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Diagnostics and Drug Delivery

# Nanomedicine—Challenge and Perspectives

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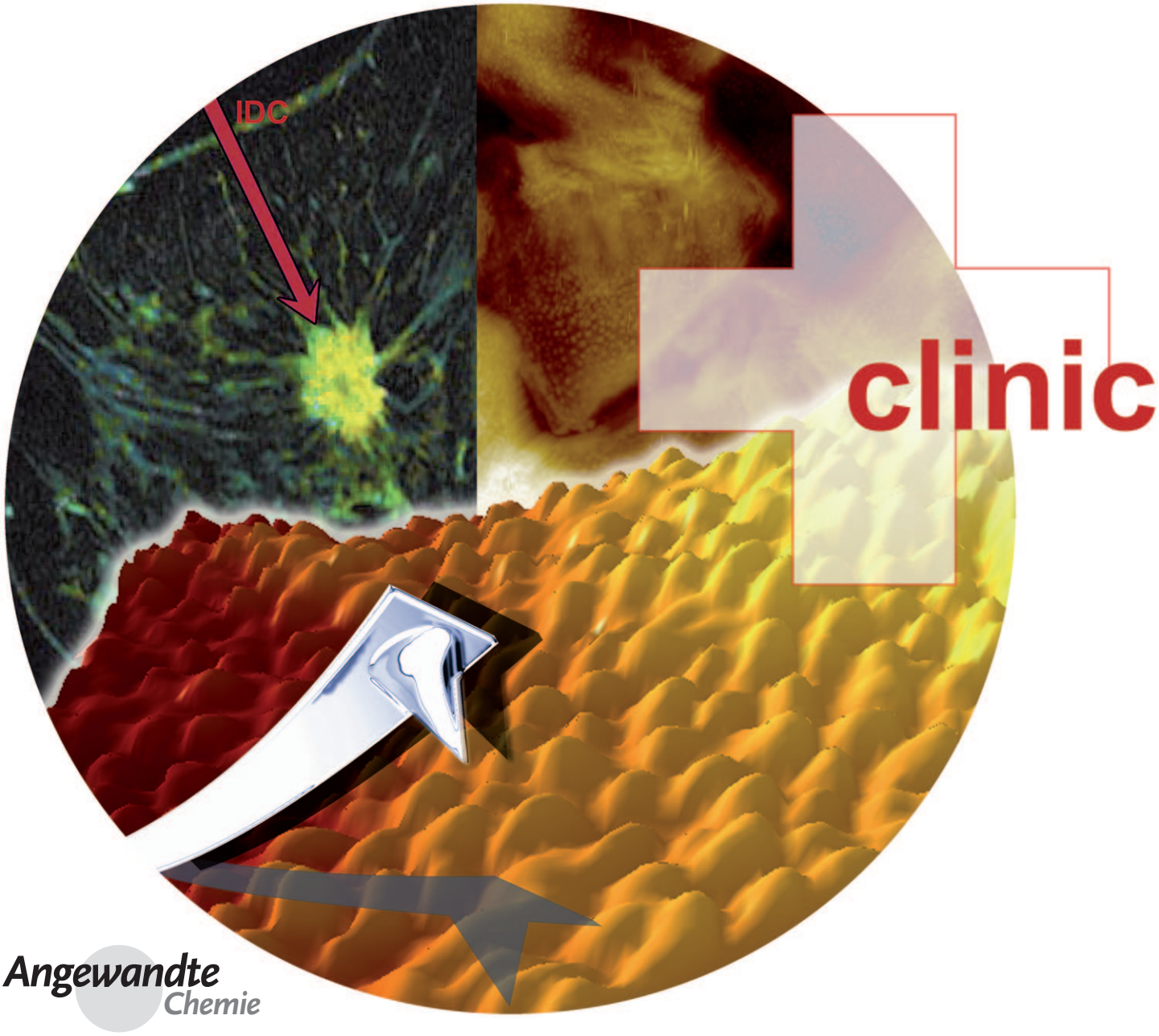




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*The application of nanotechnology concepts to medicine joins two large cross-disciplinary fields with an unprecedented societal and economical potential arising from the natural combination of specific achievements in the respective fields. The common basis evolves from the molecular-scale properties relevant to the two fields. Local probes and molecular imaging techniques allow surface and interface properties to be characterized on a nanometer scale at predefined locations, while chemical approaches offer the opportunity to elaborate and address surfaces, for example, for targeted drug delivery, enhanced biocompatibility, and neuroprosthetic purposes. However, concerns arise in this cross-disciplinary area about toxicological aspects and ethical implications. This Review gives an overview of selected recent developments and applications of nanomedicine.*

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## 1. Introduction

The manipulation of matter locally and deliberately on the atomic or molecular scale is an old dream of natural science. Starting in 1959 with the famous talk by 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, with the discovery of molecular beam epitaxy in 1968 in the Bell Laboratories, the generation of nanoparticles, and the invention of the scanning tunneling microscope, into a robust and well-accepted scientific field.[1–3] The old dream has already become true in the fields of nanoscience and nanotechnology. New opportunities have been realized in virtually all branches of technology ranging from optical systems, electronic, chemical, and automotive industries, to environmental engineering and medicine. "Smart" surface coatings, intelligent nanoscale materials, faster electronics, unprecedented optics, biosensors, and nanomotors are just a few examples from this transdisciplinary area. Although nanotechnology is still in its infancy, these first practical applications clearly demonstrate its enormous potential.

The field of medicine, on the other hand, faces very complex scientific as well as societal and ethical challenges. In particular because of the increased life expectancy of the population, some specific diseases have been identified as having a very high socio-economic impact over the next few years. Below we will discuss some specific areas, which we consider as promising applications of nanomedicine.

### 1.1. Definition

The term nanotechnology [from the Latin *nanus*, Greek *nanos* dwarf] is defined in the literature in a variety of ways, all of which have their advantages and limitations. In general, nanotechnology is concerned with dimensions and tolerance limits of 0.1–100 nm ($1\,\text{nm} = 10^{-9}\,\text{m}$), as well as with the manipulation of single atoms and molecules. A more specific definition was 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". The reduction in magnitude apparently leads to different, and qualitatively new and advantageous properties in the nanometer-scale materials.[4–6] A more general and operational definition involves the following interrelated constituents: nanoscale dimensions of the whole system or its vital components, synthetic materials, and unique characteristics that arise because of its nanoscopic size.[7]

Thus, nanotechnology includes the following key physical and chemical issues:

- The occurrence of novel physical properties characteristic of the nanoscale,
- analysis at the atomic and molecular scale at predefined positions,

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- control of matter at the atomic scale, that is, addressing individual preselected atoms and molecules, and
- the generation of complex functional systems with qualitatively novel properties (emergence).

To define the area of nanomedicine, discussed below, we first have to introduce a differentiation to the fields of molecular medicine, biochemistry, as well as nanobiotechnology.

Nanomedicine means essentially applying nanotechnology to medicine. While being related in certain aspects, the field of nano-biotechnology differs from nanomedicine, since the latter focuses on the applications of nanotechnology concepts to medical applications, while the former encompases all basic research at a nanoscopic level on biological systems, for example, investigations on plants. Molecular medicine, on the other hand, starts from a more conventional biochemical approach.

In contrast to conventional therapies—surgery, radiation, and chemotherapy—where the basic approach is to remove diseased cells faster than healthy cells, nanomedicine attempts to use sophisticated approaches to either kill specific cells or repair them one cell at a time by using a biosensor to detect, for example, when a drug should be released. Thus nanomedicine needs not only to apply and adopt nanotechnology concepts but will, at the same time, need to feedback information to nanotechnology such that the two fields can cross-fertilize and develop jointly.

One goal is the design of multilevel molecular aggregates that have novel functional and dynamic properties that are desirable for applications in medicine. Both the size- and site-specific properties of these systems which are characteristic of the nano- and mesoscale are made use of. This approach also offers new possibilities towards the development of personalized medicine, 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, efficient medications need to be established by using selective targeting. This field is currently under intensive investigation. Nanomedicine promises alternatives to molecular medicine by having the following general advantages: local processes at the nanometer length scale, such as diffusion, intermixing, and sensoric response, become ultrafast. Furthermore, nanotechnology can provide the opportunity of directly probing local properties. Physical and chemical processes can be controlled and intensified, the precision enhanced, and direct access to biomarkers becomes possible. Finally, new results can be achieved in real time. These concepts together with a combination of the research areas such as systems biology and systems medicine will contribute significantly to form the route to personalized medicine.

How is personalized medicine related to nanomedicine? Similar to existing medical diagnosis and therapeutics, and as dictated by economic reasons, mass applications of new screening and diagnostic tools in medicine have to be fast, convenient, and inexpensive. Therefore, miniaturization,

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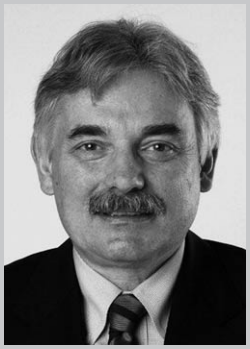

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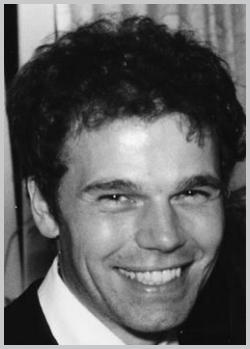

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parallelization, integration, as well as automation are mandatory. The demand for large amounts of routine in vitro measurements on patients so as to retrieve sufficient and comparable data dictates the development of smart integrated devices, such 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 and nanostructured surfaces as well as nanoanalytical techniques for molecular diagnostics, treatment, follow-up, and therapy of diseases (theranostics). Integrated medical nanosystems are also needed which, in the future, may perform monitoring and complex repairs in the body at the cellular level. Nanotechnology considers cells as a complex system of interacting nanomachines. Visionary concepts envisage the construction and control of artificial cells by using engineered nanodevices and nanostructures for medical applications (Figure 1).

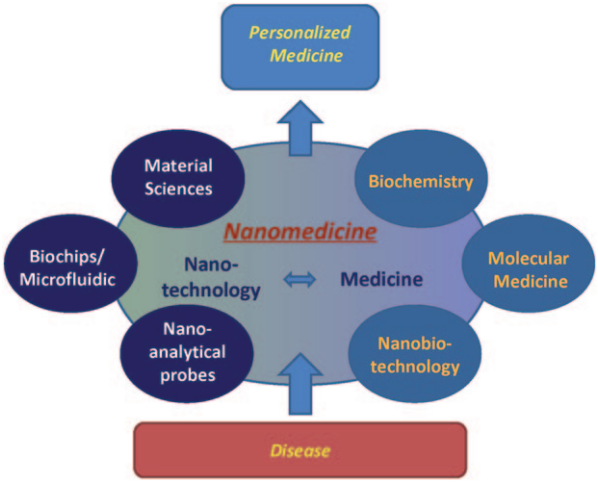


***Figure 1.*** Technologies involved in the field of nanomedicine.

## 2. Nanotechnology in Medical Diagnostics

Diagnostics play a key role in medicine for the successful prevention and efficient treatment of diseases. Taking cancer as an example of a widespread disease, and that is still the leading cause of death in the industrial countries, it will be difficult to achieve a significant increase in the cure rate unless more information about the molecular mechanisms of the pathophysiology can be obtained, which will build the basis for the development of new anticancer drugs.[9] The advantage of nanostructure-based diagnostics lies in their potentially higher sensitivity and selectivity compared to classical methods.

