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Nanomedicine – challenge and perspectives

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Abstract

Nanomedicine introduces nanotechnology concepts into medicine and thus joins two large cross disciplinary fields with an unprecedented societal and economical potential arising from the natural combination of specific achievements in the respective fields. The common basis evolves from the molecular scale properties relevant in the two fields. Nanoanalytical tools such as local probes and molecular imaging techniques, allow us to characterize surface and interface properties at a nanometer scale at predefined locations, while elaborated chemical approaches offer the opportunity for the control and addressing of surfaces e. g. for targeted drug delivery, enhanced biocompatibility and neuroprosthetic purposes. This commonality opens a wide variety of economic fields both of industrial and clinical interests. However, concerns arise in this cross disciplinary area about toxicological aspects and ethical implications. This review gives an overview of selected recent developments of nanotechnology applied on medical objectives.

Keywords

disease; nanoprobes; theranostics; personalized medicine; nanotechnology

1. Introduction

To manipulate matter locally and deliberately on the atomic/molecular scale is an old dream of natural science. Starting in 1959 with the famous talk of Richard Feynman at the annual meeting of the American Physical Society where he developed the vision of manipulating and controlling things on a small scale, nanoscience developed over the discovery of the molecular beam epitaxy in 1968 in the Bell Laboratories, the generation of nanoparticles and the invention of the Scanning Tunnel Microscope (STM) to a robust and well accepted field in the scientific community.[1–3] The old dream became already true in the field of today's nanoscience and nanotechnology, opening novel opportunities in virtually all branches of technology ranging from optical systems, electronic-, chemical- and automotive industry to environmental engineering and medicine. Smart surface coatings, intelligent nanoscale materials, faster electronic, unprecedented optics, biosensors, and nanomotors are just a few examples from this transdisciplinary area, and although nanotechnology is still in its infancy, these first practical applications clearly demonstrate its enormous potential.

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The field of medicine, on the other hand, enfaces very complex scientific as well as societal and ethical challenges. In particular due to the progressing aging of the population some specific diseases are identified to have a very high socio-economical impact in the next years. Below we will discuss some specific areas, which we consider as promising applications of nanomedicine.

1.1 Definition

To start with *nanotechnology*, literature delivers a variety of definitions of *nanotechnology* [f. L. *nanus*, Gr. *nanos* dwarf] which all have their advantages and limitations. While the prefix "nano" is often used just for a description of the length scale between 0.1 to 100 nanometer (1 nm=10^{-9} m), this size regime does not imply per se a new quality of materials or devices. A more specific definition has been given in 2000 by the US National Nanotechnology Initiative: "Nanotechnology is concerned with materials and systems whose structures and components exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes due to their nanoscale size". With the reduction of magnitude, apparently different, and qualitatively new and advantageous properties emerge from the respective material at the nanometer scale.[4–6] A more general and operational definition involves the following interrelated constituents: nanoscale dimensions of the whole system or its vital components, man-made nature and the unique characteristics of a new material that arise due to its nanoscopic size.[7]

Thus, *Nanotechnology* includes the following physical and chemical key issues:

1. Occurrence of novel physical properties characteristic of the nanoscale
2. Analysis at the atomic and molecular scale at predefined positions.
3. Control of matter, at the atomic scale, i.e. addressing individual preselected atoms and molecules
4. Generation of complex functional systems with qualitatively novel properties (emergence).

To define the area of nanomedicine (NM) to be discussed below we first have to introduce a differentiation to the field of molecular medicine (MM), biochemistry as well as nanobiotechnology.

Nanomedicine means essentially applying nanotechnology to medicine. While being at certain areas related, the field of *nanobiotechnology* differs from nanomedicine, the latter focusing on the applications of nanotechnology concepts to medical applications, while the former encloses all basic research at a nanoscopic level on biological systems, e.g. investigations on plants. *Molecular medicine*, on the other hand, starts from a more conventional biochemical molecular approach.

Unlike conventional therapies – surgery, radiation and chemotherapies – where the basic approach is to remove diseased cells faster than healthy cells, *NM* attempts to make smart decisions to either kill specific cells or repair them one-cell-at-a-time based on biosensor information that controls, for example, drug release. For that it is going to apply and adopt

NT-concepts and will, at the same time, feedback novel challenges and requirements to NT such that the two fields can cross fertilize and develop jointly. Thus, it is about the design of multilevel molecular assemblies that have novel functional and dynamic, i.e. emergent properties for application in medicine using the size- and site-specific properties of systems characteristic of the nano- and mesoscale. This approach offers also new possibilities towards the development of *Personalized Medicine (PM*), which is defined as: "the concept which marks the expected reform in medicine that is projected to arrive at the clinic in coming decades, harnessing genomics and proteomics technologies for tailoring the most suitable pharmacotherapy for each patient; based on individual profiling, it is also projected to allow improved treatment efficacies for many diseases".[8] To avoid side effects and overdosing of drugs, most efficient medications were established applying selective targeting. This field is currently under intensive investigation. Nanomedicine promises here alternatives to molecular medicine with the following general advantages inherent to the nanometer scale: local processes at the nanometer length scale, such as diffusion, intermixing and sensoric response become ultra fast. Further, NT can provide direct probing of local properties, processes can be controlled and intensified, the precision is enhanced, and direct access to biomarkers becomes possible. Finally, new results can be achieved from real time control. These concepts together with a combination of the research areas like *systems biology* and *systems medicine* will contribute significantly to form the pathway to PM.

How is *Personalized Medicine* related to *Nanomedicine*? Similarly, as in existing medical diagnosis and therapeutics, and as dictated by economical reasons, mass applications of new screening and diagnostic tools in medicine have to be fast, convenient and inexpensive. Therefore, miniaturization, parallelization, integration as well as automation are mandatory. The demand of large amounts of routine *in vitro* measurements on patients to retrieve sufficient and comparable data dictates the development of new smart integrated devices such as biosensors and decentralized actuators and drug release concepts – requirements that can only be fulfilled with the help of nano-and microsystem technologies.

Nanomedicine includes the development of nanoparticles, nanostructured surfaces and nanoanalytical techniques for molecular diagnostics, treatment, follow up and therapy of diseases (*theranostics*), as well as integrated medical nanosystems, which, in future, may perform monitoring and complex repairs in the body at the cellular level. N*anotechnology* considers cells as a complex system of interacting nanoengines. Visionary concepts suggest the construction and control of artificial cells using engineered nanodevices and nanostructures for medical applications.

2. Nanotechnology based medical diagnostics

Diagnostics as a means for successful prevention and efficient treatment of diseases plays a key role in medicine. Taking cancer as an example for a widespread disease that is still the leading cause of death in the industrial countries, a significant increase in cure rate would be unlikely to achieve unless more information about molecular mechanisms of the pathophysiology can be obtained which will build the fundament for new anticancer

drugs.[9] The advantage of nanostructure based diagnostics lies in its potentially higher sensitivity and selectivity as compared to classical methods.

Of special importance is here the generation of nanoscale materials, a major topic in the field of NT. For diagnostic purposes quantum confinement effects may be used which are characteristic of the nanometer scale. Nanoparticles may be embedded in other crystalline or amorphous nanoscale materials to guarantee better functionality and bioavailability. In this area the worldwide development of metallic and semiconductor quantum dot (Q-dot) structures, nanoclusters as well as nanopowders is intense. For medical applications (*molecular imaging)* some types of these particles can be used *in vivo* as markers in various imaging techniques such as Infrared (IR) or Nuclear Magnetic Resonance (NMR) methods to significantly increase the resolution and sensitivity, thus enabling earlier diagnosis of diseases. With increased resolution and sensitivity cheaper clinical measures are expected in therapy. Molecular recognition, i.e. the modification of nanoparticle surfaces with chemical recognition groups allows identifying complementary groups on cell surfaces which are indicative, for example, of cancer or other severe diseases (see Figure 1). The same concept can then be applied for site specific drug delivery.[10–12]

2.1 *In vitro* diagnostics

The purpose of the extracorporeal (*in vitro*) diagnostics of cells is manifold. It is necessary to protect the blood supply for transfusion reasons, to monitor the level of drugs applied to patients and to provide information to assist diagnosis and treatment of disease. The ultimate goal of any diagnostic procedure is a non-invasive, early and accurate detection of the biological disease markers in the process of routine screening, enabling to choose the appropriate treatment regimen. Various nanotechnology platforms are developed to allow for simultaneous real-time evaluation of a broad diversity of disease markers by non-invasive techniques. Interestingly, historically originated in 1980s as the microtechnological platforms two classes of devices, microarray DNA-chips, and microfluidic systems for lab-on-chip diagnostics, have now been transferred to the nanotechnology arena. This “miniaturization” was possible due to a development in the fundamental enabling technique in both cases, namely photolithography, which now allows for the lateral resolution in the 10–100 nm range, three orders of magnitude lower than at the time when these platforms were first generated. As a result the information put on a biochip could be increased by 1–100 million fold demonstrating the powerful capabilities of nanoscaling in biomedical applications. Using photolithography, photolabile groups are selectively illuminated and removed leading to exposure of reactive moieties. The technique can be used for very precise patterning of various chemical and biological moieties and diverse textures on the substrate enabling surface attachment of biomolecules to specific molecular segments, e.g. single stranded DNA for hybridization or different substrates for proteomic analysis.[14–20]

A central position in the medical diagnostic is occupied by the goal to analyze *single cells*. Nanotechnology, with the opportunity to investigate even single molecules, opens the door also here. The added value of this work becomes clear taking into consideration that larger amounts of primary cells are usually mixtures either of different cell types, or of healthy and tumorous cells, making the acquisition of statistically significant results difficult.[21]

Another motivation for single cell analysis matters the dilution of effects. In the case of disease this means that small differences between cell types or weak effects of drugs are not detectable using complete tissues. Biochemical methods often fail to give the tools for appropriate investigations because the large amount of cells needed e.g. for electrophoresis purposes leads to the analysis not of cells but of tissues or cell mixtures, i.e. systems, which give no insight into the condition of defined building blocks. Being able to describe one specific cell(type), the role of this building block in the tissue and the organism can be defined and the function of cell interaction, the effect of differentiation and diseases can be characterized.[22]

For the isolation of single adherent cells, different selection techniques such as cloning rings, limiting dilution, laser microdissection, live cell catapulting or microfabricated pallets are used.[23–25] Fluorescent activated cell sorting (FACS), magnetic sorting, column chromatography, panning, limiting dilution and by isolation of cells via microfluidics are commonly used for isolation of nonadherent cells.[24;25]

The classical analysis of those cells is performed by biochemical methods such as PCR or patch-clamp techniques. Nanotechnology is used for biochip analysis as performed with photolithographic technology (see section 2.1.1). Together with the development of smart surfaces, semiconductor manufacturing, and combinatorial chemistry and bioinformatics gave new impact in the expression analysis of single cells.[26–30] Biochip analysis, on a multicell level, is now well accepted in clinical diagnostics in several fields. For example, expression chips for the follow up of bacterial infections in the mouth made significant progress such that they are up to Point-of-Care-Diagnostics. The modification of biochip surfaces by nanotechnological methods opens the gateway to ever smaller probes for the analysis of RNA, retrieved from a single cell.

The success of expression profiling encouraged the protein investigating community to adopt some of the methods. As the differences between the expression of proteins and their biochemical appearance (e.g. folding structure or secondary modification) is remarkably high, the analysis of proteins on a single cell level is coming into the focus of industrial and scientific research because the results obtained reflect much more the biological processes within a cell than the expression profiling. Different kinds of biochips like antibody arrays or other protein arrays are available (see Section 2.1.1). Antibody arrays are known since 2002 where the application of such arrays where first published.[31;32] Clinical applications for such protein chips include the disease marker discovery for diagnosis, prognosis, and drug response and allow a follow up of disease development and progression. Antibody arrays are high-throughput tools that improve the functional characterization of molecular bases for disease. Furthermore, the characteristic of cancer progression and tumour subtypes – information gained from the protein array – may intervene and improve therapies of patients.[33–41]

Atomic Force microscopy (AFM; see Section 2.1.3.1) techniques are explored for single cell analysis. They are used for high resolution *in vitro* investigations for the analysis of cell surfaces and physical properties like mechanical compliance of single cells, useful parameters for the analysis on a single cell level. Force Spectroscopy provides locally direct

quantitative data on intra- and intermolecular forces at a single molecular basis.[42–48] As a manipulation tool it was used, for example, as a microdissection device. Offering the possibility to isolate organelles and to cut chromosomes in a precise way, this technique was applied together with subsequent PCR amplification of dissected DNA fragments, for analysis and even mechanical re-implantation of the isolated fragments back into its original position.[49;50]

2.1.1 Microfluidics and Nanoarrays—A microfluidic unit can be identified as a device comprising one or more channels with at least one dimension measuring less than 1 mm whith channels sizing below a few micrometers down to several hundreds of nanometers, thus allowing to control minute fluidic volumes of nanoliters and picoliters. Microsystem technologies developed for microfluidic chips enable just about any biological assay working on a molecular level to be incorporated onto a chip, known as *lab-on-a-chip* systems (Figure 2). These approaches not only offer the possibility to isolate and manipulate living cells, but also to perform toxicity assays, enzyme-linked immunosorbent assay (ELISA), PCR amplification, blood separation or for the genotyping of cytokine genetic polymorphism.[51]

The flow of fluidics in a microfluidics chamber is characterized by the Reynolds number which is defined as

$$\begin{aligned} Re &= (\rho u^2)/(\mu u/L) \\ &= \rho u L/\mu \\ &= \mathrm{uL}/\nu \end{aligned}$$

Re = Reynolds Number (non-dimensional), ρ = density, u = velocity, μ = dynamic viscosity, L = characteristic length, ν = kinematic viscosity

The Reynolds number should be less than 100 to maintain laminar flow, necessary to provide the means by which molecules can be transported in predictable manner through microchannels. Using materials like e.g. Poly(dimethylsiloxane) (PDMS), microfluidic chips became a conventional easy-to-produce and easy-to-use technology. The ability to tailor the material for single cells gives a strong push to this research area.[52–54] Another big advantage of PDMS is its biocompatibility. It is assumed to be a suitable biomaterial for the biomedical devices because it causes minimal endotoxin contamination, leukocyte activation, and complement activation.[55] Du et al. showed in 2006 that in a mixture of normal human glandular epithelial cells (HGEC), human cervical stromal cells (HCSC) and cervical cancer cells (HCCC) flowing through an antibody-based microfluidics platform, more than 30% of the cancer cells were captured by such a unit.[56]

