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Back to Basics: Exploiting the Innate Physico-chemical Characteristics of Nanomaterials for Biomedical Applications

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In nanomedicine design, emphasis is centered on the engineered impacts of the nanomaterials (NMs). However, failure to understand the unintended effects of nanomaterials on the cell biology can affect the overall performance, approval, and adoption in the clinic. Much of these unintended effects arise from unique physico-chemical properties of the NMs. This feature article discusses some of the key physico-chemical parameters of NMs and highlights how they could cause unexpected and novel biological responses, with some insights into their underlying mechanisms.

1. Introduction

Nanomedicine can be broadly defined as the application of nanotechnology to address or improve healthcare-related issues. Ever since the seminal lecture given by Richard Feynman "There's plenty of room at the bottom" in 1959,[1] many researchers worldwide have worked feverishly to realize the notion of "swallowing the doctor" for the treatment of illnesses and diseases by incorporating various small-scale technologies. Some 55 years on, building on the rapid development in nanoscience and nanotechnology, we are now able to manipulate materials with almost atomic precision to possess novel electronic, optical, magnetic and mechanical properties. This empowering has expanded beyond the therapeutic role of nanomedicine to branch into other emerging areas such as diagnostics,[2–4] imaging[4–6] and tissue engineering.[7,8]

Nanomaterials (NMs) can be broadly defined as materials with at least one dimension in the size range of 1–100 nm. Operating at a length scale of one-billionth of a meter, the properties of NMs are significantly different from their atomic constituents and bulk substances due to the high surface-to-volume ratio and quantum size effects.[9,10] Consequently, there are many novel nanospecific properties that could be useful for biomedical applications. For example, inorganic colloidal NMs can possess intrinsic physical properties that are governed by their size, such as fluorescence,[11–16] superparamagnetism,[17] and plasmonic properties.[18,19] While our ability to control and engineer nanosized materials to have these properties is fairly recent, the serendipitous exploits of these nanoparticle (NP)-derived properties date back centuries, as seen in plasmonic gold and silver NPs impregnated in the glass of the famous *Lycurgus* cup.[20,21] Of more recent exploits, superparamagnetic NMs are used as contrast agents for magnetic resonance imaging (MRI)[22–24] or for magnetic separation,[25] whereas plasmonic gold NMs have been used for agglutination assays,[26,27] contrast agents for immunostaining,[28] sensitizers for cancer radiotherapy,[29–31] and as "bullets" for gene guns.[32] Biology itself has been working at the nanoscale for far longer. Many essential proteins that drive almost all biological activities are in the range of tens of nanometers.[33] Cells are also equipped with nanosized surveillance apparatus to help sense and assimilate extracellular information; guiding fundamental biological fates such as survival, migration, and differentiation.[34] Therefore, the ability to fabricate and tailor NMs to the level of precision at a length scale that mimics the fundamental tenets of life would offer researchers unprecedented opportunities to probe, influence, and even rewire cellular behaviors.

At the forefront of nanomedicine is the utilization of NMs as carriers to deliver therapeutic drugs to the diseased site.[35] Currently, much of the technological efforts have been devoted to further enhancing the therapeutic efficacy by adding innovative modalities to the existing nanosized drug-carrier platforms.[36–38] This has given rise to the concept of "multifunctional" NMs, where the NMs are conferred with added

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DOI: 10.1002/adfm.201401664

competencies such as the ability to target and deliver multiple drugs.[36,37,39–43] On the flip side, additional functionalities may inevitably lead to an increase in the complexity and cost to synthesize these NMs. The therapeutic benefits-to-cost ratio would therefore need to be evaluated.[44] Furthermore, the newly acquired physico-chemical identity of NMs may also differ drastically compared to their pristine counterparts, and thus may result in unforeseen biological responses.[45] Recently, there has been mounting evidence that the intrinsic properties of multiple types of inorganic NMs potentially hold some therapeutic value.[46,47] However, the bench-to-bedside translation of these engineered NMs would also require a better understanding of how NMs interact with biological systems, for the purpose of evaluating their safe usage and any discovery of novel nanospecific phenomenon that may be of significance for biomedical applications.

In this feature article, we will first briefly review some of the physico-chemical properties of colloidal NMs that critically influence how they interact with biological systems. Following which, an in-depth analysis on some new observations of NM–bio interactions and insights into their biological mechanism and possible applications will be given.

2. Physico-chemical Parameters that Matter at the Nanoscale

Even before the onset of "nanoscience" in the context known nowadays, around the 90s in the last century, many basic properties, synthesis protocols[19,48,49] for size and shape control,[50] and linked applications of NMs were reported. However, sometimes these reports are hard to find, as terminology has changed over the years, and NPs have been referred to as colloids, particle sols, or monolayer-protected clusters; to name some specific examples: gold NPs have been referred to as colloidal gold or immunogold, and iron oxide NPs have been referred to as ferrofluids or immunomagnetic particles. Also, basic interactions of dispersed NPs with media, such as the adsorption of counter ions (the Debye–Hückel model),[51] or their spontaneous in vitro internalization by cells[52,53] and localization in intracellular endocytic vesicles, have already been investigated. Whereas the above-mentioned functional physical properties (such as fluorescence, superparamagnetism, and plasmonic properties) originate from the electronic band-structure of the NPs, the physico-chemical properties depend on more physical and mechanical parameters (such as size, shape, stiffness, etc.) or surface chemistry parameters (such as charge, hydrophobicity, surface functional groups, etc.). Though physico-chemical properties are paramount in determining the interaction of NPs with biological building blocks, and despite the fact that NPs have been heavily investigated, a comprehensive understanding is still lacking.[54,55] This is in particular due to the fact that precisely defined NPs with intentionally tuned properties have only been recently available. In the following sections, some of these physico-chemical properties of NPs will be discussed in more detail.

Though one might not expect it, inorganic NPs are complex objects, which comprise much more than their inorganic particle core. While the inorganic core provides the materialistic identity for the NP that will govern its mechanical and chemical properties, it is the NP's surface that determines much of its physico-chemical properties. Assuming plain particles containing a homogeneous inorganic core, it is easy to understand that, with van der Waals forces, the NPs would agglomerate. In order to prevent agglomeration, the NPs need to be stabilized. This can be done by charge, either by using the charged surface states of the NPs,[56,57] by the adsorption/attachment of charged molecules to their surface,[58] steric hindrances,[58] or by a combination of both effects.[59] Thus, even in pure water, the NP surface needs to be different from its inner volume in order for colloidal stability to be attained. In biological media further modifications occur, such as the afore-mentioned adsorption of counter-ions[60,61] and the repulsion of like-charged ions, as well as the adsorption of proteins, forming a protein corona.[62] In this manner, the environment changes the NP surface, as the NP surface modifies its immediate environment.[63] Thus, there are no homogeneous inorganic NPs, but in fact always complex hybrid structures (**Figure 1**).[64] In the same way, it can be easily appreciated that it is not trivial to experimentally determine the distinct physico-chemical properties of NPs, as they are often highly entangled.[64]

Agglomeration is a key parameter, as it influences virtually all the other NP physico-chemical parameters. The interaction of NPs with biological building blocks is strongly influenced by

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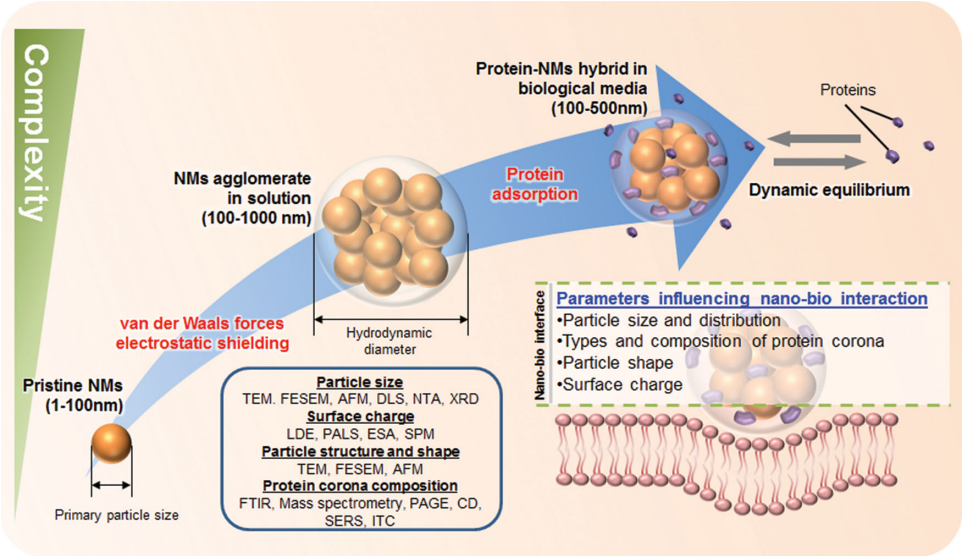