An important area in nanotechnology is the generation of nanoscale materials. For diagnostic purposes, quantum confinement effects, which are characteristic of the nanometer scale, may be used. Nanoparticles may be embedded in other crystalline or amorphous nanoscale materials to guarantee better functionality and bioavailability. In this area, research into the development of metallic and semiconductor quantum 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 IR or NMR methods to increase significantly the resolution and sensitivity, thus enabling earlier diagnosis of diseases. The increased resolution and sensitivity is expected to lead to cheaper clinical applications in therapy. Modification of the nanoparticle surfaces with chemical recognition groups allows the identification of complementary groups on cell surfaces which are indicative, for example, of cancer or other severe diseases (see Figure 2). The same concept can also be applied to site-specific drug delivery.[10–12]

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### 2.1. In Vitro Diagnostics

The purpose of extracorporeal (in vitro) diagnostics for cells is manifold. In vitro diagnostics are necessary, for example, to protect the blood supply for transfusion reasons, to monitor the level of drugs applied to patients, and to provide information to assist the 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, thus enabling the appropriate treatment regimen to be chosen. Various nanotechnology platforms have been developed to allow for the simultaneous real-time evaluation of a broad range of disease markers by non-invasive techniques. Inter-

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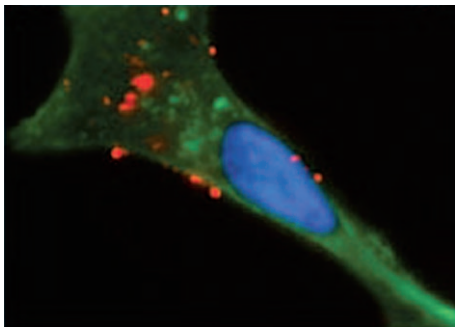

*Figure 2.* Prostate cancer cells have taken up fluorescently labeled nanoparticles (shown in red). RNA aptamers binding to the prostate-specific membrane antigen (PSMA; a well-known transmembrane protein, which is overexpressed on prostate cancer epithelial cells) were used as the targeting molecules on the nanoparticles. 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 destroy the tumors. Reprinted with permission from the American Association for the Advancement of Science (AAAS).[13]

estingly, two classes of microtechnological devices, microarray DNA chips and microfluidic systems for lab-on-chip diagnostics, which were developed in the 1980s, have now been transferred to the nanotechnology arena. This "miniaturization" was possible in both cases because of a development in the fundamental enabling technique, namely photolithography. This technique now allows for lateral resolution in the 10–100 nm range, which is three orders of magnitude lower than at the time when these approaches were first developed. As a result, the information that can be put on a biochip has increased by a factor of $10^6$–$10^8$, thus demonstrating the powerful capabilities of nanoscaling in biomedical applications. The use of photolithography allows the selective illumination and removal of photolabile groups, thereby leading to the exposure of reactive moieties. The technique can be used to pattern various chemical and biological moieties or diverse textures very precisely on the substrate, thus enabling the surface attachment of biomolecules to specific molecular segments, for example, single-stranded DNA for hybridization or different substrates for proteomic analysis.[14–20]

Another goal of medical diagnostics is to analyze single cells. Nanotechnology approaches offer the opportunity to even investigate single molecules, and opens up the possibility for new methods for analysis and detection. The added value of this approach becomes clear when it is taken into consideration that large amounts of primary cells are usually mixtures of either different cell types or healthy and tumorous cells, thus making the acquisition of statistically significant results difficult.[21] Another motivation for single-cell analysis concerns 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 are often inappropriate for investigations because the large amount of cells needed, for example, for electrophoresis purposes, leads to the analysis not of cells but of tissues or cell mixtures, that is, systems, which give no insight into the definite basic structure. The ability to describe one specific cell (type) leads to the role of this building block in the tissue and the organism being defined, and thus the function of cell interaction, the effect of differentiation, and diseases can be characterized.[22]

Different selection techniques such as cloning rings, limiting dilution, laser microdissection, live-cell catapulting, or microfabricated pallets are used for the isolation of single adherent cells.[23–25] Fluorescent-activated cell sorting (FACS), magnetic sorting, column chromatography, panning, limiting dilution, and the isolation of cells by microfluidic approaches are commonly used for the isolation of non-adherent cells.[24,25] The analysis of those cells has until now been performed by classical biochemical methods such as the polymerase chain reaction (PCR) or patch-clamp techniques. A nanotechnology alternative is now commonly used for biochip analysis performed with photolithographic technology (see Section 2.1.1). Together with the development of smart surfaces, semiconductor manufacturing and combinatorial chemistry as well as bioinformatics have made significant advances 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 have made such significant progress that they are now used as point-of-care diagnostics. The modification of biochip surfaces by nanotechnological methods offers the possibility for ever smaller probes for the analysis of RNA retrieved from a single cell.

The success of expression profiling encouraged protein researchers to adopt some of the methods. As the differences between the expressed form of proteins and their biochemical appearance (for example, folding structure or secondary modification) is remarkably high, the analysis of proteins at a single-cell level is coming more into the focus of industrial and scientific research because the results obtained reflect much more the biological processes within a cell than does the expression profiling. Different kinds of biochips with protein arrays are available (see Section 2.1.1). The first application of antibody arrays were reported in 2002.[31,32] Clinical applications for such protein chips include the discovery of disease markers for diagnosis, prognosis, and drug response, and allow the disease development and progression to be tracked. Antibody arrays are suited to high-throughput methods for the functional characterization of disease at a molecular level. Furthermore, the information gained from the protein array on cancer progression and tumor subtypes may enable intervention and therapy optimization.[33–41]

Atomic force microscopy (AFM; see Section 2.1.3.1) techniques have been explored for single-cell analysis. They allow high-resolution in vitro investigation of cell surfaces and analysis of physical properties such as mechanical compliance of single cells at a single-cell level. Force spectroscopy provides locally direct quantitative data on intra- and intermolecular forces of a single molecule.[42–48] This approach was also used for manipulation, for example, as a microdissection device. With the possibility to isolate organelles and to cut chromosomes in a precise way, this technique was applied together with subsequent PCR amplification of

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dissected DNA fragments for the analysis and even mechanical reimplantation of the isolated fragments back into its original position.[49,50]

#### 2.1.1. *Microfluidics and Nanoarrays*

A microfluidic unit can be defined as a device comprising one or more channels with at least one dimension measuring less than 1 mm. The channels, which can have a width below a few micrometers, allow control over minute fluidic volumes in the nanoliter and picoliter range. Microsystem technologies developed for microfluidic chips enable just about any biological assay working at the molecular level to be incorporated onto a chip (lab-on-a-chip systems; Figure 3). These approaches not only offer the possibility to isolate and manipulate living cells, but also to perform toxicity assays, enzyme-linked immunosorbent assays (ELISA), PCR amplification, blood separation, or for the genotyping of cytokine polymorphism.[51]

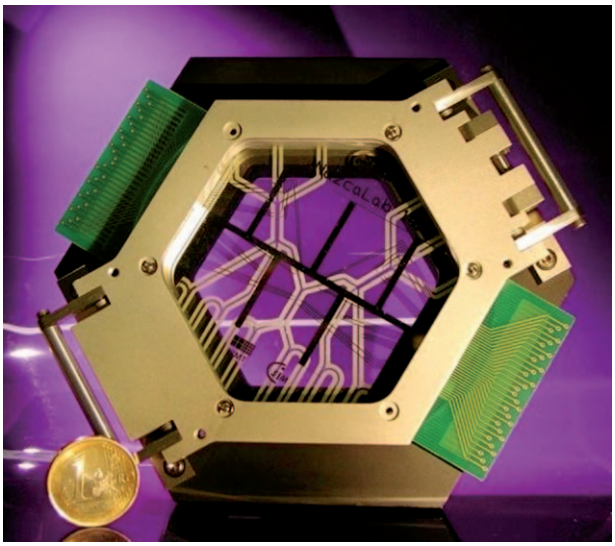

***Figure 3.*** Lab-on-a-chip technology for biological applications. A system of many branched microfluidic channels (between white double lines) with a variety of different electrode layouts (black fine lines) allow applications such as cell imprinting, cell fusion, or cell separation. Fluidic connection is realized on the backside, with electronic connection through the 2×30-pole interfaces (green boards). For size comparison a one Euro coin is shown. Kindly provided by M. Jäger, Fraunhofer IBMT, Potsdam, Germany.

The flow of fluidics in a microfluidics chamber is characterized by the non-dimensional Reynolds number Re [Eq. (1)].

$$\begin{aligned} \mathrm{Re} &= (\rho u^2)/(\mu u/\mathrm{L}) \\ &= \rho u L/\mu \\ &= u L/\nu \end{aligned} \tag{1}$$

$\rho$ = density, $u$ = velocity, $\mu$ = dynamic viscosity, $L$ = characteristic length, $\nu$ = kinematic viscosity

The Reynolds number should be less than 100 to maintain laminar flow, which is necessary so that molecules can be transported in a predictable manner through microchannels. Materials such as poly(dimethylsiloxane) (PDMS) enabled microfluidic chips to become a conventional easy-to-produce and easy-to-use technology. The ability to tailor the material for the analysis of single cells gave a strong push to this research area.[52–54] Another important advantage of PDMS is its biocompatibility. It is assumed to be a suitable biomaterial for biomedical devices because it causes minimal endotoxin contamination, leukocyte activation, and complement activation.[55] Du et al. showed in 2006 that an antibody-based microfluidics system captured more than 30% of the cancer cells from a mixture of normal human glandular epithelial cells (HGEC), human cervical stromal cells (HCSC), and cervical cancer cells (HCCC).[56]

Another approach was the development of a microfluidic cell chip for monitoring allergic response. A basophilic leukemia cell line (RBL-2H3) was cultivated on a PDMS chip containing a cultivation chamber and microfluidic channels. Molecules marked with a fluorescent dye were secreted after allergic stimulation and observed by using a photomultiplier tube (PMT) fitted onto a microscope. The technique of photolithography was adapted from the microelectronic industry. The lateral resolution was originally of the order of 100 μm (or 100000 nm). The linear spatial resolution of lithography is now 1000 times better, that is, up to a one-million-fold increase in information density can now be packed in "nanoarrays". Various nanochannel structures can be imprinted for the selective fractionating of proteins on the basis of their molecular weight. As a result, different patterns could be produced simultaneously by treating the chip with dissimilar biological samples (Figure 4).[57]

Specific serum markers for the early diagnosis of diseases such as cancer are currently unavailable. Nanotechnology offers the possibility to evaluate a wide range of molecular markers simultaneously. The information obtained can be helpful for early and efficient diagnostics as well as for monitoring and selecting therapeutic strategies. Single clinical markers that are now used for the diagnosis of carcinogenic conditions, for example, the prostate specific antigen (PSA), cannot provide this knowledge because of the broad variability in the basal expression in individuals. Thus, ex vivo diagnosis on biological fluids such as serum, saliva, urine, or tissue exudates obtained from non- or minimally invasive procedures remains an unmet need.[59]

Fluidic devices based on other concepts have in the meantime become commercially available. One of these is a device based on dielectrophoretic field cages, which give the possibility to combine the isolation and the manipulation of non-adherent cells in one device. This principle was introduced by Fiedler et al. in 1998.[61] Certain electrode configurations were constructed to function as a funnel, as liners to break aggregates of cells, or as electrical octodes to trap cells electrostatically for manipulation. Recently it was shown that moderate warming induced by the electrical field can be neglected in cultures under appropriate experimental conditions. Moreover, possible side effects of dielectrophoretic manipulation such as membrane polarization and Joule heating were excluded, thus making the method appropriate for medical applications.[60–62]

