rapidly through advancements in lab-on-a-chip devices, and at the same time a patient's vital signs could be monitored more closely through similar devices. Damaged body parts could be replaced through advances in tissue engineering (with physiological tissues and organs grown in the clinic in bioreactors) and improved implants will allow patients to regain sight and hearing.

This report gives an overview of the pharmaceutical and medical device sectors of the healthcare market and the impact that nanotechnology is having. Future developments utilising nanotechnology are discussed and links supplied to European funding sources, web and literature resources, and companies that are actively using nanotechnologies to develop products for healthcare.

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# Chapter 1 – Introduction

Mark Morrison, Institute of Nanotechnology.

## 1.1 Scope of this report

This report will describe recent advances in medicine and healthcare, and in particular the contribution of nanotechnologies to these. The report is written without assuming a detailed technical knowledge from the reader and in each chapter an overview of the area will be presented, followed by key examples, companies that are working with the technologies, future prospects, funding opportunities and literature sources.

In the context of this report medicine and healthcare can be divided broadly into pharmaceuticals and medical devices. These are explored in several key areas, specifically diagnostics, drug discovery and delivery, surgery, tissue engineering and implants.

This report focuses on the European arena, however examples from other countries (mainly the US) are cited to illustrate points. In terms of overall funding for nanotechnology, the US and Japan lead (with approximately 607 and 812 million euros allocated respectively in 2003). Many European countries do not have specific nanotechnology programmes, making the overall situation in Europe less clear, however combining known national and EU figures the total budget is somewhere in the region of 450-500 million euros for 2003.

## 1.2 What is nanotechnology?

The definition of nanotechnology is based on the prefix "nano" which is from the Greek word meaning "dwarf". In more technical terms, the word "nano" means $10^{-9}$, or one billionth of something. For comparison, a virus is roughly 100 nanometers in size. The word nanotechnology is generally used when referring to things with the size of 0.1 to 100 nanometers, beyond that limit, things are considered to be of the larger, microtechnology or the micron ($10^{-6}$) range.

Nanotechnology is the manipulation or self-assembly of individual atoms, molecules, or molecular clusters into structures to create materials and devices with new or vastly different properties. Nanotechnology can work from the top down (which means reducing the size of the smallest structures to the nanoscale e.g. photonics applications in nanoelectronics and nanoengineering) or the bottom up (which involves manipulating individual atoms and molecules into nanostructures and more closely resembles chemistry or biology).

## 1.3 The global medicine and healthcare market[1]

The medicine and healthcare markets are worth hundreds of billions of euros every year. Pharmaceuticals account for the vast majority of this with global sales amounting to $400.6 billion US in 2002 (see table).

| World Audited Market | 2002 Sales ($bn) | % Global sales ($) | % Growth (constant $) |
|---|---|---|---|
| North America | 203.6 | 51% | +12% |
| European Union | 90.6 | 22 | +8 |
| Rest of Europe | 11.3 | 3 | +9 |
| Japan | 46.9 | 12 | +1 |
| Asia, Africa and Australia | 31.6 | 8 | +11 |
| Latin America | 16.5 | 4 | -10 |
| | | | |
| **TOTAL** | **$400.6bn** | **100.0%** | **+8%** |

Source: IMS World Review 2003. Sales cover direct and indirect pharmaceutical channel purchases in US dollars from pharmaceutical wholesalers and manufacturers. The figures above represent 52 weeks of sales data, and include prescription and certain OTC data and represent manufacturer prices.

One of the largest sectors in global pharmaceuticals are the blockbuster drugs, which are unique entities each generating in excess of $1 billion US per annum (approximately $120 billion in total in 2002). Generic drugs constitute a smaller but rapidly rising segment of the market (due to loss of patent rights) e.g. in Europe, generic drugs account for 50% of all prescriptions but this is forecast to reach 75% by 2007. Drug delivery systems account for $40 to 50 billion US of the global pharmaceutical sales and it is thought that by 2007 this share will have increased to 39% of total global sales.

Nanobiotechnology is a relatively new segment of these markets however it is expected to grow rapidly to reach over $3 billion US by 2008. The current estimated worldwide market breakdown is: U.S. at 65%, Europe at 20%, Japan at 10%, and the rest of the world at 5%.

In terms of investment the medical device sector is smaller, e.g. in Europe, an average of 8.6% of GDP is spent on healthcare and of this, only 6.37% goes to medical technology (i.e. 0.54% of GDP).[2] The sales generated in the medical device markets however, is more considerable and estimated at $55 billion US in 2003.[3] This includes for example the wound closure devices market (which include sutures, staples, sealants and glues) which in Europe and the U.S. is valued at approximately $3.4 billion US, and the *in vitro* diagnostics market (which includes microarrays, sensors etc) which is predicted to grow to $25 billion by 2004.

The main growth area is age-related health care. A poll of delegates at the annual 3i Healthcare CEO conference in Barcelona, revealed that over 70% felt that the increasing elderly population (and associated long-term care) would be the major impetus in the medical devices sector. According to Jo Taylor, Director and Head of Technology for 3i in the UK: "We expect to see increased investment in the healthcare and biotechnology industry over the next three years, innovation will continue to fuel the sector and provide new opportunities for the market."[4] Of these, nearer-the-patient testing is one of the fastest-growing segments.

## 1.4 Barriers to nanotechnology in medicine and healthcare

It is easy to look at all the advantages that nanotechnology offers the medicine and healthcare markets and forget that there are still barriers to its implementation. These fall into the following broad categories: cost, manufacturing scale and consumer perception.

Cost is a major consideration for the exploitation of nanotechnology, e.g. high purity nanotubes (+95%) cost $400 per gram. Manufacturing advances however are decreasing these costs all the time e.g. five years ago, one gram of low-grade nanotubes cost $1 000, and nowadays the same nanotubes can be bought for $30. In this respect the scaling up from lab to industry manufacturing processes must be taken into account when assessing time-to-market. This is an important consideration- many nanotechnologies can take years to develop to a marketable product, in which time patents can become invalid, which in turn will have a negative effect on initial venture capital. Although some of the nanotechnology applications presented in this report are at or near market, it will be over the next 10 to 15 years that the major impact will be seen. We have tried to address this in the chapter construction by discussing the present state of affairs, the effect of nanotechnology on these and what future developments are coming.

Consumer perception is a key issue to the success of nanotechnologies and this is particularly true in the medical and healthcare sectors. There has been much discussion in the media of how nanotechnology may evolve in the future, some of which is fantastical. However the development of implantable smart medical devices that can respond to varying parameters within a patient, and for example alter drug dosage, monitor vital signs such as blood biochemistry, cardiac function and the presence of pathogens, and communicate remotely with a pc will soon be a reality. However, will people accept such implants? And who will govern access to such data, which potentially will be more comprehensive than that measured for most people at present, and ensure that it is used solely for medical treatment and not, for example, passed on to insurers?

People in most countries are living longer. According to European Commission forecasts, by 2020 there will be 40% more people aged 75 and above living in Europe than in 1990. According to the WHO "life expectancies equal or exceed 70 years in 24 countries, and 60 years in over half the Member States of WHO. At the other extreme are 32 countries where disability-adjusted life expectancy is estimated to be less than 40 years. Many of these are countries with major epidemics of HIV/AIDS, among other causes. All of the bottom 10 countries were in sub-Saharan Africa, where the HIV-AIDS epidemic is rampant and the overall life expectancy has dropped precipitously over the past 10 years."[5]

It is important that the advances in nanotechnology that will bring great benefit to medicine and healthcare, do not do so at the expense of humankind and the environment. The technology gap that may widen between developed and developing countries is a concern, nanotechnology will bring such improvements to healthcare that it should be available for all.

Finally the environmental concerns of nanotechnology must be debated from an early stage to avoid such public rejection as was witnessed with GM crops and to address any potential long-term concerns from the breakdown of nanomaterials. It is outwith the scope of this report to deal with these issues in any depth, however these ethical questions will be discussed more fully in the next Nanoforum report.

# Chapter 2 Diagnostics

Michael Gleiche, Holger Hoffschulz and Volker Wagner, VDI.

## 2.1 Introduction

The ongoing miniaturisation of electrical components in the semiconductor industry has led to an enormous price-reduction, an increased performance and a strong dissemination of integrated circuits. The ability to control and to guide extremely small quantities of liquids may evoke a similar progress in biochemical analysis and clinical diagnosis. Operating with tiny volumes results in an increased processing speed, reduced costs and thus a higher efficiency, especially when handling rare and expensive materials such as DNA samples. In this regard, microfluidic devices or biochips with analytical and functional capabilities and high sample throughput are receiving increased attention.

Two different basic approaches can be distinguished for this "miniaturised" biochemical analysis. The first is based on a parallel processing technique, where a huge number of miniaturised test tubes or wells are placed side by side on a plate. These plates or arrays are commonly named microarrays[6], for spot sizes below 200 micrometres. Microarrays can be used for diagnostic purposes such as DNA analysis (DNA array, DNA chip or genetic chip) [7] and protein detection (protein array or chip). They offer a high sample throughput due to the simultaneous sample detection, but are limited in their functionality to a single chemical reaction / well (e.g. Yes/No result). The other approach is based on a serial processing technique, with complex functions integrated on a miniaturised "lab" chip. According to their design, they offer a sequence of different chemical processing and analysis steps, such as mixing of different compounds, synthesis, electrophoreses or chromatography. These systems are called Lab-on-a-chip devices (LOC) and they perform several processes with a minimum material usage. Furthermore, combinations of microarrays and LOC are possible.

Besides the common concepts of miniaturised chemistry mentioned above, other microarray systems exist, such as the cell chip or tissue microarrays[8]. These make use of living cells or tissues which are immobilised in discrete spots on an array and permit rapid drug testing or screening experiments.

LOCs may be used for *in vitro* diagnostics (also called the near patient or Point-of-Care diagnostic) in the future. "Point-of-care" refers to a diagnosis, which is performed on the patient at the bedside in a clinical environment or at the patients home. In the ideal case, the patient would benefit from this near patient diagnosis immediately, as the LOC, which is effectively a functional miniaturised laboratory, would enable the comprehensive analysis of bodily fluids and allow medical staff to determine the optimal therapy. Therefore, point-of-care biochips may help to reduce or even prevent problems arising from drug side-effects and from antibiotic resistance in individuals. In addition LOCs may reduce costs further by removing the need for sample handling (sending to a diagnostic lab) and reporting.

Taking more than a decade and a tremendous international effort, the decryption of the human genome (the human genome project, HGP) [9] brought the field of genomics, the study of the functional dependency of genes, to the forefront of science. This rapid development was based on the capability of processing biological data with high throughput and low costs. In this regard, it is believed that biochips will boost the scientific field of genomics in the future.

The next step in understanding cellular biochemistry can be found in proteomics, the study of the functionality of all proteins in cells and tissues. The human genome is the "manual" for synthesising proteins, but it is proteins that provide functions, therefore a comprehensive analysis of disease requires a knowledge of both protein identity and function. Unfortunately, the human proteome is significantly larger than the human genome. A total of one million human proteins has been estimated, whereas the genome consists of about 30000 to 35000 genes. Thus, proteomics is dependent on the availability of high throughput protein-arrays or chips at moderate costs.

## 2.2 Array technology

### 2.2.1 Overview of array technology

Arrays and microarrays are used to perform a large number of different experiments simultaneously. They have microscopic and functionalised test tubes or spots, where each spot is designed to perform a distinct test. These reaction spots are arranged in arrays with up to several 100000 different spots on a single chip and a density of up to 100000 spots per $cm^2$. Microarrays can be used to perform several tasks including DNA sequencing, genetic disease diagnosis, drug efficiency tests (high-throughput screening) and gene expression studies.

### 2.2.2 DNA microarrays/DNA chips

DNA functionalised microarrays represent one of the most prominent biochips exploiting the correlation of cell function and the expression of genes. In Figure 2.1 a GeneChip®, Affymetrix Inc. is depicted. Such DNA arrays are available for different genomes including human, mice, rats and micro-organisms, and are commonly referred to as DNA chips. The operation principle of such DNA-chips can be understood by considering the process of events within a cell that converts genetic information into protein, i.e. gene expression.

The information to create a protein, which is stored in a sequence of base pairs in DNA, is copied first into messenger RNA (mRNA), a process called transcription. The mRNA then acts as a template for the synthesis of the particular protein (a process called translation), which fulfils the functional role of the gene. The first stage of gene expression is exploited by gene chips to examine differences between healthy and morbid tissue.

**Figure 2.1**. Affymetrix GeneChip® probe array. Image courtesy of Affymetrix.

Each spot of a DNA chip contains a molecule of single-stranded DNA. Samples of mRNA to be tested are either labelled (usually with a fluorescent dye) before being applied to the chip or are first converted into cDNA (complementary DNA, i.e. DNA that has been copied from a mRNA) before being labelled and applied to the chip. At this stage, mRNA/cDNA molecules (probe) that match molecules of single-stranded DNA fixed to the chip (target) will bind to these in a process called hybridisation (this is where complementary single-stranded DNA or RNA molecules interact to form a duplex according to base pairing rules i.e. A binds to T, G binds to C), see Figure 2.2. Probes which do not have a complementary, single-stranded DNA target fixed to the chip, will not bind. Thus, only those spots where hybridisation takes place give rise to fluorescence, which can be read by optical means (fluorescence imager). The location and the intensity of fluorescent spots provide information about which genes have been expressed by the cell and to what degree, respectively.

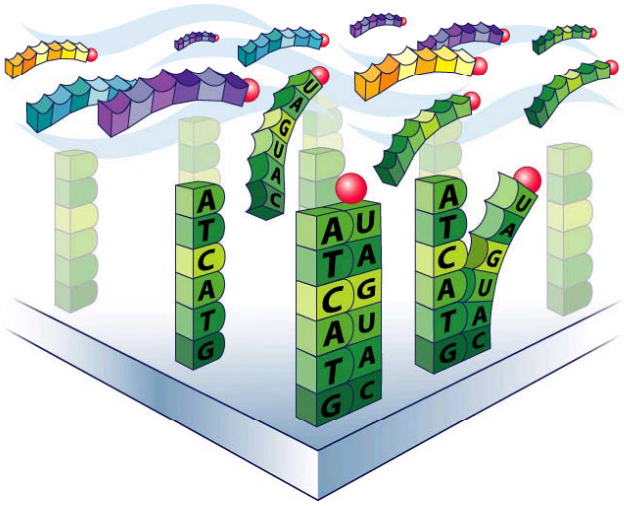

**Figure 2.2**. Schematic representation of the hybridisation of fluorescent marker tagged mRNA with their complementary single stranded DNA. Each spot on the biochip contains a huge number of identical molecules. Image courtesy of Affymetrix.

In order to carry out a comparative study, the mRNA of healthy and morbid tissue is isolated, then copied into cDNA and finally each is tagged with different fluorescent dyes (e.g. red and green). These are introduced to the DNA chip; hybridisation is allowed to occur and then investigated by fluorescence imaging.

In this scenario four different results can occur (depicted in Figure 2.3): i) the red-tagged molecules hybridise, ii) the green-tagged molecules hybridise, iii) both molecules hybridise (giving a yellow colour), iv) no hybridisation takes place (no colour). By calculating the relative contribution from each dye, investigators can determine whether a gene from a diseased cell is being expressed to the same, higher or lower levels than in a normal, healthy cell. This allows the investigation of thousands of genes on a single chip and the identification of which genes are involved in specific diseases.

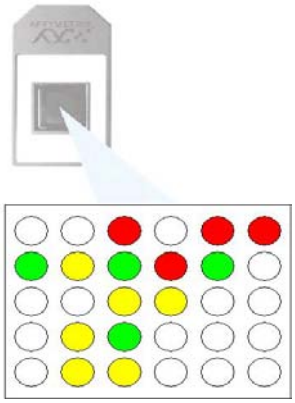

**Figure 2.3**. Schematic of an experimental result from a gene expression study.

Microarrays are also being exploited for the study of DNA polymorphisms or mutations. Single nucleotide polymorphisms (SNP) are the most common variations in the human genome[10]. These represent a difference of a single nucleotide at a defined point in the genome, for instance when AGGC changes to AGGT. A variation is termed a SNP when it occurs in more than 1 % of the human population. SNPs can be used as a marker in order to identify disease genes and occur on average every 1000 base pairs.

Only 3-5% of the human genome has the potential to encode proteins i.e. are genes. Although most SNPs occur outwith these regions, it is believed that the comprehensive analysis of SNPs within genes will improve medication in the future. If each patient has their own individual SNP map, this could provide detailed information about specific incompatibilities or possible side effects with a given drug.

In this regard, a high-throughput method, as can be found in microarrays, is essential. The format of these arrays is similar to that for gene expression studies. A specific oligonucleotide (single-stranded DNA fragment) exhibiting a distinct SNP is immobilised at each reaction spot on the microarray. mRNA is extracted from the patient, introduced to the array where it hybridises to those spots on the microarray with a matching SNP, thus providing information about which SNPs are present in the patient's genome.

#### 2.2.2.1 Production of DNA arrays

DNA microarrays are based on providing a specific "address" for different oligonucleotides (or oligos). Different single-stranded DNA fragments, representing the variety of genes under investigation, are immobilised on a substrate in discrete spots. Each spot contains a large number of identical molecules increasing the sensitivity.

The substrate can be made of silicon-wafer, glass, nylon or other coated materials. Basically, there are two different methods to create the spots containing the oligos. One method is based on the in-situ synthesis of the oligos directly onto the chip surface. The second method takes pre-synthesised oligos and immobilises them onto the chip.

Affymetrix uses the first method to create its GeneChip®. Oligos with a length of up to 25 nucleotides are synthesised using photolithography at each spot of the DNA-chip. The first step involves the immobilisation of a nucleotide at each spot. This, and subsequent, nucleotides have a light-sensitive group protecting the end where further nucleotides are added. In the second step, individual spots are selectively bleached through suitable masks, removing the light-sensitive group and allowing the next nucleotide to be added. By repeating this procedure a tailor-made DNA chip with individual oligos at each spot can be created. Using this method spot sizes of about 20 µm can be obtained, although the costs per chip are comparatively high depending on the number of different oligos synthesised and the number of spots.

Agilent use a second method, similar to inkjet printing, to immobilise molecules inexpensively onto glass chips. The inkjet method deposits the nucleotides sequentially onto treated glass wafers and uses established technologies to elongate these oligos to 60 nucleotides. Spot sizes from 150 to 200 µm can be obtained. However this produces a chip with a considerably lower density than the GeneChip® described above.

The ex-situ synthesis methods make use of commercially available printing or spotting methods. These methods can use longer cDNA fragments of up to several thousand nucleotides, but offer a rather limited density. There are several contact and contact free printing methods, such as contact tip printing, micro contact printing, microsyringe pump printing and piezoelectric printing. The latter two are contact free methods that spray the molecules in small droplets onto the surface (similar to the inkjet method).

The DNA chip read out process after hybridisation is done predominantly using optical techniques, such as fluorescence microscopy. Alternatives to fluorescent tags include chemiluminescent/luminescent or radioactive markers, and in the future the use of electrical signals is a possibility. However, fluorescent tags have the advantage of multiple colours.

### 2.2.3 Protein microarrays/protein chips

The exploitation of genomics has enormous capability, however proteomics[11]- the study of proteins and their functionality, will be more powerful in the discovery of disease pathways. Despite the progress that has been made in the investigation of the human genome, little is known about proteins involved in pathogenesis. From the genomics perspective this can be attributed in part to the weak correlation between the concentration of mRNA and that of the corresponding protein. Thus, in order to illuminate the function of a given gene in the course of a certain disease, the protein(s) encoded by that gene have to be detected themselves[12]. Considering the complexity and the tremendous number of proteins that are involved in human biology, proteomics seems to be a rather challenging task.

The human proteome is more complex than the genome encoding it would suggest and has been estimated to consist of about 0.5 to 1 million proteins. Unlike the comparatively simple composition of DNA with its four basic building blocks, proteins consist of 20 different amino acids. As a result of interactions between these amino acids, proteins adopt a specific 3-dimensional structure that is a major contribution to their functionality. Proteins can be further modified through the addition of carbohydrate and lipid groups, which adds to the variety issuing from a single gene. Proteins with identical chemical composition but different spatial structures can evoke different reactions. The 3D structure of a protein can be changed when it interacts with other materials including other proteins. Furthermore, proteins are difficult to synthesise and there is no simple way of amplification as can be found in PCR (polymerase chain reaction) for DNA.

At present, the quantitative analysis of proteins is achieved mainly with assistance of two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Proteins are isolated from cells and initially separated based on charge (first dimension, this is called isoelectric focussing), followed by separation based on mass (second dimension, perpendicular to the first). This method can effectively separate thousands of different protein species on a single gel. Protein "spots" on the gel can be visualised using stains that can either be colourimetric or fluorescent, excised from the gel and analysed by mass spectrometry.

Immunoassays[13], which are based on the specific interaction between an antibody and antigen, heralded the beginning of protein-detecting microarrays. Potential applications for protein chips can be found in diagnostics, drug screening, disease monitoring, drug discovery, and medical research[14]. The market for protein chips is expected to grow to at least 500 million dollars by 2005[15].

### 2.2.3.1 Production of protein arrays

At present, the production of protein microarrays is rather difficult and thus in an early stage of development. Several obstacles have to be overcome in order to produce protein microarrays for reliable high-throughput methods. Firstly proteins exhibit an enormous chemical diversity, ranging from hydrophilic to hydrophobic structures and having huge differences in their size and spatial structure. In order to maintain spatial structure, which is crucial to protein functionality, distinct physical conditions may be required, such as a certain temperature and pH range. Thus, the printing or spotting of proteins has to be achieved without conformational change of the molecules. Furthermore, a specific interaction between the target molecule (spotted onto the array) and the proteins under investigation is required. Unlike the hybridisation of DNA strands, protein-protein interaction is rather complex and driven by their 3D structure. A common approach to protein microarrays is based on antibody arrays, exploiting antigen-antibody interactions.

The basic mode of operation of a protein microarray is similar to that of the DNA microarrays described above. Target molecules are immobilised in discrete spots on the chip surface providing a specific "address" for labelled proteins. The spots where labelled proteins bind targets can be read by optical means (e.g. fluorescence microscopy) providing information about which protein has been expressed (protein expression study).

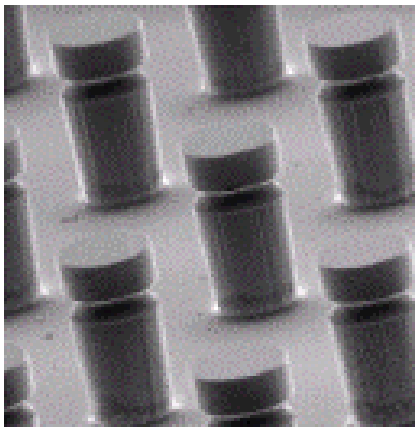

**Figure 2.4**. A protein profiling biochip system: Antibody functionalised 3D pillars on a silicon-based chip architecture permit a high assay sensitivity, and reduced non-specific binding. Courtesy of Zyomyx Inc., http://www.zyomyx.com/

Suitable substrates are glass, silicon or polymer surfaces, which have been chemically treated or coated with appropriate molecules, such as poly-L-lysine, which can bind proteins. The application of the target molecules is performed by a printing method such as inkjet or contact printing. High precision contact printing robots are able to deliver nanoliter quantities of protein samples and to produce spot sizes of 150 to 200 µm and densities of about 1600 spots per square centimetre[16]. Similar to DNA chips, fluorescent or radioactive marking is possible. Although protein chips are still in the initial stage of development, commercial products for protein arrays are available on the market.

Pioneering work by the Zyomyx Inc. company offers a protein profiling biochip system, which is based on a silicon substrate. The target molecules (antibodies) are immobilised on small three-dimensional pillars for sensitivity reasons (depicted in Figure 2.4). Other approaches rely on polymer coated glass slides, which are covered with a thin (4 µm) HydroGel$^{TM}$ layer (PerkinElmer Inc.). This coating can be used for spotting target proteins in sub-nanoliter quantities using piezoelectric tip technology (Piezoarray, PerkinElmer, Inc.).

Since the production of large quantities of proteins is rather difficult, ultra sensitive detection methods are required to measure tiny volumes. Other approaches, besides the common fluorescent and radioactive tagging methods, exist which allow the label-free detection of binding events. One method is based on SPR spectroscopy (surface plasmon resonance spectroscopy), which measures small variations in the surface refractive index of a metallic surface when a protein binds to it. This ultra-sensitive method is capable of detecting the kinetics of biomolecular interaction in real time.

### 2.2.4 Impact of nanotechnology on array technology

The advantages of microarray technology emanate from their miniaturization. In this regard, nanoscaled arrays or nanoarrays should have superior properties considering their processing speed and material consumption. Nevertheless, problems arise from the handling of ultra-small quantities of liquids. The reliable processing of volumes down to femtoliters ($1x10^{-15}$ l) is still a challanging task at present and cannot be performed on a routine base.

One approach to miniaturize array size can be found in scanning or atomic force microscopy (SFM/AFM). The AFM is a prominent tool in nanotechnology allowing the visualisation and manipulation of surfaces at the nanometre scale. An atomically sharp tip, which is depicted schematically in Figure 2.5 left, is used to obtain local topographical information by scanning the area of interest line by line and detecting the forces acting upon the tip through deflection of a microfabricated spring, induced by the scanning process.

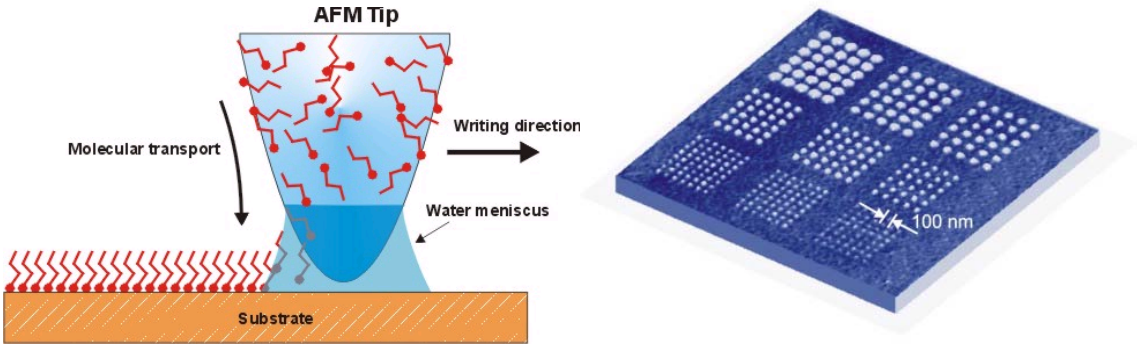


**Figure 2.5**. Dip-Pen Nanolithography (DPN). Left: The tip of an atomic force microscope (AFM) scans the surface and deposits molecules through the water meniscus. Right: DPN written arrays; the smallest spot size is about 100 nm. Mirkin et al., Image adapted from http://www.chem.nwu.edu/~mkngrp/dippen.html

The AFM can be used as a nanolithographic tool in order to modify the surface structure or chemistry. A common approach is based on the application of a voltage between tip and sample. A different method of AFM nanolithography can be used to produce arrays of nanometre dimensions by the deposition of molecules from the tip through a liquid meniscus (or "ink") onto the substrate during the scanning process. This technique is called dip pen nanolithography (DPN)[17] and can be used to functionalize surfaces in discrete spots with a user-defined chemistry (this is depicted in Figure 2.5 right). The smallest spot size which has been obtained by this method so far is about 100 nm and the ability of DPN to create protein nanoarrays has been demonstrated recently[18]. The inherent problem of all scanning techniques is low throughput which is a result of their serial nature. This can be overcome by intensive parallelisation using several thousands of ink-wetted tips. The advantage of this system can be seen in the label-free detection possibility, which can be done with the same instrument. A commercial DPN-writer was released in 2003 by NanoInk, Inc., Chicago.

A similiar approach has been taken by Bioforce, Nanoscience Inc. They manufacture the NanoArrayer™ and the NanoReader™, which are SFM based tools that can create and read nanoarrays.

Nanotechnology offers advantages not only for array production but also for sample detection on the array. The common approach of fluorescent staining suffers from the disadvantage of bleaching i.e. the fast degradation of fluorescent molecules. In contrast, nanoparticles exhibit improved stability and sensitivity for detection purposes, e.g. resonance light scattering (RLS) of distinct nanosized metal colloidal particles provides an ultra sensitive detection method. A substantial 10-fold enhancement in sensitivity compared to common fluorescent markers has been accomplished through the use of gold and silver particles of uniform dimensions in the range of 40 nm to 120 nm[19].

With regard to protein detection, a new strategy based on functionalised metal nanoparticles, in combination with a magnetic detection method, has been reported recently[20]. This so-called bio-bar code method increases sensitivity and offers a strong multiplexing and simultaneous detection of many proteins.

### 2.2.5 Research that will impact future development

From the point of view of nanotechnology, the current microarray technology is rather macroscopic. Smaller spots can be achieved with scanning probe techniques as mentioned above, however these require a higher detection sensitivity due to the small number of molecules involved. With force spectroscopy[21] the possibility of detecting single molecules by mechanical means can be realised. A conventional AFM with a functionalised tip can be used to measure specific interactions such as antibody/antigen. Typical experiments probe the mechanical properties of single molecules and measure the energy needed to unfold molecular domains.

The miniaturisation of DNA microarrays brought a significant progress in genomics, where the number of experiments covered by a single microarray has been steadily increased. The ultimate gene chip has been produced recently by Affymetrix and has all human genes on a single chip[22].

A better understanding of the functionality of proteins will have a strong impact on future diagnostics and therapies. The development of new drugs allows a more precise targeting with less side-effects. Highly specialised molecules offer an increased effectiveness in the therapy of cancer for example. Such intelligent active agents may require diagnostic tests to determine which patient is able to benefit from the drug e.g. due to the presence of a certain receptor[23]. These tests can be performed using microarray technology in the future, bringing a new approach to diagnosis, the so called personalised medicine, where patients benefit from highly specific developed drugs, which have their effectiveness ensured through a diagnostic test. These molecular diagnostic tools, with precise targeting ability, are referred to as molecular medicine.

## 2.3 Lab-on-a-chip

### 2.3.1 Overview of lab-on-a-chip

In contrast to micro- and nanoarray technology, which allow the accomplishment of a single experiment, the basic idea of lab-on-a-chip (LOC) is the integration of an entire chemistry lab on a small substrate. Resembling the integrated circuits in the semiconductor industry, a LOC can perform sample isolation, mixing, separation, detection and data analysis on one highly integrated platform. In general, a LOC device is able to perform several different production steps, which can vary dependent on the purpose of the LOC, on a miniaturised base. This is achieved by a combination of microfluidic and microelectronic devices creating a small lab, which allows the realisation of a complete experiment with minimum material consumption, and provides data analysis in real-time. In the future, LOC devices may replace the expensive and time-consuming use of clinical laboratories. Therefore, LOCs are promising candidates for medical analysis at the point-of-care.

It was the advent of microfluidics that rendered the miniaturisation of fluid devices possible. Guiding fluids in micrometer-sized channels can be achieved in several ways such as electrohydrodynamic pumping, electroosmotic flow, electrowetting and thermocapillary pumping or temperature gradients[24]. Such an integrated LOC requires micro-engineered surface topographies or a chemical wetting contrast in combination with electronics, which are commonly referred to as micro electromechanical systems (MEMS). LOCs can be fabricated on glass, silicon or polymer substrates, which offers the advantage of a fast and inexpensive production of disposable LOCs. Polymer based microfluidic devices can be manufactured by replication methods such as casting, imprinting or embossing and injection molding[25], making the mass production of LOCs feasible.

The first commercially available product was the Agilent 2100 Bioanalyzer, representing a versatile LOC platform for multiple forms of RNA, DNA and protein analysis. Figure 2.6 shows the Agilent LabChip™ for DNA (left) and protein (right) analysis, which can be inserted into the bioanalyzer (not depicted). The sample material is injected and moves through microchannels, where DNA fragments or proteins are separated by mass through electrophoresis and different components are detected by their fluorescent tags.

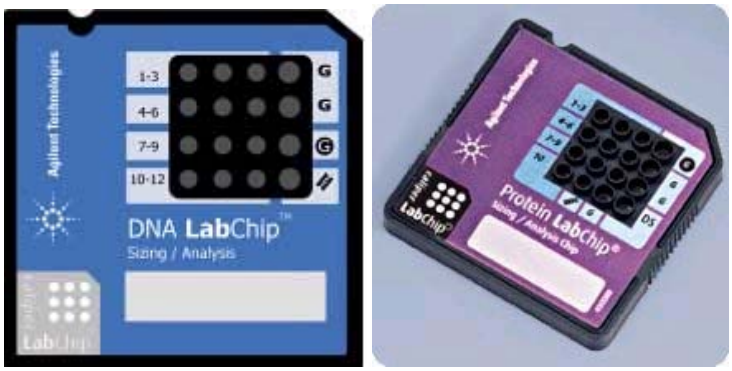


**Figure 2.6**. A DNA and a protein LabChip™, Agilent Technologies, http://www.agilent.com/

Microfluidics and LOC devices represent a comparatively dynamic field of research. Over 2000 papers concerning LOCs have been published to date[26]. The worldwide market for microarrays, microfluidic devices and other biochips is estimated to grow 65% per year and reach 3.3 billion dollar by 2004[27].

### 2.3.2 Impact of nanotechnology on lab-on-a-chip

A further miniaturisation of micro-electromechanical systems (MEMS) is leading to the so-called nano-electromechanical systems (NEMS). Although the creation of nanostructured surfaces can be performed on a routine basis at present, problems arise from the guidance of ultra-small quantities of liquids in the pico- and attoliter range.

Nevertheless, nanotechnology provides several approaches for LOC systems. The analysis of nanoscaled objects, such as proteins or viruses, is commonly done by dielectrophoresis. Dielectrophoresis (DEP) uses non-uniform alternating electrical fields to separate and to guide small objects through field gradients. This manipulation requires high electrical field strength, which can be obtained using nanosized electrodes with feature sizes well below 100 nm. Such DEP devices can be implemented in microfluidic systems for the detection of viruses for example.

Nanopore DNA sequencing is another example of a nanotechnology that can be integrated in a LOC. A nanopore, with a diameter of several nanometres, is created in a membrane, which separates two liquid compartments. Single-stranded DNA is passed through the nanopore and as it does it causes variations in electrical current that differ and are specific to each of the four bases of DNA, thus allowing the DNA sequence to be read accurately.[28]

### 2.3.3 Research that will impact future development

LOC devices could serve as point of care devices that would remove the need for the expensive analytical setup of a whole laboratory. In the ideal case, the LOC would be the only instrument needed for diagnosis and it could be carried out near the patient and in real time. However, the common detection method is based on fluorescent markers linked to the sample material. Therefore a fluorescent microscope is still needed to perform the analysis.


**Figure 2.7**. DNA-chip from Infineon. This biochip is fabricated on standard Complementary Metal Oxide Semiconductor (CMOS) technology. Images courtesy of Infineon Technologies AG, Munich.

A different approach may be found in electrical read-out systems, based on standard CMOS technology that is widely used in the semiconductor industry. Figure 2.7 represents an example of a DNA chip, with an electrical readout analysis from Infineon Corporate Research.The Infineon DNA chip consists of an array of 8x16 gold spots with diameters of 100 - 250 µm. Single stranded DNA (target molecule) can be immobilised on the gold spots using a microspotter.

The sample under investigation is applied to the whole chip allowing hybridisation to occur. Read-out is achieved by the addition of a substance that induces a small redox current in those spots where there was a hybridisation. The sensitivity for current measurement is in the range of pico- to femtoamperes.

## 2.4 Cell chips

### 2.4.1 Overview of cell chips

The cell chip is a comparatively new type of biochip, 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 drug screening, cell membrane ion channels and electrical signals in neurones. A pioneer in the field of cell chips is the company Cellomics Inc., Pittsburgh. They are developing a cell chip with a fluorescent detection system, which will be suitable for drug discovery and screening experiments.

For the analysis of the neuronal electrical activity, living neurones have been connected to a sensor array, developed by Infineon and Fromherz et al.[29]. This CMOS based array consists of 128x128 sensors per mm². Figure 2.8 represents a neurone (green) placed on the sensor array (pink). Each cell covers at least one sensor, because the intersensor distance is about 8 µm, whereas the diameter of a neurone is 10 to 50 µm.

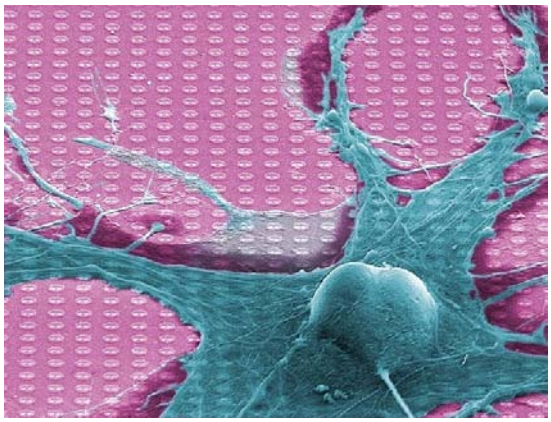

**Figure 2.8**. A living cell (neurone) on a neuro-chip exhibiting several thousands sensors recording the electrical activity of the cell with spatial resolution. http://w4.siemens.de/en2/html/press/innovation_news/2003/images/ifo_022_03_hr.jpeg.

### 2.4.2 Impact of nanotechnology on cell chips

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. The patch clamp technique is used to measure the ion current through the membrane pore. This technique is referred to as scanning ion conductance microscopy (SICM)[30]..

### 2.4.3 Research that will impact future development

Cell chips are at a very early stage of research making it difficult to forecast their future development. They may provide a detailed and comprehensive analysis of specific cell interactions, such as antibody-antigen, and are promising candidates for drug screening and drug discovery. Issues that must still be addressed are the sourcing of cells (ensuring that defined, homogeneous populations of cells are used) and reproducibility.