Another approach was the development of a microfluidic cell chip for monitoring allergic response. On a PDMS chip which contains a cultivation chamber and microfluidic channels a basophilic leukemia cell line (RBL-2H3) was cultivated. Fluorescence marked dye molecules were secreted after allergic stimulation and observed using a photomultiplier tube (PMT) fitted onto a microscope. Various nanochannel structures can be imprinted for selective fractionating of proteins based on their molecular weight. As a result, different

patterns could be simultaneously produced by treating the chip with dissimilar biological samples (Figure 3).[57]

Specific serum markers for early diagnostic of diseases such as cancer are currently unavailable. The capabilities of nanotechnology in this area are enormous due to the possibility to evaluate a wide multiplicity of molecular markers at once and to integrate this information producing reliable techniques for early and efficient diagnostics as well as for monitoring and selecting therapeutic strategies. Single clinical markers that are now used for diagnosis of carcinogenic conditions, for example prostate specific antigen (PSA), cannot provide this knowledge due to the broad inter-individual variability of their basal expression. Thus, *ex vivo* diagnosis using biological fluids such as serum, saliva, urine or tissue exudates obtained from non- or minimally invasive procedure remains an unmet need.[59]

Meanwhile other concepts of fluidic devices are on the market. One of these is a device on the basis of dielectrophoretics. Dielectrophoretic field cages give the possibility to combine the isolation and the manipulation of nonadherent cells in one device. This principle was introduced by Fiedler et al. in 1998. Certain electrode configurations were constructed to function either as a funnel, as ligners to break aggregates of cells or as electrical octodes to trap cells electrostatically for manipulation. Recently it was shown that in culture moderate thermal effects induced by the electrical field can be neglected under appropriate experimental conditions. Moreover, possible side effects of dielectrophoretic manipulation such as membrane polarization and Joule heating were excluded making the method appropriate for medical applications.[60–62]

An urgent need was the follow up on the systemic inflammatory responses that could be induced in patients following a Cardiopulmonary Bypass (CPB). As a general rule, the ability to clinically intervene in inflammation is limited by the lack of timely measurements of inflammatory responses, while blood analysis performed in medical laboratories can take from several hours to days. Thus, there is a need for a system that can separate plasma from whole blood and measure the concentration of the clinically relevant proteins in real time. A microfluidics device was fabricated to follow up the development of inflammation markers by real time blood plasma separation, which may be integrated with downstream plasma analysis device.[63] Here, microfluidics offer the chance to intervene at early stage in an inflammatory process which if untreated could be life-threatening. Recently, a new microchip with an anisotropic nanofluidic sieving structure to separate and sort biomolecules as DNA or proteins was developed at the MIT. With an extremely tiny sieve structure, the system can sort through continuous streams of biological fluids and separate proteins by size, providing an appropriate tool for the identification of small molecules for early diagnostics and follow up of medical treatment.[64]

One of the recent promising approaches in diagnostics is based on the specific recognition of the biomolecular interactions by using the appropriately selected nanosensors. This concept, proposed by the group of Gimzewsky, encounters for the nanoscale forces and deformations produced as a result of ligand-substrate binding.[65] Micro- and nano-cantilevers, the devices based on this principle, deflect and change resonant frequencies as result of affinity binding of biomarker proteins or DNA hybridization events occurring on their free surfaces (Figure

4a). The deflections can be monitored by lasers or electronically detected, enabling to rapidly and simultaneously sense a variety of biomarkers. These structures were shown to detect target oligonucleotides without fluorescent or radio labeling and serum markers at clinically significant levels.[59;65–67] Other examples of sensor technologies based on nanofabrication are nanowires and nanotubes. Nanowires placed in a microfluidic system can specifically bind or absorb various sensors, causing shift in their conductance as a function of electrical charges of the bound molecules (Figure 4b).[67–72] These changes can be electronically detected and precisely quantified. Though not yet in clinical practice, the multiplexing capabilities of these systems are fascinating and hold promise for simultaneous determination of wide array of proteomic profiles.

2.1.2 Fluorescent labels and imaging—Fluorescent dyes represent another important class of *in vivo* imaging tools mainly used for the visualizations of cells and molecules. A big disadvantage of those dyes is their photo instability with the fluorescent yield rapidly fading within less than 1 minute. The bleaching of the dyes restricts the range of their applications. Inorganic quantum dots exhibit a much higher photostability. However, selenides and sulfides mostly applied for that purpose are cytotoxic and can, therefore, be only used for diagnostics of biological samples outside the human body. Due to their biocompatibility, high photoluminescence quantum efficiency and stability against photo bleaching, silicon quantum dots are ideal candidates for replacing fluorescent dyes in biological assays. Si nanocrystals (NCs) can be fabricated using wet chemsitry or electron-beam lithography and reactive ion etching resulting in Si nanopillars that were subsequently oxidized to produce luminescent silicon cores.[73] They are so small that the addition or removal of a single atom changes their optical appearance significantly. Other unique properties of quantum dots are size- and composition-tunable emission, wide large absorption spectra and narrow emission spectra (Figure 5).[74;75]

The improved brilliance and photostability of Q-dots makes them appropriate systems for targets like cells, or for the detection of low abundance antigens.[76;77] Tests revealed that they are not stable *in vivo* because of degradation effects that lead to quenching of the fluorescence. Gao et al. demonstrated recently that a hydrophobic exterior protects the Q-dot from this effect and optimized it for medical applications. The dyes allow e.g. to distinguish simultaneously different types of cells within a tumor *in vivo*.[78–81]

2.1.3 Local probes and high resolution imaging

2.1.3.1 Chemical sensitive probes: Profiling of mammalian cellular components by matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry is known to offer also a way to characterize cells and tissues on a biochemical level. Downscaling this technology opens meanwhile the possibility to characterize proteins of one single cell.[82–87] Pioneering work in the field of time-of flight mass spectrometry as applied to medical questions was done by Benninghoven and colleagues.[88;89] An approach to characterize isolated cells was first described by Colliver et al. in 1997. They performed single cell analysis using time-time-of-flight secondary ion mass spectrometry (TOF-SIMS) providing chemical information. Preparing the cells by freeze fracture techniques, TOF-SIMS enabled characterization of the surface of *Paramecium multimicronucleatum*.[90] In a combined

technique optical microscopy (OM), ion induced electron (IIE)and *Laser post-ionization secondary neutral mass spectrometry* (Laser SNMS) were recently used for mapping native biomolecules within mouse kidney cells (Figure 6).[91;92]

2.1.3.2 Tip probe analysis: Since many biomedical and nano-medical processes occur on the molecular scale the ability to image nano-structures at predefined positions and to perform, in addition, local spectroscopy is becoming more and more important. Scanning probe microscopy opened a completely new area of surface imaging technologies complementing conventional methods such as electron and light microscopy. In particular, dynamic force microscopy operational modes are well suited for investigating soft systems such as biological cells, and it also allows tracking of individual proteins and imaging of biological macromolecules in liquids.[93–95] For example, cytoskeletal structures like stress fibres can be imaged with the AFM and the dynamics of nuclear pores after treatment with dexamethasone were imaged by this technology.[96;97] The visualization of cells is possible without damaging their surface as it was shown for renal A6 cells using AFM. Focal adhesion plaques imaging was successfully done as well as high-resolution imaging of membrane transport.[98–100]

A novel experimental approach combining AFM with quantum-dot-labeled antibodies used as surface markers, to detect the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) involved in the development of cystic fibrosiss (CF) was recently proposed. Comparison of erythrocyte plasma membranes taken from healthy donors and CF patients revealed that erythrocytes reflect the CFTR status of the organism and that quantification of CFTR in a blood sample could be useful in the diagnosis of CFTR related diseases.[101]

Promising developments in the AFM technology enabled its utilization for *in vivo* imaging. As Imer et al. demonstrated, AFM technology can be used as a minimal invasive tool in clinical diagnostics of rheumatoid arthritis to demonstrate disease related developments in the particular cartilage.[102] It is has also been proven as a suitable instrument to analyze the cell surface morphology in intact native human stratum corneum (SC). SC is composed of cornified keratinocytes (corneocytes) organized within the whole SC layer as bricks in a wall. In between the single corneocytes are linked together by a complex matrix composed of lipids and proteins. Skin diseases or aging of the skin has been shown to change the composition of the SC and corneocyte morphology. AFM has been presented as a suitable tool for the nanometer scaled analysis of native SC in terms of morphology and quantification of the volume and surface of single corneocytes.[103] Figure 7 shows representative images of SC of atrophic and healthy skin. An influenced composition of the corneocyte surrounding matrix was indicated by prominent intercelluar gaps. While atrophic skin SC surface was covered by a strongly pronounced filamentous network healthy skin SC surface was characterized by a homogenous distribution of regular shaped hump-like structures. Moreover, single corneocytes flatten upon aging, as indicated by an increased single cell surface area and a decreased cell height. The application AFM for physiological questions was recently comprehensively reviewed in a special issues of Pflugers Archiv (European Journal of Physiology).[104]

A specialized method denoted as visualization at the nanoscale level offers the Force sensing Integrated Readout and Active Tip (FIRAT).[105] It is much faster and more sensitive than the regular AFM and it also is able to record movies and to simultaneously capture several physical properties of nanostructures, such as stiffness, elasticity or viscosity. The features of this method may open the door to new much more sensitive presentation of cellular membranes than it was possible before.

2.1.3.3 Plasmonic and optical techniques: A method based on Surface Plasmon Resonance (SPR) to describe the interaction of biomolecules in a label free matter was developed by Rothenhäusler and Knoll in 1988.[106] SPR monitors changes in the refractive index in the vicinity of a surface. This effect occurs when light is focused at a certain angle on the glass/metal interface of a thin metallic film to excite the surface plasmons, i.e. the collective oscillations of free electrons propagating along the film's surface. When the biomolecules immobilized at the free metal surface are bound by their ligands, an alteration of the interfacial optical conditions occurs affecting the plasmons propagation on the free surface of the film. The binding of biomolecules is measured by changes in the refractive index. SPR imaging offers the possibility to measure the binding force of interacting biomolecules. In fact, the kinetic analyses of most biomolecular interactions like protein-protein, protein-lipid, protein-nucleic acid and protein- drug is accessible by SPR techniques. Recently, the method was used to detect the effects of plasma exchange in the blood. It was described as an accurate, time-saving method for measuring anti-A/B IgG titers that can be easily standardized and which can be used e.g. for the analysis of blood necessary during transplantations. Another development is presented by SPR *microscopy* which made high throughput imaging analysis of binding events possible.[107;108]

Laser-optical techniques have recently experienced a dramatic development towards nanoscopic medicine, as summarized by Peters.[109] The group of Bräuchle has demonstrated that a special confocal laser optical method for Single Virus Tracing (SVT) allows the direct investigation of the entry pathway of viruses into living cells using fluorescence labeled Adeno Associated Virus (AAV) particles (Figure 8).[110;111]

Biomedical information can also be retrieved from digital holography. This method, allows marker free quantitative analysis in the cellular and sub-cellular range.[112–114] With a vertical resolution of less than 8 nm holographic interferometry provides information about thickness/shape variations as well as for the analysis of volume changes and micro motion detection of cellular samples. Using this technique the differences in dynamic process of living invasive and noninvasive pancreatic cell lines was shown.[115–117] The characterization of fast movement of cells by digital holography can be taken as a predictive tool for the metastatic properties of a tumor.

Hell et al. developed a pioneering and very promising tool in the field of digital imaging. They used Stimulated Emission Depletion (STED) to reduce the focal spot area by about an order of magnitude below the optical diffraction limit, thereby resolving individual vesicles in the synapse (Figure 9). This opens completely new perspectives for high resolution optical imaging in the nanomedical field. Though not yet used for clinical applications the technique allows to retrieve nanoscopic optical information within living cells which was

hitherto obtained only by electron microscopy methods, the latter not allowing to keep cell bioactive. Recently the group published a dual-color STED with the ability to resolve about 25–35 nm in two channels. Nonlinear iterative (Richardson-Lucy) deconvolution leads to a further increase of the resolution (Figure 9). The technology was applied to the imaging of nanometer-sized features inside cells.[118–121]

The group examined neurofilaments (NFs) of neuroblastoma cells. These proteins belong to the major constituents of the axonal cytoskeleton and consist of three different subunits, the light, medium and heavy NF. In a two colour experiment the light NF was stained green whereas α-internexin, also a component of the mature filament, was marked in red. The different localization of the proteins was clearly shown (Figure 9).[121] Thus, STED provides complementary information to electron microscopy, with the added value of allowing investigations on living cells.

Also recently, Juette et al. demonstrated sub-100 nm optical resolution of thick samples using Biplane-Fluorescence Photo Activation Localization Microscopy (BP-FPLAM). This far-field techniques allowed to generate images with 30 × 30 × 75 nm resolution over a depth of several micrometers.[122]

Complementary to advanced optical techniques various types of Electron Microscopy play an important role for imaging of a biological specimen, providing an enormous amount of useful information. In recent years, numerous fascinating high-resolution EM structures obtained by cryo-Electron Microscopy (cryo-EM) were revealed. The technique is currently being developed for a comprehensive three-dimensional analysis of complex structures including viruses or molecular landscapes within whole cells. This paves the way for ‘Visual proteomics’ aiming to complement and extend mass-spectrometry-based inventories, and to provide a quantitative description of the macromolecular interactions that underlie cellular functions.[123–125]

2.2 In vivo diagnostics

The evolution of nanotechnology and the need for personalized medicine give the drive to foster development of point-of-care diagnostics with higher sensitivity, specificity and reliability. *In vivo* diagnostics creates data instantaneously from the patient and allows the follow up of the disease development and therapy. The *find*, *fight* and *follow* concept (‘*theranostics’*) of early diagnosis, therapy and its follow up will take a new turn with developments in nanotechnology. Appropriate contrast agents for imaging on the level of a single cell (‘find’), delivery of therapeutic drugs (‘fight’) and monitoring of the therapeutic development (‘follow’) are key issues of future medical care.