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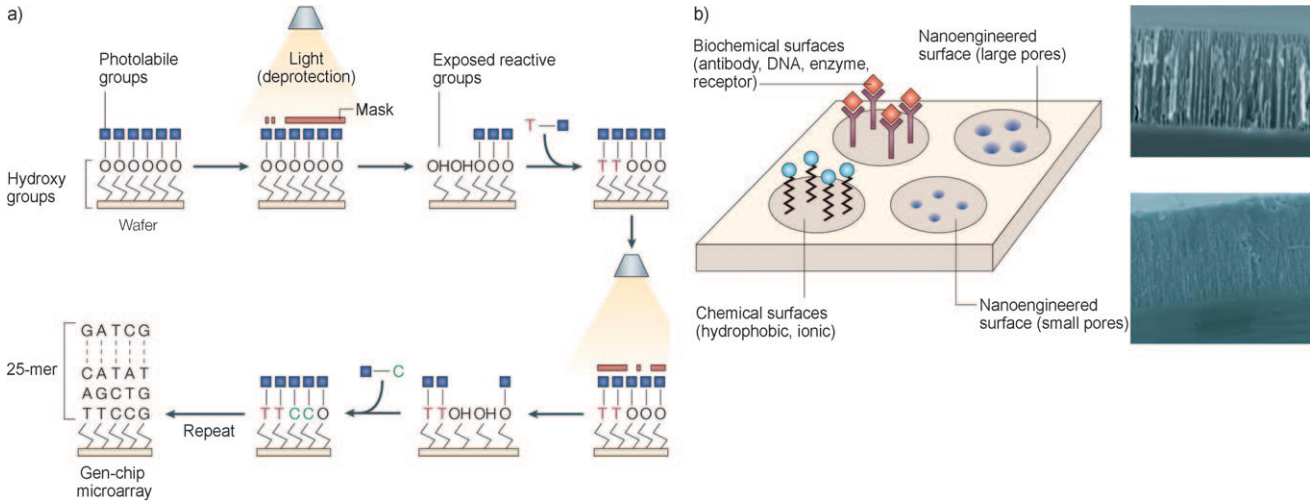


***Figure 4.*** Photolitographic techniques for manufacturing micro- and nanoarrays for DNA analysis (a) and (b) proteomics. a) A microarray with a pattern of biological molecules on the surface to obtain DNA sequencing by hybridization, for example. Blue squares represent photolabile groups, which are selectively illuminated through a mask (photolithography) and removed to expose reactive groups. Sequential application of the procedure yields single-stranded hybridization probes with specific sequences. b) Photolithography can be used to pattern different chemicals, biological moieties, and physical textures on substrates, for the purpose of prefractionation of protein mixtures before investigation by time-of-flight mass spectrometry. Different proteomic patterns are produced by different substrate treatments. The pictures 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 with permission from Macmillan Publishers Ltd.[58]

An urgent need was the investigation of the systemic inflammatory responses that can develop 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 on inflammatory responses; 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 monitor the development of inflammation markers in real time by using a plasma analysis device.[63] Here, microfluidics offer the chance to intervene at an early stage in an inflammatory process, which if untreated could be life-threatening. Recently, a new microchip was developed with an anisotropic nanofluidic structure to separate and sort biomolecules as DNA or proteins. With an extremely tiny sieve structure, the system can sort through continuous streams of biological fluids and separate proteins by size. This system thus provides an appropriate tool for the identification of small molecules for early diagnostics and the 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 appropriate nanosensors. This concept, proposed by the research group of Gimzewsky, is based on the nanoscale forces and deformations produced as a result of ligand–substrate binding.[65] Micro- and nanocantilevers, devices based on this principle, deflect or change their resonant frequencies as a result of the affinity binding of biomarker proteins or DNA hybridization events occurring on their free surfaces (Figure 5a). The deflections can be monitored by lasers or detected electronically, thus enabling the rapid and simultaneous sensing of a variety of biomarkers. This technique allowed the detection of target oligonucleotides at clinically significant levels without fluorescent or radiolabeling and serum markers.[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 analytes, thus resulting in a shift in their conductance as a function of the electrical charges on the bound molecules (Figure 5b).[67–72] These changes can be detected electronically and quantified precisely. Although these systems are not yet in clinical practice, their multiplexing capabilities hold promise for parallel analyses.

#### 2.1.2. *Fluorescent Labels and Imaging*

Fluorescent dyes represent another important class of in vivo imaging tools which are mainly used for the visualization of cells and molecules. A big disadvantage of those dyes is their photo instability, with the fluorescent yield rapidly fading within less than one minute. The bleaching of the dyes restricts the range of their applications. Inorganic quantum dots have a much higher photostability; however, selenides and sulfides, which are mostly applied for this purpose, are cytotoxic and can, therefore, only be used for diagnostics of biological samples outside the human body. The biocompatibility, high photoluminescence quantum efficiency, and stability against photo bleaching makes silicon quantum dots ideal candidates for replacing fluorescent dyes in biological assays. Silicon nanocrystals (NCs) can be fabricated by using wet chemsitry or electron-beam lithography with reactive ion etching to give silicon nanopillars that can be subsequently oxidized to produce luminescent silicon cores.[73] These are so small that the addition or removal of a

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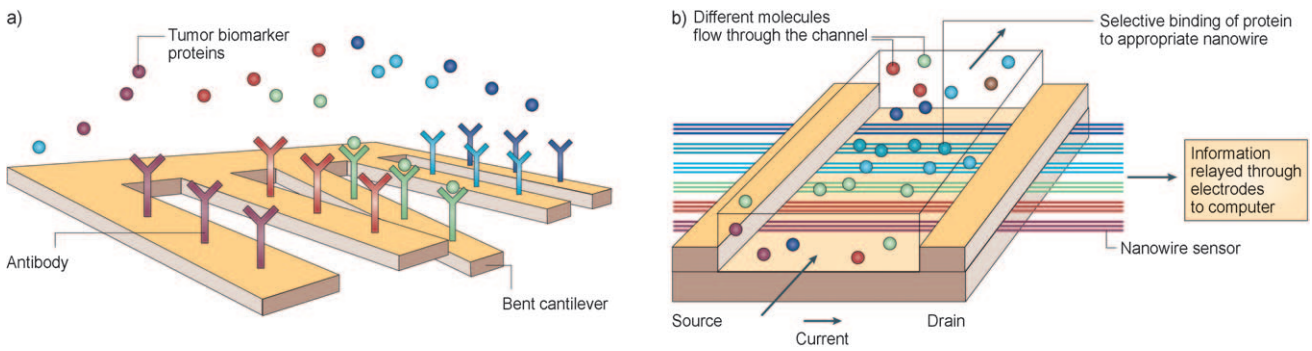


***Figure 5.*** a) Nanocantilever array: The biomarker proteins are affinity-bound to the cantilevers and cause them to deflect. The deflections can be directly observed with lasers. Alternatively, the shift in resonant frequencies caused by the binding can be detected electronically. As for nanowire sensors, a large number of different proteins can be sensed simultaneously in real time. b) Nanowires within a microfluidic system. The different colored circles represent different molecular analytes that adsorb or affinity-bind to different nanowire sensors. The binding causes a change in the conductance of the wires, which can be electronically and quantitatively detected in real time. The working principle is that of a (biologically gated) transistor. The charges on the binding protein disrupt electrical conduction in the underlying nanowire. The nanosized wire is required to attain high signal-to-noise ratios. Reprinted with permission from Macmillan Publishers Ltd.[58]

single atom changes their optical appearance significantly. Other unique properties of quantum dots are their size- and composition-tunable emission, broad absorption spectra, and narrow emission spectra (Figure 6).[74,75]

The improved luminance and photostability of quantum dots makes them appropriate for investigating cells or for the detection of low abundance antigens.[76,77] Tests revealed that they undergo degradation in vivo which leads to a quenching of the fluorescence. Gao et al. demonstrated recently that a hydrophobic exterior protects the quantum dot from this effect and optimized it for medical applications. The dyes allow, for example, different types of cells to be distinguished simultaneously within a tumor in vivo.[78–81]

### *2.1.3. Local Probes and High-Resolution Imaging*

#### *2.1.3.1. Chemical Probes*

The profiling of mammalian cellular components by matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry is a known way to characterize cells and tissues on a biochemical level. Further developments in this technology has in the meantime made it possible to characterize ever smaller structures—such as the proteins of one single cell.[82–87] Benninghoven and co-workers carried out pioneering work on the application of time-of flight mass spectrometry to medical questions.[88,89] An approach to characterize isolated cells was first described by Colliver

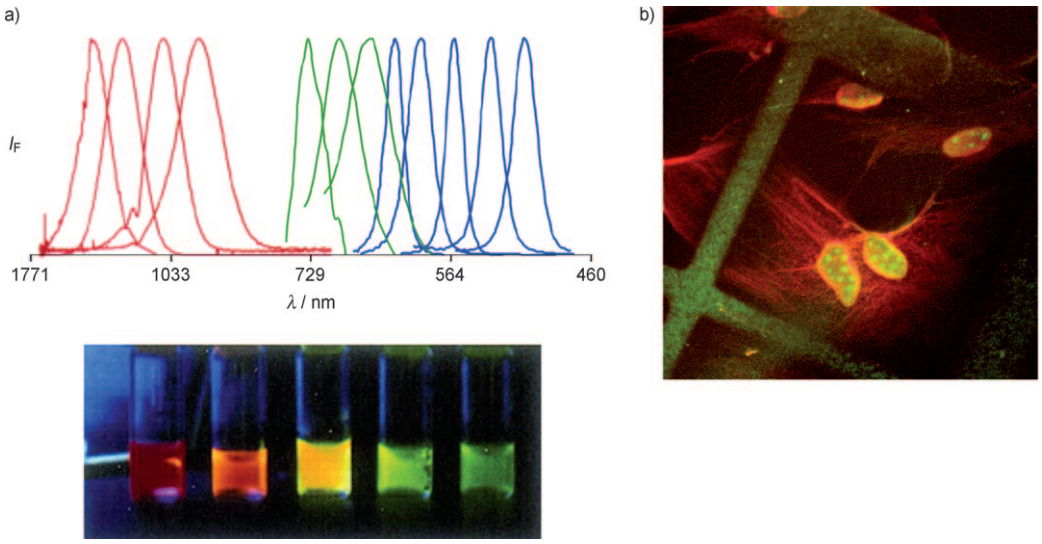


***Figure 6.*** a) Size- and material-dependent emission spectra of surfactant-coated semiconductor nanocrystals. Top: The blue series represents different sizes of CdSe nanocrystals with diameters of 2.1, 2.4, 3.1, 3.6, and 4.6 nm (from right to left). The green series is of InP nanocrystals with diameters 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. Bottom: A true-color image of a series of silica-coated nanocrystal probes with a CdSe core and a ZnS or CdS shell in aqueous buffer. The probes were all illuminated simultaneously with an ultraviolet lamp. b) Cross-section of a dual-labeled sample. Reprinted with permission from the American Association for the Advancement of Science (AAAS).[74]

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et al. in 1997. They used time-of-flight secondary ion mass spectrometry (TOF-SIMS) to analyze single cells and provide chemical information on their components. After preparing *Paramecium multimicronucleatum* cells by freeze-fracture techniques, TOF-SIMS analysis enabled characterization of the cell surface.[90] A combination of complementary techniques such as optical microscopy (OM), ion-induced electron emission (IIE), and secondary neutral mass spectrometry with subsequent laser ionization (laser SNMS) was recently used for mapping native biomolecules within mouse kidney cells (Figure 7).[91,92]

