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'Green Nanomaterial'-How Green they are as Biotherapeutic Tool

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Abstract

The emergence of nanoparticles (NPs) has attracted tremendous interest of the scientific community for decades due to their unique properties and potential applications in diverse areas, including drug delivery and therapy. These opportunities are based on the unique properties (e.g., magnetic, optical, mechanical, and electronic) that vary continuously or abruptly with changes in the size of the materials at the nanoscale. Advances in nanotechnology have significantly impacted the field of therapeutics delivery. Although the impressive progress made in the design of disease-targeted NPs allows new treatments with improved specificity, only a few NP-based medicines have reached the market. There is a need for a new discipline-nanotoxicology-that would evaluate the health threats posed by nanoparticles, and would enable safe development of the emerging nanotechnology industry related to biotherapy. Green Nanotechnology gives the opportunity in lowering the risk of using nanomaterials, limiting the risk of producing nanomaterials, and using nanomaterials to lower the risk of producing unwanted chemical intermediates and end-products.

Keywords: Green chemistry; Nanoparticle; Biotherapy; Nanotechnology; Drug delivery

Introduction

Nanotechnology is a relatively new discipline. It is the latest hype of modern technology and has applications in several human disease-related problems. Studies at the nanoscale were enabled by the practical development of instruments in the 1980s by talented visionaries including but not limited to Binnig, Roher, Gerber, and Quake working at IBM and beyond [1,2]. Nanotechnology is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale. The development of nanotechnology in its broadest and most altruistic sense seeks not only to improve the comprehension of nature through an increase in fundamental knowledge but also to provide yet another tool in tackling basic global concerns of immense moral impact. As examples, the latter include providing for a clean water supply; ensuring food security and decent human shelter; improving transportation, power, and energy systems; and reducing the weight of pollution and of greenhouse gases in industrial manufacturing processes in order to preserve a clean environment [3]. Two challenges that have slowed development have been the poor understanding of the new hazards introduced by nanotechnology and lack of appropriate policies to manage any new risks. Scientists, engineers and entrepreneurs, however, continue to move forward, grappling with challenges that range from the technical to the regulatory and everywhere in between. To this end, not surprisingly, a lot of effort has been expended towards developing nanomaterials for the destruction of environmental pollutants as well as for remediation purposes. Other groups have sought to understand the role of the structure, shape, band gap, size, morphology, and surface chemistry of the nanoparticles themselves in determining their formation and reactivity in a range of aquatic, air, and soil environments. There is an unusual opportunity to use science, engineering and policy knowledge to design novel products that are benign as possible to human and environment health. Recognition of this opportunity has led to the development of the "green nanoscience" concept [1,2]. Green nanotechnology has drawn on the field of green chemistry, and the framework of the 12 Principles of Green Chemistry [3] features significantly in work to design new nanotechnologies for joint economic, social, and health/environmental benefit [4].

Specifically, Green Nanotechnology encourages collaborations and enables a vision that uses scientific research to fundamentally move toward sustainability. In terms of rendering nano-manufacturing processes less energy and resource intensive, the principles of Green Chemistry applied to nanotechnology involve, if at all possible, (a) the use of cost-effective, nontoxic precursors; (b) minimization of carcinogenic reagents and solvents (if possible, through utilization of aqueous solvents in order to bypass potentially toxic, acidic, or basic analogues); (c) reduction of experiments carried out with either pyrophoric compounds or unstable precursors to avoid risk; (d) use of relatively few numbers of reagents, i.e. atom economy, coupled with a conscious effort to circumvent the generation of greenhouse gases; (e) minimization of reaction steps leading to a reduction in waste, reagent use, and power consumption; (f) development of reactions to generate high-purity materials with little if any byproducts through high-yield processes; (g) ambient temperature and pressure synthesis, if at all possible, so as to preclude the need for either vacuum or high temperature processes; and (h) efficiency of scale-up [5-7]. Parallel efforts in developing adequate characterization facilities as well as in promoting computer-aided nanomaterials modeling and process design tools have been of utmost importance in furthering all of these 'green' objectives with the aim of creating environmentally benign building blocks and reducing the environmental footprint of manufacturing protocols [8,9]. Optimally, green nanomanufacturing should use less material, less water, and less energy, and produce less waste as compared with conventional processes. Green Nanotechnology is based on lowering the risk of using nanomaterials, limiting the risk of producing nanomaterials, and

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using nanomaterials to lower the risk of producing unwanted chemical intermediates and end-products.

Green Nanotechnology and Bio-Therapeutics

Greener synthesis and naomaterials

Green chemistry is "the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture, and application of chemical products". The 12 principles of green chemistry (originally defined by Anastas and Warner [10] and summarized in (figure 1) have now been applied to the design of a wide range of chemical products and processes with the aims of minimizing chemical hazards to health and the environment, reducing waste, and preventing pollution. Application of these principles has reduced the use of hazardous reagents and solvents, improved the material and energy efficiency of chemical processes, and enhanced the design of products for end of life. Employing these principles toward nanoscience will facilitate the production and processing of inherently safer nanomaterials and nanostructured devices.

Nanoparticles or other nanomaterials that exhibit size and shape dependent properties are already finding application in products ranging from consumer healthcare products to high performance composites [11]. In addition, a growing number of applications of nanoscience/nanotechnology are being developed that promise environmental benefit, including new catalysts for environmental remediation [12], cheap and efficient photovoltaics [13], thermoelectric materials for cooling without refrigerants [14], lightweight (and thus energy-conserving) nanocomposite materials for vehicles [15], miniaturized devices that reduce material consumption, and sensors that eliminate the need for (often) wasteful wet-chemical analyses. Nanoscale sensors [16] can also offer faster response times and lower detection limits, making on-site, real-time detection possible. New manufacturing strategies that are additive, rather than subtractive, such as functional group directed processes involving self-assembly can reduce energy requirements and waste generation. The use of self-assembly methods also enables materials disassembly, incorporating a potential design for end-of-life. To realize new nanotechnologies that pose little harm to human health and to develop technologies that can be used to improve or protect the environment, it is desirable to design and use greener nanomaterials and develop greener nanoproduction methods.

