REVIEW ARTICLE

Significance of nanotechnology in medical sciences

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Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the molecular level in scales smaller than 1 µm, normally 1-100 nm, and the fabrication of devices within that size range. Two main approaches are used in nanotechnology. In the "bottom-up" approach, materials and devices are built from molecular components, which assemble themselves chemically by principles of molecular recognition. In the "top-down" approach, nano-objects are constructed from larger entities without atomic-level control. In addition, as the need for the development of new medicines is pressing, and given the inherent nanoscale functions of the biological components of living cells, nanotechnology has been applied to diverse medical fields such as oncology, cardiovascular medicine, and in treatment of other chronic diseases. Indeed, nanotechnology is being used to refine discovery of biomarkers, molecular diagnostics, and drug discovery and drug delivery, which could be applicable to management of these patients. In this review, we will focus upon significance of nanotechnology in medical sciences, as well as the plausible side effects related to their use.

Key words: *Drug discovery, medical sciences, nanotechnology, nano-objects, new medicines*

INTRODUCTION

Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the molecular level in scales smaller than 1 μm, normally 1-100 nm, and the fabrication of devices within that size range. It is a highly multidisciplinary field, drawing from fields such as pharmaceutical sciences, applied physics, materials science, colloidal science, device physics, supramolecular chemistry, and even mechanical and electrical engineering. Nanotechnology can be seen as an extension of existing sciences into the nanoscale, or as a recasting of existing sciences using a newer, more modern term. The impetus for nanotechnology comes from a renewed interest in colloidal science, coupled with a new generation of analytical tools such as the atomic force microscope, and the scanning tunneling microscope. Nanotechnological techniques preceded the nanotech era, and are extensions in the development of scientific advancements rather than techniques that were devised with the sole purpose of creating nanotechnology and which were results of nanotechnology research.

METHODOLOGY OF NANOTECHNOLOGY

The following approaches are used in nanotechnology:

1. Bottom-up approaches: To arrange smaller components into more complex assemblies.
2. Top-down approaches: To create smaller devices using larger ones to direct their assembly.
3. Functional approaches: To develop components of a desired functionality without regard to how they might be assembled.

Applications of nanotechnology in medical sciences

As the need for the development of new medicines is pressing, and given the inherent nanoscale functions of the biological components of living cells, nanotechnology has been applied to diverse medical fields such as oncology and cardiovascular medicine. Indeed, nanotechnology is being used to refine discovery of biomarkers, molecular diagnostics, and drug discovery and drug delivery, which could be applicable to management of these patients. To achieve these aims, nanotechnology strives to develop and combine new materials by precisely engineering atoms and molecules to yield new molecular assemblies on the scale of individual cells, organelles or even smaller components, providing a personalized medicine.[1,2] Personalized medicine is individualized or individual-based therapy, which allows the prescription of precise treatments best suited for a single patient.[3]

As nanotechnology is undergoing such explosive expansion in many areas, even poorer developing

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countries have also decided that this new technology could represent a considered investment in future economic and social well-being that they cannot ignore.

Because of increased use of nanotechnology, the risk associated with exposure to nanoparticles, the routes of entry, and the molecular mechanisms of any cytotoxicity need to be well understood. In fact, these tiny particles are able to enter the body through the skin, lungs or intestinal tract, depositing in several organs and may cause adverse biological reactions by modifying the physiochemical properties of living matter at the nanolevel.[3-6] In addition, the toxicity of nanoparticles will also depend on whether they are persistent or cleared from the different organs of entry and whether the host can raise an effective response to sequester or dispose of the particles. A number of investigators have found nanoparticles responsible for toxicity in different organs.[7-11]

Overview of different classes of nanoparticles

Liposomes are nanoparticles comprising lipid bilayer membranes surrounding an aqueous interior. The amphilic molecules used for the preparation of these compounds have similarities with biological membranes and have been used for improving the efficacy and safety of different drugs.[12-14] Usually, liposomes are classified into three categories on the basis of their size and lamellarity (number of bilayers): Small unilamellar vesicles or oligolamellar, large unilamellar vesicles, and multilamellar vesicles. Recently, a new generation of liposomes called 'stealth liposomes' have been developed. Stealth liposomes have the ability to evade the interception by the immune systems, and therefore, have longer half-life.[15] Emulsions comprise oil in water-type mixtures that are stabilized with surfactants to maintain size and shape. The lipophilic material can be dissolved in a water organic solvent that is emulsified in an aqueous phase. Like liposomes, emulsions have been used for improving the efficacy and safety of diverse compounds.[16] Polymers such as polysaccharide chitosan nanoparticles have been used for some time now as drug delivery systems.[17] Recently, water-soluble polymer hybrid constructs have been developed. Polymer conjugation to proteins reduces immunogenicity, prolongs plasma half-life, and enhances protein stability. Polymer-drug conjugation promotes tumor targeting through the enhanced permeability and retention effect and, at the cellular level following endocytic capture, allows lysosomotropic drug delivery.[18] Ceramic nanoparticles are inorganic systems, with porous characteristics that have recently emerged as drug vehicles.[19] These vehicles are biocompatible ceramic nanoparticles such as silica, titania, and alumina that can be used in cancer therapy. Metallic particles such as iron oxide nanoparticles (15-60 nm) generally comprise a class of superparamagnetic agents that can be coated with dextran, phospholipids, or other compounds to inhibit aggregation and enhance stability. The particles are used as passive or active targeting agents.[20]