Figure 1. Challenges to characterize physico-chemical properties of NMs. Proper characterization of the NMs at different stages of application is highly complex, yet essential to establish the nano–bio relationship. Conventional methods such as microscopy, DLS, and zeta potential measurements provide simple but useful information regarding the basic physico-chemical properties of the NMs in well-defined, simplified environments. However, to adequately characterize the NMs in biologically relevant liquids, additional techniques to distinguish the composition, conformation, amount, and type of adsorbed proteins on the NM surface will be required, as they constitute an important component when interacting with biological systems. A non-exhuastive list of suitable characterization methods to examine particle size, surface charge, structure and protein corona is shown in the box. TEM: Transmission Electron Microscopy; FESEM: Field Emission Scanning Electron Microscopy; AFM: Atomic Force Microscopy; DLS: Dynamic Light Scattering; NTA: Nanoparticle Tracking Analysis; XRD: X-Ray Diffraction; LDE: Laser-Doppler Electrophoresis; PALS: Phase Analysis Light Scattering; ESA: Electronic Sonic Amplitude; SPM: Scanning Probe Miscroscopy; FTIR: Fourier Transform Infrared Spectroscopy; PAGE: Polyacrylamide Gel Electrophoresis; CD: Circular Dichroism; SERS: Surface-Enhanced Raman Sepectroscopy, ITC: Isothermal Titration Calorimetry.

their size. While it is obvious that the adsorption of counterions as well as proteins increases the effective particle size (typically quantified in terms of hydrodynamic diameters d_h, in contrast to the diameters of the inorganic particle cores, d_c), even bigger changes are caused by agglomeration. Upon agglomeration, the effective particle size is no longer the size of an individual NP, but the size of the NP agglomerate. As size is known to have a profound influence on particle uptake by cells (small particles are typically internalized at a higher rate than bigger ones),[65] agglomeration is an important determinant that has to be considered. Other effects, such as toxicity caused by surface-catalytic effects, depend on the total surface of all individual particles, and thus agglomeration would also play an important role in this. In addition to the NP's intrinsic properties, agglomeration depends also on the composition of the dispersing medium,[66] and thus measurements of hydrodynamic diameters should always be performed in the biological medium in which they are studied (**Figure 2**). For the determination of hydrodynamic diameters (of single NPs in case of no agglomeration, and of the agglomerate size in case of agglomeration) many different methods exist,[67,68] though all need to be interpreted differently, as they are based on different detection principles. Dynamic light scattering (DLS) is probably the most frequently used technique for determining hydrodynamic diameters, as commercial machines are easily available in many laboratories. However, caution should be exercised if the machines were to be used without a clear understanding of the underlying measurement principles, as it may possibly lead to erroneous or misinterpreted results. This has been demonstrated in round-robin studies in which different hydrodynamic diameters were reported by different laboratories working on the same NP sample.[69,70] The working principle of DLS relies on how light is scattered by the NPs in a liquid medium. An autocorrelation function from the fluctuations of this signal can be fitted with simple diffusion models, which yield the diffusion constant of the particles (or diffusion constants, in the case of multiple particle species). From the diffusion constant D, the hydrodynamic diameter d_h is derived on

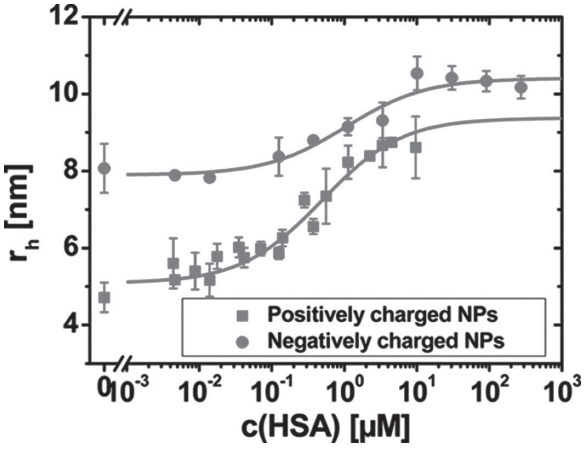

Figure 2. Surface charge-dependent adsorption of human serum albumin (HSA) onto Au NPs can be detected as an increase in hydrodynamic radius with increasing HSA concentration. Reproduced with permission.[66] Copyright 2013, American Chemical Society.

the basis of the Stokes–Einstein model $d_h \propto D^{-1}$.[71] Even with this simplified version, one can see that the resultant computed hydrodynamic diameter is dependent on the chosen fit model, which typically is hidden as a black-box in the machine. Problems arise when measurements are made for NPs that are non-spherical, display multimodal size distributions, or interfere with scattering signals from other species in solution such as proteins, etc. Despite the fact that accurate measurement of NP size is of high importance, in practice, the determination of a NP's hydrodynamic diameter is hard to standardize, though the principal foundations have been laid.

The interaction of NPs with biological units has also shown a strong dependence on NP shape.[65,72,73] For inorganic NPs, shapes are typically determined from transmission electron microscopy (TEM) images (in which only the inorganic core provides contrast), and quantified in terms of aspect ratio. However, the actual aspect ratio of the NPs may in some cases be affected by, for example, the adsorption of organic surface molecules or proteins onto NPs with very small size.[74] For a particle core of 5 nm diameter and 20 nm length, the aspect ratio is AR = 20 nm/5 nm = 4. In the realistic case of a 5 nm organic coating, the aspect ratio would be only AR = (20 + 2 × 5) nm/(5 + 2 × 5) nm = 2, which is significantly lower. Thus, even a simple parameter such as the aspect ratio cannot be unequivocally determined, which again is due to the complex hybrid nature of NPs. Shape agglomeration also plays an important role: an agglomerate of NPs with a high aspect ratio would result in an overall aspect ratio close to 1.

The surface charge of the NPs is also expected to greatly affect any potential NP–biology interaction. Unfortunately, there is no standard experimental procedure available to directly measure the surface charge of NPs. The Poisson equation states that the charge and potential of a colloidal particle are related, and thus the surface charge could be inferred from a surface potential measurement. Surface potentials are well defined for smooth surfaces, but what is the "surface" of a hybrid NP with organic surfactant molecules bound to the hard core? In addition, the NP surface interacts with the ions of the surrounding medium. There can be specific adsorption of ions,[75–77] which adsorb in a tightly bound monolayer directly at the NP surface[78] followed by a diffused region of adsorbed secondary counter-ions,[79,80] as explained by the Debye–Hückel model.[81] A first unified model of the electrical double layer of tightly bound and diffusely attached ions was proposed by Stern,[82] and later refined by Bockris and others.[83] The outer layer of adsorbed counter-ions is bound via electrostatic attraction, and the accumulation of counter-ions decays towards the bulk solution with increasing distance from the NP surface. In this static picture, the NP charge is screened by the adsorbed counter ions and, outside of the diffusive layer, there is no net charge or potential. In order to measure the counter ion adsorption, one can disturb the static equilibrium by application of an electric field, E. The charged NPs will migrate in this field with a velocity, v. Similarly, the counter ions that are found in close proximity to the NP surface are strongly bound electrostatically and will move as a collective entity with the NP under the effect of the applied electric field (as discussed above, this is one of the reasons why $d_h > d_c$). Counter ions that are found further away from the NP surface, however, are not attached sufficiently strongly to the NPs and will move in the opposite direction, resulting in the formation of a slipping plane. Within distances closer to and further from the NP surface than the slipping plane, adsorbed counter ions move with the NP and in the opposite direction, respectively, and thus create a potential difference due to charge separation. The potential at the slipping plane is called the zeta potential ζ, and can be experimentally determined.[84–87] The zeta potential determines how strongly counter ions are bound to the NP surface. This binding energy $e \cdot \zeta$ (with the elementary charge $e = 1.6 \times 10^{-19}$ C) is in competition with the thermal energy k_BT (with the Boltzmann constant $k_B = 1.38 \times 10^{-23}$ J/K), whereby thermal fluctuation would diffuse the cloud of counter ions. At room temperature (T = 293 K) the thermal energy is around $k_BT \approx 25$ meV. Thus, zeta potentials $|\zeta| > k_BT/e$ and $|\zeta| < k_BT/e$ indicate a strong and weak surface charge of the particles, with strongly attached counter ions and with loosely attached counter ions, respectively. A zeta potential value bigger ca. 25 meV is required for NPs to be stabilized by electrostatic repulsion. The zeta potential is thus a very important parameter to investigate colloidal stability. For the determination of the zeta potential of NPs, their electrophoretic mobility μ is determined (defined by $\mu = v/E$). Thus, for an experimental determination of ζ, an electric field E is applied, and the resulting velocity v of the migrating NPs needs to be measured. This can be done by making use of the Doppler Effect, and the method is called laser Doppler velocimetry (LDV), or laser Doppler anemometry (LDA). Based on simple electrophoresis models, the electrophoretic mobility is directly proportional to the zeta potential,[88,89] and therefore the zeta potential can be experimentally determined.[90] Thus, while in principle one would like to know the surface charge or surface potential of charged NPs, the experimentally accessible and also highly useful parameter is the zeta potential. Adsorption of proteins (Figure 2) typically screens part of the original surface charge of the NPs, which results in a reduction of the zeta potential.