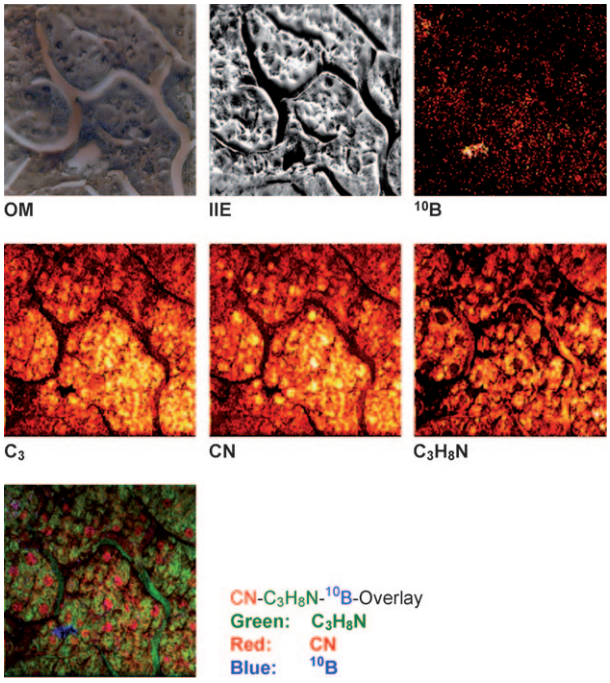


***Figure 7.*** Top row: Optical microscopy (OM), ion-induced electron emission (IIE) and boron ($^{10}$B) distribution detected by laser SNMS. The bottom row shows dispersion signals from molecular labels such as the biological fragments $C_3$, CN, and $C_3H_8N$ which are characteristic of lipids, proteins, and nucleic acids. The samples were taken from a kidney of a NMRI nude mouse and treated with a combination of sodium mercaptoundecahydro-*closo*-dodecaborate (BSH) and *p*-boronophenylalanine (BPA). Area size: 120×120 μm$^2$; lighter colors correspond to higher intensity. Reprinted with permission from Elsevier.[91]

#### 2.1.3.2. *Scanning Probe Analysis*

Since many biomedical and nanomedical processes occur on the molecular scale, the ability to image nanostructures at predefined positions and to perform local spectroscopic studies is becoming more and more important. Scanning probe microscopy opened up a completely new area of surface-imaging technologies which complement conventional methods such as electron and light microscopy. In particular, dynamic force microscopy is well suited for investigating soft systems such as biological cells, and also allows the tracking of individual proteins and the imaging of biological macromolecules in liquids.[93–95] For example, cytoskeletal structures such as stress fibers can be imaged by atomic force microscopy (AFM), and the dynamics of nuclear pores after treatment with dexamethasone can be imaged by this technique.[96,97] The visualization of cells by AFM is possible without damaging their surface, as was shown for renal A6 cells. Similarly, focal adhesion plaques as well as membrane transport was successfully imaged.[98–100]

A recent experimental approach combined AFM with quantum-dot-labeled antibodies as surface markers to detect the cystic fibrosis transmembrane conductance regulator (CFTR). This protein is frequently mutated in hereditary cystic fibrosis or not expressed in the cell membrane. 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 AFM technology has enabled its utilization for in vivo imaging. As Imer et al. demonstrated, AFM technology can be used as a minimally invasive tool in clinical diagnostics of rheumatoid arthritis.[102]

AFM has also been proven to be a suitable method to analyze the cell-surface morphology in intact native human stratum corneum (SC), the outermost layer of the epidermis. The SC is composed of cornified keratinocytes (corneocytes) organized within the whole SC layer like bricks in a wall. 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 the corneocyte morphology. AFM has been shown to be a suitable tool for the nanometer-scaled analysis of native SC in terms of its morphology as well as quantification of the volume and surface of single corneocytes.[103] Figure 8 shows representative images of the SC of atrophic and healthy skin.

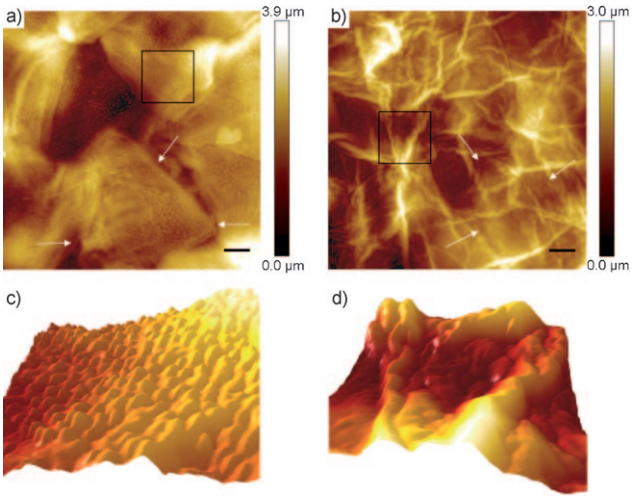


***Figure 8.*** AFM analysis of native human stratum corneum (SC). Comparison of atrophic skin (a and c) and healthy skin (b and d) reveals a reduced SC integrity in atrophic skin, as indicated by enlarged intercellular gaps between the individual corneocytes (white arrows in a and b). While the surface morphology of healthy SC is characterized by filamentous structures forming a dense network across the SC (b and d), the surface of corneocytes of atrophic skin is characterized by a regular-shaped undulating structure (c). The black bars (a and b) correspond to 5 μm. The black squares mark the surface region presented as a three-dimensional image (c andd).[305]

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The changes in the composition of the SC (corneocytes and surrounding matrix) was indicated by prominent intercelluar gaps. While the SC surface of healthy skin was covered by a strongly pronounced filamentous network, the SC surface of atrophic skin was characterized by a homogenous distribution of regular-shaped undulating structures. Moreover, single corneocytes flatten upon aging, as indicated by an increased surface area of a single cell and a decreased cell height. The application of AFM for physiological questions was recently comprehensively reviewed in a special issues of *Pflugers Archiv* (*European Journal of Physiology*).[104]

A specialized form of the method is force sensing integrated readout and active tip (FIRAT) analysis.[105] It is much faster and more sensitive than regular AFM, and is movies can also be recorded and several physical properties of nanostructures, such as stiffness, elasticity, and viscosity, determined simultaneously. This method may lead to a more sensitive understanding of cellular membranes than was possible before.

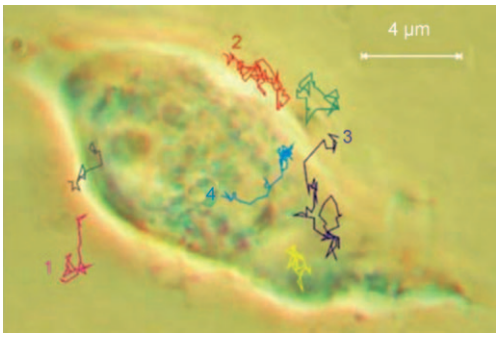


***Figure 9.*** Trajectories of single AAV-Cy5 particles on entry into a living cervical cancer cell line (HeLa). The traces showing single diffusing virus particles were recorded at different times. They describe various stages of AAV infection, for example, diffusion in solution (1 and 2), touching 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 to the nucleoplasm (4). Reprinted with permission from the AAAS.[111]

#### *2.1.3.3.* ***Plasmonic and Optical Techniques***

A method based on surface plasmon resonance (SPR) microscopy and spectroscopy was developed by Rothenhäusler and Knoll in 1988 to investigate the interaction of biomolecules without the need for additional labels.[106] SPR is very sensitive to 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—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, which affects the propagation of the plasmons. The binding of biomolecules is measured by changes in the refractive index. SPR microscopy offers the possibility to measure the binding force of interacting biomolecules. In fact, the kinetic analyses of most biomolecular interactions such as 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 which can be easily standardized and used, for example, for the analysis of blood (such as during transplantations). Another development is SPR microscopy which has made high-throughput analysis of binding events possible.[107,108]

Laser-optical techniques have recently experienced a dramatic development in regard to nanoscopic medicine, as summarized by Peters.[109] The research 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 (Figure 9). Their analysis method was based on fluorescence-labeled adeno-associated virus (AAV) particles.[110,111]

Biomedical information can also be retrieved from digital holography, which allows marker-free quantitative analysis in the cellular and subcellular range.[112–114] Holographic interferometry provides information about variations in the thickness/shape (with a vertical resolution of less than 8 nm) as well as about volume changes and the micromotion of cellular samples. The differences in dynamic processes of living invasive and non-invasive pancreatic tumor cell lines was shown with this technique.[115–117] The characterization of the movement of cells by digital holography can be used as a predictive tool for the metastatic properties of a tumor.

Hell and co-workers developed a pioneering and very promising digital imaging method. 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 10). This method opens up completely new perspectives for high-resolution optical (far-field) imaging in nanomedicine. Although not yet used for clinical applications, the technique allows nanoscopic optical information within living cells to be retrieved under physiological conditions. Such information was hitherto only obtained by electron microscopy methods, but the cells could not be analyzed under physiological conditions. Recently, the Hell research group developed a dual-color STED method with a resolution of about 25–35 nm in two channels. Nonlinear iterative (Richardson–Lucy) deconvolution leads to a further increase of the resolution (Figure 10). The technique was applied to the imaging of nanometer-sized features inside cells.[118–121]

The Hell research group examined neurofilaments of neuroblastoma cells by this method. These proteins belong to the major constituents of the axonal cytoskeleton and consist of three different subunits: the light, medium, and heavy neurofilaments. In a dual-color experiment, the light neurofilament was stained green, whereas α-internexin, also a component of the mature filament, was marked in red. The different localization of the proteins are clearly shown in Figure 10.[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 optical resolution of samples under 100 nm by using biplane-fluorescence photoactivation localization microscopy (BP-FPLAM). This

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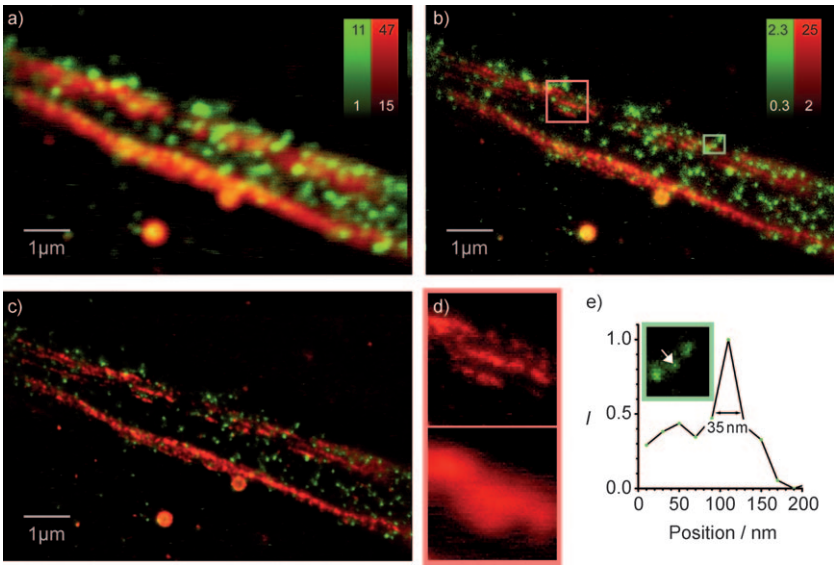


*Figure 10.* Comparison of fluorescence imaging techniques: a) Confocal, b) STED, and c) Richardson–Lucy deconvolved STED images of neurofilaments (green: light subunits, red: α-internexin). d) In contrast to the confocal image, STED reveals three well-separated α-internexin strands of the axon. e) Structures of the light subunits exhibit a full-width at half maximum (FWHM) value of < 40 nm. Note the different organization of the light subunits and α-internexin. Reprinted with permission from Ref. [121].

far-field technique allowed the generation of images with 30 × 30 × 75 nm resolution over a depth of several micrometers.[122]