Advancement in this research area will also rely on imaging of single molecules and on implantable devices. In molecular imaging, the aim is to create detection agents that can also deliver and monitor therapy. Especially the detection of diseases in an earlier stage is a central goal. Nanotechnology displays a unique perspective to produce a new generation of biosensors and medical imaging techniques with higher sensitivity and precision of recognition. This target can be reached by new nanoparticles developed for a more specific and a more sensitive imaging. In addition, miniaturizing of biosensors gives a chance for

implantation of diagnostic devices which send continuous information to a reader outside of the body, e.g. to detect the amount of cholesterol in blood – a big improvement of living conditions of people who need permanent medical monitoring.[126]

2.2.1. Targeted Imaging—Optical and electronic effects originated from spatial confinement are not observable in macroscopic systems. Developments in this area include quantum dots, metallic and semiconductor nanoclusters, and nanopowders.[127] Some of these particles can be used within the human body as markers in nuclear imaging techniques *(molecular imaging)*, thereby increasing resolution and sensitivity dramatically while enabling earlier diagnosis of disease.[75;128] As a consequence, cheaper clinical measures can be applied also in therapy. Functionalized nanoparticles exhibit vectorial character as discussed in more detail below. They can specifically identify complementary groups on cell surfaces that are indicative for diseases. As an example, superparamagnetic iron oxide nanoparticles (SPION) linked to a phosphorothioate-modified oligodeoxynucleotide (sODN) complementary to c-fos mRNA (SPION-cfos) were developed to trace neurodegenerative diseases via magnetic resonance (MR).[12]

A well established application of Superparamagnetic Iron Oxide (SPIO) – or Ultrasmall Superparamagnetic Iron Oxide (USPIO) – labeled cells in combination with MR imaging (MRI) is the follow up of immune cells (monocytes/macrophages) during the development of an inflammation. This tool is used for diagnostics of e.g. cardiovascular diseases or for multiple sclerosis. Additionally, the blood brain barrier can be passed by this iron oxide particles using macrophages as carriers, opening possibilities for the investigation of e.g. neurodegenerative brain diseases.[129–133] As reviewed by several authors MRI with nanoparticle tracers are also applied for the detection of apoptosis, angiogenesis and tissue infiltration during development of cancer.

Other applications for targeted imaging are SPIO particles which are used for stem-cell tracking, multimodal perfluorocarbon nanoparticles for visualization of angiogenesis, liposomes for targeting atheroma components, and micro-bubbles for imaging transplant rejection.[134–138]

In elaborate systems diagnostic particles have to display different specific properties and functions like magnetic behavior, stimulated optical emission and targeted binding (see Section 3). However, multiple functionalities embedded into a single system could inhibit each other leading to a loss of the desired function. For example, nanobodies used for targeting may inhibit the attachment of dyes to the system. On the other hand, NPs offer a better surface-to-volume ratio, and consequently ever smaller sized particles offer more of their reactive sites at the surface. Quantum dots (Q-dots) belong to this class of systems.[139]

Targeted imaging techniques are currently developed in many laboratories worldwide in close collaboration between physicists, medical specialists, biochemists and chemists as well as engineers. This will lead to much faster specific co-development of this kind of methodology and clinical imaging technologies including positron emission tomography (PET) and nuclear magnetic resonance imaging (MRI).[140;141] Together with Computer Tomography (CT) and Single Photon Emission CT (SPECT) these clinical imaging

techniques belong to the rapidly developing area of molecular imaging techniques enabling physicians to display ever finer details of *in vivo* tissues independently from the organ. For example, to visualize organs by PET bioactive radiotracer molecules are required. The application of 18-fluorodeoxyglucose (18F-FDG) for the detection of different types of cancer is here well established and reviewed by several authors.[142–145] The tracer must be appropriately chosen for the relevant application e.g. the detection of an inflammation or a specific cancer. Thus, the true power of this functional imaging relies on the availability of tracers that are specific to the biological question pursued.[146] The challenge for nanotechnology is to develop tracers for new applications e.g. the *in vivo* detection of gene expression.

Though materials developed for MRI application have a size mostly far beyond the nanoscale, this method strongly depends on the development of new nanosized contrast agents which may significantly improve its applications area and resolution power. For example, Au_3Cu_1 hollow nanoclusters with an average diameter of 48.9 ± 19.1 nm and a shell thickness of 5.8 ± 1.8 nm were developed.[147] These bimetallic agents enhances the contrast of blood vessels and suggests their potential use in MR angiography as blood-pool agents. Colloidal magnetic nanoparticles represent another group of agents for the visualization of organs by MR. They combine a small size, strong magnetism, high biocompatibility, and the possession of active functionality for desired receptors. Coupled to cancer-targeting antibodies nanocrystals show big advantages for monitoring of *in vivo* targeting events of human cancer cells implanted in live mice. Other MRI contrast agents are Gadolinium-based dendrimers which can be effective at a very low concentration. A number of different dendrimers are existing that have different sizes and, as a consequence of this, different target organs.[148;149] Winter et al. characterized a iodinated oil nanoparticle (NP) for the imaging of atherosclerotic plaques via CT.[150] With a size of about 160 nm, iodine oil particles used in these experiments are also not within the limitations of the strict definition of “nano” (up to 100 nm) but the group was one of the first who described specific nanometerscale targeted agents for CT.

3. Nanotechnology in therapy – research and development

The pronounced anticipated advantage of using *nanovectors* (NPs capable of transporting and delivering one or more bioactive molecules, including therapeutic agents and imaging contrast enhancers) for biomedical applications is their ability to overcome various biological barriers and to localize into the target tissue when systemically administered. Currently used and investigated nanovectors could be generally classified into three main groups or “generations” as graphically summarized in Figure 10.[151]

The first generation (Figure 10a) comprises a passive delivery system that localizes into the target site. In case of tumor as a target tissue and liposomes as a nanovector, the mechanism of action accounts for Enhanced Permeation and Retention (EPR) effect, which drives the system to home into tumor through the fenestrations in the adjacent neovasculature.[152] These systems are generally decorated on their surface by a “stealth” layer (e.g. polyethylene glycol, PEG) preventing their uptake by phagocytic blood cells and thus substantially prolonging their circulation time.[153–155] The most pronounced representatives

of this generation in clinical use are liposomes. Other systems in this category include metal NPs for use in diagnostics and albumin paclitaxel NPs approved in early 2005 for use in metastatic breast cancer.[156] The localization in this case is driven only by the particles nanodimensions and is not related to specific recognition of the tumor or neovascular targets.

The second generation in this taxonomy (Figure 10b) could be thus defined as having specific additional functionalities on each individual particle allowing for molecular recognition of target tissue or for active or triggered release of the payload at the disease site. The best examples of the first subclass of nanovectors in this category are antibody-targeted liposomes and NPs.[157–159] Various targeting moieties besides the antibodies are under investigation worldwide. These include ligands, aptamers and small peptides binding to specific target cell surface markers or surface markers expressed in the disease microenvironment.[160–162] The nanovectors in the second subclass of this generation include responsive systems e.g. pH-sensitive polymers or those activated by the disease site-specific enzymes as well as a diverse group of remotely activated vectors. Among the interesting examples are gold nanoshells activated by Near Infrared (NIR) light or iron oxide NPs triggered by switching magnetic fields.[163;164] Other techniques used to remotely activate the second generation particulates include ultrasound and radiofrequency.[58;165–167] Linking nanoshells to antibodies that recognize cancer cells, enables these novel systems to seek out their cancerous targets prior to applying NIR light for heating them up. For example, using prostate cancer in a mouse model, nanoparticles activated with 2′-fluoropyrimidine RNA aptamers that recognize the extracellular domain of the prostate-specific membrane antigen (PSMA), and loaded with docetaxel as a cytostatic drug, were used for targeting and destroying cancer cells.[168;169] Another new approach is based on the coupling of nanoparticles to small interfering RNA, used to silence specific genes responsible for malignancies. By using targeted nanoparticles, it was shown that delivered siRNA can slow down the growth of tumours in mice without eliciting the side effects often associated with cancer therapies.

Though the representatives of the second generation have not yet been approved by FDA, there are numerous ongoing clinical trials involving targeted nanovectors especially in cancer applications.

As already described, the negotiation of drug delivery nanovectors with a variety of biological barriers provides tremendous potential advantage in medicine.[58;151] Following the brief introduction of the first two generations of nanovectors in their wide variety above, we will focus here on these barriers, which the drug or vector encounter when introduced to the body, and which significantly lessen the probability of reaching the target tissues at concentration required for obtaining therapeutic efficacy, as well as on the third generation of the particulates that are aimed to successfully negotiate these barriers (Figure 10c). Considering the whole picture, it becomes clear that the molecular recognition between the vector and the affected or target tissue by use of antibodies, for example, plays only a small role in the overall myriad of bio-barriers that the vector should bypass to efficiently deliver the drug to the target site. This observation is sustained by reports that only a small portion of a targeted moiety (e.g. antibody) administrated systemically reaches the aimed tissue,

which does not reflect its *in vitro* specificity.[170] The plethora of obstacles which the agent observes on its way to the target tissue includes metabolic clearance and chemical instability of the drug, endo/epithelial barriers, osmotic pressure gradients within the affected tissue and hemodynamical aspects of particle margination.[151;171] Mathematical modeling, recently applied to nanoparticulate objects in the blood stream, demonstrated that a spherical shape of about 50–100 nm in diameter is the worst from the margination point of view when compared to other sizes and shapes.[171–174] The term margination dynamics is used in this context to describe the lateral movement of the vectors to the vascular endothelium. This characteristic is important to allow the vector drifting in the close proximity of blood vessel walls, possibly within the cell-free layer and thus enabling firmer attachment to the vasculature. Based on the hemodynamic forces acting on the particles, spheres of 50–100 nm diameter tend to stay in the center of blood vessel without proper margination toward the vessel walls where the recognition with molecular targets can occur. It is important to emphasize here that the majority of nanovectors in clinical use and biomedical research could be included under this least favorable geometric category. Thus, vectors enabling utilization of multiple synchronized modalities responsible for overcoming various sequential bio-barriers, could highly improve drug therapeutic efficacy.

As mentioned earlier, multiple and sequential mechanisms are responsible for preventing a therapeutic or contrast agent from reaching its target. The contribution of particle geometry has been overlooked, mainly, because it has been traditionally limited by the fabrication/synthesis skills of each individual laboratory and by the type of application. Recent advances in the nanofabrication technology open new avenues for the developments of alternative geometries for injectable vectors.[175] The integration of various functions requires carrying and delivering a sufficiently large amount of various agents for therapy, imaging, thermal ablation, remote guidance and possibly other functions, which can only be allocated in a sufficiently large particle. In theory, the ideal nanovector will be capable of circulating in the vasculature following intravenous administration, reaching the required target tissues at enhanced concentrations, affecting the disease site, while not having any adverse effects. It is important thus to understand that for designing this multitasking modality the new “multistage” approach is required. Such a system was recently reported.[176;177] The nanovector is based on biodegradable and biocompatible silicon microparticles with pores sizes of up to 50 nm, where this first stage carrier can be loaded with second stage nanocarriers (e.g. quantum dots, carbon nanotubes, iron oxide particles, nanoliposomes). Moreover, the dimensions and the hemispherical geometry of the system were rationally designed based on the mathematical modeling of particles margination in the blood.[171;178;179] The basic principle of the system encounters for targeting the first stage microparticles to the molecular disease markers on the vasculature walls. When these carriers tightly attach to the vascular endothelium targets, the second stage nanoparticles loaded with therapeutic or diagnostic agent(s) are being released to facilitate the delivery of active agent into the affected cells and provide enhanced therapeutic effect (Figure 11).

Exciting developments can also be reported from the area of nanotechnology applied on *regenerative medicine*. In clinical research regenerative medicine includes the manipulation of stem cells by nanoparticles and nanostructured surfaces as well as tissue engineering to

treat organs lost due to diseases and trauma e. g for skin substitution. At an application level this field includes targets like the reversal of paralysis or blindness through spinal cord or retina regeneration, heart regeneration after infarcts and minimization of stroke dysfunction through neuron repair just so mention few. The function of nanomaterials in this regards is to support the reconstitution of healthy tissues. Results obtained by Stupp et al. indicate that the regeneration within the central nervous system can be reached by applying self organized nanofibers. A peptide amphiphile (IKVAV) which self-assembles to a nano network and recognizes α3β1 integrin was used for this purpose (Figure 12). The induced signalling elongates axons and promotes neuron development. In parallel the inhibition of axon regeneration by scar forming astrocytes was blocked. In a similar approach heparin coated nanoparticles promotes angiogenesis.[180–182]

4. Clinical applications

Nanomedicine enters different fields of clinical application including tissue engineering and targeted drug delivery. The appliance here is fairly broad but mainly focuses cancer.

Known to be a cause of the development of diseases like cancer, arthrosclerosis and age related illnesses chronic inflammation (CI) takes a central position in clinical investigations. The mechanisms of this correlation are reviewed by several authors, who discussed how the immune status in humans affects the risk of cancer development in an etiology-dependent manner. The molecular machinery underlying the development of CI makes it an expanding research focus for nanomedicine.[183–187]

Therapies for CI address cell-mediated or humoral immunity by blocking mediators like interleukines (IL) or targeting receptors. For an overview of immunological mechanisms see references.[188–197] The classical treatment of CI is based on drugs like e.g. glucocorticoids, cyclosporine A, sulfasalazine/5-aminosalicylic acid (5-AZA), or calcinneurin inhibitors. Immunotherapies by means of antibodies specific for certain cells, such as anti-CD20 or anti-CTLA4 are also used. These commonly used therapies specifically or unspecifically suppress the cellular or humoral immune response, causing a variety of sometimes life-threatening side effects, such as hyperglycemia (steroid diabetes), osteoporosis, lymphopenia, sepsis, liver failure, hepatitis, skin atrophy or adrenal insufficiency. Calcineurin inhibitors as an alternative to those unspecific immunosuppressants represent an important regulator of IL-2 and activator of T-helper cells. However, following systemic application of calcineurin inhibitors potentially severe side effects such as infection and sepsis were also reported. In summary, current treatments of CI are associated with a risk of severe side effects. In addition, administration routes are often problematic and inefficient (e.g. drug degradation may occur during oral administration).

Similar problems of low efficiency, severe side effects and inefficient application routes were identified a while ago in cancer treatment. Therefore, successful efforts have been made in this field to develop targeted drug delivery and diagnostic approaches and bring them to clinical application.

The benefit of nano-sized drug delivery systems for the application on CI is to improve already existing drug application in terms of reduced side effects, enhanced efficacy, better

bioavailability and reduced health care costs. Another advantage of nanocarriers is the capacity for medical exploitation of highly toxic, poorly soluble and unstable compounds.[198;199] Nano-scaled drug or gene delivery systems are supra- and supermolecular assemblies of simple components with varying size, shape and composition. These characteristics hold true for the main part of all nano-scaled particles applied in nanomedicine. In general, the carrier is characterized by certain parameters such as a high drug or gene loading capacity or another feature such as superparamagnetism as in the case of iron oxide nanoparticles. Independently of the composition, nanovectors are usually further modified based on their individual application such as surface decoration with polyethylene glycols (PEG) for intravenous injection to prevent early clearance and to increase blood circulation time.[58;200]

Lipid-based vehicles

Liposomes are the most clinically established nanometer-scale systems used for drug delivery. Biocompatibility, biodegradability, and flexibility of size and surface manipulations comprise the outstanding profile that liposomes offer as compared to other nanoparticulate delivery systems. Liposomal nanotherapeutics for cancer treatment are on the market for more than a decade, whereas other liposomal drugs are in various stages of clinical development. Introduced to increase the solubility of hydrophobic chemotherapeutics and to enable trapping of drug molecules with a high potency, liposomes have been shown to be effective in reducing systemic side effects and toxicity, as well as in attenuating drug clearance.[201;202] Some available drugs that have shown high efficacy and less toxicity compared to non-liposomal preparation are: liposomal amphotericin B (brand names: AmBisome, Amphotec, Abelcet), stealth liposomal doxorubicin (brand names: Doxol/Caelyx), liposomal daunorubicine (brand names: DaunoXomo) and liposomal cytosinbe arabinoside (brand name: DepoCyt). These are just some representative examples to demonstrate the great impact of nanomedicine in ongoing therapies.[203]

Meanwhile an enormous amount of diverse synthetic, semi-synthetic and natural polymers are available, especially those prepared from biodegradable polymers such as Poly-Lactic-Acid (PLA), poly (D,L-lactide-co-glycolide) (PLGA), poly (ε-caprolactone), gelatin and chitosan. These systems have far reaching clinical applications. PLGA nanoparticles, represent an established biodegradable and biocompatible carrier system. Polymeric micelles, based on block copolymers that form thermo- and pH-sensitive or enzyme-sensitive structures, have raised interest for delivery applications, in particular for hydrophobic compounds. Preferably these systems are designed in such a way that they allow for self assembly in the presence of the drug to be incorporated. This will significantly facilitate their applicability in a clinical environment.

