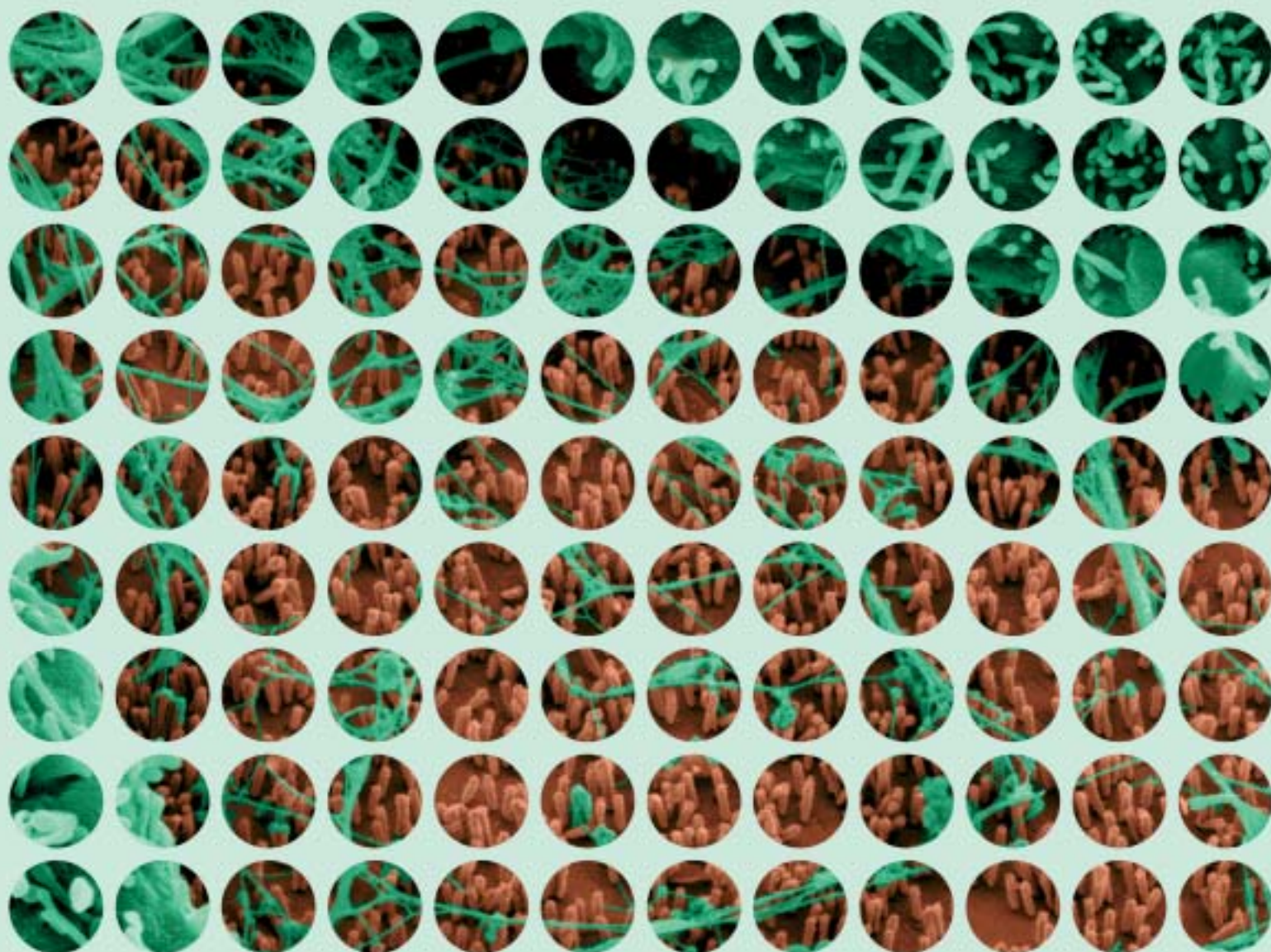


European Technology Platform on NanoMedicine

Nanotechnology for Health

Vision Paper
and Basis for a Strategic Research Agenda
for NanoMedicine

September 2005



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September 2005

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Executive Summary

The ageing population, the high expectations for better quality of life and the changing lifestyle of European society call for improved, more efficient and affordable health care.

Our improved understanding of the functioning of the human body at the molecular and nanometre scale as well as our ability to intervene at pre-symptomatic, acute or chronic stages of an illness are of utmost importance to meet these expectations. Diseases like cancer, diabetes, Alzheimer's and Parkinson's disease, cardiovascular problems, inflammatory and infectious diseases and depression are serious challenges to be dealt with. Nanotechnology applied to medical problems can offer impressive solutions. Early diagnosis, 'smart' treatments and the triggering of self-healing mechanisms are crucial targets on the way to regained health.

At present Europe has a strong position in the emerging field of NanoMedicine that has a high potential for technological and conceptual breakthroughs, innovation and creation of employment. NanoMedicine is an area that would benefit from co-ordination at European level. Thus, close cooperation between industry, research centres, academia, hospitals, regulatory bodies, funding agencies, patient organisations, investors and other stakeholders could dramatically boost this promising field.

In response to these challenges, scientific experts from industry, research centres and academia convened to prepare the present vision document regarding future research priorities in NanoMedicine. In particular, the following three research areas have been identified as a basis of a Strategic Research Agenda (SRA) in this field:

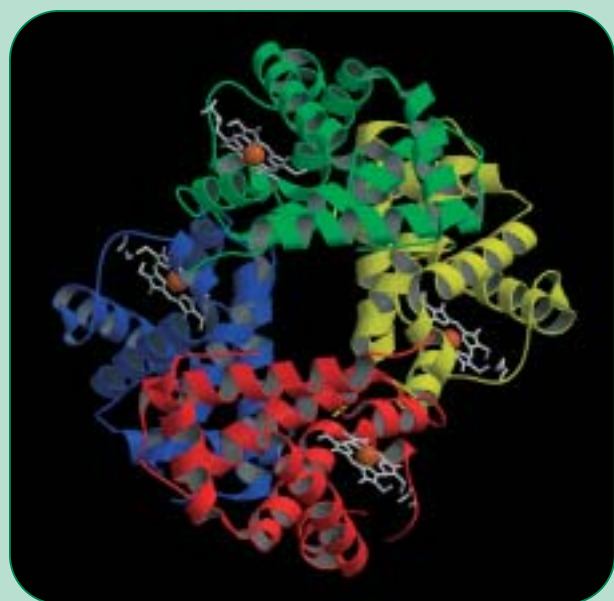
- Nanotechnology-based Diagnostics including Imaging
- Targeted Drug Delivery and Release
- Regenerative Medicine

A key conclusion of the preparatory group that elaborated this vision paper was the recommendation to the EU to set up a European Technology Platform

(ETP) on NanoMedicine. This ETP will identify the major socio-economic challenges facing Europe, in providing high standards of healthcare across the population, ensuring high quality of life, and focusing on breakthrough therapies, in a cost-effective framework.

Dissemination of knowledge, regulatory and intellectual property issues, ethical, environmental and toxicological aspects as well as public perception in general have also to be addressed by the ETP. Therefore, input from other stakeholders such as insurance companies, non-governmental organisations or patient organisations will play an important role in shaping up the final objectives of the ETP on NanoMedicine.

This European Technology Platform addresses ambitious, responsible research, development and innovation in Nanotechnology for Health to strengthen the competitive scientific and industrial position of Europe in the area of NanoMedicine and improve the quality of life and health care of its citizens.



Crystal structure of human deoxyhaemoglobin
© InformationsSekretariat Biotechnologie, 2005

1. Introduction

Definition: NanoMedicine, for the purpose of this vision document, is defined as the application of Nanotechnology to Health. It exploits the improved and often novel physical, chemical, and biological properties of materials at the nanometric scale. NanoMedicine has potential impact on the prevention, early and reliable diagnosis and treatment of diseases.

Artificial nanostructures, such as nanoparticles and nanodevices, being of the same size as biological entities, can readily interact with biomolecules on both the cell surface and within the cell (see Figure 1).

Nanomedical developments range from nanoparticles for molecular diagnostics, imaging and therapy to integrated medical nanosystems, which may perform complex repair actions at the cellular level inside the body in the future.

For the purpose of this Vision Paper, NanoMedicine encompasses the three interrelated themes of:

- nanodiagnostics including imaging
- targeted drug delivery and controlled release
- regenerative medicine.

In **nanodiagnostics**, the ultimate goal is to identify disease at the earliest stage possible, ideally at the level of a single cell. To achieve this goal, research and development activities in nanotechnology need to be undertaken to improve the effectiveness of in-vivo and in-vitro diagnostics. Nanotechnology can offer diagnostic tools of better sensitivity, specificity and reliability. It also offers the possibility to take different measurements in parallel or to integrate several analytical steps from sample preparation to detection into a single miniaturized device. Such a device could, thanks to nanotechnology, contain enough hard wired intelligence and robustness to be used by the patient and deliver a multitude of data to the practitioner. Furthermore, the use of nanoelectronics will improve the sensitivity of sensors based on already established methods.

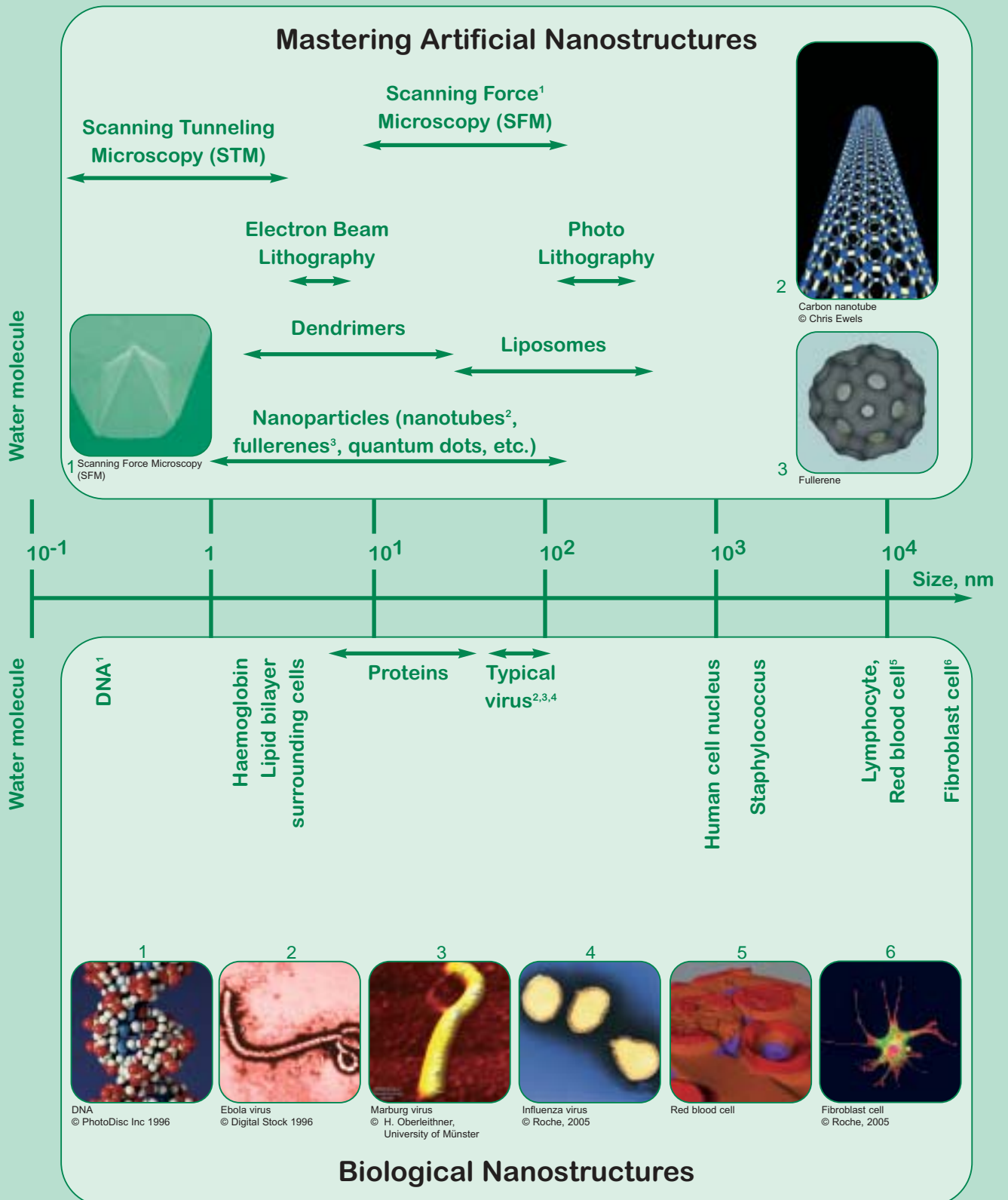
Improvements of microscopic and spectroscopic techniques towards ultra-high spatial resolution, molecular resolution and ultra-high sensitivity will provide a better understanding of the cell's complex "machinery" in basic research. The resulting progress should pave the way to more innovative and powerful in-vivo diagnostics tools. In general terms nanotechnology will have great impact on the methodologies available for both disease and drug discovery and consequently impact on the scope and throughput of pharmaceutical developments.

Advancement in in-vivo diagnostics will also rely on molecular imaging and on minimally invasive, implantable devices. In molecular imaging, the goal is to create highly sensitive, highly reliable detection agents that can also deliver and monitor therapy. This is the "find, fight and follow" concept of early diagnosis, therapy and therapy control, sometimes also known as theranostics. The tissue of interest is firstly imaged, using target-specific contrast nanostructures. Then, the targeting nanostructures, combined with a pharmacologically active agent, can be used for therapy. Finally, monitoring of the results of this therapy over time is possible by sequential imaging. Earlier and more reliable disease detection will be achieved by using better tracers and contrast agents in combination with better detection systems - progress in which is expected to come through combining existing imaging techniques.



