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The present and future of nanotechnology in human health care

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Abstract Nanotechnology is a multidisciplinary field that covers a vast and diverse array of devices derived from engineering, physics, chemistry, and biology. The burgeoning new field of nanotechnology, opened up by rapid advances in science and technology, creates myriad new opportunities for advancing medical science and disease treatment in human health care. Applications of nanotechnology to medicine and physiology imply materials and devices designed to interact with the body at subcellular (i.e., molecular) scales with a high degree of specificity. This can be potentially translated into targeted cellular and tissue-specific clinical applications designed to achieve maximal therapeutic efficacy with minimal side effects. In this review the chief scientific and technical aspects of nanotechnology are introduced, and some of its potential clinical applications are discussed.

Key words: Nanotechnology; Nanomedicine; Drug delivery; Nanodiagnostic; Molecular imaging

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale (one-billionth of a meter) [1,2]. In the past few years nanotechnology has grown by leaps and bounds, and this multidisciplinary scientific field is undergoing explosive development [3-6]. It can prove to be a boon for human health care, because nanoscience and nanotechnologies have a huge potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger, lighter materials. Human health-care nanotechnology research can definitely result in immense health benefits. The genesis of nanotechnology can be traced to the promise of revolutionary advances across medicine, communications, genomics, and robotics. A complete list of the potential applications of nanotechnology is too vast and diverse to discuss in detail, but without doubt, one of the greatest values of nanotechnology will be in the development of new and effective medical treatments [1,7-10]. This review focuses on the potential of nanotechnology in medicine, including the development of nanoparticles for drug and gene delivery and diagnostics. These technologies will extend the limits of current molecular diagnostics and permit accurate diagnosis as well as the development of personalized medicine.

Background of nanotechnology

The prefix "nano" derives from the Greek word for "dwarf." One nanometer (nm) is equal to one-billionth of a meter, or about the width of 6 carbon atoms or 10 water molecules. A human hair is approximately 80,000 nm wide, and a red blood cell is approximately 7000 nm wide. Atoms are smaller than 1 nm, whereas many molecules including some proteins range between 1 nm and larger [11].

The conceptual underpinnings of nanotechnologies were first laid out in 1959 by the physicist Richard Feynman in his lecture, "There's plenty of room at the bottom." Feynman explored the possibility of manipulating material at the scale

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of individual atoms and molecules, imagining the whole of the *Encyclopedia Britannica* written on the head of a pin and foreseeing the increasing ability to examine and control matter at the nanoscale. The term "nanotechnology" was not used until 1974, when Norio Taniguchi, a researcher at the University of Tokyo, used it to refer to the ability to engineer materials precisely at the nanometer level. The primary driving force for miniaturization at that time came from the electronics industry, which aimed to develop tools to create smaller (and therefore faster and more complex) electronic devices on silicon chips. Furthermore, at IBM in the United States, a technique called electron beam lithography was used to create nanostructures and devices as small as 40 to 70 nm in the early 1970s.

Anticipated economic impact on nanotechnology

In recognition of the enormous scientific and commercial potential for nanotechnology, President Clinton established the National Nanotechnology Initiative (NNI) in 2000. NNI is a multiagency umbrella program to build, characterize, and understand nanoscale devices. The NNI lists medicine, manufacturing, material sciences, information technology, energy, and environmental sciences as target beneficiaries. The program is slated to spend nearly $1 billion in fiscal year 2005 as compared with $464 million in 2001 [12,13]. The National Science Foundation receives $305 million for fiscal year 2005, reflecting its broad mission across scientific disciplines [14]. The Department of Defense will spend $276 million, whereas the Department of Energy receives $211 million. The National Institutes of Health meanwhile will spend $89 million on nanotechnology in 2005, including nearly $30 million for the National Cancer Institute's new Alliance for Nanotechnology in Cancer, a 5-year program announced in September.

Estimates of the impact from advances emerging from nanotechnology developments over the next 15 to 20 years have been estimated to be approximately $1 trillion by studies conducted at the National Science Foundation. In anticipation of this economic impact, nanotechnology research programs in several countries have increased substantially in recent years. In fiscal year 2005 federal agencies are slated to spend more than $1 billion on nanotechnology. They are attracting rapidly increasing investments from the government and from various business communities across many parts of the world. It has been estimated that the total global investment in nanotechnologies is currently around € 5 billion, € 2 billion of which comes from the private sector. In addition, the number of published patents in nanotechnology increased fourfold from 1995 (531 parents) to 2001 (1976 patents). Although many people believe that nanotechnologies will have an impact across a wide range of sectors, a survey of experts in nanotechnologies across the world identified hype ("misguided promises that nanotechnology can fix everything") as the issue most likely to result in a negative backlash [15,16].