Liposomal drug carrier in chemotherapy

Doxorubicin—Doxorubicin is an anti cancer drug that is widely used for the treatment of different types of tumors such as breast cancer, Kaposi-Sarkoma or ovarian cancer. Doxorubicin is a highly toxic compound affecting not only tumor tissue but also heart and kidney, a fact that limits its therapeutical applications. Therefore, intense research was done to establish a more biocompatible formulation of doxorubicin. Early development of

liposomal enclosure of doxorubicin culminated in a today approved nano-medical drug delivery system.[204;205] Liposomal formulation results in a reduced delivery of doxorubicin to the heart and renal system while the accumulation in tumor tissue is elevated.[206;207] Nanovectors of this type accumulate in tumors due to the EPR effect, the characteristic hyperpermeability of tumor tissue resulting in a selective drug delivery to tumors.[208;209] The cutoff size of the blood-tumor barrier depends on the location of the tumor and the modulation of the microenvironment but is usually in the range between 300 and 800 nm that corresponds to the size of liposomal carriers.[210]

Particles larger than 200 nm elicit, however, the complement system and provoke clearance by phagocytosis. Early clearances of nanomaterials by phagocyte activity prevent long circulation of the carrier and subsequently a long termed controlled release of the load. Improved circulation behaviour of liposomes was achieved by liposomal surface modification with PEG.[198] PEG reduces the clearance of the liposome by phagocytes in liver and spleen considerably, since opsonisation of the liposomal surface is strongly hindered.[211] A reduced clearance increases the circulation period of the carrier in the blood and prolongs the drug release, enhancing the probability of the EPR phenomena. Interestingly, a lipid composition itself is unable to modulate the clearance of PEGylated liposomes – as opposed to non-PEGylated liposomes.[212;213]

Recent studies revealed an increased clearance rate of PEGylated liposomal carrier upon multiple injections.[214–216] Here, clearance is supposed to be mainly governed by liver and spleen macrophages and might depend on a soluble factor that prime the so called enhanced clearance effect. The enhanced clearance effect is diminished with time and seems to be related to the life time of the macrophages that came directly in contact with the injected liposomes.[214;217] Therefore, injection of liposomes should be adapted to the macrophages life periode.

A disadvantage of liposomal drug delivery is the release of the drug into the extracellular fluid since liposomes usually cannot enter the cells.[214] A more specific targeting of the liposomal drug carriers or a specific cellular uptake is therefore supposed to reduce toxicity and increase effectiveness of the carried drug (second and third generations of nanovectors).

In contrast to an indirect targeting governed by the EPR phenomena an improved tumor specific drug delivery is achieved by coupling antibodies to the surface of liposomes. The advantages of these immunoliposomes are the potential cellular uptake by the target tissue accompanied by an increased tumor cell toxicity and a reduced clearance rate since the delivery to kidney and spleen is reduced. For example, anti-2C5 monoclonal antibodies were coupled to a liposomal surface in order to transfer the loaded doxorubicin to brain tumors. The 2C5 directed antibody was shown to bind specifically to human astrocytoma cell surface *in vivo*.[218] The antibody is directed against nucleosomes localized on living tumor cell surfaces originated from apoptotic neighbouring tumor cells.[219] Another approach to treat human brain tumor *in vivo* is the application of sulfatide-containing liposomes (SCL), which bind to certain glycoproteins upregulated in tumor cells. Anti-CD19 labelling of liposomes was shown to improve targeting to murine B-cell lymphoma cells and an intracellular release of liposomal Doxorubicin.[220] These examples show that vectorial, i.e.

site directed drug transport and release will revolutionize the medical therapy of brain tumors, since the present therapy is of limited success due to an insufficient drug delivery. However, toxicity does not depend only on the targeting but was proven previously to be strongly related to the release characteristic of the injected liposomal formulation.[220;221]

Other approaches are currently under investigation to enhance specificity of the drug transport. A recent study reports thermosensitive liposomes that release doxorubicin when heated. Specific release of the anti-tumor drug was achieved by selective heating of the attacked tumor. Hyperthermia was induced in this case by heated water delivered *in vivo* by small catheters.[222]

AmBisome/amphotericin B

AmBisome is a liposomal formulation of an antifungal agent, amphotericin B is indicated for different fungal infections and as an empirical therapy for presumed fungal infection in febrile neutropenic patients. It can also be used for treatment of visceral leishmaniasis. AmBisome was designed as very rigid, small unilamellar liposomes with a mean diameter of <100 nm with amphotericin B intercalated within the membrane. Such liposomes are known to have long circulation times and accumulate in areas where needed. In preclinical and clinical studies AmBisome showed less toxicity and less side effects than amphotericin B retaining the full spectrum of antifungal activity of conventional amphotericin B.[223] Therefore, it can be used in patients suffering from kidney damage, a contraindication for classical amphotericin B therapies. In animal experiments it was shown that AmBisome did not distribute evenly throughout the kidney tissue, but rather tended to localize near the areas of fungal infection. Moreover, AmBisome was found to be attached to the fungal wall and penetrate inside of fungi. In summary, liposomal amphotericin B accumulating the infection sites, show higher stability and less side effects and toxicity than the free drug. The sustained release of amphotericin B by AmBisome may also serve as a prophylaxis as shown in Histoplasma capsulatum challenged mice.[224]

Polymer based delivery

Natural polymers such as proteins or polysaccharides tend to be internalized and degraded rapidly enabling a moderate intracellular release of the drug or gene.[225] Blood circulation time or clearance is controlled by surface modification or polymer conjugate formations with Polythylenglycol (PEG).[226] A current example of clinically used polymeric nanoparticles are paclitaxel albumin bound nanoparticles (brand name: Abraxane) for the treatment of patients with breast cancer refractory to conventional therapy.[227;228] These nanoparticles are water dispersible, therefore avoiding the use of Cremophor, a solvent commonly used to solubilize and formulate free paclitaxel, a very hydrophobic drug. However, Cremophor was reported to be responsible for allergic reactions, limiting the drug dosing.[229–231] Abraxane demonstrates significantly higher response rate, longer time to tumor progression and absence of hypersensitivity reactions.[227] However, a severe side effect was recently published and demonstrates the ongoing debate on safety and drug metabolism of nanoparticles (see also Section 6).[232]

A promising anticancer treatment based on passive targeting of drug-polymer conjugates was suggested by the group of Duncan. As in the case of first generation nanovectors, it makes use of the fact that neovascular systems close to tumors are permeable for certain particle sizes, in contrast to those supplying healthy tissue. The group of Vicent reported about anticancer agents based on apoptosis induction which are coupled to nanoparticles for the enhancement of efficacy.[233;234]

Possibilities of coupling drugs to polymers are presented in Figure 13, which also nicely demonstrate that the generation and optimization of nanovectors is forming an important interdisciplinary area between chemistry, biochemistry and medicine.

The coupling of proteins and also drugs to synthetic polymers especially PEG increases their plasma residence, reduces protein immunogenicity and can increase their therapeutic index. Several PEGylated enzymes (such as L-asparaginase) and cytokines (including interferon-α and granulocyte colony-stimulating factor) have now entered routine clinical use.[233;234]

Metal nanoparticles

Nano-crystalline silver for wound care—Silver, mostly in form of nitrate or sulfadiazine salt, is a well studied anti-microbial agent and a common compound for wound treatment.[235–237] Wound healing could be subdivided into distinct phases.[238] Early after injury and coagulation, wound healing is characterized by cell invasion of leucocytes causing inflammation. During inflammation the wound is cleaned and a microbial infection is prevented. Wound healing proceeds due to a stop of inflammation followed by tissue remodelling and maturation of the novel tissue. As a consequence of a latent microbial infection the inflammatory phase could be prolonged causing chronic non-healing wounds. Medical treatment of chronic wounds with dressings containing silver significantly reduce the bacterial load of the wound and allow complete healing.[239] Silver displays a promising alternative to antibiotics since a multi resistance against antibiotics develop progressively. Recent evaluation of resistance formation against silver does not indicate an increased resistance development upon silver usage.[240–242] Toxicity of silver is not specific in comparison to antibiotics potentially affecting wound tissue, though the antimicrobially active doses of silver are low (nM-μM range) and commonly well tolerable.[242]

The advantage of *nanocrystalline* silver over silver salts is not only due to an increased anti-microbial activity but also due to its anti-inflammatory properties.[243] However, the mechanism of action remains to be elucidated. Application of nanocrystalline silver during wound management demonstrates the entrance of nanobiotechnology into experienced medical therapy. Presently, these types of dressings were applied in case of first- and second-degree burns and several types of chronic non-healing wounds.

Magnetic nanoparticles for diagnosis and therapy—Iron or iron oxide nanoparticles have a high potential for various nanobiomedical applications including drug delivery. A manifold surface chemistry allows surface coating of the iron based nanoparticles with hydrophilic polymers such as PEG or dextrane to prevent or to increase cellular clearance of the particle, respectively.[244] Cell specific transport is also possible due to the coating with antibodies, receptor specific peptides or aminosilane. In addition to

these more general properties of various nanocarrier systems the superparamagnetic character of the particle and their dimensions between 2–20 nm pave the way for further applications beside drug delivery.

With respect to the physical characteristic of iron compound nanoparticles, current research and applications mainly involve *in vitro* cell labelling and cell separation, *in vivo* drug delivery, magnetic resonance imaging (MRI) (see Section 2) and hyperthermia.[245] The latter is the most popular application of iron nanoparticles in medicine. The term describes the destruction of tumours by locally over heating the tissue. Hyperthermia is an effective and specific anti-cancer treatment since an increased temperature of the treated tissue up to 44°C is less tolerant for cancer cells than for healthy cells. This approach is usually applied in combination with other traditional therapies such as chemotherapy. Hyperthermia by iron oxide nanoparticles is induced by exposure of the particles to an alternating magnetic field.[203;246] A local deposition of nanoparticles allows the tissue specific hyperthermia that addresses preferentially the tumor tissue (Figure 14).

The benefits over classical cancer therapies are minimal invasiveness, accessibility of hidden tumours and very low side effects. By a conventional heating of a tissue (microwaves, laser light etc.) the healthy tissue surrounding the tumour is destroyed as well. However, targeted paramagnetic particles provide a new powerful tool for highly localized energy absorption and heating mainly of the cancerous cells. Several kinds of nanoparticles differing in material, composition and size are available for that purpose, e.g. hyperthermia can also be applied by magnetite cationic liposomes (MCLs) as carrier systems combined with heat shock proteins. Because of its low side effects, the treatment is well accepted by patients.[203;247–249] The superparamagnetic properties of the iron oxide particles were also exploited for MRI, as mentioned above.[245] Various iron particle-based products for MRI are commercially available (brand names: Resovist, Feridex). This dextrane coated particles were mainly used for *in vivo* MRI of liver tumour tissue. The dextrane coating increases the intracellular deposition of the particles into the cancer cells enabling diagnosis and monitoring the progression of the tumor. Iron particles are cleared by the liver macrophages, entering the reticuloendothelial system (RES) to join the physiological iron pool.

Nanoshells

In other hyperthermal concepts metal silica-gold nanoshells consisting of a spherical dielectrical nanoparticle surrounded by an ultrathin conductive metal layer are used, which can be activated by tuneable optical resonance. The nanoparticles absorb light in the NIR region guaranteeing that an optimal optical transmission through the tissue is achieved. A moderately extracorporeal near infrared light exposure (820 nm, 4 W/cm^2) resulted in a heating of the tumour tissue, producing an irreversible tissue damage displayed by coagulation, cell shrinkage, and loss of nuclear staining.[164] The big advantage of nanoshells is a tuneable plasmon resonance from the visible to infrared regime by varying the composition and dimension of the layers.

Nanoshells are not only investigated for the treatment of cancer but also for diagnostic purposes, such as acquiring higher resolution images in optical coherence tomography (OCT). The OCT applications reach from ophthalmology up to the reconstruction of whole

brain specimens. Some other noteworthy NIR imaging applications are confocal imaging, iridotomy, and photothermal coagulation, all of which take advantage of increased transparency of the tissue within this region.[250]

Non-injectable nanovectors

The most preferred way of introducing drugs into the body is via oral route; therefore pharmaceutical industry puts much effort in the development of appropriate delivery systems improved by nanotechnology. Nanosphere carriers derived from hydrogels, highly stable organic compounds that swell when their environment becomes more acidic, have been successfully formulated into controlled-release tablets and capsules, which release active compounds in a pH dependent manner.