## 2.5 Imaging

### 2.5.1 Overview of imaging

Imaging techniques are widely used in medicine and biochemical research. Techniques such as x-ray, computer tomography (CT), ultrasound (US), magnetic resonance imaging (MRI) and nuclear medicine (NM) are well established for the diagnosis of disease without surgery. The information derived from each method is complementary, so that often several methods are needed for a full diagnosis.These methods are used mainly to analyse anatomy and morphology and not changes at the molecular level. Nanotechnology has no great influence on this traditional use of imaging techniques, but new developments in biochemistry and molecular biology extend some of the above-mentioned methods to a higher sensitivity and specificity at the molecular level. This falls under the banner of molecular medicine whose aim is to detect the early biological markers of disease and diagnose illnesses before the onset of the first symptoms. The field of molecular imaging is part of a paradigm shift to the maintenance of health instead of the treatment of disease. Information on the molecular level is of great importance to an early diagnosis and a more accurate characterisation of disease, as well as early indicators of the success of treatment.

The principle of molecular imaging[31] is demonstrated in Figure 2.9. A 'payload' of either a diagnostic 'signal giver' and/or a therapeutic agent is tagged to a delivery agent. The delivery agent can be a small molecule (e.g. receptors, ligands, peptides), or artificially created carriers such as nanoparticles or microbubbles. The targeting system refers to a specific binding process or molecule, characteristic of the biochemical process to be imaged and/or treated. This basic concept is not restricted to nuclear medicine. It can also be applied to MRI, ultrasound and optical methods.

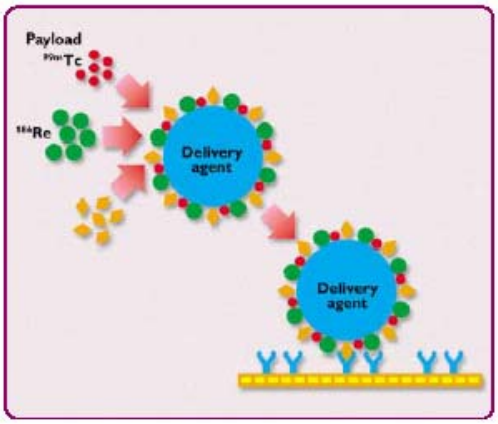


**Figure 2.9**. Schematic principle of Molecular Imaging/Treatment. This example shows targeted contrast design for Nuclear Medicine. Other payloads can be used for the different modalities.

### 2.5.2 Nuclear imaging techniques

Nuclear imaging techniques (Figure 2.10) require the injection of a radioactive substance, which emits gamma rays. The absorption of radionuclides in tissues is dependent on metabolism and allows information about physiological processes, anatomy and organ function to be gathered. The main application areas are oncology and cardiology. However the disadvantage of nuclear imaging is that it delivers limited morphological information, which makes it difficult to precisely localise regions with higher metabolism.

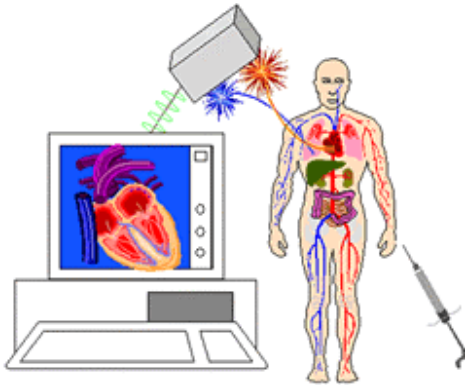

**Figure 2.10**. Principle of Nuclear Imaging (Source: http://apps.gemedicalsystems.com/geCommunity/nmpet/education/nm_intro/why.jsp)

A special method of nuclear imaging is positron emission tomography (PET). By using two opposing detectors the sensitivity is increased strongly which makes PET a promising method for molecular imaging.

### 2.5.3 Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) scanners, as depicted in Figure 2.11, require strong magnets to align the nuclear spins of hydrogen atoms in tissue in one direction. High frequency coils excite the nuclear spins and analyse the weak signals of their relaxation process, with gradient coils used for further, local resolution. MRI is known for its ability to generate high-resolution images, displaying fine anatomical details at sub-millimeter dimensions.

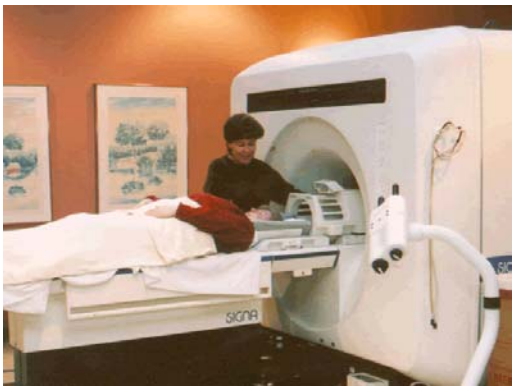


**Figure 2.11**. A Magnetic Resonance Imaging (MRI) scanner in the Brain Imaging Center of the McLean Hospital, Belmont, Massachusetts. http://research.mclean.org/background/pic-mri1.html.

MRI scanning is generally regarded as being harmless to the body, and can be applied repeatedly, with almost no restrictions, making it the ideal modality for the evaluation of treatment. Its limitations arise from similar proton densities in different tissues. Therefore, various contrast agents (gadolinium, iron or manganese compounds) are used, which are concentrated in certain tissues. Smart design of the contrast agents allow molecular signatures to be visualised down to picomole levels.

The 2003 Nobel prize in physiology or medicine was awarded to Paul C. Lauterbur (U.S.) and Sir Peter Mansfield (UK) for their discoveries concerning MRI.

### 2.5.4 Ultrasound (US)

In ultrasound diagnostics, the intensity of the ultrasound wave is reflected at tissue or organ boundaries. The reflected intensity is measured and displayed as an image on a monitor. The contrast between healthy and diseased tissues is usually weak. However, over the last two decades, several contrast agents have been developed, along with contrast-specific imaging methods, to enhance their discrimination. Current research is looking for contrast agents that bind to molecular markers. Traditional contrast agents consist of microscopic gas bubbles (microbubbles) surrounded by a stabilising coating to protect against dissolution. This well-established method offers a high sensitivity– it is even possible to detect a single microbubble. When the microbubbles are injected intravenously, they act as blood pool agents (i.e. remain within the vasculature), which limits their application to targets within the vascular system.

### 2.5.5 Optical Imaging

Optical imaging is an important tool in life sciences for the detection of gene expression and protein-ligand interactions. Although many of the techniques are restricted to *in vitro* applications due to problems with optical access or labelling, optical imaging is being used increasingly for *in vivo* imaging as well. Absorption, reflectance, fluorescence, or bioluminescence can be used as the source of contrast. Currently, most of these techniques are primarily restricted to microscopic or surface imaging, or to experimental imaging in small animals, since the penetration depth of the light is limited. However, light within a small spectral window of the near infrared region (600–900 nm) can penetrate more than 10 cm into tissue, due to the relatively low absorption coefficient at these wavelengths. The lowest wavelength in this window is determined by the relatively high absorption of blood (haemoglobin), whereas absorption above 900 nm is due to water. Near-infrared (NIR) fluorescence and luminescence imaging make use of this optical window.

Usually, optical contrast agents have to be used to obtain high specificity and sensitivity. In fluorescence imaging a fluorescent probe (optical contrast agent) is activated by an external light source, and a signal is emitted at a different wavelength. The fluorescence signal can be captured with a high-sensitivity charge-coupled-device (CCD) camera. The sensitivity of fluorescence detection is very high, and in microscopic setups it is possible to detect single molecules. Although *in vivo* optical imaging is a powerful tool in cell culture studies or animal models, its current application to human health is generally limited to 'close-to-surface' structures, as in optical imaging of the eye and skin, or optical mammography.

In addition, the widely used dyes, called fluorophores, have drawbacks. They fade quickly, and only two or three colours can be used simultaneously to label different cellular components.

### 2.5.6 Electron Microscopy (EM)

Pathology represents a completely different field of diagnosis, e.g. where analytical methods are used to search for viruses in biopsy samples. Viruses have sizes of around 100 nm or less and therefore their detection is suited instruments such as the electron microscope (EM), which is used for analyses in the nanometre region.

For example, polyomaviruses (PV) are pathogens which have tropism to the human urinary tract and have been reported with increasing frequency in renal transplant recipients.[32] It is difficult to distinguish the effect of PV on graft rejection from patient immune responses against transplanted renal antigens. An accurate diagnosis is of great importance because the therapeutic approaches to both are very different. PV appear as 40 to 50-nm, non-enveloped particles within epithelial cell nuclei and can be readily identified using EM.

The EM was also the "first line of defence" during the SARS (Severe Acute Respiratory Syndrome) breakout. SARS is a viral respiratory illness that was first reported in Asia in November 2002. It is caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV). The virus has a diameter of about 100 nanometers and was identified through an electron microscopic analysis.

## 2.5.7 Impact of Nanotechnology on Imaging Techniques

### 2.5.7.1 Nuclear Imaging Techniques

In a 3-year joint project, funded by the National Cancer Institute, Philips, Dow Chemical and Kereos Inc. intend to facilitate the development and testing of novel molecular imaging agents. These, in combination with advanced imaging systems, should enable earlier, non-invasive detection of certain cancers, as well as acting as agents that can provide highly targeted therapy and the ability to assess the therapeutic effect.[33] The contrast agents in development start with tiny perfluorocarbon nanoparticles suspended in an emulsion. Researchers then attach agents such as Technetium-99m to the nanoparticles, which provide the contrast that allows for imaging. In addition, the nanoparticles are labelled with a specific ligand that targets the agent to newly developing blood vessels. When injected into the body, the targeted nanoparticles will find and illuminate these vessels. Anti-cancer drugs and therapeutic radionuclides may also be incorporated into the nanoparticles to deliver therapy directly and selectively to areas of tumour development.

### 2.5.7.2 MRI

A new contrast agent based on nanoparticles has been described by Lanza et al.[34] In this approach, gadolinium chelates are concentrated in perfluorocarbon nanoparticle emulsions (Figure 2.12). The nanoparticles are surrounded by a lipid layer, into which antibodies or proteins can be inserted to target the particle to specific tissues. The particles are being investigated for their application in the diagnosis of arteriosclerosis plaques. Animal experiments have shown that these nanoparticles can bind to and allow the visualisation of early stage blood clot formation. Further investigations have indicated their applicability in the diagnosis of angiogenesis and cancer metastases.

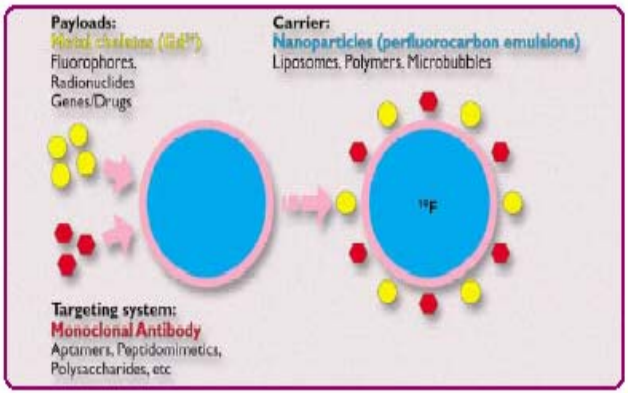


**Figure 2.12**. Targeted contrast agents based on nanoparticle technology. The core consists of perfluorocarbon compounds. This example has a payload of Gd3+ chelates and antibodies. Other payloads can be fluorophores, radionuclides, genes or drugs. Source: http://www.medical.philips.com/main/news/assets/docs/medicamundi/mm_vol47_no1/08_lanza.pdf

Other particles that are being investigated for use as MRI contrast agent are fullerenes, which are hollow cages of carbon shaped like soccer balls. The inside of fullerenes can be filled with different atoms or small molecules. Water-solublized forms like [Gd@$C_{82}(OH)_{30}$] (Figure 2.13) are being explored for use as MRI contrast agents[35]. The degree of contrast provided by this molecule is comparable to that of ordinary MRI agents. By using holmium instead of gadolinium the fullerenes can be used as x-ray contrast agents.

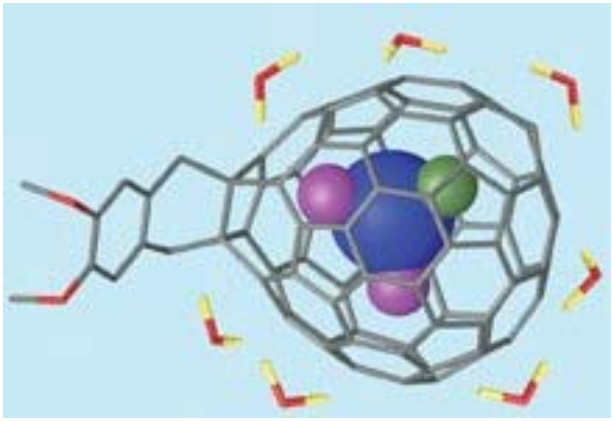

**Figure 2.13**. Water-soluble contrast agent being developed for magnetic resonance imaging encapsulates two gadolinium metal atoms (purple) and one scandium metal atom (green) that are attached to a central nitrogen atom (blue). The molecule's tail (gray and red) makes the cage water-soluble. Water molecules (red and yellow Vs) surround the molecule. (Source: http://www.sciencenews.org/20020713/bob10.asp).

### 2.5.7.3 Ultrasound

The same formulations of perfluorocarbon nanoparticles, which are used for MRI, are also being developed as contrast agents for ultrasound. The present application is the diagnosis of pathogenic changes in blood vessels. Although perfluorocarbon nanoparticles do not reflect ultrasound nearly as strongly as microbubbles, their reflection increases significantly when they are targeted and concentrated on the surfaces of tissue or cells, without increasing background noise. Another characteristic of perfluorocarbon nanoparticles is that they cannot be destroyed by acoustic pulses, which avoids the risk of ultrasound-induced cavitation. By incorporating gadolinium in the lipid layer, these nanoparticles can be used as both ultrasound and MRI contrast agents.

### 2.5.7.4 Optical methods

**Quantum dots**[36] are nanometre scale particles that are neither small molecules nor bulk solids. Their composition and small size (a few hundred to a few thousand atoms) give these dots extraordinary optical properties that can be readily customized by changing the size or composition of the dots. Quantum dots absorb light, and then quickly re-emit the light but at a different wavelength. Although other organic and inorganic materials exhibit this phenomenon i.e. fluorescence, quantum dots closely fit the profile of an ideal fluorophore: bright, non-photobleaching with narrow, symmetric emission spectra, and have multiple resolvable colours that can be excited simultaneously using a single excitation wavelength. Quantum dots can operate in a liquid environment and therefore can be applied to biological imaging (Figure 2.14). The semiconductor particles that researchers are grooming for biological imaging are generally made of a cadmium selenide core surrounded by a shell of zinc sulphide. They are of the nanometre scale and when illuminated, the quantum dot emits a particular colour based on its size. Smaller dots fluoresce at shorter wavelengths, such as blue, while larger dots emit longer wavelengths, like red.

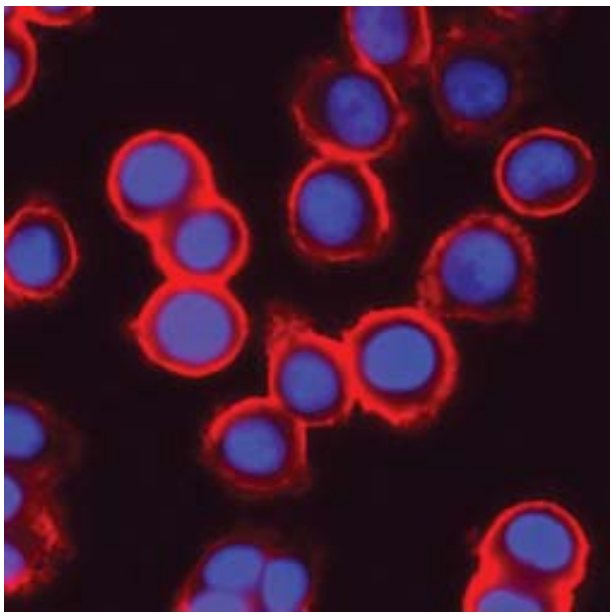

**Figure 2.14**. Red light-emitting quantum dots tag proteins on the surfaces of breast cancer cells, while a conventional blue dye stains the cells' nuclei. (Source: Quantum Dot Corp. http://www.sciencenews.org/20030215/bob10.asp).

Quantum dots have significant advantages over earlier technologies. Researchers can view typically no more than three colours at once with traditional fluorescence labelling, using proteins, such as green fluorescent protein, or organic dyes, such as rhodamine. Each of the fluorophores must first be excited with a specific wavelength of light, which can block the emitted colour of other fluorophores in the experiment. To overcome this problem, researchers can mark multiple proteins in a cell with several different colours, photograph them at different times, and then superimpose the pictures. Alternatively one protein can be tagged with one fluorophore in one cell and another with a different fluorophore in a similar cell. Generally for quantum dots, the colour is very specific to the size and composition, allowing the simultaneous fluorescence of many different, specific colors and therefore allowing the easy discrimination between tagged targets, even in a single cell.

Another limitation of conventional fluorophores is their short life span. They can fade in a couple of hours. In contrast, quantum dots remain stable for days to months.

**Dendrimers** (greek *dendron* = tree) are hyperbranched molecules, composed of a central core, to which repetitive dendritic branches are attached (Figure 2.15). These branches are synthesized from identical building blocks via a stepwise reactional synthesis strategy.

**Figure 2.15**. Scematic view of a dendrimer. Source: http://wunmr.wustl.edu/~wooley/dend.html

NASA and NCI are financing a project to develop a method using dendrimers for the detection of cell death (apoptosis) *in vivo*, for future use in the detection of cellular damage induced by ionising radiation in space. In this project dendrimers are coupled to sugar molecules, which allows them to be taken up by white blood cells. Each dendrimer contains two tightly bound molecules, linked by a bond that can be broken by an enzyme (caspase-3), which is produced during apoptosis. One of these molecules can fluoresce, however the other molecule absorbs this fluorescence due to its close proximity (a phenomenon known as fluorescence resonance energy transfer, or FRET). In normal, healthy white blood cells the bond is intact and no fluorescence is detected, however when a cell undergoes apoptosis (which can be induced by ionising radiation), caspase-3 is produced and cleaves the FRET bond. This separates the two molecules allowing fluorescence to be observed.[37]

### 2.5.8 Research that will impact future development

The above-mentioned applications of nanoparticles are completely in the development phase. They have to prove their applicability in clinical tests, before they can be routinely used in the clinic.

## 2.6 Conclusions

At present the biochip technology is mainly governed by processes on the micrometer scale. However, a further miniaturisation in microarray technology can be expected in the future. It is here that a nanotechnological influence is apparent. The standard tool of nanotechnology, the scanning probe microscope, may provide a means for the further miniaturisation of microarrays. Microfluidics, are unlikely to undergo further size decreases in the near future due to the difficulty in reliably analysing sub-picoliter volumes. However lab-on-a-chip devices have great potential as diagnostic devices at the point-of-care, due to their inexpensiveness and fast (real-time) diagnosis. The benefit would be an inexpensive and fast diagnosis of diseases for example which can be performed in real-time near the patient.

The impact of nanotechnology on imaging techniques is negligible at the moment. In addition, an increasing influence of nanotechnology on the traditional imaging methods cannot be expected at this stage. On the other hand, diagnosis in molecular medicine relies on highly specific and sensitive methods. In this regard, nanoparticles are believed to be of high significance for the progress of diagnostics in the future. At present, research is focused on the development of better contrast agents for nearly all imaging methods. Perflourocarbon nanoparticles especially are being investigated for use in different imaging methods by connecting them to different markers. Moreover, other approaches using fullerenes, quantum dots or dendrimers show promising properties.

## 2.7 Companies working in the field

### 2.7.1 Array technology

| Company/Link | Product/Description |
|---|---|
| Advion Bioscience Inc., www.advion.com | Microfluidics chips, MS |
| Affibody AB, www.affibody.com | Affibody® molecules, antibody substitutes |
| Affymetrix, www.affymetrix.com | GeneChip |
| Biacore AB, www.biacore.com | SPR-based biosensors |
| Bioforce Nanoscience Inc., www.bioforcenano.com | Nanoarrays |
| Ciphergen Inc., www.ciphergen.com | Protein-Chips |
| Cytonix Inc., www.cytonix.com | Nanoarrays and related tools |
| | |
| Invitrogen, www.invitrogen.com | Gene expression, labelling and detection |
| MolecularReflections Inc., www.molecularreflections.com | Nanoarrays |
| NanoInk, Inc.www.nanoink.net | Nanoarrays, Dip Pen Nanolithography. |
| PerkinElmer Inc., http://las.perkinelmer.com | DNA and protein microarrays |
| Zyomyx, www.zyomyx.com | Protein Chips |
| www.biochipnet.de | Link to over 345 companies and 134 institutions world-wide |
| www.cato.com/biotech/bio-genomics.html | World-wide links to companies working in the field of genomics, proteomics, HTS, drug discovery and drug screening. |

### 2.7.2 Lab-on-a-chip

| Selected companies: | Related products: |
|---|---|
| Agilent Inc., www.agilent.com | DNA and protein LabChip, Cell chips |
| Coventor Inc., www.coventor.com/microfluidics | MEMS, microfluidic devices |
| Infineon Technologies AG, www.infineon.com | DNA chip, cell chip |
| LioniX, www.lionphotonix.nl | MEMS, microfluidics |

### 2.7.3 Cell chips

| Selected companies: | Related products: |
|---|---|
| Cellomics Inc. www.cellomics.com | Cell based assays |
| Infineon, www.infineon.com | DNA chip, cell chip |

### 2.7.4 Imaging

| Selected companies: | Related products: |
|---|---|
| Micromod Partikeltechnologie GmbH www.micromod.de | Nanoparticles for diagnostics, e.g. fluorescent or magnetic particles |
| Nycomed Imaging AS, www.nycomed.no | Iron oxide nanoparticles as contrats agents |
| Schering AG, www.schering.de | Iron oxide nanoparticles for MRI |

## 2.8 Overview of European projects, literature and web-sites

### 2.8.1 European projects

#### Fifth Framework Programme (FP5)

MAGNANOMED (Magnetic nanoparticles for medical and biological diagnostics and devices) makes and evaluates new types of magnetic nanoparticles of special shape and precise size with tailored surface chemistry and topography for biomedical purposes. Histology is one application. Coordinator is Dr. Ian Carter, University of Glasgow, Institute of Biomedical and Life Sciences, United Kingdom. (http://dbs.cordis.lu/fep/FP5/FP5_PROJl_search.html search for MAGNANOMED)

#### Expressions of Interest for FP6

NANOBIOMED (Bio-Nanotechnology Next generation Nanomaterials for Biomedical applications) deal with diversified applications of nanotechnology in biotechnology and medicinal applications, including imaging applications. Coordinator is Prof. Muhammed, KTH-KI Nano- and Micro Technology Centre, Royal Institute of Technology, Stockholm, Sweden.

FUNC (Designed Functional Nanostructures) deal with the design of functional Nanostructures. Applications include semiconductor quantum dots as fluorescent probes. Coordinator was Prof. Samuelson, Department of Physics, Lund, Sweden.

NATBIO (Development of innovative nanotechnology for imaging, detecting and addressing biosystems) initiative is dedicated to the understanding of basic mechanisms involved in biology at the molecular level using imaging, detection and manipulation of biosystems at the nanometer scale making use of nanotechnologies. NATBIO is coordinated by Prof. Vieu, CNRS, Toulouse, France.

EMI-CARE (European Molecular Imaging Consortium for Advanced Research) aimed to integrate scientific and industrial research to develop Molecular Imaging as a new procedure in "healthcare of the future". The objectives of the integrated project included advances in medical diagnosis and treatment. Coordinated by Dr. Schaeffter, Philips Research Technical Systems Hamburg, Germany, the consortium includes: Amersham Health, Novartis Pharma, Siemens Medical Solutions, Center Résonance Magnétique des Systèmes Biologiques (Bordeaux), German Cancer Research Center (Heidelberg), University of Florence, University of Valencia, University of Leuven

"Nanoparticles in Medicine", coordinated by Prof. Eva Sykova, Institute of Experimental Medicine, Prague, Czech Republic, was a proposal for a network, which includes superparamagnetic nanoparticles as contrast agents in MRI.

### Sixth Framework Programme (FP6)

The Network of Excellence FRONTIERS is directed at instrumentation for the manufacturing and analysis of single molecules, individual nanostructures and 2-3D architectures of them, targeted at life sciences. The focus is on analysis and manipulation of the bio environment. Organizer is Prof. Reinhoudt, MESA Research Institute, Enschede, Netherlands.

The Network of Excellence NANO2LIFE is focused on the understanding of the nanoscale interface between biological and non-biological entities and its applications, e.g. for novel sensor technologies. Coordinator is Prof. Boisseau, CEA, Paris, France.

### National programmes and activities

Nanospecific aspects are covered by some projects in the framework of a Nanobiotechnology programme (http://www.nanobio.de/). Projects include using AFM for the analysis of ion channels in the inner ear, the use of a force sensor on DNA bases, and the use of magnetoresistive Biochips.

**Table 2.1** Links

| | |
|---|---|
| www.biochem.mpg.de/mnphys | Cell chip with neurones. |
| www.biochipnet.de | Link to over 345 companies and 134 institutions world-wide. |
| www.cato.com/biotech/bio-genomics.html | World-wide links to companies working in the field of genomics, proteomics, HTS, drug discovery and drug screening. |
| www.chemsoc.org/networks/locn/ | UK-based LOC network. |
| www.diagnostic-arrays.com | Diagnostic microbial microarray projects |
| www.gene-chips.com | DNA microarrays |
| www.hgmp.mrc.ac.uk | Human Genome Mapping Project |

| | |
|---|---|
| www.lab-on-a-chip.com | Internet forum concerning microarrays, microfluidics and lab-on-a-chip systems. |
| www.labonachip.org.uk | The Laboratory on a Chip Consortium |
| http://www-leti.cea.fr/ | Laboratoire d'Electronique de Technologie de l'Information, Biochips |
| www.microarrays.org | Microarray protocols and software |
| www.microfluidiccenter.com | Support to European organizations involved in microfluidics. |
| www.ornl.gov/TechResources/Human_Genome | Human Genome Project, U.S. Department of Energy |
| www.rsc.org/is/journals/current/loc/locpub.htm | The journal "Lab on a Chip", the Royal Society of Chemistry |

# Chapter 3 – Drug Discovery

Mark Morrison, Institute of Nanotechnology.

## 3.1 Introduction

Drug discovery requires an identified disease, a knowledge of the disease mechanism, thereby identifying a point of intervention (target), and a model system to test efficacy (summarised in Figure 3.1).

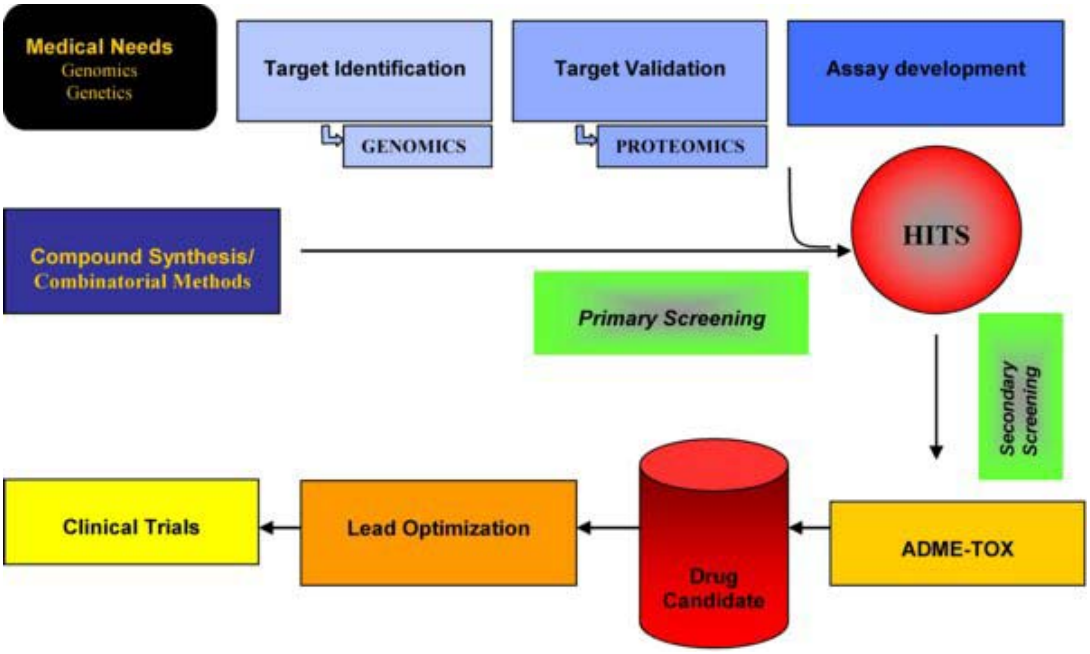


**Figure 3.1.** Flowchart of the processes involved in drug discovery. http://www.tecan.com/index/com-ou_ma/com-ou_ma-drug_discovery.htm

Most drug targets are proteins. These can be human proteins that are either defective or abnormally expressed (e.g. the drug Claritin targets the histamine receptor and reduces allergic reactions), or can be proteins within a pathogen (e.g. nucleotide analogues such as gancyclovir, which inhibits the action of the DNA polymerase of herpes viruses, or antibiotics which inhibit the action of bacterial proteins). The site of interaction of the drug with target is often at the protein's active site. This can be likened to the functional part of the protein, e.g. for DNA polymerases this is where bonds between nucleotides are catalysed to make DNA.

Novel drugs have been discovered by a variety of different routes ranging from serendipity (e.g. penicillin), the screening of natural products (e.g. digoxin isolated from foxgloves [*Digitalis lanata*] for the treatment of congestive heart failure) to combinatorial chemistry where chemicals are systematically modified to produce large numbers of variants and then screened against targets. By understanding the process involved in a disease and approaching this rationally, compounds with known beneficial effects can be chemically modified to make them more effective, safer or more easily administered (e.g. the histamine receptor antagonist, cimetidine, which is used for the treatment of gastric ulcers, was designed in this manner). Mass screening of chemicals against targets can be used to identify which ones have the desired effect.

Traditionally the pharmaceutical industry followed an empirical route to drug discovery. By understanding the mechanism of a disease compounds could be synthesised to evaluate their ability to interact with targets and alleviate disease symptoms. This approach relies on a one drug one target cure, with pharmaceutical companies hoping to produce a so-called "blockbuster drug", each of which can generate in excess of $1 billion per annum. However this has identified fewer and fewer new drugs or new molecular entities (NMEs) over the last 10 years, and of those that have been produced the majority fall into the "me too" category. Such drugs are novel formulations against established targets for which another company already markets an effective drug.

With the sequencing of the human genome novel approaches to drug discovery have become evident. The human genome contains approximately 35,000 genes each of which can be copied into messenger RNA (mRNA), which acts as a template for the translation of the genetic code into proteins, which in turn exert the biological (or phenotypical) effects of the gene. However for more than 50% of human genes this biological effect has yet to be identified. The complexity of the human genome is raised further through tissue- and developmental-specific expression of genes, the potential for some genes to express multiple forms of mRNA and through modifications of translated proteins, all of which leads to the differential expression of proteins and forms of the same protein (isoforms) within different tissues and even the same tissue under different physiological conditions. All-in-all this increases the total number of encoded proteins to more than 100000[38], and at present, potential targets for drug intervention have been estimated at anything between 600 and 10000[3940].

Genomics looks at all the genetic information in an organism and feeds into an investigation of the transcriptome (or the total complement of mRNA in a cell under the studied conditions) and ultimately proteomics, which is the study of the total cellular protein complement. The technology allows an investigation and a comparison of each of these (between normal and diseased states) in terms of mutations at the DNA level, and differential mRNA or protein expression.

Nanotechnology is becoming key to the success of these endeavours through the miniaturisation (and therefore requirement for less reagents), automation, speed and reliability of assays. In this chapter we will discuss how novel technologies are contributing to the identification of targets and drugs that can interfere with these targets.

## 3.2 Target identification

The first stage of drug discovery is the identification of a target that can be subsequently acted on by a future drug to bring about an improvement in disease. This can be achieved more quickly and reliably now through the use of array technologies.

### 3.2.1 DNA Microarray Technology

As discussed in Chapter 2, DNA microarrays were first introduced by Affymetrix in 1994 and consist of small segments of DNA (oligonucleotides or oligos) that have been chemically linked to a glass slide (wafer) in a defined order (see Figure 3.2).

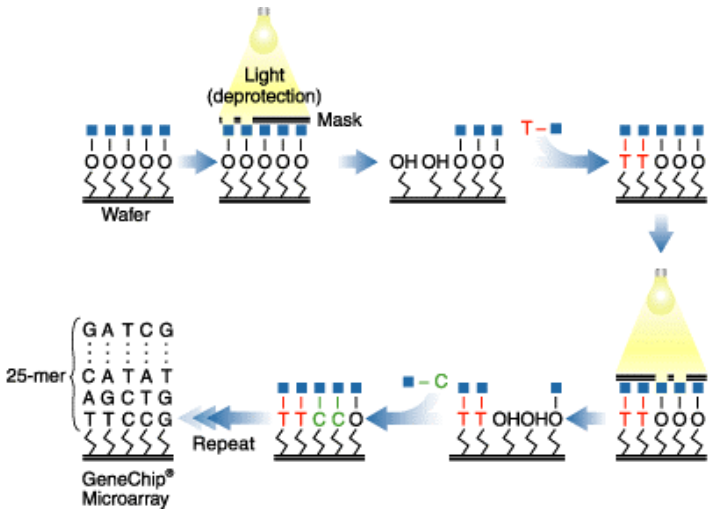


**Figure 3.2**. Use of photolithography and combinatorial chemistry to generate GeneChip® Arrays.
http://www.affymetrix.com/technology/manufacturing/ind

Up to 500000 oligos per square cm can be packed onto slides, allowing the detection of thousands of genes or mRNA species on a single chip. Differences in gene expression between normal and diseased cells can be determined by incubating microarrays with cDNA (mRNA which has been copied back into DNA) isolated from both sources that have been labelled with different fluorescent tags. As each spot is associated with an attached oligo of known sequence, the relative fluorescent intensities from each tag at each of these spots allows the determination of the relative expression of different genes in each source. By establishing whether the transcription levels of specific genes are the same, higher or lower in the diseased cell compared with normal, novel targets for drug intervention can be identified (see Figure 3.3).

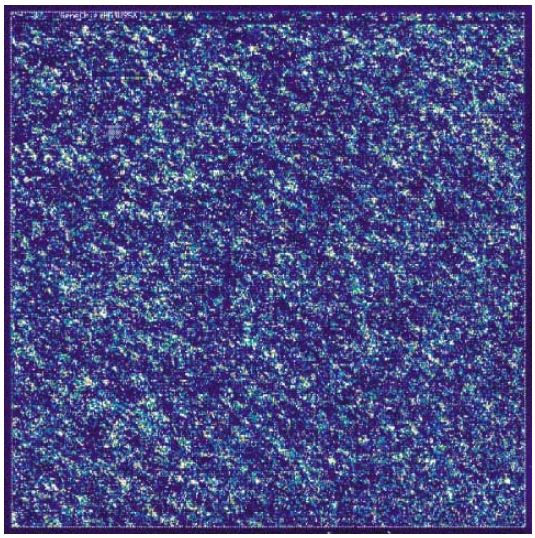

**Figure 3.3**. Data from an experiment showing the expression of thousands of genes on a single GeneChip® probe array. Image courtesy of Affymetrix.

For example oestrogen has been known to play a key role in the development of breast cancer since it was observed that a side-effect of chemotherapy was inhibition of oestrogen production in the ovaries.[41] Earlier this year Frasor and colleagues used DNA microarrays to identify oestrogen responsive genes in breast cancer cells, and therefore potential targets for drug intervention.[42]

According to analysts the market for DNA microarrays was $400-550 million in 2000, and this is expected to grow to $1-2.2 billion by 2005 [4344]

### 3.2.2 Protein Chips

DNA microarrays provide a robust platform for the screening of multiple candidate drug targets, however as has been mentioned previously the diversity of proteins in any cell is not restricted to the number of genes that it expresses. Therefore to identify all possible targets, and in some cases to identify any targets, it is necessary to look at the protein complement of the cell. Proteomics has been defined as "the branch of genetics that studies the full set of proteins encoded by a genome." Proteomic tools have been traditionally less robust and more time-consuming than those for DNA. However recent advances in protein chips and a fusion of DNA and protein technologies may change all that.

The enabling technologies have been discussed in depth in Chapter 2, but to recap briefly, consist of 2D-polyacrylamide gel electrophoresis (2D-PAGE) followed by mass spectrometry. 2D-PAGE separates proteins based on charge and mass, with it being possible to analyse two populations of proteins (e.g. from healthy and diseased tissue) stained with different fluorescent markers analysed on the same gel (Figure 3.4). Following electrophoresis, protein "spots" are excised and identified by mass spectrometry. This technology has been bolstered recently through the production of protein chips.

