Chapter 1
The Biological Significance of "Nano"-interactions

Abstract In the recent decade, the fabrication of nanoparticles and exploration of their properties have attracted the attention of all branches of science such as physicists, chemists, biologists, engineers, and even medical doctors. Interests for nanoparticles arise from the fact that their mechanical, chemical, electrical, optical, magnetic, electro-optical, and magneto-optical properties of these nanoparticles are completely different from their bulk properties and the predetermined differences are depended on the physicochemical properties of the nanoparticles. There are numerous areas where nanoparticles are of scientific and technological interest, specifically for medical community, where the synthetic and biologic worlds come together and lead to an important concern for design of safe nano-biomaterials. In this chapter, we review and discuss the major biomedical applications of nanoparticles.

1.1 Nanoscience in Medicine

Nanomedicine is the application of nanosciences to health and exploits the physical, chemical, and biological properties of nanomaterials. The advent of nanoscience and nanotechnologies is shaping the face of industrial production and economics. As a matter of fact, nano-based products now include electronic components, paint, sports equipment, fabrics, sunscreens, and other cosmetics [1]. However, the most exciting nano-innovations reside in the conception of new medical products such as heart valves, drug-delivery systems, and imaging techniques [1], which will surely obliterate the long-established boundaries amidst chemistry, physics, and biology.

It is anticipated that nanotechnology will have substantial economic impacts by encouraging productivity and competitiveness, converging different disciplines of science and technologies, and stimulating education and human development [2]. Experts predict market growth to hundreds of billions of dollars in the next decade. The worldwide market for products exploiting nanotechnology reached

about US$254 billion in 2009, with nanomedical products accounting for a margin of US$72.8 billion in 2011 [3].

The US government has granted more than US$20 billion to the US National Nanotechnology Initiative for nanotechnology research and development activities, facilities, and workforce training since 2000 [4]. In 2011, the Canadian Institutes of Health Research (CIHR) and the Canadian Space Agency (CSA) have granted US $16 million in funding to seven new research projects on regenerative medicine and nanomedicine [5]. The European Framework Program [6] will invest about 600 million euros per year for nanotechnology research until 2013, with a supplementary, comparable sum provided by individual countries [7]. The economic landscape is thus being dramatically altered by nanotechnology. For instance, in 2004, worldwide corporations spent US$3.8 billion on research and development [8]. More importantly, there is a shift from the discovery stage to applications on nanotechnology, as demonstrated by the ratio increased corporate patent applications to scientific publications from 0.23 in 1999 to 1.2 in 2008 [2]. Additionally, analysts estimate that by 2014, nanotechnology will be responsible for 15 % of all manufactured merchandise, valuing approximately US$2.6 trillion and will create 10 million jobs globally [1].

Physicochemical properties of nanoparticles such as their small size, large surface area, and kinetics of adsorption make them particularly interesting as tools for molecular diagnostics, in vivo imaging, and improved treatment of disease. Metal oxides have been introduced in the early 1960s as ferromagnetic separation moieties and have brought about the use of nanoparticles for magnetic resonance imaging (MRI) in the late 1970s. More recently, application of nanoparticles to medicine has expanded to cellular therapy [9], tissue repair [10], drug delivery [11], hyperthermia [12], (MRI) [13], magnetic resonance spectroscopy [14], magnetic separation [15], and as sensors for metabolites and other biomolecule [16]. Moreover, the unique magnetic properties and small size of magnetic nanoparticles (MNPs) make them appealing for biomolecule labeling in bioassays, as well as MRI contrast agents [17]. Superparamagnetic iron oxide (SPIO) can also be used as magnetic gradients for cell sorting in bioreactors [18], as well as absorbing material in radio-frequency hyperthermia. Moreover, the exceptional physical, mechanical, and electronic properties of carbon nanotubes (CNTs) allow them to be used as biosensors, probes, actuators, nanoelectronic devices, drug-delivery systems, and tissue-repair scaffolds within biomedical applications [19–21]. Recent research has focused on conjugating nanocarriers to specific ligands such as peptides, antibodies, and small molecules and subsequently directing them to sites of interest [22]. These techniques can prove to be appealing alternatives for current cancer and cardiovascular applications.

Thus, a vast array of nanotechnologies can be applied to medical devices, materials, and processes that will affect the prevention, early diagnosis, and treatment of diseases. However, the risk–benefit balance for these materials, with regard to their toxicological profile and any potential adverse pathogenic reactions from exposure, will ultimately define their clinical outcome.

1.2 Nanotechnology and Medical Applications

The applications of nanoscience and nanotechnology to medicine will profit patients by offering new prevention assays, rapid and accurate diagnosis, personalized nanoscale monitoring, and targeted treatment. Rapid advances in fields such as microelectronics, microfluidics, microsensors, and biocompatible materials allow for the elaboration of implantable biodevices such as lab-on-a-chip and the point-of-care devices [23]. Applications of nanotechnology include novel fields such as tissue replacement, transport across biological barriers, remote control of nanoprobes, integrated implantable sensory nanoelectronic systems, and multifunctional chemical structures for targeting of disease. Here we describe budding nanomedical techniques such as implantable biosensors, nanosurgery, tissue engineering, nanoparticle-enabled diagnostics, and targeted drug delivery.

