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ROLE OF NANOMATERIALS IN BIOTECHNOLOGY

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Nanotechnology is research and technology development at the atomic, molecular, and macromolecular scale, leading to the controlled manipulation and study of structures and devices of length scales in the 1- to 100-nanometers range. Objects at this scale, such as “nanoparticles,”take on novel properties and functions that differ markedly from those seen in the bulk scale. The small size, surface tailorability, improved solubility, and multifunctionality of nanoparticles open many new research avenues for biologists. The novel properties of nanomaterials offer the ability to interact with complex biological functions in new ways. This rapidly growing field allows cross-disciplinary researchers the opportunity to design and develop multifunctional nanoparticles that can target, diagnose, and treat diseases such as cancer. This article presents an overview of nanotechnology for the biologist and discusses “nanotech” strategies and constructs that have already demonstrated in vitro and in vivo efficacy.

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1. Introduction

Nanotechnology has achieved the status as one of the critical research endeavors of the early 21st century, as scientists harness the unique properties of atomic and molecular assemblages built at the nanometer scale. Our ability to manipulate the physical, chemical, and biological properties of these particles affords researchers the capability to rationally design and use nanoparticles for drug delivery, as image contrast agents, and for diagnostic purposes. By operating in the nanoscale realm, at the very scale of biomolecules, nanotechnology offers a wide range of tools and applications (see Table 1). Near-term applications include drug-delivery platforms [1], enhanced image contrast agents [2], chip-based nanolabs capable of monitoring [3] and manipulating individual cells [4], and nanoscale probes that can track the movements of cells [5] and individual molecules [6] as they move about in their environment. Such an unprecedented ability to observe and influence complex systems in vivo and in real time provides detailed information about the fundamental mechanisms and signaling pathways involved in the progression of disease and greatly extends the existing toolset for drug delivery and noninvasive drug monitoring. By providing constructs capable of combining multiple functionalities into a single nanoscale entity, nanotechnology also offers the opportunity to monitor and detect molecular and cellular changes associated with disease states [7]. Given this multifunctional capability, one can imagine building a nanoparticle that can target a specific tissue or cell type, delivering a contrast agent that allows for noninvasive imaging and a therapeutic payload to the target. A nanoparticle might even contain a reporter, such as an apoptotic marker, which signals that the payload has been delivered and is having the desired therapeutic effect. Such combinatorial nanostructures may eventually provide the means to achieve “personalized medicine” by tailoring drug delivery to individual response. Although this may seem futuristic, several groups have already created multifunctional nanodevices and are testing them in in vitro and in vivo systems [1, 8–18]. Such constructs must also have novel properties and functions because of their small size. For example, carbon nanotubes and gold nanoshells, two different types of nanomaterials, have physical properties different from carbon [19] or gold [20] on the

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macro scale. Other examples of nanotechnology include Dendrimers [21], liposomes [22], and semiconducting quantum dots [23]. In contrast, particles such as DNA, bacteriophage, and monoclonal antibodies (mAb) may have nanometer-sized dimensions but would not be considered examples of nanotechnology for the purposes of this review. Nanotechnology manifests itself in a wide range of materials that can be useful to the biologist [24], a sample of which is Listed in Table 1. Virtually all of these materials have been designed with chemically modifiable surfaces to attach a variety of ligands that can turn these nanomaterials into biosensors, molecular-scale fluorescent tags, imaging agents, targeted molecular delivery vehicles, and other useful biological tools. The unprecedented freedom to design and modify nanomaterials to target cells, chaperone drugs, image bimolecular processes, sense and signal molecular responses to therapeutic agents, and guide surgical procedures is the fundamental capability offered by nanotechnology, which promises to impact drug development, medical diagnostics, and clinical applications profoundly.

