IMPLICATIONS AND EFFECTS OF NANOTECHNOLOGIES IN MEDICAL RESEARCH

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Nanotechnology is a broad description that is given to processes and technologies used to produce materials which are purposely engineered through the manipulation of atoms. There are four basic categories of nanoscale materials that may need to be regulated. The metal oxides—such as ceramics from oxides of zinc, iron, cerium, and zirconium; chemical polishing agents from semiconductor wafers; scratch resistant coatings for glass. Another group of nanomaterials used in commerce is nanoclays that improve strength, harness, heat resistance and flame retardancy of materials and are used to produce barrier films in plastic beverage bottles, paper juice cartons, and tennis balls. The third group is nanotubes that are used in coatings to dissipate and minimize static electricity in fuel lines and hard disk handling trays; they can also be found in electrostatically paintable car exterior components, flame-retardant fillers for plastics, and field emitter sources in flat panel displays or even for neuronal cells repairs. The fourth group is quantum dots used in exploratory medical diagnostics and therapeutics and self assembly of nanoelectronic structures. Five types of NT applications are known: drugs (intelligent delivery systems; gene therapy; nano-bio robots), medical devices (Sensors, new systems and material for imaging), biologics, cosmetics and food (Materials in tissue engineering for improved biocompatibility and/or for biofunctionality; systems for implants). This paper will discuss pro and contra aspects of nanomaterials and advanced nanotechnologies in medical applications.

Key Words: nanomaterials; nanoscale materials; nanotechnology; risk assessment; toxicology.

1. Introduction

A first definition equates the term nanotechnology with a material which at atomic, molecular, or macromolecular levels, in the length scale of approximately 1–100 nanometer range. Additional definitions vary in terms of specifying the size dimensions of nanoscale materials, with some including sizes over 100 nm, and others identifying nanoscale materials as "those which have structured components with at least one dimension less than 100 nm". Due to their size, nanoscale materials often exhibit unique physical/chemical properties and can impart enhancements to engineered materials, including better magnetic properties, improved electrical and optical activity, and increased structural integrity. In addition, these materials are often much more reactive than their bulk material counterparts as a result of their larger surface area. The current advances of nanotechnology in modern medicine are presented and discussed in this paper and comparative effects of them. The development of specific guidance documents at a European level for the safety evaluation of nanotechnology products in medicine is strongly recommended and the need for further research in nanotoxicology, is identified. Ethical and moral concerns also need to be addressed in parallel with the new developments.
2. Type of nanomaterials in nanomedicine

While most of the existing studies on manufactured nanoscale materials have focused on a very limited range of materials, including carbon nanotubes, fullerenes, and quantum dots, the data derived from these studies provides useful information for evaluating the potential human health and environmental effects that could result from exposure to nanoscale materials.

There are different kinds of nanoparticles which are suitable to be applicable in drug- and gene- delivery, probing DNA structures, etc, and are categorized as: liposomes, polymer nanoparticles (nanospheres and nanocapsules), solid lipid nanoparticles, nanocrystals, polymer therapeutics such as dendrimers, fullerenes (most common as C60 or buckyball, similar in size of hormones and peptide a-helices), inorganic nanoparticles (e.g. gold and magnetic nanoparticles).

Fullerenes (Fig.1), a type of nanoscale material that could have utility in several areas, including in the development of novel drug delivery systems, have been shown to induce oxidative stress in juvenile largemouth bass. Exposure to uncoated C60 fullerenes caused lipid peroxidation in the brain tissue of juvenile largemouth bass. While the specific mechanism(s) responsible for this effect was not clear, it was postulated to be associated with a selective transport mechanism from the olfactory nerve into the olfactory bulb.

These data underscore the need to develop a more comprehensive understanding of the translocation specificity of fullerenes, and other lipophilic nanoscale materials, into lipid-rich tissues and cellular organelles and any subsequent effects on biological function. Additional studies are needed to determine the reversibility of the observed translocation, and to determine whether similar effects would be seen in humans. The characteristics of the underlying nanoscale material therefore must be understood in combination with the relevant surface coating or treatment.

Carbon nanotubes (diameter of 1-20 nm, as shown in Figure 2) and inorganic nanowires exhibit extraordinary mechanical, electric, electronic, thermal, and optical properties offering the electronic industry properties that few materials platforms could ever hope to match. Carbon nanotubes, and magnetic iron oxide nanoparticles, gold-coated silica nanoshells, can transform electro-magnetic energy into heat, causing a temperature increase lethal to cancer cells merely by increasing the magnetic field or by irradiation with an external laser source of near infra red light at the very location where these nanoparticles are bound to or internalised within tumour cells.