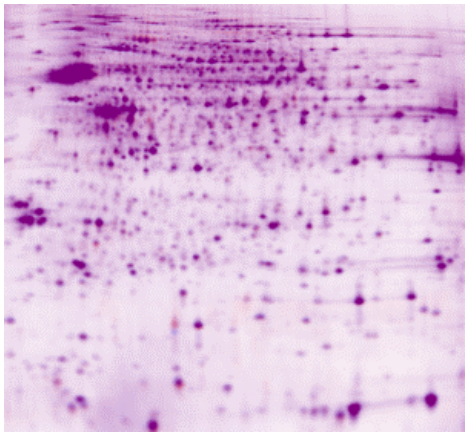

**Figure 3.4.** Example of 2D gel where proteins from different samples were labelled with fluorescent dyes cy3 and 5 prior to separation on gel. Picture from Amersham Biosciences. http://www1.amershambiosciences.com/APTRIX/upp00919.nsf/Content/Proteomics+DIGE+Technical+Tips

The identification of a protein(s) which is differentially expressed between diseased and normal tissues is the first step. If the protein has been previously characterised and its biological function is known, then perhaps intervening drugs are already available or can provide a starting point for the elaboration of more effective drugs. If however, the protein is unknown then its function must be either determined experimentally through expression and *in vitro* studies, or through the relatively new field of bioinformatics.

The "fusion" of DNA and protein technologies has come from the development and use of aptamers. Single-stranded DNA and RNA molecules can form complex 3D shapes through intramolecular interactions, and in some cases these formations (or aptamers) can interact specifically with proteins (much in the way that antibodies do). Somalogic have incorporated bromodeoxyuridine into single-stranded nucleic acids to generate photoaptamers which can crosslink to proteins when irradiated. This provides a handle for the detection of specific proteins within a sample. Photoaptamers can be arranged in array format, different populations of proteins can be incubated on matching arrays, proteins UV-crosslinked and differences in individual protein expression levels determined by applying a universal protein fluorescent stain to the plates.

## 3.3 Target validation

The genomic and proteomic methods described above can identify differences gene expression and protein levels between normal and diseased cells. The use of DNA microarrays in particular has lead to an explosion in the number of candidate targets. But how do you tell whether these candidates are significant? How do you tell whether the target that has been identified will be amenable to intervention and whether it is the sole factor in the disease or is part of a complex pattern of differential protein expression? These questions must be answered by target validation.

According to David Szymkowski, director of biotherapeutics at the biopharmaceutical company Xencor in Monrovia, California, drugs fail in the clinic for two basic reasons: they either don't work or they prove to be unsafe. "Both of these are often the direct result of sloppy early target validation." It is important to ensure that the correct target is identified before candidate drugs are tested against it, as it can take 12 years and up to $1 billion dollars to bring a drug to market. [45] Traditionally, target validation was achieved *in vivo*, however recently *in vitro* models have been developed which, although not available for more complex disease syndromes (e.g psychological diseases), offer a more ethical, quicker and cheaper alternative to animal models.

### 3.3.1 Target validation *in vivo*

The targets of many of the blockbuster drugs were validated using gene knockouts. Gene knockouts (KO) involve deleting or disrupting expression of a gene in early embryogenesis in an animal model (usually mouse, but more recently zebrafish have been used). The rationale for this is that removing the gene encoding a protein that has been identified as a candidate will effectively mimic the application of a strong repressor for that protein, allowing investigators to determine the protein's normal cellular function. For example KO mice for the histamine $H_2$ receptor (which is the target of Zantac) are unable to secrete gastric acid into the stomach in response to histamine, thereby preventing lowering of stomach pH and validating the receptor as a target for the treatment of gastric ulcers.[46]

KO animal models have to be taken in context. Some mutations may be lethal, some may have differential effects depending on developmental stage and others may have multiple effects.

### 3.3.2 Target validation *in vitro*

Using tissue culture systems is quicker and cheaper than animal models. Genomic approaches include antisense oligonucleotides and RNA interference.

The principle of antisense technology is the binding of an oligonucleotide to complementary mRNA in the cell. This can prevent gene expression in one of 3 ways: 1) directed degradation of the DNA-RNA duplex by a cellular enzyme RNase H, 2) blocking protein translation (see Figure 3.5) or 3) preventing RNA processing, prior to translation of protein.[47]

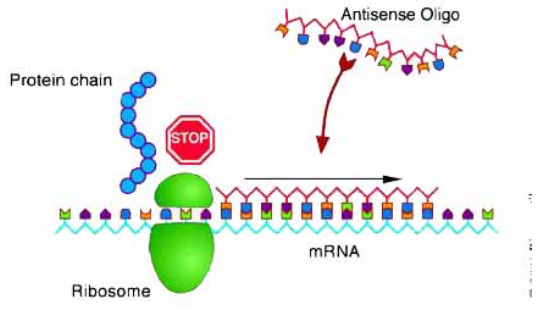


**Figure 3.5**. Mechanism of action of antisense RNA. Taken from BIOGNOSTIK: http://www.biognostik.com/

BIOGNOSTIK manufacture phosphorothioate antisense oligonucleotides (ODN) that are capable of entering cells in tissue-culture and binding to complementary mRNAs preventing their translation (Figure 3.5).

In the last few years RNA interference (RNAi) has received much attention for its ability to silence genes.[48] This technology makes use of a natural cellular defence mechanism against double-stranded RNA (dsRNA) molecules, which can arise during virus replication for example. Short dsRNA molecules that are identical in sequence to the target mRNA are transfected into cells where they are cleaved into smaller fragments by a cellular enzyme called Dicer. As a result of this recognition, Dicer also cleaves the cognate mRNA, silencing gene expression. It is estimated that the market for RNAi will be US $38 million by the end of 2003.[49]

As discussed in the introduction to this chapter, there are many more proteins than genes, and so validating targets at the DNA or mRNA level may not always present an accurate physiological picture. Proteomics approaches are now appearing in the form of solid phase and solution arrays.

Xerion Pharmaceuticals use a novel procedure to functionally inactivate target proteins. First the target is "panned" against a phage display library that expresses single chain antibody fragments (scFv) which bind proteins with high specificity. Specific scFv's to the target are identified, enriched and labelled with UV-reactive dyes. These labelled scFv's are allowed to interact with the target protein within the cell and then irradiated using a laser. This causes the breakdown of the dyes into active compounds which in turn cause the inactivation of the target protein. This procedure can also be used for a disease-based approach, combining target identification and validation. Proteins are isolated from healthy and diseased cells and scFv's identified that only bind proteins from diseased cells. These are used to validate the target as described above, and can then be used to isolate the target for identification by mass spectrometry.[50]

Oxford Glycosciences used a proteomics approach to validate targets in respiratory disease. Their collaboration with Bayer AG was one of the first to successfully combine identification and validation of target proteins on a proteomics platform.[51]

## 3.4 Drug design and validation

Following identification and validation of a target in a disease, drugs must be designed and validated that will affect that target (usually through inhibition) to alleviate disease symptoms. First of all lead compounds must be generated. This can be achieved through modelling or molecular manipulation. These then must be screened to confirm their efficacy and determine which compounds will be developed further into marketable drugs.

### 3.4.1 Structure guided discovery- molecular modelling

In the 40 or so years since Perutz and Kendrow determined the 3D structure of haemoglobin and myoglobin, X-ray crystallography has proven invaluable in the generation of data on the 3D structure of proteins. From this work it has become clear that proteins that perform similar functions but have different substrates (e.g. kinases, proteins which phosphorylate and thereby modify the activity of other proteins) have similar structures around their active sites. More recently, bioinformatics (defined as the merging of biology, computer science, and information technology into a single discipline) has been used to predict the 3D structures of targets and potential chemicals that could interact with them (ligands) and inhibit protein function, by comparing primary amino acid sequence of novel proteins with the determined 3D structure of known proteins. This approach has been heralded as the new way forward in drug discovery. It is estimated that only 1 out of 5000 screened compounds is approved as a new medicine. By using modelling systems many compounds can be eliminated from screening before they are synthesised.

De Novo Pharmaceuticals uses complex algorithms in its Site Explorer™ software to characterise the active site of a target and determine potential structures of ligands which can interact with it. Candidates are then screened "in silico" using the company's EasyDock programme to determine relative binding affinities.[52] Using this approach thousands of compounds can be screened and only the best candidates synthesised for screening.

A unique approach is employed by Momenta Pharmaceuticals. 80% of known proteins are modified through the addition of carbohydrate molecules, and it has been known for a long time that these "post-translational" modifications can influence protein function. Momenta Pharmaceuticals have developed a technology which allows the identification and functional characterisation of these sugar groups, which in turn can feed into the development of new therapeutics.[53]

### 3.4.2 Molecular manipulation- combinatorial chemistry

Traditionally drugs were designed and modified in what could be considered a linear approach. A compound was identified and if for whatever reason it had drawbacks, it was modified in a rational manner based on reactive groups within the molecule. This approach however, can take a long time to generate better compounds (e.g. it took 12 years from the concept of an anti-gastric ulcer drug to the manufacture and release of cimetidine).

Combinatorial chemistry however can be likened to taking many different LEGO building blocks and putting them together randomly to create many different structures. The building blocks are small reactive chemical species and the larger compounds that they combine to form are candidate drugs which can be generated in their thousands and then assayed on the target for functionality.

Alantos use a procedure termed Target Amplified drug Candidate Evolution (TACE) to generate libraries of compounds that "can evolve via interaction with the biological target to produce preferentially or exclusively only those compounds that bind tightly to the target." (Figure 3.6)

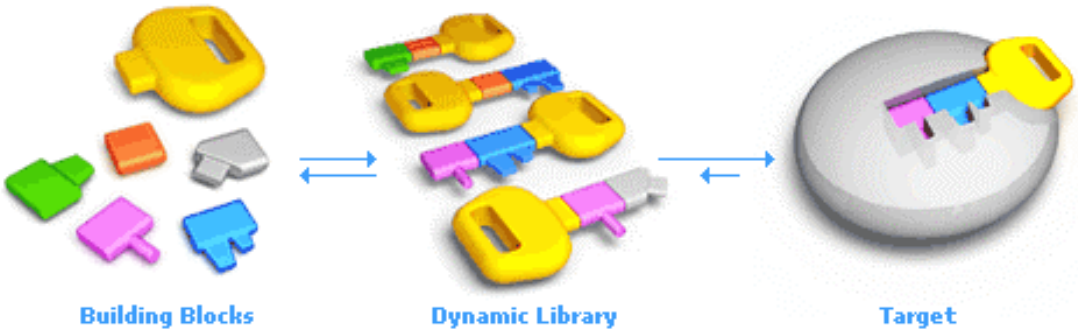


**Figure 3.6**. Target Amplified drug Candidate Evolution (TACE). http://www.alantos.com/english/technology/tace.shtml

Pharmacopeia also generate vast chemical libraries and have designed high-throughput screening assays to test compounds on different target proteins.[54] By generating libraries of molecules, companies are not only maximising their chances of finding an effective drug, they are also going some way to fending off other companies future efforts to produce and market "me too" drugs.

An alternative method is to combine smaller molecules in the presence of the target. This has been successfully used to develop inhibitors against the influenza neuraminidase protein, which is a major target for anti-influenza therapies. Hochgürtel and colleagues mixed specific aldehydes and amines in the presence of neuraminidase, allowed these to chemically react and products, which resulted in enzyme inhibition, were identified by chromatography and mass spectrometry.[55]

Astex-Technology use bioinformatics to pre-select low molecular weight compounds for assay with target proteins. Their fragment-based discovery method allows these small molecules (which are typically only part of the final drug) to interact with the active site of the protein target. Interactions are evaluated using X-ray crystallography. This technology can be expanded to investigate the simultaneous interactions of multiple fragments with the target.[56]

### 3.4.3 Existing libraries of drug candidates

As a result of research that has been undertaken in drug discovery there exists many libraries of potential drug candidates. Some of these are held privately by companies but some are available publicly. For example researchers at the Stockwell lab (http://staffa.wi.mit.edu/stockwell/) have compiled an annotated compound library (ACL) of some 2000 biologically active compounds which include 514 US FDA-approved drugs. By understanding the biological action of compounds pre-screening, it is possible to significantly increase the likelihood of success for those that enter validation.[57]

The Developmental Therapeutics Program (DTP) is the drug discovery and development division of the US National Cancer Institute and acts as a repository of synthetic and natural products, which can be evaluated as anticancer and AIDS agents. DTP provides information on more than 600000 compounds, which have been supplied from a variety of sources worldwide.[58]

Biofrontera Discovery offer an alternative based on natural compounds. This company specialises in screening compounds isolated from microorganisms, marine invertebrates and plants against protein targets. The potential for creating novel libraries of compounds to be screened against targets is immense: "Only a minute part (less than 5 %) of all naturally-occurring microorganisms have been successfully isolated and cultured…There is strong evidence that those microorganisms contain biosynthetic genes for unknown and innovative bioactive natural compounds."[59] Biofrontera Discovery uses chromatographic techniques to isolate compounds from different organisms and then characterises these via a number of methods including mass spectrometry and X-ray crystallography. The result is that they have approximately 140000 sub-fractions containing over 2 million compounds.

## 3.5 Models- validation in the real world

The systems described above have created a culture of high-throughput screening of targets and potential drug compounds that can interact with these targets. Ensuring that a drug can interact with the target protein of a disease and inhibit its function, may seem like the end-point for drug discovery. Unfortunately this is not the case. Taking a single target protein out of its cellular context and screening for compounds that have the desired effect on the target can result in the development of drugs that interact with other proteins and either become ineffectual or cause unwanted side-effects.

It is essential to determine at the earliest stage whether a drug will be ineffectual (or dangerous) when administered to a patient. This validation is summarised by the acronym ADMET, which stands for **A**bsorption, **D**istribution, **M**etabolism, **E**xcretion and **T**oxicity. This ensures that a drug will be absorbed into the body and penetrate the target tissue through the chosen route of administration (e.g. intravenous, nebulizer, enteric-coated pill), and that it will be metabolised and excreted from the body, without any toxic side-effects. Ultimately this must be determined in human volunteers (phase I clinical trials), but prior to this all drugs are evaluated in animal models. New *in vitro* developments however, allow pre-animal testing of drugs and a further validation of lead candidates, and therefore earlier elimination of unsatisfactory compounds.

To highlight the importance of this, it is worthwhile considering the impact of the cytochrome P450 (CYP450) family of enzymes on drug development. CYP450 enzymes are key to the metabolism of many drugs, making them more water-soluble and therefore able to be excreted through the kidneys. In fact three family members CYP2D6, CYP2C9 and CYP3A4 are responsible for the metabolism of approximately 75% of all prescribed drugs.[60] The family extends to 57 genes, which altogether encode more than 1000 different proteins. The structures of these proteins are similar, but the endogenous substrates of most are presently unknown.[61] Many potential drug candidates have been shown to have a higher affinity for the CYP450 family enzymes than for the target protein, making it essential that this is evaluated early on.

A number of *in vitro* assays have been developed for pre-animal screening of drugs. These range from computational methods, to protein arrays, to cell-based systems.

### 3.5.1 Protein chips

Adaptive screening use the Surrogate Proteome™ on a chip to evaluate potential drug candidates with a number of specific and non-specific cellular proteins and compare these interactions with those of other successful drugs and compounds that are known to have adverse effects. They also manufacture the CypChip™ which acts as a "molecular sensor" to evaluate the interaction of drugs with CYP450 and plasma proteins. Data from these platforms is evaluated using the company's Adaptive Software Environment (ASE™) to determine which compounds will be taken further.

### 3.5.2 Cell- and tissue-based assays

Although protein chips allow the interaction of the candidate drug with a number of proteins to be investigated, they cannot represent the complexity of real-life. Proteins are present in cells at massively different concentrations, they are restricted to sub-cellular compartments (e.g. the nucleus, cytoplasm and cell membrane) and some form complexes with others to exert a biological effect, any of which can have a direct bearing on the efficacy of a drug. To address this a number of companies have developed cell- and tissue-based systems which allow investigators to assay a candidate drug against the total cellular protein complement.

Cell-based systems are employed by a number of companies. Genesis workstations manufactured by TECAN allow the automation of many of drug validation tests including *in vitro* drug metabolism, cell permeability, toxicity and mutagenicity. Some of these tests can be combined on a single platform thus facilitating and accelerating the validation process (Figure 3.7).

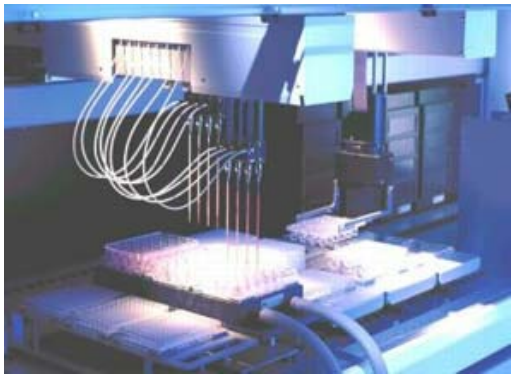

**Figure 3.7.** Genesis cell permeability workstation. http://www.tecan.com/index/com-pr-ap_so-entry/com-pr-ap-entry-drugdiscovery/com-pr-app-dd-adme_tox-entry/com-pr-ap-dd-adme-cell_perm.htm

Cellomics have a high-content screening platform that combines fluorescent tagging of subcellular structures and different cell populations with an optical system that can detect morphological changes in cells in real-time. This allows the effects of a drug on the cell to be monitored and any adverse reactions to be detected early on.

In addition to manufacturing protein chips, Adaptive Screening manufacture the CytoFlux™ cell assay chip. This utilises integrated on-chip optics, electrical-field stimulation microelectrodes and electrical microsensors to evaluate the effects of drug compounds on cells.

Advances in polymer chemistry and membranes have allowed the generation of synthetic 3D "tissues" which can be used for the evaluation of candidate drugs. These novel materials are discussed in greater depth in chapter 6, but in essence they support the growth of physiologically normal cells into appropriate structures. For example the MatTek Corporation has synthesised *in vitro* models that mimic respiratory epithelium and skin, allowing researchers to investigate the effects of drugs in physiologically relevant systems.[62]

An alternative to synthetic systems is to remove tissue from a patient to evaluate the efficacy of a drug. This is known as *ex vivo* and can be as straightforward as removing leukaemic white blood cells for investigating the effect of cytotoxic drugs on these cells or can involve transplanting human tissue into an animal model (which allows maintenance of tissue viability) as has been used for the investigation of cervical cancer.[63]

### 3.5.3 *in vivo* testing

Ultimately all drugs must be tested in an animal model before they can enter clinical trials. Many human diseases have counterparts in the animal kingdom and this, to a large extent, dictates the model to be used.

Zebrafish (Figure 3.8) have recently come into vogue as a model for many human diseases due to their relatively short reproductive cycle and ease of maintenance.

**Figure 3.8**. Zebrafish. (http://www.zygogen.com/)

Zygogen use zebrafish as a model for the investigation of cardiovascular and neurodegenerative disease, and are developing a high-content screening system based on zebrafish embryos (which are transparent and can be sustained for several days in microtitre plates).

## 3.6 Personalised drugs/Pharmacogenomics

Adverse reactions to prescribed drugs cause at least 100000 deaths per year in the US alone.[60] Small differences in genes between individuals can radically affect their responses to drugs in terms of efficacy, tolerance and metabolism. Pharmacogenomics is the study of how an individual's genetic inheritance affects the body's response to drugs. If this is understood then instead of following traditional routes of dosing based on body weight or mass, patients will be treated according to their ability to absorb, respond to and metabolise the drug (which is based primarily on their genetic makeup, but also on age, diet, general health, and other drugs taken).

Many genetic characteristics are determined by single changes of bases in DNA. As discussed in Chapter 2 these single nucleotide polymorphisms (SNPs) can be used to determine different susceptibilities to drugs between individuals, and may be of special use in diagnosis when a disease is affected by multiple genes.

The importance of SNPs can be realised by looking at the CYP450 family. Polymorphisms in this family have been linked to failure to metabolise prodrugs to the active form (e.g. codeine is oxidised to morphine by CYP2D6) or metabolise active forms to products which can then be excreted by the body. These variations can occur between individuals in a population (e.g. approximately 1% of people have multiple copies of the 2D6 gene which allows them to metabolise drugs recognised by the enzyme much faster and can lead to 100-fold differences in the blood concentration of a drug between individuals) or between different ethnic groups (e.g. the incidence of poor metabolism of CYP2C19 substrates is much higher in some asian populations (15% to nearly 100%) than in caucasians (3 to 6%)). By correlating the activity of individual members with SNPs it will be possible to use DNA arrays for screening individuals before starting a drug regime.

## 3.7 Impact of nanotechnology on drug discovery

Nanotechnology is already impacting on drug discovery. This can be seen in nanolithography techniques which are feeding into computing and array plate technologies. Faster and more powerful computers will allow molecular models of both target proteins and candidate drugs to be assessed more rapidly and to much higher levels of accuracy, while nanotechnological advances in array technologies will aid in the identification and validation of both targets and drugs, improve *in* vitro models and accelerate SNP screening.

### 3.7.1 Impact of nanotechnology on arrays

Nanotype have developed a system called C-FIT™ (congruent force intermolecular test), which they use to measure the interaction between biomolecules on separate chips (Figure 3.9). This uses two arrays: a capture array, which has "binder" molecules covalently attached to its surface (and to which the sample is added); the second (reference) array has complementary spots of "labelled force sensor complexes" or "binder" molecules that have been fluorescently tagged and are linked to the array surface by single molecules. It is these molecular bridges that are the key to the system: separation of the two arrays causes the molecular bridges to break only if binders on the reference array interact with sample molecules on the capture array. The "labelled force sensor complex" remains bound to the sample molecule on the capture array and can easily be detected. Binder molecules can be DNA or antibodies, but in the future will include RNA and other proteins.

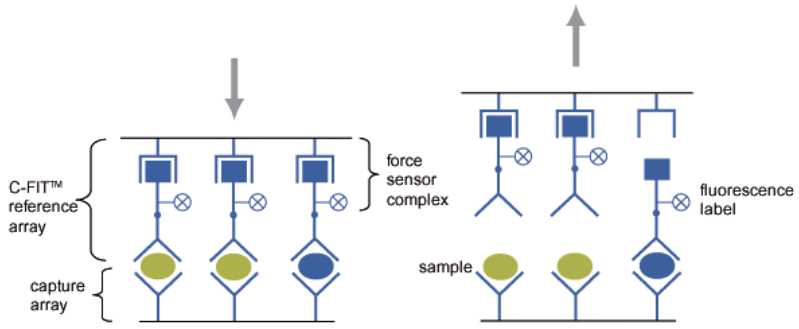


**Figure 3.9**. Mechanism of C-FIT™. ( http://www.nanotype.de/)

Surface plasmon resonance (SPR) is an electron charge density wave phenomenon that occurs at the surface of a metallic film when light is reflected under specific conditions. SPR is altered by the binding of molecules to the opposite side of the film from the reflected light. This allows the detection of molecular interactions without the use of labels. This technology is used by Biacore, in a number of platforms, to determine the binding and dissociation kinetics, and therefore the affinity, of a candidate drug with target.

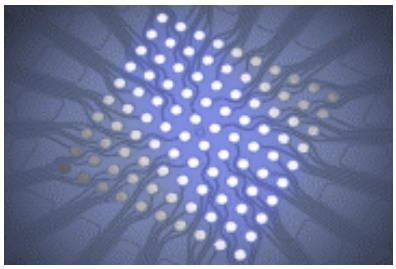

**Figure 3.10**. NanoChip®. http://www.nanogen.com/technology/core_technology.htm

Nanogen Recognomics have developed the NanoChip® with 100 test sites (each of which can be individually controlled) to test for genetic mutations. Test sites are covered by a permeable membrane and the sample is loaded above. This system accelerates molecular interactions 1000 times compared with other array platforms and has great potential for SNP screening (Figure 3.10).

Nanotechnology has allowed arrays to move into the third dimension- solution arrays. This technology uses beads that have distinct dyes attached (e.g. quantum dots) or nanoparticles that have been "barcoded" by serial electroporation with different metal ions (e.g. gold, silver, platinum- see Figure 3.11). Both methods produce populations of individually identifiable particles that can be linked to different reporter molecules allowing screening of target molecules in solution.[64].

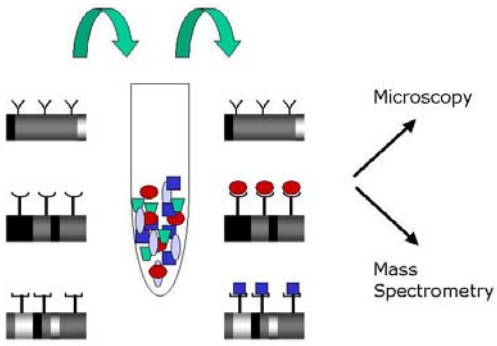


**Figure 3.11**. Barcoded nanoparticles for solution arrays.

### 3.7.2 Impact of nanotechnology on models and pharmacogenomics

Biosensors may someday replace some of the drug validation models. A nanosensor developed by Dr Bruce Cornell is sensitive to picomolar concentrations of proteins. This artificial sensor uses a bacterial protein (gramicidin) to form ion channels in a synthetic lipid membrane. The ion channel consists of one gramicidin molecule in the outer lipid layer (which is linked to a specific receptor, e.g. antibody or oligonucleotide) and a second gramicidin molecule in the inner layer (linked to a gold electrode). When the receptor molecule binds a target the ion channel opens, membrane conductivity drops and this is detected as an electrical signal.[65]

Nanobiosensors that use piezoelectric surface acoustic wave and cantilever techniques to screen DNA, proteins and viruses are being developed by the Nanotechnology Institute (NTI) in Pennsylvania (USA).

Nanosphere use nanoparticles of gold that are decorated with oligonucleotides (Figure 3.12). These are used as probes for the detection of e.g. SNPs. The company have developed different platforms for different assays and these include silver deposition onto bound gold particle (to amplify the detection signal) and a colourimetric test (Spot Test) where the nanoparticle is coloured red when in solution, but changes to blue when it binds complementary DNA.

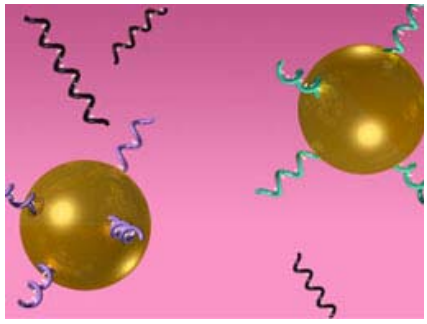

**Figure 3.12**. Nanosphere's gold nanoparticles with oligonucleotides attached. (http://www.nanosphere-inc.com/2_tech/1_nanoprobes.html)

## 3.8 Research that will impact future development

The buzzword for biotechnology at the moment is BioMEMS- the production of array chips that utilise microfluidics to assay samples and targets for specific interactions. In the future it will be BioNEMS (bio-nanomechanical systems). Techniques that are being developed such as near-field scanning optical microscopy (NSOM) and chemical-force microscopy (CFM), and the fusion of nanobiosensors and nanofluidics, could allow the exploration of candidate drug and target interactions at the single molecule level.

Nanotechnology is being incorporated into mass spectrometry techniques- making array chips that can feed directly into mass spectrometry analysis, using nanocapillaries to concentrate low abundance proteins before analysis and using nanoelectrospray to allow high-throughput screening of drug-target interactions.

As discussed in chapter 6, nanotechnology is having a major influence in tissue engineering. At present many *in vitro* cell systems for physiological organs are prone to de-differentiate (i.e. lose the unique cellular characteristics of the organ) when cultured more than a few days. By using nanotechnologies to engineer more biocompatible materials (e.g. synthetic extracellular matrices that more closely resemble those found in the organ being modelled) it is believed that this de-differentiation can be avoided allowing more accurate responses between drugs and target tissues to be monitored. In the future this may allow synthetic organs to be assembled, perhaps with their own blood supply, allowing researchers to investigate the effects of candidate drugs at the organ level and therefore decreasing (or perhaps one day negating) the need for animal models.

## 3.9 Conclusions

The advances in genomics, proteomics and bioinformatics are allowing an unprecedented push in drug discovery. At all stages of the process from identifying the protein "culprit" in a disease to developing candidate drugs and then verifying their action, systems are becoming smaller and more powerful. The traditional disciplines of biology, engineering and microelectronics are fusing and moving to the nanoscale, to provide systems that can identify and characterise disease and provide solutions based on an individual's unique genotype.

## 3.10 Companies working in the field

**Aclara Biosciences** have developed *eTag* assays to enable an efficient, high throughput systems biology approach for developing new drug targets and leads. This approach seeks broad understanding about the interaction of genes and proteins within and among cells rather than simply focusing on the activity of a particular single gene or protein. In other words, the approach looks at the whole system rather than individual components.

**Adaptive screening limited**. Mission statement: "Adaptive aims to become the leading supplier of integrated bionanotechnology systems for advanced in-vitro drug profiling. The Adaptive platforms will enable users to improve the number and quality of new medicines."

**Advion** manufacture the NanoMate 100 with the ESI Chip™, which is the world's first and only fully-automated chip-based nanoelectrospray system suitable for most API mass spectrometers.

**Affymetrix** manufactures GeneChip® arrays, instruments and analysis software.

**Agilent Technologies** manufacture a number of systems for use in drug discovery, most notably DNA arrays and Lab-on-a-chip.

**Alantos Pharmaceuticals** is a biopharmaceutical company which applies its unique and exclusive drug discovery technology - Target Amplified Drug Candidate Evolution (TACE) - to increase the speed and efficiency of small molecule drug discovery, both for its own account and in collaboration with partners.

**Astex Technology** is the leading fragment-based drug-discovery company, designing and developing new pharmaceuticals for unmet medical needs in a range of disease areas, including cancer, inflammation and Alzheimer's disease. It is pioneering fragment-based drug discovery using high-throughput X-ray crystallography.

**Avantium** employs state-of-the-art technology in providing integrated process solutions to the pharmaceutical and biotech industry. High throughput experimentation, by improving the effectiveness of process R&D, can reduce significantly the time-to-market of new chemical entities.

**Biacore AB** uses surface plasmon resonance (SPR) for sensitive detection of molecular interactions in real time, without the use of labels. The company introduced the first commercial biosensor based on SPR in 1990, and has since maintained a leading position as a supplier of the technology for investigating interactions between biomolecules.

**Biofrontera Discovery** discovers and identifies of novel active compounds for drug discovery from natural sources (bacteria, fungi, plants, marine organisms). They achieve this by using innovative isolation techniques, state-of-the-art high-throughput screening technology, intelligent screening systems with target-oriented assays from academic and biotechnology collaborations, and an extensive basic research program driven by disease-related molecular targets.

**BIOGNOSTIK** was founded in 1991 as a technology-driven company, focussing on the development of inhibitors and diagnostics of gene expression, primarily based on synthetic nucleic acids. Their research efforts centre around the .R.A.D.A.R.® software-based development of therapeutic ANTISENSE oligonucleotides.

**Cellomics®** is automating drug discovery through a unique, cell-based product platform that combines ultra high throughput primary screening with complete systems for high content screening, including fluorescent reagents, kits, and cell lines, multiparametric assays, cell analysis instrumentation, informatics and cellular bioinformatics.

**Celltech** is a leading European biotechnology company, with a substantial long-term commitment to innovative drug discovery and development. Celltech's extensive product pipeline provides excellent prospects for sustained growth, driving its goal of becoming a global biotechnology leader.

**De Novo Pharmaceuticals** use bioinformatics, structure guided drug discovery and combinatorial chemistry to create novel drugs.

**DeveloGen AG** use proprietary phenotype-first technology and stem cell expertise to create novel drugs to satisfy unmet medical needs thereby returning quality of life to the patient.

**Evotec OAI** discovers and develops new drugs through an integrated service which covers the entire process.

**Febit** is the first company in the world to develop an all-in-one system (geniom® one), which performs oligonucleotide microarray synthesis, addition of labeled sample, hybridization, and fluorescence detection.

**Fluidigm:** mission statement "Fluidigm's proprietary MSL™ microfluidics offers a dramatic competitive advantage over current drug discovery technologies through integral pumps and valves that truly automate complex experimental processes within a miniaturized system. We seek visionaries and industry leaders who want to join us in shaping the future of scientific discovery through the creative deployment of Fluidigm's empowering microfluidic technology."

**GeneTrove** is the functional genomics division of Isis Pharmaceuticals. GeneTrove uses the power of antisense technology to rapidly determine the pharmacological impact of inhibiting the expression of gene targets.

**Gyros** miniaturises and integrates laboratory applications to identify more proteins with less sample through mass spectrometry.

**Incyte** is a drug discovery company applying its expertise in genomics, medicinal chemistry and molecular, cellular and in vivo biology to the discovery and development of novel small molecule and protein therapeutics.

**Lexicon Genetics** is a biopharmaceutical company focused on the discovery of breakthrough treatments for human disease. They use proprietary gene knockout technology to systematically discover the physiological functions of genes in mice and to identify which corresponding human genes encode potential targets for therapeutic intervention, or drug targets.

**MatTek Corporation** is a world leader in the production of bio-engineered human tissue constructs. Their standardized production process, in place for over 10 years, allows MatTek to create the most reproducible *in vitro* tissue models available today.

**Momenta Pharmaceuticals, Inc**. applies breakthroughs in the understanding of complex sugars (polysaccharides) to the discovery, development, and commercialization of drug products. In its short history, Momenta has built a diversified pipeline of novel development candidates and near-term marketed products based on improvements to existing blockbuster drugs by leveraging its innovative product engine and unique understanding of the basic biology and chemistry of sugars.

**Nanogen** mission statement: to become the leading provider of molecular diagnostic products. The NanoChip® Molecular Biology Workstation, utilizing their unique electronic microarray technology, creates a platform that offers certainty, control and consolidation in genetic testing and molecular diagnostics.

**Nanosphere** combines nanotechnology-based nanoparticle probes, assays and instruments to offer superior DNA, RNA and protein detection on one platform. Its systems advance clinical diagnostics and target verification with orders-of-magnitude greater sensitivity and selectivity over standard technology as well as improved efficiency and cost.

**Nanotype** have developed the C-FIT™ (Congruent Force Intermolecular Test) technology which has applications in drug development and diagnostics.

**Oxford Gene Technology (OGT)** was founded by Prof Edwin Southern and have been instrumental in the development of DNA array technologies.

**Oxford GlycoSciences** Plc, is a biopharmaceutical company applying proteomics technologies and glycobiology to the discovery, development and commercialisation of novel therapeutic products.

**Peakdale** is the fastest growing contract chemistry company in the UK and is dedicated to supporting the research and development activities of pharmaceutical and biotechnology companies worldwide.

**Pharmacopeia** applies its proprietary ECLiPS combinatorial chemistry technology, computational modeling, discovery biology, medicinal chemistry, ultra high-throughput screening and applied engineering to identify, enhance, and optimize potential drugs.

**Quantum Dot Corporation** manufacture quantum dots for fluorescent tagging.

**Sequitur** are developing proprietary antisense and RNAi technologies as therapeutic compounds that specifically inhibit gene expression and are employed for gene function and target validation research.

**SomaLogic** manufacture photoaptamers which can be assembled into arrays so that large numbers of proteins—eventually, thousands—can be measured simultaneously.

**Syagen** manufacture mass spectrometers. Their Radiance Pro® high-throughput photoionization mass spectrometer (PI MS) technology provides crucial capabilities for meeting the demands of high-throughput drug discovery. The detection system achieves near-universal detection of many classes of pharmaceutical drug compounds and offers the best combination of both accurate and high-speed analysis compared to existing methods of high-throughput characterization.

**Tanabe Seiyaku** is an R&D-oriented pharmaceutical company, whose strategy is to continually discover new drug candidates that will improve medical care, develop them as efficiently and rapidly as possible, and market them around the world. In new drug development, they are making progress with disease-oriented drug discovery, following an original approach in which they advance only those candidates for which medical needs are high to the discovery research stage.

**Tecan** is a leading player in the fast growing Life Sciences supply industry that specializes in the development, production and distribution of enabling solutions for the discovery of pharmaceutical substances, as well as for genomics, proteomics, and diagnostics.

**Xencor** has developed "Protein Design Automation®" (PDA®) technology re-engineer proteins and create *de novo* mechanistic designs. They have used PDA® to develop inhibitors of Tumor Necrosis Factor (TNF), a clinically validated target in arthritis, psoriasis, and other autoimmune disorders.

**Xerion** have an integrated approach to the discovery and evaluation of drug targets and the development of therapeutic antibodies.

**Zygogen** uses zebrafish and fluorescent tagging technology for preclinical drug discovery, including target identification, validation and drug screening.