© PhotoDisc, Inc. 1996

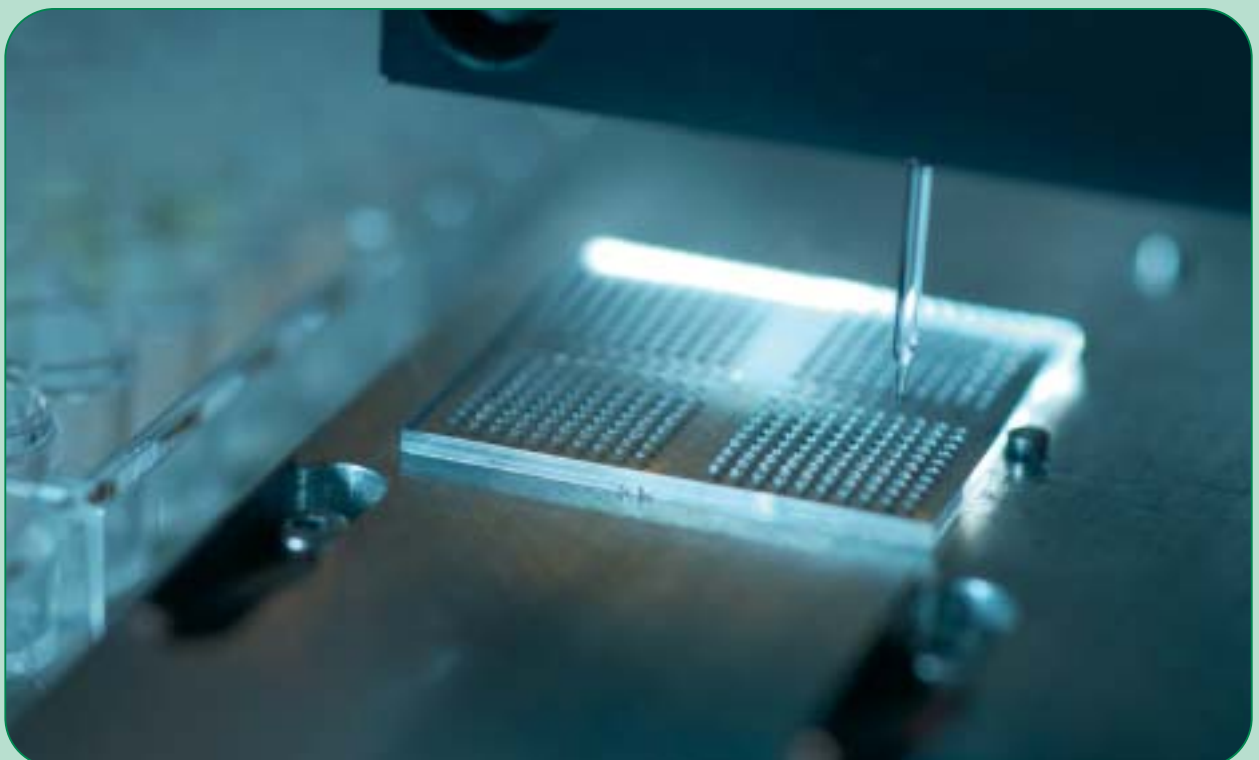
Figure 1. Artificial (top) and biological (bottom) nanostructures



1. INTRODUCTION

The long-term objective of **drug delivery systems** is the ability to target selected cells and/or receptors within the body. At present, the development of new drug delivery techniques is driven by the need on the one hand to more effectively target drugs to the site of disease, to increase patient acceptability and reduce healthcare costs; and on the other hand, to identify novel ways to deliver new classes of pharmaceuticals that cannot be effectively delivered by conventional means. Nanotechnology is critical in reaching these goals. Already now nanoparticle formulations make use of the fact that an enlarged surface/volume ratio results in enhanced activity. Nanoparticles are also useful as drug carriers for the effective transport of poorly soluble therapeutics. When a drug is suitably encapsulated, in nanoparticulate form, it can be delivered to the appropriate site, released in a controlled way and protected from undergoing premature degradation. This results in higher efficacy and dramatically minimises undesirable side effects. Such nanoparticulate delivery systems can be used to more effectively treat cancer and a wide range of other diseases, which call for drugs of high potency.

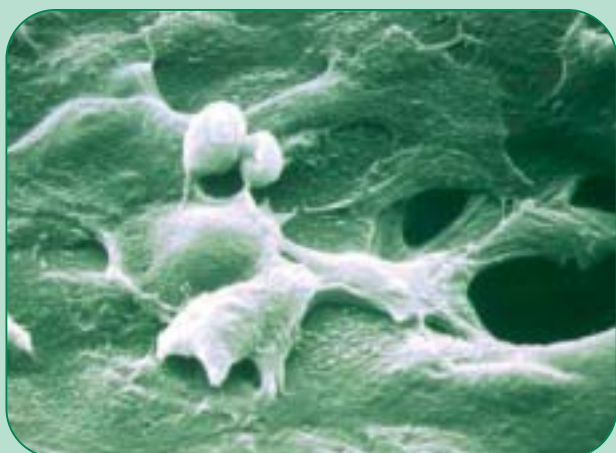
Drug-delivering microchip technology, resulting from the convergence of controlled release and fabrication technologies evolved for the electronics industry, is also benefiting from the application of nanotechnology. Further miniaturization and the ability to store and release chemicals on demand offer new treatment options in the fight against disease. A future vision is that nanoparticles will carry therapeutic payloads or genetic content into diseased cells, minimising side effects as the nanoparticles will only become active upon reaching their ultimate destination. They may even check for overdose before becoming active, thus preventing drug-related poisoning. In the past three decades, the number and variety of controlled release systems for drug delivery applications has increased dramatically. Many utilize polymers that have particular physical or chemical characteristics, such as biodegradability, biocompatibility or responsiveness to pH or temperature changes. In spite of many successful examples, the notion of combining polymer science with concepts from structural biology to provide new strategies and opportunities in the design of novel drug delivery systems adapted to



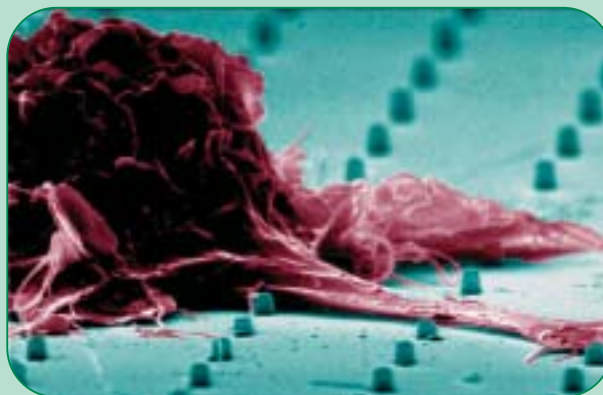
Droplets (50nl) dispensing robot
© P. Stroppa, CEA

today's demands, has not been fully embraced. In part progress has been slowed by regulatory submissions. It is critical for all nanoparticulates that drug safety is considered in parallel with efficacy.

The focus of **regenerative medicine** is to work with the body's own repair mechanisms to prevent and treat disabling chronic diseases such as diabetes, osteoarthritis, and degenerative disorders of the cardiovascular and central nervous system and to help victims of disabling injuries. Thanks to nanotechnology, a cellular and molecular basis has been established for the development of innovative disease-modifying therapies for in-situ tissue regeneration and repair, requiring only minimally invasive surgery. Rather than targeting the symptoms or attempting to delay the progress of these diseases, future therapies will be designed to rectify chronic conditions using the body's own healing mechanisms. To name some examples: facilitating the regeneration of healthy cartilage in an osteoarthritic joint, re-establishing a physiological release profile in diabetic pancreatic islets, or promoting self-repair mechanisms in areas of the central nervous system and of the heart. Nanotechnology can play a pivotal role in the development of cost-effective therapies for in-situ tissue regeneration. This involves not only a deeper understanding of the basic biology of tissue regeneration, but also identifying effective ways to initiate and control the regenerative process. This 'nanobiomimetic' strategy depends on three basic elements: intelligent biomaterials, bioactive signalling molecules, and cells.



Tissue engineered epithelium
© Fidia Advanced Biopolymers

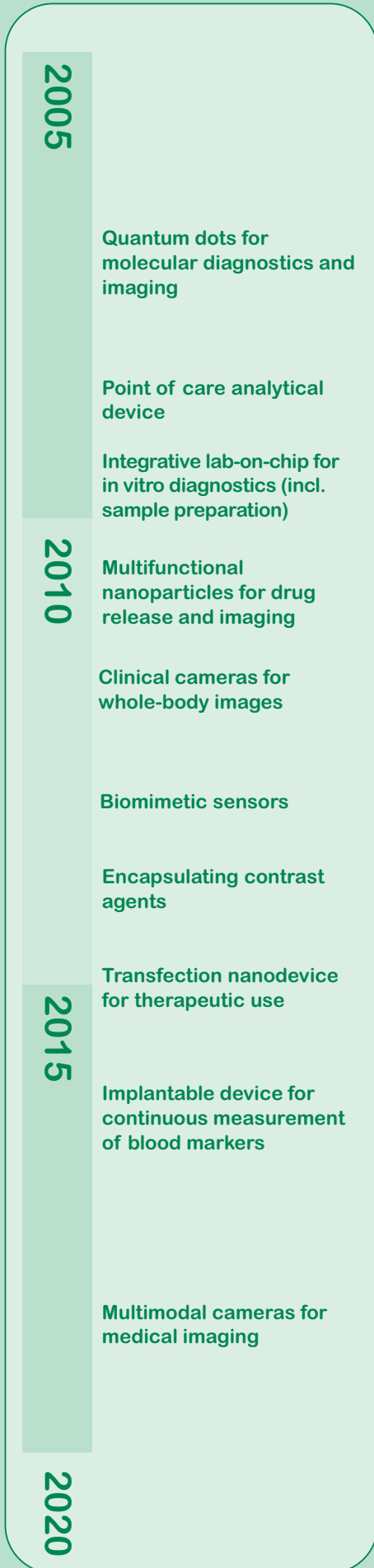


Fibroblast cell on nanostructured substrate
© Fraunhofer IBMT, St. Ingbert

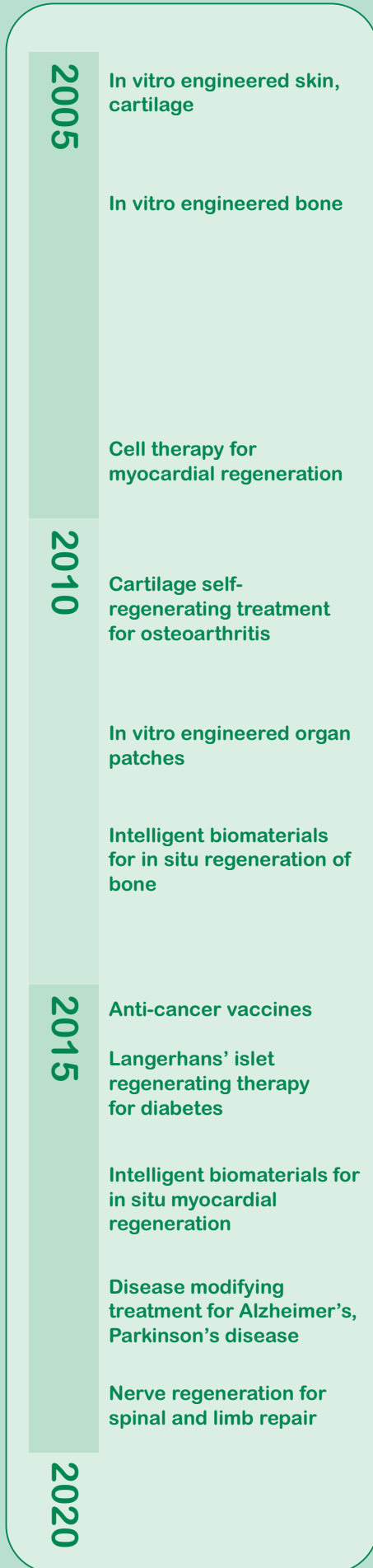
By 'tailoring' resorbable polymers at the molecular level for specific cellular responses, nanotechnology can assist in the development of biomimetic, intelligent biomaterials. These biomaterials are designed to react positively to changes in the immediate environment, stimulating specific regenerative events at the molecular level, directing cell proliferation, cell differentiation, and extracellular matrix production and organization. The sequential signalling of bioactive molecules, which triggers regenerative events at the cellular level, is necessary for the fabrication and repair of tissues. Nano-assisted technologies should enable the sequential delivery of proteins, peptides and genes to mimic nature's signalling cascade. As a result, bioactive materials are produced, which release signalling molecules at controlled rates that in turn activate the cells in contact with the stimuli.

Finally, a major focus of ongoing and future efforts in regenerative medicine will be to effectively exploit the enormous self-repair potential that has been observed in adult stem cells. Nano-assisted technologies will aid in achieving two main objectives – to identify signalling systems, in order to leverage the self-healing potential of endogenous adult stem cells; and to develop efficient targeting systems for stem cell therapies. Of huge impact would also be the ability to implant cell-free, intelligent bioactive materials that would effectively provide signalling to stimulate the self-healing potential of the patient's own stem cells.