As mentioned several times, another important phenomenon which has to be accounted for regarding the physicochemical characterization of NPs is the adsorption of proteins to their surface. This effect has been known for decades,[91–94] due to the fact that (nano-)particles are internalized slower by cells in the presence of serum proteins.[66,95] It is also known that charged objects in general are internalized by cells to a higher extent than uncharged ones. In this way, reduced NP uptake in serum-containing media might be attributed, in part, to the change in the NP's zeta potential, due to nonspecific protein adsorption. It is only recently that protein corona formation surrounding the NPs was intensively examined,[62,96] again mainly because detailed investigation required advanced protein and protein–NP complex analysis techniques, as well as state-of-the-art NP synthesis and measurement techniques.[97]

Protein adsorption to NP surfaces is an entropy–enthalpy driven process. Spontaneous formation of the protein corona will only occur when there is a net reduction in the Helmholtz free energy F of the system: $\Delta F = \Delta U - T\Delta S < 0$. Helmholtz free energy is a function of internal energy U and entropy S at a specific temperature T. Upon binding, there are adhesions (and thus a binding energy), and therefore the resulting energy is reduced: $\Delta U < 0$. Charge plays an important role, which has

been carefully investigated using proteins with tuned surface charges (this indicated that proteins are typically adsorbed in an oriented way to the NP surface[98]). However, protein adsorption onto the NP surface can also be driven by an overall entropy gain. As proteins bind to the surface of the NP, the total amount of protein area exposed to water is reduced, and thus entropy is increased (water close to proteins is ordered, and in the case where less protein surface is exposed to water, there will be less order in the system and entropy increases): $\Delta S > 0$. The entropy contribution can be seen to be directly dependent on the temperature at which the protein corona was formed. At higher temperatures, the entropy term (the negative of $T \cdot \Delta S < 0$ as $\Delta S > 0$) gains importance. Since, at higher temperatures, there are fewer bound proteins, there is a higher gain in entropy. Thus, entropically driven protein adsorption is favored at higher temperatures, which has also been experimentally demonstrated.[99]

An increase in NP hydrodynamic diameter may indicate the adsorption of proteins onto the surface of the NPs.[100] However, a dramatic increase in hydrodynamic diameter also most likely suggests that the NPs are agglomerating. Interestingly, proteins adsorbed onto particles was shown to enhance colloidal stability instead of diminishing it.[101] Thus, a large increase in hydrodynamic size in the presence of proteins is an indicator for NPs with poor colloidal stability. For colloidally stable NPs, the protein corona should instead form a thin layer on top of the NP surface. Serum proteins are designed to confer colloidal stability; otherwise, upon the formation of big agglomerates, they would clog the blood vessels. It is known that, under certain conditions, some serum proteins such as serum albumin can form small assemblies, such as dimers.[102–104] However, under ideal experimental conditions (highly stable NPs with only one type of protein) the formation of a protein monolayer around the NP surface was demonstrated for several different types of proteins.[105] In the case where the protein–NP interaction is bigger than the protein–protein interactions (which are typically weak), single proteins and nonassembled proteins will be attached to the NP surface. It has been also demonstrated, however, that protein adsorption onto the NPs may change the conformation of the proteins,[106,107] which might lead to altered protein–protein interaction profiles. Consequently, there is a body of work in which loosely bound additional layers,[106] the so-called "soft corona"[108] are claimed, which, upon rinsing, disintegrate. Nonetheless, even in this case, the resulting protein corona formed should exist as a rather thin layer. When thermodynamic equilibrium is attained, the affinity of different proteins to different NP surfaces can be conveniently quantified in terms of dissociation constants.[109] By knowing the diffusion constant, one can effectively predict, for a certain protein–NP system, the degree of protein coverage on the NP surface that is independent of the protein concentration.

However, under realistic exposure conditions, protein adsorption onto NPs is a highly dynamic and complex process.[110,111] For instance, proteins can bind weakly to a given substrate with fast binding kinetics, only to be displaced by proteins that have a stronger affinity for the surface which, in the case of planar surfaces, is known as the Vromann effect.[112] It is very important to also consider kinetics, as they may have completely different effects on different organisms. For instance, the average number of heart beats is different for different animal species. For example, the pulse rate of mice (≈500 beats per minute) is much faster that of a human (≈70 beats per minute), and the lifetime of mice (≈3 years) is shorter than the one of humans (≈75 years). In other words, the time needed for the NPs to complete one circulation cycle as they biodistribute to other parts of the organs, is a lot shorter in mice than in men. In the situation where the kinetics of protein corona modification is slower than the time for one circulation cycle, the "instantaneous" protein corona can predetermine the biodistribution of the NPs. All these facts have to be considered in appropriate pharmacokinetic models as knowledge of the kinetics of the protein corona formation and re-modeling is important.[113] These results demonstrate that, at present, there are significant technological gaps that are impeding nano-scientist from providing a full description of all the important NPs physico-chemical properties with a set of a limited number of parameters. Further effort is required to develop more advanced characterization techniques that could accurately measure the physico-chemical properties of NPs in biological relevant dispersants.

3. Some New *Yin* and *Yang* Relationships of NMs with Biological Partners

The rapid development in nanofabrication techniques in recent years has yielded a wide spectrum of NMs that can be synthesized or prepared with a high batch-to-batch consistency. With the momentum gained in the nanotechnological sector comes the need to better appreciate how these NMs may interact with biological systems. Understanding the complex interplay of NM-triggered biological events and interrelations is not only important for the safe implementation of NMs in commercial products, but is also the rationally derived key to unlock their full potential for a diverse range of biomedical applications. Therefore, in this section, an up-to-date review of some of the latest and sometimes surprising effects of nano–bio interactions impacting developmental, physiological, and pathological processes. Special emphasis will be placed on the unique biological responses that are triggered as a consequence of the physico-chemical interactions of NMs with cellular components. Implications of distinct biochemical signalling pathways will also be discussed, which could provide critical insights into nanobiology.

3.1. Interactions of NMs with Extracellular Spaces and Tissue Barriers

3.1.1. Breaking Bonds between Cells: NM Interactions with Cell–Cell Adhesion

Intercellular junctions are important for the mutual binding of endothelial and epithelial cells so as to render proper spatial organization of the cells in tissues. Adhesion of cells to one another is usually mediated by a group of specialized cell-adhesion molecules to form discrete interconnected junctions. Among the major classes of cellular junctions are adherens junctions (AJs), tight junctions (TJs) and communicating gap junctions.[114] Collectively, these specialized junctional proteins

are critical to regulate the movement of water and nutrients and to facilitate cell–cell communication. A breach in the epithelial or epithelium protective barrier in the lung may provide foreign entities with unimpeded access to the systemic circulation and would undoubtedly lead to dire consequences. However, an increase in vascular permeability may likewise present an opportunity to enhance delivery of therapeutic agents to hard-to-reach organs such as the brain via the blood–brain barrier (BBB). In recent years, there has been mounting evidence to suggest NMs are inherently biologically active and can influence expression and binding dynamics of cell–cell junctions to bring about altered barrier functions in the epithelium and endothelium.

The endothelial cells lining the blood vessels are critical to the maintenance of barrier functions. Neighboring endothelial cells are mechanically coupled by the homophilic interaction between the extracellular domains of the vascular-endothelial (VE) cadherin molecules that are associated intracellularly with the actin cytoskeleton. Stable VE-cadherin plays an important role in the preservation and control of endothelial contacts.[115,116] Therefore, loss of VE-cadherin function is widely believed to be the underlying cause of many vascular pathological conditions, and since the blood stream constitutes one of the major delivery routes commonly utilized by NPs, there is considerable interest in trying to understand how NMs may affect this special class of intercellular junction. Recently, developments have revealed that metal oxide NMs such as Fe_2O_3,[117] TiO_2,[118] ZnO,[119] SiO_2,[120] carbon nanotubes,[121] CuO,[122] and Al_2O_3[123] are capable of impairing endothelial functions, as monitored by a decrease in transepithelial or transendothelial electrical resistance (TEER) or increase in transwell permeability. While NM-mediated cytotoxicity has often been singled out as the primary cause leading to increased endothelial permeability,[122,123] there are several lines of evidence to suggest that cell death is not a prerequisite and may involve distinct NM-triggered biological signalling activities.

Broadly speaking, NMs can disrupt endothelial barrier functions via two modes of action, namely the "inside-out" and "outside-in" mechanisms. The "inside-out" framework refers to a series of NM-mediated intracellular signals, that eventually leads to the indirect modification of VE-cad binding between adjacent endothelial cells. The biological roles of oxidative stress in the regulation of endothelial cell dysfunction have been well established.[124] Therefore, this places NM-triggered intracellular reactive oxygen species (ROS) expression as a "prime suspect" that increases endothelial permeability. When human microvascular endothelial cells (HMVECs) were treated with 50 μg/mL of Fe_2O_3 NPs for 1 h, there was an increase in intercellular gap formation with a concomitant increase in ROS expression. It was later revealed that the upregulated oxidative state of HMVECs is responsible for the engagement of the Akt/GSK-3β signalling cascade, impairing microtubule remodelling and thus compromising barrier function.[117] In the same way, ROS was also postulated to have a negative impact on cell membrane integrity of human umbilical vein endothelial cells (HUVECs), provoking the global re-arrangement of the actin cytoskeleton and the loss of cell–cell contacts.[119]

Recently, Leong and co-workers reported that negatively charged TiO_2 NPs (~25 nm), but not micrometer sized TiO_2, could similarly induce endothelial leakiness (NanoEL) to a confluent layer of HMVECs at noncytotoxic concentrations, via an "outside-in-signalling" mechanism (**Figure 3**).[118] With an even shorter exposure of 30 min TiO_2 NP treatment, there was a significant increase through distinct and large gaps between endothelial cells. However, a significant upregulated state of ROS expression was only detected at a time point (24 h) that far exceeds the onset of intercellular gap formation, suggesting that NanoEL may occur via a unique mechanism that is not dependent on oxidative stress. Using a series of modified biomolecular techniques such as protein pull-down assays and a proximity ligation assay, further evidence was presented to suggest that TiO_2 NPs physically interacted and disrupted homophilic pairing of VE-cadherin molecules. The physicochemical binding of TiO_2 NPs with intact VE-cadherin presupposes that TiO_2 NPs are required to navigate their way to the intercellular niche. In support of this notion, negatively charged CdSe quantum dots (QDs) were also shown to preferentially localize as early as 5 min post-QD exposure along the cell–cell boundary of epithelial cells, whereas positively charged QDs were found to traverse through the epithelial lining via the transcellular route.[125] Intracellularly, the binding of TiO_2 NPs to VE-cadherin then triggers phosphorylation of VE-cadherin at site Y658, followed by rearrangement of the cytoskeleton. The proposed "outside-in" signalling mechanism provides critical insights into the diverse approach NPs may undertake to invoke a common biological phenomenon. More importantly, this study highlighted the possibility that NPs may possess the ability to mimic the pharmacological action of vasoactive agents such as vascular endothelial growth factor (VEGF) and histamine, and thus may hold therapeutic value.

