

Nanomedicine: Application of Nanobiotechnology in Medical Practice

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Key Words

Nanoparticles · Nanobiotechnology · Nanodiagnostics · Nanomedicine · Nanosurgery · Nanooncology · Nanopharmaceuticals

Abstract

Nanomedicine is the application of nanobiotechnologies to medicine. This article starts with the basics of nanobiotechnology, followed by its applications in molecular diagnostics, nanodiagnostics, and improvements in the discovery, design and delivery of drugs, including nanopharmaceuticals. It will improve biological therapies such as vaccination, cell therapy and gene therapy. Nanobiotechnology forms the basis of many new devices being developed for medicine and surgery such as nanorobots. It has applications in practically every branch of medicine and examples are presented of those concerning cancer (nanooncology), neurological disorders (nanoneurology), cardiovascular disorders (nanocardiology), diseases of bones and joints (nanoorthopedics), diseases of the eye (nanophthalmology), and infectious diseases. Safety issues of in vivo use of nanomaterials are also discussed. Nanobiotechnology will facilitate the integration of diagnostics with therapeutics and facilitate the development of personalized medicine, i.e. prescription of specific therapeutics best suited for an individual. Many of the developments have already started and within a decade a definite impact will be felt in the practice of medicine.

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Introduction

Nanotechnology (Greek word *nano* means ‘dwarf’) is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-length scale, i.e., at the level of atoms, molecules, and supramolecular structures. It is the popular term for the construction and utilization of functional structures with at least one characteristic dimension measured in nanometer scale – a nanometer (nm) is one billionth of a meter (10^{-9} m). This is roughly four times the diameter of an individual atom. Width of DNA is approximately 2.5 nm and protein molecules measure 1–20 nm. Given the inherent nanoscale functional components of living cells, it was inevitable that nanotechnology would be applied in biotechnology, giving rise to the term nanobiotechnology. Nanomedicine is the application of nanobiotechnologies to medicine as shown in table 1.

Nanobiotechnology is already starting to have an impact on healthcare. During the past 50 years, early concepts of nanotechnology have developed into numerous technologies and a few medicines based on nanotechnology are on the market. Numerous applications in the pharmaceutical industry such as drug discovery and drug delivery can be covered under the term ‘nanopharmaceuticals’. The invention of the microscope revolutionized medicine by enabling the detection of microorganisms and study of the histopathology of disease. Microsurgery was a considerable refinement over crude

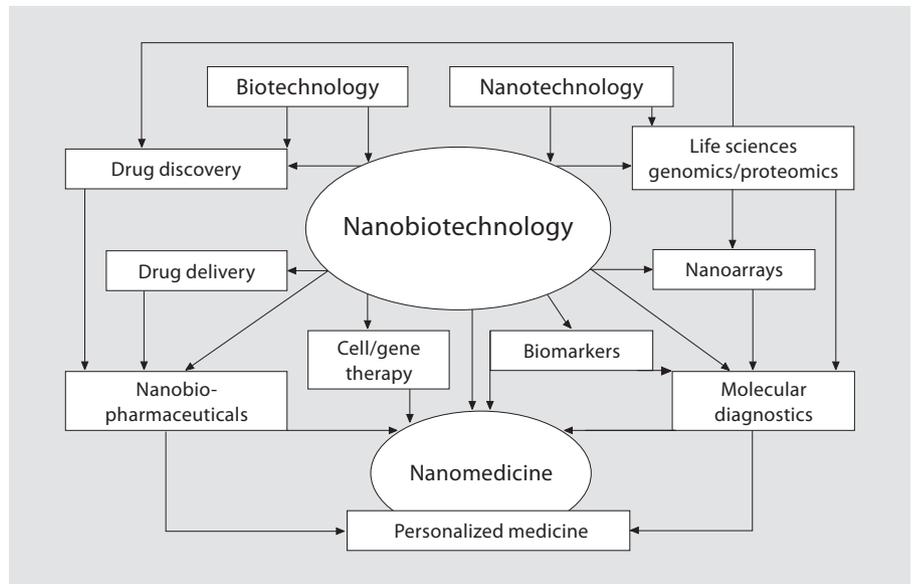


Fig. 1. Relationship of nanobiotechnology to nanomedicine and other biotechnologies.

macrosurgery and opened the possibilities of procedures that were either not carried out previously or had high mortality and morbidity. Nanotechnologies, by opening up the world beyond the microscale, will have a similar impact on medicine and surgery. This is because physiological and pathological processes at cell level occur on a nanoscale. Nanomedicine can also be considered a refinement of molecular medicine and integrates advances in genomics and proteomics to facilitate the development of personalized medicine. The relationship of nanobiotechnology to nanomedicine and other technologies is depicted graphically in figure 1. This schematic drawing shows how nanobiotechnology will have an impact on the development of nanomedicine both directly as well as by improving other disciplines such as the delivery of nanopharmaceuticals and molecular diagnostics. The same technologies facilitate the development of personalized medicine in parallel with nanomedicine.

Important applications for molecular diagnostics and drug delivery will be described briefly before discussion of the role of nanobiotechnology in various diseases.

Nanomolecular Diagnostics

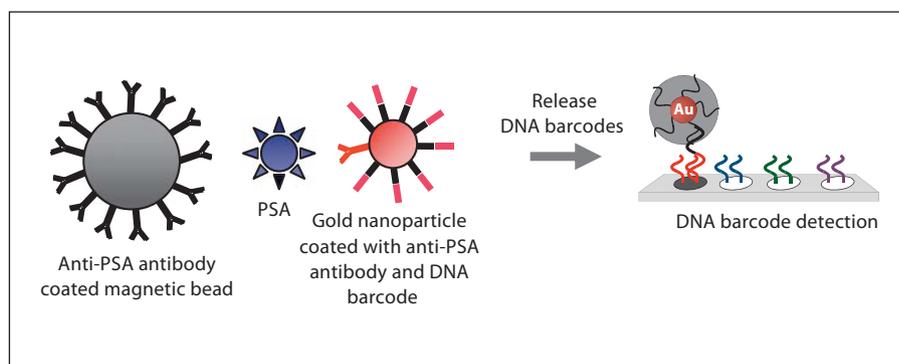
Nanomolecular diagnostics is the use of nanobiotechnology in molecular diagnostics and can be termed 'nanodiagnostics', the development of which has been covered in several publications [1, 2]. Because of the small dimension, most of these applications fall under the