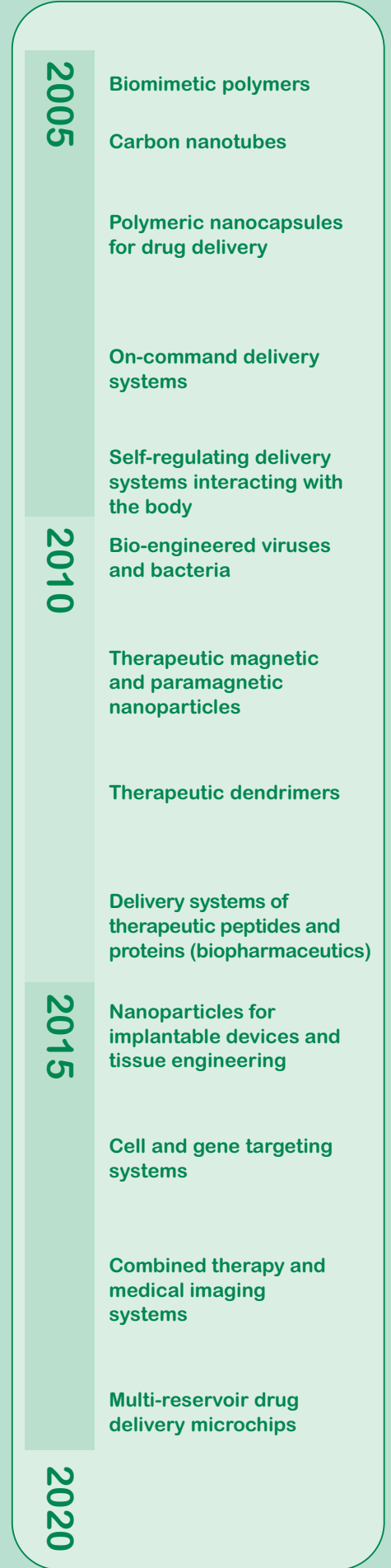
Nanodiagnostics timeline



Regenerative Medicine timeline

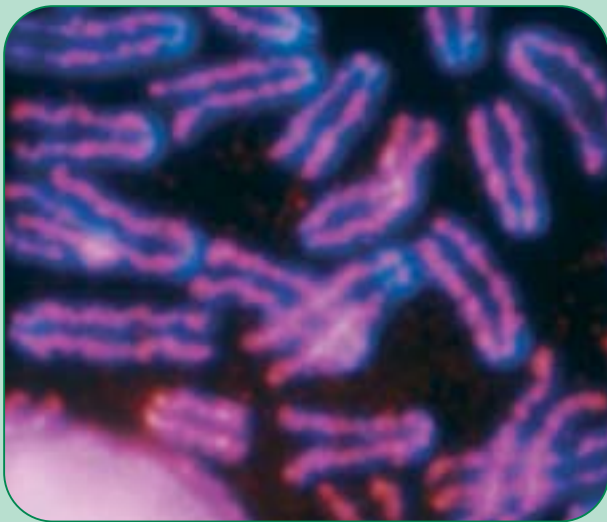


Targeted drug delivery timeline



2. Societal Impact

Disease areas which can be expected to benefit most from nanotechnology within the next 10 years are cancer, diseases of the cardiovascular system, the lungs, blood, neurological (especially neuro-degenerative) diseases, diabetes, inflammatory/infectious diseases and orthopaedic problems.



Fluorescence microscopy of labeled chromosomes
© P. Stroppa, CEA

Cancer is a complex disease involving a multitude of molecular and cellular processes, arising as the result of a gradual accumulation of genetic changes in specific cells. Nanotechnology-based highly efficient markers and precise, quantitative detection devices for early diagnosis and for therapy monitoring will have a wide influence in patient management, in improving patient's quality of life and in lowering mortality rates. Devices capable of bypassing biological barriers to deliver therapeutic agents with accurate timing and at locally high concentrations directly to cancer cells will play a critical role in the development of novel therapeutics.

Applications of nanotechnology to diseases of the cardiovascular system include the non-invasive diagnosis and targeted therapy of atherosclerotic plaque. Devices to monitor thrombotic and haemorrhagic events can have a high impact, e.g. in the diagnosis and treatment of stroke and embolisms. Multifunctional devices could detect events, transmit

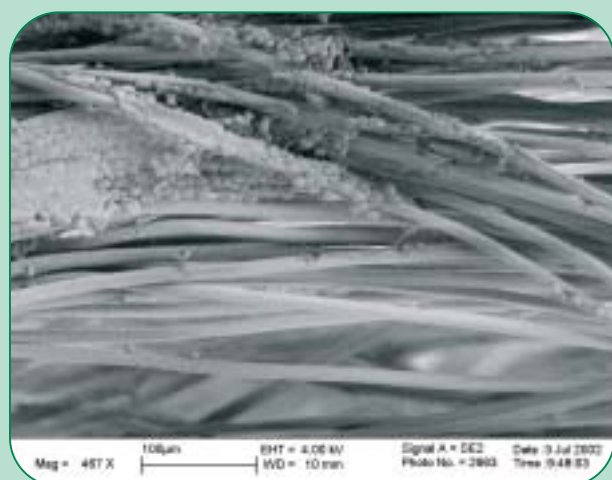
real-time biological data externally, and deliver anti-coagulants or clotting factors while the patient seeks further treatment.

Nanotechnology could also have a large impact in the area of blood purification/decontamination, based on intelligent sorbents and hemocompatible and immuno-tolerated implantable nanodevices, or on novel separation techniques using for example magnetic nanoparticles or carbon nanotubes.

Tissue repair and regeneration are other areas where nanotechnology could have great impact. For example, biodegradable nanoparticles which release appropriate growth factors and angiogenic factors could improve the bioengineering of heart or lung tissue or the production of vascular grafts to build functional tissue.

Lung inflammatory disease represents another likely target for diagnosis and therapy utilizing nanotechnology. Therapeutic nanoparticles capable of sensing alveolar function could release drugs only when needed, restricting drug delivery to disease-affected areas.

The brain represents one of the most complex systems in biomedicine. With an improved understanding of brain function, nanotechnology offers better

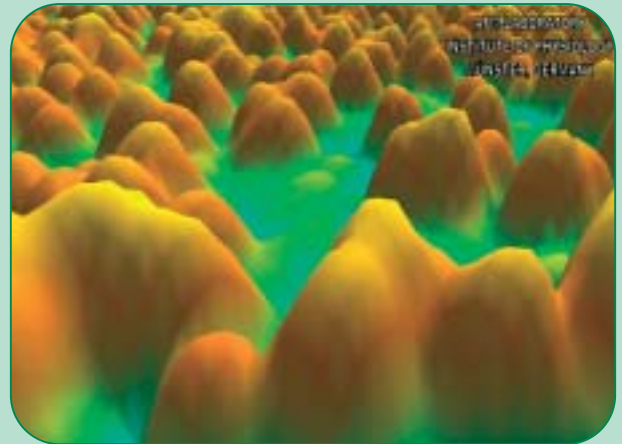


Engineered tendon
© Fidia Advanced Biopolymers

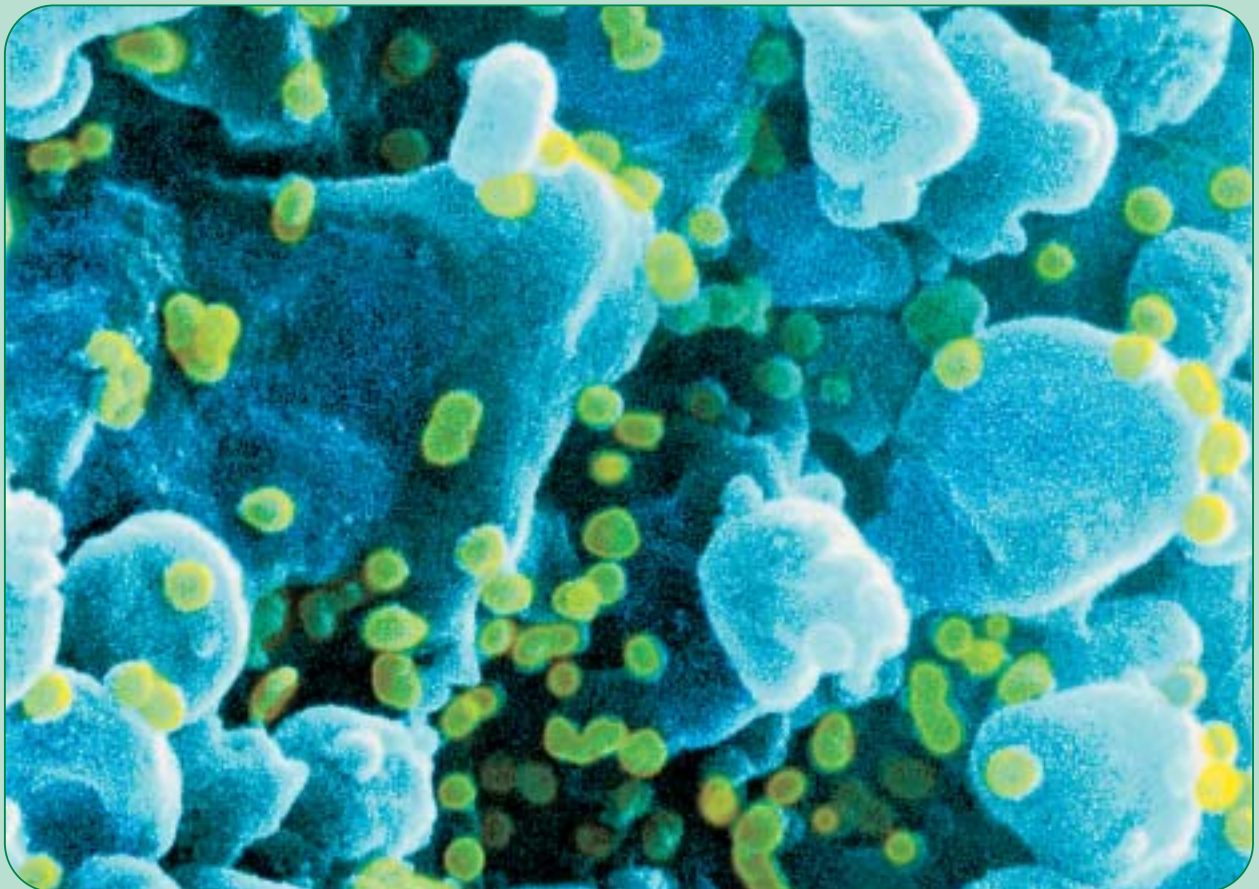
2. SOCIETAL IMPACT

diagnosis and treatment for neurodegenerative diseases like multiple sclerosis, Alzheimer's disease and Parkinson's disease.

The risks and challenges of NanoMedicine comprise issues of toxicity and carcinogenicity, as well as long-term stability and excretion pathways for artificial nanostructures, and technological challenges in molecular manufacturing, quality assurance and eventually, the programmability of nanodevices. There are challenges in managing the interdisciplinary requirements that span traditional industries. The experts identified regulatory challenges in the areas of approval times, intellectual property protection and harmonization. As medical technology advances in general, many legal and ethical challenges will also need to be addressed.



Membrane Proteins, imaged by Scanning Force Microscopy
© H. Oberleithner, University of Münster



Human Immunodeficiency Viruses budding out of T-cells, which play a large role in the immune response.
Transmission electron microscopy.
© Roche, 2005

3. Economic Impact

NanoMedicine is not only important to Europe from the social and welfare aspects, but also for its economic potential. It includes all products that can be defined as '*systems and technologies for healthcare, aimed at prevention, diagnosis or therapy*'. Little market data is published specifically about NanoMedicine at present. However, an analysis of the market segments for **medical devices** and **drugs & pharmaceuticals** gives an idea about the leverage of NanoMedicine on the markets. These two market segments represented in 2003 an end-user value of € 535 billion, of which the drugs segment is the most important, with a value of € 390 billion. Globally this market has been growing at a 7 to 9% annual rate, with variations according to country, technologies and market segments.

The **medical devices** market is expected to grow in value by about 9% annually at present. The introduction of novel nanotechnologies can be expected to give rise to a much higher rate, by providing innovative solutions and more precise care and new information for preventive medicine. The market can be further segmented into areas where NanoMedicine might have the highest potential of penetration, such as in-vitro diagnostic products, patient monitoring systems, imaging systems or imaging contrast agents.

In a medical devices market of € 145 billion in 2003, in-vitro diagnostic systems represented € 18 billion, or 13% of the total. It can be expected that nanotechnology will have an impact on this expanding market in the coming years, as it offers the potential of faster and more accurate analyses of smaller and smaller samples.

Medical imaging systems represent € 14.5 billion, or 8% of the total devices market. Imaging tools and imaging agents (including contrast media and radiopharmaceuticals) represent € 4 billion, or 3%. These segments will benefit from the application of techniques developed from an understanding both of materials and cellular activities at the nanoscale. Already the sale of tools dedicated to molecular clinical and preclinical imaging represents € 0.8 billion

out of the € 14.5 billion total, and the patient monitoring market represents € 1.5 billion.

NanoMedicine can also potentially affect aspects of all medical devices, for example new materials for surgical implants, nanometric systems for monitoring cardiac activities or minimally invasive surgery sensors.

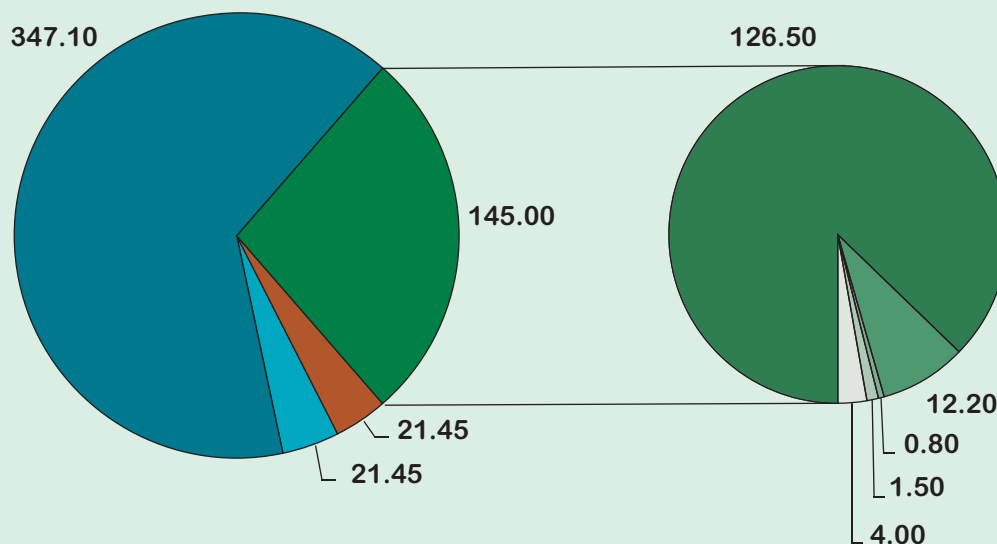
The worldwide market for **pharmaceutical drugs** has been growing at a rate of 7% in 2004. The drug market can be segmented, with the global market for advanced drug delivery systems accounting for € 42.9 billion or 11% of the total. Approximately half of this market is in controlled release systems, with needle-less injection, injectable/implantable polymer systems, transmucosal, rectal, liposomal drug delivery and cell/gene therapy responsible for the rest, and is estimated to reach € 75 billion in 2005. Developments in this market are rapid; especially in the sector of alternatives to injected macromolecules, as drug formulations seek to cash in on the € 6.2 billion worldwide market for engineered protein and peptide drugs and other biological therapeutics.