Advantages of drug delivery to disease sites with nanotechnology

The pathophysiological condition and anatomical changes of diseased or inflamed tissues offers many advantages for the delivery of various nanotechnological products. Drug targeting can be achieved by taking advantage of the distinct pathophysiological features of diseased tissues [17]. Actually, the physiology of diseased tissues may be altered in a variety of physiological conditions and can be exploited for passively targeting of drugs. Thus, it exploits the anatomical differences between normal and diseased tissues to achieve site-specific and targeted delivery of drugs. Nanotechnological products thus have an advantage over other normal drugs. An ideal targeting system should have long circulating time, it should be present at appropriate concentrations at the target site, and it should not lose its activity or therapeutic efficacy while in circulation. Various nanosystems, as a result of their larger size, are accumulated at higher concentrations than normal drugs [18]. In addition, the increased vascular permeability coupled with an impaired lymphatic drainage in tumors allows an enhanced permeability and retention effect of the nanosystems in the tumors or inflamed tissues [19,20]. Thus, this pathophysiological opportunity allows extravasation of the nanosystems and their selective localization in the inflamed tissues [21]. The tendency of nanosystems to specifically localize in the reticuloendothelial system also presents an excellent opportunity for passive targeting of drugs to the macrophages present in the liver and spleen. Thus, this natural system can be used for targeting drugs for intracellular infections [18].

The therapeutic value of many promising drugs for the treatment of various neurological disorders is diminished by the presence of the blood-brain barrier [22]. The blood-brain barrier is a unique membrane that tightly segregates the brain from the circulating blood [23]. Thus, drug delivery to this organ is a challenge, because the brain benefits from very efficient protection. Nanotechnology offers a solution for using the numerous chemical entities for treating brain disorders that are not clinically useful because of the presence of the blood-brain barrier. Nanoparticles can be effectively used to deliver relevant drugs to the brain [24,25]. Drug loading onto nanoparticles modifies cell and tissue distribution and leads to a more selective delivery of biologically active compounds to improve drug efficacy and reduces drug toxicity [26-28]. Thus, various nanosystems can be successfully used as new drug carriers for brain delivery.

Applications of nanotechnology

Nanotechnology in drug delivery

From nanotechnology there is only one step to nanomedicine, which may be defined as the monitoring, repair, construction, and control of human biological systems at the

molecular level, using engineered nanodevices and nanostructures [1,9,10,29]. It can also be regarded as another implementation of nanotechnology in the field of medical sciences and diagnostics. One of the most important issues is the proper distribution of drugs and other therapeutic agents within the patient's body [7,14,29,30].

During the past two decades, however, researchers involved in the development of pharmaceuticals have understood that drug delivery is a fundamental part of drug development, and a wide range of drug delivery systems has thus been designed. Ideally, all these systems would improve the stability, absorption, and therapeutic concentration of the drug within the target tissue, as well as permit reproducible and long-term release of the drug at the target site [31,32]. In addition to reducing the frequency of drug administration and thus improving patient comfort, novel drug delivery systems would offer protection and improve the pharmacokinetics of easily degradable peptides and proteins, which often have short half-lives in vivo [33]. For the pharmaceutical industry the field of drug delivery represents a strategic tool for expanding drug markets, because new delivery technologies could repackage classical drugs, offering a competitive edge after the expiry of patents and avoiding competition from generics. Demonstrating this advantage clearly, 13% of the current global pharmaceutical market is related to the sale of products that include a drug delivery system [34].

The final aim of pharmaceutical research is the delivery of any drug at the right time in a safe and reproducible manner to a specific target at the required level [2,17]. For many drugs, however, these ideal requirements constitute hype rather than hope. For example, although the oral route is one of the preferred methods of drug delivery, because it is noninvasive, adequate peptide or protein drug delivery has not yet been attained via this route [35,36]. This is partly due to the acidic conditions of the stomach, the first-pass effect of the liver (i.e., the loss of drug as a result of metabolic processes that occur before it enters the systemic circulation), and the resistance exerted by the intestine—all of which alter, destroy, or reduce absorption of nearly all macromolecules, thus reducing their bioavailability. As a result, millions of diabetics worldwide have to self-administer insulin injections daily, provoking a high percentage of negligence in this treatment.

Applications of nanotechnologies in medicine are especially promising, and areas such as disease diagnosis, drug delivery targeted at specific sites in the body, and molecular imaging are being intensively investigated and some products undergoing clinical trials [7,9,37,38]. Nanotechnology is relatively new, and although the full scope of contributions of these technological advances in the field of human health care remains unexplored, recent advances suggest that nanotechnology will have a profound impact on disease prevention, diagnosis, and treatment [2,4,10,29,38].

The current generation of drugs is based chiefly on small molecules with a mass of 1000 Da or less that circulate systemically. Common deleterious consequences of systemic biodistribution include toxicity to nontarget tissues, difficulty in maintaining drug concentrations within therapeutic windows, and metabolism and excretion of drugs—all of which can reduce efficacy [39]. Drug solubility and cell permeability issues are also common with small molecules and biologically active compounds. Nanotechnology-based delivery systems could mitigate these problems by combining tissue- or organ-specific targeting with therapeutic action. Multifunctional nanodelivery systems could also combine targeting, diagnostic, and therapeutic actions [32,40,41].