2. Nanotechnology applied to biological systems

2.1 Size matters

An obvious advantage of nanotechnology as it relates to biological systems is the ability to control the size of the resulting particles and devices. Nanoscale devices and components are of the same basic size as biological entities, as shown in. Nanoscale constructs are smaller than human cells (10,000 –20,000 nm in diameter) and organelles and similar in size to large biological macromolecules such as enzymes and receptors. Nanoparticles smaller than 20 nm can transit through the blood vessel walls. Magnetic nanoparticles, for instance, can image metastatic lesions in lymph nodes because of their ability to exit the systemic circulation through the permeable vascular epithelium [25]. Nanoparticles also offer the ability to penetrate the blood-brain barrier or the stomach epithelium [14, 26–29] barriers that make it difficult for legacy therapeutic and imaging agents to reach their intended targets. To be suitable as a drug-delivery platform, the size of nanoparticles must be small enough to avoid rapid filtration by the spleen, with filaments spaced at roughly 200 nm [30], which serve as a meshwork for phagocytotic cells [31]. Similarly, to traverse the liver, the particles must be small enough to pass through the organ's 150–200 nm-sized fenestrae and avoid the Kupffer cell-lined sieve plates [32]. Drug-carrying liposomes are believed to have increased lifespan, related in part to their ability to extravasate through splenic and liver fenestrate [33]. The size of nanoscale devices also allows them to interact readily with biomolecules on the cell surface and within the cell, often in ways that do not alter the behavior and biochemical properties of those molecules [34]. Such ready access to the interior of a living cell affords the opportunity for unprecedented gains on the clinical and basic research frontiers. The ability to interact with receptors, nucleic acids, transcription factors, and other signaling proteins at their own molecular scales should provide the data needed to better understand the complex regulatory and signaling networks and transport processes that govern the behavior of cells in their normal state [35] and as they undergo the changes that transform them during the disease process [36]. In particular, nanotechnology will provide an important role in integrating efforts in proteomics (identifying and measuring key cellular proteins and peptides) with systems biology (the integration of cellular pathways and networks) and other scientific investigations into the molecular nature of disease [37–39]. By virtue of their size, nanoparticles such as quantum dots can be endocytosed and used for intracellular imaging [40–42]. Despite their small size, nanoparticles can accommodate tens of thousands of atoms or small molecules, such as the magnetic resonance imaging (MRI) contrast agent gadolinium [2], creating the opportunity for improved detection sensitivity of diseases such as cancer in its earliest stage.

2.2 Solubility matters

To fully appreciate the powerful use of nanotechnology and nanoparticles in particular one must understand the surface chemistry of the particles. Modification of the nanoparticle's outer layer allows a large variety of chemical, molecular, and biological entities to be covalently or otherwise bound to it. Manipulation of this corona confers advantageous properties to the particle, such as increased solubility and biocompatibility. Attaching hydrophilic polymers to the surface, such as PEG, greatly increases the hydration (i.e., solubility) of the nanoparticles and can protect attached proteins from enzymatic degradation when used for in vivo applications [43]. Nanoparticles with hydrophilic polymers such as PEG attached to their surface can act as a platform for lipophilic molecules and overcome the solubility barrier. Insoluble compounds can be attached, adsorbed, or otherwise encapsulated in the hydrated nanoparticles [44, 45]. Solubility of the composite entity subsequently becomes a function of the nanoparticles carrier rather than being strictly dependent on the drug itself.

The surface addition of PEG (“pegylation”) and other hydrophilic polymers also increases the in vivo compatibility of nanoparticles. When injected intravascularly, uncoated nanoparticles are cleared rapidly from the bloodstream by the reticuloendothelial system (RES) [46]. Nanoparticles coated with hydrophilic polymers have prolonged half-lives, believed to result from decreased opsonization and subsequent clearance by macrophages [47]. This represents a slight paradigm shift from classical pharmacology; plasma protein binding (e.g., to albumin and α-1-acid glycoprotein) can be a desired attribute for traditional therapeutic drugs, as it serves to increase bioavailability by limiting first-pass hepatic extraction. Blood components implicated in the RES clearance of nanoparticles include fibronectin, C3, albumin, fibrinogen, immunoglobulin G (IgG), Ig light chains, and the apolipoproteins (apo) A-I and apoE [48–50].