Table 1. Nanomedicine in the 21st century

Nanodiagnostics
Molecular diagnostics
Imaging with nanoparticle contrast materials
Nanobiosensors
Nanopharmaceuticals
Nanotechnology-based drugs
Targeted drug delivery to site of action
Drug delivery from implanted nanopumps and nanocoated stents
Reconstructive surgery
Tissue engineering with nanobiotechnology scaffolds
Implantation of rejection-resistant artificial tissues and organs
Nanorobotics
Vascular surgery by nanorobots introduced into the vascular system
Remote controlled nanorobots for detection and destruction of cancer
Nanosurgery
Nanosensors implanted in catheters to provide real-time data during surgery
Nanolaser surgery

broad category of biochips/microarrays but are more correctly termed nanochips and nanoarrays. Nanotechnology-on-a-chip is a new paradigm for total chemical analysis systems [3]. Protein nanobiochips in development can detect traces of proteins in biological fluids that are not detected by conventional immunoassays.

Fig. 2. Use of Biobarcode assay for detection of PSA (courtesy of Nanosphere Inc.). Note: Anti-antibodies coated onto magnetic beads capture target protein PSA. Gold nanoparticle probes, coated with anti-PSA antibody and DNA barcodes, then bind immobilized PSA. The resulting complexes are separated and washed to remove excess probe. The DNA barcodes are then released from complex and detected via hybridization to a surface immobilized DNA probe and an oligonucleotide functionalized gold nanoparticle.



Nanobiosensors based on nanobiotechnology are portable and sensitive detectors of chemical and biological agents, which are useful for point-of-care testing of patients. The sensors can be electronically gated to respond to the binding of a single molecule of a nucleic acid or proteins. It may be possible to develop implantable detection and monitoring devices based on these detectors.

Nanoparticle-Based Diagnostics

Various nanoparticles are used for diagnostics. Small pieces of DNA can be attached to gold particles no larger than 13 nm in diameter. The gold nanoparticles assemble onto a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used, the technique can detect millions of different DNA sequences simultaneously. Quantum dots (QDs) are inorganic fluorophores that offer significant advantages over conventionally used fluorescent markers. The most important potential applications of QDs are for cancer diagnosis. Luminescent and stable QD bioconjugates enable visualization of cancer cells in living animals and enable integration of diagnostics with therapeutics. Another application of QDs is for viral diagnosis. Rapid and sensitive diagnosis of respiratory syncytial virus (RSV) is important for infection control and efforts to develop antiviral drugs. Current RSV detection methods are limited by sensitivity and/or time required for detection, which can take 2–6 days. This can delay effective treatment. Antibody-conjugated nanoparticles rapidly and sensitively detect RSV and estimate relative levels of surface protein expression [4].

Scientists at Nanosphere Inc. have developed Biobarcode assay – an ultrasensitive method for detecting protein analytes. The system relies on magnetic microparticle probes with antibodies that specifically bind a target of interest. These probes are encoded with DNA that is

unique to the protein target of interest and antibodies. Detection of prostate-specific antigen (PSA) using this assay is schematically illustrated in figure 2. In this assay increased detection sensitivity is derived from: (1) capturing and concentrating protein targets with an antibody-coated magnetic bead; (2) releasing multiple DNA barcodes per captured protein target (hundreds of barcodes are attached to a 30-nm diameter gold particle), and (3) ultrasensitive DNA detection via silver-amplified gold nanoparticles.

Nanotechnology-Based Cytogenetics

Cytogenetics, a part of molecular diagnostics, has been used mainly to describe the chromosome structure and identify abnormalities related to disease. Localizing specific gene probes by fluorescent in situ hybridization combined with conventional fluorescence microscopy has reached its limit. Molecular cytogenetics is now enhanced by nanobiotechnology. Nanotechnology has facilitated the development of methods for detection not only of a single cell but also of a few molecules.

As stem cell therapies are developing, there is a need for tracking of the stem cells introduced into the body. A superparamagnetic iron oxide nanoparticle is emerging as an ideal probe for noninvasive cell tracking. Endothelial progenitor cells, taken from human umbilical cord blood and labeled with perfluorocarbon nanoparticles, 200 nm in size, can be detected by magnetic resonance imaging (MRI) in vivo following administration [5].

Nanotechnology-Based Drug Delivery

Drug delivery is an important consideration in therapeutics. One of the reasons for lack of efficacy of some currently available medicines is poor delivery to the de-

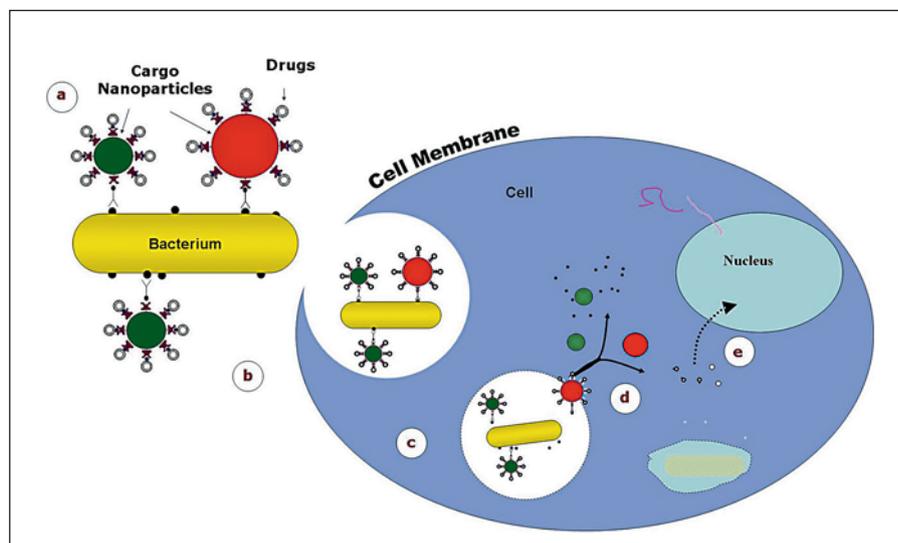


Fig. 3. Bacteria plus nanoparticles for drug delivery into cells. (Reproduced from ref. 8, by permission of Nature Publishing Group.) The delivery system begins with a cargo-carrying bacterium (a) that is 'swallowed' by the receiving cell (b). The bacterium then dissolves the cell membrane (c) and releases its cargo (d), which may also be sent to the nucleus at will to make useful proteins or regulate genes (e).