In the near term, the most important clinical applications of nanotechnology are likely to be in pharmaceutical development. There are already an astonishing number of emerging applications [42,43]. These applications either take advantage of the unique properties of nanoparticles as drugs or components of drugs per se or are designed for new approaches to controlled release, drug targeting, and salvage of drugs with low bioavailability. For example, nanoscale polymer capsules can be designed to break down and release drugs at controlled rates and to allow differential release in certain environments, such as an acid milieu, to promote uptake in tumors versus normal tissues. Substantial research is now designed for creating novel polymers and exploring specific drug-polymer combinations [44,45].

Drug bioavailability is a related problem with potential nanotechnology solutions. Nanotechnology is opening new therapeutic opportunities for many agents that cannot be used effectively as conventional oral formulations because of their poor bioavailability. In some cases, reformulation of a drug with smaller particle size may improve oral bioavailability [46,47]. Nanoparticles formulations provide protection for agents susceptible to degradation or denaturation in regions of harsh pH, and also prolong the duration of exposure of a drug by increasing retention of the formulation through bioadhesion [48,49].

Another broad application of nanotechnology is the delivery of antigens for vaccination [50-52]. Mucosal immunity is extremely important in disease prevention but continues to be limited by both degradation of the vaccine and limited uptake. Recent advances in encapsulation and development of suitable animal models have demonstrated that microparticles and nanoparticles are capable of enhancing immunization. It has been shown that M cells in the Peyer's patches of the distal small intestine are capable of engulfing large microparticles, and recent studies have explored the benefits of nanoencapsulation. Lutsiak et al have recently demonstrated uptake of poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles by human dendritic cells in vitro. PLGA nanoparticles loaded with tetramethylrhodamine-labeled dextran were prepared using a solvent evaporation technique and administered to cultures of dendritic cells and macrophages established from peripheral blood leukocytes. After 24 hours, confocal microscopy revealed the internalization of these nano-

spheres by dendritic cells as well as macrophages, with the same level of uptake in each cell type. This work has implications in selective activation of a T cell–mediated immune response [53].

Nanotechnology in gene delivery

Gene therapy is a recently introduced method for treatment or prevention of genetic disorders by correcting defective genes responsible for disease development based on the delivery of repaired genes or the replacement of incorrect ones [54-57]. The most common approach for correcting faulty genes is insertion of a normal gene into a nonspecific location within the genome to replace a nonfunctional gene. An abnormal gene could also be swapped for a normal gene through homologous recombination or repaired through selective reverse mutation, which returns the gene to its normal function [58-60].

Three main types of gene delivery systems have been described: viral vectors, nonviral vectors (in the form of particles such as nanoparticles, liposomes, or dendrimers), and the direct injection of genetic materials into tissues using so-called gene guns [30,54,55,61,62]. Viral vectors are attractive in terms of the scientific strategy exploiting the natural mechanisms. However, such systems could suffer from the inherent difficulties of effective pharmaceutical processing and scale-up, and the possibility of the reversion of an engineered virus to the wild type. There are also serious questions about the immunogenicity of the viral vectors [62,63].

Applications of nanotechnological tools in human gene therapy has been reviewed widely by Davis, who described nonviral vectors based on nanoparticles (usually 50-500 nm in size) that were already tested to transport plasmid DNA. He emphasized that nanotechnology in gene therapy would be applied to replace the currently used viral vectors by potentially less immunogenic nanosize gene carriers. So delivery of repaired genes or the replacement of incorrect genes are fields in which nanoscale objects could be introduced successfully [64].

Liposomes

Liposomes are small artificial vesicles of spherical shape that can be produced from natural nontoxic phospholipids and cholesterol [2]. One of the most investigated approaches to gene therapy uses liposomes as submicron-scale delivery vehicles consisting of a lipid shell surrounding a core containing a therapeutic molecule or gene. Liposomes are particularly useful as gene therapy devices because of their ability to pass through lipid bilayers and cell membranes, and several groups have recently reported convincing results following local delivery [65,66].

