INTRODUCTION

NanoBioInterface: a multidisciplinary challenge

C. James Kirkpatrick1,* and William Bonfield2

1Institute of Pathology, Johannes Gutenberg University, Langenbeckstrasse 1, D-55101, Mainz, Germany
2Department of Materials Science & Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK

Keywords: biomaterials; nanotechnologies; biological reactions; model systems

Nanotechnologies are being applied to many fields of science and engineering and represent a broad spectrum of methods, some of which have been known for a long time. However, the public interest as well as the promotion of this topic by funding agencies has led in some cases to a simple renaming of known fields by attaching the ‘nano‐’ prefix, without initiating a true innovation. One field that indeed stands as being novel is the study of how the biosystem reacts to nanostructures. The latter covers a broad spectrum from nanoscale topographical features on a biomaterial surface (Thian et al. 2006; Wood 2007) through a three-dimensional, interconnecting porous nanofibre scaffold for tissue engineering (Dong et al. 2008; Martins et al. 2009) to biodegradable engineered nanoparticles (Son & Kim 2010; Yang et al. in press), designed for delivery of medication, or membranes formed by molecular self-assembly of nanostructures (Capito et al. 2008). Considering the multidisciplinary nature of this subject matter, it becomes apparent that the NanoBioInterface is not only a study of how nanostructured materials interact with biological systems, but on a higher level embodies the interfacial interactions between the so-called exact sciences and the life sciences which are the essence of the remit of the Journal of the Royal Society Interface. On the threshold of the 2010 celebrations of the Royal Society’s 350 year anniversary, it is thus especially appropriate that such a topic, which can only be approached with the expertise of markedly differing disciplines, should be chosen for a Focus issue of the journal.

The space limitation of a single issue does not permit a systematic approach to the topic, nor can it do justice to the diversity of disciplines involved in its research activity. Thus, manuscripts have been chosen mostly from the latter half of the current year’s publications, with a view to highlighting some of the basic scientific questions involved in interactions between nanostructures and biological systems, as well as presenting some of the essential model systems developed to address them.

Although the order of presentation of the manuscripts does not correlate with the gravity of the scientific question posed or the novelty of the methodology employed, it is appropriate that the first paper (Jiang et al. 2009) should address a universal phenomenon in the interaction between a material and the living intact organism, namely the highly dynamic process of protein adsorption to the surface (Vroman et al. 1980). This much neglected issue in general biomaterial research is compounded by the fact that in nanostructures surface area is dominant over mass. Failure to take into consideration the high surface reactivity, for example of nanoparticles, when setting up laboratory methods can often lead to spurious results, as particle aggregation is a common endpoint. In addition, the type of proteins adsorbed determines the subsequent biological reaction, and this phenomenon is convincingly demonstrated by Jiang et al. (2009) using human transferrin to modify the surface of small diameter (5 nm), polymer-coated FePt nanoparticles. The authors used fluorescence correlation spectroscopy (FCS) to quantify the so-called protein corona adsorbed onto the nanoparticles and showed that this protein layer modulates particle uptake by cells of a permanent cell line. Thus, protein-coated nanoparticles were less easily taken up by the cells than non-coated particles.

Other molecular interactions of nanoparticles concern the interface between materials and lipids, this being the initial reaction when nanoparticles enter the deepest air spaces (alveoli) of the lung. These lipids are present in the form of surfactant molecules, a family of lipid–protein complexes that regulate surface tension and...
permit lung expansion. This organ has become an important focus of attention, as nanoscale particles, whether inhaled from the environment or in the form of a delivery system in an aerosol to gain entrance to the body for therapeutic purposes, will come into contact with the complex cellular and molecular structure of the alveolar lining. The importance of this interface for the interaction of nanoparticles with the biosystem is reflected by three consecutive papers addressing different aspects of these interactions, all of which present relevant models to study interactions at the molecular or cellular level. Harishchandra et al. (2009) review the literature on molecular models of the surfactant layer of the alveolar spaces and combine this with data generated in their laboratory using polyorganosiloxane nanoparticles of approximately 22 nm diameter. The situation encountered in the alveolar spaces with nanoparticles in contact with an air–liquid (water and surfactants) interface was simulated in a film balance combined with video-enhanced fluorescence microscopy. The authors were able to show that nanoparticles exert marked changes in domain structures and appear to be coated with lipid. Moreover, hysteresis effects indicate the possibility that the nanoparticles can also escape into the aqueous phase, which would make them accessible for cellular uptake.