sired site of action. One of the major problems with drug formulation is solubility, which is an essential factor for drug effectiveness, independent of administration route. It is also a major challenge for pharmaceutical companies developing new pharmaceutical products since nearly half of new chemically based drugs are insoluble or poorly soluble in water. New technologies are applied for constructing innovative formulations and delivering them. A detailed account of technologies for drug delivery is presented elsewhere [6]. The current focus is on targeted drug delivery. Nanobiotechnology improves drug delivery by the following approaches:

- Particle size is reduced to nanometer size range to increase the surface area, thereby increasing the rate of dissolution.
- Development of novel nanoparticle formulations with improved stability and shelf-life.
- Development of nanoparticle formulations for improved absorption of insoluble compounds and macromolecules enables improved bioavailability and release rates, potentially reducing the amount of dose required and increasing safety through reduced side effects.
- Nanoparticle formulations that can provide sustained-release profiles up to 24 h can improve patient compliance with drug regimens.
- Nanoparticles can be combined with ligands for targeted drug delivery.
- Nanotechnology is particularly useful for delivery of biological therapies.

Bacteria-Mediated Delivery of Nanoparticles and Drugs into Cells

Nanoparticles and bacteria have been used independently to deliver genes and proteins into mammalian cells for monitoring or altering gene expression and protein production. Harmless strains of bacteria could be used as vehicles, harnessing the natural ability of bacteria to penetrate cells and their nuclei. Nanoparticles and bacteria have been used simultaneously to deliver nucleic acid-based model drug molecules into cells in mice [7]. In this approach, the gene or cargo is loaded onto the nanoparticles, ranging in size from 40 to 200 nm, which are attached to the bacteria with linker molecules. The bacteria successfully deliver the molecules, and the genes are released from the nanoparticles and expressed in cells. When the cargo-carrying bacteria attach to the recipient cell they are engulfed by its outer membrane, forming 'vesicles', or tiny spheres that are drawn into the cell's interior. Once inside the cell, the bacteria dissolve the vesicle membrane and release the cargo as shown in figure 3. This technique may be used to deliver different types of cargo into a variety of cells in live animals for gene therapy without the need for complicated genetic manipulations. This delivery system is also more efficient than techniques using viruses, which usually incorporate only one copy of a gene cargo to a virus particle. In this approach, bacteria can carry hundreds of nanoparticles, each of which can in turn carry hundreds of drug molecules, depending on the size of the nanoparticles. Released cargo can be designed to be transported to different locations in the cells to carry out disease detection and treatment simultaneously.

Role of Nanobiotechnology in Biological Therapies

Biological therapies mean the application of molecular biology in therapeutics. Broadly speaking, biological therapies include vaccines, cell therapy, gene therapy, antisense therapy and RNA interference. Some of these involve use of nucleic acids and proteins, whereas others involve genetic manipulation. Biological therapies, particularly their delivery, can be refined by use of nanobiotechnology. Uses of nanobiotechnology-based biological therapies are briefly introduced in this section and will be described later under various therapeutic areas.

Vaccination

DNA vaccines have potential as new vaccines for important pathogens such as HIV, hepatitis C, tuberculosis, and malaria, but methods for their delivery are not satisfactory. Nanoparticles are promising as delivery systems for DNA vaccines [8]. Nanoemulsions or nanoparticle aerosol vaccines are also under development. Components of the immune system recognize particles more efficiently than soluble proteins. Proteosomes™ (GlaxoSmithKline) serve as vaccine delivery vehicles by virtue of their nanoparticulate nature, forming vesicles and vesicle clusters comparable to the size of small viruses. Nanoencapsulating potent viral antigens in biodegradable polymer nanospheres for controlled release can induce the production of protective and neutralizing antibodies. This controlled release vaccine delivery technology has the capability to deliver different types and combinations of vaccines including whole inactivated virus particles, DNA plasmids and/or antigens.

Cell Therapy

Cell therapy is the prevention or treatment of human disease by the administration of cells that have been selected, multiplied and pharmacologically treated or altered outside the body. The scope of cell therapy can be broadened to include methods, pharmacological as well as nonpharmacological, to modify the function of intrinsic cells of the body for therapeutic purposes. Now that stem cell-based therapies are in development, nanotechnology plays an important role in tracking stem cells in vivo.

Nanobiotechnology is well suited to optimize the generally encouraging results already achieved in cell transplantation [9]. The small size of nanomaterial constructs provides an increasing number of options to label, transfect, visualize, and monitor cells/tissues used in transplantation. Nanoparticles are by their very nature well

suited to interact with cells. Antibodies (10 nm) and viruses (100 nm) can easily interact with cells and transport across cell membranes. Nanosized constructs that are 20 nm in size can even cross the endothelial barrier.

Gene Therapy

Gene therapy can be broadly defined as the transfer of defined genetic material to specific target cells of a patient for the ultimate purpose of preventing or altering a particular disease state [10]. Vectors are usually viral but several nonviral techniques are being used as well. Genes and DNA are now being introduced without the use of vectors and various techniques are being used to modify the function of genes in vivo without gene transfer. A variety of nanoparticles and other nanostructures can be used for nonviral gene delivery. These include nanoliposomes, gelatin nanoparticles, calcium phosphate nanoparticles, dendrimers and various composites. Biocompatible, inorganic nanoparticles of carbonate apatite have the unique features essentially required for smart delivery, as well as for the expression of genetic material in mammalian cells [11]. Apatite nanoparticles are promising candidates for nonviral gene delivery and are superior to polymer- or lipid-based systems that are generally nonbiodegradable and inefficient.