Similar to the endothelial cells, paracellular transportation of molecules through densely packed epithelial cells is tightly regulated by adheren junctions known as E-cadherin and TJs.[126] A number of studies have shown that engineered NMs can also perturb epithelial cell barrier function by altering the TJs.[127–129] For instance, carboxylated single-walled carbon nanotubes (SWCNTs) were documented to trigger transient opening of TJs as shown by a significant drop in TEER measurements and ZO-1 immunofluorescence staining.[130] Moreover, it was suggested that the impact of NMs on epithelial cell permeability is dependent on the geometry of the NM. Apart from a higher cell uptake and reduction of mitochondrial activity, spherical Au NPs were documented to severely impair cell–cell contacts in an MDCK cell line, with concomitant overexpression of ROS levels, compared to rod-shaped Au NPs.[131]

In addition to its regulatory role of the barrier function, E-cadherin is also known to play a pivotal role in regulating tumour metastasis via the process of epithelial-mesenchymal-transition (EMT).[132,133] Treatment of pulmonary epithelial cell lines with multi-walled CNTs (MWCNTs) with high aspect ratios was shown to down-regulate E-cadherin expression and promote expression of other EMT markers.[134] Conversely, a recent study suggested that Au NPs synthesized by citrate reduction may possess the innate ability to exert anti-tumor and anti-metastatic effects in ovarian cancer cells by promoting re-expression of E-cadherin, thus suppressing EMT in ovarian cancers.[135] Furthermore, this therapeutic effect of Au NPs appears

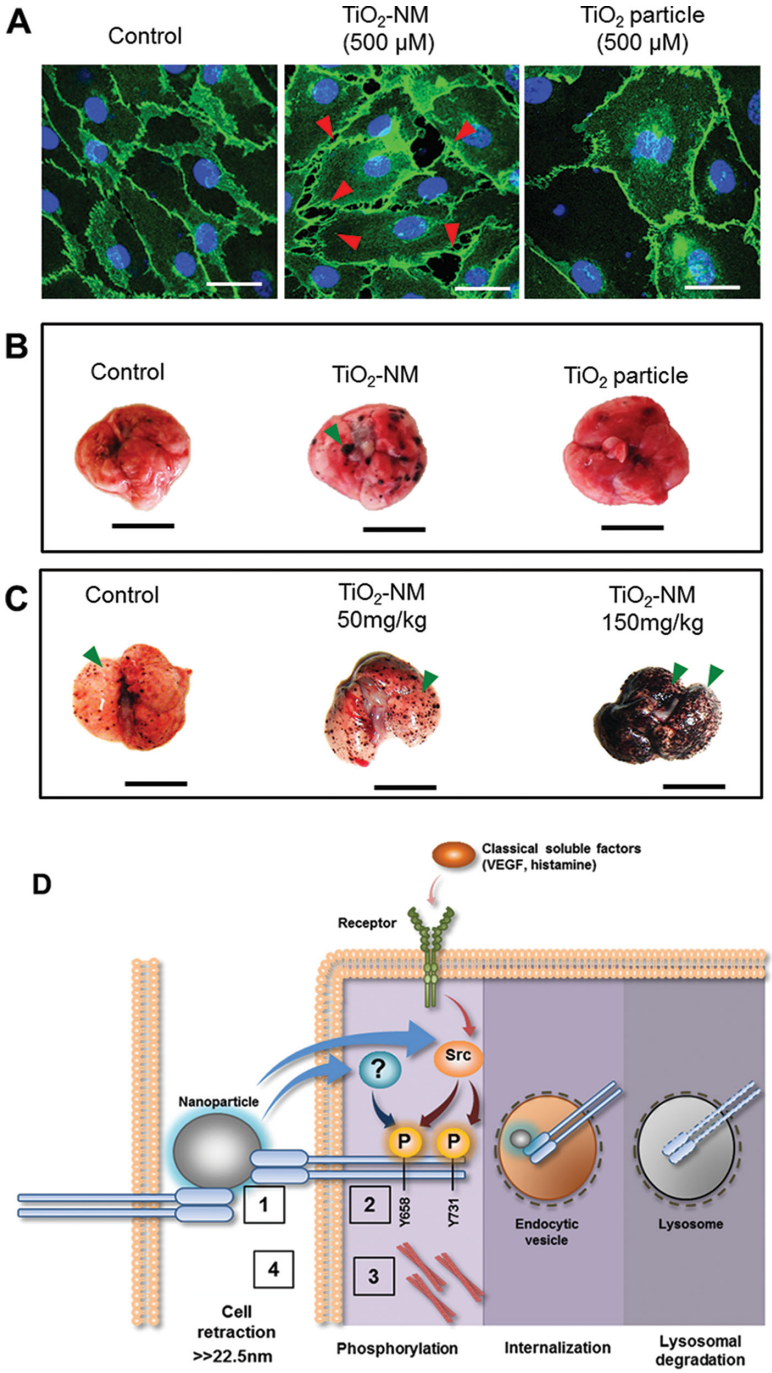

Figure 3. (A) Treatment with TiO_2 NPs (25 nm), but not with TiO_2 particles (680 nm), caused endothelial cell leakiness (NanoEL) in monolayers of endothelial cells. Scale bar = 50 μm. Adheren junctions VE–cadherin and cell nuclei are stained green and blue, respectively. (B) Consistently, in in vivo models TiO_2 NPs but not TiO_2 particles induced the blood vessels to be more leaky and promoted metastasis of murine melanoma cells (B16F10). Scale bar = 1 cm. (C) Increasing the administered dosage of TiO_2 NPs led to higher colonization of B16F10 in the lungs. Scale bar = 1 cm. (D) The TiO_2 NanoEL occurred as nanosized TiO_2 migrated to adheren junctions, allowing them to bind and disrupt homophilic interaction of VE-cadherin (1). The disruption induced VE-cadherin to be phosphorylated at the sites of Y658 and Y731, which led to the loss of interaction between VE-cadherin and β-catenin and p120 (2). This loss of interaction between VE-cadherin and its interacting partners further destabilized the actin and induced the cytoskeleton remodelling (3) with the end result of cell retraction and the occurrence of leakiness (4). Reproduced with permission.[118] Copyright 2013, Nature Publishing Group.

to be size dependent, with 20 nm Au NPs observed to exhibit the highest efficacy in suppressing pro-metastatic signalling pathways. The dissimilarities in the effects of NMs on the EMT programme between the two aforementioned studies may in part be attributed to the differences in NM composition and the cell types that were examined. Further studies would therefore be required to identify a larger panel of anti-metastatic NMs so that this tantalizing property of NMs to curb EMT can be exploited clinically.

3.1.2. Gaining a Foothold at the Nanoscale: Modulation of Cellular Adhesion Patterns with Nanocues

The rapid advancement of nanotechnology and surface science has also permitted the fabrication of a wide variety of novel nanostructures that have been shown to exert profound influence over cellular fates. Thus far, our discussion has been limited to the biological effects of discrete NMs. However, evidence is accumulating to suggest that cells can also respond to nanotextured surfaces. Apart from soluble biochemical factors, cells also take their cues from the physical nanoscale interactions between the cells and the underlying substrate. The inherent sensitivity of the cells to sub-micrometer features is perhaps not entirely unexpected, considering that cells are in constant contact with the extracellular matrix (ECM) composed of a complex blend of nanoscale (5–200 nm) pores, pits, grooves, and ridges. Furthermore, collagen fibrils, being one of the most abundant ECM structural proteins was shown to possess an average diameter of 20 nm with a D-periodicity of 67 nm.[136] The defining characteristics of nano-level hierarchical organization in biological systems thus strongly support the notion that the spatiotemporal organization of nanofeatures is an important regulator of cell function in vivo.