Cationic liposomes are composed of positively charged lipid bilayers that can form complexes with negatively charged naked DNA by simple mixing. The resulting cationic liposomes-DNA complexes (lipoplexes) formed by a combination of electrostatic attraction and hydrophobic interaction have been used extensively as nonviral vectors for the intracellular delivery of reporter or therapeutic genes in culture and in vivo [62,67,68]. Most lipoplexes are thought to be taken up via endocytosis, followed by their release from an early endosomal compartment. Liu et al [69] have shown that composite liposomes containing poly (cationic lipid) and cholesterol showed much higher transfection in the liver than naked DNA alone. This work focused on lipoplexes consisting of poly(cationic lipid), cholesterol, and DNA injected directly into the portal circulation following a partial hepatectomy. The reporter gene expression (luciferase) was observed to be much higher in these lipoplexes than in naked DNA alone. Considerable research has been directed toward delivery of the tumor suppressor gene *p53* via cationic liposome-based vectors [70-72]. The *p53* gene has been shown to be involved in the control of DNA damage-induced apoptosis, and malfunction of this *p53*-mediated apoptotic pathway could be one mechanism by which tumors become resistant to chemotherapy or radiation. Transferrin (Tf)-lipoplex has demonstrated high efficiency in tumor-targeted gene delivery and long-term therapeutic efficacy in systemic *p53* gene therapy in humans for both head and neck cancer and prostate cancer [71,73]. It has been shown that Tf significantly increased the transfection efficiency for JSQ-3 cells, established from a squamous cell carcinoma of the head and neck, in culture (a 6- to 10-fold increase) when compared with the liposome alone even in the presence of high levels of serum [74]. The intratumoral delivery of *p53* gene to a mouse tumor xenograft model of human prostate PC-3 carcinoma cells using a Tf-lipoplex vector resulted in inhibition of tumor growth and an increase in animal survival. Injection of Tf-liposome-*p53* via the tail vein to nude mice bearing DU-145 subcutaneous tumors resulted in a high level of extrogenous wild-type p53 expression. In contrast, no significant extrogenous p53 expression was observed in tumors from the mouse injected with non-targeted liposome-*p53*. The in vivo efficacy of *p53* gene therapy mediated by Tf-lipoplex was further investigated and resulted in improved efficacy in systemic *p53* gene therapy of human prostate cancer. Moreover, when combined with radiation, the group treated with Tf-lipoplex-*p53* showed complete tumor regression and had no signs of recurrence 6 months after treatment. The long-term efficacy of Tf-liposome-*p53* radiosensitization was also observed in a head and neck cancer animal model. Therefore, a novel strategy combining current molecular medicine with conventional chemotherapy and radiotherapy has the potential in the clinical treatment of cancer [70-72,75].

Targeted therapy can also be achieved using liposomes. Zhang et al showed that PEGylated (treated with polyethylene glycol) liposomes linked to a monoclonal antibody for the human insulin receptor led to widespread reporter expression in the brains of rhesus monkeys [76]. Plasmids encoding luciferase or β-galactosidase were administered via the “immunoliposome” through the common circulation

and were localized primarily to the brain. Such complexes give a hint at the future of targeted therapy and the importance of nanometer-sized constructs for the advancement of molecular medicine.

Nanoparticles

Nanoparticles are colloidal particles having a size of 10 to 1000 nm [77]. Nanoparticles and microparticles formulated using PLGA and PLA polymers are being investigated as a nonviral gene delivery system because of their sustained-release characteristics, biocompatibility, biodegradability, and ability to protect DNA from degradation in endolysosomes [78]. Although PLGA/PLA nanoparticles are under extensive investigation for drug and protein delivery, their application as a gene expression vector is recent. Recently it has been demonstrated that rapid escape of nanoparticles takes place from the endolysosomal compartment to the cytoplasmic compartment following their intracellular uptake via an endocytotic process. The rapid escape of nanoparticles from the endolysosomal compartment could protect nanoparticles as well as the encapsulated DNA from the degradative environment of the endolysosomes [79]. Prabha et al investigated the gene transfection levels of different size fractions of nanoparticles. Nanoparticle fractions were separated by membrane filtration (100-nm size cutoff), and the transfection levels of the different fractions were evaluated in cell culture. These workers showed that smaller size produced 27-fold higher transfection in COS-7 cells and 4-fold higher transfection in HEK-293 cells for the same dose of nanoparticles. Higher transfection efficiency of the smaller fraction was not related to the differences in the DNA loading, cellular uptake, or the release of DNA from the two fractions. These results suggest that smaller particle size and uniform size distribution are important to enhance the nanoparticle-mediated gene expression [80].

Recently, the same group has demonstrated that nanoparticles formulated using PLGA polymer demonstrated greater gene transfection than those formulated using PLA polymer in breast cancer (MCF-7) and prostate cancer cell lines (PC-3), and this was attributed to the higher DNA release from PLGA nanoparticles [81]. PLGA with a higher molecular weight resulted in the formation of nanoparticles with higher DNA loading, which demonstrated higher gene expression than those formulated with lower molecular weight PLGA. In another study these workers have shown that cells transfected with wild-type (WT) *p53* DNA-loaded nanoparticles demonstrated a sustained and significantly greater antiproliferative effect than those with naked WT-*p53* DNA or WT-*p53* DNA in complex with a commercially available transfecting agent (Lipofectamine, Gibco BRL, Life Technologies, Inc., Gaithersburg, MD). Cells transfected with WT-*p53* DNA-loaded nanoparticles demonstrated sustained p53 mRNA levels as compared with cells that were transfected with naked WT-*p53* DNA or the WT-*p53* DNA–Lipofectamine complex, thus explaining the sustained antiproliferative activity of nanoparticles. Studies with fluorescently labeled DNA using confocal microscopy and quantitative analyses using a microplate reader demonstrated sustained intracellular localization of DNA with nanoparticles, suggesting the slow release of DNA from nanoparticles localized inside the cells. Cells that were transfected with naked DNA demonstrated transient intracellular DNA retention [82].