Nanodevices for Medicine and Surgery

Apart from laboratory tests, several devices based on nanobiotechnology are being developed for clinical diagnostics. Biosensor systems based on nanotechnology could detect emerging disease in the body at a stage that may be curable. This is extremely important in management of infections and cancer. Nanosensors would enable body functions and responses to treatment to be monitored without cumbersome laboratory equipments. Some examples are a nanoradiotransmitter small enough to put into a cell and nanoacoustical devices to measure and record the heart sounds.

Nanodiagnostic Devices

An X-ray device is based on carbon nanotubes that emit a scanning X-ray beam composed of multiple smaller beams while also remaining stationary [12]. As a result, the device can create images of objects from numerous angles without mechanical motion, which is a distinct advantage for any machine since it increases imaging speed, can reduce the size of the device and requires less maintenance. This technology will enable the construc-

tion of smaller and faster X-ray imaging systems for medical tomography such as CT scanners, which will produce higher-resolution images.

Early diagnoses would involve using nanotechnology to improve the quality of images produced by one of the most common diagnostic tools used in physicians' offices – the ultrasound machine. In a study, mice were injected intravenously with silica nanosphere (100 nm) suspensions dispersed in agarose and imaged by a high-resolution ultrasound imaging system [13]. B-mode images of the liver were acquired at different time points after particle injection. An automated computer program was used to quantify the gray scale changes. Ultrasonic reflections were observed from nanoparticle suspensions in agarose gels. The image brightness, i.e. mean gray scale level, increased with particle size and concentration. The mean gray scale of mouse livers also increased following particle administration. These results indicated that it is feasible to use solid nanoparticles as contrast-enhancing agents for ultrasonic imaging. The long-term goal is to use this technology to improve the ability to identify very early cancers and other diseases. The ultimate aim is to identify disease at cellular level, at the very early stage.

Nanodevices for Drug Delivery

Some drug delivery devices are implanted in the body for release of therapeutic substances. The lining of these devices can be improved by nanotechnology. Formation of microcapsules by depositing coatings onto the particle surface will make it possible to control drug release kinetics by: (a) diffusion of the drug through a polymeric coating, (b) degradation of a biodegradable polymer coating on the drug particles, releasing the core drug material. DNA-containing polymeric nanocontainers or nano-traps can preserve the full activity of an encapsulated enzyme against hostile outside environments and the release can be controlled according to demand. A self-assembling cube-shaped perforated microcontainer, no larger than a dust speck, could serve as a delivery system for medications and cell therapy [14]. Because of their metallic nature, the location of cubic containers in the body can be tracked easily by MRI. These therapeutic containers can be inserted directly at the site of an injury or an illness. The microcontainers could someday incorporate electronic components that would allow the cubes to act as biosensors within the body or to release medication on demand in response to a remote-controlled radio frequency signal. A nanovalve has been devised that can be opened and closed at will to trap and release molecules [15].

Tools for Nanosurgery

Historically, surgery was macrosurgery and most of general surgery still involves gross manipulation of organs and tissues by human hands and hand-held instruments. Some branches of surgery such as ophthalmology and otorhinolaryngology have started to miniaturize early and are now using microsurgery. In the last quarter of the 20th century, miniaturization started to develop in most branches of surgery. The basic feature was minimization of trauma to the body tissues during surgery. Trends were small incisions, laparoscopic surgery by fiber-optic visualization through tubular devices, vascular surgery by catheters and microsurgery under operating microscopes to refine the procedures and reduce trauma. Many of the devices such as robotics and implants will be a part of this miniaturization process.

Nanorobotics

Robotics is already developing for applications in life sciences and medicine. Robots can be programmed to perform routine surgical procedures. Nanobiotechnology introduces another dimension in robotics leading to the development of nanorobots also referred to as nanobots. Instead of performing procedures from outside the body, nanobots will be miniaturized for introduction into the body through the vascular system or at the end of catheters into various vessels and other cavities in the human body. A surgical nanorobot, programmed by a surgeon, could act as an autonomous on-site surgeon inside the human body. Various functions such as searching for pathology, diagnosis and removal or correction of the lesion by nanomanipulation can be performed and coordinated by an on-board computer. Such concepts, once science fiction, are now considered to be within the realm of possibility. Nanorobots will have the capability to perform precise and refined intracellular surgery which is beyond the capability of manipulations by the human hand.

NanoRobotics Laboratory at the Carnegie-Mellon University (CMU, Pittsburgh, Pa., USA) is developing endoscopic microcapsules that can be ingested and precisely positioned to enhance existing commercially available passive capsule endoscopes used by clinicians to diagnose diseases in the small intestine. A control system will allow the capsule to attach to the walls of the digestive tract and move within its lumen. Several different methods are being researched for the attachment of microcapsules including both dry and wet adhesion as well as mechanical methods such as a set of tripod legs with adhesive on feet. A simple model with surface characteristics

similar to that of the digestive tract will be constructed to test these methods. Precisely positioned microcapsules would enable physicians to view any part of the inside lining of the digestive tract in detail resulting in more efficient, accurate, and less invasive diagnosis. In addition, these capsules could be modified to include treatment mechanisms as well, such as the release of a drug or chemical near an abnormal area. The so-called 'gutbot' in development at CMU is based on nanotechnology including nanosensors and sticking devices. If this device is successful, its use may be extended to the large intestine. Although the colon is currently examined by colonoscopy, physicians might be interested in introducing a pill-sized camera through the anus to visualize the suspicious area. Similar nanorobots are under development for other parts of the body.

Nanorobotics may be applied in the future for early detection as well as treatment of cancer. A nanodevice for combined diagnosis and therapy could be implanted as a prophylactic measure in persons who do not have any obvious manifestations of cancer and its location followed by external remote monitoring. It would circulate freely and could detect cancer at the earliest stage and deliver appropriate therapeutic intervention. Such a device should be biodegradable and its safety will need to be established before implantation. This would be the ultimate in preventive personalized management of cancer. Early detection would increase the chances of cure. Such a device will have advantages over the detection of biomarkers in specimens of body fluids as such examinations can be performed only periodically and would be less accurate than analyses conducted continuously in vivo [16].