Gold shell nanoparticles, other metal-based agents, are a novel category of spherical nanoparticles consisting of a dielectric core covered by a thin metallic shell, which is typically gold. These particles possess highly favorable optical and chemical properties for biomedical imaging and therapeutic applications.[21] Carbon nanomaterials include fullerenes and nanotubes. Fullerenes are novel carbon allotrope with a polygonal structure made up exclusively of 60 carbon atoms. These nanoparticles are characterized by having numerous points of attachment whose surfaces also can be functionalized for tissue binding.[22] Nanotubes have been one of the most extensively used types of nanoparticles because of their high electrical conductivity and excellent strength. Carbon nanotubes can be structurally visualized as a single sheet of graphite rolled to form a seamless cylinder. There are two classes of carbon nanotubes: single-walled (SWCNT) and multi-walled (MWCNT). MWCNT are larger and consist of many single-walled tubes stacked one inside the other. Functionalized carbon nanotubes are emerging as novel components in nanoformulations for the delivery of therapeutic molecules.[23]

Quantum dots are nanoparticles made of semiconductor materials with fluorescent properties. Crucial for biological applications quantum dots must be covered with other materials allowing dispersion and preventing leaking of the toxic heavy metals.[24]

Use of nanotechnology in diagnostics, pharmacology, and therapeutics

Nanotechnology is being applied to biomarker-based proteomics and genomics technologies. Nanoparticles can be used for qualitative or quantitative *in vivo* or *ex vivo* diagnosis by concentrating, amplifying, and protecting a biomarker from degradation, in order to provide more sensitive analysis.[25] Initial studies with magnetic nanoparticle probes coated with antibodies and single 'bar code' DNA fragments are able to amplify signals of small abundant biomolecules. This amplification is comparable to polymerase chain reaction (PCR) amplification of nucleotide sequences, and can theoretically be used to detect hundreds of protein targets at a time in patient samples. Such analysis would enable physicians to properly diagnose disease at very early stages and begin treatment before severe cellular damage, improving patient prognosis. For instance, *in vitro* streptadivin-coated fluorescent polystyrene nanoparticles have been used to detect the epidermal growth factor receptor in human epidermoid carcinoma cells by flow cytometry.[26] In addition, a nanoparticle oligonucleotide bio-barcode assay has been used to detect small levels of the cancer marker prostate-specific antigen (PSA) in serum. The use of this new technique offers a high ratio of PCR-amplifiable DNA to labeling antibodies that can considerably enhance assay sensitivity. Therefore, a low amount of free serum PSA could be detected in patients suffering from prostate cancer or even women

suffering from breast cancer with a great improvement in tumor screening and diagnosis.[27]

Molecular diagnosis

Nowadays, imaging diagnosis is not only limited to a gross description of anatomic structures, but can also involve imaging of cellular signaling. Nanoparticles are currently being tested for molecular imaging to achieve a more precise diagnosis with high-quality images. In fact, contrast agents have been loaded onto nanoparticles for tumor and atherosclerosis diagnosis. Different nanoparticles have been used for molecular imaging with magnetic resonance images (MRI), ultrasound, fluorescence, nuclear, and computed tomography imaging.[28,29]

Gadolinium complexes have been incorporated into emulsion nanoparticles, resulting in a dramatic enhancement of the signal compared to usual contrast agents.[30] In addition, it has been shown that ultrasmall superparamagnetic iron oxide particles enhanced the MRI signal of the thrombus in an experimental animal model developed in rabbits.[31] In the last few years, an emerging area of great interest is stem cell imaging with MRI. This new technique allows treating stem cells *in vitro* with superparamagnetic nanoparticles and afterwards these cells can be injected to a specific localization in the body. The stem cells can ingest the nanoparticles by endocytosis, which results in the intracellular accumulation of nanoparticles that can exert a local effect for detection *in vivo*.[32,33]