Green nanoscience/nanotechnology involves the application of green chemistry principles to the design of nanoscale products, development of green nanomaterial production methods, and application of green nanomaterials (Figure 2). This approach aims to develop an understanding of the properties of nanomaterials, including those related to toxicity and specially ecotoxicity, and to design nanoscale materials that can be incorporated into high-performance products that are safer to human health and the environment. It strives to discover synthesis/production methods that eliminate the need for harmful reagents and enhance the efficiency of these methods, while providing the necessary volume of pure material in an economically viable manner. It also provides proactive design schemes for assuring the inherently safer nanomaterials by assessing the biological and ecological hazards in tandem with design. Finally, it seeks applications of nanoscience that maximize

Figure 1: *Green chemistry principles.*

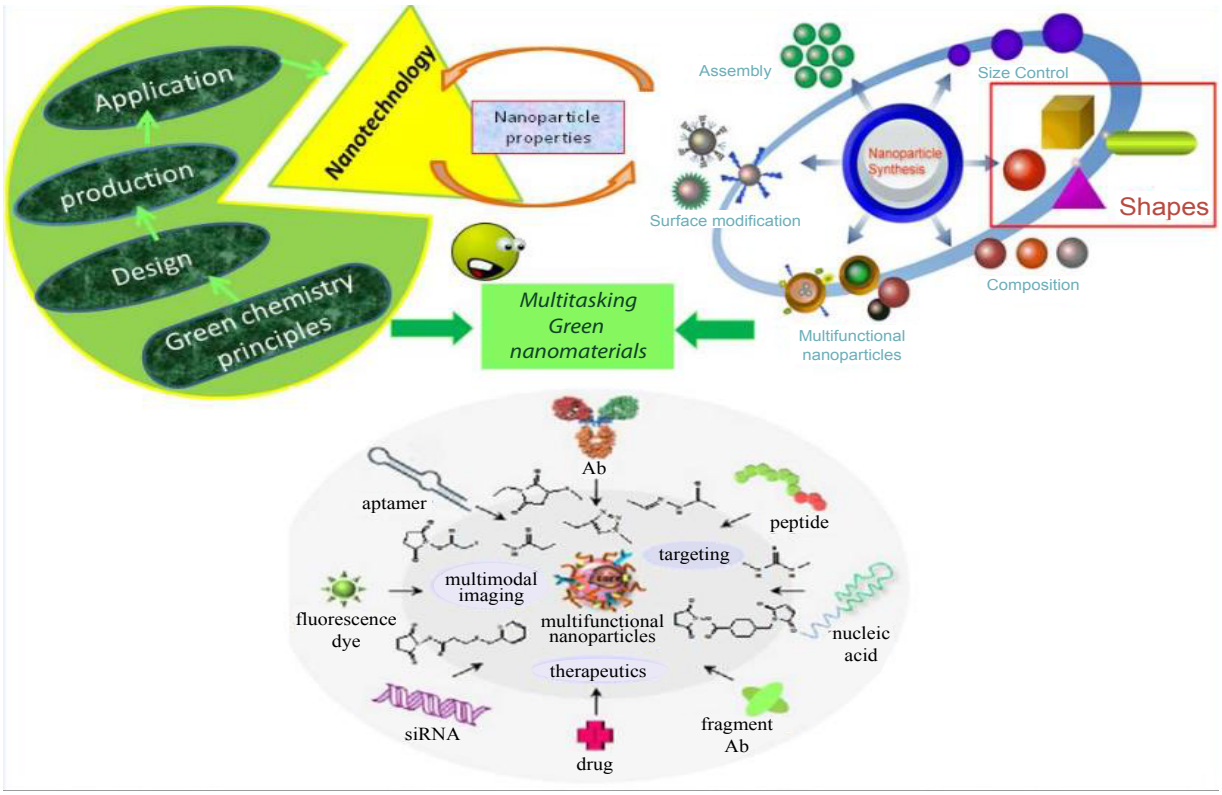

Figure 2: Schematic illustration of multifunctional nanoparticle.

societal benefit while minimizing impact on the ecosystem. In this way, green chemistry principles in combination with nanoscience guides the development, processing, and application design of green-nanomaterials throughout the life cycle, starting with raw material selection through end-of-life.

Green Nanomaterials in therapeutic use

Current clinical diagnostics and therapeutics platforms are often limited by borderline sensitivity or efficacy levels. These limitations result from low or minimal specificity for the intended target cell or organ, span a multitude of physiological disorders and result in nominal success rates for diagnosis or treatment in many cases. Diagnosis and treatment of diseases such as cancer or viral infections require next generation medical methods. Nanotechnology has the potential to significantly address diagnostics and therapeutics sensitivity and resulting unwanted side effects by providing extremely precise reagents and tools that allow for unparalleled detection and treatment at the clinical level.

Advances in green-nanotechnology and molecular biology are rapidly enabling the development of multifunctional NPs with different specific functional properties for better disease diagnosis and therapy (Figure 3). Many nanosyntheses processes have been developed in recent years, in an effort to produce structures that have a specific form and function relevant to a given application. The preparation of functionalized nanoparticles within a green context poses interrelated challenges in terms of maintaining product integrity (such as structure, shape and size disparity, functionality, purity and stability) while employing greener methods whenever possible. For example, control over particle size and disparity may reduce purification requirements by eliminating the need for extensive separations, while the ability to control surface functionalization, intended to enhance particle stability and dictate surface chemistry, solubility, and the degree of particle interactions, helps to better define the safety and reactivity of nanoparticles. Nanosynthesis methods are being refined in such a way that they are convenient and scalable, whether it involves the direct synthesis of a functionalized material or the preparation of a versatile precursor particle whose surface properties can be easily modified to meet the demands of a given application. This is accomplished through extremely controlled nanofabrication methodologies which result in the generation of molecularly defined nanoscale materials and devices that harbor known physical properties unique to each material in question and useful for particular medical applications. Concurrently, thanks to careful nanostructure construction (tailored drug and gene release characteristics, and low immunogenicity, etc.), NPs are being developed as drug and gene carriers, improving treatment efficacy and reducing side effects. The imaging, delivery facilities and other functions have been integrated during NP formulation, enabling simultaneous in vivo diagnostic imaging and drug or gene delivery for real-time treatment tracking.

This further precise targeting of these materials to specific sites within the body allows for an added layer of accuracy and potency. Research in this area is quickly advancing to the point of providing a comprehensive portfolio of green-nanotechnology-based diagnostic and therapeutic platforms (Figure 4) that will be unparalleled in sensitivity, specificity and elimination of unwanted side effects.