Nanoscale Laser Surgery

The introduction of lasers in surgery more than a quarter of a century ago refined surgery and laser microsurgery was used both for ablation and repair of tissues. Further developments are leading to manipulation of cellular structures at the nanometer scale. This is opening up the field of nanoscale laser surgery. Femtosecond (one millionth of a billionth of a second) laser pulses can selectively cut a single strand in a single cell in the worm and selectively knock out the sense of smell. One can target a specific organelle inside a single cell (a mitochondrion, e.g., or a strand on the cytoskeleton) and zap it out of existence without disrupting the rest of the cell. The lasers can neatly zap specific structures without harming the cell or hitting other mitochondria only a few hundred nanometers away. It is possible to carve channels slightly

less than 1 μm wide, well within a cell's diameter of 10–20 μm . By firing a pulse for only 10–15 fs in beams only 1 μm wide, the amount of photons crammed into each burst becomes incredibly intense: 100 quadrillion W/m^2 , 14 orders of magnitude greater than outdoor sunlight. That searing intensity creates an electric field strong enough to disrupt electrons on the target and create a microexplosion. But because the pulse is so brief, the actual energy delivered into the cell is only a few nanojoules. To achieve that same intensity with nanosecond or millisecond pulses would require so much more energy that the cell would be destroyed. Near-infrared femtosecond laser pulses have been applied in a combination of microscopy and nanosurgery on fluorescently labeled structures within living cells [17]. Femtolasers are already in use in corneal surgery.

Nanomedicine

Nanobiotechnology has applications in practically every branch of medicine and surgery. Some important therapeutic areas will be described briefly here and detailed descriptions are given in the *Handbook of Nanomedicine* [18]. Although nanomaterials have been available for a number of years and several structures in molecular biology were measured on nanoscale, further research on the systematic application of this knowledge to life sciences and particularly in healthcare is being vigorously pursued in recent years. This parallels advances in other biotechnologies. Historically new technologies are slowly absorbed into mainstream medical practice. Decision to use a new technology depends on the clinical judgment of the physicians taking care of their patients. Many of the new technologies are applied in challenging areas, where either no satisfactory treatments are available or nanobiotechnology-based methods have been shown to be more effective than the conventional approaches. Cancer is one area where rapid advances have been made in the application of nanobiotechnology.

Nanooncology

Application of nanotechnology in cancer can be termed nanooncology. This includes both diagnostics and therapeutics. Two nanotechnology-based products are already approved for the treatment of cancer – Doxil (a liposome preparation of doxorubicin) and Abraxane (paclitaxel in nanoparticle formulation). Nanoparticles can deliver chemotherapy drugs directly to tumor cells and then give off a signal after the cells are destroyed.

Efficient conversion of strongly absorbed light by plasmonic gold nanoparticles to heat energy and their easy bioconjugation suggest their use as selective photothermal agents in molecular cancer cell targeting [19]. Two oral squamous carcinoma cell lines and one benign epithelial cell line were incubated with anti-epithelial growth factor receptor (EGFR) antibody conjugated gold nanoparticles and then exposed to continuous visible argon ion laser at 514 nm. Malignant cells required less than half the laser energy to be killed than the benign cells after incubation with anti-EGFR antibody conjugated Au nanoparticles. In the absence of nanoparticles, no photothermal destruction was observed for all types of cells at four times the energy required to kill the malignant cells bonded with anti-EGFR/Au conjugates. Au nanoparticles thus offer a novel class of selective photothermal agents using a CW laser at low powers. The ability of gold nanoparticles to detect cancer was demonstrated previously. Now it will be possible to design an 'all-in-one' active agent that can be used to find cancer noninvasively and then destroy it. This selective technique has a potential in molecularly targeted photothermal therapy in vivo.

Metal nanoshells belong to a class of nanoparticles with tunable optical resonances that have been used for thermal ablative therapy for cancer. The ability to control both wavelength-dependent scattering and absorption of nanoshells offers the opportunity to design nanoshells which provide both diagnostic and therapeutic capabilities in a single nanoparticle. A nanoshell-based all-optical platform technology can integrate cancer imaging and therapy applications. Immunotargeted nanoshells, engineered to both scatter light in the near-infrared range enabling optical molecular cancer imaging and to absorb light, enable selective destruction of targeted carcinoma cells through photothermal therapy [20]. Immunotargeted nanoshells were used to detect and destroy breast carcinoma cells that overexpress HER2, a clinically relevant cancer biomarker. This approach has some significant advantages over alternatives that are under development. For example, optical imaging is much faster and less expensive than other medical imaging techniques. Gold nanoparticles are also more biocompatible than other types of optically active nanoparticles, such as QDs. Nanoshells, e.g. AuroShell™ (Nanospectra Biosciences Inc.), are in commercial development for the targeted destruction of various cancers. Nanoclusters (gold nanobombs) can be activated in cancer cells only by confining near-infrared laser pulse energy within the critical mass of the nanoparticles in the nanocluster [21]. Once the

nanobombs are exploded and kill cancer cells, macrophages can effectively clear the cell debris and the exploded nanotube along with it.

Nanosystems are emerging that may be very useful for tumor-targeted drug delivery: novel nanoparticles are preprogrammed to alter their structure and properties during the drug delivery process to make them most effective for the different extra- and intracellular delivery steps [22]. This is achieved by the incorporation of molecular sensors that are able to respond to physical or biological stimuli, including changes in pH, redox potential or enzymes. Tumor-targeting principles include systemic passive targeting and active receptor targeting. Physical forces (e.g., electric or magnetic fields, ultrasound, hyperthermia or light) may contribute to focusing and triggered activation of nanosystems. Biological drugs delivered with programmed nanosystems also include plasmid DNA, siRNA and other therapeutic nucleic acids.

