Nanotechnology: Towards the detection and treatment of inflammatory diseases

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Introduction

Inflammatory diseases comprise of a whole list of conditions such as rheumatoid arthritis, multiple sclerosis, asthma and various other life-altering diseases including myocarditis. Inflammation begins as a defensive process in which our body is equipped to protect itself from harmful pathogens and chemicals. When this defense mechanism is uncontrolled, the integrity of tissue may be breached resulting in damage to the vital organs, nervous, musculoskeletal systems and blood vessels. This breach permits soluble mediators of inflammation to be produced at the site, which causes leukocytes, monocytes and lymphocytes to migrate towards the site and to activate the immune system in a dysregulated manner, effecting hyper-reactivity of lymphocytes eliciting Th1 and Th2 type immune responses. As result of this abnormal immune activation, the surrounding tissue expresses variety of enzymes, adhesion molecules, cytokines, proteins, lipid mediators, and growth factors that participate in tissue destruction and repair [1]. Sometimes this abnormal immune response attacks self, resulting in further tissue damage. Although inflammatory reactions on the skin and other open areas on the body can be superficially visualized, in many cases when vital organs are affected, clinicians await until overt signs and symptoms are presented, and by that time destruction of the tissue in those vital organs has already progressed. Thus, in many cases it remains difficult to clearly diagnose these diseases.

New technologies are needed to speed the diagnostic processes and help the basic scientist and clinician in the initiation of targeted treatments and to follow up treatment responses. An important milestone in this process has been the advances made by researchers in biochemistry, immunology and drug discovery fields in the identification of molecular signatures of inflammation and cancer, using complicated and cumbersome wet laboratory techniques. The objective now is to exploit those initial

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accomplishments, combining them with available new technologies to identify the earliest signatures of inflammation and cancer. Such developments will allow us to provide immediate and specific intervention and monitor the progress before it cascades into chronic inflammation and malignancy. To fulfill this objective, it requires the development of technologies of 1–100 nm size, which display unique mechanical, electrical, chemical, and optical properties and assist in visualizing or interacting with receptors, cytoskeleton, specific organelles and nuclear components within the cells. It will be very rewarding when many of these technologies can migrate into monitoring the disease condition through non-invasive methods *in vivo* in a physically undisturbed state, thus minimizing the influence of artifacts induced by physical methods while securing biological samples.

The integration of nanotechnology with biology and medicine has given birth to a new field of science called "Nanomedicine". The ultimate goal of nanomedicine is to develop well-engineered nanotools for the prevention, diagnosis and treatment of many diseases. In the past decade, extraordinary growth in nanotechnology has brought us closer to being able to vividly visualize molecular and cellular structures. These technologies are beginning to assist us in our ability to differentiate between normal and abnormal cells and to detect and quantify minute amounts of signature molecules produced by these cells. Most of these represent real time measurements, relating to the dynamic relationship among structures in the damaged area and also to repair of damaged tissues. Novel pharmaceutical preparations have been developed to fabricate nanovehicles to deliver drugs, proteins and genes, contrast enhancement agents for imaging, and hyperthermia agents to kill cancer cells. Several of these inventions have already transitioned into basic medical research and clinical applications. Because of this, some social, ethical, legal and environmental issues have emerged. Thus, regulatory and educational strategy needs to be developed for the society to gain benefit from these discoveries. The focus of this chapter is to provide an overview of the state-of–the-art in nanotechnology with particular reference to detecting and treating inflammation and cancer at the earliest settings.

Nanotechnology for inflammation scientists

Nanotechnology encompasses multiple scientific disciplines, which exploit materials and devices with functional co-assembled molecules or sensors that has been engineered at the nanometer scale typically ranging from 0.1 to 100 nm [2]. In medical fields, it offers a wide range of tools that can be used as drug delivery platforms [3], better contrast agents in imaging [4], chip-based bio-laboratories [5] and nanoscale probes [6] that are able to track cell movements and manipulate molecules. Multifunctional nanostructures can combine diagnostic and therapeutic modalities and target cellular events.