Complementary to the advanced optical techniques developed over the years, various electron microscopy techniques play an important role in imaging a biological specimen and can provide an enormous amount of useful information. In recent years, numerous fascinating high-resolution structures were obtained by cryo-electron microscopy (cryo-EM). The technique is currently being developed to enable a comprehensive three-dimensional analysis of complex structures, including viruses and molecular landscapes within whole cells. This will pave the way for a "visual proteomic", which aims to complement and extend mass spectrometry based methods, 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 have provided the impetus to develop point-of-care diagnostics with higher sensitivity, specificity, and reliability. In vivo diagnostics provide data instantaneously from the patient and allows disease development and therapy to be tracked. The "find, fight, and follow" concept ("theranostics") of early diagnosis, therapy, and follow-up will take a new turn with developments in nanotechnology. Appropriate contrast agents for imaging 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 single molecules and on implantable devices. The aim of molecular imaging is to create detection agents that can also deliver and monitor therapy. In particular, the detection of diseases at an earlier stage is a central goal. Nanotechnology offers a unique possibility to produce new biosensors and medical imaging techniques with higher sensitivity and precision of recognition. This goal can be reached, for example, through the development of new nanoparticles for more specific and more sensitive imaging. In addition, the miniaturizing of biosensors gives a chance for the implantation of diagnostic devices which send continuous information to a monitor outside of the body (for example, to detect the amount of cholesterol in blood). Such devices will result in a big improvement in the living conditions of people who need permanent medical monitoring.[126]

#### 2.2.1. Targeted Imaging

Optical and electronic effects originating from the size of the nanoparticles are not observable in macroscopic samples of the same materials. 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 (for example, magnetic resonance imaging). These particles enhance the resolution and sensitivity dramatically while enabling earlier diagnosis of disease.[75,128] As a consequence, cheaper clinical measures can also be applied in therapy. Functionalized nanoparticles exhibit vectorial character (see Section 3). They can specifically identify complementary groups on cell surfaces that are indicative of 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 by magnetic resonance (MR) techniques.[12]

A well-established application of cells labeled with superparamagnetic iron oxide (SPIO), or ultrasmall superparamagnetic iron oxide (USPIO), in combination with magnetic resonance imaging (MRI) is the tracking of immune cells (monocytes/macrophages) during the development of an inflammation. This method is used for the diagnosis of, for example, cardiovascular diseases or multiple sclerosis. Additionally, these iron oxide particles can pass through the blood–brain barrier by using macrophages as carriers, which offers the possibility for the investigation of, for example, neurodegenerative brain diseases.[129–133] MRI with nanoparticle tracers can also be applied to the detection of apoptosis, angiogenesis, and tissue infiltration during the development of cancer. Other applications of targeted imaging use SPIO particles for stem-cell tracking, multimodal perfluorocarbon nanoparticles for visualization of angiogenesis, liposomes for targeting atheroma components, and microbubbles for imaging transplant rejection.[134–138]

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In elaborate systems, diagnostic particles have to display different specific properties and functions, such as magnetic behavior, stimulated optical emission, and targeted binding (see Section 3). However, multiple functionalities embedded into a single system could inhibit each other, thereby 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, nanoparticles offer a better surface-to-volume ratio, and consequently smaller particles present more of their reactive sites at the surface. Quantum dots belong to this class of system.[139]

Targeted imaging techniques are currently developed by close collaboration between physicists, medical specialists, biochemists, and chemists as well as engineers. This approach will also benefit the development of 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 that give ever finer details of tissues in vivo. For example, bioactive radiotracer molecules are required to visualize organs by PET. The application of [$^{18}$F]fluorodeoxyglucose (18F-FDG) for the detection of different types of cancer is well established in this field.[142–145] The tracer must be appropriately chosen for the relevant application, for example, for 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.[146] The challenge for nanotechnology is to develop tracers for new applications, for example, for the in vivo detection of gene expression.

Although the 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 range of application and resolution power. For example, $Au_3Cu$ hollow nanoclusters with an average diameter of $(48.9 \pm 19.1)$ nm and a shell thickness of $(5.8 \pm 1.8)$ nm have been developed.[147] These bimetallic agents enhance the contrast of blood vessels and offer great potential for use as intravascular contrast agents in MR angiography. Colloidal magnetic nanoparticles represent another group of agents for the visualization of organs by magnetic resonance. They combine a small size with strong magnetism, have a high biocompatibility, and can bind to the desired receptors through an active functional group. When coupled to cancer-targeting antibodies, nanocrystals show huge advantages for monitoring in vivo targeting events in 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 of different sizes exist, which target different organs.[148,149] Winter et al. characterized an iodinated oil nanoparticle for imaging atherosclerotic plaques by CT.[150] With a size of about 160 nm, the nanoparticles used in these experiments are not within the limitations of the strict definition of "nano" (up to 100 nm), but this was one of the first studies to describe specific targeted nanometer-scale agents for CT.

## 3. Nanotechnology in Therapy—Research and Development

One advantage of nanovectors—nanoparticles 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. The nanovectors currently used and investigated can be classified into three main groups or "generations" (Figure 11).[151]

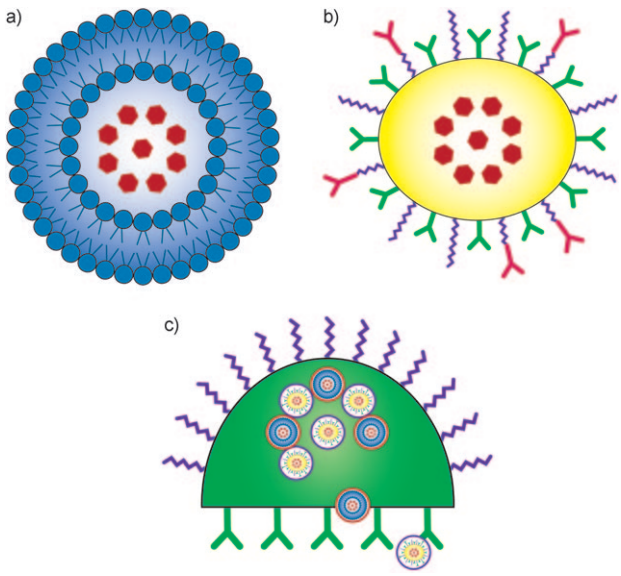


*Figure 11.* a) First-generation nanovectors (for example, currently clinical used liposomes) comprise a container and an active principle. They localize in the tumor by enhanced permeation and retention (EPR), or through the enhanced permeability of the tumor neovasculature. b) Second-generation nanovectors possess the ability to target their therapeutic action through antibodies and other biomolecules, remote activation, or responsiveness to the environment. c) Third-generation nanovectors (such as multistage agents) are capable of more complex functions, such as time-controlled deployment of multiple waves of active nanoparticles across different biological barriers and different subcellular targets. Reprinted with permission from Macmillan Publishers Ltd.[58]

The first generation (Figure 11 a) comprises a passive delivery system that localizes into the target site. In the case of a tumor as a target tissue and liposomes as the nanovectors, the mechanism of action leads to an enhanced permeation and retention (EPR) effect, which drives the system to home in on the tumor through the fenestrations in the adjacent neovasculature.[152] These systems are generally decorated on their surface by a "stealth" layer (for example, polyethylene glycol, PEG) which prevents their uptake by phagocytic blood cells, thus substantially prolonging their circulation time.[153–155] The most well-known representatives of this generation in clinical use are liposomes. Other systems in this category include metal nanoparticles, for use in diagnostics, and albumin–paclitaxel nanoparticles, which were approved in early 2005 for use in metastatic breast


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cancer.[156] The localization in this case is driven only by the size of the particles and is not related to specific recognition of the tumor or neovascular targets.

The second generation of nanovectors (Figure 11 b) are defined as having specific additional functionalities which allow for molecular recognition of the 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-functionalized liposomes and nanoparticles.[157–159] Various targeting moieties besides antibodies are under investigation worldwide. These include ligands, aptamers, and small peptides that bind 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, for example, pH-sensitive polymers or those activated by enzymes specific to the disease site, as well as a diverse group of externally activated vectors. Among the interesting examples are gold nanoshells activated by near-infrared (NIR) light, and iron oxide nanoparticles triggered by switching magnetic fields.[163,164] Other techniques used to remotely activate the second generation vectors include ultrasound and radiofrequency stimulus.[58,165–167] Linking nanoshells to antibodies that recognize cancer cells enables these systems to seek out their cancerous targets prior to applying NIR light or heating them up. For example, 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 prostate cancer cells in a mouse model.[168,169] Another new approach is based on the coupling of nanoparticles to small interfering RNA (siRMA), which can silence specific genes responsible for malignancies. By using targeted nanoparticles, it was shown that siRNA can slow down the growth of tumors in mice without eliciting the side effects often associated with cancer therapies.

Although the representatives of the second generation have not yet been approved by the American Food and Drug Administration (FDA), there are numerous ongoing clinical trials involving targeted nanovectors, particularly in cancer applications.

As described earlier, the easy access of drug-delivery nanovectors to cells and tissue provides tremendous potential advantage in medicine.[58,151] Following the brief introduction of the wide variety of the first two generations of nanovectors, we will focus here on the barriers which the drug or vector encounter when introduced into the body. Such barriers significantly reduce the probability of reaching the target tissues at a concentration required for obtaining therapeutic efficacy. The construction of nanoparticles of the third generation are aimed to successfully negotiate these barriers (Figure 11 c). When the whole picture is considered, it becomes clear that the molecular recognition between the vector and the affected or target tissue plays only a small role in the overall myriad of biological barriers that the vector need to bypass to efficiently deliver the drug to the target site. This observation is supported by reports that only a small portion of a targeted moiety (for example, an antibody) administrated systemically reaches the targeted tissue, which does not reflect its in vitro specificity.[170] The plethora of obstacles which the agent encounters on its way to the target tissue includes metabolic clearance and chemical instability of the drug, endoepithelial barriers, osmotic pressure gradients within the affected tissue, and hemodynamical aspects of particle margination.[151,171] Mathematical modeling studies applied recently to nanoparticulate objects in the blood stream demonstrated that a spherical shape of about 50–100 nm in diameter is the worst from a margination point of view 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 wall. This characteristic is important to allow the vector to drift in proximity to the blood vessel walls—possibly within the cell-free layer—thus enabling firmer attachment to the vascular endothelium. From the hemodynamic forces acting on the particles, spheres of 50–100 nm diameter tend to stay in the center of the blood vessel, without proper margination toward the vessel walls, where the molecular targets can be recognized. It is important to emphasize here that the majority of nanovectors in clinical use and biomedical research possess the least favorable geometric form. Thus, the use of vectors with multiple functionalities to overcome the various biological barriers could highly improve the therapeutic efficacy of drugs.

As mentioned previously in this Section, 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 and by the type of application. Recent advances in nanofabrication technology open up new avenues for the development of alternative geometries for injectable vectors.[175] The carrying and delivery of a sufficiently large amount of various agents for therapy, imaging, thermal ablation, remote guidance, and possibly other functions can only be achieved with a sufficiently large particle. In theory, the ideal nanovector will be capable of circulating in the vascular system following intravenous administration, reaching the required target tissues at high concentrations, and treating the disease site, while not having any adverse effects. This goal will only be reached by a "multistage" approach, and 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. This first stage carrier can be loaded with second-stage nanocarriers (for example, quantum dots, carbon nanotubes, iron oxide particles, nanoliposomes). Moreover, the dimensions and the hemispherical geometry of the system were rationally designed on the basis of mathematical modeling studies on particle margination in the blood.[171,178,179] The basic principle of the system involves the first stage microparticles targeting 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 released to facilitate the delivery of active agent into the affected cells so as to provide an enhanced therapeutic effect (Figure 12).