Therapeutic nanoparticle platforms

Nanoshells: Nanoshells are nanoparticle beads that consist of a silica core coated with a thin gold shell [17]. Manipulation of the thick-

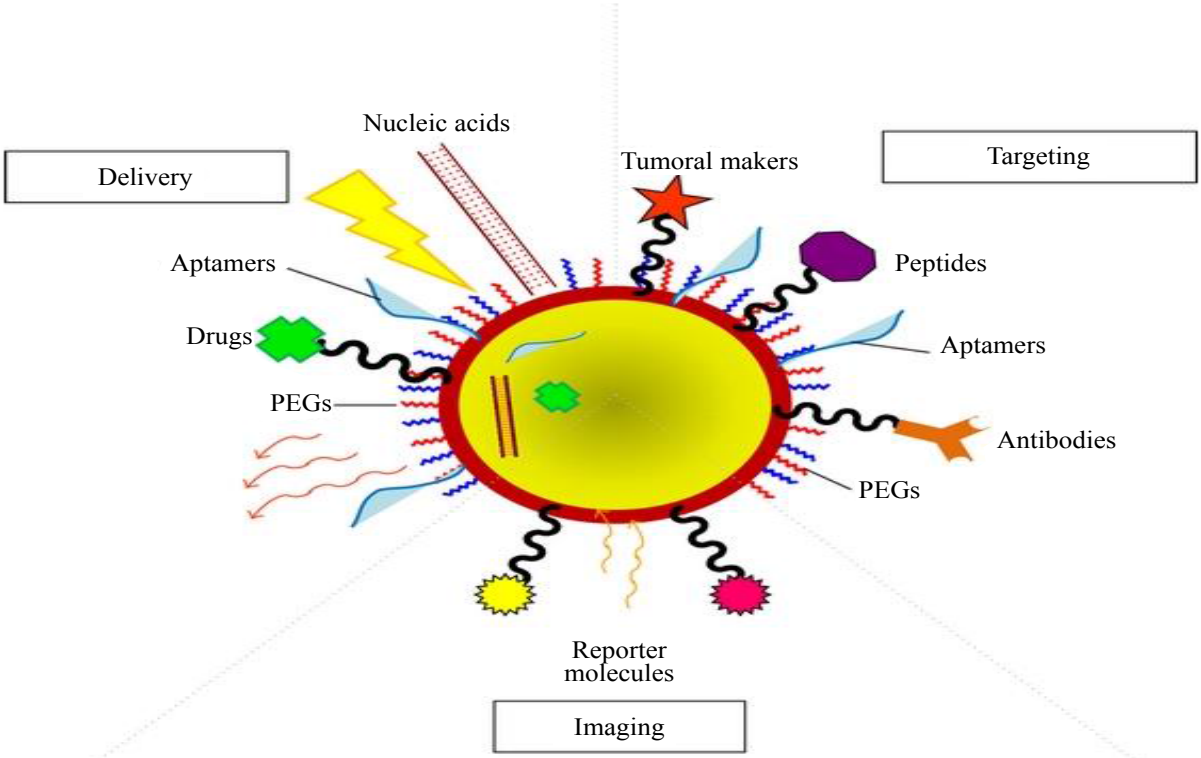

Figure 3: Schematic illustration of green chemistry principles combined with unique nanoparticle properties to design, develop and apply green nanomaterials

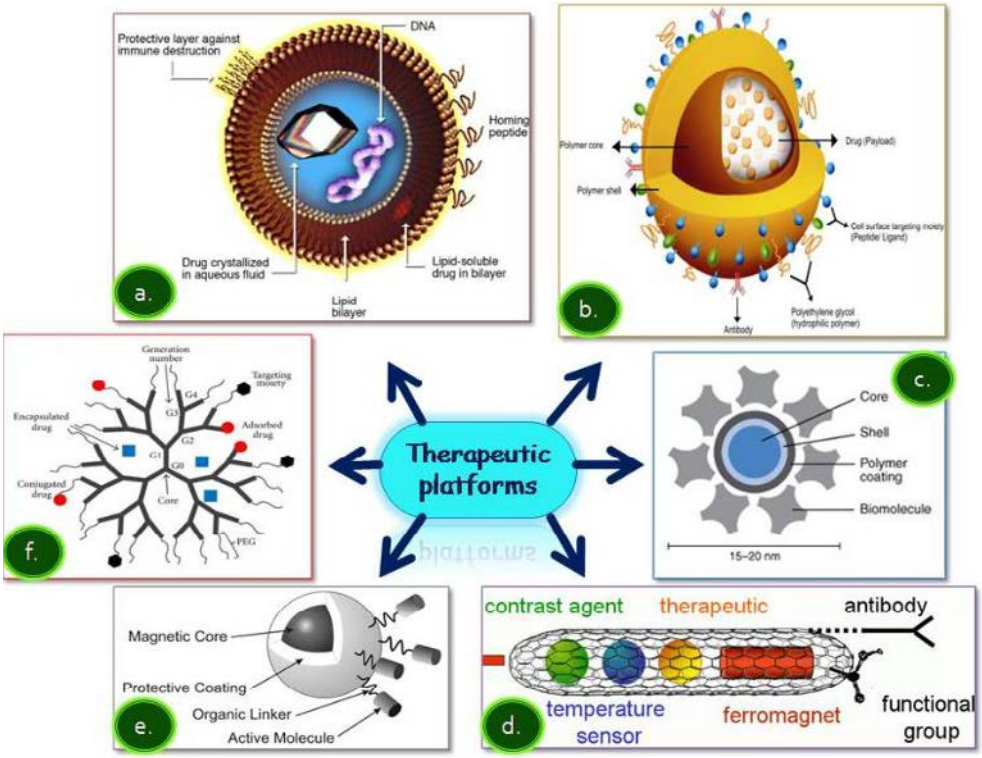

Figure 4: Schematic illustration of different therapeutic platforms; a. liposome, b. polymeric nanoparticle, c. nano shell, d. carbon nanotubes, e. magnetic naoparticles, f. dendrimer.

ness of the core and the outer shell permits these beads to be designed to absorb and scatter specific wavelengths of light across the visible and near-infrared (NIR) spectrum. Their primary application is in thermal ablation therapy by exploiting their ability to absorb light. Meanwhile, their ability to scatter light has potential for cancer imaging. The most useful nanoshells are those that have a silica core diameter of ~120 nm with a 10 nm layer of gold shell, because these strongly absorb NIR light (~800 nm) and can create intense heat that is lethal to cells. This NIR light can penetrate several centimeters of human tissue without causing harm, because tissue chromophores do not absorb much energy in the NIR range [18].

Dendrimers: Dendrimers are spherical polymers that are normally less than 5 nm in diameter. Their key useful feature is the polymer branches that provide vast amounts of surface area to which therapeutic agents and targeting molecules could be attached. The prototypical dendrimer starts with an ammonia (NH_3) core that is reacted with acrylic acid to produce a tri-acid molecule. This molecule is then reacted with ethylenediamine to produce a tri-amine, and this is known as generation 0 (G0) products. This tri-amine is reacted with acrylic acid to produce a hexa-acid, and then reacted with ethylenediamine to produce a hexa-amine (G1), and so on. This alternation of reaction with acrylic acid then with ethylenediamine continues until the desired generation is reached. Sugars or other molecules can also be used as the starting core, so long as they have multiple, identical reaction sites. Thus, it is possible to create a surface consisting of multiple amines or multiple acids, and these two kinds of surfaces provide the means of attaching different functional components. In nanomedicine, dendrimers had been found to be an invaluable tool in attaching fluorescent dyes, enzymes cell identification tags and other molecules because of the many molecular "hooks" present on their surface and it makes them a very important medium in target drug delivery agent in therapeutics.