Quantum dots (nanometer sized semiconductor nanocrystals with superior fluorescent properties that possess remarkable optical and electronic properties. They can be made to emit light at any wavelength in the visible and infrared ranges, and can be inserted almost anywhere, including liquid solution, dyes etc. Quantum
dots can be attached to a variety of surface ligands, and inserted into a variety of organisms for in-vivo research.

**Figure 2.** Structure of some nanomaterials: (a) carbon nanotubes; (b) quantum dots inside of human breast cancer cells; (c) dendrimers

**Dendrimers** (complex almost spherical macromolecules with diameter 1-10 nm, shown in Fig.2c have improved physical, chemical, and biological properties compared to traditional polymers. Some unique properties are related to their globular shape and the presence of internal cavities offering the possibility as medical nanovehicles. Dendrimers have a tree-like structure with many branches where a variety of molecules, including drugs can be attached. Less than 5 nm in diameter, dendrimers are small enough to slip through tiny openings in cell membranes and to pass vascular pores and tissues in a more efficient way than bigger polymer particles.

**Calixarenes** which exist in a “cup” like shape rigid conformation with a defined upper and lower rim and a central annulus, enabling them to act as host molecules. By modifying either the upper and/or lower rims it is possible to prepare new functionalized calixarenes (Fig.3). New synthetic calixarenes for the evaluation of their photodynamic activity - p-sulfonato-calix[6]arene (calix[6]) and p-sulfonato-calix[8]arene (calix[8]), have been studied. Discovered in the 1940’s, [1n]metacyclophanes with the common name calix[n]arenes which is derived from the molecule's shape, enjoyed a remarkable interest in almost all fields of chemistry since the 1980’s and recently in biomedical application.

**Fig.3** The calixarenes structure

Some calixarenes are able to react with C60 forming a complex 1:1, Fig. 4 or even with porphyrin (Fig.5).
3. Molecular medical machines and devices

In the medical context, nanoscience is expected to facilitate the development of, among other things: improved pharmaceutical products; implantable materials for tissue repair and replacement; implantable devices (including sensing devices, implantable medical devices and sensory aids; improved surgical tools; improved diagnostic imaging methods.

3.1. Implantable devices (including sensing devices, implantable medical devices and sensory aids).

Novel biocompatible materials can be used to make permanent implants or temporary structures that can be reabsorbed by the body following surgery. For example, bone and dental implants can be made from biocompatible nano-materials characterized by their increased surface area and improved adhesion characteristics. Tissue regeneration scaffolds made from nanomaterials are being developed with a view to growing a variety of complex human organs. In addition, biodegradable polymers can be used to make surgical sutures and orthopedic fixation devices that are designed to biodegrade at appropriate rates to facilitate bone healing in a variety of circumstances. One possible application is that a mesh device may be infused with antibiotics, painkillers and/or other medications and implanted around the heart muscle during surgery. Smart materials may include polymers that can mimic muscle contraction or hydrogels that dissolve according to body chemistry to deliver drugs as needed. Microprocessors and miniature devices can be paired with sensors to diagnose disease, transmit information and to administer treatment automatically (and remotely) if required. Implantable sensors can be used to detect a vast array of chemical or physical properties.

It is anticipated that nanotechnology will inspire an array of improved surgical tools that will allow surgeons to operate on human subjects with greater precision and safety and to monitor patients more accurately. Nanotechnology is being used in the development of smart instruments and surgical robotics for use
in laparoscopic or “minimally invasive” surgical procedures. Smart instruments can be made with an ability to interpret the in-vivo surgical terrain and assist the surgeon in performing surgical procedures. Robotic systems are already being used to give surgeons remote control over highly precise instruments that are inserted into laparoscopic ports in the patient.

3.2. Medical imaging

Nanotechnology is also spawning a new wave of innovation in the area of medical imaging. For example, nanoparticle probes are being developed for use in magnetic resonance imaging (MRI). Magnetic nanoparticles can be simultaneously attached to antibodies that specifically bind to known antigens on cancer cells26 or other molecules of biological interest (i.e. fibrin27 and labeled with a dye that can be visualized on MRI images. Following administration of labeled nanoparticles, images can be taken to assess a patient’s tumour burden. Cancer therapy can similarly be specifically targeted to cancer cells in-vivo. Magnetic nanoparticles can also be targeted to proteins or other molecules of biological relevance and used in functional MRI imaging to gain insight into a variety of human disease processes. Radiolabelled carbon nanoparticles have been used for over 15 years to assess lung ventilation in human patients. A variety of miniaturized wireless medical devices are being developed that can provide high quality images that are not possible with traditional imaging devices. Pills are being developed that contain miniature video recording devices. Researchers are attempting to develop miniature x-ray devices that can be inserted into the human body.