The latter issue of how nanoparticles interact with cells in the alveolar space is at the heart of the study by Müller et al. (2009), who investigated how relevant lung cells react to exposure to diesel exhaust nanoparticles and engineered nanoparticles (titanium dioxide and single-walled carbon nanotubes). To achieve this, a sophisticated triple culture of a lung epithelial cell line was established in the presence of human monocyte-derived macrophages and monocyte-derived dendritic cells, the last two cell types having a major role to play in antigen presentation and the immune response. The authors present novel data on how nanoparticles modulate the inflammatory response, which was studied from the point of view of oxidative stress, anti-oxidant capacity and the production of proinflammatory cytokines, such as interleukin 8 and tumour necrosis factor-α. Of special significance for the development of model systems in vitro is the observation that these responses in the triple culture exhibited important differences compared with nanoparticle interactions with the individual cell types.

The second in vitro model of the lung to study nanoparticle interactions seeks to reconstruct the alveolar–capillary (or air–blood) barrier (Hermanns et al. 2009), as this is the essential portal of entry for inhaled nanoparticles to traverse the lung and gain access to the systemic circulation. Using a permeable membrane filter support, a bilayer was established using a human lung epithelial cell line, H441, on the upper side of the membrane, co-cultured with primary human pulmonary microvascular endothelial cells growing as a monolayer on the lower surface of the membrane. This builds a functional barrier with both polarized cell layers expressing essential cell adhesion molecules, which guarantee a trans-bilayer electrical resistance. Polyethyleneimine was used as a model nanocarrier for gene transfection and its uptake was monitored by fluorescent labelling, this being studied in the presence and absence of pro-inflammatory stimuli. Uptake was investigated in monolayer as well as in co-culture, with the cytokines, tumour necrosis factor-α and interferon-γ being added either apically (epithelial side) or basolaterally (endothelial side). The results demonstrate that this model can be used to study systematically how nanoparticles can be taken up and transported by the alveolo-capillary barrier. Moreover, the barrier system can be monitored under stable (physiological) as well as impaired (pathological) conditions. In addition, the system lends itself to the study of all types of nanoparticles, whether environmental or those engineered for drug and gene delivery.

Physiological barriers are affected in various forms of inflammatory disease, as the process of inflammation, involving the release of a variety of so-called inflammatory mediators, causes an increased permeability of blood vessels in the microcirculation. This latter phenomenon could be advantageous for a drug delivery strategy using nanocarriers. This approach is adopted by Ulbrich & Lamprecht (2009), who discuss both intravenous and topical application routes in the context of inflammatory diseases, including rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease, all major diseases in our society. In addition, the differences between a passive and active targeting approach are presented. In the latter, the necessity for specific targeting, involving exact molecular recognition, still remains a major hurdle.

The challenges associated with the use of polymer gene delivery vectors is addressed by Grigsby & Leong (2009). This exciting review highlights the still unresolved problem of adequately protecting DNA from degradation while promoting its release in the desired compartment within the cell at the right time. Poly-cationic polymers electrostatically combined with DNA to form so-called polyplexes represent a very versatile molecular family, as they can be synthesized to be stimuli responsive. This phenomenon opens up the possibility to tune the nanocarrier to be released in an optimal manner to enter the transcription pathway. The authors demonstrate from the literature and their own research work how different polymer characteristics, such as molecular weight, charge density and structural rigidity, can be used in concert with stimuli-responsive strategies to achieve the delicate balance between protection and degradation of the DNA payload. Among these responsive systems are pH or temperature sensitivity, as well as redox and light reactivity.

The latter part of the review addresses the important issue of methodology to track gene nanocarriers and includes fluorescence resonance energy transfer and FCS or their combination. Grigsby & Leong (2009) conclude with a discussion of the cellular systems which are necessary to investigate not just intracellular transport and unpackaging, but also the influence of the extracellular microenvironment on the delivery systems. To this end, three-dimensional cultures and co-culture models can and should be developed to simulate the complexity of the interaction between polyplexes and the biosystem both extra- and intracellularly. Although the authors emphasize the promise contained in these
technological advances, they do stress that progress towards clinical translation using non-viral gene delivery remains slow.

While much attention is being paid to the development of nanoparticles for controlled release, whether it be a drug or gene, medical imaging is another clinical setting which is highly attractive for this technology. In his contribution, Schellenberger (2009) describes the use of superparamagnetic iron oxide nanoparticles, and in particular very small iron oxide particles (VSOP), as sensors for molecular magnetic resonance imaging (MRI). Enzymes are among the useful candidate molecules for diagnosis and this is well illustrated by measuring circulating levels of liver enzymes to help diagnose and monitor hepatic disorders. However, there are numerous enzymes whose fluctuations in activity are interpretable only in the context of their immediate tissue microenvironment. Therefore, their detection requires a sensor system that has specific targeting as well as responsiveness to the enzyme. Using various molecular coatings, the VSOP can be electrostatically stabilized by a peptide-PEG (poly(ethylene glycol)) outer shell, the peptide moiety of which contains the cleavage site for a metalloproteinase (MMP), such as MMP-9. In the presence of such an active enzyme, the peptide cleavage also releases the protective PEG from the nanoparticles, thus leading to aggregation to yield particles in the micrometre size range. The clustering phenomenon is associated with increased $T_2^*$ relaxation effects and is therefore detectable by MRI. While proof of concept was demonstrated in vitro, the in vivo efficacy still has to be proven.