1.2.1 Implantable Biosensors

Unusual physicochemical phenomena at the nanoscale, such as enhanced plasticity [24], marked variations in thermal [25] and optical properties [26], heightened reactivity and catalytic activity [27], speedier electron transport [28], and novel quantum mechanical properties [29], allow for miniaturization, biocompatibility, sensitivity, and accuracy of implantable biosensors for real-time monitoring.

For example, the incidence and prevalence of diabetes is rising worldwide, echoing lifestyle changes, such as obesity and aging populations. The World Health Organization estimates that the number of people afflicted with diabetes will surpass 350 million by 2030, creating a significant unmet need for better monitoring as well as market opportunities [30]. In spite of recent advances in glucose sensors, many obstacles still need to be overcome to achieve a downscaled, portable, and implantable device, such as biocompatibility, stability, selectivity, calibration, miniaturization, and power.

Advances in nanobiosensors offer proper technological solutions in the field of glucose screening [31]. Low cost, low power, and ease of miniaturization make label-free electrical biosensors ideal candidates for glucose monitoring. These sensors can exploit either voltmetric, amperometric, impedance, or optical systems [32]. In the case of glucose monitoring, the appropriate device needs to detect and differentiate multiple targets and should be capable of functioning in a closed-loop feedback [31]. Current management of diabetes is dependent on data acquired from blood drawn from finger pricking and analyzed on test strips. This procedure can be painful and rely on patient's diligence. It does not take into account the daily habits of the patient nor the appropriate insulin dosage required. It is thus important that such implantable sensors have the ability to continuously monitor metabolite levels without patient's intervention and regardless of its physiological state. Moreover, this sensor needs to be implanted and readily explanted without the need for

complicated invasive surgery. In this light, miniaturization of all the components of the sensor, such as the power source, signal processing units, sensory elements, and electrodes, becomes essential. Currently, carbon nanofibers and ultrathin Pt wires are used for the fabrication of miniaturized electrodes [33, 34]. The electrocatalytic properties of these electrodes can be further improved by incorporating metal nanoparticles [35], furthering neuroscience research on nerve stimulation [36], acute pain [37], and implantable drug-delivery systems [38]. Another prospect for sensor miniaturization resides in top-down nanofabrication techniques such as photolithography, dip-pen nanolithography, and micromachining. Etching processes and photolithography permit the creation of needle-shaped biosensors for glucose monitoring [39, 40] that can be produced on an industrial scale. What is more, carbon nanotubes [41], nanorods [42, 43], nanowires [44], and semiconducting polymers [45] are used to develop sensors based on changes to gate conductance [46], hysteresis [47], or threshold voltage [48].

Conclusively, it is imperative to develop implantable biosensors for the simultaneous detection of multiple interdependent metabolites in order to increase confidence in the results obtained and to assist in early disease detection. Multidisciplinary fields of nanotechnology can bring about the development of highly sensitive, multi-analyte sensors.

1.2.2 Nanosurgery

The advent of lasers in the early 1960s changed the face of surgery by making it possible to ablate biological tissue with high precision and minimal invasiveness. It is now possible to perform highly targeted manipulation and ablation at the nanoscale impacting the fields of developmental biology, cellular biology, and assisted reproductive technologies. Ultrashort laser pulses at the picosecond and femtosecond scale are increasingly used in biological applications, such as manipulation and dissection of individual cells in tissue [49–51], ablation of structures and organelles inside a living cell [52, 53], or modification of a medical implant [54]. Recently, femtosecond lasers in combination with gold nanoparticles have been used as a means for virus-free transfection method of human cancer melanoma cells [55].

Moreover, an array of fuel-powered and fuel-free microscale motors have recently been developed for multiple biomedical applications, such as directed drug delivery, biopsy, and precision nanosurgery [56, 57]. Chemically powered nanoscale motors based on the catalytic breakdown of a solution fuel, such as hydrogen peroxide, have gathered much attention [58–60]. Motion control of nanomotors has been enabled by magnetically managing their directionality and adjusting their speed using different stimuli [61, 62]. Fuel-free nanometers are based on externally applied magnetic fields and include helical microstructures and flexible or tumbling nanowires. While remarkable progress has been made regarding the development of nanoscale engines, much improvement needs to be

made with respect to their efficiency, performance, versatility, and biocompatibility. Moreover, effective drug-delivery applications may require a device with autonomous self-adaptive properties with the ability to interact with other motors in order to deliver heavy therapeutic cargoes. As the sophistication of these nanomachines becomes significant, their potential applications in drug delivery, cell sorting, nanosugery, biopsy, and bioassays become considerable. The advent of acoustically driven nanomachines opens up the prospect of controlling the micromotors harmlessly albeit in a deeply penetrative fashion permitting the navigation through physiological fluids and performing targeted therapies in places with reduced accessibility.