3.3. Drug-delivery systems

First, gold nanoparticles (3-20 nm), that are gold composites with dielectrical cores and golden shells. By choosing the right ratio of core to shell diameters the particle can be tuned to absorb highly in the near infrared, and by irradiation with such wavelength can be heated, even in deeper skin areas. If the particles are embedded in a temperature sensible hydrogenlmatrix, the matrix will collapse and the included agents will be released at a critical temperature.

Second, magnetic nanoparticles, with controllable sizes between 2-30 nm that can be coated with biological molecules to make them interact with or bind to a biological entity. Due to their magnetism they can be manipulated by an external magnetic field gradient, thereby providing a controllable means of “tagging” or addressing the biological entity. They can be made to deliver a package (an anticancer drug, or a cohort of radionuclide atoms) to a targeted region of the body. The magnetic particles can be provided with energy from the exciting external field, and can be heated up making them good hyperthermia agents, delivering toxic amounts of thermal energy to targeted bodies, such as tumours. The magnetic nanoparticles can exist as usual or can be prepared as Fe₂O₄, Fe₃S₄, γ-Fe₂O₃, ferrites (MeO•Fe₂O₃ unde Me = Ni, Co, Mg, Yn, Mn....), Fe, Ni. They can functionalized as vehicles of Fe₂O₄, sometimes porphyrins either alone or in PVA hydrogels.

For applications to medicine and physiology, these nanomaterials, nanoparticles and devices can be designed to interact with cells and tissues at a molecular (i.e., subcellular) level with a high degree of functional specificity. Molecular imaging should detect the corresponding molecular signatures of diseases and use it for medical diagnosis. This should ideally lead to diagnose and therapy before occurrence of symptoms. In
molecular imaging, an imaging molecule is coupled to a transport molecule or particle, which possesses a targeting unit (e.g. special receptors, ligands or peptides). The target finding system should be a specific molecular marker of a certain disease thus the contrast medium accumulates within the sick tissue. Molecular imaging is developed for several diagnostic procedures such as magnetic resonance, ultrasonic imaging, as well as nuclear and optical imaging technologies.

3.4. “Nanorobots” and nanodevices:

Such future devices are, for example, the artificial mechanical red blood cell or “respirocyte” (spherical shape of 1Å–m diameter) and an artificial mechanical white blood cell of microscopic size, called a “microbivore” (3.4 Å major axis diameter and 2.0Å minor axis diameter). The “respirocyte” is expected to be able to deliver more oxygen to the tissues than natural red blood cells and to manage carbonic acidity. Primary medical applications of respirocytes would include transfusable blood substitution; partial treatment for anemia, lung disorders, enhancement of cardiovascular/neurovascular procedures, tumor therapies and diagnostics, prevention of asphyxia, artificial breathing, and a variety of sports, veterinary and battlefield. The primary function of “microbivore” is to destroy microbiologic pathogens found in the human bloodstream using a digest and discharge protocol. Nanorobots are nanodevices that will be used for the purpose of maintaining and protecting the human body against pathogens. They will have a diameter of about 0.5 to 3 microns and will be constructed out of parts with dimensions in the range of 1 to 100 nanometers.

Medical “nanorobots” may also be able to intervene at the cellular level, performing in-vivo cytosurgery. The most likely site of pathologic function in the cell is the nucleus — more specifically, the chromosomes. In one simple cytosurgical procedure called “chromosome replacement therapy”, a “nanorobot” controlled by a physician would extract existing chromosomes from a particular diseased cell and insert new ones in their place, in that same cell. If the patient chooses, inherited defective genes could be replaced with non defective base-pair sequences, permanently curing a genetic disease.

The controlled aggregation of sulphonated porphyrins as \((\text{H2TPPS4}^-)\) with a size of 5-6 nm in solution was tested for neurons and glial cells. The application of medical nanorobots for brain aneurysm is presented in this work on dissociated cortical cultures from mice. Neurons and glial cells were incubated for several days to investigate the effectiveness of the method. Connections between the islands are clearly apparent and interconnected networks are formed following the exact pattern of the NT (nanotubes) templates. The bridging consists either of an axon or bundles of axons and dendrites. In some cases the bridge is covered with clusters of cells. These bridges form very efficiently over quartz surfaces which are apparently very poor surfaces for cell attachment.

![Fig.6. Neuronal cells before (a) and after PDT treatment with TSPP nanotubes 20 microg/ml, laser 440 nm (b,c)]
Fig. 6 shows the evolution of a networkover after laser irradiation three days after incubation and irradiation with different power (7 mW and 25 mW). The data show that cells first aggregate at the NT islands. As they complete this step axons and dendrites begin to form and to build connections, Fig.7.