Nanoparticles can also provide an efficient delivery tool for drugs that have to bypass the blood brain barrier, such as chemotherapeutic agents for brain malignancies, antiepileptics and anesthetics (e.g. Dalargin). Polysorbate 80-coated nanoparticles loaded with doxorubicin (5 mg/kg) achieved very high brain levels of 6 μg/g brain tissue while all the controls, including uncoated nanoparticles and doxorubicin solutions mixed with polysorbate, did not reach the analytical detection.[251] Another newly designed delivery system is based on chitosan coupled to antibodies through a PEG linker. This immune nanoparticles have on the one hand the ability of cationic (with a full positive charge) chitosane to interact with the negative charges of the brain endothelium and on the other side the affinity of the monoclonal antibody OX26 for the transferrin receptor, which makes them perfectly designed to cross the blood brain barrier. The nanospheres loaded with the peptide Z-DEVD-FMK, an inhibitor of the caspase-3, were investigated. Inhibition of this enzyme is known to increase neuronal cell survival following cerebral ischemia.[252]

Implantable drug delivery systems improved by *nanotechnology* are often preferred to the use of injectable drugs, because the latter frequently display side effects. For example, the blood concentration may increase rapidly, but decreases slowly over time. This can diminish drug efficacy as the drug concentration falls below the therapeutically relevant level. In contrast, implantable time release systems may help in minimizing peak plasma levels reducing the risk of adverse reactions and the frequency of re-dosing, thus improving patient compliance. The benefits of nanotechnology in this regards could be exemplified by biodegradable porous silicon (pSi) products. This kind of nanostructured material effectively stores an active compound or second stage nanoparticles in nanosized pockets that release minute amounts of drug as the silicon dissolves. pSi is currently explored for tissue engineering and ophthalmic delivery.[253;254]

Nanotechnology also refines the *transdermal delivery*, a safe, noninvasive method of administering drugs. Applied directly onto bare skin, the transport of large-molecular weight proteins like vaccines across the skin is relative inefficient. Recent evidence has shown that this barrier can be overcome by properly structured nanosized particles.[255]

Finally, nanotechnology could also be used for *toxin removal*. Colloidal dispersions have already been shown to remove potentially lethal compounds from the bloodstream,

including high concentrations of lipophilic therapeutics, illegal drugs, and chemical and biological agents.[256;257]

5. Nanocoatings and nanostructured surfaces for medical application

In parallel to the development of nanoparticles the knowledge about the nanostructuring of surfaces develops rapidly. Driven by the search for environmentally benign fouling control, nanostructuring of surfaces displays a big challenge for application in medicine. The main research in this field focuses on optimization the interaction of prostheses like artificial joints with the organism, aiming at producing materials which are undergoing a close connection to the body tissues, while avoiding side effects, such as chronic inflammations or allergies. Nanostructuring of a surface coating controls properties such as charge, conductivity, roughness, porosity, wettability, friction, physical and chemical reactivity, and compatibility with the organism. Especially in the area of artificial organs and prosthetics there is a growing need for smart surfaces which show a high biocompatibility.[258–263]

Another potential application of nanotechnology resides in the possibility to mimic a variety of compound materials and self-organized systems found ubiquitously in nature. Complex structures like complete cells, as well as substructures such as folded proteins and molecular motors, represent the kind of self-organized nanomachines that currently cannot be prepared in a synthetic way. Nature, however, making use of informed dynamic molecular systems, has demonstrated that self organized complex molecular systems are indeed extremely successful. These concepts will be partly transferred into synthetic systems in the future, and their implementation may lead to new developments that cannot be achieved by conventional large-scale manufacturing processes.

Nanostructuring can be done physically, chemically or by self assembly. Probably, the most popular natural occurring example is the surface structure of the leaves of a lotus flower. The special structured surface, with a typical bi-modal size distribution in the micrometer and submicrometer regime leads to a self cleaning behavior as a result of structure and surface chemistry of the material. Another example is the manipulation of cell behavior by changing the surface structure while keeping their chemical composition approximately the same. This was recently demonstrated by the group of Spatz. By altering the distance between functionalized gold particles attached to surface a different growth and attachment behavior of fibroblasts has been observed (Figure 15).[264–266] In another approach Sun et al. used *N*-Isobutyryl-L(D)-cysteine (NIBC) enantiomers to change successfully the adsorption characteristics of surfaces.[267]

The understanding of the basic principle of these effects opens technical ways for the generation of surfaces with non-fouling properties, and also surfaces representing optimized template structures for specific cell growth. For example, applied to the development of implant materials nanostructuring of titanium alloy by calcium phosphate (CP) in the form of hydroxyapatite coatings apparently result in enhanced mechanical properties and in promoting the proliferation of osteoblast cells.[268]

By changing the surface into a nanotubular structure it was found that artificial joints were better incorporated without inducing chronically inflammation.[269;270] The tailored surfaces

have the advantage of mimicking the surface of natural structures not only by coating with inorganic materials but also by adhering proteins or peptides to mimic natural conditions. The next natural step of this development is its application to the field of bionics. The exchange between life forms and synthetic constructs is a most promising attempt because evolution already selected appropriate materials and processes. Next to prosthetics this approach is most promising in neural applications.

The application of nanostructured surfaces with specific well defined properties finds their way also to diagnostic approaches. Often the diagnostic of cells is failing because of unfavorable interaction between device and cells. This is especially the case when immune cells are investigated. Receptors on the surface of leucocytes interact unspecifically with artificial structures which results in an unwanted activation or differentiation of the cells. Here surfaces are needed that do not induce any kind of activation after contact. This is important for all microfluidic devices and surfaces used for biochips and proteomics. The progress in the development of microelectromechanical systems gives nanostructuring of surfaces a new level of importance.[271–273]

Using responsive molecular systems it is possible to switch between different states such as superhydrophobicity and superhydrophilicity by external stimuli, e.g. electrical or optical fields, pH etc., acting on purpose on the functionalized surfaces. These coatings are of interest for diagnostic purposes with miniaturized lab-on-a-chip systems or the coating of artificial blood vessels and implants, thus, mimicking biomolecular systems.

6. Biocompatibility and Toxicity – Safety issues related to nanotechnology implementation

The generation of small particles may be a major issue for discussion in nanotechnology with respect to toxicology. Due to the potentially high reactivity based on the large surface-to-volume ratio of nanoparticles as compared to bulk systems there is a latent risk for all new nanosystems which must be carefully considered. While the frame work of existing laws for new chemicals and pharmaceutical materials seems to be currently sufficient to treat also this kind of materials, nanoparticle synthesis has to be investigated carefully for each new system developed with respect to its potential side effects within the human body and the environment. Fortunately enough, the public and scientific awareness of nanotechnological laboratories is high and there is an increasing intensity of discussion on these ethical and societal issues with different institutions and the public.

New technological developments will provide us in addition a variety of ethical issues which have to be discussed. The clinical application of nanotechnology requires also a number of regulatory guidelines to ensure the appropriate use of new medical devices and drugs originating from nanoscience.[274–277] The potential of molecular diagnostics and analysis possible by nanotechnology and nanomedicine also deserve attention from the political side. This includes the above mentioned toxicological aspects but also the question of the improvement of the quality of life in the cases of severe diseases, cost effective treatment of patients, the artificial extension of our natural senses, neural-electronic interface systems etc. which might be only available for a limited number of people.

The toxicological risk for human health enfolds effects during interaction with medical devices. According to the definition of the EU Medical Devices Directive, “medical devices” comprise tools for

- diagnosis, monitoring, treatment, or alleviation of or compensation for an injury or handicap;
- investigation, replacement or modification of the anatomy or of a physiological process;
- control of conception which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means.

This indicates that nanoparticles exhibit a broad range of applications in human.[278;279]

A drawback is their potential toxicity and their possible incompatibility which may result in the generation of disorders like inflammation, immunoreaction or cancer. Mechanisms of those effects are not well studied yet but might be due to an enhanced hydrophobic interaction to biological material or an increased generation of free radicals by surface catalysis.[198] Recent experimental data have shown that inhalation of air pollution derived nanoparticles (ultrafine particles) with a size below 100 nm are particular critical for the induction of pulmonary inflammation. It has been demonstrated that in this case the individual expression of glutathione S-transferase (GST) determine the strength of inflammation. Interestingly, the physiological task of GST is the detoxification of reactive oxygen species indicating the generation of reactive oxygen species on a particle surface.[280;281] Comparable data were obtained related to the inhalation of single-wall carbon nanotubes (SWCN). In mice SWCN are shown to elicit inflammation in lungs. In rat alveolars they cause small, focal interstital fibrotic lesions.[282;283]

The discussion of the risk of carbon nanotubes became recently new input by Poland et al., who reported that this kind of nanoparticles act, according to their needle like structure, in the same way like asbestos, indicating the same risk for applicants.[284] In other investigations highly purified carbon nanotubes seemed not to possess short-term toxicity and can be considered biocompatible with cardiomyocytes in culture, while the long-term negative effects that are evidenced after reseeding were suggested to be due to physical rather than chemical interactions. This effect was investigated by the group of Krug, who demonstrated that these nanoparticles induce no acute cytotoxicity or inflammatory markers like nitric oxide or interleucine-8. Observed side effect were associated with metal traces coming along with the commercial nanotubes inducing the biological effects reported.[285] The area of cytotoxicity of several kinds of nanoparticles was recently reviewed by Lewinski et al. who showed in an impressive way the manifold of interactions between foreign bodies with cells.[286]

Nanoparticles that had entered organisms and are not excreted are accumulating in cells and tissues developing there a still unknown potential of causing diseases on a long term. It has been shown that nanomaterials can enter the human body through several ports. Accidental or involuntary contact during production or use is most likely to happen via the lung from

where a rapid translocation through the blood stream is possible to other vital organs as demonstrated in animal models.[287] On the cellular level an ability to act as a gene vector has also been demonstrated for nanoparticles.[288] Carbon black nanoparticles have been implicated in interfering with cell signalling.[289;290]

Nanoparticles used for oral drug delivery have been found accumulated in the liver and excessive immune responses may cause permanent damage there.[291] This accumulation effect in cells is also well documented in the cases of pulmonary fibrosis caused by asbestos fibres (asbestosis) and silicosis, a disease that comes from breathing in silica, or quartz dust.[292–294] Meanwhile it is shown that a (high) concentration of nanoparticles may result in the transformation of cells into the tumorous state causing cancer. Investigations on hepatic and renal tissues affected by cryptogenic granulomatosis by scanning electron microscopy (SEM) and X-ray microanalysis via an energy-dispersive (EDS) detector showed a correlation of the presence of inert, non-biodegradable, exogenous micro- and nano-particles with diseases that traditional histopathology could not account for (Figure 16).[295]

It is well known that debris produced by the wear of hip prostheses could induce an inflammatory reaction and a local foreign-body granulomatous reaction. I addition, the migration and dissemination in other parts of the body, far from their origin, has been documented, with its possibility causing further pathologies. So far, no efficient gastrointestinal barrier for inert particles with a diameter below 20 μm is known. Such a finding attracted the attention to those particles. More than once, the source of those minute foreign bodies was found in dental materials like porcelain or over-worn alloys like gold/ruthenium. The migration of barium sulphate particles into liver tissue (cells), a very common contrast medium used in gastro-endoscopy, was a further indication that small particles, in principle, may cross the intestinal barrier.[159;296]

Nanoparticles used for drug delivery are exposed to biomolecules in the lung, the gastro intestinal tract, or to the endothelial barrier. The contact may result in the uptake of nanoparticles via endocytosis, mediated by receptors, membrane penetration in the case of hydrophobic particles or in the case of very small nanoparticles (<5 nm) by transmembrane channels.[297]

A strategy to prevent cellular internalisation and, therefore, uncontrolled cytotoxicity of nanoparticles in the regime below 100 nm is their surface modification with hydrophilic polymers.[244] In an aqueous environment different types of biomolecules adsorb to nanoparticles as well chemicals like pesticides. Adsorbed molecules dictate biological interactions, especially bio-uptake and the activation of cells. As an example, the interaction of nanoparticles with biomolecules was shown for the binding of C_{60} fullerenes to antibodies. Recent reports describe the cytotoxic effect of C_{60} fullerene over lipid peroxidation. In organisms all extracellular proteins like complement proteins or antibodies can adsorb on nanoparticles. During the adsorption proteins possibly change their conformation and, as a consequence, their reactivity may change as well, resulting in an auto immune response.[298;299]

To investigate potential risks of nanotechnology tools and methods are developed and adapted to perform high throughput and standardized testing of nanoparticles interacting with e.g. biological barriers. An established method of proving the intactness of biological barriers via the measurement of the transepithelial electrical resistance (TER)/impedance was adopted for the testing of the toxicity of nanoparticles and developed for routine application.[300–303] First results show, for example, no initial effect of silica based NP on Madin Darby Canine Kidney (MDCK) cells but a decrease of the TER after 150 hours, indicating a disrupture of the cell monolayer. (Riehemann et al., unpublished results). The studies will be repeated and expanded over a broader spectrum of NP, it will help to understand the interaction mechanisms of nanoparticles and biological systems in more detail. Nevertheless, they show the importance of long term studies in the investigation of the toxicity of NP.

These few examples demonstrate that the effects of nanotechnology on human health could be two edged, similar to conventional drug exposure, but possibly based on completely different schemes. Many of the investigated systems, so far, seem to exhibit relatively little short time risks. Nevertheless, since all new technologies may bear hidden risks, systematic risk assessment in parallel to the technological development has to be done to keep the hazardous potential as small as possible.

7. Summary and Perspectives

The potential applications of nanotechnology for diagnosis, prevention and treatment of diseases are currently very broad. Practical application of nanomedicine requires, therefore, besides creativity and visionary power, down-to-earth approaches and systematic development, essential to obtain real progress allowing to reach new frontiers.

In this review we provided an overview on some fascinating developments in the area of nanomedical research and applications. Since the field is currently expanding in a very fast pace, this review cannot include all aspects of present nanomedicine in detail. Our aim was mainly to demonstrate the highly transdisciplinary character on the one side and to give a view on developments and research topics in chemistry, biology, physics and engineering that can revolutionize clinical therapies and diagnostics.

Nanotechnology has already provided an important impact on clinical applications, which are expected to exponentially grow during the next years. Located on an intersection of a number of fundamental disciplines, nanomedicine relies on

- chemical knowledge to provide required modifications to the nanovector surface and to enable conjugation of drug/contrast agent;
- detailed understanding of disease biology and pathophysiology to enable efficient targeting and therapy;
- awareness of physical properties of multilevel nanosystems to be able to finely engineer and manipulate matter for the design of new nanoscale detection and drug delivery systems.

Main efforts and the majority of nanomedical clinical applications are currently focused on treatment and efficient diagnostics of cancer. In order to efficiently detect malignancies, molecular alterations must be detected as early as possible. This means that extremely sensitive techniques have to find their way to early diagnostics. Nanotechnological concepts, having, for example, the potential to enter and analyze single cells, could meet this challenge. The comprehension currently being gained in this field will be a major pillar to establish personalized medicine.

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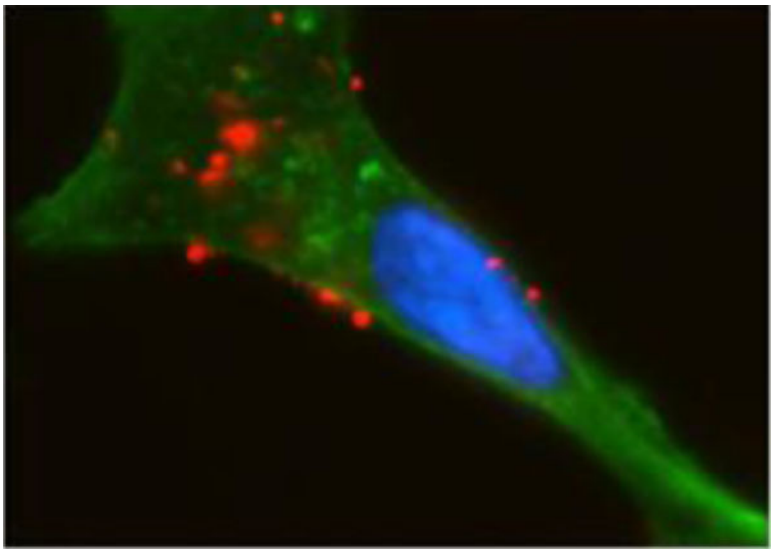


Figure 1.