The concept of molecular medicine to develop personalized treatments can be made possible with the information available with the developed nanotools. These devices, systems and functionalized structures contain unique properties as a result of their nanosize. For example, gold nanoparticles and carbon nanotubes possess different properties [7] at the micron scale. Semiconductor particles exemplified by quantum dots exhibit quantum confinement effects, hence they fluoresce at various wavelengths compared to the semiconductor particles at micron size which do not exhibit the same optical or magnetic properties [8]. Macromolecular structures such as dendrimers and liposomes at the nanoscale are also considered valid nanotools [9], while biological molecules of nanometer size in their native state such as DNA and monoclonal antibodies are not examples of "Nanotools". Thus, nanometer size is of critical importance to the cell and the living organisms. Interestingly, fabricated nanoscale devices are of the same size as subcellular organelles. Some nanoscale structures are of the order of enzymes, receptors and key molecules within the cell membrane or cytoplasm. For example, the lipid bilayer surrounding cells is 6 nm and hemoglobin is 5 nm in size. By modifying the surface chemistry of these nanostructures, which permits covalent or ligand-receptor (lock-key-type) or electrostatic interaction with key molecules, we can identify biomolecules on the cell surface and in the cytoplasm. An advantage of this will be our ability to map the transport of those molecules from the cytoplasm across the cell membrane so we can understand the cellular behavior in health and disease. Nanoparticles smaller than 20 nm can pass through blood vessel walls [10], which opens opportunities of diagnostic imaging and targeted delivery of drugs when non-toxic nanoparticles are used. What is critical for scientists engaged in inflammation science and engineering is that these technologies should be applicable to detect or monitor:

- Host biochemical and immune responses
- Bacterial and viral pathogens interacting within the local immune system
- Effect of noxious chemicals and pharmaceutical agents
- Thrombosis
- Neurogenic inflammation
- Wound healing and remodeling
- Imaging
- Diagnosing and treating vulnerable plaques
- Drug delivery and therapeutics for local delivery and retention

Nanostructures and nanosystems

Nanotechnology in the medical field offers a wide range of tools that can be used as drug delivery platforms, better contrast agents in imaging, chip-based biolabs and nanoscale probes able to track cell movements and manipulate molecules [10].

Combination of these multifunctional nanostructures through cross-disciplinary interactions may further enhance our diagnostic and therapeutic capabilities and to monitor intra- and extracellular cellular events in inflammation and cancer.

Existing and emerging technologies, which may impact on early detection of inflammation, prevention and early detection of cancer, include several diverse technological innovations. They are bio-mimicry self-assembling peptide systems, which serve as building blocks to produce nucleotides, peptides and phospholipids, which support cell proliferation and differentiation and give insights into protein-protein interactions [11]. Microchip drug release systems, micromachining hollow needles and two-dimensional needle arrays from single crystal silicon for painless drug infusion, intracellular injections, microsurgeries and needle-stick blood diagnosis form another group of tools [12, 13]. All of these inventions could one day lead to develop personalized treatments [14].

The creation, control and use of structures, devices and systems with a length scale of 1–100 nm is the domain of Nanotechnology. Macromolecular structures such as dendrimers and liposomes at the nanoscale are also considered valid nanotools [9, 15]. The application of various nanotools in various areas of medicine is depicted in Figure 1. This list in the figure is by no means exhaustive as nanotechnology is continuing to grow with new technologies emerging each day.

Nanopore technology

Biomolecular nanopore detector technology was first developed to rapidly discriminate between nearly identical strands of DNA thereby replacing the tedious process of running billions of copies of DNA through sequencing machines and minimizing errors and saving time [16]. In this technology single molecules of DNA are drawn through pores that are 1–2 nm in size and serve as a sensitive detector. The detection system through its electronic signature process can sequence more than one base pair per millisecond. This technology has the potential to detect DNA polyploidy, and DNA mutations.

Nano self-assembling systems

This field includes biomimetics encouraged to mimic nature and create biomolecular nanomachines to handle various biological problems. Many biological systems use self assembly to assemble various complex molecules and structures [17]. Numerous man-made self-assembling systems that mimic natural self assembly of molecules are created to snap together fundamental building blocks of complex polymer molecules structured easily, and inexpensively, on beads, tubes, wires, and flat supports, and in suspensions and liposomes. These assemblies can have geneti-