When reviewing the economic potential of NanoMedicine, all the biotech companies must be considered as they are directly involved in the development of new molecules, and also in the development of new tools for accelerating the discovery of



Camera for magnetic resonance imaging.
© C. Boulze, CEA

Drugs and Medical Devices Market (€ billions)



Pharmaceutical drugs (€ 390 billion) and Medical devices (€ 145 billion)

- Controlled release systems
- Other advanced drug delivery systems
- Other pharmaceutical drugs
- Medical devices
- Other medical imaging systems
- Molecular pre-clinical and clinical imaging
- Patient monitoring market
- Imaging tools and imaging agents
- Medical devices other than imaging

appropriate molecules. Today half of the new molecules discovered worldwide come from biotech companies. There are more than 4,000 worldwide, with over 300 companies in the US actively working on developing drug-delivery platforms, including therapies targeted to the site of the disease, as well as drug-containing implants, patches and gels.

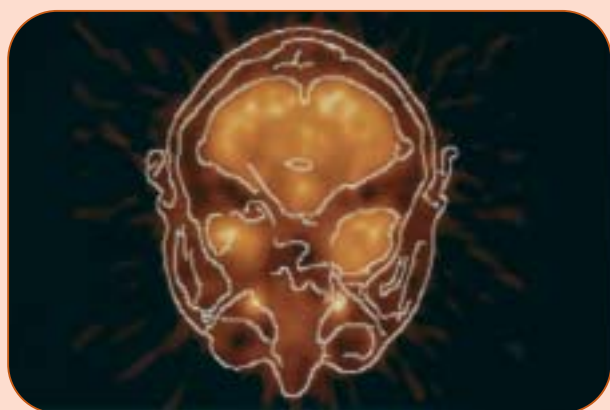
Europe has acknowledged strengths particularly in medical devices development and in drug delivery research, and these are clearly areas where the establishment of a European NanoMedicine Platform would contribute to maintaining and improving European competitiveness.

4. Nanotechnology-based Diagnostics including Imaging

4.1 Introduction

The application of micro- and nanobiotechnology in medical diagnostics can be grouped into two areas, in-vitro (biosensors and integrated devices) and in-vivo (implantable devices, medical imaging) applications.

The basis of modern medicine was laid already in the middle of the 19th century by the recognition that the cell is the source of health and disease. It followed that basic research to provide a better understanding of the highly complex working of cells is mandatory for medicine. Improvement and combination of methods to characterize cells or cell compartments in-vitro (like novel optical microscopy, scanning probe microscopy, electron microscopy and imaging mass-spectrometry) will be of importance for NanoMedicine.



Brain pictures with PET (Positron Emission Tomography) and NMR (Nuclear Magnetic Resonance). © CEA

In-vitro diagnosis for medical applications has traditionally been a laborious task; blood and other body fluids or tissue samples are sent to a laboratory for an analysis, which could take hours, days or weeks, depending on the technique used, and be highly labour intensive. The many disadvantages include sample deterioration, cost, lengthy waiting times (even for urgent cases), inaccurate results for small sample quantities, difficulties in integrating parameters obtained by a wide variety of methods, and poor standardisation of sample collection. Steadily, miniaturisation, parallelisation and integration of different functions on a single device, based on techniques derived from the electronics industry, have led to the

development of a new generation of devices that are smaller, faster and cheaper, do not require special skills, and provide accurate readings. These analytical devices require much smaller samples and will deliver more complete (and more accurate) biological data from a single measurement.

The requirement for smaller samples also means less invasive and less traumatic methods of extraction. Nanotechnology enables further refinement of diagnostic techniques, leading to high throughput screening (to test one sample for numerous diseases, or screen large numbers of samples for one disease) and ultimately point-of-care diagnostics. These technological advancements pave the way towards major changes in the way drugs can be prescribed in future, by enabling the goal of personalised medicine that is tailored to individual needs.

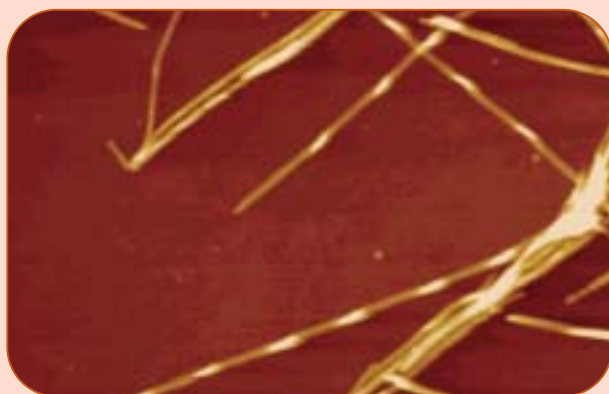
It is interesting to note that many new in-vitro techniques developed for medical testing are also finding important diverse applications, such as in environmental monitoring and security.

Medical imaging has advanced from a marginal role in healthcare to become an essential tool of diagnostics over the last 25 years. Molecular imaging and image-guided therapy is now a basic tool for monitoring disease and in developing almost all the applications of in-vivo NanoMedicine. Originally, imaging techniques could only detect changes in the appearance of tissues when the symptoms were relatively advanced. Later, contrast agents were introduced to more easily identify and map the locus of disease. Today, through the application of nanotechnology, both imaging tools and marker/contrast agents are being dramatically refined towards the end goals of detecting disease as early as possible, eventually at the level of a single cell, and monitoring the effectiveness of therapy.

The convergence of nanotechnology and medical imaging opens the doors to a revolution in molecular imaging (also called nano-imaging) in the foreseeable future, leading to the detection of a single molecule or a single cell in a complex biological environment.

4. NANOTECHNOLOGY-BASED DIAGNOSTICS INCLUDING IMAGING

One of the challenges has been to define research partnerships between the imaging industry and the contrast agent industry, which bring different, complementing competencies to the table. The proposed European Technology Platform in NanoMedicine would be timely to accelerate their integration.



Scanning force micrograph of amyloid fibrils formed from the Parkinson's disease-related protein alpha-synuclein. © V. Subramaniam, Biophysical Engineering, University of Twente

4.2 In-vitro Diagnostics

An in-vitro diagnostic tool can be a single biosensor, or an integrated device containing many biosensors. A biosensor is a sensor that contains a biological element, such as an enzyme, capable of recognising and 'signalling' (through some biochemical change) the presence, activity or concentration of a specific biological molecule in solution. A transducer is used to convert the biochemical signal into a quantifiable signal. Key attributes of biosensors are their specificity and sensitivity. Nanoanalytical tools like scanning probe microscopy or imaging mass spectrometry offer new opportunities for in-vitro diagnostics, like molecular pathology or reading out highly integrated ultra-sensitive biochips.

Techniques derived from the electronics industry have enabled the miniaturisation of biosensors, allowing for smaller samples and highly integrated sensor arrays, which take different measurements in parallel from a single sample. Higher specificity reduces the invasiveness of the diagnostic tools and

simultaneously increases their effectiveness significantly in terms of providing biological information such as phenotypes, genotypes or proteomes. Several complex preparation and analytical steps can be incorporated into 'lab-on-a-chip' devices, which can mix, process and separate fluids, realising sample analysis and identification. Integrated devices can measure tens to thousands of signals from one sample, thus providing the general practitioner or the surgeon with much more complementary data from his patient's sample. Some nanobiodevices for diagnostics have been developed to measure parts of the genome or proteome using DNA fragments or antibodies as sensing elements and are thus called gene or protein chips. 'Cells-on-chips' use cells as their sensing elements, employed in many cases for pathogen or toxicology screening.

Integrated devices can be used in the early diagnosis of disease and for monitoring the progress of therapy. New advancements in microfluidic technologies show great promise towards the realisation of a fully integrated device that directly delivers full data for a medical diagnosis from a single sample. Recent developments aim at developing in-vitro diagnostic tools to be used in a standard clinical environment or e.g. as 'point-of-care' devices.

4.3 In-vivo Nano-Imaging

In-vivo diagnostics refers in general to imaging techniques, but also covers implantable devices. Nano-imaging includes several approaches using techniques for the study of molecular events in-vivo and for manipulation of molecules. Imaging techniques cover advanced optical imaging and spectroscopy, nuclear imaging with radioactive tracers, magnetic resonance imaging, ultrasound, optical and X-ray imaging, all of which depend on identifying tracers or contrast agents that have been introduced into the body to mark the disease site

Targeted molecular imaging is important for a wide range of diagnostic purposes, such as the identifica-

tion of the locus of inflammation, the visualisation of vascular structures or specific disease states and the examination of anatomy. It is also important for research on controlled drug release, in assessing the distribution of a drug, and for the early detection of unexpected and potentially dangerous drug accumulations. The ability to trace the distribution of a drug leads to the possibility of activating it only where needed, thus reducing the potential for toxicity (see chapter on Targeted Drug Delivery and Release).

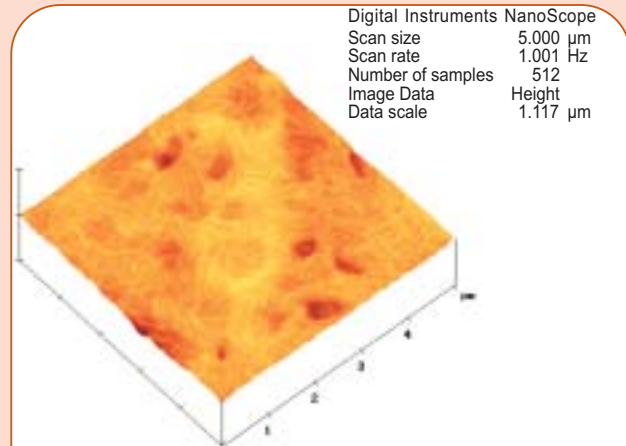
A wide range of particles or molecules is currently used for medical imaging. Some recent developments focus on using nanoparticles as tracers or contrast agents. Fluorescent nanocrystals such as quantum dots are nanoparticles which, depending on their coating and their physical and chemical properties, can target a specific tissue or cell and be made to fluoresce for imaging purposes. They offer a more intense fluorescent light emission, longer fluorescence lifetimes and increased multiplexing capabilities compared to conventional materials. Quantum dots are expected to be particularly useful for imaging in living tissues, where signals can be obscured by scattering. Toxicological studies are being undertaken to precisely study their impact on humans, animals and the environment. New developments are focusing on the nanoparticle coating, to improve its efficiency of targeting and biocompatibility.

The main benefits of nano-imaging for in-vivo diagnostics are the early detection of disease, the monitoring of disease stages (e.g. in cancer metastasis), in patient selection leading to individualised medicine and in the real-time assessment of therapeutic and surgical efficacy.

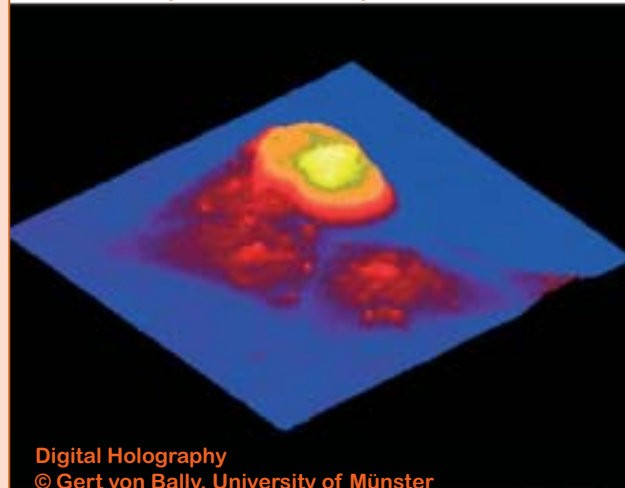
4.4 Basis for a Strategic Research Agenda

In-vitro diagnostics

The ultimate goal is the fast, reliable, specific and cost-effective detection of a few molecules (or even a single molecule) in a complex, non amplified and



Scanning Force Microscopy
© Boris Anczykowski, nanoAnalytics GmbH



Digital Holography
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Scanning-Electron-Microscopy
© Marcus Schäfer, University of Münster

Novel diagnostic approaches: cell surface characterization of pancreatic tumor cell lines with complementary methods
© J. Schneckeburger, Department of Medicine B, University of Münster

unlabelled biological sample. The improvement of in-vitro diagnostics towards this goal requires:

- nanoanalytical instruments of the highest spatial resolution, sensitivity and range of information, and integrated, combined instruments;
- better sensitivity of screening methods, enabling the sample size to be decreased, or for the early detection of low concentrations of disease markers;
- higher specificity, for quantitative detection of markers in complex samples,
- stronger reliability, simplicity of use and robustness;
- faster analysis;
- integration of different technologies to provide data for complementary multi-parameter analyses.

The commercialization of low cost, user-friendly lab-on-a-chip devices for point-of-care and disease prevention and control at home is driving research into areas of:

- user-friendly sample preparation techniques, enabling the detection of minute amounts of disease marker in a fairly concentrated blood drop and also minute quantities diluted in a relatively huge sample volume, e.g. 5 to 10 cancer cells in 100 ml of urine;
- ultra-sensitive and label-free detection techniques aiming at a faster and more compact direct detection, using for example, cantilevers or conducting polymers;
- synthetic recognition elements as sensors, in order to increase the sensitivity, specificity and ruggedness of recognition. This requires advancement in deposition techniques and surface chemistry including self assembly of biomolecules, hybrid conjugates of biomaterials with nanoparticles or Molecularly Imprinted Polymers;
- integrated, complex devices based on advanced micro- and nanofluidics, using for example active functionalised channel walls, and complex analysis protocols;
- biomimetic sensors using molecules as sensors.