Other nanoscale devices, such as nanoneedles and nanotweezers, for controlled fluid handling and cell interrogation have attracted a large amount of interest. Intracellular injections and electrophysiological measurements rely on nanodevices usually based on atomic force microscope (AFM) cantilevers with electrically or mechanically interfaced silicon or carbon-nanotube tips [63]. Nanoneedles, produced by etching a silicon AFM tip by means of a focused ion beam, can pierce membranes and reach the cell nucleus with negligible deformation and damage [64]. Moreover, multiwall carbon nanotubes can be connected to AFM tips and used to deliver molecules into the cell [65]. Recently, a multifunctional endoscope-like device was developed for prolonged intracellular probing at the single-organelle level, without metabolically disturbing the cell. Using individual carbon nanotubes, the endoscopes can transport fluid, record cellular signaling, can be manipulated magnetically, and allow for intracellular fingerprinting using surface-enhanced Raman spectroscopy (SERS) [66].

1.2.3 Tissue Engineering

Regenerative medicine is impacted by the introduction of biocompatible nanostructured scaffolds enabling the replacement, regeneration, and repair of impaired tissues, such as cardiac, bone, cartilage, skin, bladder, nervous, and vascular tissues [21]. These nanomaterials improve the biological properties of the cell by enhancing cell adhesion, motility, and differentiation [67, 68]. It is imperative to develop nanoscaffolds that mimic the three-dimensional microenvironment of the cell in order to permit specific cell interactions and adequate cell behavior. The production of nanofibers by electrospinning offers great flexibility over the scaffold's properties and geometry [69]. Moreover, complementary functionalities can be brought about by chemical conjugation of signaling molecules or protein coatings improving tissue engineering therapies and regenerative medicine.

1.2.4 Nanoparticle-Enabled Diagnostics

The emergence of nanotechnology has refocused the research effort on the remarkable nanoscale properties of several noble metal nanoparticles, such as highly tunable spectral behavior, high surface to volume ratios, and astounding optical properties. An example of these optical properties is localized surface plasmon resonance (LSPR), which is the collective oscillations of free electrons at a metal-dielectric interface when the frequency of incident light matches with the frequency of electron oscillation. Recently, noble nanoparticles, such as gold and silver, have been intensively researched for use in biomedicine and more specifically for the development of inexpensive, highly sensitive detection assays.

Colloidal gold nanoparticles have been intensively explored for the purpose of biosensing due to their optical and physical properties. Gold nanoparticles can easily be synthesized via salt reduction or laser ablation techniques and functionalized with thiol-modified oligonucleotides, permitting the detection of a vast array of biomolecules, nucleic acid sequences, and pathogens. There are fewer reports in the literature on the use of functionalized silver nanoparticles compared to their gold counterparts. This is mainly due to the difficulty of synthesizing silver nanoparticles with a homogeneous size distribution and a heightened difficulty for thiol functionalization.

The signal enhancement brought about by noble metal nanoparticles permits the development of detection assays that are more sensitive, faster, simpler, and cost-effective. These diagnostic platforms can be based on electrochemistry, luminescence, target labeling, and SPR biosensors and may be further combined to allow for early identification of diseases of clinical relevance.

For example, pathogen detection is of utmost importance in multiple sectors, such as in the food industry, environmental quality control, clinical diagnostics, biodefense, and counterterrorism. Failure to appropriately and specifically detect pathogenic bacteria can lead to serious consequences and ultimately be lethal. Conventional methods for the detection of infectious agents are based on standard microbiological methods such as plate-counting or biochemical assays. Although these methods are accurate, they are time consuming as isolation and culturing of large quantities of bacteria can take up to 7 days. In recent years, major breakthroughs in biosensor technology reduced the time required to detect bacteria. However, the majority of techniques currently employed to require some type of radio, enzymatic, or fluorescent labeling to report biomolecular interaction. Other techniques such as direct impediometric detection is limited by the fact that the media utilized needs to be optimized for electrical measurements and that not all microorganisms generate an adequate amount of ionized metabolites to allow for their detection. LSPR is a method that can be suitably modified for bacterial detection as it is designed for real-time monitoring of all dynamic processes without labeling and complex sample preparation.

1.2.5   Targeted Drug Delivery

The majority of current commercial applications of nanotechnology to medicine are dedicated to drug delivery [70]. The aim of nano-enabled drug delivery is to improve the interaction of the drug and its target in order to better locally combat the disease. Delivery of a large proportion of novel drugs is difficult because they are water insoluble. These drugs are either dispersed throughout the nanospheres or confined in the aqueous or oily cavity of a nanocapsule, which is surrounded by a single polymeric membrane. Nanoparticles used in drug delivery include virus-based nanoparticles, lipid-based polymers, and dendrimers. Nanoparticles impact drug delivery by improving medication uptake, altering exposure time and clearance, site-specific targeting, allowing predetermined drug release, reducing side effects, and allowing for immunoisolation.