Nanoparticles are very attractive structures for those disciplines seeking novel imaging techniques, but the methodology available to nanostructure a bulk material, irrespective of its chemical composition (e.g. synthetic polymer, ceramic and metal), is also varied and highly developed. Thus, not only topographical features can be changed, but also the surface chemistry can be tuned using nanotechnologies. This has become especially important as modern material scientists have learned from the life sciences that a desirable characteristic of biomaterials in contact with the biosystem should be their similarity to the physico-chemical microenvironment encountered by cells under physiological conditions. This so-called biomimetic approach involves simulating physical and chemical features of the extracellular matrix (ECM). Beutner et al. (2009) used the surface functionalization of titanium-based biomaterials to illustrate the spectrum of technologies available in the ‘tool-box’ of modern biomaterials science. The first part of the paper takes the form of a concise review of available methods in which bioactive signal molecules of various molecular classes can be immobilized to surfaces and includes techniques such as covalent immobilization, self-assembled monolayers and electrochemical methods. This is followed by a description of the authors’ newly developed modular system to attach aptamers to titanium surfaces and how this technology offers flexibility based on the high specificity of nucleic acid recognition, combined with the ability to build two- and three-dimensional structures.

Numerous research groups are also currently investigating how cells detect and react to other nanotopographic features, such as nanofibres, and how these reactions differ from those, for example, to microtopography (Bondar et al. 2008). It is evident that the parameters which can be studied are legion and very often dictated by the specific research interests of the individuals designing these studies. While recognizing the validity of this approach, there are also advantages in adopting a more global view of such comparisons. The field of proteomics provides such a platform. In the present issue, McNamara et al. (2009) review the specific technique of fluorescence two-dimensional difference gel electrophoresis (DIGE), which permits relative alterations in the amount of proteins and their post-translational modifications to be compared between two biological conditions. A particular feature is the ability to use DIGE for small protein samples, such as might be obtained from laser microdissected tissue samples or cell growth on restricted areas of nanotopography. As surfaces of interest often require pre-coating with a pro-adhesive protein and, in a serum milieu, have adsorbed serum proteins, strategies, such as the use of affinity columns, can be designed to remove high abundance serum proteins like albumin. This is of particular importance if the functional comparisons are intended to include ECM, as well as intracellular proteins. In the last part of the review, the authors discuss the methods employed to validate the DIGE results and identify specific proteins. Thus, mass spectrometry (MS) and its variations, including matrix-assisted laser desorption ionization/time-of-flight MS, are well-established techniques. Finally, the recent application of DIGE to study cellular behaviour on biomaterials is illustrated principally from the authors’ own laboratory.

The Focus Theme Supplement, ‘NanoBioInterface: crossing borders’, concludes with a topic that indeed typifies the multidisciplinary challenges encountered in applying nanotechnologies, namely health and safety issues, which in turn impinge on regulatory legislation. (Seaton et al. 2009). It is significant that the Royal Society, together with the Royal Academy of Engineering, took a lead in a 2004 publication of their joint working party by addressing both the significant opportunities as well as the possible hazards of nanotechnologies (Royal Society and Royal Academy of Engineering 2004). Seaton et al. (2009) review the topic of nanoparticles with respect to possible human health hazards and discuss the arduous task of initiating regulation in a situation of inadequate scientific evidence. The authors place the challenges of nanotechnologies in a historical context, including that of the hazards of air pollution. In doing so, they stress the important lesson from medical science’s failure in past centuries to take adequate health and safety measures with respect to quartz and asbestos exposure. This was a result of a pre-occupation with uncovering the mechanisms of particle and fibre toxicity before initiating protective measures. A critical review is also presented of toxicity paradigms, such as the ultrafine hypothesis and the role of shape and size of nanostructures as well as of the inflammatory response. Further
important topics discussed by the authors are mechanisms of toxicity of, and exposure to, manufactured nanoparticles, exposure metrics and the complex issue of risk assessment. Seaton et al. (2009) conclude this very comprehensive article by regarding codes of conduct and legislation, as well as the desired focus of future research, in the light of lessons learned from the issues presented.

REFERENCES