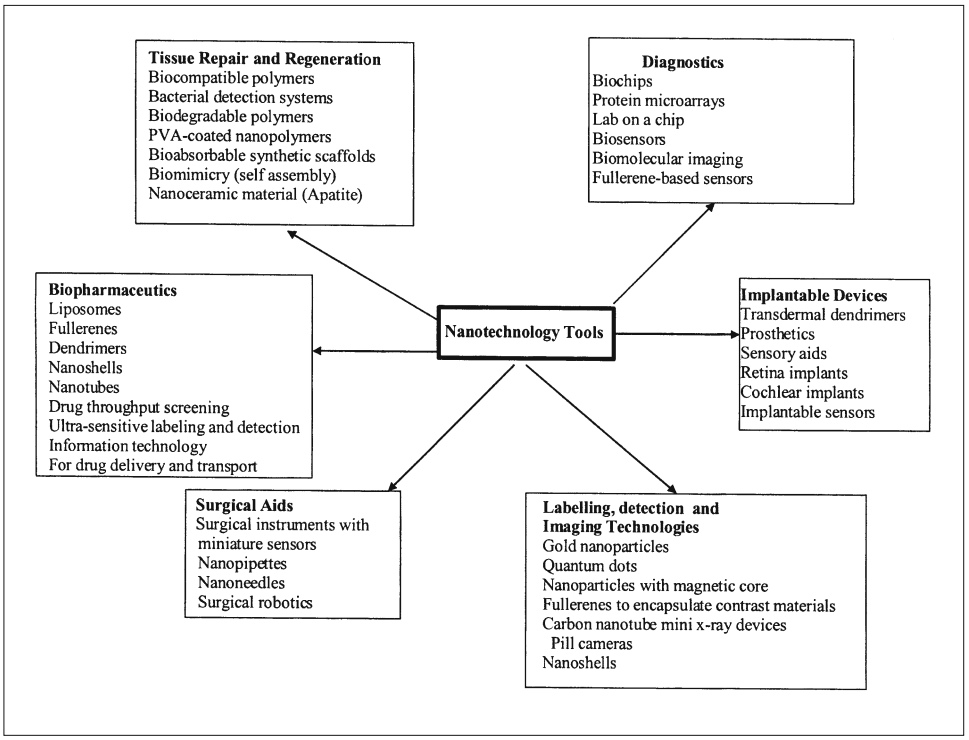

Figure 1
The application of nanotools in various areas of medicine.

cally introduced bifunctionality so that nonspecific molecules are repelled from fusing with the cell membrane fusion layers. DNA, lipid bilayers, ATP synthase, peptides and protein foldings are target candidates for self assembly. Liposomes are an example of a human-made supramolecular structure.

Cantilevers

Nanoscale cantilevers are about 50-mm-wide flexible diving board-like beams that can be coated with antibodies and DNA complementary to a specific protein or a gene. When molecules come in contact with these substrates coated on the surface of cantilevers, they bind to the substrate and make the cantilevers resonate or bend as a result of this binding event [18]. This bending deflection is proportional to the quantity of binding, thus making it a quantitative technique. Multiple cantilevers

can be used simultaneously to differentiate between bound and unbound molecules. Likewise, multiple antibodies can be used in the same reaction set up to quantify several markers at a time. An important advantage of this technique is that there is no need to add fluorescent tags to detect and quantify the molecule. Any biological sample containing biomolecules of interest can be tested. Nanoscale cantilevers, constructed as part of a larger diagnostic device, can provide rapid and sensitive detection of inflammation and cancer-related molecules and to evaluate how various drugs bind to their targets at a concentration 20 times lower than clinical threshold.

Carbon nanotubes

Carbon nanotubes, also known as "bucky tube" and "buckyballs" are a member of fullerene structural family potentially useful in a number of biological applications. They could be cylindrical (nanotubes), spherical (buckyballs) or branched (fullerenes). Nanotubes could be single-walled nanotubes (SWNT) or multi-walled nanotubes (MWNT).

The usefulness of nanotubes in drug delivery and cancer therapy is accomplished through the transporting capabilities of carbon nanotubes via suitable functionalization chemistry and their intrinsic optical properties. Proper surface functionalization is necessary to make carbon nanotubes biocompatible. In their most recent applications, SWNTs have been used to transport DNA inside living cells [19]. Intracellular protein transport has also been accomplished [20], although they are suspected to cause severe immune responses. Most SWNTs have diameters close to 1 nm, with a tube length that can be many thousands of times larger. SWNTs with lengths up to the order of centimeters have been produced [21]. SWNTs are a very important class of carbon nanotubes because they exhibit important electrical properties not shared by the MWNT variants. On the other hand, MWNTs are fabricated as multiple concentric nanotubes precisely nested within one another for perfect linear or rotational bearing. The technology has now advanced into merging these MWNTs with magnetic nanomaterials like magnetite, which can be functionalized.

Gadofullerenes offer the ability to concentrate more gadolinium at the site of disease, than traditional Gd-DTPA. This is the result of the shielding that the carbon structure provides and its ability to link more gadolinium per conjugate. Gadofullerenes also take advantage of the gadolinium-water interactions as the gadolinium is brought along the periphery of the structure and can maintain its interaction with water, which is the basis of traditional proton density magnetic resonance imagine (MRI). These properties lead to a greater signal, which can increase sensitivity to small lesions.

Recently, the technology has been further improved by developing smart bionanotubes that could be manipulated to produce open or closed end nanotubes to

encapsulate drugs or genes to deliver them in a particular location [22]. Thus, possibilities exist for using nanotubes to improve gene sensing, gene separation, drug delivery and detection of biomarkers to improve health care, protection against bioterrorism and other areas of molecular sensing.

Nanoparticles

They are mostly spherical particles with specific properties that allow their detection, analysis and quantification. Fundamentally, they are nanoprobes where these particles are complexed with biomolecules, drugs and other reagents. They exhibit various physical and optical properties. For example, iron oxide nanoparticles exhibit super paramagnetic properties [23], and gold nanoparticles specific optical absorption properties depending on their size [24]. It is important to note that particles made from the same materials but of micron dimensions do not exhibit such unique optical or magnetic properties [8]. One can thus combine the immense surface to volume ratio of these nanostructures to deliver higher loads of compounds encapsulated or linked to their surface, while their presence can be measured due to their characteristic magnetic or optical properties.