Liposomes: Liposomes are spherical vesicles with an aqueous core and a vesicle shell. They contain a single or multiple bilayered membrane structure composed of natural or synthetic lipids. Depending on design, they can range in size from tens of nanometers up to micrometers in size. Liposomes have been widely used as pharmaceutical carriers in the past decade because of their unique abilities to (a) Encapsulate both hydrophilic and hydrophobic therapeutic agents with high efficiency, (b) Protect the encapsulated drugs from undesired effects of external conditions, (c) Be functionalized with specific ligands that can target specific cells, tissues, and organs of interest, (d) Be coated with inert and biocompatible polymers. such as polyethylene glycol (PEG), in turn prolonging the liposome circulation half-life in vivo, and (e) Form desired formulations with needed composition, size, surface charge, and other properties [19,20]

More recently, many smart liposomes have been used for drug and gene delivery. For example, Kaiden et al. [21] fabricated a novel liposome modified with hyperbranched poly (glycidol) derivatives having N-isopropylamide and carboxyl groups, whose destabilization can be triggered by combination of temperature and pH value.

Polymer nanoparticles: Polymeric NPs are engineered from biocompatible and biodegradable polymers. Most of these NPs are formulated through a self-assembly process using block-copolymers consisting of two or more polymer chains with different hydrophilicity. These copolymers spontaneously assemble aqueous environment whereas the hydrophilic blocks form the shell to stabilize the core. The resulted structure is well suited for drug delivery. Many studies and reviews about polymer NPs for drug and gene delivery have been reported [22,23]. For example, Xu et al. [24] reported that the micelles self-assembled from amphiphilic dendritic poly(glutamic acid)-β-polyphenylalanine copolymers could release the loaded drug for 60 h and efficiently inhibit the proliferation of HepG2 liver cancer cells. However, the lack of specificity of the normal delivery systems can negate or significantly reduce the benefit. To this end, recent innovative approaches in design of polymer particles have leaded to targeted localization, changes in delivery kinetics, and triggered drug release. Therefore, functional or responsive systems are being pursued in order to improve local or systemic drug delivery, as the release of therapeutic compounds can now be tailored to respond to specific extrinsic stimuli such as temperature, light, pH and ultrasound [25]. Recently, Du et al. [26] designed a tailor-made dual pH-sensitive polymer drug conjugate NPs system for efficient anticancer drug delivery [26]. The NPs are capable of reversing their surface charge from negative to positive at tumor extracellular pH (~6.8) to facilitate cell internalization. Subsequently, the significantly increased acidity in subcellular compartments such as endosome (pH~5.0) further promotes doxorubicin (DOX) release from the endocytosed drug carriers. Besides self-assembly, other methods can formulate polymeric NPs too. For example, particle replication in non-wetting templates (PRINT) is a technique in which particles can be formulated with precise size, shape, and composition using films of tiny molds.

Inorganic nanoparticles

i. Carbon nanotubes: Carbon nanotubes are a distinct molecular form of carbon atoms that was discovered in the late 1980s. There has been tremendous enthusiasm over carbon nanotube applications in many industrial sectors, in part because they have been actively promoted as possessing the advantages of being 100 times stronger than steel with only one-sixth of its weight, and with unusual heat and conductivity properties. In the area of therapeutics, carbon nanotubes have primarily been used for transporting DNA cargoes into the cell and for thermal ablation therapy, in much the same way as the nanoshells described above. Kam et al have shown that single-walled carbon nanotubes 1 to 2 nm in diameter and carrying a cargo of 15 mer DNA adsorbed onto their surfaces can be internalized by cells and accumulate in the cytoplasm without causing cytotoxicity [27]. At 48°C, there was minimal cellular uptake of DNA-carrying carbon nanotubes, suggesting an energy dependent uptake mechanism. Exposing the DNA-nanotube containing cells to several 10 second pulses of NIR caused endosomal rupture, unloading of the DNA from the carbon nanotubes, and translocation into the nucleus. Again, the cells showed normal morphology and no apparent death under these conditions. Like nanoshells, carbon nanotubes can absorb NIR light to generate intense heat. For example, continuous irradiation with NIR (808 nm laser at 1.4 W/cm^2) for 2 minutes will heat up a 25 mg/L solution of single-walled carbon nanotubes to 708°C and lead to boiling of the solution with longer exposures [4]. Kam et al have shown that folic acid can be adsorbed onto the carbon nanotubes to allow specific binding to cancer cells that overexpress folate receptors and subsequent receptor-mediated endocytosis. Tumor cells that had internalized the folic acid–bound carbon nanotubes were selectively destroyed upon irradiation with NIR, whereas receptor-free normal cells that had not internalized these carbon nanotubes were not harmed by NIR irradiation. The localization of carbon nanotubes, and whether or not they were internalized by cells, could be visualized by attaching fluorescent tags to the carbon nanotubes. Recently, Z. Zhang et al have demonstrated that carbon nanotubes carrying short (or small) interfering RNA (siRNA) can rapidly enter tumor cells, then release the siRNA to exert RNA interference on target gene expression [28]. They have shown that the delivery of siRNA via carbon nanotubes

into tumor cells not only silenced the target gene (i.e., reduced both its mRNA and protein levels), but also inhibited the proliferation of cancer cells in vitro and suppressed tumor growth in mouse models, upon intralesional injection of siRNA-conjugated carbon nanotubes. The use of carbon nanotubes as a vehicle for delivery of drug, DNA, siRNA presents great promise.

ii. Quantum dots: Quantum dots are frequently referred to as nanocrystals. They range from 2 to 10 nm in diameter and are made of semiconductors. Quantum dots are composed of 10–50 atoms, and they confine electron-hole pairs to a discrete quantized energy level. When excited with ultraviolet light, they fluoresce in different neon colors depending on their size, which determines the energy level of the quantum dot. Larger particles emit light in the red end of the visible spectrum, whereas smaller particles emit in the blue range. When quantum dots were first developed some 20 years ago for electronics and optics, no one realized their potential for application in biomedicine. However, their use as research tools has expanded markedly in the last few years, and they are currently being used as probes for high resolution molecular imaging of cellular components and for tracking a cell's activities and movements inside the body.