Nanotechnology and medicine. Prostate cancer cells have taken up fluorescently labeled nanoparticles (shown in red). As targeting molecules on the nanoparticles RNA aptamers binding to the prostate-specific membrane antigen (PSMA; a well-known transmembrane protein, which is overexpressed on prostate cancer epithelial cells) was used. The cell nuclei and cytoskeletons are stained blue and green, respectively. Similarly designed targeted nanoparticles are capable of getting inside cancer cells and releasing lethal doses of chemotherapeutic drugs to eradicate tumors. Reprinted with permission from American Association for the Advancement of Science (AAAS).[13]

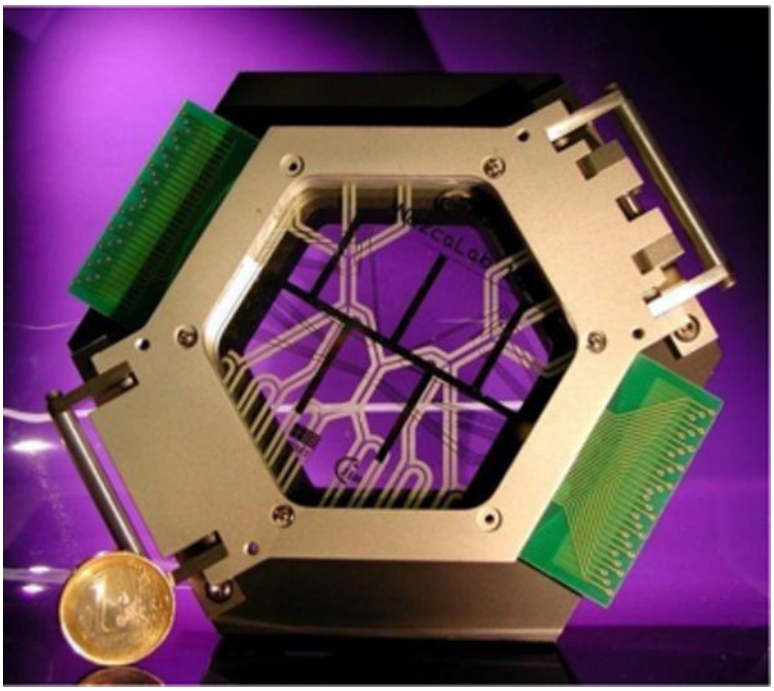


Figure 2.

Example of a lab-on-chip technology for biological applications. A multiplicity of branched microfluidic channels (between white double lines) bear a variety of different electrode layouts (black fine lines) for applications like cell imprinting, cell fusion or cell separation. Fluidic connection is realized on the backside, electronic connection via the 2×30-pole interfaces (green boards). For size comparison a one Euro-coin at the lower left corner. Kindly provided by M. Jäger, Fraunhofer IBMT, Potsdam, Germany.

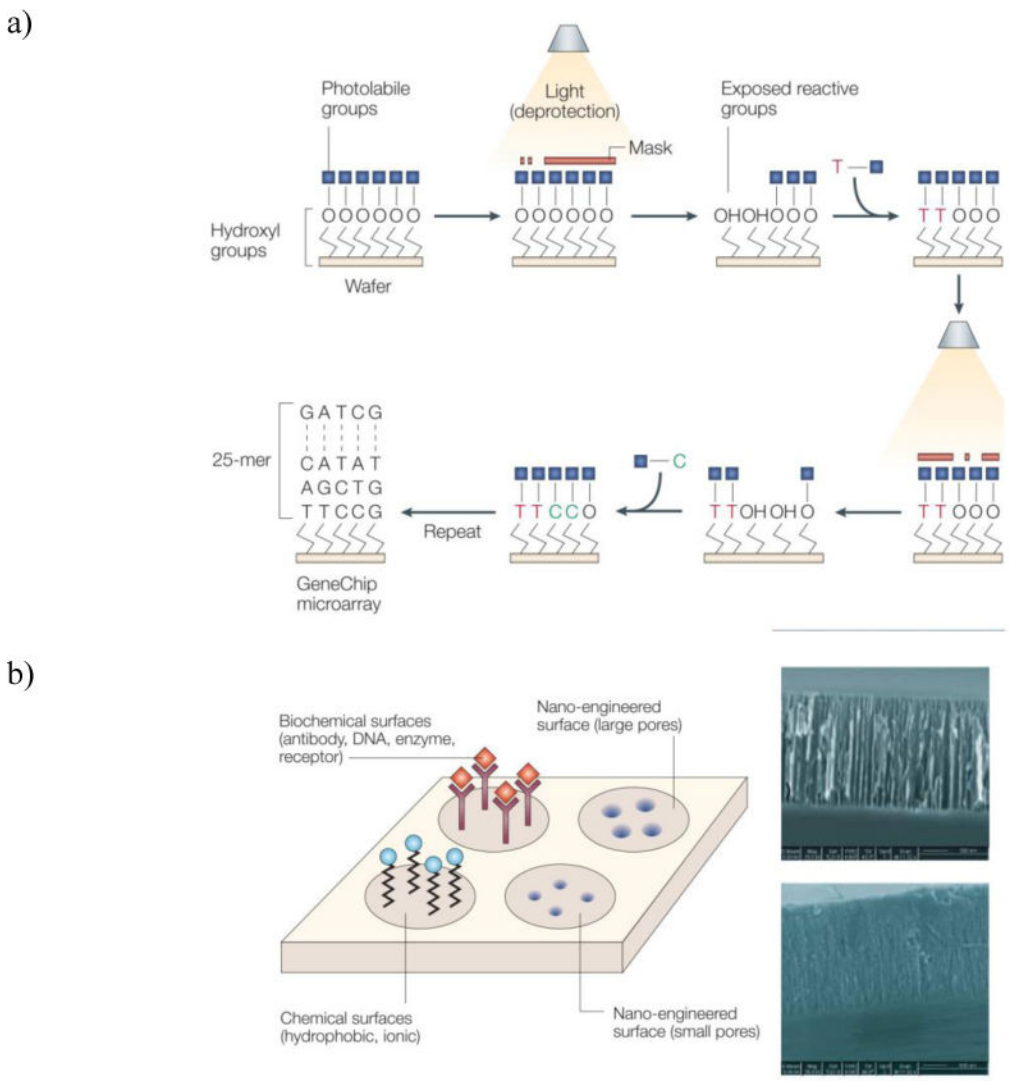


Figure 3.

Photolitographic techniques for manufacturing of a) DNA and b) proteomics micro and nano-arrays: a) Microarrays exemplify the patterning of biological molecules on surfaces, with exquisite control over their spatial placement, for instance to obtain DNA sequencing by hybridization on a chip. In the figure, blue squares represent photolabile groups, which are selectively illuminated through a mask (a process known as photolithography) and removed to expose reactive groups. Sequential application of the procedure yields single-stranded hybridization probes of preselected vertical sequences at predetermined locations on the microarray. The technique of photolithography was adapted from the microelectronic industry. The ability to control the lateral dimensions of each square in the checkerboard of a microarray was originally of the order of 100 microns (or 100,000 nanometres). Now, the linear spatial resolution of lithography is 1,000 times better, indicating that up to a one-million-fold increase in information density could be packed in ‘nanoarrays’; b) photolithography can be used to pattern different chemistries, biological moieties and physical textures on substrates, for the purpose of prefractionation of protein mixtures before investigation by time-of-flight spectrometry. Different proteomic patterns are produced by

different substrate treatments, on contact with the same biological sample. The panels to the right illustrate different nanochanneled surfaces, which selectively retain proteins and proteolytic fragments. This has the effect of ‘focusing’ the resulting protein profiles in different molecular-weight ranges. Reprinted by permission from Macmillan Publishers Ltd.[58]

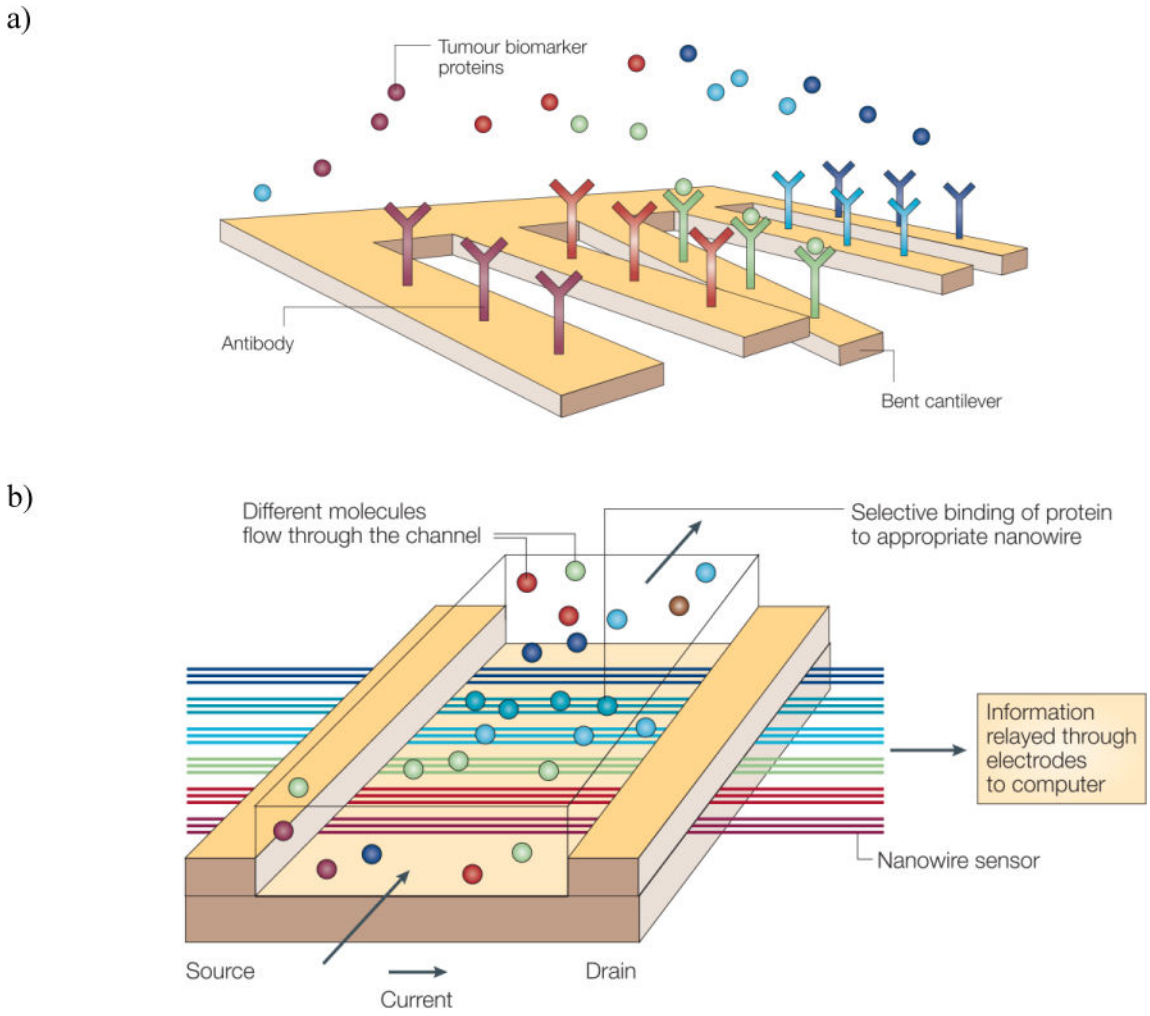


Figure 4.

Presentation of working principle of a) nano-cantilevers and b) nano wires: a) Nanocantilever array. The biomarker proteins are affinity-bound to the cantilevers and cause them to deflect. The deflections can be directly observed with lasers. Alternatively, the shift in resonant frequencies caused by the binding can be electronically detected. As for nanowire sensors, the breakthrough potential in nanocantilever technology is the ability to sense a large number of different proteins at the same time, in real time; b) Nanowires deployed within a microfluidic system. Different colors indicate that different molecules (colored circles) adsorb or affinity-bind to different nanowire sensors. The binding causes a change in conductance of the wires, which can be electronically and quantitatively detected in real time. The working principle is that of a (biologically gated) transistor and is illustrated in the insert. The charges of the binding protein disrupt electrical conduction in the underlying nanowire. The ‘nano’ size of the wire is required to attain high signal-to-noise ratios. Reprinted by permission from Macmillan Publishers Ltd.[58]

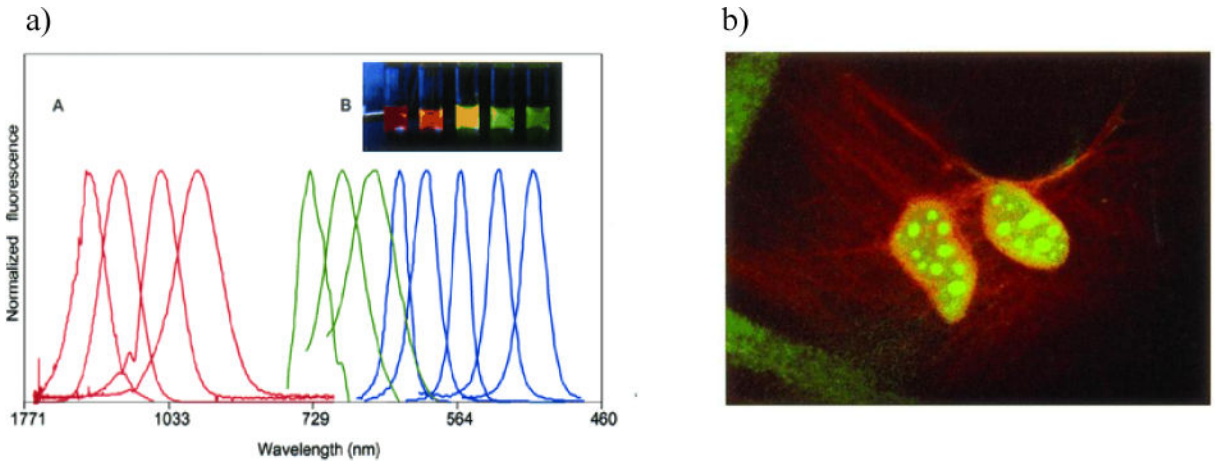


Figure 5.