The major difficulty of nanoparticle-mediated drug delivery is the poor penetration of the NP and the release of its therapeutic cargo. Powerful propulsion and enhanced navigation capabilities are required for the efficient delivery of the payloads to their site-specific targets. Fuel-free magnetically driven nanomotors are an attractive solution for drug nanoshuttles [71]. However, despite recent progress in drug nanoshuttle research, much challenges need to be overcome in order to translate the technology to in vivo applications. Namely, these challenges comprise biocompatibility of the nanocarriers, autonomous release of the drugs carried, swimming against blood flow, and limited tissue penetration. Independent unloading of the therapeutic drugs could be brought about by use of cleavable linkers reactive to tumor microenvironments, such as acidic pH and protease enzymes. Moreover, new research in ultrasound-triggered microbullets [72] allow for the transportation of the therapeutic payloads for site-specific discharge while overcoming cellular barriers and blood flow. Finally functionalization of the nanocarriers with targeting ligand could confer tissue specificity, reducing substantially the side effects of toxic drugs in cancer therapy.

1.3   Bridging Nanoscience and Nanomedicine

More than 40 years of research in biomedical engineering has brought about revolutionary medical instruments, such as endoscopes for surgical practice. Effective biomedical research and successful development of medical instruments rely on the ability to understand the requirements of the medical practitioner and the unmet medical need. The main actors involved in the production of novel technologies, namely, universities and industry, must cooperate extensively to assure the process of knowledge flow between the various stakeholders.

Improving the individual sectors of education, research, and innovation is imperative for the convergence of nanoscience and technology. Bridging medicine and nanoscience requires an efficient transfer of knowledge between laboratories

and the market and subsequent successful commercialization of the products. Moreover, this necessitates close collaboration between multiple disciplines such as engineering, medicine, and computer science. Therefore, multidisciplinary research groups and technology transfer offices are playing a crucial role in the development of novel medical technologies through a higher comprehension of the nanostructure, physicochemical properties, and biocompatibility and their influence on the performance of these devices.

1.4 The Nanoparticle Interface

Although the use of nanoparticles can significantly improve the way illnesses are diagnosed and treated, it is primordial to shed light on the correlations between nanoparticles' unique properties and the biological response they will evoke. In effect, the present paradigm in environmental epidemiology holds that exposure to materials in the nano-size range could cause significant public health problems, such as pulmonary and cardiovascular disease [73]. These observations put forward the need to assess the potential risk of newly engineered nanoparticles in terms of various physicochemical properties to properly assign their mechanisms or causes for toxicity both outside and within the biological environment. To study the safe use of nanomaterials at the nano–bio-interface, it is essential to examine the dynamic physicochemical interactions, kinetics, and thermodynamic exchanges between the surfaces of the nanomaterial and the biological components with which it interacts. Examples of such components are proteins, membranes, phospholipids, endocytic vesicles, organelles, DNA, and biological fluids.

Complete characterization includes several measurements, such as size and size distribution, chemistry of the material, surface area, state of dispersion, surface chemistry, and others [74, 75]. Most importantly, the material's chemical composition, surface functionalization, shape and curvature, porosity and surface crystallinity, heterogeneity, roughness, and hydrophobicity or hydrophilicity will greatly influence the nanoparticle surface properties. These characteristics will shape the interaction of the nanomaterial with its surrounding medium through (1) ions, proteins, organic materials, and detergents adsorption; (2) double-layer formation [73]; (3) dissolution; or (4) reducing free surface energy by surface restructuring [76].

1.4.1 Interaction of Nanoparticles with Environmental Biomolecules

Characterizing the interface between the nanoparticle and its liquid environment is fundamental to the understanding of the nano–bio-interface. However, interaction mechanisms between nanoparticles and living systems are not yet fully understood.

Although steady-state behavior is often assumed when evaluating the bulk properties of nanoparticulate suspensions, the nano–bio-interface is exposed to an inhomogeneous and dynamic environment. This is a direct result from the distribution and spatial localization of proteins, lipids, and glycosylated structures of the nanoparticles' microenvironment. Moreover, the interface experiences constant fluctuations as a result of cellular turnover and environmental variations, namely, secreted cell products. Furthermore, the nature of the particle influences the binding of protein's surface ligands, and alterations to free surface energy may induce conformational changes or oxidative damages. The microenvironments of the particle can also chance as these particles can be engulfed inside the cell.