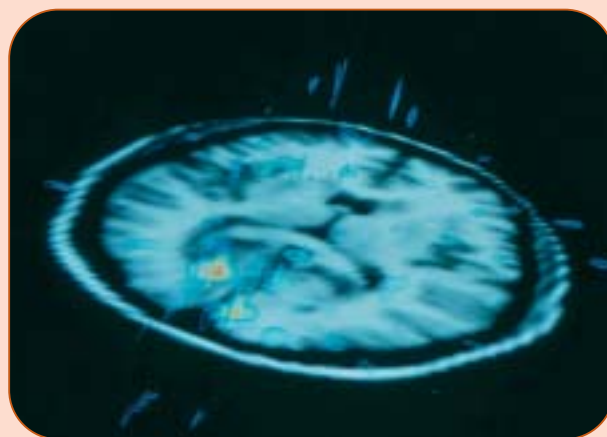
In-vivo nano-imaging

The goal of in-vivo diagnostics research is to create highly sensitive, highly reliable detection agents that can also deliver and monitor therapy. This is the

'find, fight and follow' concept of early diagnosis, therapy and therapy control, that is encompassed in the concept of theranostics. With this strategy, the tissue of interest can firstly be imaged, using target-specific contrast nanostructures. Then, combined with a pharmacologically active agent, the same targeting strategy can be used for applying therapy. Finally, monitoring of treatment effects is possible by sequential imaging. All developments related to the drug release and drug targeting aspect can be found in Chapter 5: Targeted Drug Delivery and Release.

Improved Detection

Research is needed to improve the efficiency and reliability of detection systems. A major objective for the coming years is to develop efficient, reasonably priced clinical cameras capable of acquiring whole-body images in one step and undertaking multi-isotope studies. The benefit would be a drastic increase in the throughput of whole body imaging, particularly important for cancer screening. Developments in nuclear



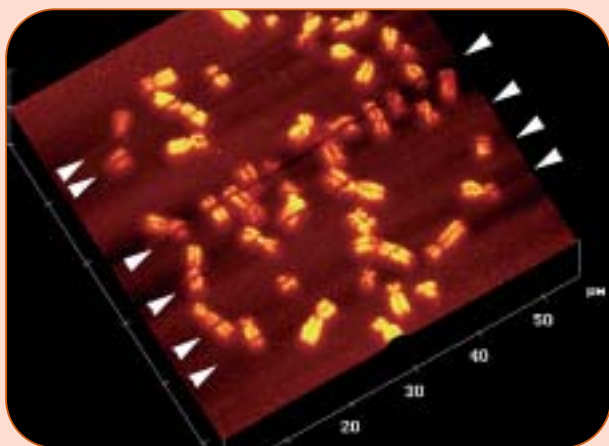
Brain pictures with PET (Positron Emission Tomography) and NMR (Nuclear Magnetic Resonance).
© LABORATOIRE CYCERON, CEA

imaging (which remains an expensive technology) require new detector architectures and new crystal growing methods to reduce manufacturing costs.

Combining different imaging modalities is a promising approach; for example, positron emission tomography with magnetic resonance imaging,

magnetic resonance imaging with ultrasound or with electroencephalogram-based brain mapping, ultrasound with optical technologies, and will lead to the possibility of benefiting from the advantages of each system. The fusion of magnetic resonance imaging and optical imaging modalities remains a challenge. In principle, this will require use of fluorescent nanoparticles as signal emitters, which function in both paramagnetic and infrared modes. Once this is achieved, nanotechnology may lead to the miniaturisation of detection devices or the remote transduction of signals.

In high-resolution measurements of electromagnetic fields, the development of new interfaces with nano-structured and/or biological functionalised surfaces, would improve continuous monitoring of biological parameters dramatically. Research is also required to improve methods of image analysis and visualisation, such as 3-D optical reconstruction, real-time intracellular tomography, stereo-imaging, virtual and augmented reality, holography, in-vivo imaging from optical catheters, and better endoscopic tools.



Scanning Force Microscopy analysis of chromosomes, which were processed with a laser as a tool for nanosurgery. © Fraunhofer IBMT, St. Ingbert

Furthermore, the ability to measure small local variations in temperature using radio frequency detectors has applications in identifying the onset and locus of many diseases, especially cancer.

Several other research issues are to be taken into consideration if molecular imaging is to become a reality. When dealing with the identification of tiny changes, achieving gains in the signal-to-noise ratio is critical, so also is the validation of the delicate measurements that images appear to provide for drug and therapy developments, as well as improvements to measurement techniques and data processing software. Lastly, effort has to be dedicated to the management of large amounts of data in order to fully gain advantage of nano-imaging results.

Nano Probes

The development of probes is an extremely active field where the miniaturization of complex reporting devices adapted to in-vivo imaging would be extremely beneficial. The major issues are specificity and the ability to penetrate the cell.

The very earliest manifestations of disease in the body are indicated by changes in living cells, including defective cell adhesion, cell mis-signalling, mitotic errors, intercellular communication errors, and abnormal cytoplasmic changes. Major benefits are envisaged from being able to image and identify these changed states. Different imaging techniques require different reporting devices; for example, quantum dots may 'report back' by fluorescing on contact with diseased cells. It is not difficult to make the leap from a reporter device to a device that not only indicates the locus of the disease, but also delivers a cure; for example nanomagnetic particles 'report back' by providing increased contrast and also take part in the therapeutic process (in addition, this concept underpins the need for research into gene and cellular therapy and drug delivery).

To improve reporting, research is required into the design and composition of nanoparticles, enabling them to better target diseased cells, including those situated behind barriers such as epithelial tissues. Further research is required into the creation of an 'all-purpose nanoparticle' that can be imaged by the variety of existing instruments (e.g. optical, acoustic, magnetic, etc.). Apart from identifying disease and delivering therapy,

4. NANOTECHNOLOGY-BASED DIAGNOSTICS INCLUDING IMAGING

the non-toxic labelling of specific cell types is important for the imaging of intracellular trafficking.

Work is also urgently required to improve the biocompatibility of reporter devices and for minimising any potential toxicity, allergic or inflammatory response, taking into account natural elimination to prevent possible long lasting effects. Research is also required on encapsulating contrast agents to facilitate their delivery to the target site, or on attaching specific linkers to provide new properties.

Essentially, the development of new probes for molecular imaging requires a multidisciplinary approach, with the transfer of discoveries in material science (new nanostructures such as particles, tubes, capsules, fullerenes, dendrimers, new polymer structures, etc.).

Combined Techniques

Nanobiotechnology offers significant inputs to the improvement of detection devices and in the tagging of disease indicators administered in-vivo, which will lead to advancement in imaging. Potent driving forces include synergies, such as those between in-vitro diagnostics (probes and markers) and in-vivo imaging; and between contrast / probe development (in drug delivery and/or toxicology studies) and imaging technology (medical instrumentation). The combination of in-vitro diagnostics and in-vivo nano-imaging could lead to targeted tumor disruption or removal: Tagging tumor cells with functionalized nanoparticles, which react to external stimuli, allows for in-situ, localized 'surgery' (breaking up or heating of particles by laser, magnetic fields, microwaves, etc.) without invasiveness within the human body.

Surgery

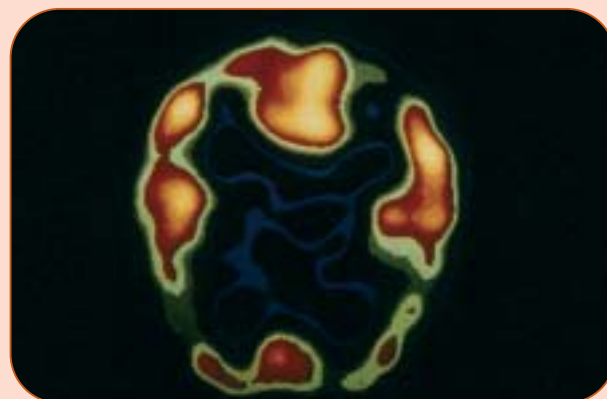
In the clinic, the use of nanoparticles for diagnosis and manipulation may lead to an improvement of surgical techniques. This may be achieved, for example, through cancer distribution mapping using near-infrared quantum dots, and applying thermotherapy or heat treatment, the characterisation and non-destructive removal of cells or tissue in a specific area, the tracking of specific cell types used in therapy, as well as the visualisation of bio-therapeutic agents.

Nanotechnology has further application in transfection devices for therapeutic uses. An example would be the development of devices that can cross biological barriers (like the blood-brain barrier) to deliver multiple therapeutic agents at high local concentrations directly to cancer cells and the neighbouring tissues that play a critical role in the spread of the disease.

Implantable devices for in-vivo diagnostics

Nanotechnology also has many implications for in-vivo diagnostic devices such as the swallowable imaging 'pill' and new endoscopic instruments. Monitoring of circulating molecules is of great interest for some chronic diseases such as diabetes or AIDS. Continuous, smart measurement of glucose or blood markers of infection constitutes a real market for implantable devices. Miniaturisation for lower invasiveness, combined with surface functionalisation and the 'biologicalisation' of instruments will help increase their acceptance in the body. Autonomous power, self-diagnosis, remote control and external transmission of data are other considerations in the development of these devices.

Nanosensors, for example used in catheters, will also provide data to surgeons. Nanoscale entities could identify pathology/defects; and the subsequent removal or correction of lesions by nanomanipulation could also set a future vision.



Localisation of benzodiazepine receptors by Positron Emission Tomography (PET).
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5 Targeted Drug Delivery and Release

5.1 Introduction

The very slow progress in the treatment of severe diseases has led to the adoption of a multidisciplinary approach to the targeted delivery and release of drugs, underpinned by nanoscience and nanotechnology. New drug delivery systems (DDS) combine polymer science, pharmaceuticals, bioconjugate chemistry and molecular biology. The aim is to better control drug pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity and biorecognition of systems in the quest for improved efficacy.



Zoom on a virus head, computer model.
© E. Hewat, IBS-CEA

Drug delivery and targeting systems under development aim to minimize drug degradation and loss, prevent harmful side effects and increase the availability of the drug at the disease site. Drug carriers include micro and nanoparticles, micro and nanocapsules, lipoproteins, liposomes, and micelles, which can be engineered to slowly degrade, react to stimuli and be site-specific. Targeting mechanisms can also be either passive or active. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the differences in the vascularization of the tumor

tissue compared with healthy tissue. Active targeting involves the chemical 'decorating' of the surface of drug carriers with molecules enabling them to be selectively attached to diseased cells.

The controlled release of drugs is also important for therapeutic success. Controlled release can be sustained or pulsatile. Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate, by diffusion out of the polymer or by degradation of the polymer over time. Pulsatile release is often preferred, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g. exposure to light, changes in pH or temperature).

Other nano-based approaches to drug delivery are focused on crossing a particular physical barrier, such as the blood-brain barrier; or on finding alternative and acceptable routes for the delivery of a new generation of protein-based drugs other than via the gastro-intestinal tract, where degradation can occur. Nanoscience and nanotechnology are thus the basis of innovative delivery techniques that offer great potential benefits to patients and new markets to pharmaceutical and drug delivery companies.

For over 20 years, researchers in Europe have used nanoscale technology as the basis of vast improvements in drug delivery and targeting, and Europe is now well placed to build on this body of knowledge.

5.2 Drug Delivery Systems

Drug Carriers

A successful drug carrier system needs to demonstrate optimal drug loading and release properties, long shelf-life and low toxicity. Colloidal systems, such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10-400 nm diameter show great promise as carriers in drug delivery systems.

Micelles: Drugs can be trapped in the core of a micelle and transported at concentrations even greater than their intrinsic water solubility. A hydrophilic shell can form around the micelle, effectively protecting the contents. In addition, the outer chemistry of the shell may prevent recognition by the reticuloendothelial system, and therefore early elimination from the bloodstream. A further feature that makes micelles attractive is that their size and shape can be changed. Chemical techniques using crosslinking molecules can improve the stability of the micelles and their temporal control. Micelles may also be chemically altered to selectively target a broad range of disease sites.

Liposomes are vesicles that consist of one to several, chemically-active lipid bilayers. Drug molecules can be encapsulated and solubilised within the bilayers. Certain (channel) proteins can be incorporated in the membrane of the liposome, which act as size-selective filters only allowing the diffusion of small solutes such as ions, nutrients and antibiotics. Thus, drugs encapsulated within a liposome 'nanocage' that has been functionalized with channel proteins, are effectively protected from premature degradation. The drug molecule, however, is able to diffuse through the channel, driven by the concentration difference between the interior and the exterior of the 'nanocage'.

Dendrimers are nanometre-sized, polymer macromolecules. They consist of a central core, branching units and terminal functional groups. The core chemistry determines the solubilizing properties of the

cavity within the core, whereas external chemical groups determine the solubility and chemical behavior of the dendrimer itself. Targeting is achieved by attaching specific linkers to the external surface of the dendrimer which enable it to bind to a disease site, while its stability and protection from phagocytes is achieved by 'decorating' the dendrimers with polyethylene glycol chains.

Liquid Crystals combine the properties of both liquid and solid states. Liquid crystals can be made to form different geometries, with alternate polar and non-polar layers (i.e., lamellar phases), within which aqueous drug solutions can be incorporated.

Nanoparticles, including nanospheres and nanocapsules, can be amorphous or crystalline. They are able to adsorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. In nanocapsules, the drug is confined to a cavity surrounded by a polymer membrane, while nanospheres are matrix systems within which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles have attracted considerable attention in the controlled release of drugs in targeting particular organs/tissues, as carriers of DNA in gene therapy and in their ability to deliver proteins, peptides and genes by the oral route.