Cells are imbued with a plethora of nanoscale sensory apparatus such as integrins to enable cells to survey and respond to the submicrometer ECM features. Integrins belong to a specialized class of transmembrane cell adhesion molecules, consisting of two noncovalently bound α and β subunits that bind specifically to distinct motifs found on ECM molecules.[137] Once the integrins are engaged with ECM adhesion proteins such as fibronectin or collagen, integrin clustering will follow and subsequently trigger a series of highly orchestrated signalling events and cytoskeletal remodelling to drive maturation of the focal adhesions (FAs). FAs not only play an important role in cellular adhesion, but also regulate vital mechanotransduction signalling pathways via FA-actin coupling, governing key functions such as

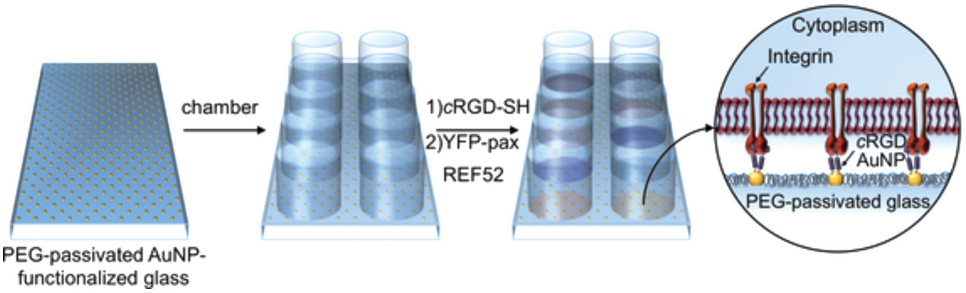

Figure 4. Schematic to portray preparation of a multi-well array of surface-patterned 8 nm Au nanodots conjugated with thiolated *c*RGD (cRGD-SH) peptides to study cell adhesion dynamics. Murine REF52 cells were stably transfected with paxillin (focal adhesion protein) (pax) and fused to the yellow fluorescent protein (YFP) to monitor spatiotemporal expression of the FAs. Reproduced with permission.[141] Copyright 2014, Wiley-VCH Verlag.

motility, proliferation, and differentiation.[138,139] Therefore, integrin-mediated adhesion on a given surface can be considered the defining step that will critically determine the performance of the biomaterials.[140] Significant progress in surface patterning techniques has now made it possible to engineer the spatiotemporal organization of integrins with nano-resolution. For example, using block copolymer micelle lithography, one is able to pattern arrays of 8 nm Au nanodots conjugated with cyclic-RGD (cRGD) peptides on a 2D surface.[141] Against a nonbiofouling background comprising polyethylene glycol (PEG), cell adhesion was restricted to cRGD motifs that were spatially defined by the position of the Au nanodots (**Figure 4**). It was revealed that a lateral spacing >73 nm between integrin ligands severely hindered focal adhesion formation and cell spreading.[142,143] Consistent with this study, human mesenchymal stem cells (hMSCs) on vertically standing TiO_2 hollow nanotubes with diameter >100 nm similarly impaired cell adhesion by preventing integrin clustering and focal complex formation.[144] Unravelling the spatial detection limit of the integrins will have important implications to the design of advanced biosurfaces that are geared to either strengthen or weaken cell–matrix adhesion.

Once adhesion is initiated, the cells will begin the process of mechanosensing, in which fine cellular processes, termed filopodia,[145] are employed to sense and interrogate the physical surroundings, guiding cell adhesion and morphological adoption. In fact, as early as 1912, Harrison alluded that the physical structure of micrometer-thick spider webs can guide adhesion and the spreading of cells via a process termed "contact guidance".[146,147] Using nanoscale architectures, researchers are now able to manipulate cell morphology, adhesion, motility, and differentiation.[148] Some of the more commonly studied nanotopography configurations include nanogratings,[149,150] nanopits,[145] and nanopillars.[151–153] Evidence is mounting to suggest that nanotopography plays an important role in the guidance of cellular development.[154]

Nanogratings with periodicities ranging from 200 nm up to 800 nm are by far one of the most commonly used architectures for the control of cell adhesion and to induce cellular elongation. Given that the cell body is considerably larger than the nanoscale topography, the cell is still capable of reacting to the directionality of the topographical cues. This is a true example of "contact guidance" taking place at the nano level. Cells respond to the architectural cues by adopting a highly stretched morphology in the direction of the gratings and the focal contacts are typically redistributed to the apical ends of the cell body. The resultant atypical cellular morphology and guided adhesion pattern also makes such nanostructures particularly useful to guide the direction of neurite extension, which will be useful to construct neural circuits.[155] Furthermore, the use of nanogratings to direct large-scale cellular alignment and polarity not only recapitulate in vivo myocardial tissue organization, but also promote cell–cell coupling and action potential conduction velocity of neonatal rat ventricular myocytes.[150] There is also increasing evidence to demonstrate that cellular adhesion-based modulation such as cell geometry[156–159] and matrix stiffness[160–162] can be exploited as an important biophysical impetus to steer stem cell differentiation. Using electron beam lithography, controlled disordered arrangement of 120 nm nanopits of 100 nm depth can be used to promote osteogenesis of hMSCs without the need for any additional soluble chemical cues.[163] Osteo hallmark markers such as osteopontin and osteocalcin were clearly visible after 21 days on the osteo-inductive surface, suggesting that stem cell differentiation can be modulated through precise design of the nanoscale features. Interestingly, long-term maintenance of mesenchymal stem cell multipotency can also be attained when the 120 nm nanopits were arranged with a high degree of symmetry.[164]

3.2. Interaction of NMs with the Cell Surface

3.2.1. Breaching the Outer Defense Line: NM Interactions with Cell Membrane

The function of the cellular plasma membrane is analogous to that of medieval city walls, offering vital protection from foreign threats and structural support to maintain the cell shape.[165] Therefore, the initial engagement of the NMs with the cell membrane and the cellular changes that follow can be considered a critical biological event that may affect the performance of the cells. NMs may, for example, disrupt the integrity of the phospholipid bilayer, thus leading to compromised cellular viability. Early descriptions of NM-induced cell membrane damage reported magnesium oxide (MgO) NPs causing punctated cell walls in *Bacillus subtilis*.[166] Subsequently, other studies observed that the physical interaction of Ag,[167–169] MgO,[166] ZnO,[170,171] and carbon[172] NMs with the cell membrane can significantly disrupt membrane integrity. Electrostatic forces

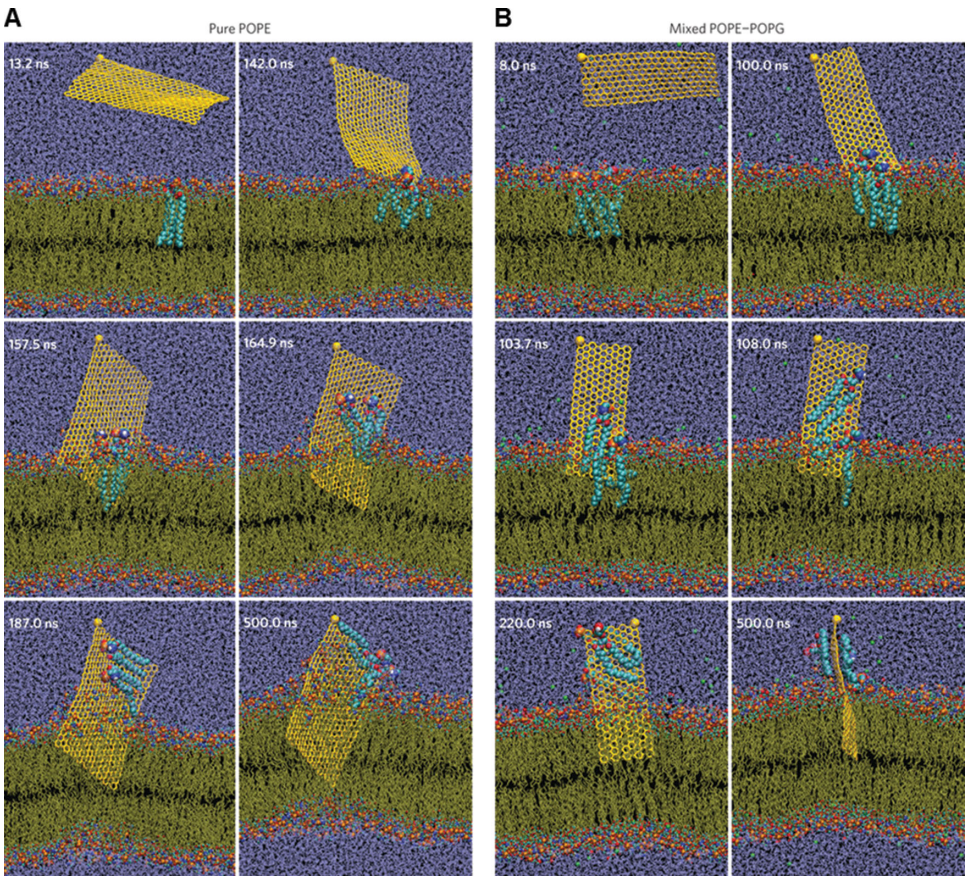

Figure 5. Time course computer simulated path of graphene nanosheet as it approaches the cell membrane of *Escherichia coli* (*E. coli*). It was shown that the graphene nanosheet mimics the action of a nanoscale "blade" by inserting through the (A) outer membrane consisting of pure POPE and (B) inner membrane made up of 3:1 mixed POPE-POPG of *E. coli* and in the process extracting the lipid content from the phospholipid membrane. Reproduced with permission.[175] Copyright 2013, Nature Publishing Group.

appeared to be responsible for this disruption due to the oppositely charged nature of the interacting NMs and the cellular membrane.[167] For example, positively charged ZnO NMs were shown to bind to the negatively charged *Pseudomonas aeruginosa* membrane, leading to acute cytotoxicity.[170] Besides electrostatic interactions, it was also suggested that NM interactions with cell membrane components are facilitated by other types of interactions such as van der Waals forces, hydrophobic interactions, and receptor ligand interactions.[173] A recent study showed that interaction between 2D graphene nanosheets with the cell membrane was initiated by strong van der Waals and hydrophobic forces. In a computer simulation experiment, it was shown that these nanosheets act as "mechanical blades", cutting/piercing into the cell's lipid membrane (**Figure 5**). The effect of NMs causing physical injury to the cell membrane was also documented with the use of nanoscale protrusions formed on the surface of black silicon for antimicrobial applications. These rigid nanoprotrusions pierced several types of bacteria cells that were attached to the silicon surface.[46] A similar observation was also made for graphene-based nanowalls, where the sharpened edges possessed the capability to penetrate and slice the bacteria cell membrane, leading to extensive cytotoxicity.[174]