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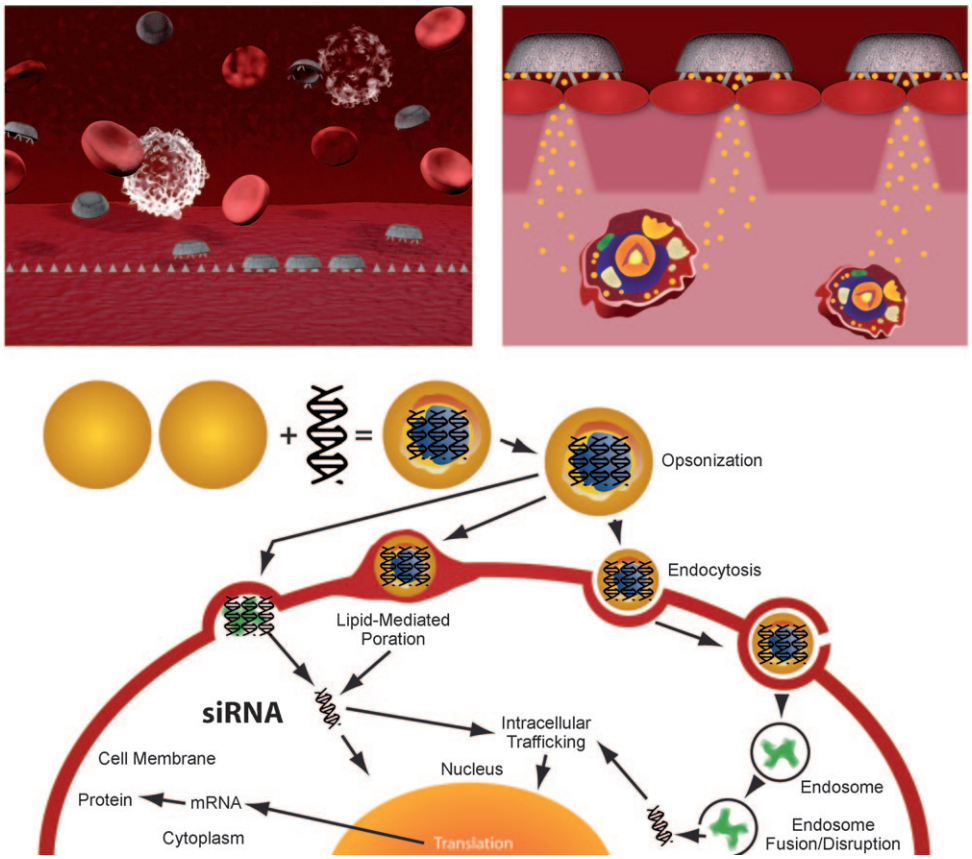


***Figure 12.*** Mechanism of action of multistage (3rd generation) nanovectors. Top left: Rationally designed stage one nanoparticles marginate to the vessel wall and adhere to the endothelium. Top right: Stage one nanoparticles release a reagent to break down tight junctions and the basement membrane. Finally, stage two particles—in this case, liposomes—will be released. Bottom: the stage two liposomes interact with the target cell membrane, and then deliver the intended payload—in this case, siRNA. Reprinted with permission from Ref. [151].

Exciting applications of nanotechnology have also been reported in 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 as a result of disease and trauma. This includes skin substitution after burn injuries, the reversal of paralysis or blindness through spinal cord or retina regeneration, heart regeneration after infarcts, and minimization of stroke dysfunction through neuron repair. The nanomaterials support the reconstitution of healthy tissues. Results obtained by Stupp and co-workers indicate that the regeneration within the central nervous system can be reached by applying self-organized nanofibers. An amphiphilic peptide (IKVAV) which self-assembles into a nanonetwork and recognizes $\alpha_3\beta_1$ integrin was used for this purpose (Figure 13). The induced signaling appears to stimulate the axons to grow longer 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

As described in Section 3, nanomedicine has entered many different fields including tissue engineering and targeted drug delivery. Clinical application is fairly broad, but mainly focuses on cancer. Known to be a cause of the development of diseases such as cancer, arthrosclerosis, and age-related illnesses, chronic inflammation takes a central position in clinical investigations. The mechanisms of this correlation have been 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 chronic inflammation makes it an expanding area of research for nanomedicine.[183–187]

Therapies for chronic inflammation address cell-mediated or humoral immunity by blocking mediators such as interleukines (IL) or targeting receptors (for an overview of immunological mechanisms see Refs. [188–197]). The classical treatment of chronic inflammation is based on drugs such as glucocorticoids, cyclosporine A, sulfasalazine/5-aminosalicylic acid (5-AZA), or calcinneurin inhibitors. Immunotherapies by means of antibodies, such as anti-CD20 or anti-CTLA4, specific for certain cells are also used. These commonly used therapies specifically or unspecifically suppress the cellular or humoral immune response, thus 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 are important regulators of IL-2 and activators of T-helper cells, and are thus an alternative to unspecific immunosuppressants. However, potentially severe side effects such as infection and sepsis were also reported following systemic application of calcineurin inhibitors. Furthermore, administration routes are often problematic and inefficient (for example, 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 classical cancer treatment. Therefore, successful efforts have been made in this field to develop targeted drug delivery and diagnostic approaches,

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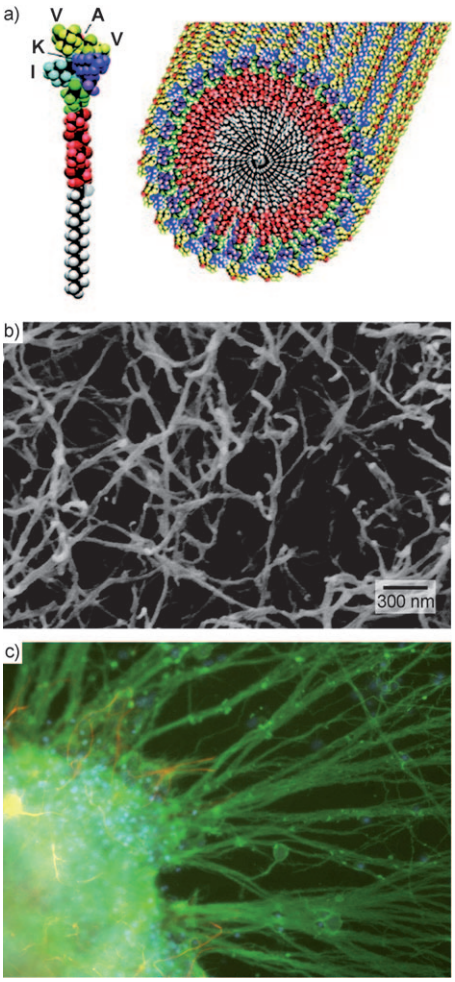


*Figure 13.* Functionalized nanoparticles for nerve regeneration: a) Schematic representation of an IKVAV-containing amphiphilic peptide; b) SEM image of a self-assembled network of IKVAV amphiphiles; c) supported by a nanofiber network, progenitor cells differentiated to functioning neurons instead of the scar-forming astrocyte. Reprinted with permission from the AAAS.[182]

and bring them to clinical application. Nanosized drug-delivery systems for the treatment of chronic inflammation can improve on the already existing application of the drug 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]

Nanoscale drug- or gene-delivery systems are supra- and supermolecular aggregates of simple components with various sizes, shapes, and composition. These characteristics hold true for the majority of the nanoscale particles applied in nanomedicine. In general, the carrier is characterized by certain parameters such as a high drug or gene loading capacity, or are superparamagnetic as in the case of iron oxide nanoparticles. Independently of the composition, nanovectors are usually further modified depending on their individual application, such as surface decoration with polyethylene glycol (PEG) for intravenous injection to prevent early clearance and to increase blood circulation time.[58,200]

### 4.1. Lipid Vehicles

Liposomes are the most clinically established nanometer-scale systems used for drug delivery. Biocompatibility, biodegradability, and flexibility of size and surface manipulations are the important features that liposomes offer compared to other nanoparticulate delivery systems. Liposomal nanotherapeutics for cancer treatment have been 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 examples of available drugs that have higher efficacy and lower toxicity than nonliposomal preparations are: liposomal amphotericin B (brand names: AmBisome, Amphotec, Abelcet), stealth liposomal doxorubicin (brand names: Doxol/Caelyx), liposomal daunorubicine (brand names: DaunoXomo), and liposomal cytosine β-arabinoside (brand name: DepoCyt). These are just some representative examples to demonstrate the great impact of nanomedicine in current therapies.[203] An enormous number of diverse synthetic, semisynthetic, and natural polymers are now available, particularly 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 are 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 of hydrophobic compounds. These systems are preferably 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.

#### 4.1.1. Liposomal Drug Carriers in Chemotherapy
##### 4.1.1.1. Doxorubicin

Doxorubicin is an anticancer drug that is widely used for the treatment of different types of tumors such as breast cancer, Kaposi sarkoma, and ovarian cancer. Doxorubicin is a highly toxic compound affecting not only tumor tissue but also heart and kidney, a fact that limits its therapeutic applications. Therefore, intense research was done to establish a more compatible formulation of doxorubicin. The development of doxorubicin enclosed in liposomes culminated in an approved nanomedical drug-delivery system.[204,205] Liposomal formulation result 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 because of the EPR effect, that is, the characteristic hyperpermeability of tumor tissue which results in a selective delivery of the drug to the tumors.[208,209] The cutoff size of the blood–tumor barrier depends on the location of the tumor and the modulation of

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the microenvironment, but is usually between 300 and 800 nm, which corresponds to the size of liposomal carriers.[210] Particles larger than 200 nm activate, however, the complement system and provoke clearance by phagocytosis. Fast clearances of nanomaterials by phagocyte activity prevent long circulation of the carrier and thus inhibit the long-term controlled release of the load. The circulation behavior of liposomes was improved by modification of the liposomal surface with PEG.[198] PEG reduces the clearance of the liposome by phagocytes in the liver and spleen considerably, since opsonization 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, thereby 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]

More recent studies revealed an increased clearance rate of PEGylated liposomal carrier upon multiple injections.[214–216] Here, clearance is presumed to be mainly governed by liver and spleen macrophages and dependent on a soluble heat-labile serum factor (or factors) that primes the so-called enhanced clearance effect. The enhanced clearance effect diminishes with time and seems to be related to the life time of the macrophages that come directly in contact with the injected liposomes.[214,217] Therefore, the injection intervals of such liposomes should be adapted to the life time of the macrophages.

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 envisaged to reduce the toxicity and increase the effectiveness of the drug (second and third generations of nanovectors).