A single-particle QD conjugated with a tumor-targeting MAb (anti-HER2) has been tracked in tumors of live mice [23]. The researchers used a dorsal skinfold chamber and a high-speed confocal microscope with a high-sensitivity camera to track the antibody-labeled QDs and made 30-frame-per-second movies of these nanoparticles as they traveled through the bloodstream. The HER2 MAb binds to a protein found on the surface of certain breast and other tumors. This was injected, conjugated to the QDs, into mice with HER2-overexpressing breast cancer to analyze the molecular processes of its mechanistic delivery to the tumor. The investigators identified six distinct 'stop-and-go' steps in the process involved in the antibody-labeled QDs traveling from the injection site to the cell where they bind HER2: within a blood vessel in the circulation, during extravasation, in the extracellular region, binding HER2 on the cell membrane, moving into the perinuclear region and within the perinuclear region. The image analysis of the delivery processes of single particles in vivo thus provides valuable information on antibody-conjugated therapeutic nanoparticles, which will be useful in increasing therapeutic efficacy.

Nanoneurology

Nanobiotechnology will have an impact on improving our understanding of the nervous system and developing new treatments, both medical and surgical, for disorders of the nervous system [24]. Working with platinum nanowires and using blood vessels as conduits to guide the wires, researchers have successfully detected the activity of individual neurons lying adjacent to the blood

vessels [25]. Use of noninvasive, biocompatible and biodegradable nanoprobe improves our understanding of the brain at the neuron-to-neuron interaction level.

Delivery of drugs to the central nervous system is a challenge and various strategies based on nanobiotechnology are discussed elsewhere [26]. Most of these are directed at overcoming the blood-brain barrier, which is a major hurdle in drug delivery to the brain.

Nanobiotechnology can facilitate neuroprotection. Water-soluble derivatives of buckminsterfullerene C60 derivatives are a unique class of nanoparticle compounds with potent antioxidant properties. Robust neuroprotection against excitotoxic, apoptotic and metabolic insults in cortical cell cultures has been demonstrated by use of carboxyfullerenes. Ongoing studies in animal models of neurodegenerative disorders suggest that these novel antioxidants are potential neuroprotective agents.

Nanobiotechnology has been used to facilitate neuroregeneration. Neural progenitor cells, encapsulated in vitro within a three-dimensional network of nanofibers formed by self-assembly of peptide amphiphile molecules, facilitate growth of nerve cells in tissue cultures [27]. Thin films of carbon nanotubes deposited on transparent plastic can also serve as a surface on which cells can grow and these nanotube films could potentially serve as an electrical interface between living tissue and prosthetic devices or biomedical instruments [28]. If nanotubes turn out to be sensitive enough to record ongoing electrical activity in cells, they could form the basis of a device that can both sense and deliver stimuli to cells for prosthetic control, e.g. control of an artificial arm at will by a person.

One of the major challenges of treating neurological disorders, particularly central nervous system damage resulting from trauma, is repair and regeneration. At nanoscale, there is little difference between basic building blocks of neuronal structures whether they are created artificially or occur in nature. Nanoelectronics, by improving cell-to-cell communication, may enable the creation of a bridge between severed nerves and muscles up to a meter away. This opens up the possibilities of repairing severed spinal cords and rehabilitation of stroke victims.

Nanocardiology

Perfluorocarbon nanoparticles provide an opportunity for combining molecular imaging and local drug delivery in cardiovascular disorders. The utility of targeted perfluorocarbon nanoparticles has been demonstrated for a variety of applications in animal models includ-

ing the diagnosis of ruptured plaque, the quantification and antiangiogenic treatment of atherosclerotic plaque and the localization and delivery of antirestenotic therapy following angioplasty [29]. Nanoscale particles can be synthetically designed to potentially intervene in lipoprotein matrix retention and lipoprotein uptake in cells – processes central to atherosclerosis. Nanoengineered molecules called nanolipoblockers can be used to attack atherosclerotic plaques due to raised levels of low-density lipoproteins [30]. An experimental study in rats using injectable self-assembling peptide nanofiber bound to platelet-derived growth factor demonstrated sustained delivery to the myocardium resulting in decreased cardiomyocyte death and preserved systolic function after myocardial infarction [31]. In studies on rats, cell therapy with insulin-like growth factor 1 delivery by biotinylated nanofibers improved systolic function after experimental myocardial infarction [32]. This nanobiotechnology approach has the potential to improve the results of cell therapy for myocardial infarction, which is on clinical trials currently.

Nanobiotechnology may facilitate repair and replacement of blood vessels, myocardium and myocardial valves. It may also be used to stimulate regenerative processes such as therapeutic angiogenesis for ischemic heart disease. Cellular function is integrally related to morphology, so the ability to control cell shape in tissue engineering is essential to ensure proper cellular function in final products. Precisely constructed nanoscaffolds are needed to guide tissue repair and replacement in blood vessels and organs. Nanofiber meshes may enable vascular grafts with superior mechanical properties to avoid patency problems common in synthetic grafts, particularly small-diameter grafts. Cytokines, growth factors, and angiogenic factors can be encapsulated in biodegradable microparticles or nanoparticles and embedded in tissue scaffolds and substrates to enhance tissue regeneration. Scaffolds capable of mimicking cellular matrices should be able to stimulate the growth of new heart tissue and direct revascularization.