Another interesting mechanism whereby NMs can disrupt the cell membrane entails the concept of the NM serving as a "sponge" to absorb essential biomolecules from the cell membrane. For instance, graphene nanosheets[175] were reported to siphon phospholipid molecules from the bacterial wall, thus destroying the structural integrity of the cell membrane. In addition, the damaged bacteria cell membrane could result from the extensive lipopolysaccharide and membrane proteins leaching out onto the negatively charged Ag NPs.[176,177] Interaction of negatively charged Ag NPs with the bacterial cell wall could also deplete membrane calcium content to disrupt membrane integrity.[176–178]

3.2.2. NM Interactions with Cell Surface Receptors Activate Signalling Pathways

Embedded in the cell membrane are myriad biomolecular molecules and transmembrane receptors governing important biological cascades that are essential to maintain cell function and survival. The comparable size scale of the NMs and these surface receptors' ligands brings about a promising opportunity to exploit the NMs as an extracellular signalling molecular analogue that can bind to surface receptors and modulate signal transduction (**Figure 6**). Magnetic forces applied to ferromagnetic microbeads bound to ECM surface receptors could

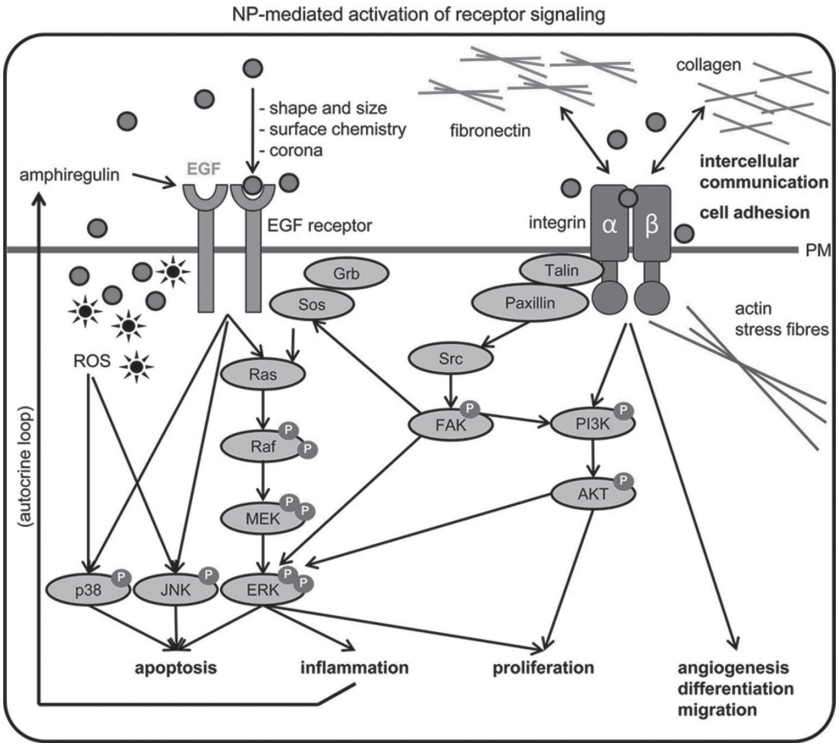

Figure 6. Physico-chemical binding of NPs of comparable length scales with cell receptors such as the EGFR or integrins can activate the RAS-MEK-ERK signalling pathway and impact on fundamental cellular functions (e.g., apoptosis, inflammation, proliferation, migration, etc.) without the need for the NPs to be internalized into the cell. The manner in which the NPs bind to the receptors is highly dependent on size, shape, charge, and protein corona, encapsulating the NPs. Reproduced with permission.[189] Copyright 2013, American Chemical Society.

remotely initiate cytoskeleton remodelling, leading to cell stiffening with higher resistance toward mechanical deformation.[179] Iron-based NMs, in conjunction with magnetic field gradients, have been used to gain spatiotemporal control of the surface reporters to regulate signalling activities, leading to engineered cell growth and differentiation.[180–182] This signalling activities were facilitated by NMs decorated with the receptor's recognition molecules such as RGD,[179] Tie2,[181] anti-TREK1 antibody,[182] or herceptin[2,183] to enhance binding specificity to the targeted receptors. However, unmodified NMs such as carbon black (14 nm) also exhibited an intrinsic capacity to bind to EGFR receptors to activate the Akt signalling cascade, stimulating proliferation of the RLE6TN cells.[184] In contrast, Ag NPs exerted anti-proliferative effects on rat airway smooth muscle cells, caused by their unintended interaction with the cells' muscarinic receptors.[185] Gadolinium endohedral metallofullerenols ($Gd@C_{82}(OH)_{22}$), purported to be potential antitumor chemotherapeutics due to their low toxicity to non-cancer cells, could still interact with Toll-like receptors (TLRs) and this interaction promoted pro-inflammation cytokine IL-1β production.[186] Similarly, the NM's capability to activate intracellular pathways due to its interactions with surface receptors have been reported for undecorated nanostructures such as graphene,[187] TiO_2, and SiO_2.[188]

In a more recent study, graphene was shown to interact with TLR4 and subsequently induced TNF-α-mediated cell necrosis.[190] Interestingly, this nano-induced phenomenon is restricted to cells treated with graphene-based NMs (i.e., graphene oxide, aminated graphene, carboxylated graphene), but not carbon black or carboxylated MWCNT structures,[190] suggesting that the process of NMs binding to specific receptors is tightly coupled to the composition of the NM and how the chemistry is presented on the interacting surface of the NM. In addition to NM's shape and size, surface charge can also exert a deterministic role on the surface receptors. For example, poly(acrylic acid)-conjugated Au NPs were demonstrated to bind to the Mac-1 receptor and the associated inflammatory response in THP-1 cells was also strikingly dependent on the size of the NMs. Further mechanistic studies revealed that the NM-induced unfolding of surface-adsorbed human plasma-derived fibrinogen, leading to promotion of Mac1 receptor activation.[191] These studies highlighted the need to understand the complex interplay between NM properties and their effects on the protein corona formation. Negatively charged SPIONs were also shown promote cellular proliferation by binding to EGFR and activating the AKT and ERK signalling pathways.[192] This SPION-EGFR ligand-free induction effect was observed for breast cells MCF10 A and in even higher levels of induction in its Ras mutagenic phenotype CA1,[192] suggesting potential growth stimulation of cancer cells. Overall, it is important for the nanotechnologist and biologist alike to consider the potential inadvertent interactions of the NMs with some surface receptors.

3.2.3. Mechanisms of NM Transport Across the Cell Membrane

Successful internalization of NMs into the cells is important for many nanomedicine application such as drug delivery, gene therapy, biosensing, and bioimaging. Targeting cell receptor-mediated pathways, which has been extensively reviewed elsewhere,[193–195] is one of the most commonly adopted strategies to facilitate NM cellular uptake. However, additional targeting functionalities are not always desirable as it will translate to more synthesis steps, a higher cost, and more uncertainties in real in vivo settings.[44]

Nature has fashioned specific pathways to transport proteins and macromolecules into the cells. Depending on the nature and the size of the molecules, the cells will activate a certain pathway to internalize these important biomolecules. Using the size of the NMs as a criterion, it is possible to postulate the endocytic pathways that the NMs may undertake to gain entry into the cells (**Table 1**). However, the inherent complexity of nano–bio interactions and life itself makes it hard to exactly pinpoint which entry mechanism the NMs will exploit based solely on their size. Phagocytosis, for instance, is traditionally

Table 1. Possible internalization routes engaged by NMs based on their size.[205]

Uptake mechanism	Description	NM size approximation
Phagocytosis	Internalization of solid particles such as bacteria and yeast by specialized cells	>1 μm
Macropinocytosis	Engulfment of large fluid pockets by formation and enclosure of plasma membrane ruffles	1–2 μm
Clathrin-mediated endocytosis	Transmembrane receptors mediated process. Ligand-receptor interaction triggers "coated pits" on plasma membrane formed by clathrin molecules	~120 nm
Caveolin-mediated endocytosis	Large flask shaped invaginations of solid particles	50–80 nm
Clathrin/Caveolin-independent endocytosis	Membrane invagination based on generic physical interaction	<200 nm
Direct penetration	Direct penetration of cell membrane	4–10 nm

considered to take in large solid particles (e.g., bacteria cells with size >1 μm). Nevertheless, recent reports indicate that phagocytosis is also involved in mediating gold,[196,197] silver,[198] NPs and QDs[199] into the cells. In addition, these studies have shown that changing the size of these NMs did not prompt any change to the entry pathway.[200] Likewise, macropinocytosis has been shown to engulf NMs independently of their size and their material composition and also activation of other endocytic pathways. NMs may also exploit multiple uptake mechanisms simultaneously to gain entry into cells. For example, monodisperse iron-based NMs (7 nm) were reported to enter MCF-7 cells via macropinocytosis and clathrin-mediated endocytosis.[201] Similarly, TiO_2 NMs (6 nm) were internalized via macropinocytosis in addition to clathrin-mediated and caveolin-mediated pathways.[202] Clathrin-mediated uptake transports NMs with sizes between 10–300 nm. Also, Au NPs with the size range of 14–74 nm entered HeLa cells via a clathrin-mediated pathway.[65] Caveolae rafts, which are approximated to be 50–80 nm in diameter, were reported to take in nano-hydroxyapatite (50 nm)[203] and nano-TiO_2 (24 nm)[203] in addition to the much larger silica NPs (178 nm).[204] While it appears to be clear cut for some particles, the concurrent activation of multiple endocytic pathways has made the general assignment of NM uptake to a particular endocytotic pathway too convoluted and confusing. The list of notoriously promiscuous endocytotic inhibitors does not alleviate the experimentally controlled discernment of which endocytic pathway is responsible for a particular NM uptake.