Events occurring at the nanoscale are still governed by Van der Waals (VDW), electrostatic, solvation, and depletion forces [77]. VDW forces are a consequence of the quantum mechanical movements of the electrons. These fluctuations result in a small nonetheless significant dipole in the nanoparticle, which induces a dipole moment in the atoms of the neighboring particle, triggering an attractive force between both particles. The electrostatic force in the system results from surface charges that inexorably occur on the particles when they come in contact with water. The ionic strength in most biological fluids is approximately 150 mM [77]. Thus, the electrostatic forces are, in all likelihood, to be screened within a few nanometers of the surface. Solvation becomes important when dealing with inorganic and hydrophilic nanoparticles. This phenomenon occurs when water molecules attach to the particles with enough energy to create steric layers on the surface of the nano-entities. This renders interactions and adherence of two particles extremely difficult. On the other hand, hydrophobic attraction can occur if the affinity of two surfaces for water is lower than that between water molecules. However, these known interactions can be complicated by nonrigid compliant cell membranes that can deform when interacting with a nanoparticle, due to the former's fluidity and thermodynamics. Moreover, the cell surface is nonuniformly charged due to the presence of surface proteins and other structures. This surface heterogeneity varies between 10 and 50 nm and thus greatly alters its interaction with nanoparticles. More importantly, cell surfaces are not passive, inducing a time-dependent dynamic interface [76].

1.4.1.1 Nanoparticle–Protein Interactions

Immediately after its introduction in a physiological environment, proteins such as apolipoproteins, fibronectin, vitronectin, and others, adhere to the nanoparticle (Fig. 1.1). Protein adsorption to various materials has been widely studied and it has been found that factors such as electrostatic interactions, hydrophobic interactions, and specific chemical interactions between the protein and the adsorbent play important roles in the characteristic of the bound protein–nanoparticle. It is argued that to understand and predict the cell–nanomaterial interaction, the particle and its "corona" of more or less strongly associated proteins from blood or other body fluids should be considered. It is important to understand how cells
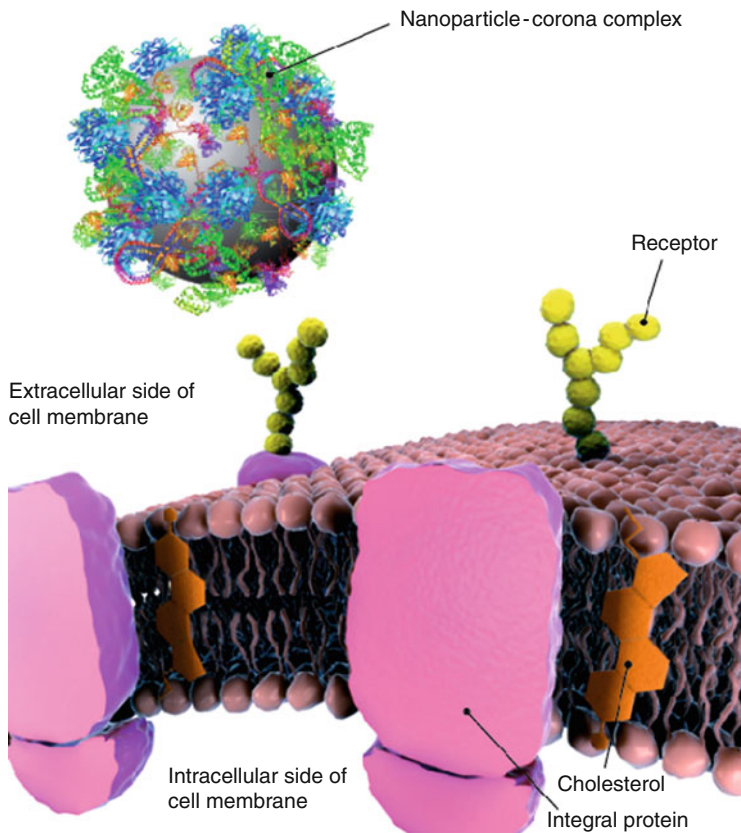
Fig. 1.1 The formation of protein corona on the surface of nanoparticle can affect the interaction of nanoparticle with the cell plasma membrane (Adapted from [78])

"read" at once the composition, the organization of this protein layers, and the exchange times of the proteins on the nanoparticles. The composition of the protein corona at any given time will be determined by the concentrations of over 3,700 proteins in plasma [79]. The organization may depend on concentrations, association rate, and affinity of the protein to the particle. Studies of protein adsorption show two forms of adsorption layers consisting of an irreversibly adsorbed fraction and a reversibly adsorbed fraction. These proteins may undergo conformational changes, leading to exposure of new epitopes, altered function, and avidity effects. Preexisting surface species prior to the introduction of the nanoparticle into the biological fluids might influence protein adsorption kinetics. These preexisting molecules can be residues from the manufacturing process, industrial chemicals, and stabilizers or originate from ambient gases and organic and inorganic biological buffers.