2.3 Targeting matters

One of the earliest examples of applying nanotechnology to solving problems in biology was the use of liposomes as drug delivery vehicles [51]. A liposomal formulation of the potent but toxic antifungal agent amphotericin B has revolutionized the treatment of life-threatening, systemic, fungal infections in immune-compromised patients by allowing patients to receive normally lethal doses of amphotericin B with minimal risk of toxicity [52]. The liposomes, 50–70 nm in diameter, are taken up rapidly by macrophages, which then carry the liposome and drug to the site of fungal colonization. Cancer therapy has benefited from the use of liposomal doxorubicin, a formulation that again increases the therapeutic index of the active agent through a combination of passive tumor targeting and reduced toxicity [53]. In this case, coating the liposome with PEG significantly decreases uptake by macrophages and allows the liposomes to concentrate in tumors by escaping from the leaky vasculature surrounding solid tumors [54] through a phenomenon known as the enhanced permeation and retention (EPR) effect [55–57]. Targeted delivery of nanoparticles can be accomplished by attaching a mAb or cell-surface receptor ligand that binds specifically to molecules found on the surfaces of targeted cells, be they cancer cells or the angiogenic micro capillaries growing around malignant cells. Targeting molecules that have been used successfully include folate [1], luteinizing hormone releasing hormone (LH-RH) [58], thiamine [26], receptor-specific peptides [59, 60], aptamers [61], and a wide variety of mAb directed against cell-surface markers, such as integrins [15]. It is interesting to note that these functionalized nanoparticles have been demonstrated to have high avidity for their target cells, believed to be the result of their multivalent interaction [1, 10]. Once bound to the target cell, the nanoparticles are readily internalized by receptor-mediated endocytosis [1, 11, 28, 62].

2.4 In vivo imaging

A variety of nanoscale particles have already demonstrated use in imaging tumors and the tumor microenvironment in animal models and human clinical trials, as listed in **Table 1**. Some of the most advanced work in this area uses dextran-coated, ultra-small superparamagnetic iron oxide (USPIO) nanoparticles to image lymph nodes containing micrometastases in patients with prostate cancer [63]. Other studies have used paramagnetic, gadolinium-labeled, nanoparticulate dendrimers to image lymphatic micrometastases in a mouse breast cancer model [64].

Table 1. Examples of Nanoparticles Used in Biological Research

Nanoparticle	**Application**
Dendrimers	Targeting of cancer cells, drug delivery, Imaging, boron, Neutrons capture therapy
Ceramic Nanoparticle	Passive targeting of cancer cells, Lipid-encapsulated per fluorocarbon Nanoemulsion Passive targeting of cancer
Magnetic nanoparticles	Specific targeting of cancer cells, Tissue imaging, LH-RH-targeted silica-coated lipid
Micelles	Specific targeting of cancer cells
Thiamine-targeted nanoparticles	Directed transfer across Caco-2 cells
Liposomes	Specific targeting of cancer cells, Gene therapy, Drug delivery
Nanoparticle-aptamer bioconjugate	Targeting of prostate cancer cells
Anti-Flk antibody-coated 90Y nanoparticles	Antiangiogenesis therapy
Gold nanoshells	Tissue imaging, Thermal ablative cancer therapy
Anti-HER2 antibody-targeted gold/silicon nanoparticles	Breast cancer Therapy
CLIO paramagnetic nanoparticles	Imaging of migrating cells
Quantum dots	Tissue imaging
Silicon-based nanowires	Real-time detection and titration of antibodies, Virus detection
Chip based biosensors	Real-time detection and titration of antibodies, Virus detection
Electronic biosensors	Noninvasive vaccine delivery, Drug delivery
Carbon Nanotubes	Bone grafting, Biosensors, Bacteria and virus Filtration
Silver Nanoparticles	Antibacterial agents, Filters