As mentioned, while it is technically challenging to resolve the endocytic pathway taken by the NMs to enter the cells based on NM size alone, there are a few studies which demonstrate a strong NM size dependence in governing the overall uptake process. For instance, it was shown that spherical Au NPs with diameters of 14, 50, 74, and 100 nm were taken up by HeLa cells in a size-dependent manner, with 50 nm being the critical size for optimal uptake.[65] Other studies have suggested that the critical size of spherical NM uptake is within the radius range of 20–30 nm.[206,207] QDs with a radius of 25 nm were also reported to be taken up more efficiently than 7.5 nm ones.[208] A more recent study identified this critical radius to be between 20–25 nm.[209]

In addition to size, NM shape has also been shown to influence cellular uptake. A study showing that transferrin-coated Au nanorods (74 nm × 14 nm) were internalized by HeLa cells to a lesser degree when compared to their spherical counterparts (two sizes: 74 nm and 14 nm). Moreover, the authors noted that the rate trend holds when uncoated NMs are used.[65,72] In contrast, higher internalization of long silica nanorods (aspect ratio 4) was measured in human melanoma cells (A375) as compared to shorter silica nanorods (aspect ratio 2) and spherical silica nanoparticles (aspect ratio 1).[210] Carbon-based nanotubes, however, do not show any propensity to follow any of the aforementioned trends. SWCNT (130, 320, 430, 660 nm in length) internalization into NIH-3T3 cells was found to follow a skewed Gaussian distribution of the tube length. It has been noted that the optimal uptake occurred for SWCNTs with a capture radius of 26.4 and length of 320 nm.[211] In a separate study, it was reported that endocytosis only occurred for NPs with intermediate aspect ratios, while those with smaller aspect ratios were not endocytosed. Those long NMs with even larger aspect ratios could not be totally endocytosed, resulting in the phenomenon of "frustrated phagocytosis".[212] This led to inflammation and eventually the formation of granulomas and pleural mesothelioma.[213]

However, the shape dependency of the NMs in effecting a particular endocytotic mechanism may not be that straight forward, because one also has to consider the intertwined effects of other possible NP parameters such as surface charge. For instance, a study where the aspect ratios of nano-hydroxyapatite were varied (but keeping surface area similar) showed similar cellular association.[214] Furthermore, in a recent simulation study, NMs with different shapes and surface charges were found to have diverging internalization rates. Negatively charged NMs showed no preference for their mode of translocation across the cell membrane, while the positively charged NMs were internalized with a strong dependency on their shape and orientation (**Figure 7**). In addition, the presence of facets on the NMs provides more planes for the NMs to interact with the cell membrane, facilitating stronger adhesion and higher internalization.[215] The interdependency of all these NM physical properties is exemplified by the positively charged, faceted, rice-shaped NMs, which undergo electrostatic reorientation near the cell membrane to maximize their contact area, resulting in the instantaneous internalization and disruption of the cell membrane.[215] Also, spherical silica nanospheres (178 nm) were internalized through a clathrin-mediated pathway, while worm-like silica nanostructures (232 nm × 1348 nm) utilized macropinocytosis and phagocytosis.[204] Conversely, indiscriminate internalization of mesoporous silica nanoparticles (MSNs) with different architectures (long nanorods, NLRs; short nanorods, NSRs; and nanospheres, NSs), utilizing non-specific endocytotic pathways was reported in A375 cells.[210] In a follow-up study, the same group found that the PEGylated versions of the same NMs were internalized into HeLa cells following remarkably different pathways. NS-PEG favored a

nanoparticle	Charge density	Barrier(kJ/mol)	k (s^{-1})	$t_{1/2}$ (s)
Rice 	0.0	223.8 ± 10.3	6.7×10^{-27}	1.0×10^{26}
	0.1	190.1 ± 9.7	4.9×10^{-21}	1.3×10^{20}
	0.2	92.6 ± 6.2	4.7×10^{-4}	1.4×10^{3}
	0.3	43.6 ± 3.6	1.6×10^{5}	4.3×10^{-6}
	0.4	barrier less		
Sphere 	0.0	408.2 ± 23.1	5.3×10^{-59}	1.3×10^{58}
	0.1	259.4 ± 12.3	4.3×10^{-33}	1.6×10^{32}
	0.2	164.1 ± 6.9	1.7×10^{-16}	4.1×10^{15}
	0.3	106.2 ± 8.2	2.0×10^{-6}	3.4×10^{5}
	0.4	60.3 ± 3.1	1.9×10^{2}	3.5×10^{-3}
Pyramid 	0.4	66.3 ± 3.2	1.8×10^{1}	0.04
Cone 	0.4	72.2 ± 4.1	1.7×10^{0}	0.4
Rod 	0.4	75.3 ± 3.9	4.9×10^{-1}	1.4
Cube 	0.4	79.8 ± 3.5	7.9×10^{-2}	8.7

Figure 7. Translocation rate of gold-based NPs is heavily dependent on shape, charge density, and orientation of the NPs as it approaches the negatively charged cell lipid bilayer membrane. 'Barrier' refers to the energy barrier encountered by the NPs and thus is a measure of resistance to pass through the cell membrane. k is the translocation rate constant and $t_{1/2}$ is the half life that provides a good estimation of the translocation efficacy of each NP type. Reproduced with permission.[215] Copyright 2012, American Chemical Society.

clathrin-mediated pathway for their uptake, NSR-PEG made use of both clathrin- and caveolin-mediated pathways to enter the cells, and NLR-PEG was internalized mainly via a caveolin-mediated pathway.[216] This discrepancy highlights the complex interplay between the physical properties of NMs and the cellular uptake mechanism.

Surface charge, too, affects cellular uptake. Positively charged NMs typically exhibit higher uptake due to the electrostatic attraction between the oppositely charged NMs and the cell membrane. For example, positively charged PAA-coated Au NPs were internalized more effectively by SK-BR-3 cells compared to citrate-coated (negatively charged) and PVA-coated (neutral charge) Au NPs.[217] Au NPs with a positive surface charge are taken up with significantly higher efficiency compared to zwitterionic and negative Au NPs.[218] Though designing NMs to carry a positive charge could improve cellular entry for any biomedical application, most of these positively charged NMs seem to also induce mitochondrial damage, cell membrane damage, and cell death.[218–223] While we are uncertain whether it is the positive charge that is responsible for the observed damage, one needs to weigh the benefits of an increased uptake with the potential cytotoxicity when dealing with positively charged NMs.

In addition, one should also consider the interplay between the physico-chemical properties of NMs and the cellular uptake mechanism to facilitate NM internalization to the target cells, such as was demonstrated by Tay et al.[224] These authors reported Au nanoclusters capped with different types of ligands of the same charge causing significant differences in the uptake of these Au nanoclusters. The authors observed that the negatively charged, MPA-coated Au nanoculsters were internalized more easily by NCM460 cells. They concluded that the NM's surface charge is not the only the deciding factor for their internalization and, more importantly, the nature of the ligand and its interplay with the cells also determines the biological response.[224]

Another important parameter to be considered is the adsorption of proteins onto the NM surface. When the NMs are dispersed in biological fluid, a layer of protein (a corona) will form around the NM. Formation of the protein corona on the NM surface is a highly dynamic and complex process in which all of the aforementioned NM parameters (i.e., material, size, shape, surface charge) have been shown to affect the composition, thickness, and conformation of the protein layer surrounding the NMs in numerous studies.[72,225–228] Within a short time span of less than 0.5 min, as many as 300 different proteins were observed to form on the NM's surface.[110] Proteins of high abundance will likely dominate the protein corona initially, only to be displaced by proteins of lower concentration but with higher affinity to the NM surface at longer time scales. Since

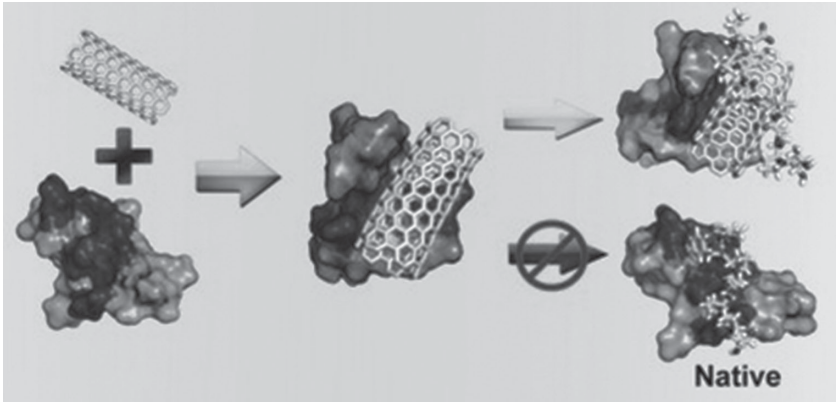

Figure 8. Illustration of SWCNTs (wheat sticks) physically binding to the hydrophobic (dark grey) domain of the YAP65WW protein (light grey). The SWCNTs not only caused the unfolding of the β-sheet of the WWW domain but also prevented binding of the proline residues to the protein. Reproduced with permission.[239] Copyright 2013, Wiley-VCH Verlag.