Quantum dots can also be attached to various proteins and receptors to monitor with which molecules they interact and in what part of the cell they are found. Because cells are impermeable to quantum dots, they must be coated with special molecules or antibodies to facilitate their uptake by cells. This property can be exploited to devise a method that uses extracellular enzymes to modulate cellular uptake of quantum dots. Because semiconductors are poisonous heavy metals, toxicity is a huge obstacle to clinical application of quantum dots for humans. Currently, their application is restricted to in vitro or animal studies, and researchers are actively trying to develop different greener ways to coat them so that they would be safe for use in people.

iii. Super paramagnetic nanoparticles: Super paramagnetic nanoparticles refer to iron oxide particles or magnetite (Fe_3O_4) particles that are less than 10 nm in diameter. They have been around for years as contrasting agents for magnetic resonance imaging (MRI). Many groups have explored the use of magnetic fields to localize magnetic nanoparticles to targeted sites, a system known as magnetic drug targeting. Magnetic nanoparticles can be remotely activated using electromagnetic fields under the influence of an alternating field. Super-paramagnetic nanoparticles undergo Brownian relaxation in which heat is generated by the rotation of particles in the field. Most recently, super paramagnetic nanoparticles have been used in clinical thermotherapy, which has many applications such as various selective bio-separations and contrast enhancing agents for MRI in drug delivery systems; magnetic hyperthermia (local heat source in the case of tumor therapy) and magnetically assisted transfection of cells.

Challenges of targeted drug-encapsulated nanoparticles delivery

Advances in nanotechnology have significantly impacted the field of therapeutics delivery. This is evidenced by the increase in the number of nanoparticle-based therapeutic products in development over the last two decades. A 2006 global survey conducted by the European Science and Technology Observatory (ESTO) revealed that more than 150 companies are developing nanoscale therapeutics, and twenty-four nanoparticle therapeutics are currently in clinical use [29]. These drugs are being developed to treat a wide range of diseases, such as fungal or bacterial infections, HIV infections, diabetes and cancers. The use of materials on the nanoscale level provides the unprecedented freedom to modify some of the most fundamental properties of therapeutic carriers, such as solubility, diffusivity, biodistribution, release characteristics and immunogenicity. One strategy to further improve the therapeutic index of nanoparticle therapeutics is to functionalize nanoparticles with targeting ligands [30,31]. The addition of targeting ligands allows the delivery of drug-encapsulated nanoparticles to uniquely identified sites while having minimal undesired effects elsewhere. Most of the success can be contributed to targeting, as targeted therapeutics can selectively treat diseases without affecting normal tissue. There has been increasing interest in applying molecular targeting to nanoparticle therapeutics and formulate biofunctionalized targeted nanoparticles [32]. Targeted nanoparticles, when compared to non-targeted nanoparticles, have several potential advantages: the ability to partition more of the nanoparticles within target tissue, increased uptake into target cells, higher therapeutic efficacy and lower toxicity. Although there is no clinically approved targeted nanoparticle therapeutics yet, many are in preclinical and clinical development. However, several challenges remain in their development. The first key challenge lies in balancing the targeting ligand density against the antibiofouling surface of nanoparticles. Therapeutic nanoparticles require an antibiofouling surface for increased circulation uptake and decreased non-specific interaction. The addition of targeting ligands increases targeted delivery but also compromises the 'stealth' surface of nanoparticles. Therefore, targeted nanoparticles should be engineered and formulated with precise control of the targeting ligand density on their surfaces. Another challenge in formulating the optimal therapeutic carrier depends on engineering small nanoparticles that can carry a high payload. The optimal therapeutic carriers' size should be around or below 150 nm to lower liver uptake. On the other hand, the size should not be too small (less than 5 nm) since the payload will be lower and the particles may be rapidly excreted by the renal system [33]. One technique to lower nanoparticle size is the utilization of microfluidic devices in formulating nanoparticles.

Hazards of nanoparticle as biotherapeutic agents

There is heightened concern today that the development of nanotechnology will negatively impact public health, and it is indisputable that engineered nanomaterials are a source of nanoparticle pollution when not safely manufactured, handled, and disposed of or recycled. A large body of research exists regarding nanoparticle toxicity, comprising epidemiological, animal, human and cell culture studies. Compelling evidence that relates levels of particulate pollution to respiratory, cardiovascular disease, and mortality, has shifted attention to particles with smaller and smaller sizes (nanometer scale). Research on humans and animals indicates that some nanoparticles are able to enter the body, and rapidly migrate to the organs via the circulatory and lymphatic systems. Subjects with pre-existing diseases (such as asthma, diabetes, among others) may be more prone to the toxic effects of nanoparticles. Genetic factors may also play an important role in the response of an organism to nanoparticles exposure. The ability of nanoparticles to enter cells and affect their biochemical function makes them important tools at the molecular level. The toxic properties of nanoparticles can in some instances be harnessed to improve human health through targeting cancer cells or harmful bacteria and viruses. These very properties that might be exploited as beneficial may also have secondary negative effects on health and the environment. For example, nanoparticles used to destroy cancer cells may cause harmful effects elsewhere in the body, or nanoparticles used for soil remediation may have an adverse impact upon entering the food chain via microorganisms, such as bacteria and protozoa.

Very little is known about the transport of substances on nanopar-

ticulate surfaces but it will clearly depend on the nature of the surfaces. Airborne nanoparticles with surface bound substances can enter the body, and nanoparticles that contain mutagenic substances, which remain in the lungs for years, increase the risk of developing cancer [34]. For cells to be able to react to any topography they must be able to sense shape. Strong responses from cells to nanotopography have been seen even though the dimensions of these shapes are much smaller than the dimensions of the cells [35]. CNTs can easily enter into cells which are the basis for some concern and this relates to the way DNA can wrap around the nanotubes. The cellular response of murine fibroblasts to nano-scale silica topography has been investigated. The cells become rounded and do not replicate. When they are placed back onto a regular surface, their activity returns to normal spreading out and proliferating [36]. This result nicely demonstrates surface effects on living cells. A cell can sense even a very small feature of object. The interplay of cells with material at the nanometer level has been demonstrated by looking at different pit sizes on silicon substrates (35, 75 and 120 nm). Human fibroblasts placed on these surfaces interact with pits of all sizes although the response for 35 nm pits is weak. Thus cells are sensitive to surface features at the nanometer scale, and designing materials with nanoscale features need to take this into account [35] in developing sensors that use biological detecting components, care must be taken in immobilization of the detector molecules so that they preserve their shape and function and hence original recognition specificity. To do this the surface needs to be pre-coated to allow sufficiently strong binding of detector molecules and sufficiently weak and gentle perturbations so that the molecules retain their shape and functionality [37].