**Links to companies working in the field:**

| **Company** | **Link** |
|---|---|
| Aclara Biosciences | http://www.aclara.com/etag_products_services.asp |
| Adaptive Screening | http://www.adaptive-screening.com/ |
| Advion | http://www.advion.com/index.php |
| Affymetrix | http://www.affymetrix.com/index.affx |
| Agilent Technologies | http://we.home.agilent.com/ |
| Alantos Pharmaceuticals | http://www.alantos.com/ |
| Astex Technology | http://www.astex-technology.com/home.jsp |
| Avantium | http://www.avantium.com/newsite/life_sciences/default.htm |
| Biacore AB | http://www.biacore.com/home.lasso |
| Biofrontera Discovery | http://www.biofrontera.com/discovery/html/intro/home.htm |
| BIOGNOSTIK | http://www.biognostik.com/ |
| Cellomics® | http://www.cellomics.com/ |

| Company | Link |
|---|---|
| Celltech | http://www.celltechgroup.com/ |
| De Novo Pharmaceuticals | http://www.denovopharma.com/default.htm |
| DeveloGen AG | http://www.develogen.com/index.php |
| Evotec OAI | http://www.evotecoai.com/ |
| Febit | http://www.febit.com/ |
| Fluidigm | http://www.fluidigm.com/ |
| GeneTrove | http://www.genetrove.com/ |
| Gyros | http://www.gyros.com/ |
| Incyte | http://www.incyte.com/control/home |
| Lexicon Genetics | http://www.lexicon-genetics.com/index.php |
| MatTek Corporation | http://www.mattek.com/ |
| Momenta Pharmaceuticals | http://www.momentapharma.com/about.asp |
| Nanogen | http://www.nanogen.com/index.htm |
| Nanosphere | http://www.nanosphere-inc.com/1_about/index.html |
| Nanotype | http://www.nanotype.de/ |
| Oxford GlycoSciences | http://www.ogs.com/ |
| Oxford Gene Technology (OGT) | http://www.ogt.co.uk/index.html |
| Peakdale | http://www.peakdale.co.uk/ |
| Pharmacopeia | http://www.pharmacopeia.com/dd/leads/index.html |
| Quantum Dot Corporation | http://www.qdots.com/new/index.html |
| Sequitur | http://www.sequiturinc.com/home.html |
| SomaLogic | http://www.somalogic.com/index.html |

| Company | Link |
|---|---|
| Syagen | http://www.syagen.com/ |
| Tanabe Seiyaku | http://www.tanabe.co.jp/english/index.html |
| Tecan | http://www.tecan.com/ |
| Xencor | http://www.xencor.com/index.html |
| Xerion | http://www.xerion-pharma.com/ |
| Zygogen | http://www.zygogen.com/ |

## 3.10 Overview of European projects, literature and web-sites

### 3.10.1 European Funded Projects

**BIOCENSAR**. The proposal is focused on several cytochrome P450 systems, which are currently under study by participating teams. Within the network activity teams will perform focused coordinated research, aimed at improvement of methods of expression and purification of recombinant P450s and partner proteins, their structure-functional and enzymatic characterization and 3D structure determination at the atomic level. This assumes regular exchange of information and research achievements (subject to due consideration of Intellectual property rights), collaborative research considering investigation of protein-protein interactions in P450 systems and their ultrastructural studies using the high-resolution techniques (X-ray crystallography, including synchrotron facilities, and scanning probe microscopy). It is anticipated that the proposed research would also lead to identification of new potential targets among cytochrome P450 systems for their practical applications in biosensor design and bioelectronics. The network activity is thought to be achieved also through exchange visits of team members to partner laboratories, performing joint experiments, organization of workshops, conferences. Essential part is devoted to focus interdisciplinary training and education of young scientists in the modern fields of cytochrome P450 bioinfomatics, biotechnology, biosensor technology and nanotechnology. It is anticipated, that the network will promote and extend the already existing scientific links and lead to new collaborative research projects.

Contact: NICOLINI, Claudio (Professor)
Tel: +39-065-7992570
Fax: +39-065-7992575
Email: victor@pst-elba.it

**MOLECULAR SIGNALING**. Signalling cascades play multiple roles in cell growth, differentiation and death. Their perturbation has profound consequences linked to diseases such as cancer, myopathy and diabetes, major health concerns in Europe. We propose to develop and implement nano-technologies, which allow monitoring and examination of intracellular signalling processes in real time at the single cell level. Our goals are to create detailed molecular maps of the temporal and spatial organization of proteins involved in signal transduction, in particular insulin signalling. With this information we can compare diseased and normal tissue to identify specific errors in signalling cascades. Moreover, we can directly develop non-animal based assays for use in drug discovery. Once established this nanotechnology can be extended to the analysis of diverse signalling cassettes in a variety of other diseases of high social and economic impact in Europe.

Contact: RETOURNA, Michel (Dr)
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
1919 Rue de la Cardonille 141
34396 MONTPELLIER
FRANCE

**CHROMATIN AND CANCER**. Acetylation/deacetylation by histone acetylases (HAT) and deacetylases (HDAC)regulates gene transcription and specific functions, such as tumour suppression byp53. Abnormalities of HAT or HDAC are frequently found in tumours. Targeting of HDAC by small compounds is effective in the differentiation treatment of a small subset of leukemias. We aim to determine whether HDACs are suitable general targets for cancer drug discovery. We will characterize the mechanisms of activities of HDAC/HAT in normal and cancer cells, and screen tumours with a panel of HDAC inhibitors. Our experimental approaches range from biochemistry, tomolecular biology, to postgenomics (proteomics and nanotechnology), and include the functional validation of compounds and demonstrated mechanisms in animal modelsystems and tumour samples. Final objective is the design of differentiation treatment protocols for leukemias and myelodysplastic syndromes.

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ISTITUTO EUROPEO DI ONCOLOGIA SRL
Via Ripamonti 435
20141 MILANO
ITALY

### 3.10.2 National Funded Programmes

**Germany**:DE-BIOTECH. Nano-biotechnology; techniques for manipulation for biological nanometre-scale systems; using of nano-scale particles as means of transportation for pharmaceutical products; using of nano-scale particles for combat cancer; new basic approach for advanced array technologies

**Contact:** BUJOK, Oliver
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**Tel:** +49-211-6214476
**Fax:** +49-211-6214484
Email: bujok@vdi.de

**Germany**: BMBF. 50 million euros to be made available until 2006 for nanobiotechnology.

**UK**: BBSRC. Research theme for nanobiotechnology. Funding available until end 2006.

**UK**: June 2003 the UK government allocated £4 million over 3 years for pharmacogenetic research into existing drugs.

# Chapter 4 Drug Delivery

Jean-Charles Guibert, Sandrine Locatelli and Carole Nicollet, CEA LETI.

## 4.1 Introduction

Drug delivery describes a process whereby a therapeutic agent is administered to the body in a controlled manner. It includes Systems administering drugs through controlled delivery so that an optimum amount reaches the target site. These processes rely on new ways and new molecules to treat disease.

As scientists research drugs and pharmaceutical giants develop pipelines, methods of administering therapeutic drugs will change and adapt to the new environment.

## 4.2 Drug Delivery Market

According to IMS World Review, in 2002 the global pharmaceutical market was $400.6 billion and is projected to grow by 5% annually. The evolution of the global pharmaceutical market is shown in Figure 4.1, and its 2002 distribution per segment is shown in Figure 4.2:

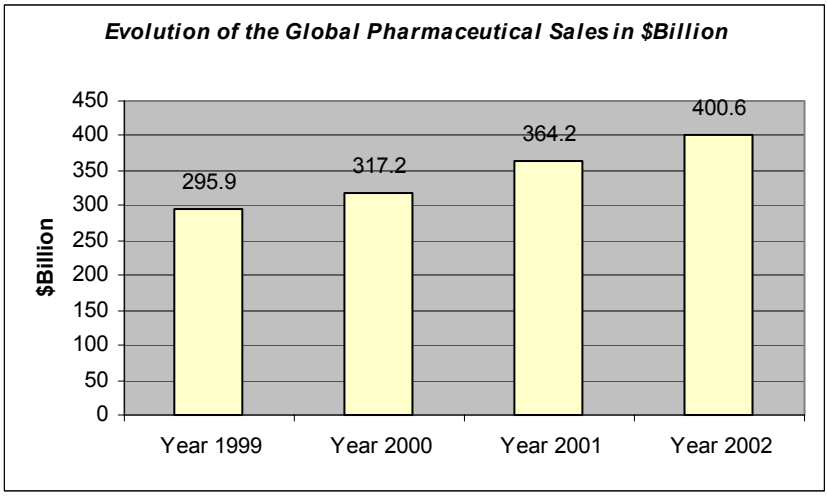



**Figure 4.1** Source : IMS World Review.

In 2002, the drug delivery market represented about 13.5% of the global pharmaceutical sales, i.e. $53.8B and by 2007 it will account for 39%[66].

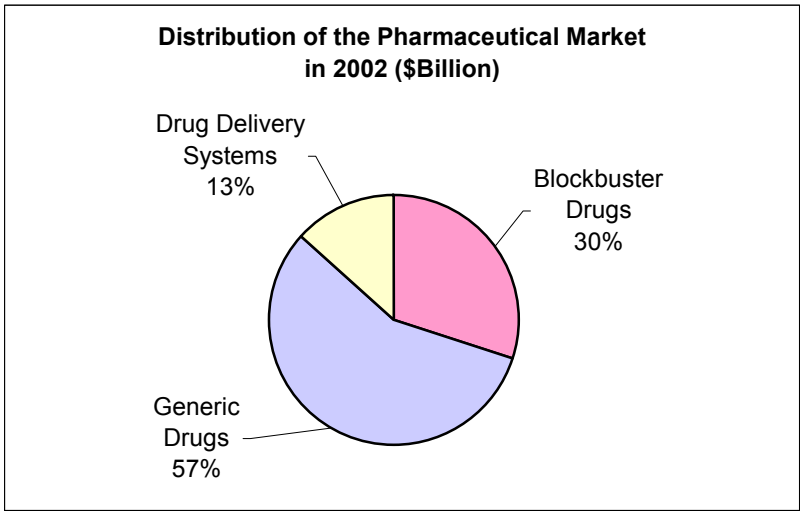



**Figure 4.2.** Source : IMS World Review.

Growth in the drug delivery market will continue at an average annual rate of 11% (Figure 4.3).

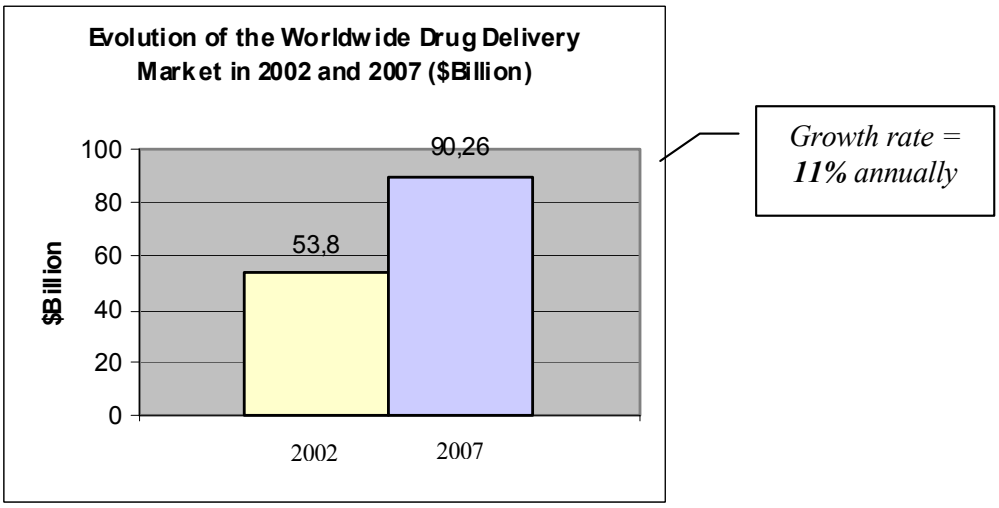



**Figure 4.3**. Source : : http://www.the-infoshop.com

The fast growth of this industrial sector can be attributed to the following major developments:

There are at present and will be in the near future a large number of drugs coming off patent. Many of these drugs are not soluble in water or have limited efficacy in their traditional delivery system, but when delivered through the appropriate system can have an increased therapeutic value. Many companies are attempting to combine novel new drug delivery technologies with existing drugs in order to extend their patent life.

The biotechnology sector of the pharmaceutical industry is continuing to make advances in breakthrough therapies for diseases such as viral infections, cancer, and hormonal disorders. But, these therapies are difficult to deliver due to the size of the molecules or are too unstable and require delivery through intravenous infusion or frequent injections. Many new drugs never make it to market because selected methods for drug delivery are ineffective. Such agents would benefit from the numerous new advanced drug delivery technologies that are beginning to crack the market.

The drug delivery sector which was simply a part of the pharmaceutical production process has become a driving force for innovation and profits. Due to this strong need for more effective delivery technologies, there is a growing competition in this area amongst companies of all sizes for the most lucrative licensing agreements with the major players of the pharmaceutical industry. Success is not easy to come by, as new technologies must offer clear biomedical advantages, do so at a reasonable cost, and provide a proprietary position in order to recoup development costs. As a result, developers of drug delivery products and technology have seen significant attention from customers and investors alike. The value of enhanced drug delivery is real in improving treatment outcomes, differentiating between products, optimizing the patented revenue potential of a drug and maximizing the Return On Investment (ROI) in new chemical entities (NCEs).

## 4.3 Main drug delivery technologies

Today, major drug delivery technologies are the following:

Oral
Pulmonary/Inhaled
Transmucosal
Implantable/Injectable Polymer systems
Transdermal
Others

The evolution of the worldwide drug delivery market between 2000 and 2005 in Billion $, per technology is shown in Figure 4.4.

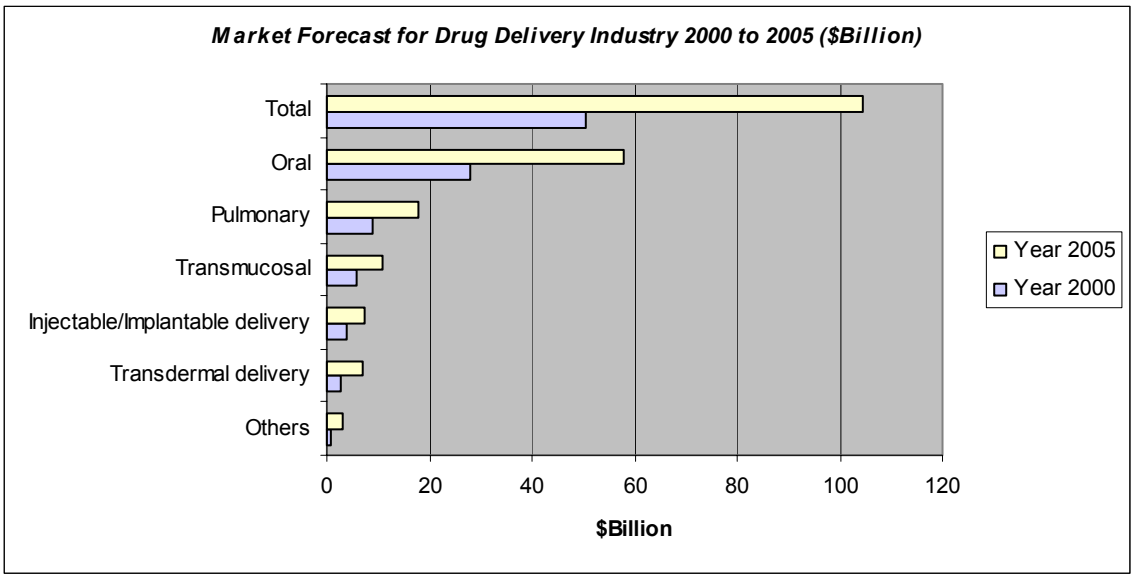



**Figure 4.4**. Source : Drug Delivery Technology

**Oral**: Oral delivery is the most valued method for the introduction of therapeutic agents. It is regarded as the safest, most convenient and most economical method of drug administration. It includes micro encapsulation, coatings, and polymer/ membrane technologies. In 2002, oral delivery technologies accounted for 40% of the world market. This represented $21.6B with 60% for the US, 25% for Europe and 10% for Japan.[1]

The growth rate for oral drug delivery systems is expected to increase by 9% annually by 2007.

**Pulmonary/inhaled** (through the lungs): Pulmonary drug delivery is also an effective and popular technology that is still growing. Pulmonary delivery systems, absorption and drug deposition through alveoli in the lungs enable to readily absorb fragile proteins and to deliver drugs directly to the heart of the system for a global distribution. Pulmonary delivery technologies can be broadly classified as:

- Metered Dose Inhalers (MDI) – these are buccal such as asthma inhalers and medicate the airways but not deep into the lungs. The particle size used varies considerably. Asthma therapies account for a large percentage of the total pulmonary market.

- Nebulisers - nasal (through the nose): fine liquid spray mainly used in hospitals. The nasal route is particularly attractive for compounds that cannot be taken orally and would normally have to be injected. It also avoids the problem of degradation by the liver as absorption into the circulatory system takes place across the highly vascularised soft tissue that lines the nose.

- Dry Powder Inhalers ('DPI'): these use solid form drugs that are unable to replicate the effect of an aerosol. Dry powder inhalers are being utilized for a growing number of indications.

Front Line calculated the pulmonary drug delivery market to be approximately $8.4B in 2001 and $17B in 2002, i.e. 32% of the global drug delivery market.[1] The growth rate for pulmonary drug delivery systems is expected to increase by 9% annually by 2007. For the year 2005, the-infoshop.com estimates the rate for pulmonary drug delivery at $22.6B. By 2013 the global pulmonary delivery market is expected to reach $40B, reflecting consumer expectations of simple drug delivery mechanisms to replace injections and pills.[1]

**Transmucosal**: More and more companies are developing transmucosal technologies for the delivery of both large- and small-molecule drugs. These methods of drug delivery are gaining favour due to their possible application in meeting the needs of biotech drugs such as monoclonal antibodies. Transmucosal delivery is particularly attractive because these membranes are thin and permeable allowing for the rapid uptake of a drug into the body. Transmucosal delivery systems offer several benefits over other methods of delivery that include direct absorption, rapid onset, and lower dosage. The delivery systems are also relatively simple and are, therefore, inexpensive to produce. In 2002, the worldwide market for transmucosal technologies reached $3B i.e. less than 6% of the global drug delivery market, with 55% for the US, 30% for Europe and 10% for Japan[1] The growth rate for transmucosal drug delivery systems is expected to increase 11% annually through 2007.

**Implantable/Injectable polymer systems**: Polymer-based drug delivery systems have an enormous impact on drug therapies. The drug is physically entrapped inside a solid polymer that can then be injected or implemented in the body. Polymer-based drug delivery systems allow the slow release or extended circulation of a therapeutic over a time span ranging from days to months. The systems are often implanted or injected, thus, therapy is initiated during office visits or through surgical procedures. Worldwide market revenues for polymer drug delivery systems were $4.2B in 2002 i.e. about 8% of the global drug delivery market, with the US at 55%, Europe at 35% and Japan at 5%.[1] The growth rate for polymer drug delivery systems is expected to increase 14% annually over the next five years.

**Transdermal** (through the skin): Transdermal systems deliver drugs through the skin into the bloodstream, making them easy to administer. Passive and active transdermal delivery systems are used to deliver medicines in even concentrations in a way that is painless and results in few adverse side effects. Worldwide market revenues for transdermal drug delivery systems reached $3B in 2002 i.e. less than 6% of the global drug delivery market, with the U.S. at 56%, Europe at 32% and Japan at 7%.[1] The growth rate for transdermal drug delivery systems is expected to increase 12% annually through 2007.

Other drug delivery systems include:

- Liposome delivery systems, vesicles composed of a phospholipid bilayer in which pharmaceutical agents can be contained, allowing controlled release, target specificity and prolonged life span of drugs.

- Needle-free injection systems which utilize pressurized gas or a coil spring for needle-free injection that shoots pharmaceutical agents through the skin for administration with reduced pain, less risk of cross contamination and capability for mass immunization.

The growth rate for other drug delivery systems is expected to increase by 20% annually by 2007.[1]

## 4.4 Main issues of Drug Delivery

Almost all current medications are delivered to the body as a whole, which is fine as long as they only become active in the areas you want them to, but this is not usually the case. The side-effects are enormous, especially when the treatment is designed to kill cells, as in the case of cancer; so evading the body's immune system while directing a therapeutic agent to the desired site is a very important factor. Moreover, the delivery of pharmaceutical agents that are water-insoluble to targets within the human body has always been a challenge. Many potentially valuable drugs that look promising are, unfortunately, not very soluble in water and their clinical uses are greatly restricted because they are unable to get into the bloodstream. Thus, pills and injections serve us well, but for a patient with a chronic illness like diabetes or heart disease, they are far from perfect. Dosages do not always match the body's fluctuating needs, and it is easy to forget to take a pill.

But what if doctors could implant a small capsule under your skin that could detect, say, changes in blood sugar levels or some heart disease-related molecule and then release the exact amount of medication needed to keep your illness in check? Drug delivery vehicles that improve drug solubility could dramatically advance medicine, particularly in detecting and treating infectious diseases. That is the idea behind a new generation of "smart" drug-delivery devices being worked on by a number of research labs. Emerging technologies in drug delivery are closely examined and insights provided into how these technologies could potentially transform drug delivery practice in the future. The key to commercial success in drug delivery will lie in the ability of companies to capitalize on an unmet need. Products utilizing unique drug delivery systems will present companies with a competitive edge and this competitive advantage will readily translate into strong sales.

## 4.5 Impact of nanotechnology on drug delivery

Nanotechnology is already having a notable impact on medicines, health and safety products. The technology enables companies to produce better, faster, cheaper and more powerful applications while offering great potential for generating revolutionary new applications. Nanotechnologies dedicated to biology are called “nanobiotechnologies”.

Nanobiotechnology refers to the ability to create and manipulate biological and biochemical materials, devices, and systems at atomic and molecular levels (billionth of a meter). In nanobiotechnology, big things are expected from really small things. The field, in fact, seems poised for a major expansion as the flow of federal research grants and other funding picks up pace. Among the most promising of these are medical devices, bio-analysis and drug delivery.

A key area of interest is accurately targeted drug delivery. Nanotechnology offers the promise of delivery of the right drug in the right place at the right time. Benefits include lower doses and lower side effects, particularly desirable for cancer drugs. But how is nanotechnology R&D being used for new drug delivery applications? Today, mechanical, electrical and biological devices built on a nanometer scale are ideal for medical applications because they can interact directly with the body at the cellular and molecular levels. There is a large amount of hyperbole in the nano-sized world, yet there are plenty of real opportunities available today in particular in nanobiotechnology.

Regarding drug delivery, every year, pharmaceutical companies give up on promising but poorly soluble pharmaceuticals, because they have low bioavailability in the bloodstream, and existing solubilization technologies are not adequate. To solve this problem, drug companies are developing new delivery systems, by using new smart drugs. In theory, these smart drugs would be able to travel through the body, "diagnosing" problems, and delivering "nano" doses of therapeutic medicine to diseased cells.

Drug delivery is an area already showing significant impact from nanotechnology, with some approaches using nanoparticles or nanocapsules to deliver drugs through the skin, lungs, stomach and eyes already in clinical trials and many more in pre-clinical trials. These approaches offer numerous advantages such as increased solubility, resistance to gastric enzymes (offering oral delivery of drugs previously needing intravenous delivery), controlled release or the ability to direct the drug though various means to the very place where it is needed.

The following sections describe different devices for drug delivery under development using nanotechnologies.

### 4.5.1 Micro and Nanosystems

#### 4.5.1.1 Micro and nano pumps and valves

These are components of micro and nano electro mechanical systems described below. The main issue with manipulating nanolitre amounts of liquid is the clogging of conduits and valves. Pumps such as the electromechanical displacement micropump have been used successfully to control analgesia in patients and can dispense volumes as small as 100 nl with an accuracy of ± 5 nl. [67] Such pumps have been designed and used successfully for drug delivery.[6869] Other groups are researching methods of moving nanoscale volumes of liquid without requiring moving parts. A group at Arizona State University have produced tiny capillaries made of light responsive molecules which, when exposed to light, attract and transport water molecules through a central channel. [70]

#### 4.5.1.2 MEMS/NEMS devices

MEMS and NEMS refer to a technology used to integrate various electro-mechanical functions onto integrated circuits. A typical MEMS device combines a sensor and a control logic to perform a monitoring function. Examples include sensing devices used to control the deployment of airbags in cars and switching devices used in optical telecommunications cables. These devices can also be employed for drug delivery (Figure 4.5).

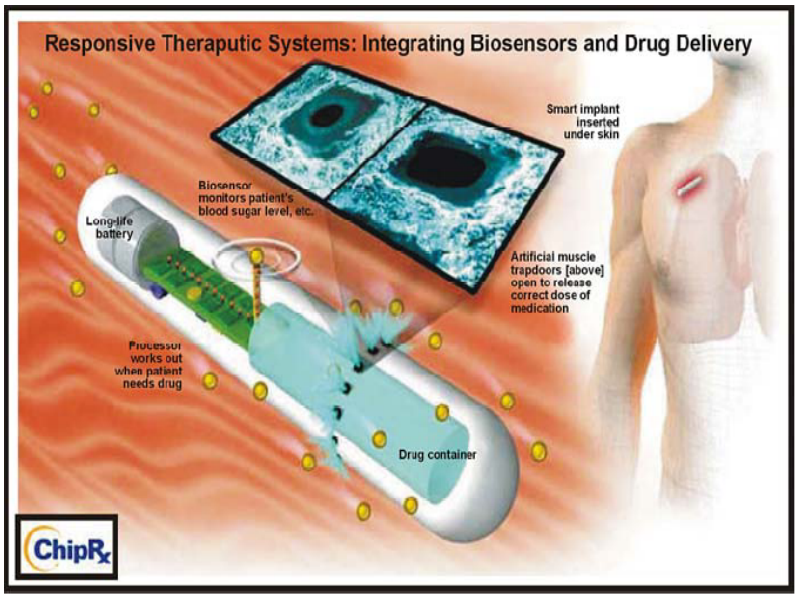



**Figure 4.5**. Source: Professor Sylvia Daunerts – Nanotechnology: a technology forecast, April 2003.

For example, microchip-based devices can deliver nano-litres of chemical substances on demand from several sealed reservoirs. This system could be coupled to sensors to create an implantable pharmacy capable of administering thousands of independent doses of a single drug, or specific combinations of several different drugs, in a pre-programmed or stimulus-controlled manner (Figure 4.6).

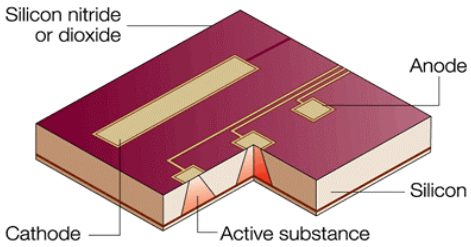



**Figure 4.6**. Source: Nature Reviews – January 2002

**Examples of achievements**

**iMEDD** is developing novel drug delivery products based on nanoscale structural features created by proprietary "top-down" micro fabrication techniques. iMEDD's lead drug delivery platform, NanoGATE, is a small subcutaneous implant that uses membranes containing pores of nanometer dimensions that control the diffusion of drugs at a molecular level.

### 4.5.1.3 Micro and Nanoneedles

Transdermal local tissue and intracellular delivery using micro or nano-machined needles opens new opportunities for painless drug delivery. For example, large molecules can be taken up transdermally with the assistance of microneedle arrays. Advances over the past few years in micro and nanofabrication technologies have allowed investigators to create minute, hollow needles that painlessly cross the uppermost layer of the skin to deliver drugs to the dermal layer. Some products under development can effectively, efficiently, and painlessly deliver drugs by bolus injection or infusion to depths of 1 mm.

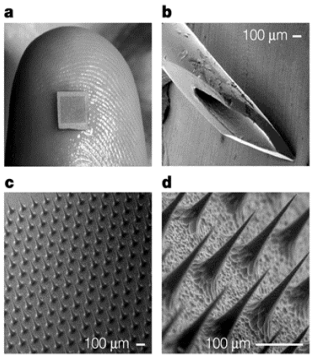



**Figure 4.7**. Nano-needles. Source: Nature Reviews – January 2002.

Nano-needles are straight, slightly bent, or kinked with sharp tips (Figure 4.7).

**Examples of achievements**
A nanobiotechnology team at the Georgia Institute of Technology have micro fabricated arrays of micro-needles that can be painlessly inserted and worn in the skin. These can deliver drugs either as an immediate injection or for slow, extended administration.

## 4.5.2 Nanostructured materials

### 4.5.2.1 Nanoparticles

Nanoparticles are made out of a wide variety of materials such as metals, metal oxide ceramics and silicates. According to the most widely-accepted definitions, at least one of their dimensions must be less than 100 nm. The nanoparticles in metal and metal oxide ceramic nanopowders tend to be roughly the same size in all three dimensions with dimensions ranging from 2-3 nm up to a few hundred, whereas silicate nanoparticles currently in use are flakes about 1 nm thick and 100 to 1000 nm across (examples are shown in Figures 4.8a and 4.8b).

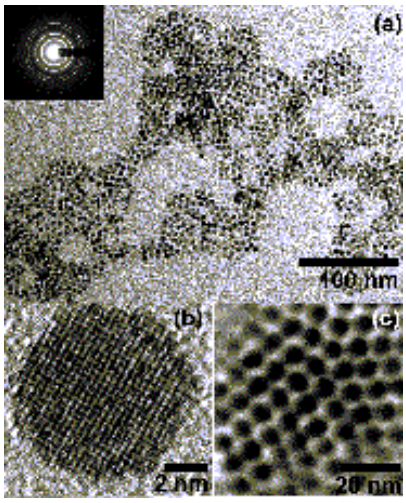



**Figure 4.8a**. Barium titanate nanoparticles. Source : Columbia University.

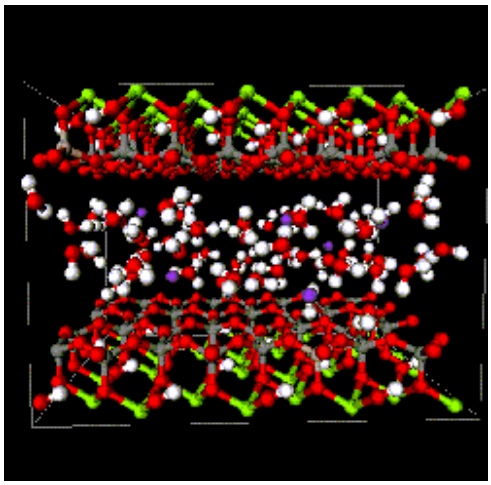


**Figure 4.8b**. Silicate nanoparticles. Source : ILL.

For drug delivery, these systems combine the advantages of high surface area, improved interfacial properties and size confinement to deliver drugs that have increased efficacy, offer more convenient dosing of regimens and have improved toxicity profiles compared to their micron-sized predecessors. With nanoparticles, it is possible that drugs may be given better solubility, leading to a better absorption. Also, drugs may be contained within a molecular carrier, either to protect them from stomach acids or to control the release of the drug to a specific targeted area, reducing the likelihood of side effects. These nanoparticles could also contain a slowly-released drug payload, be radioactive, or enhance the heating effects of a laser shone through the flesh to destroy tissue.

For example, inhalation of sub-micrometer diameter particles may offer an efficient route for the administration of therapeutic substances: these particles can be deposited deep within the lungs, whence, with a thin blood-gas barrier and copious blood supply, they can be absorbed rapidly into the body. Ultra fine particles individually contain very little mass, but if present in extremely large numbers can disperse significant amounts of material deep in the lung. Once there, their very large cumulative surface area may assist their dissolution.

It is hypothesised that engineering inhaled drug particle sized down to ultra fine particle levels will produce effective "vectors" for respiratory and other drugs, by virtue of their large numbers (and hence potential area coverage) and their penetrative and deposition properties. In order to prove that, a size-controlled ultra fine aerosol delivery system has been developed for human volunteer studies and will be used to determine the clinical efficacy of a given dose of drug administered in a variety of particle sizes. An added benefit would be a reduction in deposition to the mouth and throat, reducing some of the adverse side effects seen with current inhaled drug delivery systems. Such drugs are already beginning pre-clinical or clinical trials, adhering to the strict regulatory requirements for new pharmaceuticals.

Regarding oral drug delivery, factors that affect the efficacy of a drug include the solubility, bioavailability, biological half-life, dose and dosing regimen, and shelf-life. Technologies that can improve oral delivery of drugs by controlling the release and absorption in the gastro-intestinal tract are in great demand and improved powder processing is one strategy that can overcome these obstacles. Indeed, *in vivo* studies have shown sustained-release and improved bioavailability of pressed powders for improved tablet processing of next generation pharmaceuticals. For example, the company Nanotherapeutics has developed a system based on nanoparticles that enables oral delivery of macromolecules (peptide/protein) and improves the oral bioavailability of insoluble and poorly absorbed drugs that require injection (e.g. antibiotics, antivirals, anti-inflammatories).

Nanoparticle suspensions also offer an advanced approach in the delivery of insoluble drugs for injectable delivery. Indeed, nanoparticles for injectable delivery offer several advantages including low excipient loads/smaller dose volumes, better control of particle size and dispersion efficiency, controlled release-rates, increased efficacy and systemic bioavailability.

**Examples of achievements**

- The company **Bespak** has acquired a nanoparticle technology from the Massachusetts Institute of Technology that could lead to the development of a novel nasal delivery system.

- **Nanomat**, a manufacturer of nanopowders, produces titanium dioxide and iron nanopowders. The company develops a system to incorporate iron nanopowders in ferrofluids for fast-acting targeted drug delivery, DNA tagging and improved MRI imaging.

- **Nanotherapeutics Inc**, which applies nanotechnologies to the development of novel drug therapies, has developed two proprietary drug delivery technologies (Nanodry and Nanocoat) for oral, injectable, nasal and inhaled drug delivery. The Nanodry process is a technique that produces dry powders for efficient and reproducible delivery of large and small molecules and the Nanocoat process is a patented, solventless encapsulation system for coating micron and sub-micron size powders. For example, Nanotherapeutics is developing treatments to improve the breathing for asthmatic patients or to relief from a headache in minutes instead of hours.

### 4.5.2.2 Nanocapsules

Nanocapsules represent any nanoparticle that consists of a shell and a space in which desired substances may be placed. These nanocapsules are reservoir systems composed of a polymer membrane surrounding a liquid core (Figure 4.9).


**Figure 4.9**. Nanocapsules. Image from: http://perso.club-internet.fr/ajetudes/nano/index.html

Nanocapsules have been made for many years following the example of nature, using molecules called phospholipids, which are hydrophobic at one end and hydrophilic at the other. Inside cells, similar capsules called liposomes are used to transport materials. Liposomes are vesicles with sizes ranging from 1 nm to 20 μm, based on phospholipid bilayers which spontaneously form lipid spheres when they are hydrated, enclosing the aqueous medium and the solute (drug).

Recently, many other materials such as a variety of polymers have been used to make nanocapsules. Polymeric nanocapsules can now be made in specific sizes, shapes, and in reasonable quantities. These can then be functionalised by attaching a specific receptor to the exterior of the capsule and place the drug inside, leaving the structure unchanged. The capsule then delivers the drug to the specific destination where it is released.

Liposomes are very promising, broadly applicable, and highly researched drug delivery systems. These nanoscale devices are extremely versatile because they are easily modified, they encapsulate a large volume for carrying therapeutic drug agents and it is possible to vary their composition. For example, liposomes can prolong the duration of drug exposure acting as a slow-release reservoir or protect the patient against side effects of the encapsulated drug. Other examples are the reduction of haemolytic effects of drugs by liposome encapsulation and the protection against local irritation on intradermal, subcutaneous or intramuscular injection of a tissue-irritating drug. In some cases, liposomal therapies have been shown to accumulate at the site of a tumour or infection, delivering higher concentrations of that therapy to the disease target. Liposomes are also often attached to other molecules, like polyethylene glycol (PEG) that will prevent or lessen detection by the immune system or homing molecules to make these structures target-site-specific.

Liposomes are currently being used for intravenous delivery of small molecules or poorly water-soluble lipophilic compounds and are being investigated for oral, transdermal and sustained-release delivery of drugs. The most advanced application of liposome-based therapy is in the treatment of systemic fungal infections. Liposomes are also under investigation for treatment of neoplastic disorders. However, although many experiments describing the encapsulation of antimicrobial agents and antineoplastic agents in liposomes have been performed in research labs, there are not so many drugs developed for commercial use.

There is a large amount of research activity regarding the use of polymeric nanocapsules for drug delivery. The advantages of these polymeric carriers are the reduction of drug toxicity, the possibility to solubilize hydrophobic drugs, the improvement of biodistribution and the reduction of interactions with reticuloendothelial system, leading to an increased therapeutic efficacy. For example, poly-derivatives have been shown to entrap up to 45% by weight of a drug within particles that have extended circulation times due to decreased uptake by the mononuclear phagocyte system. Such a system could be useful for altering drug bio-distribution to deliver molecules, proteins and genes through mucosal barriers. Moreover, the functionalization of polymeric nanoparticles with specific receptors, leading to a targeted drug delivery, would allow for as much as a 10,000-fold decrease in drug dosages, reducing the harmful side effects of drugs such as those employed in chemotherapy.

These systems can play a leading role in various drug delivery means. For example, regarding transdermal drug delivery, solid lipid nanoparticles produced by exchanging a portion of the liquid oil part of an emulsion with a lipid solid at room temperature have been shown to deliver molecules deeper into the skin than traditional mixtures. Regarding injectable systems, polymeric nanoparticle suspensions offer an advanced approach in the delivery of insoluble drugs and formulations for sustained-release.

**Examples of achievements**

- **TATLYS** ("new biocompatible nanoparticle delivery system for targeted release of fibrinolytic drugs") is a current GROWTH project studying the development of a new biocompatible and bioerodable delivery system for targeted release of drugs for the dispersion of blood clots (thrombus) in the cardiovascular system.

New polymeric matrices suitable for the formulation of nanoparticles are being prepared on laboratory scale, while specific thrombus-targeting compounds are being modelled and synthesised. These are combined with appropriate drugs under experimental conditions, in order to derive effective nanocapsules systems. After optimisation of the experimental parameters, promising candidate combinations will be scaled-up to pre-industrial level. The stability and toxicity of the nanoparticles will also be investigated.

- The **University of Alberta** demonstrated that poly n-butylcyanoacrylate (PNBCA) nanocapsules can be used as vehicles for topical drug delivery in order to improve the skin permeation of anti-inflammatory and anti-tumor drugs such as indomethacin and presumably other more hydrophobic drugs.

- **Osaka University** has developed a novel nanoparticle – a hybrid of viral and nonviral vectors – that can deliver a gene or protein cargo specifically to human liver cells. The nanoparticles, a kind of stripped-down version of hepatitis B virus (HBV) particles, are hollow phospholipid vesicles, ~80 nm in average diameter, embedded with ~110 molecules of the L protein from HBV.