Nanoparticle drug delivery systems

The use of pharmacological agents, developed using classical strategies of pharmacological development, is frequently limited by pharmacodynamics and pharmacokinetics problems such as low efficacy or lack of selectivity. Moreover, drug resistance at the target level owing to physiological barriers or cellular mechanisms is also encountered. In addition, many drugs have a poor solubility, low bioavailability, and they can be quickly cleared in the body by the reticuloendothelial system. Furthermore, the efficacy of different drugs, such as chemotherapeutical agents, is often limited by dose-dependent side effects. Indeed, anticancer drugs, which usually have large volume of distribution, are toxic to both normal and cancer cells. Therefore, precise drug release into highly specified target involves miniaturizing the delivery systems to become much smaller than their targets. With the use of nanotechnology, targeting drug molecules to the site of action is becoming a reality resulting in a personalized medicine, which reduces the effect of the drug on other sites while maximizing the therapeutic effect. This goal is mainly achieved by the small size of these particles, which can penetrate across different barriers through small capillaries into individual cells. In addition, nanoparticles can be prepared to entrap, encapsulate, or bind molecules improving the solubility, stability, and absorption of several drugs, as well as avoiding the reticuloendothelial system, thus protecting the drug from premature inactivation during its transport. In fact, it has been shown that nanoparticles have the ability to carry various therapeutic agents including DNA, proteins, peptides, and low molecular weight compounds. Among all of them, liposome and polymer-based nanoparticulates are the most widely used nanoparticles as drug delivery systems, as these compounds are generally biodegradable, do not accumulate in the body, and they are possibly risk-free.[34] For instance, several anticancer drugs, including paclitaxel,[35] 5-fluorouracil,[36] and doxorubicin,[37] have been successfully formulated using polymers and liposomes as drug delivery systems. Biodegradable nanoparticle-based vaccines, for oral vaccination, are also in development and may allow targeting of antigens to specific dendritic cell receptors.[38]

Toxicity of nanoparticles

Humans have been exposed to nanoparticles throughout their evolutionary phases; however, this exposure has been increased to a great extent in the past century because of the industrial revolution. Nanoparticles constitute a part of particulate matter (PM). Epidemiological studies have shown that urban pollution with airborne PM deriving from combustion sources, such as motor vehicle and industrial emissions, contributes to respiratory and cardiovascular morbidity and mortality.[39-41] A typical ambient PM is a highly complex mix of particles with median diameter size ranging from 1 nm to 100 mm. Carbon in elemental form is a major component of these particles and the size of these particles is a determinant of their ability to cause systemic cardiovascular effects. Indeed, fine and ultrafine PM (from 0.1 to 2.5 mm in mass median aerodynamic diameter) that can more easily access the vasculature via inhalation are linked to cardiovascular dysfunctions,[41] particularly in subjects with pre-existing vascular diseases.

The growing use of nanotechnology in high-tech industries is likely to become another way for humans to be exposed to intentionally generated engineered nanoparticles. Nanotechnology is also being applied in medical sciences trying to achieve a personalized medicine. However, the same properties (small size, chemical composition, structure, large surface area and shape), which make nanoparticles so attractive in medicine, may contribute to the toxicological profile of nanoparticles in biological systems. In fact, the smaller particles are the more the surface area they have per unit mass, and this property makes nanoparticles very reactive in the cellular environment.

Therefore, any intrinsic toxicity of the particle surface will be enhanced. The respiratory system, blood, central nervous system (CNS), gastrointestinal (GI) tract, and skin have been shown to be targeted by nanoparticles.

Respiratory system

One of the most important portals of entry and organ target for nanoparticles is the respiratory system. It is well known