Nanoorthopedics

A new method of repairing bones using nanotechnology is based on bone scaffold material (nano-HA/collagen/PLA composite) produced by biomimetic synthesis. The scaffolds or 'nanobones' have been successfully implanted in patients in China for repair of bone defects after fractures or tumor removal and also for spinal fusion. Bone cells can grow and proliferate on a scaffold of carbon nanotubes, because they are not biodegradable,

A NanoViricide™ Attacking a Virus Particle: Unique, Novel, Nanotech Design

Schematic Diagram Not Intended to be Construed as the In Vivo Mechanism

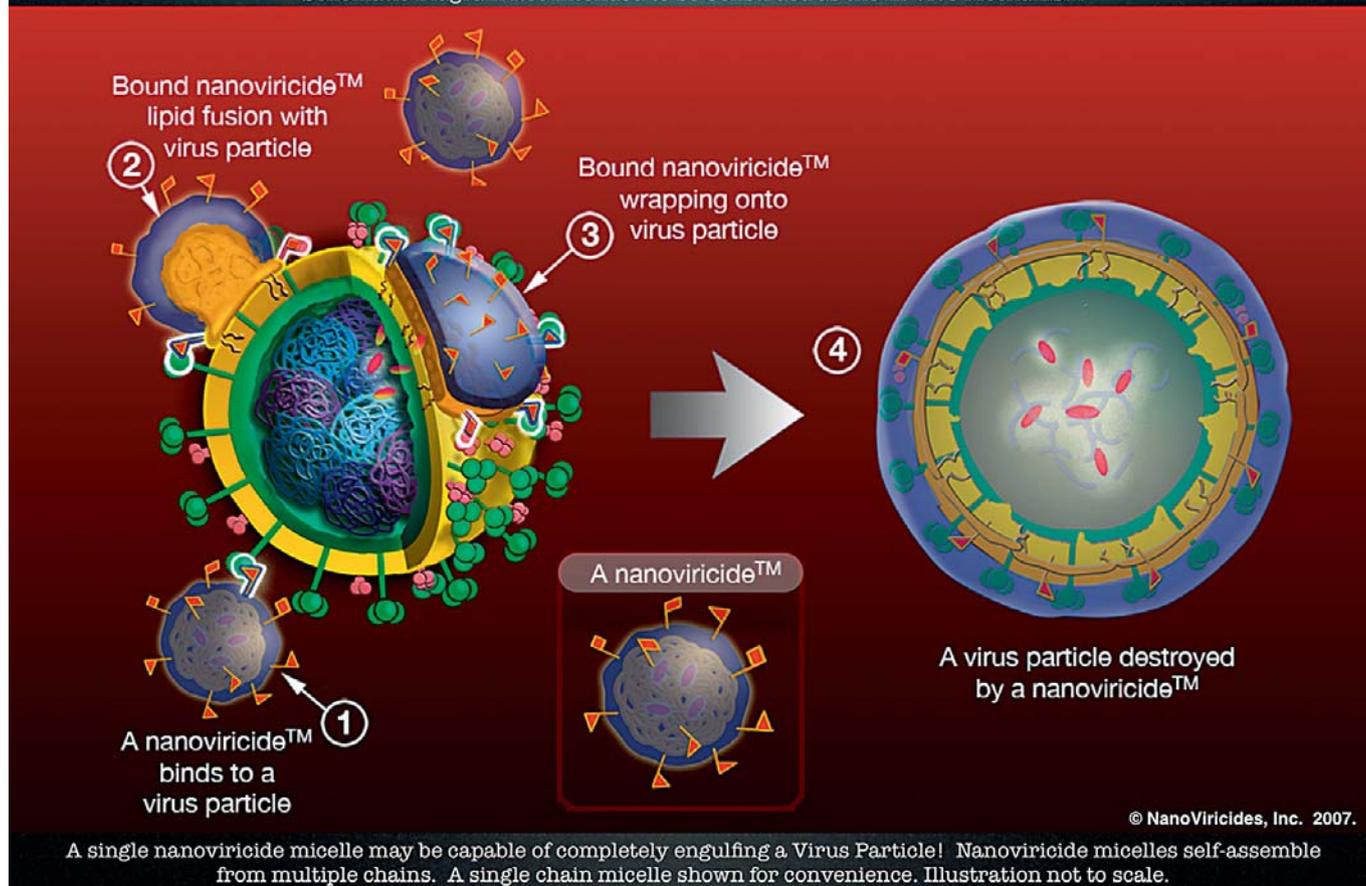


Fig. 4. Schematic representation of NanoViricide attacking a virus particle: unique, novel Nano Technique Design. (Reproduced by permission of NanoViricide Inc., schematic diagram not intended to be construed as the in vivo mechanism.) A single nanoviricide

micelle may be capable of completely engulfing a virus particle. Nanoviricide micelles self-assemble from multiple chains. A single chain micelle shown for convenience. Illustration not to scale.

and behave like an inert matrix on which cells can proliferate and deposit new living material, which becomes functional, normal bone [33].

Several methods are being developed to encourage the regeneration of cartilage defects, particularly after knee injuries. Nanotechnology and cell therapy are being used as refinements of procedures to replace the torn knee cartilage. The fine structure of an electrospun poly (l-lactide)-scaffold makes it an ideal material for tissue engineering, in particular for cartilage repair. Implanted cells showed a clear preference for growth along the nanofibers, which are both biocompatible and biodegradable [34]. Nanotechnology-based scaffolds are capable of pro-

moting the growth of mesenchymal stem cells, and differentiate these cells into viable structural and functional tissue for replacement of the medial meniscus of the knee.

Role of Nanobiotechnology in the Treatment of Infections

Nanobiotechnology is used not only for the diagnosis of infections but as a basis of microbicidal agents as well. Certain formulations of nanoscale powders possess antimicrobial properties. These formulations are made of simple, nontoxic metal oxides such as magnesium oxide (MgO) and calcium oxide (CaO, lime) in nanocrystalline

form, carrying active forms of halogens, e.g. $\text{MgO}\cdot\text{Cl}_2$ and $\text{MgO}\cdot\text{Br}_2$. When these ultrafine powders contact vegetative cells of *Escherichia coli*, *Bacillus cereus*, or *Bacillus globigii*, over 90% are killed within a few minutes. A simple molecule synthesized from a hydrocarbon and an ammonium compound can produce a unique nanotube structure with antimicrobial capability [35]. The quaternary ammonium compound is known for its ability to disrupt cell membranes and causes cell death whereas the hydrocarbon diacetylene can change colors when appropriately formulated; the resulting molecule would have the desired properties of both a biosensor and a biocide. Silver nanoparticles have been incorporated in preparations for wound care to prevent infection. Acticoat bandages (Smith & Nephew) contain nanocrystal silver, which is highly toxic to pathogens in wounds. Nanoviricides, which are nanomedicines that destroy viruses, are in development. A nanoviricide is an agent that recognizes a specific virus particle, binds to it at multiple points, neutralizes and then dismantles it (fig. 4). Targets for this approach include influenzas, HIV, hepatitis C and rabies.