Hydrogels are three-dimensional polymer networks that swell but do not dissolve in aqueous media. They are used to regulate drug release in reservoir-based



Gene delivery using ultrasound. The presence of gas in the gene-filled microbubbles allows ultrasound to burst them. © Unger et al. Therapeutic Applications of Microbubbles

systems or as carriers in swelling-controlled release devices. On the forefront of controlled drug delivery, hydrogels, as enviro-intelligent and stimuli-sensitive gel systems, can modulate drug release in response to pH, temperature, ionic strength, electric field, or specific analyte concentration differences. Release can be designed to occur within specific areas of the body. Hydrogels as drug delivery systems are very promising if combined with the technique of molecular imprinting.

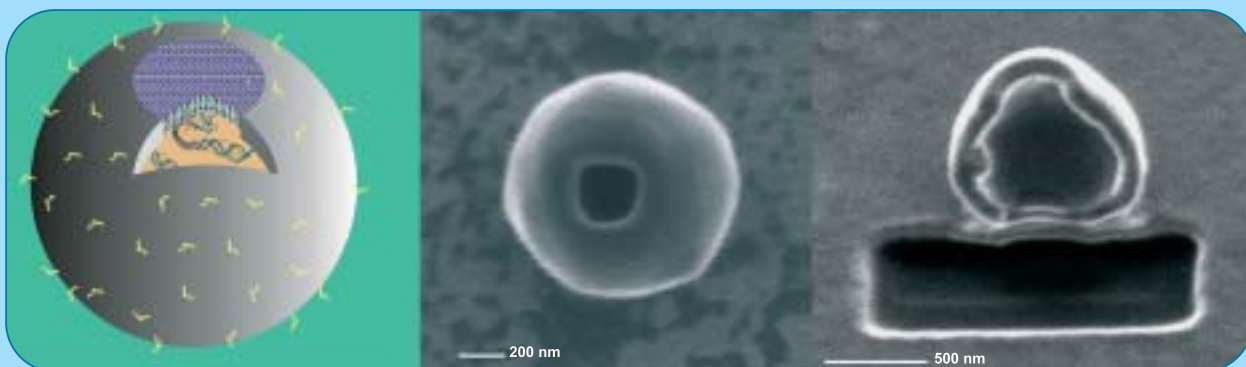
Molecularly imprinted polymers have an enormous potential for drug delivery systems. Examples include: rate-programmed drug delivery, where drug diffusion from the system has to follow a specific rate profile; activation-modulated drug delivery, where the release is activated by some physical, chemical or biochemical processes; and feedback-regulated drug delivery, where the rate of drug release is regulated by the concentration of a triggering agent, which is activated by the drug concentration in the body.

Despite already-developed applications, the incorporation of the molecular imprinting approach for the development of drug delivery systems is at an early stage. It can be expected that in the next few years significant progress will occur, taking advantage of the improvements in this technology in other areas.

The **conjugation of biological molecules (peptides/proteins) and synthetic polymers** is an efficient means of improving control over the nanoscale structure formation of synthetic polymers that can be

used as drug delivery systems. The conjugation of suitable synthetic polymers to peptides or proteins can reduce toxicity, prevent immunogenic or antigenic side reactions, enhance blood circulation times and improve drug solubility. Modification of synthetic polymers with peptide sequences, which can act as antibodies to specific epitopes, can also prevent random distribution of drugs throughout a patient's body by active targeting. The functionalisation of synthetic polymers with peptide sequences derived from extracellular matrix proteins is an efficient way to mediate cell adhesion, for example. In addition the ability of cationic peptide sequences to complex DNA and oligonucleotides offers prospects for the development of non-viral vectors for gene delivery, based on synthetic polymeric hybrid materials.

The field of **in-situ forming implants** has grown exponentially in recent years. Liquid formulations generating a (semi-) solid depot after subcutaneous injection are attractive delivery systems for parenteral (non-oral) application because they are less invasive and painful compared to implants. They enable drugs to be delivered locally or systemically over prolonged periods of time, typically up to several months. These depot systems could minimize side effects by achieving constant, 'infusion-like' drug profiles, especially important for delivering proteins with narrow therapeutic indices. They also offer the advantage of being relatively simple and cost-effective to manufacture.



Left to right:

- Schematic of nanofabricated biomimetic drug delivery vehicle. The surface of a hollow polystyrene bead containing the desired payload is functionalized for specific targeting. The payload is released through the membrane lipid bilayer covering the hole nanofabricated in the scaffold.
- Transmission electron microscopy image of a hollow polystyrene bead with a nanofabricated hole.
- Transmission electron microscopy image of the cross-section of an "artificial cell".

© A. Dudia and V. Subramaniam, University of Twente, The Netherlands



Microchromatographic column.
© P. Stroppa, CEA

The ultimate goal in controlled release is the development of a microfabricated device with the ability to store and release multiple chemical substances on demand. Recent advancement in **microelectromechanical systems (MEMS)** have enabled the fabrication of controlled-release microchips, which have the following advantages:

- Multiple chemicals in any form (e.g. solid, liquid or gel) can be stored and released;
- Chemical release is initiated by the disintegration of the barrier membrane by applying an electric potential;
- A variety of highly potent drugs can potentially be delivered accurately and safely;
- Complex release patterns (e.g. simultaneous constant and pulsatile release) can be achieved;
- Local delivery is possible, achieving high concentrations of drug where needed, while keeping the systemic concentration of the drug at a low level;
- Water penetration into the reservoirs is avoided by a barrier membrane and thus the stability of protein-based drugs with limited shelf-life is enhanced.

Administration Routes

The choice of drug is often influenced by the way it is administered, as this can make the difference between a drug's success and failure. So the choice of a delivery route can be driven by patient accept-

ability, important properties of the drug (e.g. solubility), the ability to target the disease location, or effectiveness in dealing with the specific disease.

The most important drug delivery route is the **peroral route**. An increasing number of drugs are protein- and peptide-based. They offer the greatest potential for more effective therapeutics, but they do not easily cross mucosal surfaces and biological membranes, they are easily denatured or degraded, they are prone to rapid clearance in the liver and other body tissues and they require precise dosing. At present, protein drugs are usually administered by injection, but this route is less accepted by patients and also poses problems of oscillating blood drug concentrations. So, despite the barriers to successful drug delivery that exist in the gastrointestinal tract (e.g. acid-induced hydrolysis in the stomach, enzymatic degradation throughout the gastrointestinal tract, bacterial fermentation in the colon), the peroral route is still the most intensively investigated as it offers advantages of convenience, cheapness of administration and manufacturing cost savings.

Parenteral routes (e.g. intravenous, intramuscular or subcutaneous) are very important. The only nanosystems presently on the market, liposomes, are administered intravenously. Nanoscale drug carriers have a great potential for improving the delivery of drugs through nasal and sublingual routes, both of which avoid first-pass metabolism; and for difficult-access ocular, brain and intra-articular cavities. It has been possible to deliver peptides and vaccines systemically using the nasal route through the association of active drug macromolecules with nanoparticles. In addition, there is the possibility of improving the ocular bioavailability of drugs if administered in a colloidal drug carrier.

Pulmonary delivery is also important and is effected in a variety of ways - via aerosols, metered dose inhaler systems, powders (dry powder inhalers) and solutions (nebulizers), which may contain nanostructures such as liposomes, micelles, nanoparticles and dendrimers. Aerosol products for pulmonary delivery

comprise more than 30% of the global drug delivery market. Research into lung delivery is driven by the potential for successful protein and peptide drug delivery by this route and by the promise of an effective delivery mechanism for gene therapy (e.g. in the treatment of cystic fibrosis), as well as the need to replace chlorofluorocarbon propellants in metered dose inhaler systems. Pulmonary drug delivery offers local targeting for the treatment of respiratory diseases and increasingly appears to be a viable option for the delivery of drugs systemically. However, the success of pulmonary delivery of protein drugs is diminished by proteases in the lung, which reduce their overall bioavailability, and by the barrier between capillary blood and alveolar air (the air-blood barrier).

Transdermal drug delivery avoids problems such as gastrointestinal irritation, metabolism, variations in delivery rates and interference due to the presence of food. It is also suitable for unconscious patients. The technique is generally non-invasive, well accepted by patients and can be used to provide local delivery over several days. Limitations include slow penetration rates, lack of dosage flexibility and/or precision, and a restriction to relatively low dosage drugs.



Pulmonary delivery
© Stockbyte™, 2000

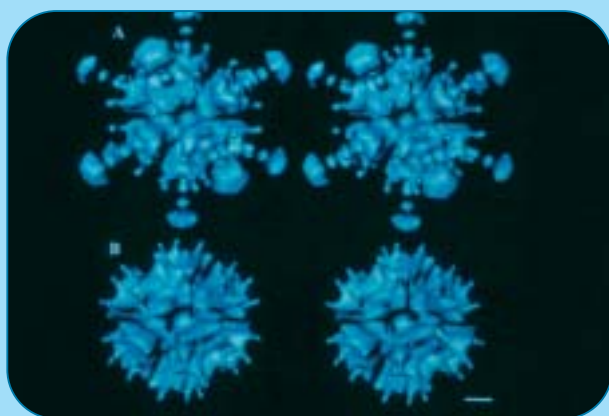
Trans-tissue and local delivery systems are systems that require to be tightly fixed to resected tissue during surgery. The aim is to produce an elevated pharmacological effect, while minimizing systemic, administration-associated toxicity. Trans-tissue systems include: drug-loaded gelatinous gels, which are formed in-situ and adhere to resected tissues releasing drugs, proteins or gene-encoding adenoviruses; antibody-fixed gelatinous gels (cytokine barrier) that form a barrier that on a target tissue could prevent the permeation of cytokines into that tissue; cell-based delivery, which involves a gene-transduced oral mucosal epithelial cell-implanted sheet; device-directed delivery - a rechargeable drug infusion device that can be attached to the resected site.

Gene delivery is a challenging task in the treatment of genetic disorders. Plasmid DNA has to be introduced into the target cells. It then needs to be transcribed, and the genetic information ultimately translated into the corresponding protein. To achieve this, a number of hurdles have to be overcome. The gene delivery system has to be targeted to the target cell, transported through the cell membrane, taken up and degraded in the endolysosomes, and the plasmid DNA trafficked intracellularly to the nucleus.

5.3 Basis for a Strategic Research Agenda

For all delivery routes **formulation** is essential and presents new challenges. Research into novel ways to introduce nanomedicines into the body is as important as the drug itself. Formulation research adds value in a competitive marketplace where change is now rapid. Present paradigms may not hold; an example is the increasing market share now occupied by needleless injections.

Nanoparticles and nanoformulations have already been used as drug delivery systems with great success, and nanoparticulate drug delivery systems have still greater potential for many applications, including anti-tumour therapy, gene therapy, AIDS



Stereographic pictures of pseudo-virus
© CEA

therapy, radiotherapy, in the delivery of proteins, antibiotics, virostatics, vaccines, and as vesicles to pass the blood-brain barrier.

Nanoparticles provide massive advantages regarding drug targeting, delivery and release, and with their additional potential to combine diagnosis and therapy, will emerge as one of the major tools in NanoMedicine. The main goals are to improve their stability in the biological environment, to mediate the bio-distribution of active compounds, to improve drug loading, targeting, transport, release and interaction with biological barriers. The cytotoxicity of nanoparticles or their degradation products remains a major problem and improvements in biocompatibility obviously are a main concern of future research.

The European Technology Platform on NanoMedicine needs to reflect on European strengths in this area in order to be competitive. In drug delivery, these are in polymer therapeutics, non-viral gene delivery, biological models for cells and tissues for in-vitro testing and in cancer targeting and therapy.

These form the basis for further developments, such as:

- Nano-drug delivery systems that deliver large but highly localized quantities of drugs to specific areas, to be released in controlled ways;
- Controllable release profiles, especially for sensitive drugs;

- Materials for nanoparticles that are biocompatible, biodegradable and non-toxic;
- Architectures/structures, such as biomimetic polymers and nanotubes;
- Technologies for self-assembly;
- New functions (active drug targeting, on-command delivery, intelligent drug release devices/bioresponsive triggered systems, self-regulated delivery systems, smart delivery);
- Virus-like systems for intracellular delivery;
- Nanoparticles to improve implantable devices;
- MEMS (improved by nanotechnology) for nanoparticle release and multi-reservoir drug delivery systems;
- Nanoparticles for tissue engineering, e.g. for the delivery of cytokines to control cellular growth and differentiation and to stimulate regeneration;
- Biodegradable layered coatings on implants for sustained release of active molecules;
- Advanced polymeric carriers for the delivery of therapeutic peptide/proteins (biopharmaceutics).

And also in the development of:

- Combined therapy and medical imaging, for example, nanoparticles for diagnosis and manipulation during surgery (e.g. thermotherapy with magnetic particles);
- Universal formulation schemes that can be used as intravenous, intramuscular or peroral drugs;
- Cell and gene targeting systems;
- Devices for detecting changes in magnetic or physical properties after specific binding of ligands on paramagnetic nanoparticles that can correlate with the amount of ligands;
- Better disease markers in terms of sensitivity and specificity;
- nanoanalytical instrumentation for improved understanding, engineering, and control of drug delivery systems;
- High throughput in-vitro and in-vivo test systems for immunological and toxicological screening of nanoparticles and nanoformulations.

6. Regenerative Medicine

6.1 Introduction

In the 1960s and 1970s, the first generation of materials was developed for use inside the human body. A common feature of most of these materials was their biological inertness. The clinical success of bioinert, bioactive and resorbable implants was an important response to the medical needs of a rapidly ageing population. The field of biomaterials subsequently began to shift in emphasis from a bioinert tissue response, to instead producing bioactive components that could elicit controlled actions and reactions within the body. By the mid-1980s, bioactive materials had reached the clinic in a variety of orthopaedic and dental applications. They included various compositions of bioactive glasses, ceramics, glass-ceramics and composites, as well as a range of bioresorbable polymers. Whereas second-generation biomaterials were designed to be either resorbable or bioactive, more advanced therapeutic approaches are now being followed to combine these two properties to develop implants, which will induce a regenerative-like healing modality. In other words, help the body to heal itself.