2.5 Enhanced In Vitro Diagnostic

The impact of nanotechnology on biology is certainly not limited to applications within the body. Indeed, the development and use of nanoscale analytical tools are the most promising areas of immediate benefit. Many of the efforts in developing nanoscale in vitro or ex vivo measurement and molecular detection systems rely on the methods being developed to construct nanoscale electronic circuits. For example, 1–2 nmwide, boron-doped silicon nanowires laid down on a silicon grid can be coated with antigens to provide real-time detection and titering of antibodies [65]. Antibody binding to immobilized antigen produces an immediate, measurable change in conductance at antibody concentrations below 10 nm. In the same study, nanowires derivatized with the calcium-binding protein calmodulin provided real-time measurements of calcium ions at physiologic levels. More recently, investigators have developed methods for chemically modifying lithographically etched silicon nanostructures to enable attachment of a wide range of molecules as the first step for creating versatile, chip-based biosensors [66]. Silicon-based arrays made of antibody-conjugated nanowire field effect transistors have also been multiplexed to simultaneously detect single copies of multiple viruses [67]. Functionalized carbon nanotubes can also function as highly specific electronic biosensors [68].

2.6 Bacteria and Virus Filtration

By using radially aligned carbon nanotube walls Monolithic, macroscopic, nanoporous nanotube filters are fabricated [69]. The freestanding filters have diameters and lengths up to several centimeters. A single-step filtering process was demonstrated in two important settings: the elimination of multiple components of heavy hydrocarbons from petroleum, a crucial step in post-distillation of crude oil, and the elimination of bacterial contaminants such as *Escherichia coli* or the nanometer-sized poliovirus from drinking water. Nanotube filters can be cleaned repeatedly after each filtration process to regain their full filtering efficiency. During the past few decades, several investigations have been carried out concerning the use of synthetic and natural zeolites, polymer films, and metal nanoparticles as bactericides for water purification. High reactivity of metal nanoparticles due to their large surface to volume ratio, expected to play a crucial role in water purification [70].

3. Concluding Remarks

Although there is a general sense that the technological base in nanotechnology is developed sufficiently to enable biologists to make ready use of these tools and materials, there are still fundamental questions about these materials that must be answered if nanotechnology is ultimately to have a significant impact extending beyond the laboratory and into the clinic. For example, there is a need for better characterization of nanotechnology constructs and production of "reagent-grade" nanomaterials, which permit comparisons between researchers. "Standardized" assays also need to be developed that facilitate rigorous evaluation of nanomaterials in terms of their toxicity and efficacy. The field would also benefit from increased interdisciplinary and international collaborations to verify more quickly and extend results to rapidly emerging areas of interest. These areas include nanomaterial development, production, environmental and health impact, bioinformatics, and modeling and simulation tools. Along with the prospects that nanotechnology holds for medical innovation comes the caveat that this is uncharted scientific territory and may have potential risks and hazards. There is evidence that certain nanoscale particles can have detrimental effects on living organisms. Carbon nanoparticles, for instance, have been shown to induce lipid peroxidation in the brain cells of fish and pulmonary inflammation in rats [71, 72]. The Royal Society and The Royal Academy of Engineering, a group that actively monitors this technology, has published a critical review of best practices for nanoparticle risk assessment [73]. On another front, the NNI has set aside $106 million in funding for research into the ethical, environmental, and health implications associated with nanoparticles [74]. Whether actual or perceived, the potential health risks associated with the manufacture, distribution, and use of nanoparticles must be balanced by the overall benefit that nanotechnology has to offer biomedical science, such as the therapeutic and diagnostic applications described in this article. Although nanotechnology is a relatively young field, it is developing rapidly, thanks to a strong foundation of material science and engineering. Biologists are using this innovative technology to overcome boundaries common to cell biology and clinical medicine.As more biologists learn about the capability of nanotechnology and develop cross-disciplinary collaborations with physicists, engineers, and material scientists, these breakthroughs will undoubtedly increase in magnitude and quantity.

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