In contrast to an indirect targeting governed by the EPR phenomena, an improved tumor-specific drug delivery can be 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 toxicity to the tumor cells, and a reduced clearance rate since the delivery to the kidney and spleen is reduced. For example, anti-2C5 monoclonal antibodies were coupled to a liposomal surface so as to transfer the encased doxorubicin to brain tumors. This antibody was shown to bind specifically to human astrocytoma cell surfaces in vivo.[218] The antibody is directed against nucleosomes localized on living tumor cell surfaces originating from apoptotic neighboring tumor cells.[219] Another approach to treat human brain tumors in vivo is the application of sulfatide-containing liposomes (SCL), which bind to certain glycoproteins upregulated in tumor cells. Anti-CD19-labeling of liposomes was shown to improve the targeting to murine B-cell lymphoma cells and the intracellular release of liposomal doxorubicin.[220] These examples show that vectorial, that is, site-directed, drug transport and release will revolutionize the therapy of brain tumors, since the present therapy is of limited success due to insufficient drug delivery. However, the 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 the specificity of the drug transport. A recent study reported on thermosensitive liposomes that release doxorubicin when heated. Specific release of the antitumor drug was achieved by selective heating of the targeted tumor. Hyperthermia was induced in this case by heated water delivered in vivo through small catheters.[222]

#### 4.1.1.2. AmBisome/Amphotericin B

AmBisome is a liposomal formulation of an antifungal agent amphotericin B, which is recommended 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 is composed of very rigid, small unilamellar liposomes with a mean diameter of under 100 nm, with amphotericin B intercalated within the membrane. Such liposomes are known to have long circulation times and accumulate in the required areas. In preclinical and clinical studies, AmBisome showed less toxicity and fewer side effects than amphotericin B, but retained the full spectrum of antifungal activity.[223] Therefore, in contrast to classical amphotericin B therapies, it can be used in patients suffering from kidney damage. It was shown in animal experiments 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. In summary, liposomal amphotericin B accumulates at the infection sites, shows higher stability, and fewer side effects and toxicity than the free drug. The sustained release of amphotericin B from AmBisome may also serve as a prophylaxis, as shown in mice challenged with *Histoplasma capsulatum*.[224]

### 4.2. Polymer-Based Delivery

Natural polymers such as proteins or polysaccharides tend to be internalized and degraded rapidly, thus enabling a moderate intracellular release of the drug or gene.[225] Blood circulation time or clearance is controlled by surface modification or the formation of polymer conjugates 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 resistant 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). Cremophor was reported to cause allergic reactions, thus limiting the drug dosing.[229–231] Abraxane demonstrates a significantly higher response rate, a longer time to tumor progression, and absence of hypersensitivity reactions.[227] However, a severe side effect was recently reported, which demonstrates the ongoing debate on the

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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 research group of Duncan. As in the case of the 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 research group of Vicent reported on the coupling of apoptosis-inducing anticancer agents to nanoparticles for the enhancement of efficacy.[233,234]

Figure 14 shows the possibilities of coupling drugs to polymers. This also demonstrates that the generation and optimization of nanovectors forms an important interdisciplinary area between chemistry, biochemistry, and medicine.

The coupling of proteins and drugs to synthetic polymers, especially PEG, increases their plasma residence, reduces protein immunogenicity, and can increase their therapeutic range. 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]

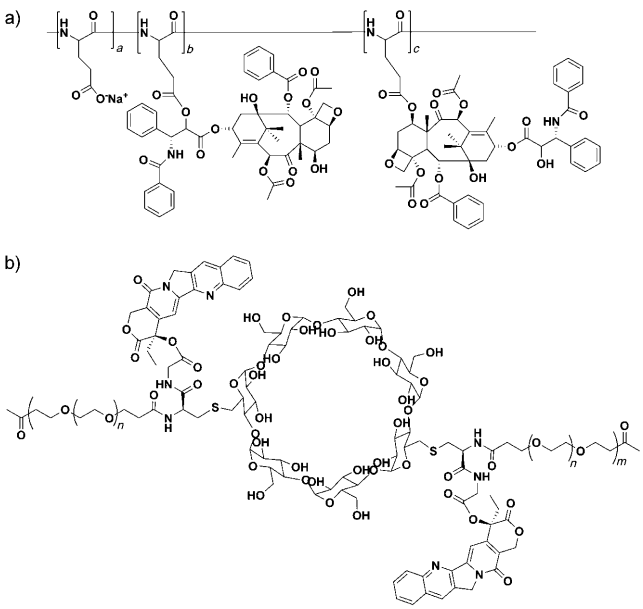


*Figure 14.* Polymer conjugates of anticancer drugs: a) Paclitaxel (PTX) is linked to the carrier polyglutamate (PGA) through an ester bond. The main drug release occurs after polymer degradation by the lysosomal enzyme cathepsin B; b) conjugate of camptothecin (CPT) and a linear β-cyclodextrin-based PEG polymer (CDP). Pharmacokinetic and preclinical studies have demonstrated that this conjugate exhibits a longer plasma half-life and better distribution to the tumor tissue than CPT alone. Reprinted with permission from Ref. [234].

### 4.3. Metal Nanoparticles

#### 4.3.1. Nanocrystalline Silver for Wound Care

Silver, mostly in the form of nitrate or sulfadiazine salts, is a well-studied antimicrobial agent and a common compound for wound treatment.[235–237] Wound healing can be subdivided into distinct phases.[238] Soon after injury and coagulation, wound healing is characterized by cell invasion by leucocytes which causes inflammation. This inflammation phase cleans the wound and a microbial infection is prevented. Wound healing proceeds, after the inflammation has ceased, by tissue remodeling and maturation of the new tissue. Latent microbial infection could cause the inflammatory phase to be prolonged, thereby causing chronic nonhealing wounds. Medical treatment of chronic wounds with dressings containing silver salts significantly reduce the bacterial load of the wound and allow complete healing.[239] Silver is a promising alternative to antibiotics since multiresistance against antibiotics develops progressively. A recent study does not indicate an increased development of resistance upon silver usage.[240–242] In comparison to antibiotics, which potentially affect wound tissue, the toxicity of silver is not specific, although the antimicrobially active doses of silver are low (nM–μM range) and is commonly tolerated well.[242] The advantage of nanocrystalline silver over silver salts is not only due to an increased antimicrobial activity but also due to its antiinflammatory properties.[243] However, the mechanism of action remains to be elucidated. The application of nanocrystalline silver during wound management demonstrates the entrance of nano-biotechnology to medical therapy. These types of dressings are currently applied in cases of first- and second-degree burns and in several types of chronic nonhealing wounds.

#### 4.3.2. Magnetic Nanoparticles for Diagnosis and Therapy

Iron or iron oxide nanoparticles have a great potential for various nano-biomedical applications including drug delivery. The nanoparticles can be coated with hydrophilic polymers such as PEG or dextrane to prevent or to increase the cellular clearance of the particle, respectively.[244] Cell-specific transport is also possible by coating with antibodies, receptor-specific peptides, or aminosilane. In addition to these more general properties of nanocarrier systems, the superparamagnetic character of the particles and their dimensions of 2–20 nm pave the way for applications other than drug delivery.

Current research and applications of iron-containing nanoparticles mainly involve in vitro cell labeling and cell separation, in vivo drug delivery, magnetic resonance imaging (MRI; see Section 2), and hyperthermia.[245] The most popular application of iron nanoparticles in medicine is hyperthermia, which is the destruction of tumors by locally over-heating the tissue. Hyperthermia is an effective and specific anticancer treatment, since an increased temperature of the treated tissue up to 44 °C is less well tolerated by cancer cells than by healthy cells. This approach is usually applied in combination with other traditional therapies such as chemotherapy. Hyperthermia treatment by iron oxide nanoparticles is induced by exposure of the particles to an alternating magnetic field.[203,246] A local accumulation of nanoparticles allows for tissue-specific hyperthermia that preferentially addresses the tumor tissue (Figure 15).

The benefits over classical cancer therapies are minimal invasiveness, accessibility of hidden tumors, and very few side effects. Conventional heating of a tissue (microwaves, laser

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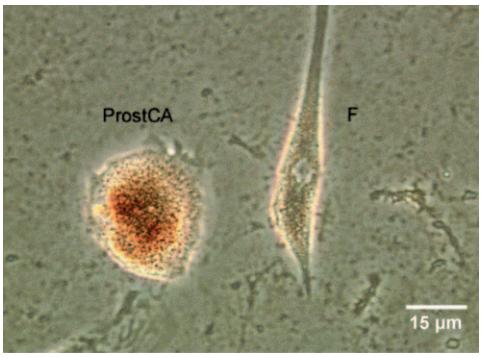


*Figure 15.* Comparison of a healthy and a tumor cell incubated with nanoparticles. The phase-contrast light microscopy image shows a prostate carcinoma cell and a fibroblast cell. While the tumor cell (left) shows a high level of pigmentation because of the uptake of a large number of nanoparticles, the adjacent fibroblast cell shows lower levels of pigmentation, that is, no or lower levels of particle uptake. Reprinted with permission from Elsevier.[246]

light etc.) results in the healthy tissue surrounding the tumor also being destroyed. However, targeted paramagnetic particles provide a powerful tool for highly localized energy absorption and heating of mainly the cancerous cells. Several kinds of nanoparticles differing in material, composition, and size are available for this purpose; for example, hyperthermia can also be applied by using magnetite cationic liposomes (MCLs) as carrier systems combined with heat-shock proteins. However, the low-levels of side effects means the treatment is well accepted by patients.[203,247–249]

Superparamagnetic iron oxide particles have also been used for MRI studies.[245] Dextran-coated particles (brand names: Resovist, Feridex) are commercially available and have mainly been used for in vivo MRI of liver tumor tissue. The dextran coating increases the intracellular deposition of the particles into the cancer cells, thereby enabling diagnosis and monitoring of the progression of the tumor. Iron particles are cleared by the liver macrophages, and enter the reticuloendothelial system to join the physiological iron pool.

### 4.4. Nanoshells

In other hyperthermal concepts, gold–silica nanoshells consisting of a spherical dielectrical nanoparticle surrounded by an ultrathin conductive metal layer are used. The nanoparticles absorb light in the NIR region, thus guaranteeing that an optimal optical transmission through the tissue. A moderate exposure to extracorporeal near-infrared light (820 nm, 4 $W cm^{-2}$) resulted in a heating of the tumor tissue and irreversible tissue damage, as evident by coagulation, cell shrinkage, and loss of nuclear staining.[164] The big advantage of nanoshells is their tuneable plasmon resonance from the visible to the infrared regime by varying the composition and dimensions of the layers.

Nanoshells have not only been 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 sections. Some other noteworthy NIR imaging applications are confocal imaging, iridotomy, and photothermal coagulation, all of which take advantage of the increased transparency of the tissue within this region.[250]

### 4.5. Non-injectable Nanovectors

The most preferred way of introducing drugs into the body is orally; therefore, the pharmaceutical industry invests much effort in to the development of appropriate delivery systems that can be 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 (for example, Dalargin). Nanoparticles coated with polysorbate 80 and loaded with doxorubicin (5 $mg kg^{-1}$) achieved very high levels of 6 $\mu g g^{-1}$ of the drug in brain tissue, while all the controls, including uncoated nanoparticles and doxorubicin solutions mixed with polysorbate, were below the analytical detection limit.[251] Another newly designed delivery system is based on chitosan coupled to antibodies through a PEG linker. These immune nanoparticles have, on the one hand, the ability to interact with the negative charges of the brain endothelium through the cationic (with a full positive charge) chitosan, and, on the other hand, an affinity for the transferrin receptor by the monoclonal antibody OX26, which makes them perfectly designed to cross the blood–brain barrier. Nanospheres loaded with the peptide Z-DEVD-FMK, an inhibitor of caspase-3, were also 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, thereby reducing the risk of adverse reactions and the frequency of re-dosing, thus improving patient compliance. The benefits of nanotechnology in this regards are exemplified by biodegradable porous silicon (pSi) materials. These nanostructured materials store an active compound or second stage nanoparticles effectively in nanosized pockets that release minute amounts of drug as the silicon dissolves. pSi is currently being explored for tissue engineering and ophthalmic delivery.[253,254]

Nanotechnology also refines transdermal delivery, a safe and non-invasive method of administering drugs. The transport of large-molecular-weight proteins (such as vaccines) is relatively inefficient when the substance is applied directly

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onto bare skin. Recent evidence has shown that this barrier can be overcome by using properly structured nanoparticles.[255]

Finally, nanotechnology can also be used for the removal of toxins. 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

Knowledge about the nanostructuring of surfaces develops rapidly with the development of nanoparticles. The main research into the nanostructuring of surfaces focuses on the optimization of the interaction of prostheses, such as artificial joints, with the organism, with the aim of producing materials which have 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. There is a growing need for smart surfaces which show a high biocompatibility, particularly in the area of artificial organs and prosthetics.[258–263]

Another potential application of nanotechnology resides in the possibility of mimicking a variety of compound materials and self-organized systems found ubiquitously in nature. Complex structures such as 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, has made use of informed dynamic molecular systems and demonstrated that self-organized complex molecular systems are indeed extremely successful. When 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. The most popular naturally occurring example is probably the surface structure of the leaves of a lotus flower. The special structured surface, with a typical bimodal size distribution in the micrometer and sub-micrometer regime leads to self-cleaning behavior. 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 research group of Spatz. A different growth and attachment behavior of fibroblasts has been observed by altering the distance between functionalized gold particles attached to a surface (Figure 16).[264–266] In another approach Sun et al. used *N*-isobutyryl-L(D)-cysteine (NIBC) enantiomers to successfully alter the adsorption characteristics of surfaces.[267]

The understanding of these effects opens up ways for the generation of surfaces with nonfouling properties, and also of surfaces with optimized template structures for specific cell growth. For example, the nanostructuring of titanium alloy by hydroxyapatite coatings apparently results in enhanced mechanical properties and in promoting the proliferation of osteoblast cells.[268] This finding is interesting for the development of implant materials.