Dendrimers

Dendrimers are macromolecular compounds that are made up of a series of branches around an inner core [83]. Dendritic polymers provide another avenue for delivery of genes. They can form extremely small particles, on the order of nanometers, and have been shown to be effective as DNA conjugates [84,85]. The resulting dendrimer-DNA complex differs from encapsulation in that the primary interaction causing gene retention is caused by electrostatic interactions between the negatively charged phosphate groups on the DNA backbone and the positively charged amino groups on the polymer [86,87]. The complex is formed by simply mixing the components in an aqueous solution. Polyamidoamines (PAMAMs) are the most often used and characterized dendrimers for gene delivery, and several groups have recently demonstrated their efficacy. Kukowska-Latallo et al used ninth-generation PAMAM dendrimers in complex with the pCF1CAT plasmid for intravascular and endobronchial delivery of chloramphenicol acetyltransferase (CAT) to eventually treat cystic fibrosis. Intravenous administration of the complex showed transgene expression in the lung with peaks at 12 to 24 hours and 3 to 5 days. The 114-Å dendrimer-plasmid complexes were completely localized to the lung, whereas the naked plasmid was randomly distributed. In comparison, endobronchial delivery of naked plasmid was more effective than the dendrimer complex [88]. Maksimenko et al have also shown improved transfection using PAMAM conjugates [87]. This study showed that the transfection of several cell lines by plasmid cytomegalovirus β-galactosidase plasmid-dendrimer complexes was enhanced by the presence of anionic oligomers including oligonucleotides or dextran sulfate.

Nanotechnology as a tool in imaging

Noninvasive imaging techniques have had a major impact in medicine over the past 25 years or so. The current drive in developing techniques such as functional magnetic resonance imaging is to enhance spatial resolution and contrast agents. Nanotechnology has a potential to transform the field of medicine, because it offers novel opportunities for sensing clinically relevant markers, molecular disease imaging, and tools for therapeutic intervention [89]. Nanotechnologies already afford the possibility of intracellular imaging through attachment of quantum dots (QDs) or synthetic chromophores to selected molecules, for example proteins, or by the incorporation of naturally occurring fluorescent proteins that, with optical techniques

such as confocal microscopy and correlation imaging, allow intracellular biochemical processes to be investigated directly [38,89,90]. QDs are semiconductor nanocrystals with unique optical and electrical properties. Among others, one of their most valuable properties is their fluorescence spectrum, which renders them optimal fluorophores for biomedical imaging. Moreover, because of their quantum confinement of charge carriers in tiny spaces, QDs show some fascinating optimal properties, which are characterized as sharp and symmetrical emission spectra, high quantum yield, broad absorption spectra, good chemical and photostability, and size-dependent emission wavelength tenability [92-95].

Thus, QDs are more appealing as in vivo and in vitro fluorophores in a variety of biological investigations in which traditional fluorescent labels based on organic molecules fall short of providing long-term stability and simultaneous detection of multiple signals [96]. QDs have also been successfully used as new fluorescent tags in many biological and biomedical fields and show definite promise as a new tool in biomedical studies, clinical diagnostics, drug delivery, and photodynamic therapy. Nanotechnology has also been identified as a field of great promise to detect and diagnose cancer. For instance, semiconductor QDs can allow the detection of tens to hundreds of cancer biomarkers in blood assays, on cancer tissue biopsies, or as contrast agents for medical imaging [96-98].

Recent advances in nanosensors includes nanosensors based on fluorescence resonance energy transfer, which is capable of detecting low concentrations of DNA in a separation-free format. It uses QDs linked to DNA probes to capture DNA targets. Actually, in this system, a fluorescence resonance energy transfer donor-acceptor ensemble forms as a result of the binding of the target strand to a dye-labeled reporter strand. In addition, the QD also functions as a concentrator that amplifies the target signal by confining several targets in a nanoscale domain [99]. However, although fluorescent QDs hold great potential for molecular imaging in vivo [96,100], the utility of existing QDs is limited because they require excitation from external illumination sources to fluorescence. This results in a strong autofluorescence background and a paucity of exciting light at nonsuperficial locations. These drawbacks can be effectively overcome by the self-illuminating QD conjugates. So et al have devised a QD technology that permits improved imaging in vivo compared with existing QDs in the absence of an external excitor [101]. The new probes rely on bioluminescence resonance energy transfer [102], which converts chemical energy into photon energy, resulting in dramatic increases in fluorophore excitation as well as reductions in the effects of tissue autofluorescence. The conjugates are prepared by coupling carboxylate-presenting QDs to a mutant of the bioluminescent protein *Renilla reniformis* luciferase. Thus, compared with existing QDs, self-illuminating QD conjugates have greatly enhanced sensitivity [101].

Moreover, nanoparticles have also been incorporated into a polymer matrix for controlled drug release. In vivo applications of nanoparticles are also starting to emerge. For example, Ruoslahti and co-workers linked QDs to a peptide for labeling tumor vasculatures in live mice; silica nanoparticles coated with gold nanoshells have photothermal capabilities for cancer treatment; and iron magnetic particles were used to track progenitor cells in vivo using magnetic resonance imaging [103]. Nie and co-workers reported the first simultaneous in vivo targeting and imaging of tumors in live animals using QDs tagged to antibodies [104]. Thus, the applications of nanotechnology to biological systems will undoubtly transform the foundations of disease diagnosis, treatment, and prevention in the future.