Quantum dots

Quantum dots (QDs) are tiny light-emitting particles on the nanometer scale. They are emerging as a new class of biological probes that could replace traditional organic dyes and fluorescent proteins. The fundamental benefit of using QDs is the high quantum yield and strong emission intensity. The emission spectrum of QDs is a function of the particle size, and hence by varying particle size QDs can emit from visible to infrared wavelengths [25]. They can be excited by UV light. Their broad excitation spectrum and narrow emission spectrum with little or no spectral overlap makes them attractive for imaging and resolving multiple species at the same time without complex optics and data acquisition systems. QDs offer higher signal to noise ratios compared to traditional fluorochromes. Their high sensitivity allows accurate detection even in the presence of strong autofluorescence signals encountered during *in vivo* imaging [26]. Their excellent resistance to photobleaching is particularly useful for long-term monitoring of biological phenomena, critical in live cell imaging and thick tissue specimens. Already QDs are finding increasing use in live cell imaging, by themselves or as fluorescence resonance energy transfer (FRET) donors combined with traditional fluorchromes, and in *in vitro* assays and live animal imaging for cancer and tumor diagnostics.

Limitations of QDs can arise from the stability of the core shell structure. Most commercially available materials comprise a core of CdSe and a shell of ZnS. To

render this inorganic structure hydrophilic, amphiphilic polymers are used to cap the shell layer and provide reactive sites for further linking to proteins. It is, therefore, the stability of this layer that controls the aggregation of QDs, as well as possible release of core materials (Cd ions) to their surroundings, which may result in toxicity [27]. This has limited the immediate clinical use of QDs, but has focused applications to animal testing and *in vitro* assay developments. Another reported problem is the blinking property of the QDs. QDs tends to blink at the single dot level and hence present some limitation in absolute fluorescence quantification. However, when used for imaging of biomarkers, this property does not have much of an effect as there are hundreds or thousands of QDs in a sample to allow proper averaging.

Paramagnetic iron oxide crystals

Paramagnetic iron oxide nanoparticles are a new class of contrast agents that are finding increasing applications in the field of diagnostics and molecular imaging based on magnetic resonance (MR) [23]. Traditional MRI agents rely on the interaction of the proton density, i.e., water molecules and the magnetic properties of the tissue. These paramagnetic agents accelerate the rate of relaxation of protons in the longitudinal direction, resulting in bright images, and hence are highly dependent on water molecules. However, the super paramagnetic iron oxide nanoparticles, by the virtue of their nanoscale properties, disturb the magnetic field independently of their environment, and hence are not dependent on presence of water molecules. They are also called negative enhancers as they act as negative contrast agents and appear dark where they are sequestered. The traditional MR agents such as gadolinium–diethylenetriamine penta-acetic acid (DTPA) enhance the signal from the vascular compartments and are nonspecific, whereas the nanoparticle-based contrast agents impact the MR signal from tissues and cells.

Iron oxide nanoparticles are classified into two types depending on their size: (1) superparamagnetic iron oxides (SPIOs) (50–500 nm), and (2) ultra-small super paramagnetic iron oxide (<50 nm). The advantage of these contrast agents lies in their ability to get sequestered anywhere within a support matrix and still generate a contrast, whereas the traditional MR agents need water in their vicinity of generate contrast. These nanoparticles can be used for both passive and active targeting. Because of the small size of these particles, tissue macrophages readily take up these agents, and hence it is possible to image liver, spleen, lymph nodes, and lungs. In addition, it is also possible to functionalize these nanoparticles using a wide variety of ligands, antibodies, peptides, aptamers, drugs, etc., to achieve site-specific or biomarker-specific targeting. This is an added advantage since traditional paramagnetic formulations are difficult to conjugate to antibodies, and even when conjugated, owing to the small number of cellular receptors, the signal intensity is not sufficient for accu-

rate imaging. Thus, as a result of their superparamagnetic properties, iron oxide nanoparticles have been used as contrast agent for imaging of cancer, brain inflammation, arthritis, and atherosclerotic plaques. Because of the small size, these iron oxide particles have been able to distinguish between the normal and tumor-bearing lymphatic nodes [28]. These nanoparticles may also distinguish very small metastases (less than 2 mm in diameter) within normal lymph nodes, a size well below the detection limit of the most sensitive imaging techniques such as positron-emission tomography (PET) available today. Using cells loaded with iron oxide nanoparticles, it has been shown that these particles are non toxic and are cleared from the cell after five to eight divisions. Lewin et al. [29] labeled stem cells with iron oxide particles using HIV TAT peptide and injected them systemically. The labeled stem cells homed on to the bone marrow, and the labeled stem cells did not cause any impairment. However, due to the small size of these particles, a long time is required (up to 24 h) to clear them from the organs and blood to reduce background signals. Thus, MRI using SPIOs may result in improved sensitivity and selectivity, and may assist diagnosis of tumors at the earliest stages of malignancy or metastasis.

