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Protein-Nanoparticle Interactions

The Bio-Nano Interface

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Morteza Mahmoudi: I dedicate this book to the following lovely people: Haniyeh Aghaverdi, Fatemeh Ardeshir, Shokrollah Mahmoudi, Sedighe Soleimani, Ali Aghaverdi, Hossein Ardeshir, Mahin Khosravi, Mahmood Mahmoudi, and Moazameh Asadi-Khoyi.

Preface

Nanoscience has been a subject of study for at least a century, although fields, such as colloid science and cellular biology, were not known by this name. Nanotechnology started in the early 1980s due to the advances made in integrated circuits and has gained drastic growth and development over the last decade. Due to the high potential for commercial product developments, today, nanoscience and nanotechnology are tremendous topics of interest for both academic communities and industrial sectors. A distinguishing feature of nanotechnology and nanoscience is the design of new physicochemical properties of nanostructured materials that cannot be attained by using bulk materials. Designed properties of nanomaterials have great potential to enhance many conventional and well-recognized matters in our modern life. The rapid launch of new products incorporating nanotechnology is showing a clear trend across a wide spectrum of fields from manufacturing and bio (nano)materials to electronics and information technology applications. One of the promising subfields of bionanoscience is "nanomedicine," which is recognized as a highly interdisciplinary field to provide precise theranostic (i.e., simultaneous diagnosis and treatment) agents for fast, high-yield, easy, and low-cost treatment of catastrophic syndromes with minimal side effects and lower patient compliance. Although nanomedicine field has been extensively developing by scientific community, these will have the longest time to successful market. The major shortcoming for commercialization of bionanomaterials is the "protein corona" effect and poor understanding of protein–nanomaterials interactions, to date. Protein corona is recognized as the protein (and other biomolecules) layers which are formed at the surface of nanomaterials, upon their entrance to the biological medium. Therefore, what a biological entity (e.g., cells, tissues, and organs) actually "sees" when interacting with nanomaterials is completely different from the original pristine surface of the nanomaterials. This new biological identity of the nanomaterials is achieved by creation of a new interface between the nanomaterials and the biological medium, the so-called bio-nano interface.

In this book, a wide scope of current and future developments of protein corona is covered by combining contributions from faculty members in materials science, chemical engineering, chemistry, biomedical engineering, and biology. Great emphasis is given to the interdisciplinary nature of the protein corona and bionanointerfaces.

After deep description of the biological significance of nanointeractions in Chap. 1, the authors dedicate Chap. 2 to protein corona; in this case, the importance of the physicochemical characteristics of nanomaterials (e.g., size, shape, charge, coatings, surface modifications with targeting ligands, crystallinity, electronic states, surface wrapping in the biological medium, hydrophobicity, and wettability) on the nature of the formed corona is discussed in details. In Chap. 3, full applications, opportunities, and challenges of protein corona, to date, are provided; in addition, a broad overview of both in vitro and in vivo data on the role of protein–nanomaterials interactions in determining nanomaterials' fate and behavior is provided. Chapter 4 presents comprehensive description of the currently available evaluation techniques for assessing the protein corona.

Readers will obtain a deep understanding of the effect of the nanomaterials' physicochemical properties and other factors (such as slight incubating temperature changes) on the final structure, composition, and function of nanomaterials–protein complexes present in biological fluids and on their possible impact on the nanomaterials' fate and behavior either in vitro or in vivo. Also, a broad overview on the major shortcomings of the protein corona effect would be achieved. In addition, the reader will realize the further steps required to fully understand the role of protein–nanomaterials interactions in determining nanomaterials' fate and behavior together with the strategies to control/predict biological fate of nanomaterials.

Finally, I would like to thank the production team of Springer for their continuous and dedicated support during the preparation of this book.

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Spring 2013

Morteza Mahmoudi

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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

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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