a): Size- and material-dependent emission spectra of several surfactant-coated semiconductor nanocrystals in a variety of sizes: A: The blue series represents different sizes of CdSe nanocrystals with diameters of 2.1, 2.4, 3.1, 3.6, and 4.6 nm (from right to left). The green series is of InP nanocrystals with diameters of 3.0, 3.5, and 4.6 nm. The red series is of InAs nanocrystals with diameters of 2.8, 3.6, 4.6, and 6.0 nm; B: A true-color image of a series of silica-coated core (CdSe)-shell (ZnS or CdS) nanocrystal probes in aqueous buffer, all illuminated simultaneously with a handheld ultraviolet lamp; b) Cross section of a dual-labeled sample. Reprinted by permission from American Association for the Advancement of Science (AAAS).[74]

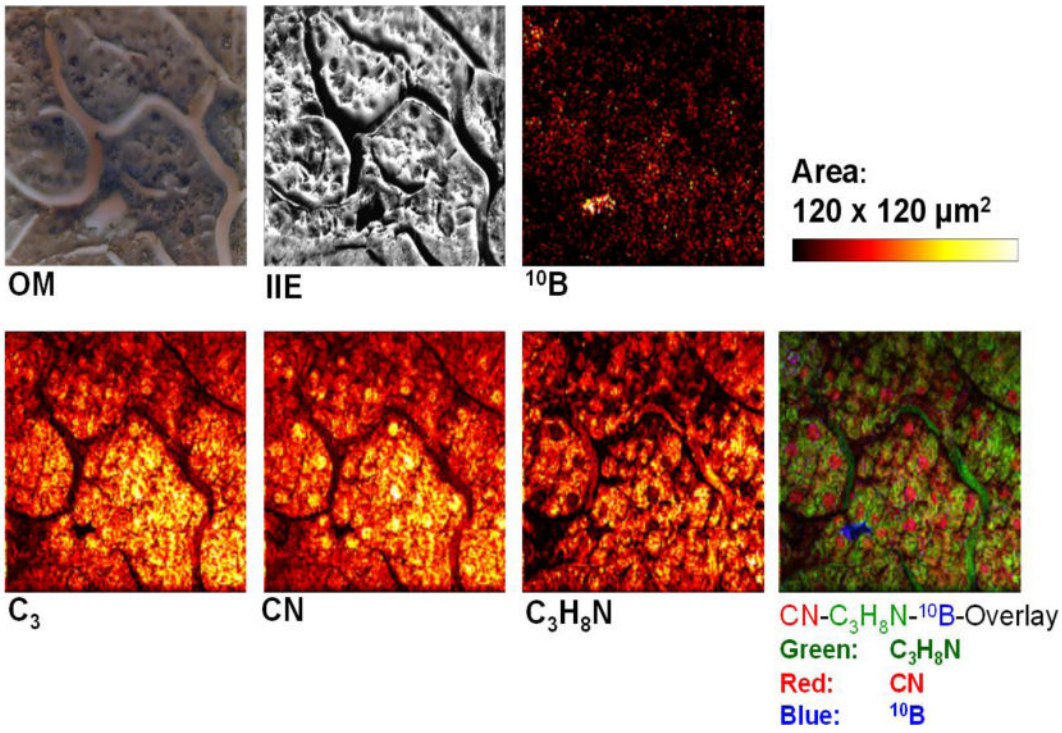


Figure 6.

Optical Microscopy (OM), ion induced electron (IIE) and boron distribution (^{10}B) detected by laser SNMS are shown in the upper row. In the bottom row intents signals from molecules like C_3, CN and C_3H_8N are observed, representing lipids, proteins and nucleic acids. Samples were taken from a kidney of a NMRI nude mouse and treated with a combination of sodium mercaptoundecahydro-closododecaborate (BSH) and p-boronophenylelanine (BPA). Reprinted with permission from Elsevier.[81]

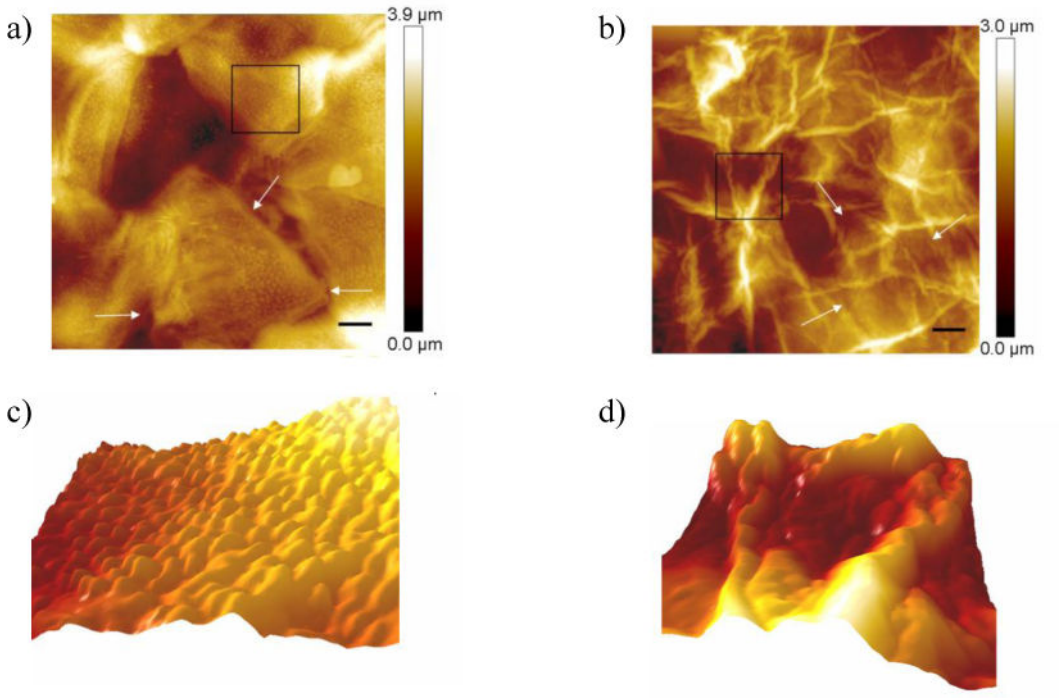


Figure 7.

Surface analysis of native stratum corneum (SC) derived from human skin applying atomic force microscopy. Comparison of atrophic skin (a and c) and healthy skin (b and d) reveals a reduced SC integrity of atrophic skin indicated by enlarged intercellular gaps between the individual corneocytes (a, b white arrows). While the surface morphology of healthy SC is characterized by filamentous structures forming a dense network across the SC (b, d), the surface of corneocytes of atrophic skin is characterized by regular shaped hump-like structure (c). Black bars (a, b) correspond to 5 µm, black squares mark the surface region presented as a three-dimensional image (c,d). (unpublished, S. W. Schneider, Department of Dermatology, Münster, Germany)

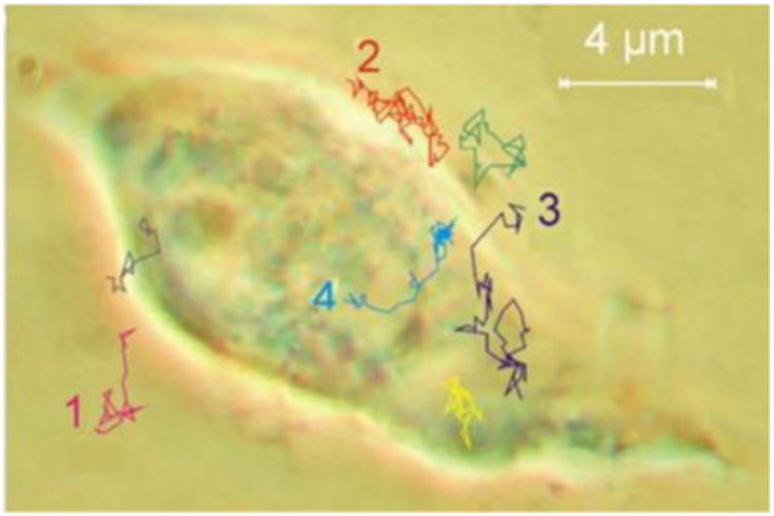


Figure 8.

Trajectories of single AAV-Cy5 particles indicating infectious entry pathways of AAVs into a living cervical cancer cell line (HeLa). The traces showing single diffusing virus particles were recorded at different times. They describe various stages of AAV infection, e.g. diffusion in solution (1 and 2), touching at the cell membrane (2), penetration of the cell membrane (3), diffusion in the cytoplasm (3 and 4), penetration of the nuclear envelope (4), and diffusion in the nucleoplasm. Reprinted with permission from AAAS.[111]

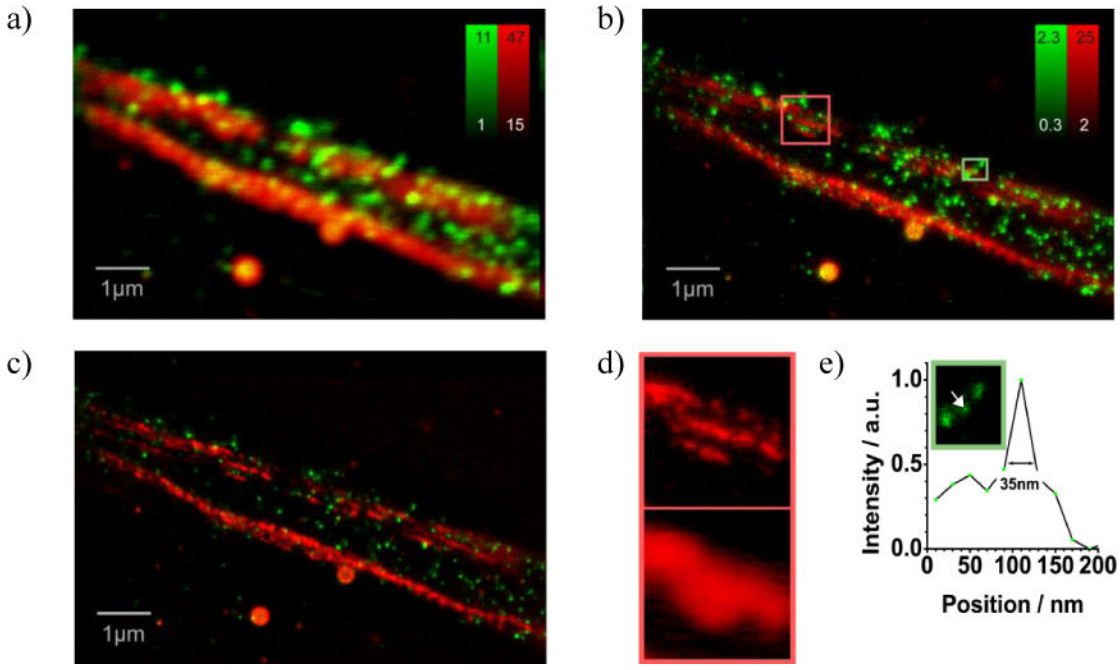


Figure 9.

Comparison of fluorescence imaging techniques: a) Confocal, b) STED, and c) Richardson Lucy deconvolved STED images of neurofilaments (green: light subunits, red: α-internexin). d) In contrast to the confocal image, STED reveals three well-separated α-internexin strands of the axon. e) Structures of the light subunits exhibit a Full Width at Half Maximum (FWHM) < 40 nm, a measure for the reolution of the imaging method. Note the different organization of the light subunits and α-internexin. Reprinted with permission.[121]

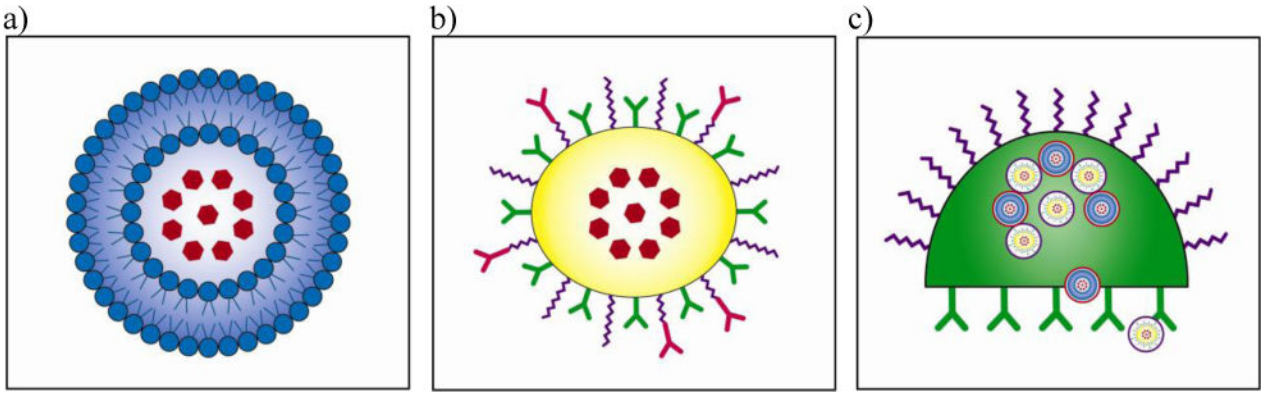


Figure 10.

Different types of nanovectors: a) First-generation nanovectors (e.g. currently clinical liposomes) comprise a container and an active principle. They localize in the tumor by Enhanced Permeation and Retention (EPR), or the enhanced permeability of the tumor neovasculature; b) Second-generation nanovectors further possess the ability for the targeting of their therapeutic action via antibodies and other biomolecules, remote activation, or responsiveness to environment; c) Third-generation nanovectors such as multistage agents are capable of more complex functions, such a time-controlled deployment of multiple waves of active NPs, deployed across different biological barriers and with different sub-cellular targets. Reprinted by permission from Macmillan Publishers Ltd.[58]

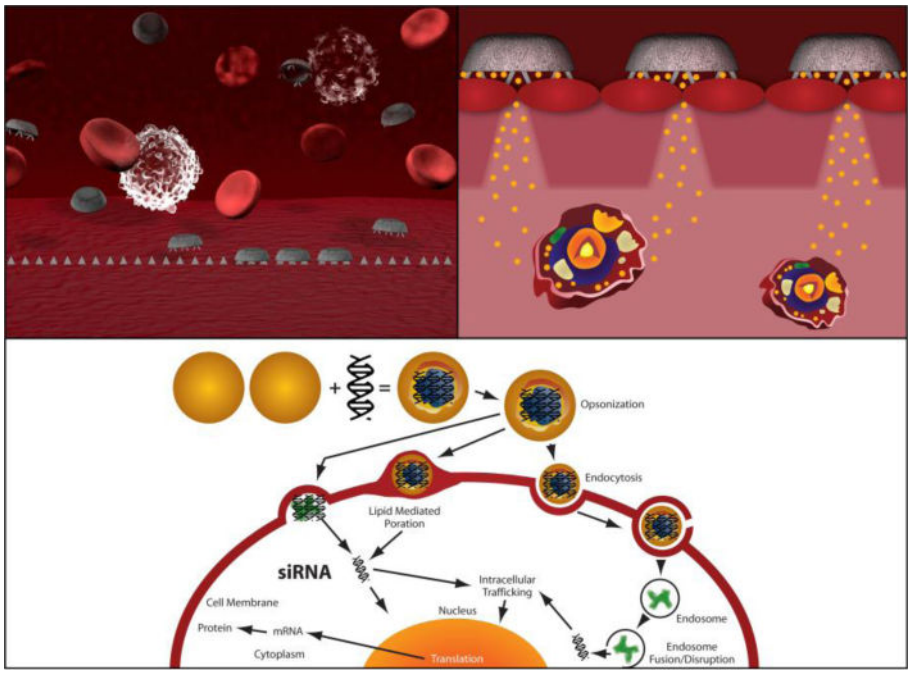


Figure 11.