Dendrimers

Dendrimers are a new class of hyper-branched polymer macromolecules that radiate from a central core with structural symmetry. They can vary in shape, size, surface, flexibility and topography, enabling fabrication of functional nanoscale materials that would have unique properties [30, 31]. They may be useful for developing antiviral drugs, tissue repair scaffolds, and targeted carriers of chemotherapeutics. Certain dendrimers are now being used commercially as immuno-diagnostic agents and gene transfection vectors. Dendrimers complexed with gadolinium (III) ions (Gadomer–17) are being tested (Phase I clinical trial) for MRI angiography [32]. It is anticipated that many exciting developments will emerge from the use of dendrimers in the near future.

Nanosomes/polymersomes

These are made up of phospholipid bilayers exhibiting multifunctioning characteristics. They facilitate encapsulating of various classes of drugs and diagnostic agents for their controlled delivery into cellular and therapeutic targets. Important drug delivery strategies utilizing these agents include polymersomes, hydrogel matrices, nanovesicles/nanofiber mats and biodegradables [33]. Both small and large molecules can be used. Biodegradeable polymersomes based on polyethylene oxide have been synthesized, and they may be used as a surface to anchor antibodies or other targeting molecules. Quite recently fluorescent materials have been embedded into

these cell-like vesicles [34] to produce near-infrared emissive polymersomes that could be used to locate areas of inflammation and deliver a load of drug to inflammation sites. Interestingly, inflammation sites deeper than 1 cm could be imaged with this technique.

Efforts are being made to target DNA complexes into hepatocytes and macrophages with the idea of enabling gene therapy and delivering genetically derived vaccines in a safe and efficacious manner. Polymeric micelles are useful as developing agents for γ-scintigraphy, MRI, and computed tomography (CT) [35]. Liposomes can be injected intravenously and when they are modified with lipids that render their surface more hydrophilic, their circulation time in the bloodstream can be increased significantly.

Another class of polymersomes, called polymer nanotubes, has been synthesized by directly pulling on the membrane of polymersomes using either optical tweezers or a micropipette [36]. These polymersomes are composed of amphiphilic diblock copolymers consisting of an aqueous core connected to the aqueous interior of the polymersome, which are less than 100 nm in diameter. They are unusually long (about 1 cm) and are stable enough to maintain their shape indefinitely. The pulled nanotubes are stabilized by subsequent chemical cross-linking. The aqueous core of the polymer nanotubes together with their robust character offer opportunities for nanofluidics and other applications in biotechnology, especially in the development of nanohyperdermic syringes [36].

In vitro diagnostics

Development and use of analytical tools in diagnostic area possibly presents immediate benefits to the user. Many diagnostic tools have been developed to improve human health. The diagnostic detection methods involve measuring antibody or antigen-based complexes, enzyme-based reaction rates, and polymerase chain reactions using micro-electro-mechanical systems (MEMS) [37]. Other methods include whole-cell bacterial sensors and biosensors which utilize aptamers, which are biomimetic synthetic bioreceptors that can complex with proteins, nucleic acids and drugs. The signal processing in these systems may be optical, electrochemical, or mass-related, creating resonance and thermal detection. Some diagnostic methods utilize nanoparticles as nanoprobes where nanoparticles are interfaced with biological molecules such as antigens, antibodies or chemicals. The nanoparticles used in diagnostics include QDs, nanobarcodes, metallic nanobeads, silica, magnetic beads, carbon nanotubes, optical fibers and nanopores [37].

In antibody/antigen-based detection methods, for example, 1–2-nm-wide, boron-doped silicon wires laid down on a silicon grid can be coated with antigens to provide real time detection of antibodies. Antibody binding to immobilized antigen gives a measurable conductance change at antibody concentrations less than 10

nM. Detection of single copies of multiple viruses has been accomplished via antibody-conjugated nanowire field effect transistors [38].

The dream of optical biopsy is closer to reality with antibody-functionalized semiconductor nanoparticles (QDs) detected by fluorescence microscopy. Multiplexed assays can be developed since the fluorescence emission of QDs is tunable by changing their size. Outstanding detection sensitivity of antibodies in whole blood (picogram per ml) has been obtained using gold nanoparticle conjugates [39].

For detection purposes classical tools can be used as well as nanobased methods, such as atomic force microscopy (AFM) and near-field scanning optical microscopy (NSOM), methods where quantum tunneling plays a key role in amplifying detection capability. Again, such phenomena are related to the nanometer distance between the instrument probe and the surface/specimen under examination. AFM is used to elucidate structures of biomolecules under physiological conditions [40], to determine antibody/antigen binding properties [41], to image the topology of viruses [42], and to image pathologies at the molecular scale [43].