- **American Pharmaceutical Partners** presented in April 2002 a new nanoparticle delivery system for an established anticancer drug. This nanoparticle named ABI- 007 is 130 nm long and consists of an engineered protein-stabilized nanoparticle that contains paclitaxel, which is used to treat breast, bladder, and more than a dozen other cancers. Such new delivery systems combine a drug with an artificial vector that can enter the body and move in it like a virus. If more advanced clinical tests are successful, ABI-007 is likely to enter the market in a few years.[71]

- **Nanotherapeutics Inc** has developed an alternative carrier system to emulsion for topical delivery, based on solid lipid nanoparticles (SLN), that offer several advantages including increase in loading capacity, potential for decreased skin irritation, controlled-release, physical and chemical long-term stability. Initial commercial products have been launched under the Leunesse family of creams, with new over-the-counter and prescription products under development.

- **NanoPharm AG** has developed nanoparticles as a drug-delivery formulation for medical applications especially diseases of the central Nervous System, the "NANODEL" technology. Depending on the method of manufacturing, drugs are either attached to the surface and/or are incorporated into (poly-butylcyanoacrylate) particles (PBCA). Subsequently, this nanoparticle/drug formulation is coated with a suitable surfactant (such as polysorbate 80). The average nanoparticle diameter is about 200-400 nm and nanoparticles can be stored either in lyophilized form or in solution. Efficacy of these systems has been demonstrated in studies with the analgesic drug, dalargin, a peptide as well as with several other drugs.

- **Flamel Technologies SA**, a French company providing tailored solutions to the biotech and pharmaceutical companies for optimised controlled-release delivery of drugs, has invented an innovative polymer-based drug delivery system, Medusa, a versatile nanocarrier for the delivery of native protein drugs. Medusa technology consists of naturally occurring aminoacids (Leu hydrophobic and Glu hydrophilic), which form stable nanoparticles spontaneously in water. The amphiphilic character of the poly-aminoacid polymers drives the self-assembling of the nanoparticles in water; the poly-Leu chains are packed inside the structure, whereas those of Glu aminoacids are exposed to water. The 100-200 nanometers diameter-sized nanoparticles, composed of 95% water and 5% Leu-Glu polymer, are robust over a wide range of pH values and can be stored as either stable liquid or stable dry forms. This system allows the controlled-release of proteins over a long period of time with high bioavailability and efficacy.

#### 4.5.2.3 Fullerenes, Nanotubes

Fullerenes (also known as "buckyballs") are a series of hollow carbon molecules made of a cage of interlocking pentagons and hexagons like the patches on a soccer ball (Figure 4.10). The basic fullerene is made of 60 carbon atoms and has a diameter of approximately 1 nm. Carbon nanotubes, made of graphite sheets of hexagonal arrays of carbon rolled into tubes, are close cousins.

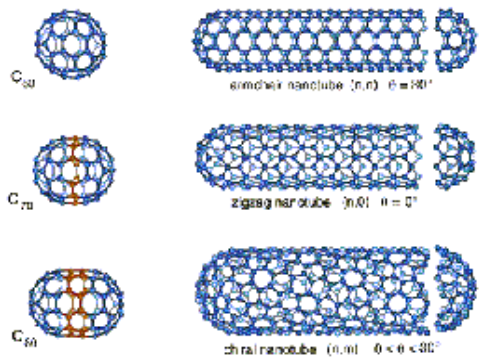


**Figure 4.10**. Fullerenes (Source: Université de Mons-Hainaut).

The application of nanotubes and fullerenes to medical practices has been explored for a number of years. More recently companies and researchers have started to investigate their use as drug delivery vehicles, as their nanometer size enables them to enter cells and they are small enough to pass through the kidneys and be excreted from the body. Nanotubes and fullerenes also have the advantages of being inert and the ability to have their structure chemically modified, which can allow an active compound to be inserted within or attached to the surface of the particle. For example, a buckyball-based anti-HIV protease treatment is just about to enter stage I clinical trials. However, nanotubes are still relatively expensive to produce in pure form and industrial-sized quantities, and it is likely that advances in production will be required before their wide-spread application in drug-delivery.

**Examples of achievements**

- Recent work by **New York State STAR Center for Biomolecular Diagnostics and Therapeutics** demonstrates that nanotubes can be opened and filled with biomolecules such as Beta-lactamase I. Once these modified nanotubes are mounted onto atomic force microscopy tips, it should be possible to inject, diffuse, or discharge encapsulated material, for example, metal ions or neurotransmitters into media such as cells. As a first step towards developing a drug delivery device capable of relatively painless, localized injections of medication at the single molecule level, the faculty is developing three-dimensional carbon nanotube arrays to be used as transdermal transport agents.

- A team from **Rice University** is studying metallofullerene materials (all-carbon fullerene cages that enclose metal ions) for various applications, including the treatment of tumours. In this particular application, the goal is to use selected metallofullerenes to deliver radioactive atoms directly to the cancer. It is hoped that these will increase the therapeutic potency and decrease the adverse effect profile for radiation treatments.

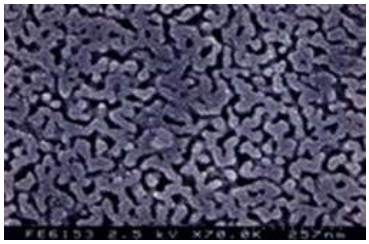
- **C Sixty**, a Canadian nanobiotech company that studies and modifies fullerenes to develop drug-delivery systems for cancer, AIDS and other diseases, is currently applying to the FDA for Phase I clinical trials and fast-track status on a novel fullerene drug for HIV that has shown great promise in animal studies, even against drug-resistant forms of the virus. Even better, it seems nontoxic, it is absorbed well orally and excreted unaltered by the kidneys. The concept is to insert drug-coated fullerenes inside the virus that causes AIDS to prevent it from reproducing. Unlike traditional HIV therapies, the fullerenes are also effective in combating the virus as it mutates during treatment.

The company also reported progress on another fullerene-based approach – this one for Lou Gehrig's disease, a degenerative disorder that affects nerve cells. Animal studies are encouraging. If successful, it may treat similar illnesses such as Parkinson's disease.

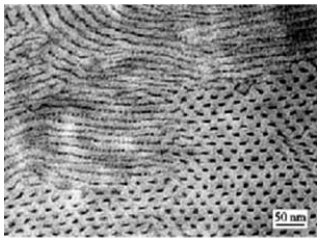
To treat cancer, C Sixty is working on the surface of fullerenes or larger carbon units known as "buckysomes" to "decorate" them with chemotherapeutic agents. Antibodies are attached to the surface of the buckysome and serve as a guidance system, by binding to surface receptors on the cancer cell. This stimulates uptake into the cancer cell where the chemotherapeutic drugs have their effect.

### 4.5.2.4 Nanoporous materials

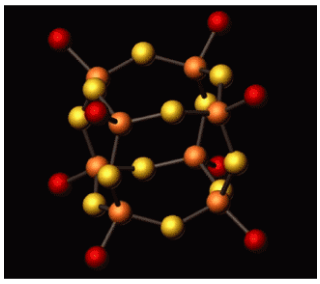
Nanoporous materials have holes that are less than 100 nm and can be thought of as diminutive sponge-like substances. Nanoporous solids are made out of a wide variety of substances, including carbon, silicon, various polymers, ceramics, various metallic minerals and compounds of organic materials and metals, or organic materials and silicon, such as the polyhedral oligomeric silsesquioxanes (or POSS), a family used in nanocomposites and other applications (Figure 4.11).



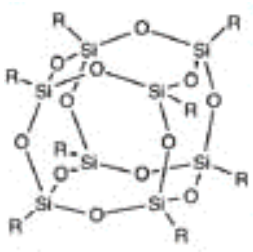
Nanoporous glass-derived material Source: www.techcentre.tde.alstom.com




lamellar-within-cylinders self- organized comb copolymer
Source: Helsinki University of Technology / OMM



POSS
Source: www.afrlhorizons.com/0001/spinoffs.html



POSS
Source: www.zyn.com/flcfw/fwtproj/Poss.htm

**Figure 4.11**. Nanoporous materials.

Potentially nanoporous materials will allow evasion of the body's immune system whilst directing a therapeutic agent to the desired site. For example, clinical trials have already begun on the use of nanoporous silica nanoparticles for delivering insulin through an inhaler. Biosanté Pharmaceuticals announced good pre-clinical results late in 2001 to deliver insulin in an inhaled form, subcutaneously or orally, using calcium phosphate nanoporous particles.

Nanoshells are another platform for nanoscale drug delivery. These are hollow spheres made of silica and coated with gold, giving them unique optical and conducting properties. The nanoshells are embedded in a drug-containing polymer and injected into the body. When heated with an infrared laser, the nanoshells make the polymer melt and release their drug payload at a specific site. This technique could be useful in treating diabetes as well. Instead of daily injections of insulin, a patient would use a ballpoint-pen-size infrared laser to heat the skin where nanoshell polymers had been injected previously. The heat from the nanoshells would cause the polymer to release a pulse of insulin.

**Examples of achievements**

- **Nanospectra Biosciences** is developing non-invasive medical therapies using patented Nanoshell particles. These nanoparticles can be tuned to absorb or scatter light at desired wavelengths, including ranges where human tissue is relatively transparent. These nanoshells will utilize antibodies or other proteins as cell targeting mechanisms to deliver the particles to specific cells or tissues.

- **pSivida** is exploring nanotechnology for medical applications through a joint venture with **QinetiQ**. The partners are developing and commercializing the nanobiomaterial BioSilicon™, a porous form of silicon with applications in orthopaedics, specialised drug delivery, neural interfacing and culture scaffolds. The core focus of this company is drug delivery and it uses its bio-material to help other companies in delivering their drugs or vaccines. The pores are typically the width of approximately 10 atoms and can be loaded with drugs, peptides, genes, proteins, radionuclides and other therapeutics or vaccines.

- **Inhale Therapeutic Systems Inc** (now named **Nektar Therapeutics**) has developed an improved propellant system for use in aerosol drug delivery. Currently, the most widely used devices for delivering drugs into the lungs are metered dose inhalers (pMDIs) and dry powder inhalers (DPIs), which deliver micronized drugs. Using hollow, porous, perforated particles, Inhale Therapeutic System Inc has discovered that drugs administered in the propellant are more likely to enter the lungs, leading to more consistent and beneficial results. The PulmoSphere process is currently undergoing clinical study.

### 4.5.2.5 Nanocrystals

Nanocrystals are also called quantum dots. These are capable of confining a single electron, or a few, into discrete energy states just as they would in an atom. Nanocrystals exhibit all the colours of the rainbow due to their unique semiconductor qualities (Figure 4.12).


**Figure 4.12**. Quantum Dots: a) 5 nm CdSe Qdot particle (Source: Quantum Dots), b) a family of Qdot particles (Source: MIT), c) a family of Qdot particles (Source : Nature Biotechnology, July 2001 Vol. 19).

Quantum dots can be coated with biological materials and used as detection agents for such things as pathogens or cancer cells. Although they are more widely used in diagnostics, quantum dots can also be employed for drug delivery where they may present the following advantages: more rapid absorption of the active drug substance, elimination of fed-fasted effects, higher dose loading with smaller dose volume, longer dose retention in blood and tumours for some compounds.

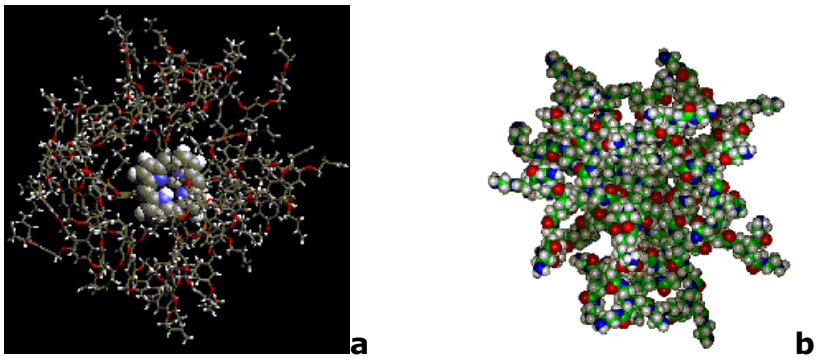
**Examples of achievements**

- The **Georgia Institute of Technology** and **Emory Medical School** have developed a process of binding quantum dots to particular genes and proteins and a method of using microbeads to incorporate various sizes of quantum dots for use in drug delivery and cancer therapy.

- The company **NTera** is seeking ways to infiltrate brain cells using nanocrystals coated with antibodies that will steer them to diseased cells. Their potential lies in treating neurological diseases (such as multiple sclerosis and Alzheimer's) as well as brain cancers because they are small enough to slip through the blood-brain barrier. After delivering drugs to the diseased brain cells, the crystals return to the bloodstream.

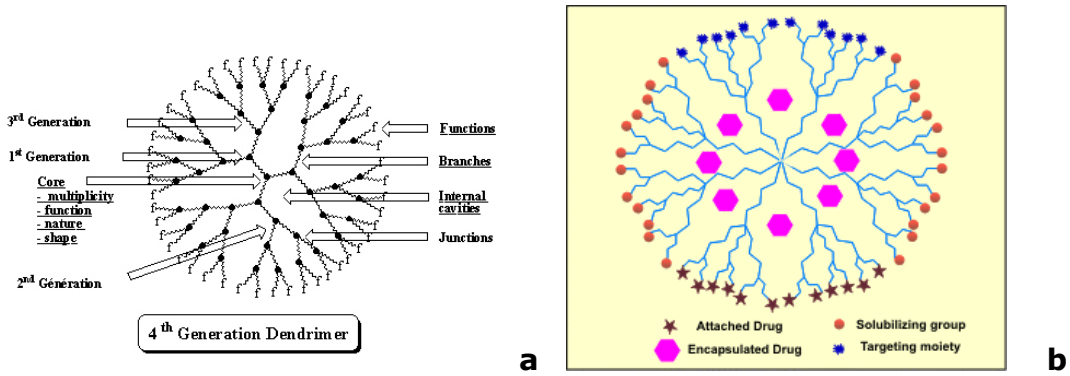
### 4.5.2.6 Dendrimers

Dendrimers are large and complex molecules with very well-defined chemical structures. From a polymer chemistry point of view, dendrimers are nearly perfect monodisperse macromolecules with a regular and highly branched three-dimensional architecture (Figure 4.13). They consist of three major architectural components: core, branches and end groups (Figure 4.14a). 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 (Figure 4.14b).



a b

**Figure 4.13**. Dendrimers. Sources: a) California Institute of Technology, b) Michigan Molecular Institute




a b

**Figure 4.14**. Composition of dendrimers (a- Calgary University) and possibilities of covalent attachment of drugs, solubilising groups, targeting groups or drug encapsulation with dendrimers (b- Centre for Drug Delivery Research, University of London).

Some of the most interesting applications for dendrimers, in a technical and commercial sense, are in the pharmaceutical and biomedical areas. Dendrimers are especially interesting for drug delivery because they are small enough to pass into cells and the cavities present in dendrimers can be used as binding sites for small drug molecules. Dendritic macromolecules also make suitable carrier systems because their size and structure can be controlled simply by synthetic means, they can easily be processed and made biocompatible and biodegradable, and they improve both tumor targeting and therapeutic efficacy due to the enhanced permeability and retention effect observed in tumour tissue.

So, dendrimers can be used to deliver substances such as a drug, document that the drug is there and report back on the cell's response. This is a real example of a multifunctional nanodevice.

To date, these delivery systems remain largely unexplored, but the polycationic polymer, polyaminodiamine, is currently being explored as potential synthetic vectors for gene delivery. Furthermore, several other polymeric networks are under investigation for DNA delivery. For example, researchers have demonstrated the usefulness of attaching the anticancer agents 5-fluorouracil to polyaminoamine dendrimers and methotrexate to hydrazide-terminated dendrimers formed from poly(aryl ether). The dendrimers incorporate protein docking domains that recognize tumour associated antigens to tether the complex following its delivery to the tumour, and additional antitumour agents thought to have synergistic effects with cytotoxins, that is, angiostatic agents, among others. Dendritic polyester systems based on the monomer unit 2,2-bis(hydroxymethyl)propanoic acid are also investigated as possible versatile drug carriers with improved solubility due to their well-defined molecular architecture and their multiplicity of surface sites.

**Examples of achievements**

- **The Centre for Polymer Therapeutics at the University of London** has developed polyamidoamine dendrimers as a novel oral drug delivery system of anticancer agents. These nanoparticulate carriers can carry drugs by covalent binding or complexation to the dendrimer surface, or by entrapment within the dendrimer core. The biocompatibility of polyamidoamine (PAMAM) dendrimers has been demonstrated and *in vitro* studies have shown that anionic PAMAM dendrimers rapidly transfer across the everted rat intestinal sac model.

- **The Center for Biological Nanotechnology at the University of Michigan** use poly(amidoamine) dendrimers to deliver anticancer agents such as cisplatin and methotrexate. The drugs are conjugated to the dendrimers using photocleavable or labile linkers, which can be made to release the drug using light or through acid cleavage. Dendrimers can be functionalized with peptides or small molecules to provide multivalent displays of ligands. These dendrimers appear to enter cells through facilitated transport. Preliminary studies have shown that they are not toxic to mice in single-doses of up to 10 mg/kg.

## 4.6 Research that will impact future development

As has been described nanotechnology is already impacting drug delivery, however this will continue apace. In the future, nanoparticles will contain far more intelligence than the entire current system of drug delivery. These will be able to target and deliver therapeutics to specific tissues and cells with no side effects. For example a nanoparticle taken orally, that passes undisturbed through the stomach and small intestine and into the colon, where it homes in directly on the tumour cells and releases a powerful anticancer drug that destroys just the cancerous cells, could soon be a reality. In this respect antibodies that bind exclusively to cancerous cells could be attached to nanoshells and injected into patients. Following infrared irradiation of the nanoshell-targeted tumour the resultant heat would destroy the cancer cells.

Another possible targeted drug delivery system involves the use of nanomagnets that can be directed to specific sites within the body using external magnetic fields. These magnets could be attached to drugs that could treat specific cellular structures. A drug payload is not even necessary: the material could just produce high temperature under heat or light to destroy the targeted cells. The advantage of such a system is that it allows very focused and intense treatment of diseased cells without harming cellular structures of non-interest.

Nanotubes may one day be used in transdermal drug delivery patches as nanoscale needles that can inject substances into the body. In fact, developing nanotubes as nanoscale, intravenous or intradermal, drug delivery devices is medically significant because a) it increases the mechanical and sensing functionality of the resultant nanoneedle, which makes it precise, and b) it is a less invasive and less painful drug administration. Nanotubes offer the potential of targeted drug delivery, for example to muscles, with molecular amounts of material, which maximizes efficiency by permitting lower doses, thereby minimizing possible toxicity and harmful side effects.

Nanotubes could even be used as nanoneedles that inject drugs directly into individual cells, as developed at Purdue University. Indeed, many drugs destroy infectious bacteria by poking holes in their cellular membranes and leaking out their nutrients, just like pricking a hole in a balloon. The nanotubes developed by Purdue University could also act in this manner, but in addition, they can be targeted and thus lure bacteria with "a bait" that guides the nanotubes to the bacterial cell membrane where they can start destroying the cell.

Scientists are currently studying methods to link quantum dots to drugs or other therapeutic agents to target cancer cells. These dots could serve as "smart bombs" to deliver a controlled amount of drug to a particular type of cell. Moreover, these particles would be able to profile a large number of genes and proteins simultaneously, allowing physicians to individualize cancer treatments based on the molecular differences in the cancers of various patients (indeed, even when cells appear to be similar under the microscope, their genes and proteins may be decidedly different, which explains why cancer patients with apparently similar cancers sometimes respond differently to the same treatment).

Other nano-devices will allow the continuous monitoring of the level of various biochemicals in the bloodstream and in response could release appropriate drugs. For example, an insulin-dependent diabetic could use such a device to continuously monitor and adjust insulin levels autonomously.

## 4.7 Conclusions

To conclude, nanotechnologies have already begun to change the scale and methods of drug delivery. Nanotechnology can provide new formulations and routes for drug delivery that broaden their therapeutic potential enormously by effecting delivery of new types of medicine to previously inaccessible sites in the body.

The use of nanocapsules and nanoparticles for drug delivery is an area with a great deal of activity that could have a major impact on the medical and pharmaceutical industry. Some of the technologies are relatively developed but will be affected by the notoriously long timescales needed for clinical testing.

The eventual success of nanotechnology in areas of medicine will require patient acceptance and careful consideration of the social and economic consequences of genetic testing and therapy.

## 4.8 Companies working in the field

**Abbott Laboratories**
- Address: Abbott Bioresearch Center ,100 Research Drive Worcester, Massachusetts 01605 (US)
- Phone: (508) 849-2500
- Fax: None
- Website: http://abbott.com/index.cfm; http://abbott.com/abbottbioresearch/
- Short profile: Abbott Laboratories is a global, broad-based health care company devoted to the discovery, development, manufacture and marketing of pharmaceuticals, nutritional, and medical products, including devices and diagnostics. The company is also working on drug delivery nanoencapsulation systems.

**Access Pharmaceuticals**
- Address: 2600 Stemmons Freeway, Suite 176 Dallas, TX 75207-2107 (US)
- Phone: (214) 905-5100
- Fax: (214) 905-5101
- Website: http://www.accesspharma.com/
- Short profile: Access Pharmaceuticals is a pharmaceutical company which has produced a new material consisting of a soft hydrogel nanoparticle network, composed primarily of water, which exhibits an ordered crystalline structure with a characteristic coloured opalescence. This colour varies in response to changes in physical and chemical environments. Access will apply this material to drug delivery of peptides and proteins.

**Advectus Life Sciences Inc.**
- Address: Suite 100, 265 – 25th Street West Vancouver, BC Canada V7V 4H9 (Canada)
- Phone: 604-926-6098
- Fax: 604-926-4398
- Website: http://www.advectuslifesciences.com/
- Short profile: Advectus Life Sciences Inc. is an emerging pharmaceutical company. This company is focused on the development and commercialization of a patented process using nanotechnology for the delivery of approved cancer fighting drugs across the blood-brain barrier (BBB) for the treatment of brain tumours. It uses a nanoparticle technology for delivering doxorubicin across the BBB.

**Alnis Biosciences**
- Address: 5764 Shellmound St., Suite A, Emeryville, CA 94608 (US)
- Phone: (510) 420-3760
- Fax: (510) 420-3761
- Website:http://www.alnis.com/

- Short profile: Alnis BioSciences Inc. is a biopharmaceutical company that is developing therapeutics from its nanoparticle technology platform. Alnis is investigating targeted delivery of bioactive entities using its nanoparticle materials. Alnis' nanoparticles are constructed through the engineering of proprietary polymeric materials that are biodegradable, non-toxic, and non-immunogenic.

**Altair Nanotechnologies, Inc.**
- Address: 204 Edison Way, Reno, NV 89502 (US)
- Phone: (775) 858-3742
- Fax: (775) 856-1619
- Website: http://www.altairnano.com/
- Short profile: Altair's Nanotechnology Inc. creates porous microstuctures consisting of high surface area nano primary particles to enable new applications for drug delivery. The Company uses TiNano Sphere powders which are new delivery forms for existing drugs and new drugs.

**Alza Corp.**
- Address: 1900 Charleston Road, P.O. Box 7210, Mountain View, CA 94039-7210 (US)
- Phone: (650) 564-5000
- Fax: (650) 564-7070
- Website: http://www.alza.com/
- Short profile: ALZA Corporation is the leading provider of drug delivery solutions including oral, transdermal, implantable and liposomal technologies. The Company uses various technologies for drug delivery, in particular nanoparticles. For example, ALZA uses a technology (STEALTH®) which is composed of lipid nanoparticles to provide the precise delivery of drugs to disease-specific areas of the body.

**American Bioscience**
- Address: (US)
- Phone: 888-884-7770
- Fax: None
- Website: http://www.americanbiosciences.com/
- Short profile: American BioSciences provides natural health products. ABI-007 is a novel protein (albumin) stabilized, cremophor-free, nanoparticle formulation of paclitaxel that may be used without premedication, and may allow the administration of paclitaxel safely at doses higher than 175 mg/m2. The ABI-007 paclitaxel nanoparticle measures approximately 130 nanometers, about the size of a virus, and about 1/80th to 1/100th the size of a single red blood cell.

**American Pharmaceutical Partners Inc. (APP)**
- Address: 11777 San Vicente Blvd., Suite 550 Los Angeles, CA 90049 (US)
- Phone: 310/826-8505
- Fax: None
- Website: http://www.appdrugs.com/

- Short profile: American Pharmaceutical Partners, Inc. (APP) is a strategically integrated pharmaceutical company that manufactures markets and develops injectable products. This Company has presented results from an early human trial ABI-007 in April 2002, a new nanoparticle delivery system for an established anticancer drug. ABI- 007 is 130 nm long and consists of an engineered protein-stabilized nanoparticle that contains paclitaxel, which is used to treat breast, bladder, and more than a dozen other cancers.

**Aphios**
- Address: 3-E Gill Street Woburn, Massachusetts 01801 (US)
- Phone: (001) (781) 932-6933
- Fax: (001) (781) 932-6865
- Website: http://www.aphios.com/
- Short profile: Aphios is a biopharmaceutical company focusing on cancer and infectious diseases. The Company uses encapsulation technologies including polymer nanospheres and phospholipid nanosomes.

**Battelle Pulmonary Therapeutics**
- Address: 1801 Watermark Drive, Suite 100 Columbus, Ohio 43215-1037 (US)
- Phone: 614-340-2325
- Fax: 614-340-2320
- Website: http://www.battellepharma.com/
- Short profile: Battelle Pharma is a specialty pharmaceutical company leveraging science and technology for more effective medicines. The company collaborates with Eiffel Technologies, who supplies sub-micron particles of a protein, testing these particles in their Mystic$^{R}$ aerosolpulmonary delivery device. The nanoparticles are used to control the size of the drops in the aerosol.

**Bespak**
- Address: 2450 Laura Duncan Road, Apex North Carolina 27502 (US)
- Phone: (919) 387 0112
- Fax: (919) 3870116
- Website: http://www.bespak.com
- Short profile: Bespak is a leading supplier of drug delivery technologies and services to the pharmaceutical, drug delivery and biotechnology industries. The company has acquired a nanoparticle technology from the Massachusetts Institute of Technology that could lead to the development of a novel nasal delivery system.

**Bioalliance Pharma**
- Address: 59, boulevard du Général Martial Valin, 75015 Paris (France)
- Phone: (+33) 01 45 58 76 00
- Fax: (+33) 01 45 58 08 81
- Website: http://www.bioalliancepharma.com/
- Short profile: BioAlliance Pharma is a private biotech company. The strategy of which is to develop a platform based on nanotechnologies. This company has implemented the Transdrug™ programme which allows pre-clinical studies. These studies have shown that Transdrug™ (which involves doxorubicin-loaded nanospheres made of polymer) can overcome the mechanisms of multi-drug resistance and is capable of restoring the sensitivity to doxorubicin of resistant cancer cells.

**Biosante Pharmaceuticals Inc.**
- Address: 111 Barclay Boulevard Lincolnshire, Illinois 60069 (US)
- Phone: 847-478-0500
- Fax: 847-478-9152
- Website: http://www.biosantepharma.com/
- Short profile: BioSante Pharmaceuticals Inc. is an emerging pharmaceutical company which develops a robust product pipeline of hormone therapy products. This company is notably developing a calcium phosphate nanoparticulate-based platform technology (CAP) for drug delivery systems (with different applications: administration orally, into muscles, under the skin and into the lungs by inhalation).

**Biotech Australia**
- Address: 28 Barcoo Street, Roseville, NSW 2069 (Australia)
- Phone: 61 2 9928 8800
- Fax: 61 2 9928 8899
- Website: http://www.bioaust.com.au/
- Short profile: Biotech Australia has developed a technology for the oral delivery of medicals based on a capsule with a diameter from 1 nm to 100 microns, and a carrier particle that adheres to it.

**C-Sixty**
- Address: 2250 Holcombe Blvd., Ste. 174 Houston, TX 77030 (US)
- Phone: 713-610-4035
- Fax: 713-797-9913
- Website: http://www.csixty.com/
- Short profile: C Sixty Inc. is a leading nanomedicine company pioneer in the development of biopharmaceutical fullerenes. This company is developing fullerenes as a drug-delivery system for cancer, AIDS and other diseases.

**Capsulution Nanoscience**
- Address: Volmerstr. 7b, D-12489 Berlin (Germany)
- Phone: +49-(0)30-63923627
- Fax: +49-(0)30-63923601
- Website: www.capsulution.com
- Short profile: Capsulution NanoScience AG develops innovative drug delivery systems for the simple, efficient and safe delivery of pharmaceutically active compounds. The company's drug delivery systems are based on a set of nano- and micron-sized capsules (between 20nm and 20 microns) designed to suit pharmaceutical and other industries.

**Ciba Specialty Chemicals**
- Address: 540 White Plains Road, P.O. Box 2005, Tarrytown, 10591 – 9005 New York (US)
- Phone: +1 914 785 2000
- Fax: +1 914 785 2211
- Website: http://www.cibasc.com/view.asp

- Short profile: Ciba Specialty Chemicals is a producer of speciality chemicals. Markets are plastic additives, coating effects, water and paper treatment, textile effects, home care and personal care. One of their product lines is based on the use of 50 nm particles (nanoparticles), "nanotopes™", to encapsulate and transport vitamins, amino acids and other active ingredients beneath the surface of the skin.

**Cytimmune Sciences Inc.**
- Address: 8075 Greenmead Drive, College Park, Maryland 20740 (US)
- Phone: 301-445-4220
- Fax: 301-445-4370
- Website: http://www.cytimmune.com/
- Short profile: Cytimmune Sciences Inc. is focused on the chemistry of colloidal gold for drug delivery in the treatment of cancer. Colloidal gold is a solution of gold nanoparticles that is manufactured from inexpensive gold salts and is currently used as a protein marker by chemists and for medical purposes.

**Eiffel Technologies**
- Address: Level 14, 50 Market Street, Melbourne VIC 3000 (Australia)
- Phone: +61 3 9629 8022
- Fax: +61 3 9629 8077
- Website: http://www.eiffeltechnologies.com.au/html/default.asp
- Short profile: Eiffel Technologies uses Supercritical Fluid (SCF) technologies to re-engineer pharmaceuticals and proteins to improve their bioavailability and delivery. The application of Eiffel's SCF technologies produces nano-sized particles with a very narrow particle size distribution. The company is working on inhalation drug delivery based on these nanoparticles.

**Elan Pharmaceutical Technologies**
- Address: 41st Floor, 345 Park Avenue, New York, NY 10154 (US)
- Phone: +1-212-407-5755
- Fax: +1 212-755-1043
- Website: http://www.elan.com/
- Short profile: Elan has an extensive range of drug delivery technologies which are non-core to its biopharmaceutical business. The technologies address the fundamental factors that define a drug's biodisposition - dissolution, transport, distribution (i.e. drug targeting), and metabolism. One of its products is 400 nm particles of pharmaceutical active ingredients with improved water solubility. This technology was licensed from Merck & Co in 1999 and is named Nanocrystals®.

**Flamel Technologies**
- Address: 33 avenue du Dr. Georges Levy, 69693 Vénissieux Cedex (France)
- Phone: +33 (0) 472 783 434
- Fax: +33 (0) 472 783 435
- Website: http://www.flamel-technologies.fr/
- Short profile: Flamel Technologies is a drug delivery company that has invented two innovative delivery systems. Flamel's systems, Micropump® for small molecules and Medusa® for proteins (which is a nanoencapsulation technique), allow improvements in the therapeutic characteristics, safety profile and patient compliance of many drugs.

**GeneFluidics**
- Address: 2540 Corporate Place, Suite B-101, Monterey Park, CA 91754 (US)
- Phone: (323)269-0900
- Fax: (323)269-0988
- Website: http://www.genefluidics.com/
- Short profile: GeneFluidics is commercializing a novel technology platform that integrates nanobiotechnology and microfluidics. Among its various activities, GeneFluidics develops, manufactures, and markets drug delivery devices. For example, this company uses nanodevices for the delivery of peptides and oligonucleotides.

**Gilead Sciences Inc.**
- Address: Foster City, CA (Headquarters), 333 Lakeside Drive, Foster City, CA 94404 (US)
- Phone: (650) 574-3000
- Fax: (650) 578-9264
- Website: http://www.gilead.com/
- Short profile: Gilead Sciences Inc. is a leading biopharmaceutical company that uses liposomes for drug delivery. The liposome carrier is playing a leading role in reducing the harmful effects of certain therapies on healthy tissues, thereby offering the potential for an improved safety profile for certain drugs. The method is to encapsulate therapeutic compounds within liposomes for enhanced drug delivery.

**Hollister Incorporated**
- Address: 2000 Hollister Drive, Libertyville, Illinois 60048 (US)
- Phone: 1-800-323-4060
- Fax: None
- Website: http://www.hollister.com/us/
- Short profile: Hollister Incorporated is a global leader in healthcare product manufacturing. The Company develops different technologies for drug delivery. For example, a buckyball-based AIDS treatment is just about to enter clinical trials for drug delivery.

**Hybrid Systems**
- Address: University of Birmingham, Vincent Drive, Edgbaston Birmingham B15 2TT (UK)
- Phone: None
- Fax: None
- Website: http://www.hybridsystemsltd.co.uk
- Short profile: Hybrid Systems is a spin-out from the UK University of Birmingham's Institute for Cancer. They own a technology that uses a nanoparticle bound with a polymer to target specific forms of cancers.

**IMARX Pharmaceutical Corp.**
- Address: 1635 East 18th Street, Tucson, AZ 85719 (US)
- Phone: (520)770-1259
- Fax: (520)791-2437
- Website: http://www.imarx.com/
- Short profile: IMARX Pharmaceutical Corp is dedicated to the development and production of systems for drug and gene delivery, using nanoparticles in some of their technologies and specializing in the use of ultrasound.

**IMEDD Inc.**
- Address: 1381 Kinnear Rd., Suite 111, Columbus, OH 43212 (US)
- Phone: 614-340-6001
- Fax: 614-340-6005
- Website: http://www.imeddinc.com/
- Short profile: iMEDD is a Drug delivery company using nanotechology and micromachines (MEMS) to address chronic afflictions like Hepatitis C. This Company is developing novel drug delivery products based on nanoscale structural features created by proprietary "top-down" micro fabrication techniques. IMEDD's lead drug delivery platform, NanoGATE, is a small subcutaneous implant that uses membranes containing pores with nanometer dimensions that control the diffusion of drugs at a molecular level.

**ITN Nanovation GmbH**
- Address: Untertürkheimer Straße 25, D–66117 Saarbrücken (Germany)
- Phone: 0681/5001482
- Fax: 0681/5001458
- Website: http://www.itn-nanovation.com/home.html
- Short profile: ITN Nanovation GmbH is a high tech company focusing on the development, production and marketing of products based on nanotechnology (ceramic nanopowders). Among other things, the nanoparticles of ITN are used in drug delivery.

**Kereos Inc.**
- Address: 4041 Forest Park Ave., St. Louis, MO 63108 (US)
- Phone: (314) 633-1879
- Fax: (314) 615-6901
- Website: www.kereos.com
- Short profile: Kereos is using medical "missiles" to deliver imaging agents and drugs to specific diseased sites in the body for diagnostic and therapeutic purposes. This company uses targeted nanoparticle technology. In fact, anticancer drugs and therapeutic radionuclides can be incorporated into the nanoparticles to deliver therapy directly and selectively to areas of tumour development.

**Merck KGaA**
- Address: Frankfurter Str. 250, D-64293 Darmstadt (Germany)
- Phone: +49-6151- 72-0
- Fax: +49-6151- 72-2000
- Website: http://pb.merck.de/servlet/PB/menu/1001723/index.html
- Short profile: Merck KGaA develops drugs that are used to cure, alleviate or prevent diseases. This Company uses lipid-based nanoparticle coupled to the integrin alphanubeta3-targeting ligand that delivers genes selectively to angiogenic blood vessels.

**MitoVec**
MitoVec is creating new therapies for cancer and other diseases using advanced drug delivery technology to target mitochondria. Mitochondria, tiny organelles found in nearly all cells, supply over 80% of the body's energy, and play key roles in metabolism and cell death. MitoVec is commercializing MitoSpheres™, a novel nanotechnology that selectively targets mitochondria in diseased cells. Many difficult-to-treat diseases, including cancer and aging-related diseases like diabetes, Parkinson's, and Alzheimer's, are associated with mitochondrial dysfunction and will benefit from MitoSphere-enabled treatments. Further, nearly 100 currently untreatable orphan genetic disorders with mutations in mitochondrial DNA (mtDNA) as their basis could be remedied using MitoSpheres to deliver mitochondrial repair treatments.

**Nanobiotix**
- Address: Toulouse (France)
- Phone: None
- Fax: None
- Website: www.nanobiotix.com/
- Short profile: Nanobiotix uses nanotechnologies for its applications in Drug delivery. In fact, Nanobiotix scientists describe how magnetic nanoclinics, less than 70 nm in diameter, can selectively destroy human breast and ovarian cancer cells *in vitro* when a magnetic field is applied. Studies are underway in animals aimed at demonstrating the selective uptake of nanoclinics by tumour cells.

**NanoCarrier Co. Ltd.**
- Address: Tokatsu Techno Plaza, 5-4-6 Kashiwanoha, Kashiwa, Chiba 277-0882 (Japan)
- Phone: +81-4-7169-6550
- Fax: +81-4-7169-6551
- Website: http://www.nanocarrier.co.jp/
- Short profile: NanoCarrier uses technology to utilize nanoparticles in the range of 20 – 100 nm with bi-layer structures comprising inner and outer cores. NanoCarrier's proprietary micellar nanoparticle technology, the NanoCap-system, involves physical entrapment of therapeutic agents into nanoparticles, which results in increased solubility and sustained drug release.