![7 mW – 900 s TSPP](image1)

![25 mW – 900 s TSPP](image2)

Fig. 7. The microscopy images of neuronal cells at 7 mW irradiation power (left) and 25 mW (right) during PDT treatment with TSPP nanotubes 40 microg/ml

### 3.5. Nanotechnology against Cancer

Nanotechnology may have an impact on the key challenges in cancer diagnosis and therapy. Diagnosing, treating, and tracking the progress of therapy for each type of cancer has long been a dream among oncologists, and one that has grown closer thanks to parallel revolutions in genomics, proteomics and cell biology. Nanotechnology’s greatest advantage over conventional therapies may be the ability to combine more than one function. Recently, there is a lot of research going on to design novel nanodevices capable of detecting cancer at its earliest stages, pointing it’s location within the human body and delivering chemotherapeutic drugs against malignant cells. The major areas in which nanomedicine is being developed in cancer involve: a) early detection of tumor (developing “smart” collection platforms for simultaneous analysis of cancer-associated markers and designing contrast agents that improve the resolution of tumor area comparing with the nearby normal tissues), and b) cancer treatment (creating nanodevices that can release chemotherapeutic agents).

Tumour diagnostics and prevention is the best cure for cancer, but failing that, early detection will greatly increase survival rates with the reasonable assumption that an in situ tumour will be easier to eradicate than one that has metastasized. Nanodevices and especially nanowires can detect cancer-related molecules, contributing to the early diagnosis of tumour. Nanowires having the unique properties of selectivity and specificity can be designed to sense molecular markers of malignant cells. They are laid down across a microfluidic channel and they allow cells or particles to flow through it.

### 3.6. Nanoparticle contrast agents

Labeled and non-labeled nanoparticles are already being tested as imaging agents in diagnostic procedures such as nuclear magnetic resonance imaging. Such nanoparticles are paramagnetic ones, consisting of an inorganic core of iron oxide coated or not with polymers like dextran. There are two main groups of nanoparticles: 1) superparamagnetic iron oxides whose diameter size is greater than 50 nm, 2) ultrasmall superparamagnetic iron oxides whose nanoparticles are smaller than 50nm. Moreover, quantum dots being the nanoscale crystals of a semiconductor material such as cadmium selenide, can be be used to measure
levels of cancer markers such as breast cancer marker Her-2, actin, microfibril proteins and nuclear antigens. Tumour treatment can be succeeded with nanoscale devices (such as dendrimers, silica-coated micelles, ceramic nanoparticles, liposomes). These devices can serve as targeted drug-delivery vehicles capable of carrying chemotherapeutic agents or therapeutic genes into malignant cells. It is worthwhile to mention that selective delivery and targeting of nanoparticles to tumours may overcome the problem of toxicity and may increase the effectiveness of drug delivery. The barriers involving this procedure and that should be under consideration, are a variety of physical and anatomical characteristics of solid tumours, such as the necrotic core with the surrounding hypoxic area, the elevated local temperature and the interstitial liquid pressure. The vascular permeability of the tumour influences the retention of intravenously administered nanoparticles.

4. Adverse effects

Multi-walled carbon nanotubes (MWCNTs) were recently examined for effects that could result from dermal exposure in workers and consumers. Simple in vitro tests using human epidermal keratinocytes indicate that MWCNTs could induce a cytokine-mediated inflammatory response, and localize in cytoplasmic vacuoles, in mammalian systems. Such results could be evaluated more comprehensively in vivo to elucidate the clinical significance of these in vitro effects.

Respiratory exposure to nanoscale materials such as carbon nanotubes has also received a considerable amount of interest. Because of the difficulties associated with generating aerosols of carbon nanotubes to facilitate an evaluation of inhaled material, and the high cost of homogeneous well-characterized material, several in vivo pulmonary studies have employed intratracheal instillation as the exposure methodology. Single-walled carbon nanotubes (SWCNTs) have been evaluated in rodents using this technique. SWCNTs instilled into the lungs of mice produced granulomas in the pulmonary interstitium of the lungs. Rats exposed to SWCNTs developed multifocal granulomas in the absence of any pulmonary inflammation or cellular proliferation which suggests that SWCNTs may act via a different mechanism of toxicity than other inhaled toxicants such as crystalline silica.

5. Selective references

5. Ion, RM, D.Boda, Rev.Chim. (Bucharest), 53(2), 205-207(2008);
6. S.Patachia, S.Varga, Ion, RM, Pollack Periodica, 2(2) 131-140 (2007);
10. RM Ion, DV Brezoi, I.Udrea, 57(8), pp. 886-889(2006);
11. RM Ion, DV Brezoi, JOAM, 4, 936-939, 2007;