Surfaces of nanoparticles of zirconia can undergo reversible adsorption-desorption of DNA depending on the pH of the solutions, with basic solutions resulting in desorption [38]. This relates to a change in charge on the surface of the zirconia particles. Surface topography at the nano- and micrometer range of titanium devices critically determines cellular attachment and subsequent biological function. There is an increase of cell attachment to micron and sub-micron patterned surfaces in comparison to smooth surfaces which are attributed to pores acting as positive attachment sites [39]. Cell migration, proliferation and differentiated function are dependent on adhesion, and cell adhesion is enhanced by a high surface to volume ratio in nano-fibrous scaffolds. Synthetic nano-fibrous scaffolds which can mimic collagen feature in tissue engineering and have the benefit of overcoming possible immunogenicity and disease transmission problems [40]. Creating nano-featured surfaces is important in the biocompatibility of artificial material. Silicon-based surfaces are prone to fouling in the presence of biological fluids, but this can be overcome by grafting PEG (polyethylene glycol) of various densities on silicon. The decrease in protein adsorption on PEG surfaces is due to its hydrophilicity and ability to shield the surface from direct contact from proteins and charged particles [41]. Chitosan is a polysaccharide which is biocompatible and does not cause allergic reactions, breaking down to harmless products which are easily removed. Chitosan-based nanoparticulate drug delivery systems have the advantages of improved efficacy, reduced toxicity and improved patient compliance over standard preparations. This is a form of green chemistry in nanotechnology-utilising a renewable resource which is non-toxic. This covers carbon based nanotubes and those of other elements, binary systems and more complex systems. Little is known on the effects of exposure of nanotubes to biological environments and the release of the entrapped particles, and how this will affect the body, and the general principles of fibre toxicology as applied to nanofibres need to be determined [42]. The health hazard of nanotubes depends on the nature of the material, how easily they can become airborne, the size of the clumps or aggregates of the material, and the ability to deaggregate to form smaller particles following entry to the lungs. A further hazard is created by the presence of other small particles or metal catalysts embedded in the tubes (which may be a consequence of the method of fabrication of the tubes as in the synthesis of CNTs) [43]. Entry into biological systems by means other than respiration can occur via (i) supramolecular complexation where the nanotubes are wrapped up by material involving non-covalent interactions, or (ii) by covalent functionalisation where polar groups are attached to the surface of the material. In this way CNTs bearing water solubilising peptide groups have been shown to cross the cell membrane and to accumulate in the cytoplasm or reach the nucleus without being toxic to the cell [44]. As a comparison, graphite is biocompatible with cells, and graphite-coated plastic valves in vivo show little or no blood clotting. This has lead to the development of artificial heart valves and dental implants comprised of carbon fibre reinforced carbon composites [45]. CNTs have been reported to be harmful to living organisms [46-48]. They come in varying lengths and a study of 220 nm and 825 nm long CNTs showed no severe inflammatory response, although the degree of inflammation was larger for the longer nanotubes. This suggests that macrophages can envelop the shorter nanotubes more readily. Thus longer nanotubes may pose more of a health issue than shorter ones. Single walled carbon nanotubes (SWCNT) can inhibit the proliferation of cells. They induce a signal inside a cell and the nucleus, resulting in decrease of cell adhesion, causing the cell to detach, float and shrink in size. Cells have a self-protection response, secreting proteins to wrap the nanotubes into nodular structures, which isolate the cells attached to the CNTs from other cells [49]. Unfunctionalised multiwalled carbon nanotubes (MWCNTs) are capable for both localising within and initiating an irritation response in human cells, but there is no information as to whether MWCNTs structures are an occupational risk [50]. A study involving mice showed that, for equal masses in the lungs, CNTs can be more toxic than quartz (a form of silica), which is known to cause silicosis following chronic exposure [51]. The hierarchies of in vitro cytotoxicity of carbon nanomaterials (and quartz) follow a sequence of order based on mass: SWCNT. MWCNT (10-20 nm diameters). Quartz. C60. Profound toxicity of SWCNT is observed after a six hour exposure with a 35% increase in cytotoxicity when the dosage of SWCNT is 11.3 mg cm^2 . In comparison there is no significant toxicity for spheroidal C60, up to a dose of 226.0 mg cm^2 [52].Thus cytotoxicity depends on the different geometric shapes of carbon nanomaterials. SWCNTs induce dose dependent epithelioid granulomas and interstitial inflammation in mice. Carbon black treated mice are normal and quartz treated mice have mild to moderate inflammation [51]. On an equal weight basis, if SWCNTs reach the lungs, they are much more toxic than carbon black, and can be more toxic than quartz, which is a serious health hazard in chronic inhalation exposures. Functionalised CNTs by themselves are not toxic, but they can help transport other molecules that are cytotoxic into cells [53]. A SWCNT-biotin conjugate causes extensive cell death. Nanotubes non-specifically associate with hydrophobic regions of the cell surface and internalize by endocytosis, and accumulate in the cytoplasm in the cells. DNA can wrap around CNTs and this is the basis of a method for separating different size CNTs. At the same time it raises concerns over the entry of CNTs into the human body [29]. Studies in animal models revealed that mesothelioma could be induced by asbestos and other related fibres, y0.25-1.5 microns in diameter and y4-8 microns in length, regardless of composition.

Green nanotechnology-a solution in nano-bio-therapeutic toxicity

Nanotechnology presents an opportunity to develop a revitalized,

sustainable chemical and materials manufacturing base. Green Nanotechnology is an application that pays attention to implications. It is based on lowering the risk of using nanomaterials, limiting the risk of producing nanomaterials, and using nanomaterials to lower the risk of producing unwanted chemical intermediates and end-products. Green Nanotechnology maintains a commitment to rigor with applications related to sustainability as well as awareness about and prevention of possible harmful consequences from its successful implementation. Moreover, responsible development of Green Nanotechnology necessarily involves balanced and measured considerations of toxicity and cost-benefit risk assessments. Indeed, the explicit goal of all of these analyses is to ensure a smart process design of materials that will eliminate hazards throughout the material's life cycle and potentially deleterious impacts on the environment. This new emerging science and associated technologies do not have to follow the typical path of many past innovations in the chemical industry that, despite providing significant benefits, also turned out to have unanticipated costs to human and environment health. But the development and commercialization of viable green nanotechnologies is difficult, and the barriers will require effort from the scientific, research and government communities. Understanding and rationally dealing with the potentially toxic effects of nanoparticles requires a multidisciplinary approach, necessitating a dialogue between those involved in the disparate aspects of nanoparticle fabrication and their effects, including but not limited to nanomaterial fabrication scientists, chemists, toxicologists, epidemiologists, environmental scientists, industry, and policy makers. While steady progress has been made in the development of green nanomaterials and the accompanying toxicology and analysis, large-scale commercialization has yet to occur. In some respects, this is not surprising. Almost all new technologies face significant barriers in moving from the laboratory and into the market. This issue has been documented by scholars and business people alike for decades. Furthermore, green chemistry, the principles of which are a core part of green nanotechnology, has also been documented to have its own, distinct challenges in terms of commercialization. However, there are some unique aspects to green nanotechnology, as it is an emerging science that must deal with the compounded challenges present in a new area of science, while at the same time breaking new ground on incorporating environmental and health considerations into research and development at the earliest stages. Green nanoscience requires new analytical techniques and toxicological protocols in order to fully understand the impacts on people and the environment. These fields need to balance the task of being able to find ways to effectively analyze new technologies as they emerge, and also to develop fundamental understanding of how different properties link to impacts, in order to provide guidance to the discovery community so that they design the most benign products possible from the start [9]. There is also a need to develop in-line process analytical and control techniques for full-scale manufacturing operations. The development and commercialization of viable green nanotechnologies is difficult, and the barriers mentioned will require effort from the scientific, research and government communities.