The nature of the particle surface (i.e., size, surface area, hydrophobicity, charge density, surface chemistry, and stability) will greatly affect its interaction with surrounding biological moieties. The particle size is usually defined as the diameter of a sphere that is equivalent in volume to the particle measured. Several methods are used to determine size distributions: light scattering, differential mobility analysis, time-of-flight mass Spectrometry (TOF-MS), microscopy, and others [80]. Reducing particle size to the nano-level can modify the physicochemical properties compared to the corresponding bulk material [73]. The size of nanoparticles (NPs) determines the path they take in the body. It has been reported that particles less than 30 nm in size are rapidly eliminated by renal excretion and that larger particles are phagocytosed by macrophages. Nanoparticles of 30–150 nm will go to the bone marrow, the heart, the stomach, and the kidneys and those of 150–300 nm will be found mainly in the liver and the spleen [81].

The surface area corresponds to the surface of the nanoparticle that is exposed to the environment. This property is of great importance when we study toxicity of NPs, because interactions with the biological organism occur at the interfacial area of the material. The area–volume ratio establishes the number of possible reaction sites on the particles. An increase in reactivity can be either advantageous (increased ability of carrying drugs, increased uptake, etc.) or negative (toxicity, induction of oxidative stress, etc.) [73]. The hydrophobicity of nanoparticles controls the adsorption of plasma proteins on the surface of NPs and may also play a role in the macrophage uptake. An augmentation in the hydrophobicity of the particles facilitates binding to the cell membrane by forming hydrophobic interactions. In fact, studies showed that the more hydrophobic the particles, the larger the total amount of bound protein [82].

Among the physical characteristics of nanoparticles, the surface charge density is an important one. It has major effects on the impact of the particle in the organism. Indeed, the concentration of electric charge on a particle will cause or inhibit some bindings and will change the dispersion of particles in the body, considering a repelling force between like charges and an attractive force between the opposite charges. Note that, although not attracted magnetically, nanoparticles agglomerate. The presence of salts and electrolytes in biological solutions may neutralize repulsion of surface charges on the nanoparticles, allowing particles to agglomerate [83]. The surface charge density has a direct effect on the binding of nanoparticles with cells. For example, macrophages present negatively charged sialic acids on their surface [83]. Thus, positively charged nanoparticles will bind to them. Consequently, these particles will be phagocytosed. Remarkably, the sign of the charge (positive or negative) of the particle is not as influential as expected. Indeed, studies show that charged particles, cations or anions, are more easily absorbed by the cells than electrically neutral particles [82].

Surface chemistry also has a great influence on the interaction of the particle with the biological environment. In order to stimulate or reduce the effects of nanoparticles on the organism, it is possible to coat its surface with various substances. To interact with specific biological targets, a coating acting as an interface can be attached to the nanoparticle. Among all possible coatings, we

found antibodies, biopolymers (such as collagen), and monolayers of small molecules [80]. For example, a coating of small polyethylene glycol (PEG) molecules has allowed NPs to be specifically absorbed by cancer cells, but not significantly by macrophages. Therefore, PEG could be used in cancer therapy. Surface modification has other benefits. Because of their surface charge density, nanoparticles tend to agglomerate. As discussed previously, the repulsion between the negative charges is offset by the impact of dissolved salts of opposite charge in the biological solution. Polymeric coatings can be used to prevent agglomeration. However, it is necessary to study the characteristics and impacts of these polymer layers, as they may have an effect on the performance of a nanoparticle.

Functionalization of nanoparticles with peptides can be used to control protein–nanoparticle interactions. Studies show that the chirality of the functional groups bound to nanoparticles affects the complex stability. This demonstrates that ligands can be used to control protein recognition [79].

The particle's interaction with biological compounds is dependent on protein association and dissociation kinetics. The nanomaterial–ligand complexes have a lifespan ranging from microseconds to days. Multiple proteins form transient complexes with nanoparticles, and it is known that protein concentration and composition of the physiological fluid will influence the formation of the corona. In the blood, human serum albumin and fibrinogen are predominant and thus dominate the particle surface for brief periods of time, whereas proteins present in a lesser extent with higher affinities and slower kinetics might, in due course, oust them. Conversely, bronchial and ocular fluids are less abundant in proteins and it is the lower affinity proteins that will dominate the nanoparticle's surface.

A detailed review of the formation of protein corona on the surface of nanoparticles, the kinetics of hard and soft corona, and parameters affecting the corona composition and structure is available in Chap. 2. The role of protein corona on nanoparticle–cell interaction, toxicity, and circulation lifetime in body is discussed in Chap. 3. Finally, Chap. 4 contains the characterization techniques for studying and analyzing the adsorbed corona on the surface of nanoparticles.

1.4.1.2   Nanoparticle–Lipid Interactions

Nanoparticle interactions with phospholipid bilayers result in a process called membrane wrapping, where the nanoparticle is often engulfed in the lipid moiety. This phenomenon is valuable for site-specific drug delivery. In order to overcome the forces obstructing particle uptake in the cell, surface ligands are cemented on the particle's surface and interact with complementary receptors on the cell, resulting in receptor-mediated endocytosis. These ligands can be chemical moieties, metallic sites, polymers, or surface functionalities. Many strategies, such as the use of amphipathic cell-penetrating peptides (CCPs), polycationic polyethyleneimine (PEI), and polyamidoamine, permit the particles to enter the cell membrane without causing cell injury. However, attention must be given to the

cationic density, which can compromise the membrane's integrity and lead to cytotoxicity.