that lungs are easily exposed to atmospheric pollutants such as PM and many other products of thermodegradation. In this regard, combustion-derived nanoparticles have been largely studied as a possible etiologic factor for several adverse health effects, including exacerbations of airways disease as well as deaths and hospitalization from cardiovascular disease.[42,43] One of the main mechanisms of lung injury caused by combustion-derived nanoparticles is via oxidative stress leading to activation of different transcription factors with upregulation of proinflammatory protein synthesis.[44] In fact, activation of mitogen-activated protein kinase and nuclear factor-kappa B signal pathways by combustion-derived nanoparticles can culminate in transcription of a number of proinflammatory genes such as *IL-8*, *IL-6*, and *TNF-a*.[45-47] Aerosol therapy using nanoparticles as drug carrier systems is becoming a fashionable method to deliver therapeutic compounds.[48] It has been found that nanoparticles can induce increased lung toxicity compared to larger particles with the same chemical composition at equivalent mass concentration. In addition, it has been also shown that nanoparticles of different diameters can induce inflammatory reactions in the lungs of experimental animals.[49-51] In fact, a significant correlation between the surface area of nanoparticles and the induced inflammation was observed via increased oxidative stress. In addition, nanoparticle-induced proinflammatory reactions have been demonstrated in several *in vitro* models of exposure.[52] Therefore, these results indicate that nanoparticles can lead to inflammatory and granulomatous responses in lungs and this could have important implications for human risk assessment. Interestingly, nanoparticles could avoid normal phagocytic defenses in the respiratory system and gain access to the systemic circulation or even to the CNS. Once inhaled and deposited, nanoparticles can translocate to extrapulmonary sites and reach other target organs by different mechanisms. The first mechanism involves passing of nanoparticles across epithelia of the respiratory tract into the interstitium and access to the blood stream directly or via lymphatic pathways, resulting in systemic distribution of nanoparticles. It showed for the first time that nanoparticles can be rapidly observed in rat platelets after intratracheal instillation of particles of colloidal gold (30 nm).[53] It was also found that inhaled (99 m) Tc-labeled carbon particles (100 nm) pass to the blood circulation 1 min after exposure.[54] In contrast, it was also not found an accumulation of the same radiolabel in the liver after exposure.[55] It was previously found that mixed carbon nanoparticles and nanotubes, both MWCNT and SWCNT, are able to induce platelet aggregation *in vitro* and, in addition accelerate the rate of vascular thrombosis in rat carotid artery.

Furthermore, it has been found that nanoparticles can directly induce cytotoxic morphological changes in human umbilical vein endothelial cells, induction of proinflammatory responses, inhibition of cell growth, and reduction of endothelial nitric oxide synthase.[55] Inhibition of cell function and induction of apoptosis have also been reported *in vitro* in kidney cells treated with SWCNT.[56] The translocation of nanoparticles to CNS may take place by the uptake of nanoparticles by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to ganglionic and CNS structures. In addition, nanoparticles can be taken up by the nerve endings of the olfactory bulb and translocated to the CNS. It has been found that C60 fullerenes can induce oxidative stress in the brain of largemouth bass via the olfactory bulb.[57] Recent studies have indicated that this translocation pathway is operational for inhaled nanoparticles. It has been shown that the exposure of rats to 13C ultrafine particles (35 nm) for 6 h resulted in a significant increase of 13C in the olfactory bulb on day 1 and this increase was even greater on day 7 post-exposure. This result contrasts with 15-day inhalation of larger-sized MnO_2 particles in rats (1.3 and 18 mm median diameter) where no significant increase in olfactory Mn was found.[58] The latter observation could have been expected, given that the individual axons of the fila olfactoria (forming the olfactory nerve) are only 100-200 nm in diameter. However, there are substantial differences between humans and rodents and therefore, these results should be interpreted with caution. In humans, the olfactory mucosa comprises only 5% of the total nasal mucosal surface, whereas in rats this amounts to 50%. Interestingly, human studies have shown that elevated levels of Mn could be associated with increased rate of Parkinson's disease.[59] Recently, it has been found that exposure of PC-12 neuroendocrine cell line to nano-sized Mn induced an increase in reactive oxygen species and dopamine depletion.

Gastrointestinal tract and skin

Other portals of nanoparticles entry in the body are GI tract and skin. Nanoparticles can be ingested into the gut by many ways, but inhaled nanoparticles can also be ingested by GI tract once they are cleared by respiratory tract.[60] It is known that the kinetics of particle uptake in GI tract depends on diffusion and accessibility through mucus, initial contact with enterocytes, cellular trafficking, and post-translocation events. The smaller the particle diameter is, the faster they could diffuse through GI secretion to reach the colonic enterocytes.[61] Following uptake by GI tract nanoparticles can translocate to the blood stream and distribute all over the body.[62] As with lungs, GI tract is easily exposed to stimuli that can induce an inflammatory response. Inflammatory bowel disease (IBD) that includes both ulcerative colitis and Crohn's disease (CD) is an inflammatory chronic condition whose etiology remains still unclear. However, several lines of evidence suggest that IBD can result from a combination of genetic predisposition and environmental factors.[63] However, yet no studies published to date showed direct toxicological effects of nanoparticles in GI tract.

CONCLUSIONS

Nanomaterials and nanoparticles are likely to be cornerstones of innovative nanomedical devices to be used for drug discovery and delivery, discovery of biomarkers, and molecular diagnostics. As nanoparticles may also exert toxicological effects, thus development of novel nanoparticles for pharmacology, therapeutics, and diagnostics must proceed in tandem with assessment of any toxicological and environmental side effects of these particles. The diversity of engineered nanoparticles and of several possible side effects represents one of the major challenges for nanopharmacology and therapeutics. Modern medical instruments alter the human body that would have been hard for people to imagine a hundred years ago. In the future, nanobiotechnology will alter the human body (on a nanoscale) in ways that we cannot now imagine.

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