**Nanomagnetics Inc.**
- Address: 108 Longmead Road, Emerald Park, East Emersons Green, Bristol, BS16 7FG (UK)
- Phone: +44 (0)1179 104160
- Fax: +44 (0)1179 104161
- Website: http://www.nanomagnetics.com/
- Short profile: Nanomagnetics is using magnetic grains within hollow spheres of the iron-carrying protein apoferritin, found in blood, as a magnetic storage material for computer hard drives. The uniform grain size of these protein nanoparticles gives high magnetic stability. NanoMagnetics' core technology can also be used in drug delivery.

**Nanomat Inc.**
- Address: 1061 Main Street, North Huntingdon, PA 15642-7425 (US)
- Phone: 724.861.6120

- Fax: 724.861.6119
- Website: http://www.nanomat.com
- Short profile: Nanomat is a leading manufacturer of nanomaterials, powders, and technologies. The firm's NanoTalc and NanoCalc powders can be used in numerous products and industries, including paper, polymers, paints, pigments and pharmaceuticals. This company produces more specifically iron nanopowders for pharmaceuticals. The process implemented is the incorporation of iron nanopowders in ferrofluids for fast-acting targeted drug delivery, DNA tagging and improved MRI imaging.

**NanoMed Pharmaceuticals Inc.**
- Address: 5265 Saddle Club Drive, Kalamazoo, MI 49009-9774 (US)
- Phone: +1 (269) 372-6256
- Fax: None
- Website: http://www.nanomedpharm.com/nanomed/
- Short profile: NanoMed Pharmaceuticals Inc. is a privately-held, early-stage advanced drug delivery company. It is focusing on the application of its proprietary Nanotemplate Engineering to develop drug delivery systems. The claims in these applications cover composition of matter, methods and processes to make novel nanoparticles, and their applications. Nanotemplate Engineering enables the manufacturing of nanoparticles < 100 nm in diameter. These nanoparticles are made using all pharmaceutically-acceptable excipients and can be engineered to contain or carry small molecules, peptides, proteins, plasmid DNA, diagnostic agents, and radio- and bio-sensors.

**Nanomix Inc.**
- Address: 5980 Horton Street, Suite 600 Emeryville, CA 94608 (US)
- Phone: 510.428.5300
- Fax: 510.658.0425
- Website: http://www.nano.com/
- Short profile: Nanomix is focused on producing chemical and gas sensors, components that are used in medical diagnostic, industrial safety and process control applications. Nanomix is also exploring the use of carbon nanotubes as the building blocks of new materials for drug delivery.

**NanoPass Technologies Ltd.**
- Address: 18 Ha'Neviim, St. Haifa (Israel)
- Phone: 972-3-6414080
- Fax: 972-4-8605807
- Website: http://www.nanopass.com/
- Short profile: NanoPass is a market-driven developer of painless nano-needle devices for drug delivery and diagnostics. This company is developing needle array to address the need for safe, efficient transdermal platform technologies that will be able to offer controlled release macromolecule transfer in sufficient quantities.

**NanoPharma Corp.**
- Address: 75 Park Plaza, 4$^{th}$ Floor, Box 35n Boston, MA 02116 (US)
- Phone: (617) 482-2333
- Fax: (617) 482-3337
- Website: none

- Short profile: Nanopharma is a drug delivery company using nanotechnologies. The Company is developing a technology platform called "Lymph Node Drug Delivery technology" (LNDD), which consists of a core nanoparticle, 15-25 nm in diameter, engineered to transport the drug substances of interest (potential applications in metastatic cancer and certain infectious diseases, e.g. HIV).

**Nanopharm AG**
- Address: Leipziger Str. 44, Haus Nr. 65 (Zenit-Gebäude), 39120 Magdeburg (Germany)
- Phone: +49 (3 91) 6 11 73 30
- Fax: +49 (3 91) 6 11 71 03
- Website: http://www.nanopharm.de/nano.html
- Short profile: NanoPharm AG has developed a method to transport drugs across the blood brain barrier: Drugs are bound to nanoparticles which are subsequently coated with a surfactant. The average nanoparticle diameter is about 200-400 nm. The NANODEL-technology as a platform product in drug delivery represents a novel vector system. Furthermore, NANODEL-Particles open a new field of targeted drug delivery compound transport in the field of gene therapy.

**Nanospectra Biosciences**
- Address: 8285 El Rio Street, Suite 130 Houston, Texas 77054 (US)
- Phone: (713) 842-2720
- Fax: (713)440-9349
- Website: http://www.nanospectra.com/
- Short profile: Nanospectra Biosciences Inc. commercializes gold nanoshells for targeted destruction of various cancers. Nanoshells utilize antibodies or other proteins as cell targeting mechanisms to deliver the particles to specific cells or tissues, especially cancerous cells. For cancer treatment, the shells would be introduced into a patient's body where they would attach to cells. These cells, which capture energy at infrared wavelengths that can penetrate tissue, would then be heated by an infrared source to destroy the cancer cell.

**Nanotherapeutics Inc.**
- Address: 12085 Research Drive Suite N, Alachua, FL 32615 (US)
- Phone: None
- Fax: 386.462.2087
- Website: http://www.nanotherapeutics.com/
- Short profile: Nanotherapeutics Inc. has made new discoveries in the area of nanotechnology and is applying them to the development of novel drug therapies. Nanotherapeutics has developed two proprietary drug delivery technologies, Nanodry™ and Nanocoat™ (using novel nanoparticles and nanopowders).

**Novavax Inc.**
- Address: 8320 Guilford Road, Suite C Columbia, Maryland 21046 (US)
- Phone: 301.854.3900
- Fax: 301.854.3901
- Website: http://www.novavax.com/delivery.html

- Short profile: Novavax, Inc is a speciality biopharmaceutical company that uses micellar nanoparticles (MNP) for drug delivery. In fact, the MNP formulations used by Novavax for the transdermal delivery of drugs have cosmetic properties similar to creams and lotions. These transdermal formulations have the advantage over injectable delivery systems of being less invasive and inconvenient and may also cause less skin irritation than patch transdermal delivery systems.

**NTera**
- Address: 58 Spruce Avenue, Stillorgan Industrial Park Co., Dublin (Ireland)
- Phone: (+353 1) 2137500
- Fax: (+353 1) 2137564
- Website: http://www.ntera.com/
- Short profile: NTera is a broad-based nanotechnology company with current applications in many areas, in particular drug delivery. In this company, scientists are seeking ways to infiltrate brain cells using minuscule, concentrated drug particles called nanocrystals. Like fullerenes and nanoshells, nanocrystals are coated with antibodies that will steer them to diseased cells.

**pSivida Limited**
- Address: Level 12 BGC Centre 28, The Esplanade, Perth WA 6000 (Australia)
- Phone: (+61 8) 9226 5099
- Fax: (+61 8) 9226 5499
- Website: http://www.psivida.com.au/
- Short profile: pSivida Limited is an Australian company committed to the biomedical nanotechnology sectors. This company is exploring nanotechnology through a joint venture with QinetiQ. The joint venture partners are developing and marketing the nanobiomaterial BioSiliconTM, a porous form of silicon with applications in drug delivery.

**PsiMedica**
- Address: Malvern Hills Science Park, Geraldine Road, Malvern, Worcs, WR14 3SZ (UK)
- Phone: +44 (0)1684 585300
- Fax: +44 (0)1684 585357
- Website: www.psimedica.co.uk
- Short profile: pSivida currently owns 42.85 % of pSiMedica Limited which owns the BioSilicon Intellectual Property (IP). It operates principally in the field of human and animal healthcare and *in vivo* diagnostic applications. BioSilicon drug delivery technology uses nanostructured porous silicon for specific cancer therapies.

**Separex**
- Address: 5, rue Jacques Monod, BP9, 54250 Champigneulles (France)
- Phone: (+33) 03 83 31 24 24
- Fax: (+33) 03 83 31 24 83
- Website: http://www.separex.com/
- Short profile: Separex is a spin-out of the ENSIC (Ecole Nationale Supérieure des Industries Chimiques) fully acquired by Lavipharm. Separex is a pioneer in the supercritical fluid technology. Its nanotechnology-related activities deal with aerogels (nanoporous materials) and the production of nanoparticles, microspheres and microcapsules with applications in drug delivery.

**Skyepharma**
- Address: 105 Piccadilly, London W1J 7NJ (UK)
- Phone: (44) 207 491 1777
- Fax: (44) 207 491 3338
- Website: http://www.skyepharma.com/
- Short profile: Skyepharma is a world's leading developer, manufacturer and provider of drug delivery technologies. One of its technologies uses nanoparticles for the enhancement of drug solubility to impart less physical stress upon the drug particles. The company uses a piston gap homogeniser to create these nanoparticles through hydrodynamic cavitation.

**Targesome**
- Address: 4030 Fabian Way Palo Alto, CA 94303 (US)
- Phone: 650.842.1820
- Fax: 650.842.1828
- Website: http://www.targesome.com/
- Short profile: Targesome Inc. is a drug development and drug delivery company utilizing its proprietary nanoparticle technology to produce novel, efficacious drugs with improved properties. Applications include the enhancement of drug properties, drug delivery and imaging. The company's nanoparticle technology provides a carrier particle that is generally 50 to 70 nm in size. They are developing their antiangiogenesis product, Vitaxin TM, in collaboration with MedImmune Inc. and are jointly developing anti-angiogenesis compounds using nanoparticles with Merck.

## 4.9 Overview of European projects, literature and web-sites

### 4.9.1 European Funded Projects

**Micro and Nanotechnologies in Advanced Delivery Systems for Patient-Friendly Insulin Therapy**. The aim of this proposal is to mobilize the European scientific expertise on insulin delivery to provide patients and health care professionals with advanced delivery systems for more effective, safe and accepted treatment of diabetes. The goal is to provide therapeutic alternatives to the current invasive modes of administration by means of non-parenteral administration of particulate vectors of insulin of nano size range. The administration routes envisaged are in order of relevance, oral, pulmonary or nasal, and transdermal.

Contact:
Organization: TEFARCO INNOVA
Address: Parco Area Delle Scienze
Post Code: 43100
City: PARMA; ITALY
URL address: http://www.unipr.it/arpa/dipfarm
Phone: +39-05-21905086

Fax: +39-05-21905085
E-mail address: farmac2@unipr.it

**Nanoparticle Delivery of Drugs to the Brain: A New Application of Nanotechnology**. Nanoparticles overcoated with polysorbate 80 have been shown to enable the transport across the blood-brain barrier of drugs, that normally do not enter the brain in significant amounts. The objective of the present project is to determine the mechanisms producing this enhanced drug delivery to the brain when nanoparticles are used as a vector. The factors that initiate internalization of the particles into brain capillary endothelial cells will be established and the route(s) by which nanoparticles and drug(s) cross the blood-brain-barrier will be elucidated. Any modification of blood-brain-barrier permeability by the modulation of tight junctions will also be investigated. A combination of *in vitro* and *in vivo* studies will be used to investigate the above objectives. The development of nanoparticles as drug vectors to the central nervous system (CNS) will open the door to advances in the treatment of other CNS diseases such as Alzheimer's disease, Parkinson's disease, Ischaemic stroke, Epilepsy, Multiple sclerosis, and Cerebral AIDS in addition to more effective therapies for brain tumours.

Contact:
Organisation: Institut für Pharmazeutische Technologie / Universität Frankfurt am Main
Address: Marie-Curie-Strabe 9
Postcode: 60439
City: Frankfurt am Main; GERMANY
Contact Person: KREUTER, Jörg (Professor)
Phone: +49-697-9829682
Fax: +49-697-9829694
E-mail address: kreuter@em.uni-frankfurt.de

**TATLYS : a new biocompatible nanoparticle delivery system for targeted release of fibrinolytic drugs**. The main objective of the project is the development of a new biocompatible- bioerodible nanoparticle delivery system for targeted release of fibrinolytic drugs directed to thrombus in the cardiovascular compartment. New polymeric-matrices, suitable for the formulation of nanoparticles containing urokinase, will be prepared on laboratory scale. To target the thrombus, specific oligopeptides, which recognize the chosen epitopes of fibrin will be modelled and synthesized. Polymers, oligo-peptides and proteic drugs will be combined under experimental conditions suitable for the attainment of nanoparticulate systems. Polymer, oligopeptide and nanoparticle productions will be scaled-up at pre-industrial level after optimisation of experimental parameters in order to achieve an effective targeting of fibrin and a suitable release kinetics of the drug from the nanoparticles. Stability and toxicity of the nanoparticles will also be investigated.

Contact:
Organisation: INSTM
Address: Via Risorgimento 35, Universita degli Studi di Pisa
Postcode: 56126
City: PISA; ITALY
Contact Person: GATTESCHI, Dante (Professor)
Phone: +39-055-410717
Fax: +39-055-410534
E-mail address: incm@chim1.unifi.it

**MAGNANOMED : Magnetic nanoparticles for medical and biological diagnostics and devices**. The objective of the project is to make and evaluate new types of magnetic nanoparticles of special shape and precise size with tailored surface chemistry and topography for biomedical purposes, especially the precise delivery of drugs by magnetic nanoparticles to the exact target tissue by application of external magnetic fields. This approach should reduce drug dosage, eliminate side effects and obtain faster action. These particles are needed as those used until now show inadequate magnetic properties, biocompatibility and surface chemistry. The novelty is to remedy these defects by making super paramagnetic nanoparticles of various shapes, appropriately functionalized, in one step in a continuous Segmented Flow Tubular Reactor. The project will deal with all aspects from particle production and surface functionalization to biological and clinical tests and will end with commercialization.

Contact:
Organisation: Institute of Biomedical and Life Sciences / University of Glasgow
Address: Joseph Black Building, University Avenue
Postcode: G12 8QQ
City: GLASGOW; UNITED KINGDOM
Contact Person: CARTER, Ian (Dr)
Phone: +44-1413-304881
Fax: +44-1413-302747
E-mail address: i.carter@enterprise.gla.ac.uk

### 4.9.2 Literature and websites


- "Buckyballs Make Fantastic Voyage", Jill Neimark, Wired News
- "Tiny weapons with giant potential", Karen Breslau, Newsweek June 24 2002
- European Pharmaceutical Review, Vol 5, Issue 4
- Future Technologies, Today's Choices – Alexander Huw Arnall
- Hollister – 2002
- IMS World Review
- Ineke Malsch, Biomedical Applications of Nanotechnology – June/July 2002
- Informa Pharmaceuticals / Jain PharmaBiotech
- MedAd News 2002 and Report on "Alternative Drug Delivery Systems Series"
- Miles & Jarvis 2001
- Modern Drug Discovery, April 2001, Vol. 4
- Nanotechnology: a technology forecast, April 2003 - Professor Sylvia Daunerts
- Nature biotechnology – August 2003
- Nature Biotechnology, Vol 21, number 8, August 2003

- Nature Reviews – Drug Delivery and targeting, April 30th 1998
- Nature Reviews – Drug Discovery, January 2002, Vol 1
- Nature Reviews – January 2002
- New dimensions for manufacturing - "A UK strategy for Nanotechnology" – UK Advisory Group, June 2002
- New Drug Delivery Report, oncologyknowledge base
- Options Seem Limitless For Nanobiotech - Ted Agres, Deputy Managing Editor, Washington Times
- Smaller, Cleaner, Cheaper, Faster, Smarter, Nanotechnology Applications and Opportunities for Australia – "Emerging Industries" 16
- The Big Down – Atomtech: Technologies Converging at the Nano-scale, ETC Group
- The Nanotechnology Opportunity Report – CMP Cientifica, Volume 1 and 2
- The Social and Economic Challenges of Nanotechnology, Economic & Social Research Council
- Tatlys project (http://dbs.cordis.lu/cordis-cgi/srchidadb?ACTION=D&SESSION=196702003-3-13&DOC=1&TBL=EN_PROJ&RCN=EP_RCN:54551&CALLER=PROJLINK)
- www.drugdeliverytech.com
- www.ims-global.com
- http://www.the-infoshop.com
- www.investaustralia.gov.au
- www.physicsweb.org
- www.smi-online.co.uk

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# Chapter 5 Surgery

Morten Bøgedal, Nordic Nanotech.

## 5.1 Introduction

Surgery is one of the most physical and human dependent techniques within health care. In addition, it is one of the most emotive aspects of healthcare, as patients undergoing surgery are at their most vulnerable.

This chapter focuses on some of the tools and techniques used by surgeons when operating on people. It will describe the medical and health issues for surgery as a result of injury or illness and does not cover the field of plastic surgery in depth.

As you will read at the end of this chapter, the European Commission has given this area lot of attention by launching a great number of projects under Framework Programme 5. New initiatives are to be created also in the 6$^{th}$ Framework Programme.

## 5.2 Overview of surgery

Surgery has been practised for a long time, with surgical tools found from cultures as long ago as ancient Rome. With simple tools and techniques "surgeons" could provide critical help for injured persons. Of course this often created infection, toxicity and inflammatory problems due to the lack of sterile clinical conditions or of knowledge in how to prepare tools. However tools were developed for almost maximum effect and surprisingly enough some of the designs from back then can be found in present instruments.

The parallel research and development of tools and equipment on one side and surgery techniques on the other, has led to massive improvements in the surgery field. Tools are now very well developed and every factor for securing a patient's health is taken care of- including the provision of a sterile environment around and within the patient.

Recent developments such as laser surgery have been used for the successful removal of cancerous tissue for example. In the following sections a set of surgical techniques and tools are described all of which incorporate a nanotechnology approach.

## 5.3 Laser Surgery

Laser surgery uses concentrated beams of light of particular wavelengths instead of instruments for surgical procedures. Lasers were first developed in 1960. Since that time, many modifications have been made and a number of different types of lasers have been developed. These differ in emitted light wavelengths and power ranges, as well as in their ability to clot, cut, or vaporize tissue. The most commonly used are the pulsed dye laser, the Nd: YAG laser, the $CO_2$ (carbon dioxide) laser, the argon laser, and the KTP laser. Each works in a different manner and each is sometimes used as part of a treatment plan. The laser works as following; when the laser beam hits tissue such as skin, a certain amount of light is reflected. The remaining light is absorbed or transmitted through the tissue. Absorbed light has specific effects, creating physical, mechanical, chemical, or temperature changes that result in the desired surgical effect. Lasers permit highly localized precise surgery with reduced blood loss. Moreover, a relatively dry operative field affords better vision for the surgeon.

## 5.4 Minimally invasive surgery

In what is generally referred to as open surgery, surgeons have traditionally had to make an incision large enough to expose the internal organs being operated on, i.e. to gain access to the body cavity, normal, healthy tissue is cut. Within the last decade, however, new minimally invasive surgical techniques have revolutionized many surgical procedures by enabling surgeons to access body cavities for procedures while reducing the trauma to overlying skin, muscles, and nerves that occurs with traditional open surgery. Most minimally invasive surgical procedures are performed with small optical instruments, such as endoscopes, and instruments placed through the body. The benefits of minimal invasive surgery are a decreased injury to skin, muscle, and nerves, fewer wound complications, easier postoperative pain management and quicker recovery and return of the patient to normal functioning.

## 5.5 Surgical tools

It is outwith the scope of this report to discuss all the surgical instruments available on the market today, varying from scalpels and knives to scissors and retractors. Usually these are made of stainless steel or tungsten but more recently titanium instruments have been introduced. Titanium benefits from its light weight and high tensile strength (45 % stronger than steel) producing a much lighter instrument and giving the surgeon better handling during an operation. Sutures are another essential medical device and have been used since the beginnings of surgery to close up tissues or compress blood vessels and prevent bleeding. Historically hair, animal gut and various textiles were used, which understandably caused many problems for the patient, however these have now been replaced by synthetic materials such as polyethylene and polypropylene.[72]

## 5.6 Impact of nanotechnology on surgery

The driving force nowadays is to make surgery progressively less invasive. By producing a new set of tools on the nanoscale one can perhaps imagine a surgeon being able to make changes to and track individual cells. This could be very beneficial for neurosurgical aspects for example and, in addition, the patient will benefit from the reduced trauma of even smaller wounds.

Gesellschaft für Diamantprodukte (GFD) have, through the application of nanotechnology, in a process termed "plasma polishing", created diamond scalpels with a cutting edge of only a few atoms (approximately 3 nm), that have applications in eye and minimal invasive surgery. The width of the scalpel blade is approximately one thousandth that of a metal blade (Figure 5.1) and makes these scalpels officially the smallest in the world (Guinness Book of Records).

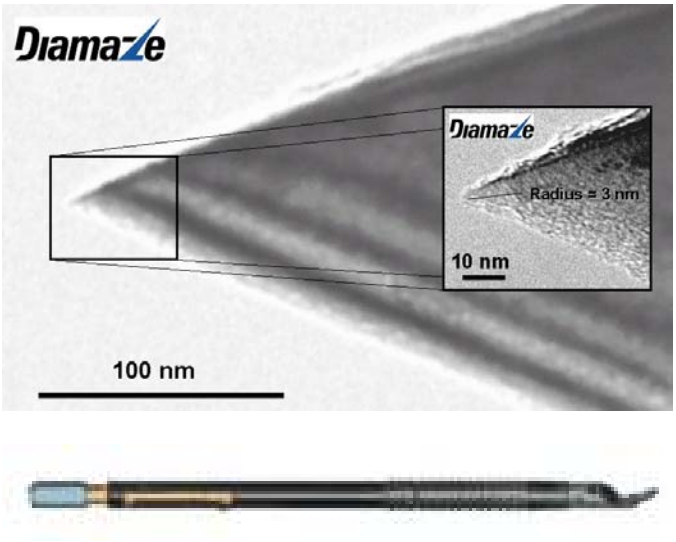


**Figure 5.1**. This picture shows the smallest diamond scalpel blade in the world (published in the Guinness book of world records) which was produced by GFD. The blade possesses 3 cutting edges and is only 0.12mm wide.

Minimally invasive surgery will also answer another demand, not only from surgeons but also patients, which is to make the operation as short and minimally traumatic as possible and reduce the length of time spent in the hospital. Another way to initiate this is to establish virtual surgical setups. The introduction of telepresence surgery has the potential to remove surgical limitations between countries and continents (and even between Earth and Space). However political, ethical and cultural differences between countries will need to be addressed to ensure that suitable procedures, acceptable to all parties, are established. A new chapter in history of medicine is Cyber Surgery (Figure 5.2).

When performing a surgical operation the surgeon works in a biological environment with corrosive or other effects. As a result it is essential that instruments are thoroughly cleaned and rinsed with de-ionised water before sterilisation, to avoid corrosion and pitting. Corrosion of an instrument can lead to its failure (e.g. not opening or closing properly) and can visually mask contamination, which in turn can spread infection to patients who are operated on. The use of titanium alloyed with nano-composites of aluminium and/or vanadium in new surgical instruments addresses this. Unfortunately such alloys are extremely difficult to machine and finish, currently making the prices for these products very high. Titanium is immune to corrosive attacks by laboratory saline solutions, blood and other bodily fluids, chlorides, oxidizing acids, alkalis etc., and it is 100% nonmagnetic, a great benefit when working with nerves, for example.

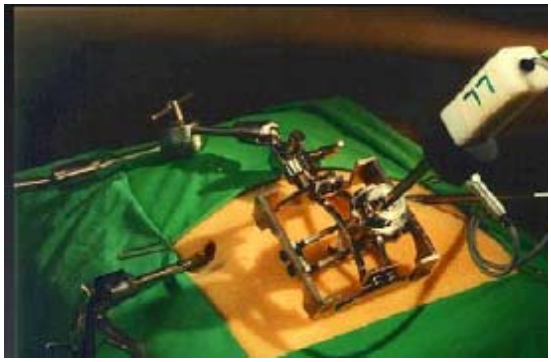

**Figure 5.2**. Typical cyber surgery setup from the VRvis Research Center.

Not all surgery techniques will be influenced through the production of better tools. For example sodium-2-mercaptoethane sulphonate (or MESNA) has been described recently as a "chemical dissection agent". This compound breaks disulphide bonds between proteins allowing tissue to be pulled apart more easily. As a result MESNA could potentially reduce surgery time, blood loss, trauma to local tissues and post-operative complications.[73]

## 5.7 Research that will impact future developments

Considerable efforts are being made in the creation of new tools for minimally invasive surgical instruments. Over the past decade, a number of ingenious instruments have been developed for manipulating individual cells, macromolecules and even individual atoms. One prime example is the scanning probe microscope, developed originally at IBM Zurich Research Labs. These tools have contributed in significant ways to advances in several fields of research, such as electron transport in molecules, protein folding mechanics and dynamic processes at cell membranes. Such research provides novel insight into the structure and function of molecules relevant to biotechnology and the surgery field.

Figure 5.3 shows silicon-chip based tweezers which have been made by microlithography and e-beam deposition. The probe spacing is less than 100nm.

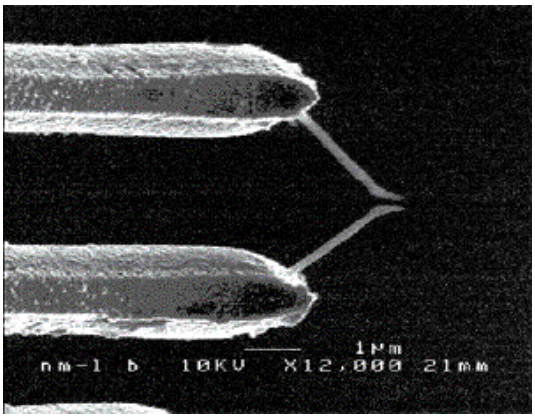


**Figure 5.3**. (MIC, Technical University of Denmark)

Virtual Reality (VR) interfaces to experiments have also evolved rapidly over the past decade, a notable example being the nanomanipulator at the University of North Carolina, the leading U.S. academic VR research center, which provides a virtual interface to a scanning probe microscope. This device has now been commercialised, and has been successfully used in teaching programs.

Another major development of the past decade has been the use of the Internet and PCs to control scientific equipment and analyse experimental data remotely. A prime example is the World Wide Web, which was originally developed by CERN for scientific purposes. Today, researchers are developing the GRID, a next generation of network applications that will greatly facilitate internet-based distributed computing and remote visualisation, including VR applications. This will be particularly important for surgical operations as it requires a lot of computer power to interpret and display images and data to allow the surgeon to make the correct decisions.

**Figure 5.4**. Center for Microscopy and Imaging microscope (University of North Carolina, Research, U.C. San Diego)

Figure 5.4 shows how telesurgery could be performed. The surgeon uses a haptic display that delivers force feedback from the operation scene. A simple example of how this works is the steering wheel found in some computer games, where a servo motor can make the wheel turn and vibrate just as a real wheel on a real car would do. In the nanomanipulator, the haptic device is a pen-like rod connected to several servos making the pen capable of 3D motion.

When connected to a computer with appropriate software, the pen can be used as a probe to "feel" the outline of simulated objects. If the device and the software are advanced enough, texture and elasticity can also be included. Instead of "feeling" a simulated landscape, the haptic device can be connected to an atomic force microscope (AFM). In this case, the AFM tip is moved in the same way as the pen, and the hand guiding the pen feels the surface magnified about a million times. If there are particles lying on the surface, the pen can be used to move them about. Once in a while, the AFM is run in tapping mode to image the surface so that the operator can see what has been created.

## 5.8 Conclusions

The role of nanotechnology in the field of surgery is still difficult to see clearly. Yet promising steps for minimizing invasive surgeries, involving nanotechnology matters, have been taken. Perhaps surgeons in the future will be able to perform wound and scar free operations. Also the field of telepresence surgical setups involving nanotechnology motors and manipulators will in the future play a significant role in society. Nanotechnology advances that produce new and smart materials, with specific useful properties, will feed into the manufacture of new surgical tools. Nanostructured tools which are more corrosion resistant and stronger, will both last longer and decrease the possibility of contamination. Improved biocompatibility and effectiveness would benefit the patient, doctor and society in general. In addition savings in cost would benefit hard-pressed health authorities.

## 5.9 Companies working in the field

**Gesellschaft für Diamantprodukte (GFD)**
GFD develops and produces diamond based products and is rapidly developing into a world wide leader in the sales of diamond micro-technology.
Contact: Dr. André Flöter
Phone: +49 (0) 731 505 4550
Email: info@gfd-diamond.com
Website: http://www.gfd-diamond.com/index_en.htm

**Kleindiek Nanotechnik**
Kleindiek Nanotechnik is entering new territories in micro and nano positioning. The Nanomotor®, invented by the company founder Dr. Stephan Kleindiek is a mini linear motor with a 3 mm diameter, 15 mm length and 10 mm stroke, and combines the highest precision with an extremely large working range.
Contact: Dr. Stephan Kleindiek
Phone: +49 7121 345 395-0
Email: kleindiek@nanotechnik.com
Website: http://www.nanotechnik.com

**NEWCO Surgical**
NEWCO Surgical is supplier of surgical instruments and accessories to Hospitals, Clinics, Medical Centres and Practitioners throughout the UK and provider of innovative products and services.
Contact: NEWCO Surgical
Phone: +44 121 3866 688
Email: sales@neco.co.uk
Website: www.newco.co.uk

**Fine Science Tools**
Fine Science Tools is a distributor of precision European surgical and microsurgical instruments to the scientific and biomedical research community.
Contact: Fine Science tools
Phone: (+49) 62 21 / 90 50 50
Email: europe@finescience.com
Website: http://www.finescience.com/

## 5.10 Research groups working in the field

**Nanohandgroup** at MIC, Danish Technological University, Denmark
The Nanohand group at the National Micro and Nano electronics Center in Denmark has many years of expertise in creating very small tweezers. They produce and develop "Nanotweezers" on microcantilevers.
A new multi-function sensing and manipulating nanotool - a nanohand - is being fabricated using microtechnology combined with direct-write freeform 3D nanofabrication, in collaboration with Lund University.
Two narrow fingers with customizable shape form the tweezer. The tweezer can be opened and closed using electrostatic voltages. By using force feedback (like atomic force microscopy) the device can image surfaces and nanostructures. The needles are conducting and can also be used for electrical measurement. A nanotweezer with a 20 nm gap has been produced.
They are collaborating with leading research groups in the fields of physics, chemistry and biology to apply the device.
Contact: Mr. Peter Bøggild
Phone: +45 4525 5723
Email: pb@mic.dtu.dk
Website: http://www.mic.dtu.dk/

**Research Group Surgical Therapy Technology**
The aim of the "Surgical Technology Research Group" is to improve the intraoperative support of the surgeon by advanced technologies and engineering methods explicitly including human factors analysis as an essential base of developmental work. In recent years they have focused their research activities in the areas: Orthopaedic Surgery, Neurosurgery, Maxillo-Facial Surgery and Minimal Invasive Endoscopoic Surgery in General Surgery, Gynaecology and Urology.
Contact: Dr.-Ing. K. Radermacher
Phone: (0241) 80 – 87626
E-Mail: radermacher@hia.rwth-aachen.de
Website: http://www.hia.rwth-aachen.de/research/cht/indexE.html

## 5.11 Overview of European projects, literature and web-sites

### 5.11.1 European projects

Project Acronym: **AB ( Algal bioadhesives)**

Objectives: Sessile marine organisms have evolved strong natural underwater adhesives, which remain attached under tensional conditions, comparable to the surgical environment. Algal adhesives could serve as tissue adhesives and could offer a safe and efficient alternative to traditional wound closure devices. The scientific objectives of this research are purification, characterization and gene expression of Algal Bioadhesives (ABs). They will be treated by a mutidisciplinary approach using physico-chemical, biochemical, molecular and cellular methodologies. The major deliverables of the study will be novel, surgical tissue adhesive compounds. The contribution to the socio-economic impact will be through improvement of the quality of life by better health care. The economic prospects include the development of mariculture and biotechnological industries.

Contact: DISHON, Josef (Mr)
Tel: +972-4-8515202
Fax: +972-4-8511911
Email: dishon@ocean.org.il

Project Acronym: **CLEANTEST**

Objectives: Testing and measurement procedure for the validation of the cleaning behaviour of reusable surgical devices.

Contact: ROTH, Klaus (Mr)
Tel: 49 (0) 7071 770 4242
Fax: +49 (0) 7071 770 4244
Email: info@smpgmbh.com

Project Acronym: **INTELLISCAF**

Objectives: With our ageing and more active population, there is an increasing demand for materials that can potentially replace, repair or even regenerate injured bone, cartilage and skin tissues. Present surgical or grafting procedures are only partly successful in restoring all the functions of damaged tissues. This RTD project is aimed at developing functional biomaterials and targeted at providing advanced nano-tailored materials via surface technologies and nanostructured particles.

Contact: STJERNQVIST, Soeren (Mr)
Tel: +45-72202001
Fax: +45-72202930
Email: info@teknologisk.dk

Project Acronym: **MENISCUS-REGENERATIO**

Objectives: A great number of Europeans are suffering from meniscus defects, which often cause persistent knee pain and may lead to osteoarthritis, knee joint instability and consequent deterioration of the quality of life of the patient. For years the standard treatment of a torn meniscus was complete resection but today orthopaedic surgeons are very keen on new techniques for replacing or regenerating this tissue. Over the past years, efforts have been made to develop a suitable meniscus substitutes to replace the degenerated meniscus, but no fully-suitable material/products exist to date. The main objective of the proposed research is to closely emulate the structure of the natural meniscus by using state-of-the-art tissue engineering technologies and highly biointeractive materials. By using a problem solving approach, the proposers will develop of a novel, bio-engineered, living meniscus reconstruction material composed of autologous cells grown on novel biodegradable and bioactive scaffold.

Contact: DE LUCA, Claudio (Dr)
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Fax: +39-049-8232752
Email: cdeluca@fidiapharma.it

### 5.11.2 Links for literature and websites

Online atlas of surgery: http://www.bgsm.edu/surg-sci/atlas/atlas.html
UK Medical and Health Website Directory: http://www.medic8.com/index.htm
Surgerydoor: http://www.surgerydoor.co.uk/
National library of medicine, Medline: http://www.nlm.nih.gov/
Cordis: http://www.cordis.lu/nanotechnolopgy
Nanoforum webpages: http://www.nanoforum.org/

# Chapter 6 Tissue Engineering

Michael Gleiche, Holger Hoffschulz and Volker Wagner, VDI.

## 6.1 Introduction

Severely damaged tissue, tissue loss or even the failure of an entire organ may lead to a life-threatening situation. Under these circumstances the basic approach in traditional medical treatment relies on the transplantation of organs (e.g. heart), artificial implants (e.g. artificial hip joint), and on the use of machines to maintain the vital functions (e.g. renal dialysis). These conventional treatments provide a basic life support, but they are not able to restore the primary tissue functions completely. Patients suffer from the low availability of donor organs, the effects of immunosuppressor medication after transplantation and the limited life-time of artificial implants, which have eventually to be replaced after a certain time due to material fatigue.

Therefore, the ability to repair or rebuild damaged tissue, or even to regrow entire organs in the future, increasingly has attracted social and economic attention. In the mid 1980s, a new field of science arose from this fundamental interest, and the idea of tissue engineering was born. According to Joseph Vacanti and Robert Langer, tissue engineering may be defined as follows[74]:

"Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function."

Tissue engineering consists of three components (see Figure 6.1):

cells to repopulate the damaged/missing tissue or organ.

growth factors, which are natural, physiological molecules that interact with cells and direct them to express specific proteins and therefore acquire specific functions.

scaffolds, which act as a support for the growth of cells and ensure that the correct form of the replaced tissue is achieved.

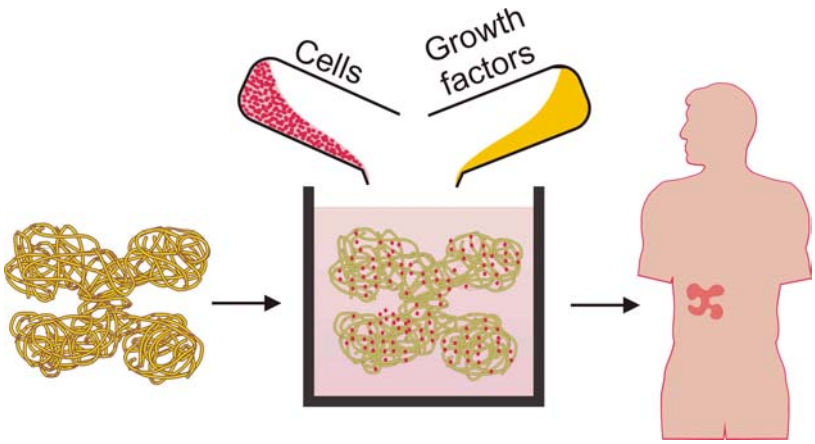


**Figure 6.12**. Schematic procedure of in-vitro tissue engineering. Cells are seeded in a suitable scaffold and cultured with the assistance of growth factors.

Taken from: http://www-2.cs.cmu.edu/People/tissue/tutorial.html

Cells can be sourced from within the patient, termed autologous transplantation, which consists of three steps: the extraction of suitable and healthy cells from the body, their culturing (proliferation) *in vitro* and finally the implantation of the *in vitro* grown tissue. Alternatively cells can be transplanted from another human (allogeneic) or another species (xenogeneic).[75] In this regard, human stem cells, embryonic and adult, have obtained increased significance, due to their self-regeneration capability and their pluripotency, or their ability to differentiate into various cell types. Actually, they represent the basic building blocks of every tissue and are thought to provide a route to the engineering of many different tissue-types in the future.

Growth factors are critical to the growth and differentiation of cells. As described in the section above, stem cells are pluripotent, however they are only able to differentiate into specific cell-types in response to their exposure to specific growth factors, at the correct concentration and in the correct order. For example the development of vascular endothelium requires the presence of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF), whereas bone development requires the presence of several insulin-like growth factors and neural tissue requires various neurotrophic factors (amongst other things).