Nanotechnology for molecular diagnostics

Because of the small dimensions, most of the applications of nanobiotechnology in molecular diagnostics fall under the broad category of biochips/microarrays but are more correctly termed nanochips and nanoarrays. Biochips constructed with microelectromechanical systems are on a micron scale and related to micromanipulation, whereas nanotechnology-based chips are on a nanoscale and related to nanomanipulation [105,106]. Even though microarray/biochip methods making use of the detection of specific biomolecular interactions are now an indispensable tool for molecular diagnostics, there are some limitations. DNA microarrays and enzyme-linked immunosorbent assays rely on the labeling of samples with a fluorescent or radioactive tag, a highly sensitive procedure that is time-consuming and expensive. The chemical modification and global amplification of the nucleic acid samples are achieved by polymerase chain reaction, which can introduce artifacts caused by the preferential amplification of certain sequences. Alternative label-free methods include surface plasmon resonance and quartz crystal microbalance, which rely on mass detection. Nanotechnologies also provide label-free detection. Nanotechnology is thus being applied to overcome some of the limitations of biochip technology [29,31,105,107].

Nanotechnology on a chip is one more dimension of microfluidic/lab-on-a-chip technology. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive, and more flexible when certain nanoscale particles are put to work as tags or labels. Magnetic nanoparticles, bound to a suitable antibody, are used to label specific molecules, structures, or microorganisms. Magnetic immunoassay techniques have been developed in which the magnetic field generated by the magnetically labeled targets is detected directly with a sensitive magnetometer. Gold nanoparticles tagged with short segments of DNA can be used for detection of genetic sequence in a sample. Multicolor optical coding for biological assays has been achieved by embedding different-sized QDs (nanocrystals of cadmium selenide) into polymeric microbeads. This spectral coding technology

is expected to open new opportunities for medical diagnostics [29,107].

Nanotechnology on a chip is a new paradigm for total chemical analysis systems. The ability to make chemical and biological information much cheaper and easier to obtain is expected to fundamentally change health care, food safety, and law enforcement. Lab-on-a-chip technology involves micro-total analysis systems that are distinguished from simple biosensors because they conduct a complete analysis: a raw mixture of chemicals goes in and an answer comes out. There are several lab-on-a-chip products. Sandia National Laboratories (Albuquerque, NM) is developing a hand-held lab-on-a-chip that will be used in the analysis of airborne chemical warfare agents and liquid-based explosives agents [29]. This development project brings together an interdisciplinary team with areas of expertise including microfabrication, chemical sensing, microfluidics, and bioinformatics. A hand-held lab-on-a-chip is in development to detect airborne chemical warfare agents and liquid-based explosives agents. Nanopore technology for analysis of nucleic acids converts strings of nucleotides directly into electronic signatures. DNA nanomachines can be used as biomolecular detectors for homogeneous assays. Nanobarcodes, sub-micronic metallic barcodes with striping patterns prepared by sequential electrochemical deposition of metal ions, show differential reflectivity of adjacent stripes permitting identification of the striping patterns by conventional light microscopy. This can be used in population diagnostics and in point-of-care hand-held devices [108].

NanoChip (Nanogen, San Diego, CA), although it carries the name "nano" is actually a microelectronic chip for analysis of nanoliter quantities of samples rather than a chip based on nanotechnology. The NanoChip system integrates advanced microelectronics and molecular biology into a platform technology with broad commercial applications in the fields of biomedical research, genomics, medical diagnostics, genetic testing, and drug discovery. The NanoChip system uses electronically accelerated hybridization under minimal salt conditions, potentially avoiding problems with DNA conformation and secondary structures, whereas most sequencing and primer extension technologies require high-salt conditions. The hybridization is not only accelerated by an electrical potential, but (after switching) this potential can be used to considerably enhance the specificity [105,106].

Nanotechnology in cardiac therapy

At present, cardiac diseases are the major cause of mortality, morbidity, and disability. Ever more people are dying of various cardiac problems including atherosclerosis, myocardial infarction, arrhythmias, ischemic heart disease, and restenosis [109]. Oral and systemic administration of drugs, though effective, does not provide appropriate therapeutic drug levels in the target arteries for sufficient periods of time. Moreover, biomedical engineers have already succeeded in developing microscale instruments to open blocked arteries and to treat other cardiovascular diseases. However, these tools are bulky, infection prone, and subject to other disorders. Currently nanotechnology offers a broad platform in the field of cardiovascular science by offering tools to explore the frontiers of cardiac science at the cellular level. Nanotechnology-based tools can be effectively used to treat the cardiovascular diseases. These tools can be used in the areas of diagnosis, imaging, and tissue engineering [110]. Miniaturized nanoscale sensors like QDs, nanocrystals, and nanobarcodes can sense and monitor biological signals such as the release of proteins or antibodies in response to cardiac or inflammatory events [90]. Nanotechnology can also help in revealing the mechanisms involved in various cardiac diseases. It also helps in designing atomic-scale machines by imitating or incorporating biological systems at the molecular level. The use of these newly designed nanomachines can have a paradigm-shifting impact in the treatment of the dreaded cardiovascular diseases. These machines have three key elements meant for sensing, decision making, and carrying out the intended purpose. For instance, abciximab, a chimeric mouse-human monoclonal antibody used to lessen the chance of heart attack in people who need percutaneous coronary intervention (a procedure to open blocked arteries of the heart), can be considered as an example of a simple nanomachine. It has sensors that bind to the GP2b3a receptor and also has an "effector" that inhibits the receptor through steric hindrance. Thus, by inhibiting the ability of the GP2b3a receptor to bind fibrinogen, abciximab changes platelet behavior, impeding platelet aggregation and activation [109]. Tenecteplase (TNK-rt-PA), another nanomachine used in cardiac therapy, is a tissue plasminogen activator (tPA) produced by recombinant DNA technology using an established mammalian cell line (Chinese hamster ovary cells). It differs from rt-PA by three sets of substitution mutations that decrease its plasma clearance rate and is used to dissolve blood clots that have formed in the blood vessels of the heart that seriously lessen the flow of blood in the heart [109].