As with protein interactions, surface charge, particle size, hydrophobicity, and surface roughness play an important role in particles' interactions with phospholipids. It is known that hydrophobic particles tend to agglomerate and are quickly removed by the reticuloendothelium. Moreover, nanoscale surface roughness significantly decreases repulsive interactions, thus promoting adhesion on NPs to lipid membranes and easing their endocytosis.

1.4.2   Biological Response to Nanomaterials

Despite intensive in vitro research on the interaction of nanomaterials with biological compounds, very little is known about the in vivo fate of nanoparticles. Current knowledge is based on few studies addressing the endocytic pathways of small quantities of fluorescent or radiolabelled nanomaterials. In order to determine the in vivo impact of nanomaterials, it is primordial to study the cellular responses relative to the physicochemical properties of the nanoparticles used. This section will explore the internalization and uptake process of nanoparticles, as well as their potential cytotoxic effect.

1.4.2.1   Internalization and Uptake

Nanoparticles cause an extensive array of intracellular responses contingent on their physicochemical properties, concentrations, time of contact, subcellular distributions, and interactions with biological molecules.

The human body recognizes all nanoparticles as foreign entities; therefore, they are quickly removed from the blood circulation [84]. Multiple in vitro and in vivo studies dealing with mechanisms of NP uptake in different cell types and NP distribution in animal models demonstrate that there is not one common uptake mechanism for NP. Endocytosis is the process by which cells absorb material from outside the cell. Endocytic pathways comprise pinocytosis, the formation of caveolae and clathrin, and caveolae/clathrin-independent uptake. Phagocytosis is the process by which cells ingest particles as they are sealed off into a large vacuole known as a phagosome. Phagosomes fuse with lysosomes in their maturation process, forming phagolysosomes. Pinocytosis is the biological process of the cell membrane to form a pocket (vesicle). The filling of the vesicle occurs in a nonspecific manner. The vesicle then travels into the cytosol and fuses with other vesicles such as endosomes and lysosomes. Caveolae consist of internalization of particles by the protein caveolin-1 with a bilayer enriched in cholesterol and glycolipids. Caveolae are pits in the membrane that resemble the shape of a cave. Clathrin-mediated endocytosis is the uptake facilitated by membrane localized receptors and ion channels. These receptors are associated with the cytosolic

protein clathrin, which initiates the formation of a vesicle by forming a crystalline coat on the inner surface of the cell's membrane.

As other factors, the importance of uptake depends on the physicochemical properties of the NP such as chemical composition, size, geometry, surface charge, coating, aggregation status, the exposed cells, and their microenvironment. Particle size and shape are believed to be key parameters for endocytotic pathways. Particle sizes of less than 120 nm are believed to adhere to endocytic uptake; however, little existent scientific data confirms this notion. While limited studies imply a correlation between particle size and endocytic mechanisms, most lack appropriate nanoparticle characterization and rely on nonspecific inhibitors to hinder endocytic uptake. Uptake mechanisms of NP in specific cells of the immune system like neutrophils, monocytes, macrophages, and dendritic cells have been studied by several investigators, and it is well known that macrophages are predominantly involved in these mechanisms [82]. Phagocytosis uptake mechanisms are believed to favor particles bigger than 500 nm. However, nanomaterials can agglomerate and are therefore capable of being phagocytosed. The scientific literature is incongruent with regard to the relationship between the sizes of primary nanoparticles, aggregation, agglomeration, and their phagocytotic potential. Obvious discrepancies in the literature corroborate the fact that our understanding of such systems is limited.

The surface modification of nanoparticles is an important issue in terms of the control of internalization and their uptake by cells and targeted tissue. For instance, binding of transferrin ligands to its receptors triggers endocytosis through clathrin-coated pits. The abundance of caveolae in mammalian cells impacts their potential for caveolae-mediated endocytosis. This type of uptake appears to play as an important role entry mechanism for viruses into cells. It is thus believed that caveolar entry and transport into cells can be favored by adopting viral coat proteins.

Lastly, the protein corona greatly impacts the fate of the nanoparticle in a biological environment. Albumin, immunoglobulins, complement, fibrinogen, and apolipoproteins tend to bind more strongly to nanomaterials and have been shown to promote opsonization, phagocytosis, and endocytosis.

1.5 Cytotoxicity

Cytotoxicity can be analyzed by different features [85]. In the literature, various studies state that the exposition of NPs to cells can affect the cellular, subcellular, and genetic behavior and induce cell's death through disruption of the plasma membrane's integrity, mitochondrial damage, and impairment of the nucleus. Exposure of the body to nanoparticles is believed to trigger an inflammatory response and provoke oxidative stress that will ultimately lead to cell death. Cytokine production and disturbance of the oxidant and antioxidant cellular processes are believed to be key factors in NP cytotoxicity.