By leveraging novel cell culture techniques and synthesis and the design of bio-resorbable polymers, tissue engineering strategies have recently emerged as the most advanced therapeutic option presently available in regenerative medicine.

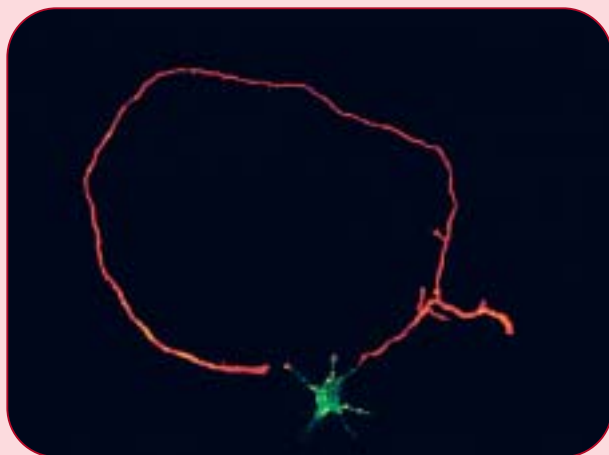


Engineered adipose tissue
© Fidia Advanced Biopolymers

Tissue engineering encompasses the use of cells and their molecules in artificial constructs that compensate for body functions that have been lost or impaired as a result of disease or accidents. It is based upon scaffold-guided tissue regeneration and involves the seeding of porous, biodegradable scaffolds with donor cells, which become differentiated and mimic naturally occurring tissues. These tissue-engineered constructs are then implanted into the patient to replace diseased or damaged tissues. With time, the scaffolds are resorbed and replaced by host tissues that include viable blood supplies and nerves. Current clinical applications of tissue-engineered constructs include engineering of skin, cartilage and bone for autologous implantation. Recent advancement in therapeutic strategies involving tissue engineering include the use of adult stem cells as a source of regenerative cells, and the use of cell-signalling molecules as a source of molecular regeneration messengers.

A complex and uneven European regulatory and funding environment has limited, to date, the extensive therapeutic use of tissue-engineered treatments in addressing different clinical needs, despite the significant advancements that have been made. The high cost, together with a limited space for significant economies of scale in the mass-production of tissue-engineered products, has hindered widespread clinical application. In addition, presently available tissue-engineered products still share some of the concepts of substitution medicine, where a laboratory grown 'spare part' is implanted in the body to compensate for lost tissue. Despite these limitations, the clinical availability of therapies based on tissue engineering represents a tremendous step forward in regenerative medicine. By building on the pioneering achievements of tissue engineering, advanced therapies in regenerative medicine can therefore address even more challenging objectives - to initiate and control the regeneration of pathological tissue, and to treat, modify and prevent disabling chronic disorders such as diabetes, osteoarthritis, diseases of the cardiovascular and central nervous system. Given the dynamics of Europe's societal growth and the need

to provide advanced and cost-effective therapies to an ageing population, it is a further challenge for regenerative medicine to deliver the disease modifying benefits of tissue-engineered products to a wide patient population, in a cost-effective way.



Growth cone of an immature hippocampal neuron developing in vitro. Fluorescence microscopy.
© Roche, 2005

6.2 A Biomimetic Strategy

The vision for nano-assisted regenerative medicine is the development of cost-effective disease-modifying therapies that will allow for in-situ tissue regeneration. The implementation of this approach involves not only a deeper understanding of the basic biology of tissue regeneration – wound healing, in its widest sense – but also the development of effective strategies and tools to initiate and control the regenerative process.

In the field of biomaterials and biotechnology, the term 'biomimetics' has been established to describe the process of simulating what occurs in nature. The biomimetic philosophy can be condensed into three basic elements: intelligent biomaterials, bioactive signalling molecules and cells.

Intelligent biomaterials and smart implants

Third-generation biomaterials that involve tailoring of resorbable polymers at the molecular level to elicit specific cellular responses show great promise as

scaffolds or matrices in tissue regeneration. These 'intelligent' biomaterials are designed to react to changes in the immediate environment and to stimulate specific cellular responses at the molecular level. Molecular modifications of resorbable polymer systems elicit specific interactions with cells and direct cell proliferation, differentiation and extracellular matrix production and organization. For example, new generations of synthetic polymers are being developed which can change their molecular conformation in response to changes in temperature, pH, electrical stimuli or energetic status.

Access to nanotechnology has offered a completely new perspective to the material scientist to mimic the different types of extra-cellular matrices present in tissues. Techniques are now available which can produce macromolecular structures of nanometre size, with finely controlled composition and architecture. Conventional polymer chemistry, combined with novel methodologies such as electrospinning, phase separation, direct patterning and self-assembly, have been used to manufacture a range of structures, such as nanofibres of different and well defined diameters and surface morphologies, nanofibrous and porous scaffolds, nanowires and nanoguides, nanospheres, nano 'trees' (e.g. dendrimers), nano-composites and other macromolecular structures.

Nanotechnology also improves non-resorbable biomaterials and effective manipulation of biological interactions at the nanometre level, which will dramatically improve the functionality and longevity of implanted materials. By applying bioactive nanoparticle coatings on the surface of implants, it will be possible to bond the implant more naturally to the adjoining tissue and significantly prolong the implant lifetime. Similarly, it may be possible to surround implanted tissue with a nanofabricated barrier that would prevent activation of the rejection mechanisms of the host, allowing a wider utilization of donated organs. Nanomaterials and/or nanocomposites with enhanced mechanical properties could replace the materials that fatigue-fail due to crack initiation and propagation during physiological loading conditions. Nanomaterials with enhanced elec-

trical properties that remain functional for the duration of implantation could replace the conventional materials utilised for neural prostheses, whose performance deteriorates over time. Third-generation bioactive glasses and macroporous foams can be designed to activate genes that stimulate regeneration of living tissues. By understanding the fundamental contractile and propulsive properties of tissues, biomaterials can be fabricated that will have nanometre-scale features representing the imprinted features of specific proteins.

In conclusion, nanotechnology can assist in the development of biomimetic, intelligent biomaterials, which are designed to positively react to changes in their immediate environment and stimulate specific regenerative events at the molecular level in order to generate healthy tissues.

Bioactive signalling molecules

Bioactive signalling molecules are defined as those molecules which are naturally present in cells (cytokines, growth factors, receptors, second messengers) and trigger regenerative events at the cellular level. Recently available therapies based on signalling molecules involve the uncontrolled delivery of a single growth factor - which is an obvious oversimplification, in light of the complexities associated with the healing cascades of living tissues, especially in chronic pathologies. Sequential signalling is obligatory in the fabrication and repair of tissues; therefore

the development of technologies for the sequential delivery of proteins, peptides and genes is critical.

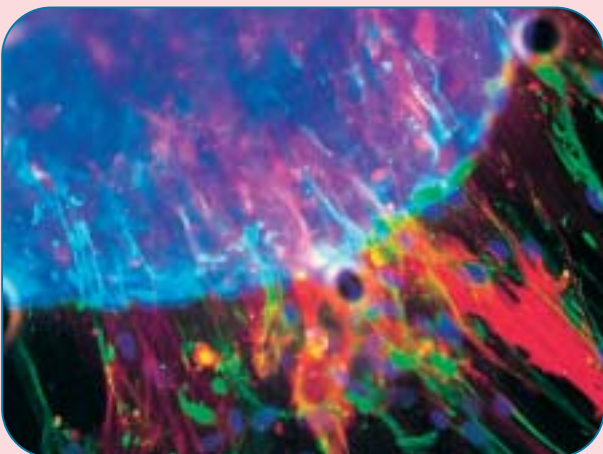
The provision of the correct bioactive signalling molecules to initiate and direct the regenerative process is being pursued by designing bioactive materials and encoding biological signals able to trigger biological events. The primary goal is to develop extracellular, matrix-like materials, by either combining natural polymers or developing structures starting from synthetic molecules combined with matricellular cues. By immobilizing specific proteins, peptides and other biomolecules onto a material, it is possible to mimic the extracellular matrix (ECM) environment and provide a multifunctional cell-adhesive surface. Cell-specific recognition factors can be incorporated into the resorbable polymer surface, including the adhesive proteins, fibronectin or functional domains of ECM components. Polymer surfaces can be tailored with proteins that influence interactions with endothelium, synaptic development and neurite stimulation.

To achieve any advancement it is essential to understand those molecular interactions that lead to regenerative pathways, and the development of technologies for the sequential delivery of proteins, peptides and genes to mimic the signalling cascade. The use of nanotechnologies is advocated in assisting in the development of therapies involving the activation and spatio-temporal control of in-vivo tissue regeneration.

In conclusion, nano-assisted technologies will enable the development of bioactive materials which release signalling molecules at controlled rates by diffusion or network breakdown that in turn activate the cells in contact with the stimuli. The cells then produce additional growth factors that will stimulate multiple generations of growing cells to self-assemble into the required tissues in-situ.

Cell based therapies

Cellular differentiation occurs in mammals as part of the embryological development and continues in adult life as part of the normal cell turnover or repair



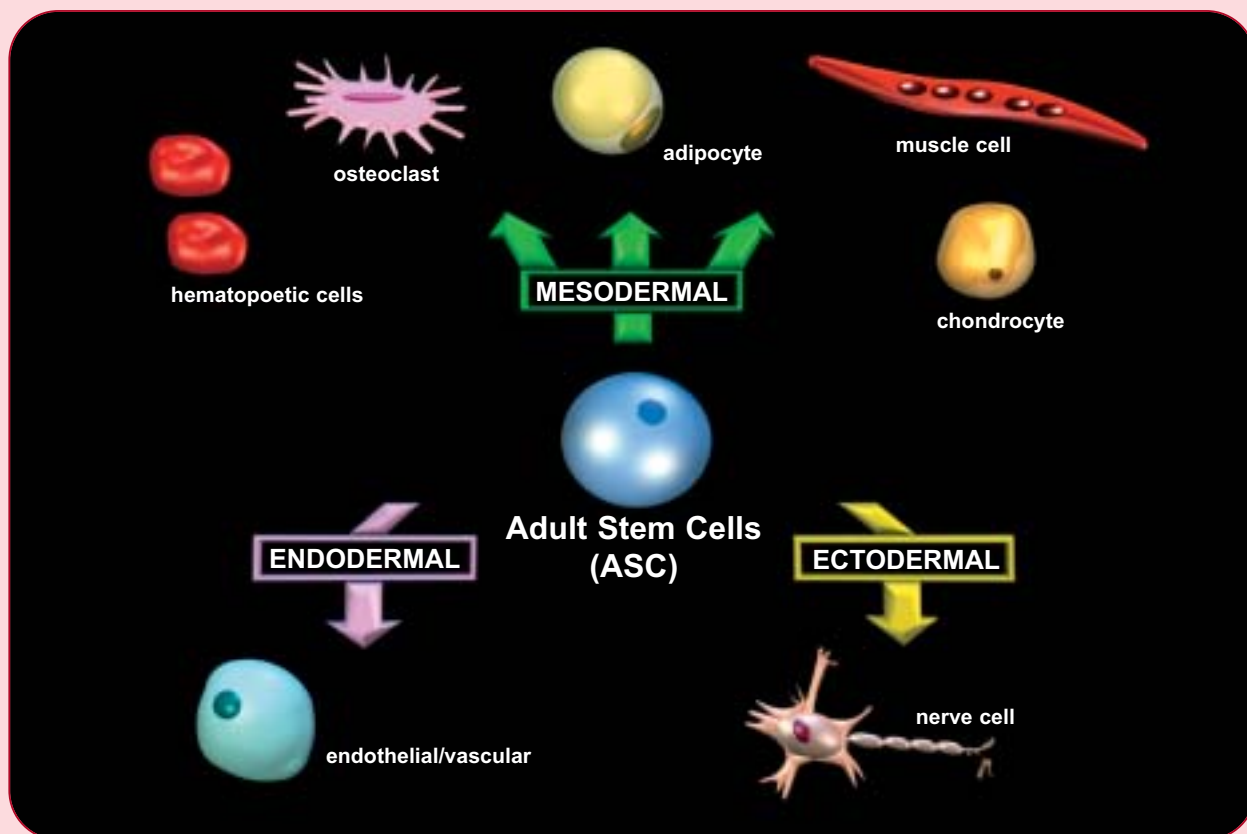
Spontaneous stem cell differentiation
© Fraunhofer IBMT, St. Ingbert

following injury. Growth, from the cellular aspect, means a continuous process of cellular turnover that is dependent on the presence of self-renewing tissue stem cells that give rise to progenitor and mature cells. Cellular turnover is known to be fast in certain tissues, such as intestinal epithelium, blood and epidermis, and slow in others, such as bone and cartilage, while it has been considered limited or non-existent in tissues such as the brain and the heart. However, scientific results in recent years have radically changed the view of the ability of even these tissues to regenerate after ischaemic injury. This paradigm shift will refocus research into the understanding of mechanisms for stem cell recruitment, activation, control and homing.

The major focus of ongoing and future efforts in regenerative medicine will be to effectively exploit the enormous self-repair potential that has been observed in adult stem cells. Given the logistical complexities and the costs associated with today's tissue engineering

therapies, which are based on the autologous reimplantation of culture-expanded differentiated cells, next generation therapies will need to build on the progress made with tissue engineering in understanding the huge potential for cell based therapies which involve undifferentiated cells. Nanotechnology will help in pursuing two main objectives – identifying signalling systems in order to leverage the self-healing potential of endogenous adult stem cells, and developing efficient targeting systems for adult stem cell therapies.

In conclusion, cell-based therapies should be aimed at the efficient harvesting of adult stem cells, to allow for a brief pre-implantation, cultivation stage, or, preferably, for immediate intra-operative administration using an intelligent biomaterial as a bio-interactive delivery vehicle. Of huge impact would also be the ability to implant cell-free, intelligent, bioactive materials that would effectively provide signalling to leverage the self-healing potential of the patient's own stem cells.