Restenosis, the obstruction of an artery after interventional procedures such as balloon angioplasty, remains a major problem, in that 30% to 50% of patients develop reocclusion, with 20% requiring additional intervention [77,111,112]. Although different therapeutic strategies have been investigated for the inhibition of restenosis, the main drug therapy approach is targeted toward inhibiting the proliferation and migration of smooth muscle cells [113]. Systemic administration of therapeutic agents has been ineffective in preventing restenosis. The main reason for the failure of drugs in clinical trials is the inefficacy of such an approach in providing therapeutic drug levels in the target tissue for a sustained period of time. Therefore, researchers have a great hope that nanotechnology-based localized drug therapy using sustained-release drug delivery systems could be more effective, because it can provide higher and

prolonged drug levels in the target tissues without causing systemic toxicity [78]. Nanotechnology could also have an impact in the diagnosis and treatment of unstable plaques and in the management of other cardiovascular problems like calcification of valves. Thus, nanotechnology could be an effective treatment modality to achieve localized and sustained arterial and cardiac drug therapy for the prevention of cardiovascular diseases.

Nanotechnology in dental care

Nanotechnology will have future medical applications in the field of nanodentistry. Nanodentistry will make it possible to maintain near-perfect oral health through the use of nanomaterials [114,115], biotechnology [116-119], and nanorobotics. Through this it will be possible to provide high-quality dental care to the millions of the world's population who currently receive no significant dental care [120,121]. In the years to come it will be possible through nanodentistry to induce local anesthesia. A colloidal suspension containing millions of active analgesic dental nanorobotic particles could be instilled on the patient's gingivae. These nanorobots, after contacting the surface of the crown or mucosa, reach the dentin by migrating into the gingival sulcus and pass painlessly to the target site. On reaching the dentin, the nanorobots enter dentinal tubule holes that are 1 to 4 μm in diameter [122-124] and proceed toward the pulp, guided by a combination of chemical gradients, temperature differentials, and even positional navigation, all under the control of the onboard nanocomputer as directed by the dentist.

Apart from this, nanodental techniques for major tooth repair may also evolve. Orthodontic nanorobots could directly manipulate the periodontal tissues (gingivae, periodontal ligament, cementum, and alveolar bone), allowing rapid and painless tooth straightening, rotating, and vertical repositioning within minutes to hours. Another pathological phenomenon that may be benefited by nanodental treatment is dentin hypersensitivity [121]. Dentin hypersensitivity is a common condition of transient tooth pain associated with a variety of exogenous stimuli. There is substantial variation in the response to such stimuli from one person to another. Except for sensitivity associated with tooth bleaching or other tooth pathology, the clinical cause of dentin hypersensitivity is exposed dentinal tubules as a result of gingival recession and subsequent loss of cementum on root surfaces [125-127]. Reconstructive dental nanorobots could selectively and precisely occlude specific tubules within minutes, offering patients a quick and permanent cure. Nanodentistry could also play a vital role in natural tooth maintenance [128]. The appearance and durability of teeth may be improved by replacing upper enamel layers with covalently bonded artificial materials such as sapphire [129] or diamond, which have 20 to 100 times the hardness and strength of natural enamel. A subocclusal-dwelling nanorobotic dentifrice delivered by mouthwash or toothpaste could patrol all supragingival and subgingival surfaces at least once a day, metabolizing trapped organic matter into harmless and odorless vapors and performing continuous calculus debridement [121].

Nanotechnology in orthopedic applications

An ageing population and an increased occurrence of sports-related injuries have made musculoskeletal disorders one of the major health concerns. Current treatment modalities include orthopedic implants used for internal fixing of fractured bones, but these are limited by the large number of implant failures. In addition, these engineered implants are stiffer than those of cortical bones, and removal of the implants require a second operation. Besides, the polymers used suffer from the drawbacks of loss of mechanical strength within a time interval and also development of a sterile sinus at the site of implantation. Biomaterials proposed as ideal scaffolds for cell growth should be biocompatible, osteoinductive, osteoconductive, integrative, porous, and mechanically compatible with native bone to fulfill their desired role as bone implants and substitutes. Current treatments have some but not all of these properties. This has prompted orthopedic surgeons and scientists to look for viable alternatives. Nanotechnology can provide an alternative platform with higher mechanical strength, enhanced bioactivity, and resorbability in improving the quality of life of patients who suffer from debilitating bone fractures.