Scaffolds have been used in tissue-engineering for a long time. Scaffolds can be defined as biodegradable polymers or other biocompatible materials, which are crafted into suitable structures or moulds in order to support the tissue regeneration. In this context they represent the extracellular matrix (ECM), which is present in every tissue and and acts as a three-dimensional support, interacting with proteins on cell surfaces. In addition, the ECM, is involved in specific interaction processes and is of significant importance for the regulation of cell growth and differentiation. Similar to the ECM, the artificial matrix or scaffold acts as a stabilizing construct which should assist the reconstruction of three-dimensional tissue by inducing and guiding cell growth, however it is unable to fulfill the more sophisticated tasks of the ECM. Scaffolds can be placed in the damaged tissue region where they degrade over time and are ideally replaced continuously by the growing tissue.

Although tissue engineering is a comparatively new sector of science, various products for different medical purposes can be found, ranging from different dermal substitutes to cartilage and simple bone implants. Nevertheless, all products, which are available at present, rely on the therapy of relatively simple types of human tissue as can be found in the human skin and cartilage[76]. In order to construct more complex human tissues several obstacles have to be overcome. Generally, living tissues are composed of a rather complex mixture of different cell types, with a specific spatial and functional dependency. These complex interactions have not been fully elucidated and cannot be copied in an *in vitro* environment. Furthermore, cells with a specific functionality tend to lose their ability to differentiate when they are cultured artificially. Finally, vascular systems are a key element in extended complex tissues (except cartilage), and progress in this area to integrate vascular systems in artificial, *in vitro* grown tissues is still at an early stage. That being said, researchers at M.I.T. have successfully implanted artificial one-layer kidney and liver systems with vascular endothelium into rats.[77]

The first tissue engineering products, such as skin and cartilage implants, are already on the market and their transplantation is almost routine, while cardiac and neurological tissues are being investigated in clinical laboratories. The potential market volume for tissue engineering is unknown, although values of about 80 billion euros for the year 2006 are found in reports [78] with annual market growth rates over 30%. Current costs are about 6000 euro for cartilage substitutes and about 1000 euro for skin substitutes[79].

Currently tissue engineered products have a restricted functionality, which allows the imitation of basic tissue properties. In this respect, nanotechnology and nanobiotechnology are thought to provide a route to improved tissue engineered products and a fundamental understanding of the elaborated functionality and complex interactions of cells.

## 6.2 Integration of materials, scaffolds and cells

As described before tissue engineering can be subdivided into cells, growth factors and the matrices that surround the cells and imitate the extracellular matrix (ECM). In accordance with the required tissue, different cells can be used for the tissue reconstruction, e.g. epithelial cells or a combination of keratinocytes and fibroblasts are suitable for a tissue engineered dermal implant, chondrocytes for cartilage and osteoblasts for bone replacements[80]. An alternative approach may be provided in the future by exploiting the pluripotency of stem cells. In this regard, an autologous possibility would be the use of adult stem cells, which can be extracted from the bone marrow of the patient. By controlling culture conditions (i.e. growth factors), these stem cells subsequently could be directed to develop the appropriate phenotype. The associated allogeneic possibility can be found in embryonic stem cells, whose tremendous capabilities have been discussed controversially[81].

Matrices or scaffolds are used to support and to frame cell growth to obtain a three-dimensional tissue. Suitable materials have to be nontoxic and biocompatible. They can be either biological or biogenous (assembled by living things) polymers such as collagen and polysaccharide, or synthetic polymers such as polylactic acid (PLA) and polyglycolic acid (PGA). Collagen is one of the most important fibrous proteins in the body and is widely used for structural enhancement in cells, cartilage and bones. It can be extracted from human or animal tissue and because of its natural origin, biogenous collagen is highly biocompatible and already in clinical use e.g. for dermal treatments. Despite these advantages, biogenous materials such as collagen can carry a risk of infections. In this regard, artificial polymers that are biocompatible, absorbable or biodegradable seem to be advantageous. Furthermore, they can be produced in large quantities without the problem of sourcing human material.

The polymers PLA and PGA are biodegradable and widely used for matrices or scaffolds. In addition, composite materials such as polylactic-glycolic acid (PLGA) may provide better conditions for cell culturing. Furthermore, the polymer degradation time can be adapted to the actual clinical indication by choosing the right material or composition.

"Bioreactors" are a means to achieve the conglomeration of cells into 3D structures *in vitro*. In their basic form bioreactors are vessels that contain cells and provide nutrients for cell growth. Cells within the bioreactor can be free in suspension or adhered to the vessel walls, or can be associated with a scaffold material. It is the latter that is receiving much interest in the field of tissue-engineering. For example, a team at the Carnegie Mellon University is developing advanced bioreactors that can incorporate the step-wise assembly of a scaffold, while simultaneously introducing cells to populate the scaffold.[82]

The following two products are examples of tissue-engineered products:

1) A dermal substitute obtained from allogeneic human fibroblast cells derived from newborn foreskin tissue is depicted in Figure 6.2. The cells were seeded onto a mesh made of PLA, which serves as a scaffold. The cells proliferate filling up the interstices of the mesh. This skin substitute is used for the treatment of diabetic foot ulcers.

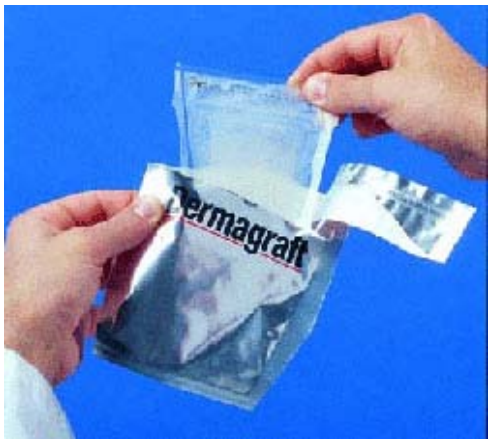


**Figure 6.13**. Dermagraft, this is a cryopreserved human fibroblast-derived dermal substitute manufactured by Smith&Nephew Inc.

2) The production of autologous cartilage requires a sample to be taken from the patient in a first step. The cells are cultured over 6 weeks and seeded onto a biodegradable polymer fleece. In a final step the fleece is implanted in the morbid cartilage region and degrades within half a year (Figure 6.3).

**Figure 6.14**. A three-dimensional cartilage implant derived from autologous chondrocytes: BioSeed®-C, BioTissue Technologies AG.

One of the issues of using autologous or xenologous cells and tissues is patient immune-induced rejection. This has been addressed historically through the use of immunosuppressors such as rapamycin, which depress the patient's immune system and decreases the likelihood of tissue rejection. However the use of such drugs can also allow adventitious infections to occur. An alternative approach can be found in the development of "biocapsules", which can be thought of as bridging the gap between tissue engineering and implants. Biocapsules consist of small clusters of cells (usually of animal origin i.e. xenogeneic) that secrete a therapeutic protein (e.g. insulin) and are enclosed by an artificial matrix. This matrix allows nutrients into the cells and the therapeutic protein to exit the biocapsule when it is implanted in a patient. The matrix also serves another important function in that it shields the cells from the host immune system and prevents rejection.

Another area of tissue engineering that is receiving a lot of interest is nerve regeneration. Recent work has shown that the co-culturing of severed neurones (nerve cells) with polymers such as poly(lactide-co-glycolide) and Schwann cells (which provide neurones with a protective, "insulating" sheath of myelin), promotes regeneration of the nerve endings. Experiments have been successfully translated into rat models, where severed sciatic nerves regenerated within a six week period.[83]

## 6.3 The impact of nanotechnology

Native tissues exhibit a complex hierarchical architecture ranging from macroscopic to nanoscopic dimensions. The arrangement of cells takes place in the micrometre scale, since a typical cell is at least several micrometres in width, whereas cell-cell interaction, which is mainly achieved by proteins (e.g. through protein expression), exhibits nanoscale features and is important for the formation of distinct tissue characteristics. Therefore, the engineering of tissues requires the ability to assemble cells in a specific three-dimensional configuration with a predefined chemical environment, with distinct functionality and nanoscopic precision.

The manufacturing of suitable scaffolds will be a key element in future tissue engineering, since the spatial arrangement of different cells, their distance and their interactions are important factors for the successful production of evolved tissues with complex functionality.

At present, the production of scaffolds is not directly affected by nanotechnology, but in the near future, the comprehensive analysis of the extracellular matrix (ECM) could be performed with the assistance of nano- and nanobiotechnological methods e.g. chemical analysis with biochips, topographical inspections with scanning probe microscopes (SPM) and transmission electron microscopes (TEM).

One approach to produce nanofunctionalised scaffolds can be found in the self-assembly (SA) of molecular systems as shown by Hartgerink et al.[84]. They describe the spontaneous aggregation of peptide-amphiphiles into cylindrical micelles at a low pH (Figure 6.4). These rod-like nanostructures resemble collagen molecules and can be used as a template for the mineralisation or crystallisation of hydroxyapatite[1] forming a composite material. A similar composition can be observed in natural bone, where collagen forms fibrils by self-assembling in triple helices, and hydroxyapatite crystals grow within these collagen fibrils[85]. Thus, the artificial scaffold shown in Figure 6.4 may be used for the production of bone substitute with nanoscaled functionality and superior quality.

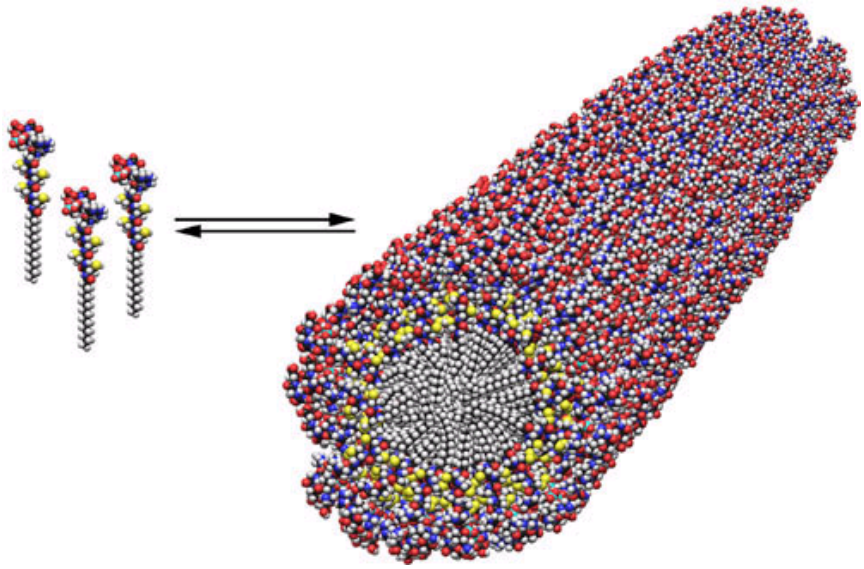

**Figure 6.15**. Left:The steric structure of the peptide-amphiphile. Right: schematic representation of the cylindrical micelle: a nanoscaled substrate for tissue growth. Hartgerink et al. 2001, http://www.matsci.northwestern.edu/stupp/jdh.html

[a] Crystallisation or the forming of minerals induced by biomolecules is commonly named biomineralisation.

Another approach to a nanostructured scaffold can be found in structured or grooved surfaces, which can influence or guide the cell growth down to nanometer dimensions. A surface with a topographical anisotropy can affect the direction of cell growth and proliferation[86]. In Figure 6.5, the impact of a nanogrooved surface on the growth of osteoblast cells is shown. The surface structures were obtained by a self-patterning coating process, in combination with the nanoimprinting technique[87] by Lenhert et al.[88].

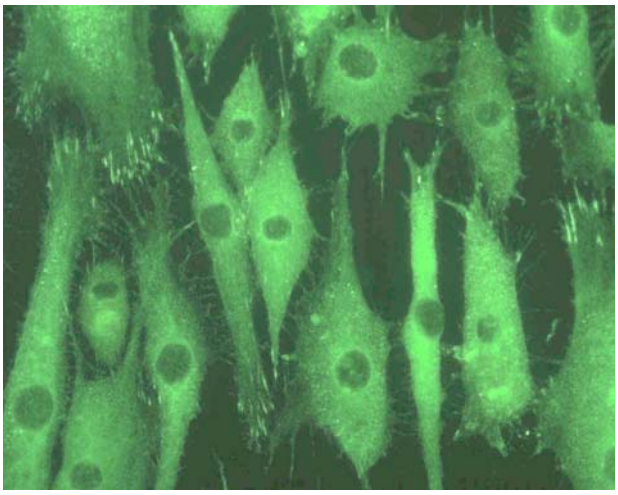

**Figure 6.16**. Osteoblast cells growing on a nanogrooved surface exhibiting elongated shapes and preferential orientations. The underlying grooves (pitch 500 nm, depth 150 nm) are not visible. S. Lenhert et al. 2003.

For the production of superior scaffolds for tissue engineering, micro- and nanostructured templates with chemical functionality will be required. Besides the analytical methods mentioned above, nanotechnology provides different approaches to produce topographical and chemical structured templates with micro- and nanoscaled features.

The molecular imprinting technique, for example, can be used to produce structured binding sites for specific proteins, with discrete distribution. As demonstrated by Shi et al.[89], this surface exhibits a structured and functionalised surface for protein recognition. Proteins incorporated in such a surface can influence cell differentiation and proliferation.

Furthermore, microcontact printing (μCP), electron beam lithography (EBL) and embossing techniques are suitable methods to produce micro- and nanostructured surfaces for biotechnology and tissue engineering[9091].

## 6.4 Research that will impact future development

Besides the lack of suitable and evolved scaffolds with micro- and nanoengineered functionality, the sourcing of appropriate cells seems to be one of the most delicate challenges in future tissue engineering. Primary cells, which are freshly sampled cells, tend to dedifferentiate when they are cultured *in vitro*, and lose their specific functionality during the continuous reproduction process, resulting in ineffective or even defective cells. Consequently, tissues cannot be engineered by proliferating a single cell line. Thus, there is a continuous and increasing demand for primary biological material. In this regard, embryonic or adult stem cells, with their unique properties of self-renewing and pluripotency, are thought to be promising candidates. There has been a long debate on the risks and ethics of stem cell research, especially on studying embryonic stem cells.[81] However by exploiting the unique properties of stem cells, the idea of producing "spare parts" for the body might become feasible.[92]

On the other hand, autologously derived cells represent an approach without ethical complications, but with the disadvantageous necessity of a relatively long production time of several weeks.

Nanostructures could form novel biomaterial coatings to improve the adhesion and biocompatibility of cells. The fabrication of nanostructures can be done by different methods like nanoimprinting or deposition of nanoparticles.

Nanotechnology can contribute also to the understanding of the self-assembling process of biocompatible materials. Areas to be investigated include advanced functional materials for interfaces to biological entities, the molecular recognition of these materials and the dynamics of growth. Templates for tissue growth need to be patterned on the nano scale. Lithography or new patterning methods have to be adapted with a high reproducibility and low tolerance to biological materials.

Nanotechnology can also contribute innovative analytical methods like AFM (Atomic force microscopy), confocal microscopy, etc. to characterize the material surfaces.

## 6.5 Conclusions

Tissue engineering is an interdisciplinary field of science covering cell biology, the chemistry of biomolecules, and the physical properties of biomaterials to medication and surgery. The main objective of tissue engineering is the reconstruction of damaged tissues and ultimately, the replacement of entire organs. Besides the use of embryonic stem cells, the autologous, or the use of the patient's own cells, approach to tissue engineering seems to be one of the most promising methods at present. Current products for tissue engineering offer a limited functionality and are restricted to the imitation of simple tissues (skin, cartilage). These limitations arise from the complexity of different cell types and their interactions in natural tissues, the dedifferentiation of cells in an *in vitro* environment and the lack of a vascular system in artificial tissues.

Although, the impact of nanotechnology on tissue engineering is in the early stages and mainly concerned with fundamental research, it is believed that it will promote the basic understanding of cell-cell interactions and enable the manufacturing of sophisticated scaffolds with nanoscaled functionality.

## 6.6 Companies working in the field

Despite the fact that the basic idea of tissue engineering is relatively long standing, it is still an emerging technology. Throughout the world, more than a hundred companies are working on all kinds of tissue-engineered products, ranging from materials for scaffolds to the final goods for medical treatment. As a result of limited knowledge, tissue engineered products are comparatively simple at present covering different dermal substitutes, cartilage and bone implants. Two examples of leading products are given below.

Apligraf®is a skin substitute from Organogenesis Inc., Massachusetts USA. Similar to the product "Dermagraft", mentioned above, Apligraf® is produced through culturing human fibroblast cells with collagen in a layered structure. The product resembles the human skin and protects the lesion from dehydration and infections, and stimulates natural cell growth. It is mainly used for the treatment of venous leg ulcers and diabetic foot ulcers.

Bioseed-S®, from BioTissue Technologies AG, Freiburg Germany is an autologous manufactured liquid skin substitute similar to the cartilage substitute mentioned above. A skin sample is taken from the patient and cultured in a laboratory for 2-3 weeks. The resulting skin is kept in a liquid environment, shipped to the patient and applied to the wound with a syringe. Besides the liquid skin product, the company provides a cartilage and a bone substitute (BioSeed®-C and BioSeed®-Oral Bone) which are manufactured in a similar way.

| Company/Link | Product/Description |
|---|---|
| Bio-Tissue, Inc., www.biotissue.com | Human amniotic membrane tissue (US) |
| BioTissue Technologies AG, www.biotissue-tec.com | Autologous skin grafts, bone and cartilage implants (DE) |
| FibroGen, Inc., www.fibrogen.com | Collagen, gelatin production (US) |
| Fidia group, www.fidiapharma.it/site/index_html.htm | Skin, cartilage and neuronal tissue regeneration (IT) |
| Geistlich group www.geistlich.com | Cartilage repair, bone substitutes (CH) |
| Isotis, www.isotis.com | Synthetic bone substitutes, fibrin-based autologous epidermal sheets (CH, NL) |
| Johnson & Johnson www.jnjgateway.com | Vast product range (US) |
| Laboratoires Genevrier, www.laboratoires-genevrier.com/Web/Public_eng | Skin substitute, autologous keratinocytes culturing (FR) |
| Minucells and Minutissue Vertriebs GmbH www.minucells.de | Culture accessories (DE) |
| Organogenesis Inc., www.organogenesis.com | Skin substitutes (US) |
| OsteoBiologics Inc., www.obi.com | Bioabsorbable tissue-engineered scaffolds (US) |
| Smith & Nephew www.smith-nephew.com | Skin substitutes, wound management (GB) |
| Synthecon, Inc., www.synthecon.com | Polymer scaffolds, culture systems (US) |
| TransTissue Technologies GmbH www.transtissue.com | Cell culturing, accessories (DE) |

## 6.7 Overview of European projects, literature and web-sites

### 6.7.1 European projects

#### Fifth Framework Programme (FP5) 1998-2002

NANOMED (Nanobiotechnology and medicine) investigates reactions of cells to polymer and other surfaces bearing nanotopography or/and nanopatterned chemistry. Upon the applications is the production of improved biomaterials for tissue engineering. NANOMED is coordinated by Susan Ferguson, University of Glasgow, Institute of Biomedical & Life Sciences, Glasgow, UK. (see: http://www.f5nanomed.org/)

INTELLISCAF (Intelligent scaffolds for tissue engineering of bone, skin and cartilage), coordinated by Soeren Stjernqvist, Danish Technological Institute, Materials Technology Department, Taastrup, Denmark. Aims are advanced nano-tailored materials via surface technologies and nanostructured particles for innovative scaffolds materials to regenerate bone, cartilage and skin tissues upon implantation. (see: http://dbs.cordis.lu/search/en/simple/EN_PROJ_simple.html, search for INTELLISCAF)

The main objective of the project ADIPOREGENERATION (Novel Technologies For Soft Tissue Reconstruction: A Tissue Engineering Solution Based On Biocmpatible Polymers And Adipocytes-Precursors Cells), coordinated by Dr. Emilio Mauro, Fidia Advanced Biopolymers SRL, Abano Terme, Italy, is to bring together new technologies of polymeric scaffold engineering and recent cell culturing techniques to develop a living and viable artificial adipose tissue to be surgically implanted for a more efficient treatment of large soft tissue defects. (http://dbs.cordis.lu/fep/FP5/FP5_PROJl_search.html search for ADIPOREGENERATION)

SCAFCART (Novel bioresorbable scaffolds and culture methods for cartilage tissue engineering) try to significantly improve the quality of tissue-engineered cartilage by combining optimised scaffolds with advanced culture methods. This project is coordinated by Dr. Pursglove, University of Sheffield, School of clinical dentistry, United Kingdom. (http://dbs.cordis.lu/fep/FP5/FP5_PROJl_search.html search for SCAFCART)

The main objective of MENISCUS-REGENERATIO (Innovative materials and technologies for a bio-engineered meniscus substitute) is to closely emulate the structure of the natural ***meniscus*** by using state-of-the-art tissue engineering technologies and highly biointeractive materials. The project is coordinated by Dr. Claudio de Luca, Fidia Advanced Biopolymers, Abano Terme, Italy. (http://dbs.cordis.lu/fep/FP5/FP5_PROJl_search.html search for MENISCUS-REGENERATIO).

**Expressions of interest, sixth framework programme 6 (FP6) 2002-2006**

BIOSMART (Smart Nanomaterials with Biorecognition Functions) was a proposal for a Network of Excellence in design and application of biomimetic materials and smart (nano)materials with biorecognition functions. One goal was new porous biorecognition materials for tissue engineering and growing of whole new organs. Coordinator was Dr. Sergey Piletsky, Institute of BioScience and Technology, Cranfield University, UK. (see http://eoi.cordis.lu/dsp_details.cfm?ID=37679)

SAM-MED-NET (Self molecular assembly in health and medicine) was a proposal for a Network of Excellence for a better understanding and predictability of self assembly. Progress was expected e.g. in tissue engineering. Coordinator: Dr. Frederic Cuisinier, Laboratoire dÓdontologie, Strasbourg, France. (see http://eoi.cordis.lu/dsp_details.cfm?ID=34635)

NA.BIO.MAT (Nano Scale Ordering in (Bio)polymeric Materials) was another proposal for self-assembling processes, here specialized to aqueous media. Within the applications are tissue engineering scaffolds. Coordinator: Prof. Gaio Paradossi, Dipartimento di Scienze e Tecnologie Chimiche- Universita' di Roma, Italy. (see: http://eoi.cordis.lu/dsp_details.cfm?ID=34559)

The Integrated Project proposal Nanoarchitecture (Biomaterials with Micropatterned Architecture for Tissue Engineering and Biomimetic Applications), coordinated by Prof. Vasif Hasirci, Middle East Technical University, Ankara, Turkey. Nanostructuring modification of biomaterials for tissue engineering and other biomimetic applications should be developed and applied to a group of tissues (cartilage, skin, ocular tissue and bone). (see: http://eoi.cordis.lu/dsp_details.cfm?ID=34835)

**Sixth framework programme (FP6)**

The Network of Excellence EXPERTISSUE (Novel Therapeutic Strategies for Tissue Engineering of Bone and Cartilage Using Second Generation Biomimetic Scaffolds) of 41 partners is selected for funding. The network is coordinated by the University of Minho, Portugal. The main aim of the proposed network of excellence (NoE) is to combat and overcome fragmentation of European Research in the field of tissue engineering of bone and cartilage.

**National programmes and activities in Europe**

The German Ministry of Research funds tissue engineering projects in its own programme (http://www.fz-juelich.de/ptj/contentory/index.lw?index=461). Three topics are mentioned in the call: design of molecules, design of cells and design of tissue and organs. The programme is not focused to Nanotechnology aspects.

The UK Centre for Tissue Engineering, UKCTE, was established in March 2001 following the award of £9.7 million from the Biotechnology and Biological Sciences Research Council, the Engineering and Physical Sciences Research Council and the Medical Research Council for a multi-disciplinary research collaboration between the University of Manchester and the University of Liverpool. Funded for a period of six years, its remit includes clinical research programmes in Skin/Wound Healing, Cartilage/Intervertebral Disc Repair and Vascular/Blood Vessel Replacement, as well as research into tissue engineering platform technologies including biomaterials, biocompatibility, haemodynamics, angiogenesis and gene transfer. The programme thus combines the best in biomaterials research with state-of-the art research in cellular and molecular biosciences. Endowed with an exceptionally strong team of scientists, engineers and clinicians, the UKCTE aims to develop internationally competitive basic science research to guide innovative tissue engineering applications in medicine.

Ireland has established the National Cell & Tissue Culture Centre (NCTCC) at the Dublin City University in 1987 as one of three specialist biotechnology centres, as part of the Government's first national biotechnology programme which later became BioResearch Ireland. It is recognised as the National Centre of expertise in animal cell biotechnology, and provides a range of research and technical services to industry, as well as delivering practical courses in animal cell culture for industry and for Irish researchers.

**Table 6.2** Links

| | |
|---|---|
| http://www.biotek.at/ | Austrian internet forum on tissue engineering |
| http://www.biomat.net/ | Biomaterials Resources on the Internet |
| http://www.tissueeng.net/ | Canadian internet forum on tissue engineering |
| http://www.shef.ac.uk/tissue-engineering | Centre for Biomaterials and Tissue Engineering, University of Sheffield |
| http://etes.tissue-engineering.net/index.php | European Tissue Engineering Society (ETES) |
| http://www.etrs.org/ | European tissue repair society |
| http://www.i-s-b.org/fokus/tissue/te.htm | German webpage related to the funding of Tissue Engineering |
| http://www.cartilage.org/ | International cartilage repair society |
| http://www.tissue-engineering.net/ | Internet forum concerning Tissue Engineering |
| http://www.med.nagoya-u.ac.jp/oral/jste/index.e.html | Japanese Society for Tissue Engineering |

| | |
|---|---|
| http://www.bioen.utah.edu/faculty/PAT/KCTE/index.html | Keck Center for Tissue Engineering, Department of Bioengineering at the University of Utah. |
| http://tissue.medicalengineer.co.uk | Part of the MedicalEngineer.co.uk site network |
| http://www.ptei.org/ | Pittsburgh Tissue Engineering Initiative |
| http://www.wmin.ac.uk/cter/welcome.htm | The Centre For Tissue Engineering Research, University of Westminster |
| http://www.fda.gov/cdrh/tisseng/TEMPS.HTML | Tissue Engineered Medical Products Standards (TEMPS) |
| http://wtec.org/loyola/te/final | Tissue engineering report, world technology evaluation center. |
| http://www.itelab.com/ | Tissue Engineering Research and Development Centre, Beijing China |
| http://www.terc.uwa.edu.au/ | Tissue Engineering Research Centre (TERC) |
| http://www.tces.org/ | Tissue and Cell Engineering Society |
| http://www.tesinternational.org/ | Tissue Engineering Society International Journal: *Tissue Engineering* |
| http://www.ukcte.org/ | UK Centre for Tissue Engineering, UKCTE, Manchester, Liverpool. |

# Chapter 7 Implants

Morten Bøgedal, Nordic Nanotech.

## 7.1 Introduction

An implant is an artificial structure whose purpose is to replace or stabilize damaged body functions and include many different types such as hip and knee joints (see Figure 7.1). Implants are classified as medical devices and as such are regulated in Europe by Directive 93/42/EEC. The technology behind implants has been achieved through years of research and development. Each year about 430 000 patients in the European Union have a total hip or knee joint replacement to provide relief from crippling joint diseases such as arthritis. However with everyday wear the implant surface degenerates, producing a tremendous number of predominantly sub-micron-sized polyethylene particles, and eventually leading to implant loosening. As a result the survival time of these implants rarely exceeds fifteen years.

**Figure 7.1**. Various implants. (National Geographic).

This chapter focuses on new technologies to develop and produce implants such as those for hip, knee and dental replacements. The Bone and Joint Decade[2] (BJD, 2000-2010) was launched on January 13, 2000 at the headquarters of the World Health Organization in Geneva, Switzerland. UN Secretary General, Kofi Annan said, "There are effective ways to prevent and treat these disabling disorders, but we must act now. Joint diseases, back complaints, osteoporosis and limb trauma resulting from accidents have an enormous impact on individuals and societies, and on healthcare services and economies."

The goal of the Bone and Joint Decade is to improve the health-related quality of life for people with musculoskeletal disorders throughout the world. Moreover BJD has to:

Raise awareness of the growing burden of musculoskeletal disorders on society.

Empower patients to participate in their own care.

Promote cost-effective prevention and treatment.

Advance understanding of musculoskeletal disorders through research to improve prevention and treatment.

All these actions by BJD may push forward the development of new types of joint materials.

[2] www.bonejointdecade.org

## 7.2 Overview of implants

Implants have been used for a long time, e.g. people have used gold for dental repair for more than 2000 years and the Aztecs used wood to fix broken bones. As early as the beginning of the 20$^{th}$ century other materials such as metals were used for implants. New developments and discoveries during and after World War II led to experiments with materials that are unfamiliar as present day implant-materials. Materials such as Dacron fabric were used for prosthesis in cardiovascular surgery and Plexiglas was used for artificial eyes. This caused many problems for users as the technology was not sufficiently advanced to minimize toxicity and infection. In addition, research and development of better implants at this time was very much on a trial and error basis. The implants were often made of regular materials on the market and not necessarily developed with the biocompatibility in mind. Later on materials such as titanium, cement and polymers were introduced to address biocompatibility problems. All of these improved the situation, however there are still problems such as wear debris and release of degradation products.

One key issue to address when developing successful implants, that remain functionally intact over a long period of time (20+ years), is how the new implant will interact with the body. This depends on the implant material, its biocompatibility and whether or not the mechanical properties will remain the same over time.

Below is a list of some of the most popular implants. These implants cover up to 85% of the total inserted implants worldwide.

### 7.2.1 Hip-joints

A hip joint consists of 2 major parts: a replacement ball and a replacement socket. The replacement ball (head) is usually made of metal or ceramic and the replacement socket is usually made of plastic (polyethylene). In younger, more active patients, one part (usually the socket) or both parts may be inserted without cement. If cement is not used, the surfaces of the implants may be roughened or specially treated to encourage bone to grow onto them. Sometimes it is possible to resurface the joint without removing much bone. For example, in metal-on-metal (MoM) hip resurfacing less of the femur is removed. This makes a future second replacement easier to carry out. As the name suggests, both the ball and the socket parts of the artificial joint are made of metal. MoM resurfacing is not suitable for people with a low bone density or osteoporosis.

### 7.2.2 Knee-joints

When having a knee joint replacement the procedure is often to put in a whole new implant. A picture of such an implant is shown in Figure 7.2. This type of joints is made of metal (usually cobalt chrome, but also stainless steel or titanium). Plastics may be used as a cartilage replacement between the thigh and shin bones. Also "half knee" (uni-compartmental) joints are available, which are less surgery intensive. Usually knee joints are cemented to the bones, although some joints are non-cemented by design.

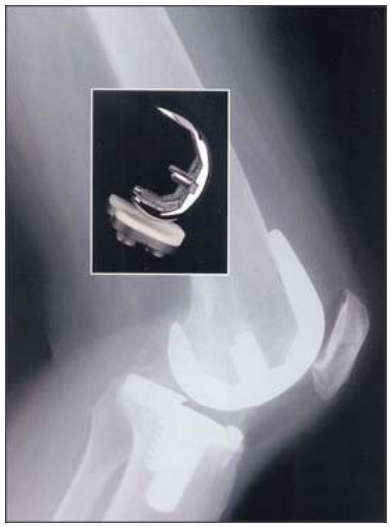

**Figure 7.2**. Artificial knee joint.

### 7.2.3 Spinal implants

Historically, spinal fusion has been performed with a bone graft without implants. However, the fusion rate with this technique is comparatively low, in the 50% range. Spinal implants consists of screws and axial connectors, which are usually made of stainless steel, however the use of titanium in spinal implants is on the increase.

### 7.2.4 Dental implants

Dental implants usually consist of metal (titanium, stainless steel) in combination with porcelain, ceramic or plastic.

## 7.3 Impact of nanotechnology on implants (products/ materials and components on or near market)

The application of nanotechnology to implants allows the structure or surface of a biomaterial to be precisely defined thus improving biocompatibilty.

Nanotechnology can also play a significant role in decreasing toxicity by creating a nano-coating on the implant, which reduces the effect of a corrosive environment. Optimising the coating not only avoids toxicity but also establishes a better interaction with the surrounding cells and tissue leading to a more stable and biocompatible implant. A nano-coated titanium or tantalum implant will therefore be better adapted to the human body and less likely to need to be subsequently removed and replaced by a new implant. It is however important that the coating does not itself cause problems by debonding

### 7.3.1 Biocompatibility

"Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application."

An implant should be biocompatible in order to stay in the body over time and interact properly with living tissue. Biocompatibility can be achieved in several ways. Either an implant can be made from a biocompatible material and then implanted directly. Otherwise, biocompatibility can be achieved by coating the implant with a biocompatible material, or modifying the surface in another way. Several technologies for surface modification are used, including self-assembled monolayers, lithography, microcontact printing, colloidal lithography and protein template imprinting. In addition, the implant can be chemically treated before use, e.g. for direct bone bonding.

A biomaterial is "A material indented to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body."

As one can imagine it is not sufficient for an implant just to be biocompatible, the implant also has to perform a physical task and function effectively in a way that does not hurt or irritate the wearer. The mechanical properties of an implant must be evaluated also when new technologies are used in development or manufacturing

### 7.3.2 Biomimetics

Biomimetics is the technology of biological growth of new bio-structures in a mould or directly in/on the injured bodypart. Researchers develop these structures from a single nano-physical or nano-chemical structure. A nano-physical structure is developed from a single nano-crystal, whereas a nano-chemical structure is developed from an array of large reactive molecules attached to a surface. The idea is to use these nanostructures as seed molecules, or seed crystals, to drive materials to grow by themselves (*in vitro* or moulding). Researchers hope that this will lead to much better biomaterials for new and better implants.

Other trends in the biomimetic field are the understanding of how specific body parts evolve and develop. Researchers are working on constructing replacement tissues such as muscle and bone using "tissue-engineering" techniques (see chapter 6).

Also the understanding of the biological functions of molecules e.g. biological motor proteins is now given lot of attention. The development of a molecular motor will aid the understanding of the biological process involved in muscle contraction and expansion.

At present biomimetic properties are often used in apatite coatings on e.g. titanium alloys to encourage bonding to natural bone. This thin layer mimics biological tissue and creates the proper bioactivity for bonding.

Other topics include research on how bonding of proteins and cells influence nanostructures and how this can be achieved for better biocompatibility.

New engineering possibilities for future biomaterials are depicted in Figure 7.3.

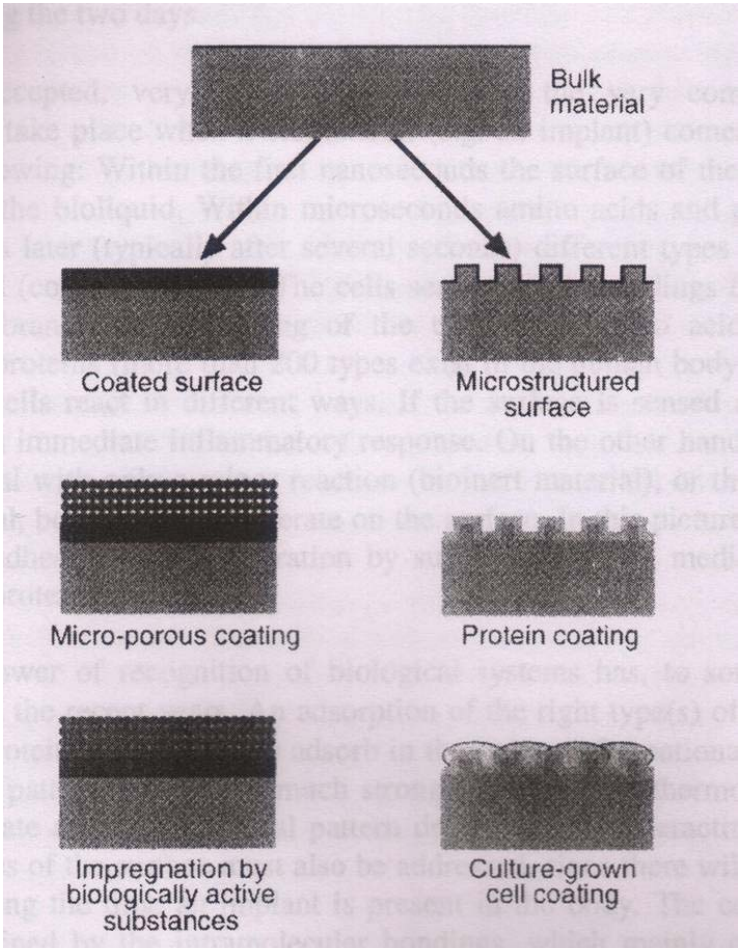


**Figure 7.3**. New engineering possibilities for future biomaterials (taken from B. Kasemo, Biological Surface science).

## 7.4 Conclusions

It is clear that nanotechnology will have a large impact on implants in the future. By developing techniques to create or prepare implants with new technology on the nanoscale we can even now see new medical devices such as implants with e.g. better biocompatibility leading to a better quality of life for patients. There are many ways of reaching these goals and therefore a lot of hot topics for researchers and companies. In the very near future this will result in new generations of implants, which, although some may be costly initially, will be less expensive in the long-term due to improved characteristics. With proper funding for research in place, society will realise these great prospects. Fortunately this funding is available in European communities through both the European Commission and many national initiatives.

## 7.5 Companies and important research groups working in the field

**NAMOS GmbH**
Nanotechnology and biomimetics on surfaces. NAMOS Ltd. uses simple procedures to produce complex and functional surface coatings. The company include a major part of their know-how not in the procedure, but in their "intelligent" material. The advantage: unique characteristics, simple production, low costs.