Future opportunities of nanotherapeutic devices

In the following we highlight important questions and research directions that should be addressed in the near future by the scientific community involved in the study of nanoparticles sciences and by government agencies responsible for regulations and funding. Advanced analysis of the physical and chemical properties of nanoparticles will be essential in revealing the relationship of their size, composition, crystallinity, and morphology with their electromagnetic response properties, reactivity, aggregation, and kinetics. It is important to note that fundamental properties of nanoparticles are still being discovered, such as magnetism in nanoparticles made of materials that are non-magnetic in bulk form. A systematic scientific approach to the study of nanoparticle toxicity requires correlation of the physical and chemical characteristics of nanoparticles with their toxicity. Existing research on nanotoxicity has concentrated on empirical evaluation of the toxicity of various nanoparticles, with less regard given to the relationship between nanoparticle properties such as exact composition, crystallinity, size, size dispersion, aggregation, ageing and toxicity. This approach gives very limited information, and should not be considered adequate for developing predictions of toxicity of seemingly similar nanoparticle materials.

Most nano-products can be designed in such a way as to either increase or decrease toxicity depending on the desired outcome. For treatment in drug delivery, minimising toxicity of the carrier is necessary and in most cases this can be done. On the other hand for chemotherapeutic agents, toxicity is designed to be magnified and targeted to specific tissues or areas. Altering the coating of many of these therapeutic agents can increase selectivity and toxicity. Care must also be taken in the profiling of the pharmacokinetics and release of the products. The nanoparticle may be designed for human consumption knowing that it will stay intact in the body. If afterwards, the coating designed to alleviate toxicity is broken down, what are the ensuing products once released from the body and exposed to a different environment? Increasing concentrations of mercapto-undecanoic acid (MUA) QDs show a decrease in cell viability in Vero cells (African green monkey's kidney cell), HeLa cells and human primary hepatocyte cells. The Vero cells are affected less than the others. Four to six hours incubation in the presence of the QDs resulted in cell death. There is a range of concentrations for QDs where cell viability can decrease without cell death depending on cell types and capping material [54]. When QDs remain intact, toxicity is dependent on the surface presented to the cell rather than what is in the core. A MUA coated QD is toxic when treated for twelve hours at 100 mg per mL and above, with DNA damage observed after two hours treatment at a dose of 50 mg per mL and above. Cysteamine coated QDs induce some DNA damage when treated for twelve hours, the other coatings showing little to no signs of toxicity [55]. Toxicity of QDs is also associated with oxidation of the surface of the nanoparticles. This leads to reduced cadmium on the surface deteriorating the CdSe lattice, releasing free Cd^{2+} which then results in cell death. The toxicity can be modified by altering the QD coating; mercaptoacetic acid (MAA) renders CdSe QDs non-toxic and coating with ZnS and bovine serum albumin molecules reduce toxicity. Water soluble CdSe/ZnS QDs induce DNA damage in the presence of UV light. This nicking of DNA is attributed to free radicals that are both photogenerated and surface oxide generated. Formation of radicals can be avoided by doping the lattice with Mn^{2+} ions to hold charge carriers in the internal structure. Michalet et al. found that most of the papers on QDs used in vivo and in vitro did not find any notable adverse effects arising from QDs at labelling concentrations, while the Se^{2-} and Cd^{2+} ions are released from the QDs and so they are not completely without risk but can be used effectively within a safe range [56]. In another study, pathogenic bacteria S. aureus were found to extract Se^{2-} and Cd^{2+} ions from CdSe QDs. The extraction is dependent on the surface conjugation; holotransferrin conjugates show a significant increase in the amount of Se^{2-} ions inside the cytoplasm compared to the original QDs and mercaptoacetic acid coated QDs. Studies on the injection of QDs into the blood stream of pigs and mice show no toxicity. Even for QDs loaded in cells growing in vitro, there is no toxicity evident after two weeks. Nano-sized selenium is less toxic than selenite and high seleni-