Nanostructure materials with sizes 1 to 100 nm can act as new and effective constituents of bone materials, because bone is also made up of nanosized organic and mineral phases. Several studies have reported improved osseointegration on nanostructure surfaces created from a wide range of chemistries including ceramics, metals, polymers, and composites. For instance, studies show that alumina nanometer fibers significantly stimulate osteoblast responses such as adhesion, alkaline phosphatase activity, and calcium deposition, when compared with conventional grain size alumina [130,131]. Greater in vitro osteoblast adhesion has also been observed on helical rosette nanotube-coated titanium compared with uncoated titanium, because these helical rosette nanotubes mimic the dimensions of the nanostructure of the bone components [132].

Nanomaterials, nanopolymers, carbon nanofibers, nanotubes, and nanocomposites of ceramics will also lead to more efficient deposition of calcium-containing minerals on the implants. Recent studies have demonstrated that the adsorption and conformation of proteins that mediate specific osteoblast adhesion (such as fibronectin and vitronectin) are enhanced on nanospaced materials like 3D nanofibrous scaffolds [133-135]. It is also estimated that nanophase materials attract more proteins to their surface because of their altered surface energetics, brought about by a higher exposed surface area and altered electron distribution as compared with conventional materials. The greater wettability and presence of surface features close to the size of the proteins (on the nanoscale) leads to alteration of the

bioactivity of the selected proteins on the nanophase material as compared with their conventional counterparts. Specifically, the peptide sequence arginine–glycine–aspartic acid is more exposed when vitronectin is adsorbed to nanophase materials than with conventional ceramics, and this leads to greater adhesion of osteoblast cells to the proteins already adsorbed to the implants.

Similarly, increased unfolding and exposure of osteoblast-adhesive epitopes was demonstrated on fibronectin adhered on nanostructure ceramics, encouraging osteoblast function. Studies also imply that cell responses are more sensitive to changes in surface roughness on the nanometer scale as compared with conventional regimes. This leads to increased osteoblast and osteoblast activity with simultaneous decreased fibroblast function, thus improving osseointegrative potentials [136]. All of this evidence demonstrates that nanostructure materials represent an important and growing area of research that may improve bonding between an implant and surrounding bone by increasing bone cell interactions, and this will certainly aid in improving orthopedic implant efficacy while drastically minimizing patient compliance problems.

Nanotechnology as a risk to human health

Although the benefits of nanotechnology are widely publicized, discussion of the potential effects of their widespread use in consumer and industrial products is just beginning. Both pioneers of nanotechnology and its opponents are finding it extremely hard to argue their case because of the limited information available to support one side or the other. Given the rapid rate of development in this area and the amount of publicity it is attracting, it is not surprising that concerns should have been raised relating to the safety of nanomaterials in a variety of products. Some have drawn an analogy between high-aspect-ratio nanoparticles and asbestos fibers [5,137].

In the United Kingdom, the Prince of Wales has requested advice on nanotechnology from the Royal Society, whereas Greenpeace and the Canadian Action Group on Erosion, Technology, and Concentration have called for a moratorium on the use of nanoparticles until the toxicological issues have been resolved. Although some concerns may be ill-founded, it remains true that the toxicology of many nanomaterials has not yet been fully evaluated. To address this issue, some companies are participating in the European Nanosafe consortium, which is starting to evaluate the possible risks presented by nanomaterials. In the United States, the Center for Biological and Environmental Nanotechnology at Rice University has begun an investigation of two popular nanomaterials systems: carbon nanotubes and TiO_2. Such environmental concerns may temper the enthusiasm of the venture capital community to a certain extent, but they are unlikely to stem the flow of venture capital funding into this exciting sector.

It has been shown that nanomaterials can enter the human body through several ports. Accidental or involuntary contact during production or use is most likely to occur via the lungs, from which a rapid translocation is possible to other vital organs through the bloodstream. On the cellular level, an ability to act as a gene vector has been demonstrated for nanoparticles. Carbon black nanoparticles have been implicated in interfering with cell signaling. There is work that demonstrates uses of DNA for the size separation of carbon nanotubes. The DNA strand just wraps around it if the tube diameter is right. Though excellent for the purposes of separation, this tendency raises some concerns over the consequences of carbon nanotubes entering the human body [3,138].

Conclusions

The multidisciplinary field of nanotechnology is bringing the science of the almost incomprehensibly small device closer and closer to reality. The effects of these developments will at some point be so vast that they will probably affect virtually all fields of science and technology. As such, nanotechnology holds the promise of delivering the greatest technological breakthroughs in history. Over the next couple of years it is widely anticipated that nanotechnology will continue to evolve and expand in many areas of life and science, and the achievements of nanotechnology will be applied in medical sciences, including diagnostics, drug delivery systems, and patient treatment.

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