Nanoophthalmology

Approximately 90% of all ophthalmic drug formulations are applied as eye drops. While eye drops are convenient, about 95% of the drug contained in the drops is lost through tear drainage, a mechanism for protecting the eye against exposure to noxious substances. Moreover, the very tight epithelium of the cornea compromises the permeation of drug molecules. Nanocarriers, such as nanoparticles, liposomes and dendrimers, are used to enhance ocular drug delivery [36]. Easily administered as eye drops, these systems provide a prolonged residence time at the ocular surface after instillation, thus avoiding the clearance mechanisms of the eye. In combination with a controlled drug delivery, it should be possible to develop ocular formulations that provide therapeutic concentrations for a long period of time at the site of action, thereby reducing the dose administered as well as the instillation frequency. In intraocular drug delivery, the same systems can be used to protect and release the drug in a controlled way, reducing the number of injections required. Another potential advantage is the targeting of the drug to the site of action, leading to a decrease in the dose required and a decrease in side effects.

Nanoparticles have also been investigated to provide controlled drug release, protect the drug against enzymatic degradation and to direct the drug to the site of action in the eye. Subconjunctivally administered 200-nm and larger polylactide nanoparticles can be almost com-

pletely retained at the site of injection. Poly(lactic acid-glycolic acid) nanospheres encapsulating pigment epithelium-derived factor have been shown to have neuroprotective effects in experimentally induced retinal ischemic injury [37].

Safety Issues of Nanomedicine

The first generation of nanomedicines (liposomal preparations) were approved more than a decade ago before a real awareness existed about a number of issues related to safety concerns of nanomaterials. These products have been used for the treatment of cancer without any toxicity of nanoparticles. However, nanomaterials such as phospholipids or biodegradable polymers, are of a completely different nature from other anticipated materials that will be produced in the near future from the research pipeline. Although in vitro use of nanoparticles in diagnostics does not pose any risk, concern has been expressed about the introduction of nanoparticles into the human body for therapeutic purposes and possible toxic effects. The small size of particles, particularly those below 50 nm, makes them versatile therapeutic tools for drug delivery and treatment of cancer but they may have undesirable effects. This topic is discussed in more detail elsewhere with review of studies on the toxic potential of nanoparticles [18].

The biological effects of various nanoparticles vary according to size, chemical composition, surface structure, solubility, shape, and aggregation. QDs may release potentially toxic cadmium and zinc ions into cells. However, because of their protective coating, QDs have minimal impact on cells. Studies using 2-nm core gold nanoparticles have shown that cationic particles are moderately toxic, whereas anionic particles are quite nontoxic [38]. A study has shown that naturally occurring gum arabic can be used as a nontoxic phytochemical excipient in the production of readily administrable biocompatible gold nanoparticles for diagnostic and therapeutic applications in nanomedicine [39]. Because several nanoparticle formulations are designed for systemic administration, the compatibility of these with blood and blood cells can be tested with a particular focus on hemolytic activity, platelet function, and blood coagulation [40]. This is no different from the requirements for testing of non-nanoparticulate formulations for systemic administration. Nanomaterials are likely to receive closer attention from regulatory bodies for toxicological potential in a number of different applications. It has been suggested

that existing nanopharmaceuticals, when administered for the same or new therapeutic indications making use of different administration routes (e.g. pulmonary), should not receive waiver of a full assessment of their potential toxicology [41].

Role of Nanobiotechnology in Personalized Medicine

Personalized medicine simply means the prescription of specific therapeutics best suited for an individual. It is usually based on pharmacogenetics, pharmacogenomics and pharmacoproteomics, but other individual variations in patients are also taken into consideration. Apart from refinements in molecular diagnostics, an important basis of personalized medicine, nanobiotechnology also helps in the discovery of biomarkers that are crucial for the development of personalized medicine. A good example of the application of nanobiotechnology for personalized medicine is that of cancer [16]: variation in the behavior of cancer of the same histological type from one patient to another is also taken into consideration. Personalization of cancer therapies is based on a better understanding of the disease at the molecular level and nanotechnology will play an important role in this area.

Future Potential of Nanomedicine

Applications of nanobiotechnology are beginning to show an impact on the practice of conventional medicine. Promoted by the National Institutes of Health of the United States, nanomedical research is providing easy access to innovative nanodevices and nanosystems based on the rational design and precise integration of functional nanomaterials for the further development of clinical nanomedicine [42]. Nanotechnology will enable de-

sign and delivery of more effective drugs with targeted delivery increasing efficacy and reducing toxicity. Although considerable progress has been made in identifying the molecular components of the mitochondrial machinery, no effective treatment for diseases caused by mitochondrial dysfunction have been developed. An impediment to manipulating mitochondria within living cells is their limited accessibility to direct physical, biochemical and pharmacological approaches. Advances in nanotechnology are providing new tools that have the potential for the diagnosis and therapy of mitochondrial disorders [43]. Nanotechnology provides the basis of computer-controlled molecular tools that are much smaller than a human cell and built with the accuracy and precision of drug molecules. Such tools will be used for interventions in a refined and controlled manner at the cellular and molecular levels. They could remove obstructions in the circulatory system, kill cancer cells, or take over the function of subcellular organelles. Instead of transplanting artificial hearts, a surgeon of the future would be transplanting artificial mitochondria.

Refinements in nanodiagnostics will enable routine detection of single particles of viruses or bacteria in minuscule samples. Nanotechnology will also provide devices to examine tissue in minute detail. Biosensors that are smaller than a cell would give us an inside look at cellular function. Tissues could be analyzed down to the molecular level, giving a completely detailed 'snapshot' of cellular, subcellular and molecular activities. Such a detailed diagnosis would guide the appropriate treatment. Although several nanomedicine-related applications of nanobiotechnology are in development or nearing commercialization, they face the usual regulatory approval hurdles encountered in the introduction of other innovative technologies and products. Judging from the progress and the increasing interest in this area during the past decade, further positive developments are predicted in nanomedicine in the next decade.

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