um protein that is administered to rats [57]. Inhaled nanoparticles are of great concern, since the most likely accidental contact with nanoparticles occurs through the lungs. The effect of environmental factors in the development of Parkinson's disease has become increasingly apparent, and the high exposure to airborne particulates in urban areas is a cause for concern. Suitable industrial hygiene should be maintained to minimise exposure during the manufacture of carbon nanotubes [58]. The onset of pathogenic effects generally requires the achievement of a sufficient lung burden. When inhaled, nanoparticles are deposited in all regions of the respiratory tract [59]. Numerous studies have found that for equal masses, nanoparticles exert a more marked effect in the detriment to respiratory function, compared with 'normal' sized particles. There are significant differences between nanoparticles and larger entities during deposition and clearance in the respiratory tract [59]. In the case of nanoparticles, the most prevalent mechanism for clearance is phagocytosis by macrophages. A study of the pulmonary toxicity of carbon nanotubes in mice showed they could be more toxic than quartz which is known to cause significant health problems after chronic exposure. Although nanoparticles often form larger clusters, such airborne aggregates can disintegrate in the lungs and this might result in increased toxicity. With normal handling of carbon nanotube material, the concentration of splinters in the air is minimal. The majority of in vivo studies into the pulmonary toxicity of nanoparticles has been conducted in rodents, but it is known that rats are especially sensitive when it comes to adverse lung responses [60]. In addition, all in vivo studies use instillation into the trachea (as a mass of particles) out of necessity. Intratracheal instillation of nanoparticles may cause oxidative stress which can lead to atherosclerosis, carcinogenesis or acute or chronic inflammatory disorders [61]. A thorough inhalation study still remains to be undertaken to investigate the respirability of nanoparticles [58], that is, their ability to pass beyond the airways and move to the low-level structures of the lungs [60]. Normal-sized TiO_2 is classified as biologically inert although more research is warranted [61]. The 'precautionary principle' should be applied to nanoparticle toxicity. Conclusions about the toxicity of nanoparticles cannot be made until the reaction of lung tissue to these particles is established [62]. Such a response may take many years to develop. Diesel exhaust contains nanoparticles which have been linked to inflammation and oxidative stress in the lungs and cardiovascular system. Inhalation of diesel particles may result in the formation of reactive oxygen species. Another substance with this ability is TiO_2, which can absorb UV light and produce various oxygen-containing, reactive species in solution [61]. Reactive oxygen species are known to attack lipids and proteins and have been implicated in carcinogenesis. A hypothesis has been suggested that smaller particles have a higher potency in inducing oxidative stress in the absence of photoactivation. Long fibers (greater than 20 microns-easily achieved by carbon nanotubes) in lungs are not cleared effectively. At the alveolar level, these long fibres result in 'frustrated' phagocytosis or the attempt by multiple macrophages to ingest the particle. These results in an inflammatory response and leads to scarring of lung tissue (fibrosis) [60]. At this stage, it remains unknown whether the asbestos mechanism of toxicity is relevant to carbon nanotubes If MWCNTs reach the lungs, they are biopersistent, and induce lung inflammation and fibrosis. However, short term tests in vitro fail to accurately model the breakdown of nanomaterials in the lungs [60]. Thus, a false positive result may be obtained when a substance is, in fact, non-persistent in the lungs. Rat tracheal explants have been used to examine how nanoparticles interact with cells in the lungs [63]. Nanoparticulates persist as aggregates but larger particle aggregates get smaller over time. It is hard to tell if the inflammatory response is caused by the smaller-sized nanoparticles, or just the large number of particles administered. Donaldson et al. have shown a very clear distinction between normal and nano-sized particles. Ultrafine TiO_2 (20 nm) and 500 nm particles induce free radical activity and break the strands of super-coiled plasmid DNA, but the ultrafine TiO_2 results in complete destruction of DNA. Some of the damage caused by TiO_2 particles can be alleviated in the presence of mannitol, which shows that hydroxyl free radicals are involved. Free radical activity is also detected on the surface of asbestos fibres, environmental particles, ceramic fibres and glass fibres [64]. Ingested nanoparticles: Gold nanoparticles are being studied for use as transfection vectors, DNA-binding agents, protein inhibitors and spectroscopic markers. Cationic gold nanoparticles (quaternary ammonium terminal groups) increase vesicle lysis while anionic (carboxyl terminal groups) are relatively harmless. The cationic particles presumably have a strong electrostatic interaction with the negatively charged cell membrane [65]. Two fullerene C60 derivatives, a dendritic C60 mono-adduct and a malonic acid C60 tris-adduct, have been tested for cell growth and viability on Jurkat cells (human T-lymphocytes).The dendritic mono-adduct inhibits cell growth (over two weeks it decreased to 19%) but has no effect on cell vitality, while the tris-malonic acid adduct has little effect on either. Growth inhibition arising from the dendritic C60 is reversible; the same cells grown without the fullerene resume normal growth. The tris-malonic (TMA) acid fullerene is more phototoxic than the dendritic derivative. The two different fullerenes possibly interact with the cell membrane in different ways. The dendritic adduct has very large branches, while the tris-malonic adduct is more compact, the branches keeping the C60 away from the cell. Also the dendrofullerene molecules aggregate lowering the interaction with cell membranes [66]. Foley et al. have demonstrated that a fullerene derivative ($C_{60}(CO_2H)_2$) could cross the cell membrane and be localized preferentially in compartments within the cell. This is thought to strengthen the case for using fullerenes for drug delivery [67]. Three C60 fullerene derivatives, namely (i) di-malonic acid, DMA, (ii) TMA, and (iii) tetra-malonic acid (QMA) C60, have been tested on HeLa cells to determine growth inhibition. The cytotoxicity of these fullerenes is dose and irradiation dependent with the hierarchy of toxicity DMA. TMA. QMA. Mannitol, which is able to prevent hydroxyl radical damage, does not prevent the damage induced by the C60 compounds [68]. 151Fullerene C60 forms stable nano-aggregates 25-500 nm, which are also stable in ionic solutions for months. There is a need, therefore, to study the properties of such nanoparticles-which are likely to be different from bulk C60 [69]. Less-derivatised, less-soluble forms of C60 are the most toxic to HDF (human dermal fibroblasts) and HepG2 (human liver carcinoma) cells. They cause oxidative damage to cell membranes which can lead to cell death, yet with a notable lack of damage to DNA, proteins and mitochondria. In water C60 aggregates and can form superoxide anions that seem to be the most likely cause of membrane damage. More soluble derivatives of C60 like $C_{60}(OH)_{24}$ and Na+2–3[C60O7–9(OH)12–15](2–3)2 are much less toxic (C60 is toxic at 0.020 ppm compared to $C_{60}(OH)_{24}$ at 5000 ppm which is limited by its solubility). Given the differential cytotoxicity, strategies can be developed for increasing toxicity to target bacteria or cancer and minimised for safety in other applications [70]. Exposure of juvenile largemouth bass fish to C60 resulted in lipid peroxidation in their brains after 48 h of exposure to 0.5 ppm of uncoated C60. This is the first study to show the oxidative effects of fullerenes in vivo. The fullerenes also keep the water clearer, possibly owing to its anti-bacterial effects [71]. Huang et al. found that chitosan toxicity depends chiefly on the degree of deacetylation. Decreasing the degree of deacetylation increases cell viability. Turning chitosan into nanoparticles does not influence the toxicity but it increases its endocytosis, and molecular weight has little effect on the toxicity. Super para-

magnetic iron oxide nanoparticles (SPIONs) have been modified with a pullulan coating. This has the desired result of reducing toxicity and increasing cellular uptake compared with uncoated SPIONs. Uncoated SPIONs are toxic (determined by cytotoxicity and adhesion studies) and their internalisation results in disruption of the cytoskeletal organisation in human dermal fibroblasts. The difference in toxicity may be due to the pullulan coating being hydrophilic which protects the surfaces from interacting with biological materials. These coatings may add additional specificity for magnetic nanoparticles in drug delivery [72].

Further studies on kinetics and biochemical interactions of nanoparticles within organisms are imperative. These studies must include, at least, research on nanoparticles translocation pathways, accumulation, short- and long-term toxicity, their interactions with cells, the receptors and signaling pathways involved, cytotoxicity, and their surface functionalization for an effective phagocytosis. Existent knowledge on the effects of nanoparticle exposure on the lymphatic and immune systems, as well as various organs, is sparse. For example it is known that nanoparticle exposure is able to modulate the response of the immune system to different diseases, however much research is needed in order to better understand to what extent this occurs and the full implications of risk groups (age, genotype). In order to clarify the possible role of nanoparticles in diseases recently associated with them (such as Crohn's disease, neurodegenerative diseases, autoimmune diseases, and cancer), nanoscale characterization techniques should be used to a larger extent to identify nanoparticles at disease sites in affected organs or tissues, and to establish pertinent interaction mechanisms.

Conclusion

Until recently the spectacular developments in nanotechnology have been with little regard to their potential effect on human health and the environment. There are no specific regulations on nanoparticles except existing regulations covering the same material in bulk form. Difficulties abound in devising such regulations, beyond self-imposed regulations by responsible companies, because of the likelihood of different properties exhibited by any one type of nanoparticle, which are tunable by changing their size, shape and surface characteristics. Green chemistry metrics need to be incorporated into nanotechnologies at the source. We foresee a future with better-informed, and hopefully more cautious manipulation of engineered nanomaterials, as well as the development of laws and policies for safely managing all aspects of nanomaterial manufacturing, industrial and commercial use, and recycling.

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