Mechanism of action of multistage (3rd generation) nanovectors. Top-left: rationally designed stage one particles marginate to the vessel wall and adhere to the endothelium. Top-right: stage one particles release a penetration enhancer to break down tight junctions and the basement membrane and release stage two particles – in this instance, liposomes. Bottom: the stage two liposomes interact with the target cell membrane, and then deliver the intended payload – in this example, siRNA. Reprinted with permission.[151]

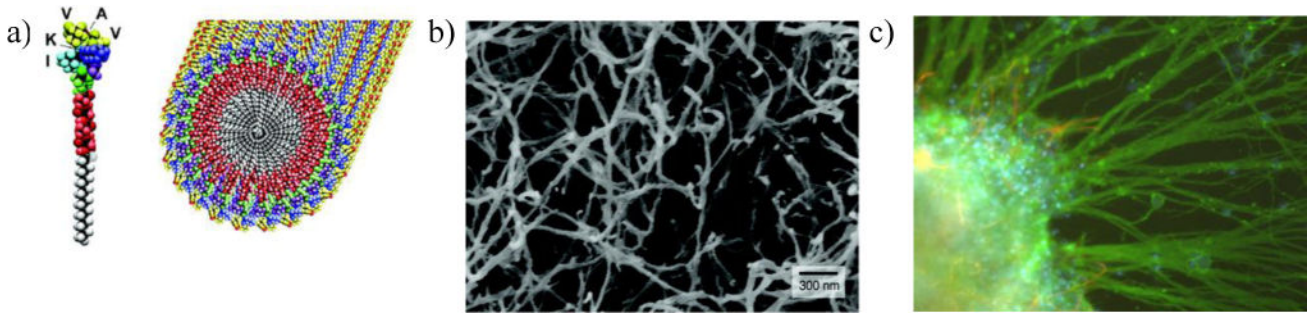
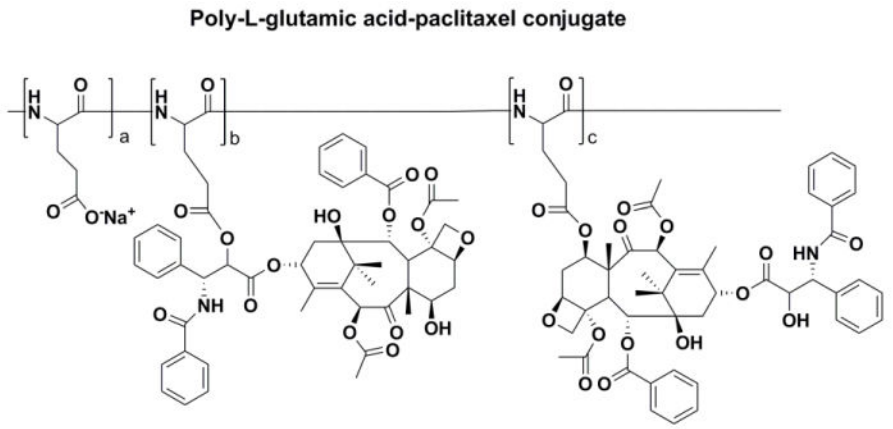


Figure 12.

Functionalized nanoparticles for nerve regeneration: a) Molecular graphics illustration of an IKVAV-containing peptide amphiphile molecule; b) Self assembled Network of IKVAV amphiphiles; c) Supported by a nanofiber network progenitor cells differentiated to functioning neurons instead of scarforming astrocyte. Reprinted with permission from AAAS.[182]

a)



b)

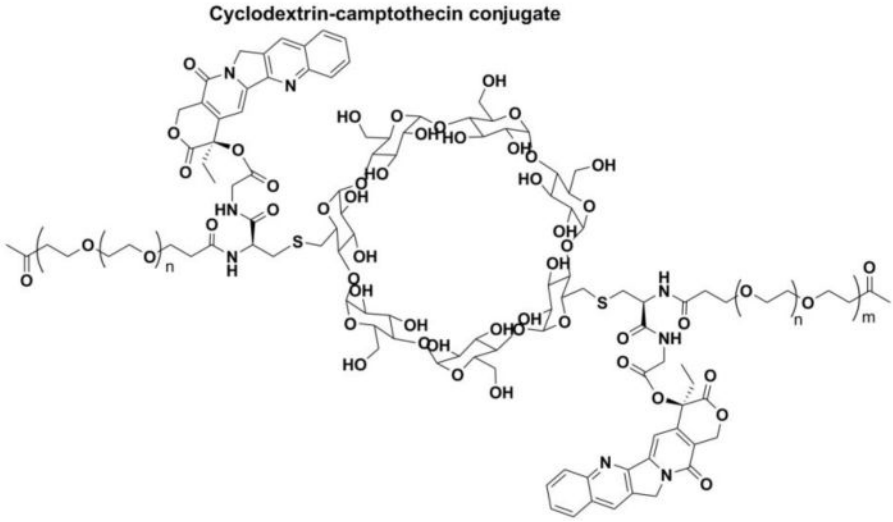


Figure 13.

Examples for polymer–anticancer drug conjugates: a) Paclitaxel (PTX), an anticancer agent, is linked to the carrier polyglutamate (PGA) via an ester bond. It was shown that the main drug release occurred subsequent to polymer degradation by the lysosomal enzyme cathepsin B; b) Conjugate of camptothecin (CPT) and a linear cyclodextrin-based polymer (CDP). The components of CDP are β-cyclodextrin and PEG. Pharmacokinetic and preclinical studies have demonstrated that this conjugate exhibits a longer plasma half-life and better distribution to the tumor tissue than does CPT alone. Reprinted with permission.[234]

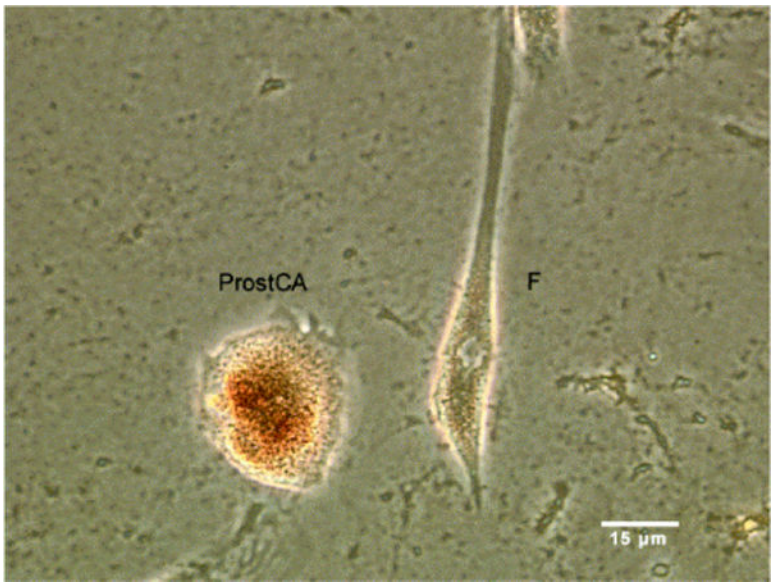


Figure 14.

Comparison of a healthy and a tumor cell incubated with nanoparticles. In a phase-contrast light microscopic picture a prostate carcinoma cell and a fibroblast cell were compared. While the tumor cell (left) shows remarkable pigmentation due to large nanoparticle uptake, the adjacent fibroblast cell depicts lower pigmentation, i.e. no or lower particle uptake. Reprinted with permission from Elsevier.[246]

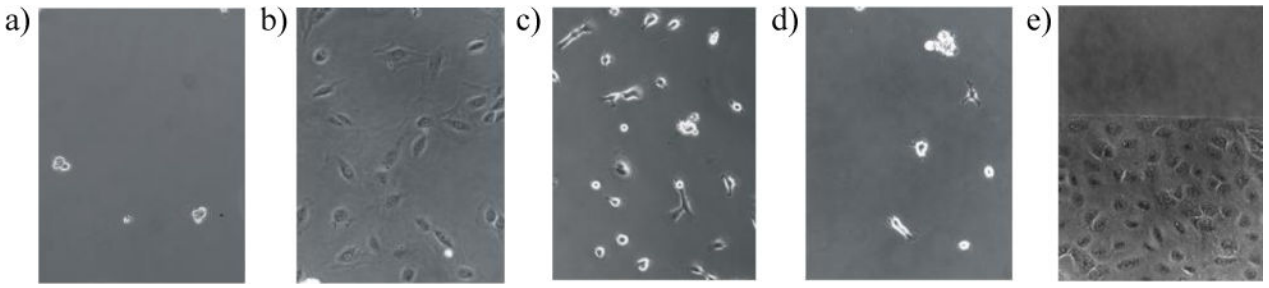


Figure 15.

Phase contrast optical micrographs of 3T3 fibroblasts on Polyethylene Glycol Diacrylate (PEGDA) 700 hydrogels. a) Cells on a non-RGD-functionalized gold nanoparticle pattern. (b–d) Cells on cyclo(-RGDfK-)-functionalized gold particles; cyclo(-RGDfK-) patches are separated by varying distances b) 40 nm, c) 80 nm, and d) 100 nm, after 24 h. in culture. e) Dense cell layer on a PEG support after 14 days in culture. The bottom part of the sample was patterned with cyclo(-RGDfK-) peptide-functionalized gold nanoparticles spaced 40 nm apart. Reprinted with permission.[266]

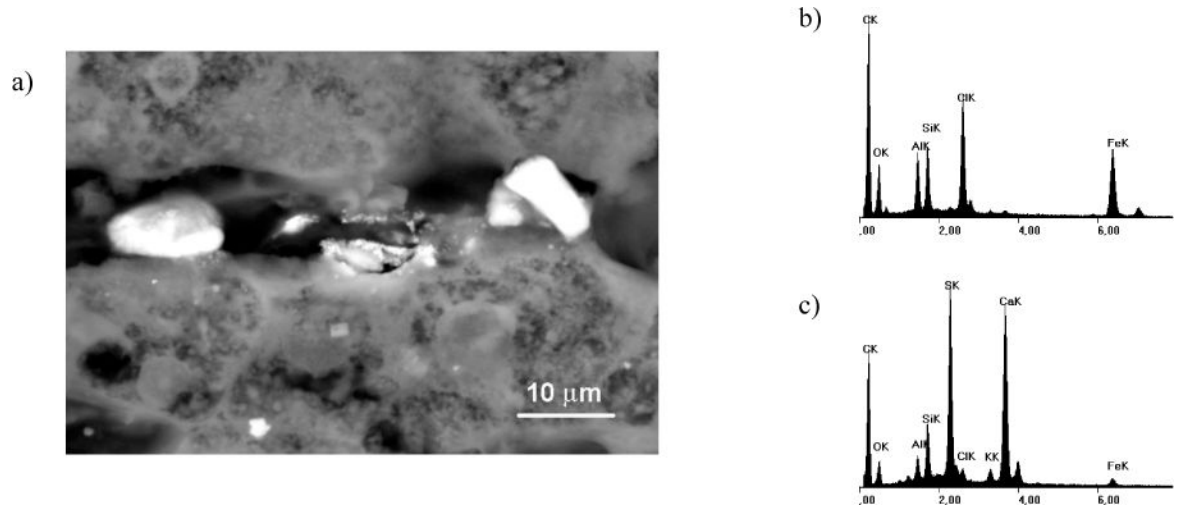
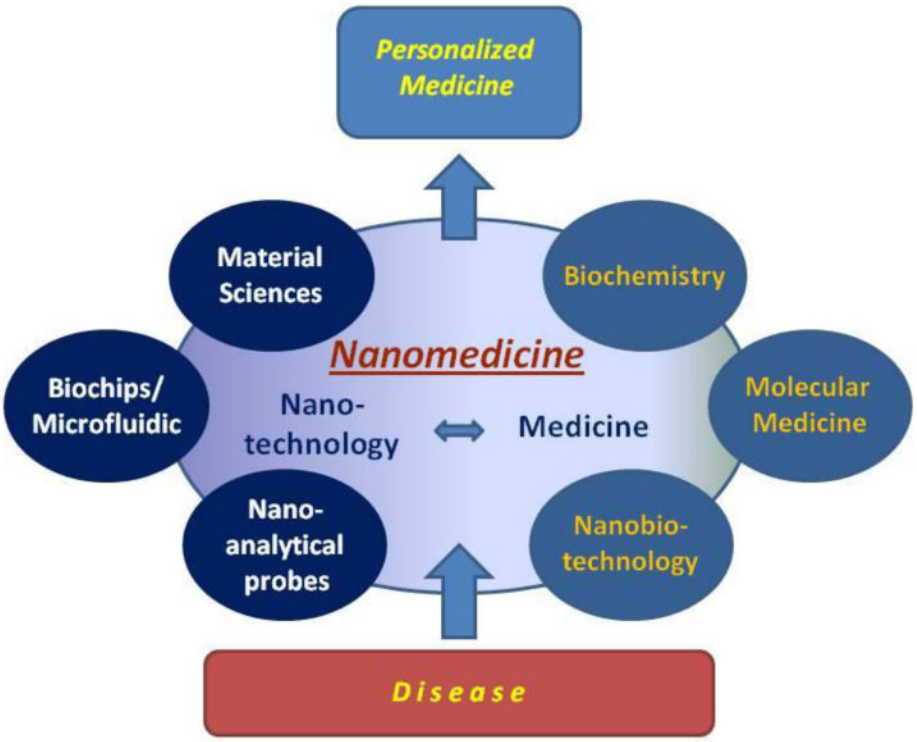


Figure 16.

SEM microphotograph of a granulomatous liver section: a) two small particles and a cluster of nanodebris in between;. b) and c) EDS spectra reveal that the debris have different compositions, and probably have different origins. Reprinted with permission from Elsevier.[295]



Scheme 1.
Technologies involved in the field of nanomedicine