6.3 Basis for a Strategic Research Agenda

Careful consideration of the high-potential for regenerative medicine leads to the conclusion that much basic and applied research must be undertaken, not only in developmental biology and stem cell research, but also in the field of biomaterials. As numerous European groups are amongst the world leaders in biomaterials and cell therapies, there are great opportunities here for European small and medium enterprises. This is a niche where the European Research Area can gain prestige and a corresponding share of the world market in the development, production and marketing of such 'intelligent' biomaterials.

These complex challenges can be addressed only by an interdisciplinary approach using international specialists, with both academic and industrial backgrounds. This will require enlarging the number, facilities and staffing of the relevant laboratories within Europe, with an emphasis on nanotechnology expertise and capability. It will be essential to set up multidisciplinary research groups which bring together chemistry and biochemistry, molecular and cell biology, materials science and engineering, as well as ensuring an adequate balance of academic and industrial researchers.

Thanks to nanotechnology, a cellular and molecular basis has been established for the development of



Chondrocyte on Hyaluronan-derivative fiber imaged by scanning electron microscopy
© Fidia Advanced Biopolymers



Left: intervertebral disc, 12 months after treatment with autologous disc chondrocytes
Right: untreated intervertebral disc
Regenerated discs mimic native disc morphology; autologous treatment promotes tissue regeneration.
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third-generation biomaterials that will provide the scientific foundation for the design of scaffolds for tissue engineering, and for in-situ tissue regeneration and repair, needing only minimally-invasive surgery. It is strongly recommended that in future planning policy, attention and resources be focused on developing these biomaterials.

Projects will also need to be highly focused towards a clearly identified clinical application, not being limited to basic research on the optimisation of 'generic' cell/artificial matrix constructs. They must be rooted in the specific characteristics of the tissue to be regenerated, and in the economic advantage of one approach over another.

It should be feasible to design a new generation of gene-activating biomaterials tailored for specific patients and disease states. Emphasis should be given to projects designed with the objective of developing disease-modifying, cost-effective treatments for chronic disabilities that mostly affect the elderly, such as diabetes, osteoarthritis, cardiovascular and central nervous system degenerative disorders.

To this end, the following research activities will need to be promoted:

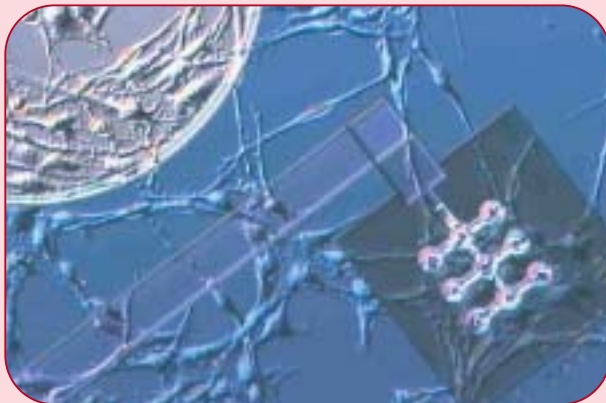
- The development of 'intelligent', multi-functional biomaterials;
- Control of the structure of materials at the micro- and nanoscale - mandatory in the design of intelligent scaffolds. This will also require research in the

6. REGENERATIVE MEDICINE

fields of micro- and nanofabrication for the creation of structures that differentially control cell adhesion, proliferation and function;

- Technologies for the development of new generations of synthetic polymers that can change their molecular conformation in response to changes in external stimuli (temperature, pH, electric field or energetic status);
- Technologies for the development of bioactive nanocoatings;
- Projects which include electronic and/or communication components in forms of nanowires and nanopores (or their equivalents) for the stimulation and biosensing of cells within an artificial matrix;
- Sensor technology for the assessment of the interface activity and the progress of implant integration;
- Novel technologies that enable the development of biomaterials for the sequential delivery of actives and/or chemo-attractants for the triggering of endogenous self-repair mechanisms;
- Stem cell research, aimed at understanding mainly the potential and plasticity of adult stem cells;
- The development of technologies for minimally-invasive, site-specific cell therapy;
- Research aiming to generate knowledge and products centred on the nanoscale interactions between different types of cells and their immediate environment;
- Monitoring tissue regeneration;
- Sensors for precise gene activation and control during cell and tissue growth;
- In-vitro and in-vivo toxicity testing of engineered nanoparticles.

Finally, a future goal for regenerative medicine is the possibility of using bioactive stimuli to activate genes as a preventative treatment; maintaining the health of tissues as they age. Only a few years ago this concept would have seemed unimaginable. But we need to remember that only 30 years ago the concept of a material that would not be rejected by living tissues also seemed unimaginable!



Gliacells on substrate with electrodes
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Engineered epithelium
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7. Regulatory Issues and Risk Assessment

The acceptance of NanoMedicine necessitates transparent and timely information of all stakeholders, including the general public. Safety aspects of NanoMedicine have to be properly and systematically addressed, and there has to be a clear positive benefit-to-risk ratio that will accompany the clinical implementation of products and procedures based on nanotechnology. This will be achieved by a combination of evidence-based public awareness programmes and the development of science-based regulatory processes, which have to take risk management into account, e.g. by adopting a system, which identifies and assesses risks at every stage of the product or process cycle from the concept to post-treatment.

All medical products in Europe are currently regulated according to well-established Directives relating to medicinal products or to medical devices, according to their principal mode of action. Discussions are also underway to provide a regulatory framework for human tissue engineered products and their associated processes. It is extremely important that any new risks that may be associated with the introduction of nanotechnology into clinical medicine are evaluated, and that the details of new risk management procedures identified as being

relevant to this assessment are properly incorporated into the existing regulatory frameworks and are fully integrated into any new regulatory developments that may emerge in the future as being necessary to complement existing European legislation.

It is necessary to put into place measures that identify the hazards associated with novel nanotechnology-based therapies, characterize the associated risks, reduce those risks as far as reasonably practicable, establish a positive risk/benefit balance, and communicate the nature of any residual risks and other relevant safety information to doctors, patients and other key stakeholders. These risks could relate to, for example, patients, medical personnel and those working in production. There is a possible role for standards in this process. A harmonized systematic risk management standard already exists for medical technology products and could form the basis for a risk management protocol for medical nanotechnology. In addition, existing harmonized standards relating to biological safety could be reviewed and revised as necessary to take account of the specific characteristics of medical nanotechnology products.

An approach to the safe, integrated and responsible introduction of nanotechnology into medical practice should thus be included at a fundamental scientific level, to assess all aspects of risk and to contribute to appropriate regulations for this new technology, so that safety for patients and others is maximised whilst, at the same time enabling the benefits of NanoMedicine to be realised in a timely manner and in a way that encourages innovation and technological development. This approach is fully in line with the Commission's European strategy for nanotechnology set out in the Communication "Towards a European Strategy for Nanotechnology" and its associated Action Plan.



Technician at a robot producing oligonucleotides
© P. Stroppa, CEA

8. Ethical Issues

Nanotechnology offers great promise for medicine, but much of this lies in the future. This future orientation has made nanotechnologies vulnerable to the current zeitgeist of over claiming in science, either the potential benefit or harm. There is a need to be careful about placing premature weight on speculative hopes or concerns about nanotechnologies raised ahead of evidence. Foresighting of breakthrough technologies is notoriously difficult, and carries the risk that early public engagement may promote either public assurance or public panic over the wrong issues.

Nanotechnology as an enabling technology for many future medical applications touches on issues such as sensitivity of genetic information, the gap between diagnosis and therapy, health care resources and tensions between holistic and functional medicine. On the other hand nanotechnology will add a new dimension to the bio (human) and non-bio (machine) interface such as brain chips or implants, which eventually might raise new ethical issues specific to NanoMedicine. This requires careful analysis of ethical aspects in view of existing standards and regulations by ethics committees at the European scale. At the same time new nanomedical inventions have to be evaluated for new ethical aspects by ethical, legal and social aspects - specialists. The most crucial point in this regard is an early proactive analysis of new technological developments to identify and discuss possible issues as soon as possible. This requires a close collaboration and co-learning of technology developers and ethics specialists assisted by communication experts to ensure open and efficient information of the public about ethical aspects related to nanomedicine. This co-evolution will ensure a socially and ethically accepted development of innovative diagnostic and therapeutic tools in NanoMedicine.

From the above it is clear that an in-depth ethical analysis is necessary in this field. Such an analysis should be based on the following principles.

Human Dignity and the derived ethical principles of:

- **Non-instrumentalisation:** The ethical requirement of not using individuals merely as a means but always as an end of their own.
- **Privacy:** The ethical principle of not invading a person's right to privacy.
- **Non-discrimination:** People deserve equal treatment, unless there are reasons that justify difference in treatment. It is a widely accepted principle and in this context it primarily relates to the distribution of health care resources.
- **Informed Consent:** The ethical principle that patients are not exposed to treatment or research without their free and informed consent.
- **Equity:** The ethical principle that everybody should have fair access to the benefits under consideration.
- **The Precautionary Principle:** This principle entails the moral duty of continuous risk assessment with regard to the not fully foreseeable impact of new technologies as in the case of ICT implants in the human body.

The last of these principles (the Precautionary Principle) is particularly important in this particular context.

The ethical analysis should also examine value conflicts

There could be conflict between the personal freedom to use one's economic resources to obtain advanced treatment such as NanoMedicine and what society at large considers desirable or ethically acceptable. Freedom of researchers may conflict with the obligation to safeguard the health of research subjects. Concern for economic competitiveness and other economic values (economic growth) may come into conflict with respect for human dignity. The unrestricted freedom of some may endanger the health and safety of others. Therefore a balance has to be struck between values that are all legitimate in our culture.

9. NanoMedicine: The bigger picture

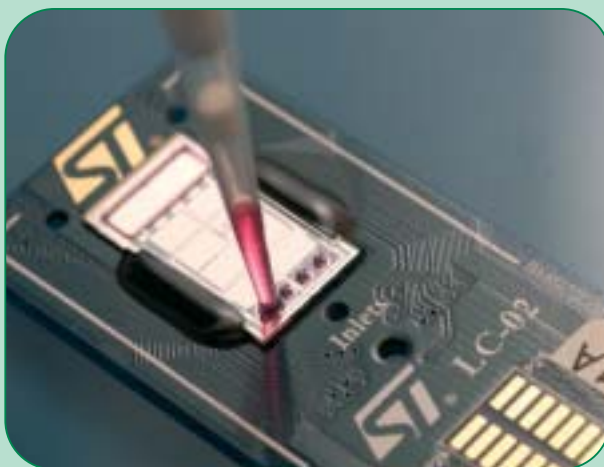
Future techniques in medical diagnosis and treatment have often been the subject of science fiction literature and cinema. What was once the stuff of science fiction is now closer to becoming reality. Nature operates at the nanoscale, and today we are acquiring an increasingly profound understanding of natural processes at this scale, enabled by a new generation of scientific instruments. From this knowledge, we are able to design devices that can either directly interact with, or influence, the behaviour of living cells. As with any nascent and rapidly developing field, there are research, technological, and ethical challenges to be considered, and the approaches to these constitute an integral part of the vision.

Nanotechnology has a trump card to play when applied to medicine. At the nanometre scale, materials often exhibit surprisingly different physical, chemical and biological properties, compared to the very same material in bulk form. The properties of nanoparticles, such as increased chemical activity and the ability to cross tissue barriers, are leading to new drug targeting and delivery techniques. In the future, a nanoparticle or a set of nanoparticles may be designed to search for, find and destroy a single diseased cell, taking us even closer to realising the ultimate goal of disease prevention.

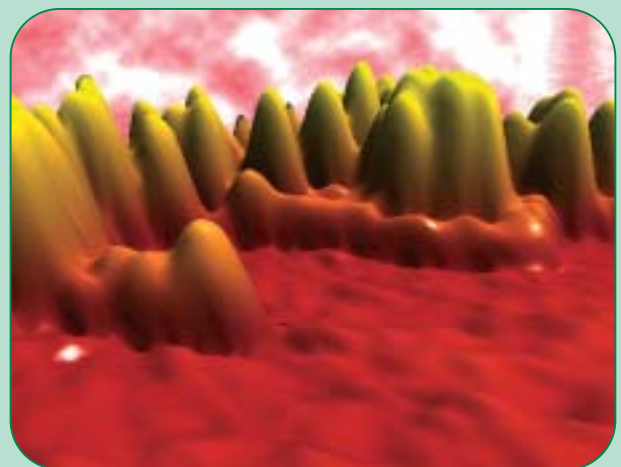
Nanotechnology is also making possible the stimulation of the body's own mechanisms to successfully repair diseased or damaged tissues, replacing the need for transplants and artificial organs. In the foreseeable future, nanotechnology as applied to medicine, will lead to advancement in remote monitoring and care, where a patient may be treated at home - a less expensive option, and one that is more conducive to a successful medical outcome than treatment in a surgery or hospital.

Continued research into disease processes at the molecular level is essential for the development of NanoMedicine, and involves teams of scientists from across 'conventional' disciplines, such as physics, chemistry, surgery and mathematics, as well as those from the 'new' fields of genomics, proteomics, metabolomics, pharmacokinetic modeling and microscope design.

Europe has already established key strengths in those nanomedical technologies which will form the basis of some future medical breakthroughs. The European Technology Platform on NanoMedicine will bring together the key stakeholders to ensure this lead is maintained, and that technologies are quickly developed to address the health needs of its citizens.



Filling channels with a mix for PCR analysis
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Plasma membrane with proteins, Scanning Force Microscopy
© H. Oberleithner, University of Münster

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The ageing population, the high expectations for better quality of life and the changing lifestyle of European society call for improved, more efficient and affordable health care.

Nanotechnology can offer impressive resolutions, when applied to medical challenges like cancer, diabetes, Parkinson's or Alzheimer's disease, cardiovascular problems, inflammatory or infectious diseases.

Experts of the highest level from industry, research centers and academia convened to prepare the present vision regarding future research priorities in NanoMedicine. A key conclusion was the recommendation to set up a European Technology Platform on NanoMedicine designed to strengthen Europe's competitive position and improve the quality of life and health care of its citizens.