**Rebone Biomaterials Co., Ltd**
Rebone produces nanocement for regrowing of bone and teeth. The company produce a self setting nano-calcium phosphate cements (CPC). The cement seeks to replace such existing methods as autograft—taking part of a rib or other bone to repair a fracture, allograft—using Bone Bank donations, and synthetic replacement. As a highly adaptable bone substitute, CPC can be applied to orthopaedics, neurosurgery, plastic surgery and even dentistry.
Website: http://www.shei.gov.cn/qycp/q0523101.htm

**Sulzer Innotec**
Sulzer Innotec. produces Bio-materials and surfaces, with applications in: fluid technology; biomechanics and implants; medical systems; materials and failure analysis; testing and metrology.
Contact: Walter Wolfer
Phone: +41 52 262 51 00
E-Mail: walter.wolfer@sulzer.com
Website: http://www.sulzer.com/

## 7.6 Overview of European projects, literature and web-sites

### 7.6.1 Research groups

**iNANO**, University of Aarhus, Denmark
The interdisciplinary Nanoscience center at University of Aarhus has two very strong groups acting in this field. The Scanning Probe Microscopy (SPM) group and the Retroviral Molecular Biology (RMB) group both collaborate with respect to studies of cell adhesion on structured surfaces. The SPM group is internationally recognized for high-resolution STM-studies, in particular of dynamic phenomena on surfaces visualized in the form of movies. Moreover, the SPM group has broad expertise with respect to surface properties and their characterization. The RMB group is internationally recognized for work on viral gene transfers and virus-host interactions in cellular and animal models. Recent studies within iNANO of the interaction of biomolecules with surfaces include water adsorption in $TiO_2$ surfaces, adsorption of amino acids and other organic molecules on metal surfaces as well as parallel studies of cell adhesion using cryo-SEM and QCM-D.
Contact: Fleming Besenbacher
Phone: +45 89 42 36 04
Email: fbe@inano.au.dk
Website: http://www.inano.dk/

**Biocompatibles international**
Biocompatibles international is an international bio-materials group with headquarter in the UK, focused on the development and commercial exploitation of its biocompatible technology in healthcare – mainly based on phosphorylcholine coatings, which should mimic the natural the natural red cell membranes.
Website: http://www.biocompatibles.co.uk/

**University of Nottingham, centre for biomaterials and medical devices.**
The Centre investigates the interface between surface coatings (e.g. HA, DLC and TiN/TiC) and bone. Also haemocompatible multilayer coatings are developed here.
Website: http://www.nottingham.ac.uk/biomaterial/cbmd/

**University of Glasgow, Centre for cell engineering.**
A core theme in the work of the centre is the study of the creation of cells to topography.
Website: http://www.gla.ac.uk/centres/cellengineering/

**The AO Research institute**
The AO Research Institute is a non profit organisation doing research and development in the field of treatment of musculoskeletal injuries. Especially there is a group with the main focus directed towards understanding the interface reaction between soft tissue cells (fibroblasts), hard tissue cells (osteoblasts), or bacteria (staphylococcus Aureus) and implant surfaces. Another group focuses on better understanding of friction and wear of artificial implants like hip implants.
Website: http://www.ao-asif.ch/

**Chalmers, Department of applied physics**
In this group several of methods for producing micro and nanostructured surfaces are examined. Furthermore the QCM-D technique is used to characterize the adsorption of proteins and vesicles.
Website: http://fy.chalmers.se/ssf/biocomp.html

### 7.6.1 European Projects

Many European Commission supported projects have been initiated in these areas over the past years. To be introduced to these and get a brief overview of the area, you can find a short list with descriptions and contact information in the sections below.

**Biocompatibility/ Implant Material:**

Project Acronym: **ALUSI**
Objectives: Designing of an alumina alloy with improved wear resistance as new biomaterial for surgical implants (hip/knee).
Contact: DISHON, Josef (Mr)
Tel: +972-4-8515202
Fax: +972-4-8511911
Email: dishon@ocean.org.il

Project Acronym: **BIOKER**
Objectives: The aim of this project is to increase the life span of ceramic-ceramic knee and hip orthopaedic implants to 30 years by using a zirconia toughened alumina ceramicnanocomposite with a clearly improved suitability. This material is obtained by means of a new and innovative processing technology using power / alcoxide mixtures and a pressure casting forming technique (patent pending).
Contact: TARRACH, Rolf (Professor)
Tel: +34-91-5855000
Fax: +34-91-4113077
Email: presidente@orgc.csic.es

**Coatings:**

Project Acronym: **IGOID**
Objectives: Target of this project is set-up of a new bi-layer treatment on dental and orthopaedic prostesis. This treatment should improve prosthesis osseointegration by producing the following effects: Faster osseintegration, longer life of implants and formation of a stable barrier against Ti corrosion by biological agents. The coatings consist of a first TiC layer deposited according to a (Pat pend.) high energy process and a second polymeric resorbable layer containing hydroxyapacite particles and growth stimulating molecules (Patent. Pending).
Contact: MISIANO, Carlo (Dr)
Tel: +90-86-39062203
Fax: +39-086-3995311
Email: cetev@ermes.it

Project Acronym: **INCOMED**
Objectives: Innovative method of coating temperature sensitive medical implant objects like plastic, foils, cables, tubes e.g. with a thin layer of hydroxylapatite, bioactive glass or mixtures of both in order to receive biocompatible surfaces with good soft tissue anchoring.
Contact: SCHULTHEISS, Christoph (Dr)
Tel: +49-724-7824384
Fax: +49-724-7826126
Email: christoph.schultheiss@ihm.fzk.de

**Others**

Project Acronym: **BIOCERARP**
Objectives: New Generation Of Multi-Functional, Cost-Effective And Quick Set-Up Time System For Processing And Forming Ceramic Part Dedicated To Single Or Small Batch Production For Medical Applications.
Contact: VANDERMARCQ, Olivier (Mr)
Tel: +33-55-5426150
Fax: +33-55-5426155
Email: vandemarcq@ceramic-center.com

Project Acronym: **MICROSPRAYMED**
Objectives: Development and optimisation of microplasma spraying for biomedical applications
Contact: MANERO, Inaki (Mr)
Tel: +34-94-3635033
Fax: +34-94-3627727
Email: iontech@adegi.es

### 7.6.2 Acknowledgements and links for literature and websites

This chapter on Implants could not have been made without the help and support from the Director of the Interdisciplinary NANOscience Center (iNANO), Denmark Dr. Flemming Besenbacher and Ph.D Morten Foss. By giving access to various reports and important knowledge within the center substantial support was available.

**References and links for further reading**

[1] M.Foss F.Besenbacher, "Surface physical aspects of biocompatibility with emphasis on alloys of titanium and tantalum" 2003.

**Web-links**

Cordis: http://www.cordis.lu/nanotechnolopgy
Nanoforum webpages: http://www.nanoforum.org/
Smalltimes Magazine: http://www.smalltimes.com/
The Arthritis Research Campaign: http://www.arc.org.uk/
**The Dental Implant Home Page:** http://www.dental-implants.com/

# Chapter 8. Active Implants.

Ineke Malsch, MalschTechnoValuation.

## 8.1. Introduction

"Active implants are implants for the diagnosis or treatment of disease containing an energy source. The cardiac pacemaker is a typical and maybe the most common active implant."

(Source:http://webferret.search.com/click?wf,active+implants,,www.kth.se%2Fstudent%2Fstudiehandbok%2F03%2FKurs.asp%3FCode%3D7E1112%26Lang%3D1,,aol ).

Active implants fall into three broad classes. Cochlear implants and retinal implants are examples of implants which aim to restore perception, by transforming an external signal into an electronic signal which is intelligible to the human brain. The cardiac pacemaker, bladder stimulator and other neural stimulator devices are examples of active implants whose purpose is to restore control over bodily functions. Finally, implantable insulin pumps and *in vivo* diagnostic devices are examples of medical therapies that are moving from the outside of the body to the inside.

Not all of these active implants include nanotechnology, nanostructured materials or nanocomponents. In this report we focus on those active implants which do include nanotechnology, or for which nanotechnology improvements are being developed, e.g. in the form of bio-active coatings.

**Table 8.1**: overview of active implants

| Organ | Implant |
|---|---|
| heart | implantable cardiac pacemakers and defibrillators |
| | cardiomyoplasty cardiac assist devices |
| brain | deep brain neurostimulators for control of involuntary limb movement, e.g. treatment of Parkinson's disease |
| other nervous system | spinal cord stimulators for pain control |
| | dynamic gracioplasty and sacral nerve stimulators for control of bowel and bladder function |
| | vagal nerve stimulators for epilepsy seizure control |
| | phrenic nerve stimulators for respiratory support |
| | peripheral nerve stimulators |
| | implantable functional electric stimulators for limb/motor function |
| | implantable drug infusion pumps for control of pain and spasticity |
| ear | cochlear implants |
| eye | electronic eye |
| | retinal implant |
| other | implantable insulin pumps |
| | microchip implant (for animals) |

According to the European medical technology association, Eucomed, nanotechnology offers great potential for innovation in medical devices in general and some elements of (active) implants in particular. The nanotechnologies for (active) implants include advanced materials with nanostructured surface treatment. This surface treatment can enhance biocompatibility, promote tissue growth around the implant, and enhance the lifetime of the implants. New smart materials incorporating slow release drugs can also be used in coatings for (active) implants to fight rejection or inflammation. Finally, nanotechnology can be applied in implanted lab-on-a-chip devices that can supply diagnostic data to active implanted medical devices. Eucomed therefore wants to stimulate networking between its members and the emerging nanotechnology industry in Europe. (Eucomed, 2002)

According to Richard Moore, Director of Scientific Affairs at Eucomed, new generations of active implantable medical devices can benefit from "the integration of several different advanced technologies into a single product, e.g. electronic miniaturisation, advanced materials science and telematics". He foresees potential benefits for patients, but also expects a need for new EU legislation to ensure patient safety and a good investment environment: "potential area of concern from the regulatory viewpoint are emerging medical technologies such as nanotechnology and where within the regulatory framework these may fit. …work at the atomic, molecular and nano-levels has the potential to profoundly challenge 'traditional' regulatory demarcation boundaries."

(Scientist LIVE, 2003, www.scientistlive.com/cgi-bin/article.cgi?id=2346&magazine=emed&issue=1 )

The European NEXUS Network of Excellence in Microsystems and nanotechnology has developed a roadmap for MEMS development for Medical Devices. They expect that by 2006, new MST pumps and valves implant development will be taken up in active implants such as pacemakers. After 2008, new power and data coupling methods will be taken up in active implants with internal electrodes and sensors. They do not explicitly mention nanotechnology, only the related biomaterials as expected technological developments. (Nexus: Medical Devices USC Meeting, 5 March 2003)

### 8.1.1 Risks and ethical aspects

Active implants can cause severe risks to the bearers. Among these is a risk of injury from the interaction between active implants and diathermy ("deep heat" therapy) treatment. In 2001, the Medical Device Agency (MDA) of the UK issued a safety notice about nerve or tissue damage. If shortwave, microwave or ultrasound diathermy is used on a patient with an active implant, this can cause heating at the tissue/stimulation electrode interface. This in turn may lead to permanent injury or even death. (TGA news, October 2001) Similar problems have been reported with strong magnetic fields, including those used in MRI scans. Of course there is always the balance of risk and benefit, and even after control measures are put in place there will be some degree of residual risk. However, unless the benefit of using a device greatly outweighs the risk, such a device will not be placed on the market.

Active implants, especially neuroprosthetics, lead to fuzzy boundaries between humans and machines (cyborgs). Kevin Warwick, University of Reading (2002), has discussed the identity and privacy issues raised by biomedical implants, and has personally experimented with an active neural implant in his arm. Such integration of machine and person may lead to changes in personality and raises issues of control, e.g. does the person control the implant or the other way around? Can people or institutions, such as states, control other people through such implants? ("Big Brother is controlling you…"). An analysis of such issues goes beyond the scope of this report, however Nanoforum will discuss these and other risks and ethical issues related to nanotechnology and its applications in a future report.

## 8.2. Retinal implants

### 8.2.1. Overview of retinal implants

The eye transforms images, which form patterns in the incoming light, into an electrical signal that is transmitted to the brain. The light passes through the cornea, lens, and vitreous humor, all of which are transparent, onto the retina. The lens focuses the light to form an upside down image on the retina, which consists of five layers of cells covering the back of the eye. The top layer is made up of pigment cells. The second layer consists of 100 million photoreceptor cells, which transform the light into an electric signal. Each photoreceptor cell is connected to a bipolar cell, which passes the signal on to amacrine cells and finally to ganglia whose axons (or processes) constitute the optic nerve. The optic nerve transfers signals to the visual cortex of the brain. (source DOG, http://www.dog.org/)

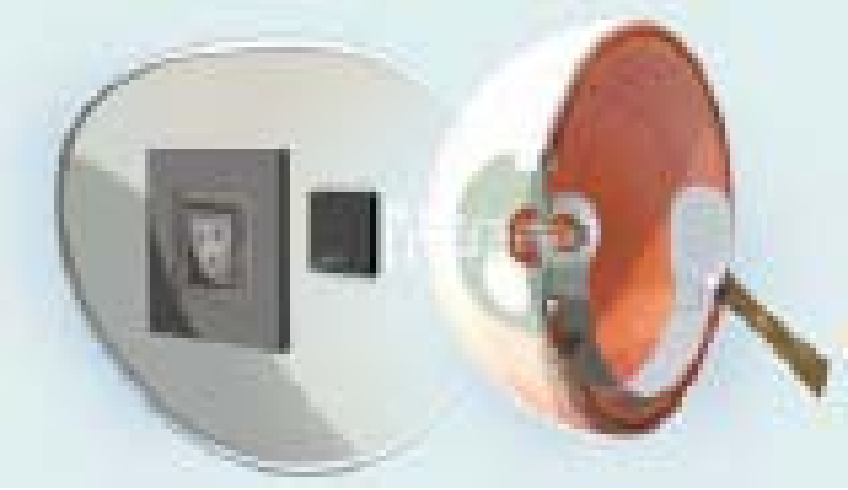

**Figure 8.1**. Image of retina implant, source IIP Technologies, http://www.iip-tec.de/

Retinal implants (Figure 8.1) are being developed to partially restore sight for blind patients suffering from diseases which destroy the photoreceptor cells of the retina at the back of the eye, but leave the visual nerve and visual cortex intact. These diseases include Retinitis Pigmentosa, Usher Syndrome, and Macular Degeneration. In the EU, there are 70-100,000 patients suffering from Retinitis Pigmentosa and 2.1 to 2.2 million patients with Macular Degeneration. (Source IIP-Technologies, http://www.iip-tec.com/english/index.php4?ID=48 )

Since the late 1990s there have been at least two fundamental retinal implant research projects in the USA and a further two in Germany, funded by the Federal Ministry for Education and Research. The German projects are the Epiret project, which aims to develop a retinal implant located at the back of the ganglion cells, that connects directly to the optic nerve; and the Subret project, which aims to develop a retinal implant located in place of the lost photoreceptor cells. The projects have been running in two phases between 1995 and 2003. (Source DOG, http://www.nero.uni-bonn.de/ri/retina-en.html, http://134.2.120.19/) PD Dr. Marlies Dorlöchter, project leader for both projects at DLR in Bonn, Germany, explains: "In the current funding period prototypes of retinal implants have been developed and successfully tested in animal eyes (Figure 8.2). A third funding phase is planned but not yet realized. In this potential funding phase the clinical application of an implant will be the main goal."

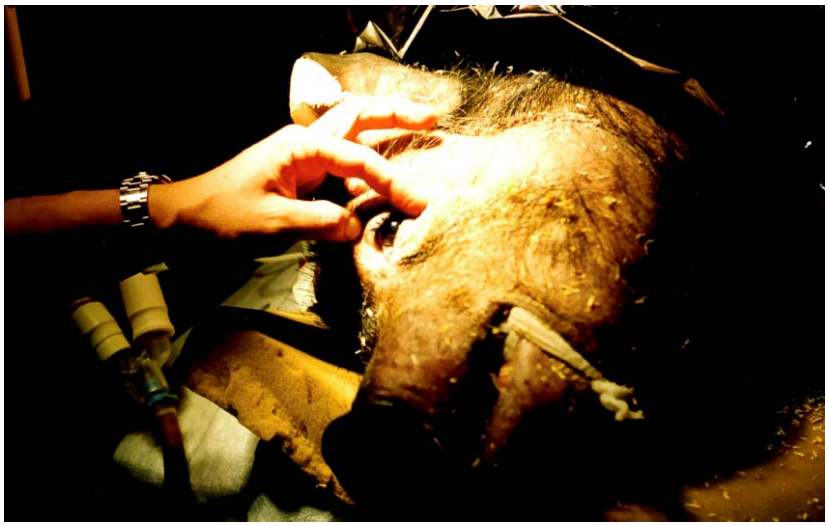

**Figure 8.2**. Surgeon controls position of retina implant after implantation in a pig's eye. Source: MPD-Array, the Subretinal Implant project, Germany, http://134.2.120.19/index_en.html

Currently at least one start up company in Germany, IIP-tec has started clinical trials of a retinal implant (Autumn 2003).

### 8.2.2. Impact of nanotechnology on retinal implants

Dr. Martin Stelzle, Head of Physical Chemistry & Sensors Group, NMI Naturwissenschaftliches und Medizinisches Institut in Reutlingen, Germany is working on the SUBRET project in Germany. He explains what nanotechnology can contribute to this technology: "We are using nanotechnology in the artificial retina implant, particularly to fabricate electrodes with enhanced charge transfer capabilities. The artificial retina implant requires highly localized application of electrical stimuli, i.e. electrodes with a small geometric area. At the same time, charge transfer requires high capacitance, which requires a large surface area of the electrode. These opposite requirements can be reconciled by employing a nanoporous electrode material, which provides for an effective surface area which may be by as much as a factor of 100 higher than the geometric area of the electrode."

### 8.2.3 Research that will/may impact future developments

Researchers at State University of New York at Buffalo and Stanford University have developed a silicon retina that can transmit high-speed optical data. The researchers aim to integrate the chip in robots, smart sensors and cameras in one to five years. Source: MIT Technology Review, 5 September 2003, http://www.technologyreview.com/articles/rnb_090503.asp

## 8.3. Cochlear implants

### 8.3.1. Overview of Cochlear implants

Cochlear implants have been available for 25 years or so. These devices, which are implanted in the inner ear can restore partial hearing in profoundly deaf people in both ears, including children over two years old. The hearing ear consists of an outer, middle and inner ear. The outer ear picks up acoustic pressure waves, which are transformed via a series of small bones in the middle ear into mechanical vibrations. These vibrations are passed to the cochlea, a fluid-filled, snail-shaped cavity, in the inner ear. The resultant pressure waves cause movement of hairs on the basilar membrane of the cochlea. The frequency and amplitude of these movements are specific to the sounds picked up and causes the release of an electrochemical substance, which in turn stimulates neurons to fire and transmit an electrical signal to the brain. The electrical signal includes information about which neurones have fired, which is correlated to the sound heard.

The cochlear implant can partly restore hearing in profoundly deaf people if the cause of deafness is the absence of the hair cells. As long as there are enough neurones that can pick up and transmit an electrical signal, the cochlear implant can replace the missing link in the hearing chain of the absent hair cells (Figure 8.3).

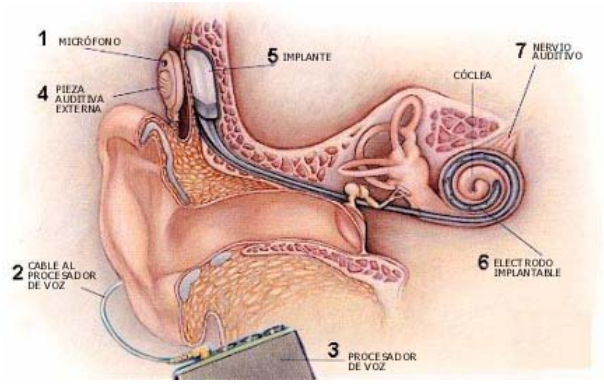


**Figure 8.3**. Cochlear implant schema, source: EURO-CIU, European Association of Cochlear Implant Users, http://eurociu.implantecoclear.org/implante_i.htm

There are different types of cochlear implants, however all consist of a microphone, a signal processor to convert the sound into electrical signals, a transmission system and a surgically implanted electrode or electrode array.

Several companies manufacture different types of cochlear implants, including Ineraid, Nucleus, Clarion and Med-El. Most of the devices use transcutaneous connections between the outside microphone and the implanted device. Following surgical implantation of the inner part, the skin is re-closed to prevent infection. Both parts of the cochlear implant are held in place by a magnet, and the signal transmitted through a radio frequency link between external and internal coils. However, there are two downsides: removing the inner part requires another operation, and the system is incompatible with external magnetic fields such as those used in MRI scanners, which could expose the patient to risk.

The Ineraid device uses a percutaneous connection whereby the implanted electrodes are physically plugged into the outside part, without extra signal processing elements. However, the device is implanted through the skin, creating the possibility of infections. (source: Loizou, 1998, www.utdallas.edu/~loizou/cimplants/tutorial/tutorial.htm )

There is an ongoing debate about the pros and cons of cochlear implants. Proponents are optimistic about the health risks of operatively implanting the device and believe that deaf children have better chances of leading a normal life and acquiring language skills as a result. Opponents highlight the risks of invasive surgery, which may cause dizziness and a mild form of eczema. They also point out hazards caused by strong magnetic fields such as applied in some jobs or involved in MRI scans. They also believe the implant and its associated medical care limit the ability of a child to lead a normal life with a high self-esteem. (See www.zak.co.il/deaf-info/old/ci-opinions.html )

The risk of developing bacterial meningitis after a cochlear implant has received attention recently from regulators and industry. A study published in the USA in mid 2002 by the Centre for Disease Control and Prevention and the FDA revealed that in a group of 4264 children, who had received cochlear implants before the age of six, 26 had contracted bacterial meningitis. Steps have been taken to mitigate this risk by ensuring prior vaccination of children, antibiotic treatment at the time of implant and removing from the market those devices that were associated with a higher probability of disease. (Source Advanced Bionics, 30 July 2003, http://www.advancedbionics.com/bionicnews/article.asp?ArticleID=120 )

### 8.3.2. Impact of nanotechnology on cochlear implants

It is apparent that the market for cochlear implants is already well established, however as described above, some problems with the technology remain. These include interference with strong magnetic fields, and the risk of infection, eczema or dizziness. According to Professor Helmut Schmidt of the Institute of Nanostructured Materials (INM) in Saarbrücken, Germany, nanotechnology can be applied in antimicrobial coatings on hearing aids including cochlear implants. His institute is developing nanostructured coatings including diffusable silver ions, which are released slowly from the coating and prevent infections in the ear. (Presentation at the 3rd NanoMed conference on Medical Applications of Nanotechnology, Berlin, February 17-18, 2003.)

There are two methods of administering antimicrobial remedies to the inner ear following cochlear implant operations: coatings or fluid-based drug delivery systems. Caroline Garnham and colleagues from Med-El have reviewed these alternatives (Garnham *et. al.*, 2002). They favour fluid-based systems because these are less invasive and easier to control, as they can be switched off from outside the body.

### 8.3.3 Research that will/may impact future developments

Cochlear and other active implants need electrical energy to function. Therefore innovation in cochlear implants would benefit from the development of better and smaller rechargeable batteries. The European LISA project has contributed to the development of new anode materials for such on-chip batteries. Project leader Professor Mino Green, Optical and Semiconductor Devices Group, Department of Electrical and Electronic Engineering, Imperial College, London explains: "An on-chip, lithium integrated battery (LiB), re-chargeable by an external coil, is highly desirable for various medical implants, e.g. cochlear. Achieving this goal is the ultimate aim of our work. In the more immediate future the objective is the fabrication of electrodes and the provision of electrolyte capable of being made into an on-chip battery, satisfying the designated specifications."

The highest charge density for the ANODE of a LiB is achieved by using silicon rather than graphite (C 837; Si12Li7 3780; Si5Li22 9280 mAhr/cc host). However, during discharge the volume of Si12Li7 expands by a factor of 2.13 and Si5Li22 by 4.12. Such volume changes cause systems with Si powder anodes to fail mechanically. Recent work (project LISA) on arrays of sub-micron diameter pillars ~1 micron high, fabricated on silicon wafers showed mechanical integrity after many insertion/extraction cycles. (see Figure 8.4, Mino Green, Elizabeth Fielder, Bruno Scrosati, Mario Wachtler and Judith Serra Moreno, "Structured Silicon Anodes for Lithium Battery Applications", Electrochemical and Solid-State Letters: 6, A75-A79, 2003).

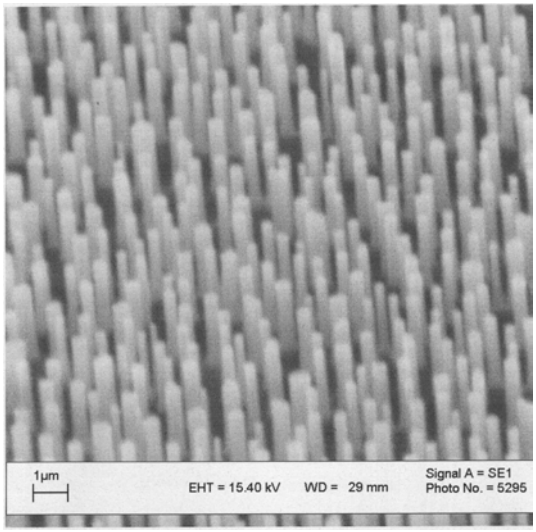


**Figure 8.4**. (Source: M. Green, Imperial College, London).

## 8.4. Neural implants

### 8.4.1. Overview of neural implants

"Neural prostheses are technical systems that partially substitute neural body functions after traumatic lesions or neurological disorders." according to Dr Thomas Stieglitz, Fraunhofer IBMT. Examples of neural implants include pacemakers, bladder stimulators, cochlear implants and implants for incontinent patients and Parkinsons patients.

Several neural prostheses currently available on the market or in development are based on microsystems technology. For neural implants which need to remain in the body for many years, long term stability of miniaturised systems is a major challenge. These active implants must be non-toxic and stable in a biological environment inside the body. They must also mimic the biological tissue near the implant to avoid irritation and rejection.

### 8.4.2. Impact of nanotechnology on neural implants

Dr Thomas Stieglitz of the Fraunhofer Institute for Biomedical Technology, in Ingbert, Germany is working on micro and nanotechnology for neural prostheses. His Neuroprosthetics group works on a broad range of fundamental research topics including bladder stimulation, upper limb prostheses (grasp), implantable stimulators, neural prostheses, retinal-stimulator and a lower limb neuroprosthesis.

According to him, "reactions between the artificial implant surface and the cells [of the human body] are mainly dependent on the material surface properties like surface energy, functional groups and on the nanostructure of the surface." His group has made topological surface modifications in the nanoscale range by Reactive Ion Etching, to change the cell adhesion on the implant. They are also focusing on other nanoscale surface modifications to improve the functionality of the electrical interfaces in neural prostheses, their fixation by biological means, and their long-term stability by defined topologies and cell-material interactions. (paper presented at the 3rd NanoMed workshop on Medical Applications of Nanotechnology, Berlin, 17-18 February 2003)

Dr Daryl R. Kipke, Department of Biomedical Engineering, University of Michigan is working on thin-film silicon and polymer neural probes for electrical and chemical interfaces to the central nervous system. "We have probe systems targeted for cortical, sub-cortical, and spinal cord targets. These are used actively in neuroscience research and we are beginning to translate the technology to medical applications." Nanotechnology aspects to this technology are nanostructured materials and nano-emulsions for bio-active coatings; micro-fluidics and device/tissue interface.

The company Biotronik in Berlin, Germany, is applying fractal coatings to heartbeat sensors and other cardiac devices such as pacemakers and defibrillators. They apply a coating of iridium to such a device, by inducing a fractal surface area through physical vapour deposition, down into the nanometre range. This way, the surface area is increased exponentially. Such a fractal surface has a higher capacity and leads to less inflammation of the surrounding tissue and a longer battery life of the device. It also does not involve any drugs.

## 8.5 Controlled drug dosage

### 8.5.1 Overview of controlled drug dosage

Patients suffering from some chronic diseases need to be injected regularly with medication. Diabetics are the best known group, and need to inject themselves several times a day with insulin to control their blood sugar levels. Other groups of patients who need frequent medication are sufferers of chronic pain and spasticity. These patients can benefit from an implanted drug dosage device which can release the drug they need in a controlled manner.

The US headquartered multinational company Medtronic, best known for their pacemakers, also manufactures drug dosage devices, in particular implanted infusion systems. The annual turnover of these devices is $100 million, equating to 12,000 items. The Swiss branch of Medtronic is collaborating with CSEM and the Institute for Microtechnology at the University of Neuchatel, both in Neuchatel, Switzerland, in a project to develop a new microsystem fluid measurement sensor, which can be included in new-generation implanted infusion systems. The prototype devices have already been developed (presented in April 2003) and Medtronic is Verifying and Qualifying the technology to prepare for inclusion in Implanted systems. In the last stage of the project, the company will conduct clinical trials together with the University Clinic of the Swiss Canton Waadt. The project receives funding from the Swiss Federal Office of Professional Education and Technology, MedTech programme (2000-2003).

The device consists of a hybrid fluid sensor that can measure a fluid throughput down to 1 ml per day with an error of 1%. The sensor is 10x6x1.2 mm in size and consists of two absolute pressure sensors at opposite ends of a capillary tube. The sensor is integrated with electronics and the whole device is 20x20x5 mm in size. The device is covered in a protective thin film to guarantee a life-span of seven years even under extreme pH conditions (2.5 to 9.5). (Source Swiss Federal Office of Professional Education and Technology, 4 April 2003, http://www.bbt.admin.ch/kti/success/d/med.htm)

## 8.6. Conclusions

Most active implants are still very much an emerging technology in themselves. Pacemakers were the first active implanted medical device and have been on the market since 1957. Cochlear implants have been around for 25 years. Others, such as the retinal implant, are still in the research or development phase and may become available for patients in the coming decade or later. Most of these medical devices utilise microsystems technology and do not include nanotechnology. However, nanotechnology can play a role in several elements of product improvement and future generations of active implants, including antimicrobial coatings, surface treatment and materials for new rechargeable batteries.

The nanotechnologies for (active) implants include advanced materials with nanostructured surface treatment. This surface treatment can enhance biocompatibility, promote tissue growth around the implant, and enhance the lifetime of the implants. One method applied in such surface treatment is Reactive Ion Etching.

New smart materials incorporating slow release drugs or particles (such as silver ions) can also be used in coatings for (active) implants to fight rejection or inflammation. Such coatings are being developed for coating cochlear implants, which may help to reduce the risk of meningitis for patients. In addition, Nano-emulsions are applied in such coatings.

Active implants include an energy source, such as a rechargeable battery. Nanostructured materials may be included in future generations of such batteries, because of their large surface to volume ratio.

Nanotechnology is applied in the artificial retinal implant, particularly to fabricate electrodes with enhanced charge transfer capabilities. A nanoporous electrode material provides for an effective surface area which may be 100x greater than the geometric area of the electrode.

Nanotechnology can also be applied in implanted lab on a chip devices which can supply diagnostic data to active implanted medical devices.

Generally speaking most active implants are highly effective devices, however post-marketing surveillance has shown risks to patients in some circumstances, which include heating of the device in an external electromagnetic field (such as in MRI scans), infections (such as meningitis for patients with a cochlear implant), and neural disorders such as headaches and dizziness. Current nanotechnology research appears to be focused only on solutions to the risk of infection (antimicrobial coatings). Other nanotechnology research is more aimed at product improvement in general.

## 8.7 Companies working in the field

### 8.7.1 Retinal Implants

**IIP-Technologies GmbH**
Niebuhrstr 1a
D-53113 Bonn
Germany
Tel: +49 228 96955-0
Fax: +49 228 96955-22
E-mail: mailto:info@iip-tec.de
http://www.iip-tec.de/
The company specialises in the development of innovative active implants. The first product they are developing is a retina implant. The company started the first phase of clinical trials in Autumn 2003, and hopes for market entrance in five years.
According to director Dr Holger Becker: "We are active in the development of a novel active medical implant, the Learning Retina Implant which will help people blinded by retinal degenerative diseases to regain modest vision by our implant. Such an implant only becomes possible with the aid of modern microtechnology, which we rely on heavily and, to a certain extend, what now constitutes a bit of a hype in nanotechnology. While the structuring methods are used typically for structural dimensions of a few micrometers, a lot of the action of the implant is determined by the interface between the technical microsystem implanted in the eye and the biological cells of the retina and many actions (particularly the electrochemistry) take place within a nanometer length scale. We do not see ourselves as a classical nanotechnology company, however effects in that range and also the development of biocompatible materials are of great interest for us."

**Second Sight, LLC**
Valencia, California, USA
www.2-sight.com
publicrelations@2-sight.com
Contact person: Dr Robert Greenberg, President and CEO

The company started in 1998 and is developing a retinal prosthesis for blind patients suffering from outer retinal degradation, e.g. Retinitis Pigmentosa or Macular Degeneration.

### 8.7.2 Cochlear Implants

**MED-EL UK Ltd**
Bridge Mills
Huddersfield Road
Holmfirth
HD9 3TW, UK
Tel: +44 1484 686223
Fax: +44 1484 686056
admin@medel.co.uk
http://www.medel.com/
MED-EL manufactures cochlear implants.

**MXMlabs**
Sofia Antipolis
France
http://www.mxmlab.com/index_en.html
http://www.mxmlab.com/digisonic/index.html
R&D and manufacturing of active implants including cochlear implants, neuro-stimulation implants, and opthalmology, since 1977.

**Cochlear Corporation**
Headquarters, Australia
http://www.cochlear.com/
Manufactures and develops Nucleus® cochlear implants. Claims to be the market leader, having achieved 50,000 cochlear implant systems in patients. The company has commercialised the cochlear implant developed by the inventor, Prof. Graeme Clarke, director of the Bionic Ear institute, University of Melbourne, Australia. He performed his first cochlear implantation in 1978, and has been awarded the Royal Society of Medicine fellowship in the UK (July 2003).

**Advanced Bionics**
USA
http://www.bionicear.com/advancedbionics/mission.html
Claims to be the only American producer of cochlear implants. They are also developing other neural implants for chronic pain and urinary urge incontinence. The company started in 1993 and manufactures the Clarion cochlear implant, which has been used in adults since 1996 and children since 1997.

### 8.7.3 Neural Implants

**ELA pacemakers**
ELA Medical BV
Bastiaan de Zeeuwstraat 8
NL-3227 AC Oudenhoorn

The Netherlands
Tel: +31 181 461455
Fax: +31 181 461527
Elamedical.holland@wxs.nl

**Biotronik GmbH & Co**
Berlin
Germany
http://www.biotronik.com/
Biotronik manufactures and develops electrical heart stimulation implants including pacemakers as well as (not active) stents. They have sold more than 1 million active implants. The company is a well established multinational (1963) and has 2700 employees worldwide. Headquarters are in Berlin, Germany. Biotronik is applying nanotechnology in fractal surface treatment of sensors for heartbeat and in defibrillators and pacemakers. They use physical vapour deposition to induce a fractal surface geometry on an iridium coated lead. (see Schaldach,1997)

**Litronik GmbH & Co**
Pirna
Germany
http://www.biotronik.de/content/detail.php?id=2565
Biotronik has a subsidiary, Litronik, which manufactures and develops Lithium Ion batteries for inclusion in pacemakers and defibrillators, applied in Biotronik's devices and for other customers. The materials used in Lithium Ion Batteries are structured in the nanometre range.
Litronik has 100 employees.

**Guidant**
MXMlabs
2720 Chemin Saint Bernard
06224 Vallauris Cedex
France
+33 493951818
+33 493953801
http://www.mxmlab.com/index_en.html
http://www.mxmlab.com/neuro/index.htm
R&D and manufacturing of active implants including cochlear implants, neuro-stimulation implants, and opthalmology, since 1977.
The company is experimenting with an active implant for the rehabilitation of paraplegia (Stand Up and Walk-project). Two implantations have been performed in 2003.

### 8.7.4 Controlled Drug Dosage

**Medtronic**
710 Medtronic Parkway
Minneapolis, MN 55432-5604
HQ USA
http://www.medtronic.com/

This multinational company manufactures and develops active implants and is active in 120 countries worldwide, with 30,000 employees. Products include pacemakers, defibrillators, neural implants and implanted drug delivery devices.

## 8.8. Overview of European projects, literature and web-sites

### 8.8.1 European projects

"NEURAL TISSUE ENG: Tissue Engineered Nerve Repair Devices: Development of European medical implantable devices and research training focus", 1 February 2000-31 January 2004; co-ordinator Ms Sarah Brooke, Kings College London, UK http://www.medinfo.dist.unige.it/nerve/

LISA: Lithium-in-silicon Nanostructured Anodes for On-chip battery applications (2001-2002). Contact: Professor Mino Green, Imperial College, London, mailto:m.green@ic.ac.uk

Joint Research Centre, Institute for Health and Consumer Protection, Biomedical Materials and Systems unit:
1) Medical Devices and Health Technology (MEDTECH)
2) Nano Biotechnologies for Health Application (Nano Biotech)
http://bms.jrc.cec.eu.int/prj.html
Consortium of companies developing drug eluting coatings on stents: http://www.biocoats.com/home.htm

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### 8.8.3. Websites

European Centre of competence for Biomedical Microdevices: http://www.medics-network.com/

Fraunhofer Institute for Biomedical Technology, http://www.ibmt.fhg.de/

Introduction to Cochlear implants: http://www.utdallas.edu/~loizou/cimplants/tutorial/

The Bionic Ear Institute, Melbourne Australia: http://www.medoto.unimelb.edu.au/index.htm

European Commission aims for co-ordinated effort in brain / neuroscience: http://europa.eu.int/comm/research/conferences/2003/brain/index_en.html

European Brain Council: http://www.europeanbraincouncil.com/

European Association of Cochlear Implant Users EURO-CIU: http://eurociu.implantecoclear.org/euroing.htm

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