Nanoarrays

Researchers in academia and the pharmaceutical industries traditionally use bioassays, which are often cumbersome and riddled with errors. The recent explosive development in the field of microfluidics, biotechnology and functional genomics has resulted in the miniaturization of these bioanalytical assays to micron scales for routine and throughput screening [44]. These assays have been used for genomic and proteomics analysis, though their application to proteomics still requires refinement since replication of proteins as opposed to DNA is yet to be fully realized. Efforts are being made to improve miniature microarrays, which are still used for analyzing proteins. These include fabrication of AFM-based Dip-pen nanolithography (DPN), which can probe complex mixtures of proteins, reactions involving the protein features and antigens in complex solutions, and can aid the study of cellular adhesion at the submicrometer scale. Protein nanoarrays generated by Dip-pen nanolithography are emerging [45].

With further advances in miniaturization techniques like DPN, it will be possible to design nanoarrays that can detect biological entities on a single particle level in a time- and cost-efficient manner and also profiling of new diagnostic biomarkers at a detection level beyond our imagination.

Application of nanosystems and nanoparticles in inflammation and cancer

The pharmaceutical industry, physicians and patients have long desired better pharmaceutical formulations to improve and extend the economic life of proprietary

drugs, to reduce the costs of preparation and treatment, and reduce toxicity or even death. Nanotechnology has already made significant inroads into the problems of improving delivery of injectibles, oral formulations, drug device implants, and topical and transdermal delivery of drugs. More is expected from nanotechnology in improving the delivery of drugs to the brain, as many of the formulations aimed at treating diseases of the brain fail to cross the blood-brain barrier. Various methods have been tested for drug delivery. For example, carbon-based materials, nanostructures, silicone-based materials, polymers and liposomes, which are capable of delivering drug molecules directly into cells, tumors and sites of inflammation either actively or passively. There is no question that there are many limitations such as opsonization, problems with encapsulation and leakiness of drug that needs to be tackled. Some of these obstacles have been overcome by the development of agents like "stealth liposomes", which escape attack by the immune system. Thus, nanotechnology is expanding our capabilities through promising approaches for delivery of therapeutic agents.

Nanosystems and nanoparticles have opened up hitherto unforeseen avenues in diagnostics and therapeutics in medicine, especially in the fields of inflammation and cancer. The previous treatment strategies in the fields of autoimmune diseases and cancer involved non-targeted treatment options with extensive "collateral damage". Nanodelivery of drugs is envisioned to reduce this collateral damage, extend a drug's availability and effectiveness at the site, and reduce toxicity, cost and storage.

The focus of this section is to highlight several nanomedicine applications that have made an immediate major impact in these fields. Biological nanostructures used in drug delivery systems include lipid-, silica-, polymer-, fullerene (carbon-based buckyballs, bucky tubes)-based nanostructures such as liposomes, micelles and nanoparticle systems.

Liposomes have been widely used as drug delivery systems, but current knowledge extends the use of any nanoparticle as an efficient carrier with necessary modifications.

Liposomal formulations for drug delivery

Liposomes are vesicles with phospholipid membranes that contain hydrophilic substances in their core. The properties vary widely based on the size, lipid composition, surface charge and method of preparation [46]. Liposomal formulations have been used as anti-cancer and anti- fungal drugs, and have helped reduce the adverse effects of these drugs, while improving the efficacy and pharmacokinetics. Conventional liposomes are short-lived *in vivo*, and are rapidly cleared by the reticuloendothelial system (RES). A novel liposomal formulation with a polyethylene glycol (PEG) coating avoids RES-mediated clearing, and is called a stealth liposome. These

stealth liposomes have favorable properties like long circulation half-life and targeted accumulation in tumor tissues [47].

Liposomes have been extensively used in cancer therapy. Some of the major classes of anti-cancer drugs in liposomal formulations that are currently available or in late stages of development include anthracyclines, camptothecins, platinum derivatives, anti-metabolites and cell-cycle-specific drugs like vincristine and doxorubicin. Liposomal formulations have been shown by clinical trials to decrease cardiotoxicity as compared to conventional formulations [48]. Current liposomal formulations include pegylated liposomal doxorubicin (Doxil® Orthobiotech, Caelyx® Schering-Plough) non-pegylated doxorubicin (Myocet® Elan Pharma) and liposomal daunorubicin (DaunoXome®, Gilead Sciences). This protective strategy to limit toxicity has aided in limiting the cumulative dose of anthracyclines and administration of dexrazoxane, a highly effective cardioprotective agent, prior to anthracycline administration [49].