The contact between cells and NPs is believed to induce the formation of reactive oxygen species (ROS) cellular signaling cascades that control cellular proliferation, inflammatory processes, and cell death [86]. Oxidative stress is caused by an unbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. Enzymes that maintain the reduced state through a constant input of metabolic energy preserve this reducing environment. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.

The main cellular substructures affected are the following:

(1) The plasma membrane with enzyme complexes such as the NADPH oxidases, (nicotinamide adenine dinucleotide phosphate-oxidases), the activity and regulation of which may be affected by interaction with nano-sized particles
(2) The mitochondria electron flow and leakage from the inner membrane
(3) The endoplasmic reticulum's calcium ion levels may be disregulated

The production of ROS by NPs is dependent on the chemical reactivity of nanoparticle materials, the chemical reactivity of impurities found in particle preparations, and the physical interaction of particles with cellular structures involved in the catalysis of biological reduction–oxidation process.

In vitro and in vivo studies have shown that among particles of different sizes, nanoparticles which have a size smaller than 100 nm are potentially the most dangerous due to their large surface area, deep penetration, and high content of reduction–oxidation cycling organic chemicals [87]. Moreover, it has been demonstrated that carbon nanotubes hinder macrophages ability to degrade and remove foreign particles. Macrophages are believed to being incapable of incorporating long and stiff nanotubes into their phagosomes. Oxygen radicals and hydrolytic enzymes are excreted in the microenvironment in an effort to obliterate the CNTs, thus leading to chronic inflammation. This chronic inflammation could lead over time to mutagenesis, a problem previously encountered with asbestos fibers.

Although oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, myocardial infarction, and Alzheimer's disease, ROS are not automatically harmful. It is only when the protective responses fail to provide adequate protection that a further increase in ROS production can result in proinflammatory and cytotoxic effects. The presence of immune system proteins and cells, such as proinflammatory cytokines (IL-1, IL-6, and TNF-α), Th1-type cytokines (IL-12 and IFN-γ), Th2-type cytokines (IL-4, IL-5, and IL-10), macrophages, and neutrophils, is indicative of an inflammatory response. Although little is known about the physicochemical factors that provoke an inflammatory response and apoptosis subsequent to NP exposure, it is believed that NP size is a determining factor. Size-dependent effects of nanomaterials have been observed in instillation and inhalation exposure studies where the inflammatory responses of experimental animals correlated specifically with nanoparticle surface area [88–90].

Apoptosis is the process of a death of a cell mediated by an intracellular program that may occur in multicellular organisms. Apoptosis occurs when a cell is damaged beyond repair, infected with a virus, or undergoing stressful conditions. Damage to DNA from ionizing radiation or toxic chemicals can also induce apoptosis. The "decision" for apoptosis can come from the cell itself, from the surrounding tissue, or from a cell that is part of the immune system. During apoptosis, cells put in place "suicide mechanism" which results in various modifications on the cellular level. The most significant modifications are alteration of the outer mitochondrial membrane, condensation of cell's cytoplasm and core, and fragmentation of DNA.

Many nanoparticle cytotoxicity researchers have identified mitochondria as a potentially relevant target organelle with regard to the cellular effects of NP. Currently, several NPs have been shown to be capable of eliciting damage of the nuclear DNA [91]. On a single-cell level, such particle-induced injuries may principally have three major consequences, usually depending on the type and extent of DNA damage, namely, induction and fixation of mutations, induction of DNA cell cycle arrest, and activation of signal transduction pathways which promote apoptosis.

Correlating the physicochemical properties of nanoparticles and their biological responses can be challenging as surface area, particle surface chemistry, biodegradability, concentration, and solubility will greatly affect the way the particle is going to be perceived by the biological environment and their subsequent pharmacokinetics and biodistribution. It is therefore imperative to understand the mechanisms governing the interactions of the aforementioned particles and the major players of the immune response. In order to do that, the physicochemical properties, adsorbed proteins, adherent cells, and inflammatory cytokines and growth factors need to be fully characterized.

1.6   Conclusion

The late Nobel laureate physicist Richard Feynman laid the first stepping stones of modern nanotechnology in 1959. In his lecture "There's plenty room at the bottom, an invitation to enter a new field of physics," he proposed to employ machine tools to manipulate and control things on a small scale. In recent years, the field of nanoscience has been rapidly evolving. Enormous amount funding has contributed to advances in diagnostics and therapeutics at the nanoscale. However, these nanodevices will ultimately need to pass many rigorous testing protocols to ultimately be approved by the regulatory agencies, such as the FDA, before being allowed on the markets. Therefore, much work is still needed in order to better understand the interface between nanomaterials and biological systems. These interactions are governed by a large number of phenomena such as the formation of the protein corona, cellular contact, particle wrapping at cell surfaces, endocytosis, and intracellular processes. A better understanding of the nano–bio-interface will permit, in a near future, for the safe use of nanotechnology.

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