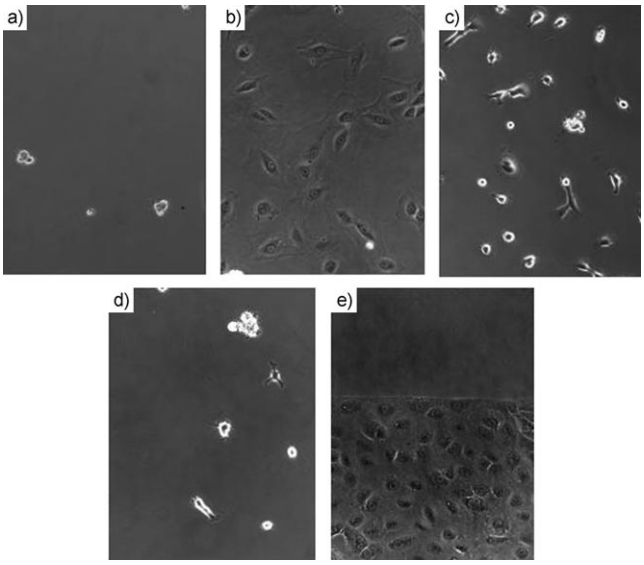


*Figure 16.* Phase-contrast optical micrographs of 3T3 fibroblasts on polyethylene glycol diacrylate (PEGDA) 700 hydrogels. a) Cells on a non-RGD-functionalized gold nanoparticle pattern. b–d) Cells on cyclo-(-RGDfK-)-functionalized gold particles after 24 h in culture; cyclo-(-RGDfK-) patches are separated by various distances b) 40 nm, c) 80 nm, and d) 100 nm. e) Dense cell layer on a PEG support after 14 days in culture. The bottom part of the sample was patterned with gold nanoparticles functionalized with the cyclo(-RGDfK-) peptide spaced 40 nm apart. Reprinted with permission from Ref. [266].

Changing the surface to a nanotubular structure resulted in artificial joints that were better incorporated without inducing chronic inflammation.[269,270] The tailored surfaces have the advantage of mimicking the surface of natural structures, not only by the coating of inorganic materials but also by adhering proteins or peptides to mimic natural conditions. The next step in 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 has already selected appropriate materials and processes. Besides prosthetics, this approach is most promising for neural applications.

Nanostructured surfaces with specific well-defined properties have also found applications in diagnostics. The study of cells often fails because of unfavorable interaction between the device and the 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.[271–273]

By using responsive molecular systems it is possible to switch between different states (such as superhydrophobicity and superhydrophilicity) by external stimuli (for example, electrical or optical fields, pH value) applied to the function-

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alized surfaces. These coatings are of interest for diagnostic purposes with miniaturized lab-on-a-chip systems or for the coating of artificial blood vessels and implants, thus mimicking biological systems.

## 6. Biocompatibility and Toxicity—Safety Issues Related to Nanotechnology Implementation

The generation of small particles may be a major issue with respect to toxicology. The potentially high reactivity arising from the large surface-to-volume ratio of nanoparticles compared to bulk systems means there is a latent risk for all new nanosystems, which must be carefully considered. While the existing laws for new chemicals and pharmaceutical materials seems to be currently sufficient for these types of materials, each new nanoparticle system has to be investigated carefully with respect to its potential side effects within the human body and the environment. Fortunately, the public and scientific awareness of nanotechnology is high and there is an increasing intensity of discussion on these ethical and societal issues. The clinical application of nanotechnology also requires 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 based on nanotechnology and nanomedicine also deserves attention from the political side. This not only includes in regard to the above-mentioned toxicological aspects but also to the question of the improvement in 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 includes 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.

If one considers nanoparticles as "medical devices", they could have a broad range of applications in the human body.[278,279] A drawback is their potential toxicity and their possible incompatibility, which may result in the generation of disorders such as inflammation, immunoreaction, or cancer. Mechanisms of those effects are not well-studied yet, but might be due to an enhanced hydrophobic interaction with biological material or an increased generation of free radicals by surface catalysis.[198] Recent experimental data have shown that inhalation of nanoparticles with a size below 100 nm from air pollution can lead to the induction of pulmonary inflammation. It has been demonstrated that in this case the individual expression of glutathione *S*-transferase (GST) determines the extent of the inflammation. Interestingly, the physiological task of GST is the detoxification of reactive oxygen species, thus indicating the generation of reactive oxygen species on the particle surfaces.[280,281] Comparable data were obtained in relation to the inhalation of single-walled carbon nanotubes (SWCNs). SWCNs have been shown to elicit inflammation in the lungs of mice, while they cause small, focal interstital fibrotic lesions in rat alveolars.[282,283]

The discussion on the risk of carbon nanotubes recently received new input from Poland et al. They reported that this kind of nanoparticle acts, according to their needle-like structure, in the same way as asbestos, thus indicating the same risk for handlers.[284] In other investigations, highly purified carbon nanotubes seemed not to possess short-term toxicity, and can be considered biocompatible with cardiomyocytes in culture. The long-term negative effects that were evidenced were suggested to be due to physical rather than chemical interactions. This effect was investigated by the research group of Krug, who demonstrated that these nanoparticles induce no acute cytotoxicity or inflammatory markers such as nitric oxide or interleucine-8. The observed side effects were associated with metal traces in the commercially available nanotubes.[285] The 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 and cells.[286]

Nanoparticles that enter organisms and are not excreted accumulate in the cells and tissues, thus developing a still unknown potential of causing diseases at these sites over the long term. It has been shown that nanomaterials can enter the human body by several means. 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, it has been shown that nanoparticles can act as gene vectors.[288] Carbon black nanoparticles have been implicated in interfering with cell signaling.[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 in cells is also well documented in the cases of pulmonary fibrosis caused by asbestos fibers (asbestosis) and silicosis, a disease that comes from breathing in silica or quartz dust.[292–294]

It has since been shown that a (high) concentration of nanoparticles may result in the transformation of cells into the tumorous state, thus causing cancer. Investigations on hepatic and renal tissues affected by cryptogenic granulomatosis by scanning electron microscopy (SEM) and X-ray microanalysis with an energy-dispersive (EDS) detector showed a correlation between the presence of inert, non-biodegradable, exogenous micro- and nanoparticles and diseases that traditional histopathology could not account for (Figure 17).[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. In addition, the migration and dissemination of debris in other parts of the body, far

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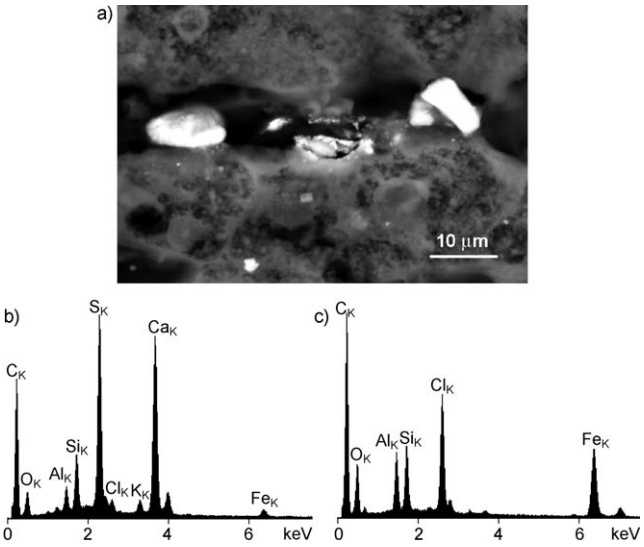


***Figure 17.*** a) SEM image of a section of a granulomatous liver showing two small particles and a cluster of nanodebris. b,c) EDS spectra showing that the debris has different compositions, and probably different origins. Reprinted with permission from Elsevier.[295]

from their origin, has been documented, which has the possibility of causing further pathologies. No efficient gastrointestinal barrier for inert particles with a diameter below 20 μm is so far known. On more than one occasion, the source of such minute foreign bodies was identified as dental materials such as porcelain or over-worn alloys such as gold/ruthenium. The migration of barium sulfate particles, a very common contrast medium used in gastroendoscopy, into liver tissue (cells) 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 gastrointestinal tract, or to the endothelial barrier. The contact may result in the uptake of nanoparticles through endocytosis (mediated by receptors), membrane penetration in the case of hydrophobic particles, or by transmembrane channels in the case of very small nanoparticles ($<5$ nm).[297] A strategy to prevent cellular internalization and, therefore, uncontrolled cytotoxicity of nanoparticles with sizes below 100 nm is by surface modification with hydrophilic polymers.[244]

In an aqueous environment, different types of biomolecules as well chemicals such as pesticides adsorb to nanoparticles. Adsorbed molecules dictate biological interactions, especially bioavailability, and the activation of cells. An example of the interaction of nanoparticles with biomolecules is the binding of $C_{60}$ fullerenes to antibodies. Recent reports attribute the cytotoxic effect of $C_{60}$ fullerene to lipid peroxidation. In organisms, all extracellular proteins such as complement proteins or antibodies can adsorb onto nanoparticles. The proteins possibly change their conformation, and as a consequence their reactivity, during the adsorption, thereby resulting in an autoimmune response.[298,299]

To investigate the potential risks of nanotechnology, tools and methods have been developed and adapted to perform high-throughput and standardized testing of the interaction between nanoparticles and, for example, biological barriers. An established method of proving the integrity of biological barriers is the measurement of the transepithelial electrical resistance (TER). This method was adopted for determining the toxicity of nanoparticles and was developed for routine application.[300–303] The initial results show, for example, no initial effect of silica-based nanoparticles on Madin Darby canine kidney (MDCK) cells, but a decrease of the TER after 150 h was observed, thus indicating disrupture of the cell monolayer.[304] Theses studies show the importance of long-term studies in the investigation of the toxicity of nanoparticles. The studies will be extended over a broader spectrum of nanoparticles, which will help to understand the interactions between nanoparticles and biological systems in more detail.

These few examples demonstrate that the effects of nanotechnology on human health could be double edged, similar to conventional drug exposure, but possibly based on completely different mechanisms. Many of the investigated systems, so far seem to exhibit relatively few short-term 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 potential hazards as low 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, simple approaches, and systematic development.

In this Review we have provided an overview on some fascinating developments in the area of nanomedical research and applications. Since the field is currently expanding at a very fast pace, we could not include all aspects of present nanomedicine in detail. Our aim was mainly to demonstrate the highly transdisciplinary character of nanomedicine 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 made an important impact on clinical applications, which are expected to grow exponentially during the next few years.

Nanomedicine relies on:

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

The main focus of clinical nanomedical applications is currently on the treatment and efficient diagnosis of cancer. To efficiently detect malignancies, for example, molecular

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changes to the cells must be detected as early as possible. This means that extremely sensitive techniques have to be used. Nanotechnological concepts have, for example, the potential to address single cells and so meet this challenge. The knowledge currently being gained in this field will provide the basis for establishing personalized medicine.

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