Liposomal platinum derivatives like cisplatin and carboplatin are used in the treatment of head and neck cancers, testicular cancer, lung cancer and many other malignancies. They have shown significantly reduced toxicity and better pharmacokinetic profiles compared to conventional formulations [50]. Some formulations have not yielded the best results. For example, SPI-077, a stealth liposomal cisplatin showed low clinical efficacy in Phase I/II clinical trials, possibly secondary to inadequate release of drug from liposomes [51, 52]. Lipoplatin, which has shown lipid bilayer fusing properties [53], has shown significant nephrotoxicity, but further clinical research is awaited to see if this translates into improved clinical efficacy.

The use of liposomes has also been extended for enhancing immunotherapeutic effects. It is now known that liposomal targeting can be achieved by passive targeting or active targeting. Passive targeting is achieved in both inflammatory and cancerous conditions taking advantage of the leakiness caused by many vascular factors that enhance permeability. This opens up a window of opportunity to increase the drug delivery, with accumulation of drug at higher concentration at the targeted site by extravasation, thereby reducing toxicity and collateral damage. On the other hand, active targeting depends on certain unique properties and molecular strategies involving monoclonal antibody-liposomal conjugates (immunoliposomes), which enables specific tumor cell targeting by antigen identification and drug delivery by internalization of the liposome by tumor cells [54]. Promising examples are the enhanced anti-tumor activity of anti-HER2 immunoliposomes containing doxorubicin [55], and the use of anti-epidermal growth factor receptor (anti-EGFR) immunoliposome, which showed increased cytotoxic effect *in vitro* against tumor cells overexpressing EGFR and enhanced efficacy *in vivo* in xenograft models [56]. The recent advent of the widespread use of monoclonal antibodies in cancer therapy promises more such targeted therapeutic agents.

Liposomal preparations have also been studied in various autoimmune and chronic inflammatory diseases. Targeted delivery of anti-inflammatory agents to

inflamed tissue is a promising approach limiting the adverse impact of these agents on healthy tissues. In animal colitis models, liposomal formulations of 5-ASA achieved significantly higher local concentrations of 5-ASA in inflamed colonic tissues compared to current treatment methods. On the other hand, liposomal preparation of Mercaptopurine (6-MP) failed to improve local delivery [57] because the drug is metabolized before it reaches the inflammation site. When PEG-liposomes containing glucocorticoids were injected in mouse collagen arthritis models, long-lasting reduction in joint inflammation was achieved with a single dose, while regular steroids needed multiple injections [58].

Weekly inhaled liposomal budesonide was as effective as daily inhaled budesonide in a mouse model of asthma [59]. If this result can be replicated in clinical trials, it can greatly enhance patient compliance. This is particularly important since treatment of chronic inflammatory diseases is hampered by patient non-compliance. Similarly, it is reported that liposomal preparations of anti-oxidants can also be used in diseases like adult respiratory distress syndrome (ARDS), sepsis, radiation lung injury and emphysema [60]. Liposomes have also been shown to be effective in diverse clinical applications such as enhanced drug delivery systems for analgesics [61–63].

Application of other nanoparticles in medicine

The carbon nanostructures that have gained most attention have been fullerene nanotubes and the geodesic dome-shaped C60 fullerenes. They have wide ranging applications as drug carriers and can also be used as vaccine delivery tools enhancing the immune response [64]. They have demonstrated neuroprotective properties in cortical cell cultures and have potential therapeutic applications in neuronal-inflammation and neurodegenerative disorders like Parkinson's disease, amyotropic lateral sclerosis (ALS) and cerebral ischemia [65]. Carbon nanotubes can cross the cell membrane without causing damage and they can act as "nanoneedles" [66].

Tectodendrimers are multicomponent dendrimers capable of multiple functions like identifying defective cells, delivering imaging and therapeutic agents to the cell and reporting the response to therapy. They can be individualized for each specific disease state and can be mass produced. Baker and colleagues [67] designed dendrimers with folic acid, fluorescein and methotrexate, and showed a 100-fold increase in the cytotoxic response of cells to methotrexate. In some cases, nanoparticles also aided in avoiding harmful adverse effects of drug vehicles, as in the case of Abraxane® (American Bioscience), nanospheres of albumin-bound paclitaxel thus avoiding the need for toxic solvents like cremophor [68].

Recently, Kriz et al. [69] described a new sensing technology platform integrating a magnetic permeability detection and a two-site heterogeneous immunoassay using monoclonal anti-CRP-conjugated superparamagnetic nanoparticles and solid-

phase polyclonal anti-CRP-conjugated silica microparticles to assay CRP in blood samples. The results were comparable to assays performed in a clinical laboratory. The methodology seems applicable for rapid screening of biomarkers and drugs in a rapid and cost-effective manner using whole blood samples.

Nanoparticles in molecular imaging and targeted radiation therapy

The field of molecular imaging has exploded in recent times. Significant advances have been made in real-time cellular imaging and for detecting cellular pathophysiology. Over the last few years, varieties of nanostructures containing novel contrast agents and nanomaterials such as QDs, gold nanoparticles or nanoshells, supramagnetic nanoparticles complexed with biological agents that can specifically bind molecular signatures of inflammation and cancer have been described [70, 71].

QDs have enabled *in vivo* live imaging, down to the level of a single QD inside a cell. QDs provide several advantages over organic fluorochromes since they are photostable permitting imaging over extended periods of time, avoid interference with cellular autofluorescence, permit tracking of multiple processes simultaneously in the cells and are less toxic than organic dyes [72]. Although there is a possibility of cellular toxicity from the metallic components of QDs, no cellular toxicity was seen, even under selection pressure, when QDs were used to track metastatic tumor cell extravasations in an animal model [73]. However, toxicity to humans is still being debated. QDs have multimodal applications as contrast agents in bioimaging, microarrays, and FACS analysis, in monitoring pharmacokinetics of therapeutic agents, and in multicolor optical coding for high throughput screening [74].

QDs have been successfully been used for sentinel lymph node sampling in gastrointestinal tract in pig models. QDs can have immediate applications in oncological surgery if the safety profile can be established for humans [75]. There are several potential pitfalls, including lack of convincing evidence for absence of cytotoxicity. Further research is needed before we move forward towards widespread use of QDs in biological systems [76].

QDs can also potentially replace conventional fluorochromes in complex fluoroimaging techniques like FRET and fluorescence lifetime imaging microscopy, but intensive research is needed before that can happen.

SPIO crystal core nanoparticles have magnetic properties that can be used to enhance current MRI techniques due to their selective activity during T2 relaxation times. They can also act as ‘negative enhancers’ [77]. Utilizing the lymphotropic properties of these nanoparticles, Weissleder and colleagues [78] showed that superparamagnetic nanoparticle enhanced high-resolution MRI. It was far superior to conventional high-resolution MRI in detecting clinically occult prostatic cancer metastasis to lymph nodes. The combined use of QDs with superoxide paramag-

netic crystals may provide additional information by targeting specific molecular targets for imaging [79]. A particularly interesting application is the use of SPIO particles for the study of nucleic acid sequences and surface topography of subcellular organelles. This is achieved by a modified AFM with a nanoneedle mounted on a cantilever beam that deflects when it comes in contact with a paramagnetic nanoparticle. This response can be quantified and mapped [80].

Metal nanoshells are nanoparticles that can serve as strong near infrared absorbers. This property has been exploited to provide targeted thermal therapy selective to tumor cells without damaging normal tissue using gold nanoshells [81]. Gadolinium neutron capture therapy has several advantages, including more efficient tumor killing effects and the potential for simultaneous MRI to assess response. Fukumori and colleagues [82] utilized cationic polymer chitosan nanoparticles that incorporated gadolinium for efficient cellular uptake, and demonstrated significant *in vitro* tumoricidal effect at relatively low concentrations.

Recently, Bankiewicz and colleagues [83] described an integrated strategy to delivery drugs to the brain. The combined technology involved convention-enhanced delivery (CED) to deliver liposomes containing Gadoteridol, with DIL-DS and MRI to obtain detailed images of drugs moving through a living primate brain following CED for imaging, and to induce better clinical efficacy.

Molecular imaging has now crossed-over into medical imaging through the use of smart imaging agents for *in vivo* molecular imaging and imaging of animal models [84–86]. A recent study showed that magnetic nanoparticle conjugated with anti-VCAM-1 antibodies can detect VCAM-1 expression through fluorescence and magnetic resonance on endothelial cells *in vivo* and *in vitro* [87]. This is an important step, opening up opportunities to use many specific markers specific for inflammation and cancer to diagnose and monitor many inflammatory diseases and cancers.

Summary and conclusions

Biological systems operate at the nanoscale. Nanomedicine is the application of nanotechnology to monitor and treat biological systems in health and disease. This is accomplished by real time monitoring of molecular signaling at the cellular and tissue level. During the past decade, there has been an explosion in this field, resulting in revolutionary advances in determining the microstructure and function of living systems. These discoveries have led to the development of powerful tools for fundamental biological and medical research. Nanotechnology has been applied to targeted drug delivery to minimize side effects, creating implantable materials as scaffolds for tissue engineering, creating implantable devices, surgical aids and nanorobotics, as well as throughput drug screening and medical diagnostic imaging. The nanoinitiatives are funded by governments and private sources throughout the world to develop or further refine the technology to provide the beyond-imag-

inable, most sophisticated tools to a physician and scientists to inflammatory diseases. No doubt, there will be many technical, regulatory and legal challenges in the deployment of these technologies. Unquestionably, there is enough desire and commitment to meet these challenges for the good of society and betterment of the quality of life.

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