biological systems usually interact with protein-coated NMs, the amount, identity, and associated structural changes to the surface-adsorbed proteins is also expected to exert a significant impact on the manner in which the NMs are internalized into the cells. As an extreme example, compared to silica NPs coated with serum-derived proteins, bare silica NPs were observed to show higher cellular association and uptake by exploiting other nonendolysosomal pathways to enter the cells.[229] Similarly, an overall decrease in cellular uptake was observed with increasing amounts of surface-adsorbed proteins on the NPs.[230] While the presence of proteins on the NM surface may limit cellular uptake, it may also trigger cell- and receptor-specific-mediated pathways to promote endocytosis, which can be exploited for targeted delivery.[231,232]

3.3. Interaction of NMs with Intracellular Components

3.3.1. Interfering from Within: NM Influences over Enzymatic Functions and Genomic Stability

Upon gaining entry into the cells, the internalized NMs can come into contact with the intracellular machinery, which may elicit diverse biological outcomes.[224,233–238] For instance, NM–enzyme association could occupy the enzyme's active site and/or block the substrate binding to the active sites (**Figure 8**), resulting in the loss of enzymatic activity.[239] In the case of Ag NPs, it was shown that the NPs were able to bind to RNA polymerase, an essential enzyme for RNA synthesis and maintaining overall cell functions. This interaction brought about a robust inhibition of RNA polymerase activity[240] in mouse erythroleukemia cells. This led to the reduction of the haemoglobin content in the embryonic erythrocytes, which accounted for embryonic anemia and embryonic developmental retardation.[240]

Consistent with other aspects of nano–bio interactions, the physico-chemical properties of NMs were also reported to be a contributing factor in determining the manner in which the NMs will interface with other essential enzymes in the human body. The activity of pepsin was shown to reduce significantly following its exposure to spherical nano-TiO_2 (60 nm), while perturbation to the enzyme activity when exposed to larger 200 nm TiO_2 particles was minimal.[241] The underlying mechanism can be best understood by the conformational changes in pepsins that were adsorbed onto the NM surface, dependent on the size of the NM. While noncovalent binding of pepsin to the 60 nm nano-TiO_2 was shown to induce unfolding of the enzyme's secondary structure, thus decreasing the enzyme activity, negligible protein structural changes were observed when pepsin was adsorbed onto the 200 nm nano-TiO_2.[241] Similarly, smaller Au NPs (8 nm) with a large surface-to-volume ratio were documented to exert a greater effect in depleting intracellular glutathione (GSH) levels as compared to their bigger counterparts (37 nm). The GSH adsorption on the surface of the 8 nm Au NPs was so immense that the cell's anti-oxidative stress mechanism was compromised, and this triggered apoptosis.[242]

TiO_2, SiO_2, ZnO, and clay-based NMs were also shown to exert an inhibitory effect on the human arylamine *N*-acetyltransferase 1 (NAT1), a cystolic enzyme that aids drug metabolism and detoxifies carcinogens. The mode of inhibition in this case involves the binding of the NM to the active site of NAT1, thus preventing NAT1 from binding to the target substrate. Interestingly, the more negative the surface charge of the NM, the lower the LD50 concentration needed to inhibit the NAT1 activity, highlighting the importance of considering NM charge as a critical design parameter to inhibit enzymatic activity.[243]

Recently, it was also suggested that NMs could directly or indirectly interact with cytoplasmic proteins to induce cellular autophagy.[244,245] In simple terms, autophagy can be defined as an evolutionary conserved process displayed by the cells to sequestrate cytosolic elements that are meant to be degraded. Autophagy has significant physiological implications and constitutes a homeostatic cellular response to cope with stress derived from oxidative stress, organelle damage, and even internalized nondegradable NMs.[237,246,247] NMs can potentially interact with mitochondria, anti-oxidative enzymes, or surface receptors to active signalling cascades, leading to increased intracellular ROS levels, triggering autophagy.[245] It was also proposed that NMs may incite autophagy by promoting NM–ubiquitin aggregate formation, which can subsequently prompt the cells to activate the proteasome pathway for clearance. This is supported by evidence showing that gold NMs can induce substantial conformational changes and protein misfolding to surface-bound human ubiquitin protein to form large NM–ubiquitin complexes.[248] α-Al_2O_3 (60 nm) was also observed to co-localize with p62/SQSTM1, an important protein that is linked to the autophagic machinery in the autophagosome of dendritic cells.[249] Because autophagy is linked to numerous pathologies, researchers are now looking into the possibility of exploiting NM-mediated autophagy for therapeutic applications such as the enhanced and selective killing of cancer cells. While the prospect appears to be promising, there is a need to better understand the underlying NM's physico-chemical properties

governing the autophagy process. Based on the available literature, it appears that NM size plays an important role, since QDs that had a tendency to aggregate to micrometer-sized particulates failed to induce autophagy compared to their smaller nano-counterparts, regardless of the surface coating.[250,251] This size-dependent phenomenon may in part be explained by the difference in the uptake level of the NMs.

Due to their small dimensions, some internalized NMs could translocate into the cell nucleus[252] via the nucleus pores, which have a reported pore size range of 80–120 nm.[205] This poses a significant concern since any potential interaction between the NMs and the genetic materials may induce genomic DNA damage, leading to impaired cellular functions and viability. Furthermore, persistent NM-mediated DNA damage could also lead to genetic mutation and epigenetic defects that could be passed on to the daughter cells. The ability of NMs to bind to DNA has been demonstrated and fully utilized for the formation of well-dispersed and stable hybrids of DNA-CNTs.[253] Recently, it was also documented that CNTs and tungsten oxide could interact physically with plasmid DNA, resulting in single-strand and double-strand breaks in *Escherechia coli* (*E. coli*) DNA. The mutagenic potential of the NMs was demonstrated by transforming *E. coli* with plasmids pretreated with CNTs and tungsten trioxide NPs. Only 10% of the total cell population was found to survive, and the entire surviving cell population carried DNA damage.[254] In a similar manner, double-stranded as well as single-stranded DNA have been reported to bind to and interface with carbon NPs[255] and silver NPs.[256] It was observed that these interactions severely compromised DNA replication fidelity and impaired cell growth.[255,256] It appears that physical binding between the NMs and the DNA may not be a pre-requisite to inflict DNA damage. For instance, Pt^{2+} ions released from Pt NPs were shown to form Pt–DNA complexes and, in turn, to cause DNA strand breaks.[257] Interestingly, chemotherapeutic platinum-based prodrugs (e.g., cisplatin, satrapplatin, oxoplatin, etc.) also entail the formation of Pt–DNA complexes as the main therapeutic mechanism to kill cancer cells.[258] Therefore, the ability of Pt NPs to replicate the same effects of these prodrugs may suggest the potential use of Pt-based NPs for cancer chemotherapy.

3.3.2. Crippling Migrating Cells: NM Interactions with the Cellular Cytoskeletal Network

'Cytoskeleton' refers to the intricate fabric-like system consisting of long chains of biopolymers that is instrumental in regulating multiple fundamental cellular functions. The cytoskeletal polymers are in a constant state of polymerizing and depolymerizing with a time scale of microseconds to minutes, so as to allow the cells to respond quickly to extracellular signals in a dynamic fashion. Broadly speaking, the cytoskeletal system consist of three subtypes of nanosized filamentous protein, namely: i) microfilaments such as the filamentous actin (F-actin), ii) microtubules (MTs), and iii) intermediate filaments (IF). The spatiotemporal organization of the cytoskeletal network is important for organelle partitioning, maintenance of cell shape, directing cell motility, and also intracellular transport.

Due to the abundance of cytoskeleton molecules in the cytoplasm in addition to their extensive network, which spans the entire cell body, substantial interactions between internalized NMs and these structural proteins can be expected. Vesicle-coated NMs can also utilize motor proteins such as dynein or kinesin to be transported along the cytoskeletal track towards the perinuclear region.[259] However, nonspecific binding of the NMs with cytoskeletal components may also result in detrimental outcomes, as NMs may interact and interfere with the dynamic remodelling of the cytoskeletal polymers.[260] Several ex vivo studies have suggested that NPs can physically bind to the MTs and induce significant conformational changes to the MTs and impair their polymerization.[261–264] For instance, fullerene derivative $C_{60}(OH)_{20}$ was shown to be able to occupy multiple binding sites between adjacent tubulin dimers with a binding constant of $1.3 \pm 0.16 \times 10^6$ M^{-1} and effectively inhibit MT polymerization.[264] Treatment of PC12 murine neuronal cells with Fe_3O_4 NPs could also induce physical conformational changes to the secondary and tertiary structures of tubulin and prevent cooperative binding between tubulin and tau protein, which then destabilizes the MT fibril structure.[262] Although the exact parameters that cause NM-mediated changes to the cytoskeletal network still needs to be fully elucidated, there are studies suggesting that both particles size and shape are important determinants.[210,263,265] Comparing Au NPs with sizes of 20, 40, and 60 nm, Au NPs with diameter 40 nm inflicted the greatest damage to the MT system by causing massive tubulin aggregation in the cytosol, resulting in G_0/G_1 cell cycle arrest and apoptosis in A549 cells.[263] The shape-dependent effect of NMs was also observed for silica NPs with higher aspect ratios, which were shown to display higher cellular internalization and to perturb cytoskeletal formation.[210]

Apart from negatively affecting cell viability, NPs can also trigger "latent" toxicity that could lead to impaired cellular function as a result of cytoskeletal network destruction. For instance, NP treatment can significantly impair directed cell migration without any apparent cell death.[266–269] Recently, using techniques such as microcontact printing (μCP) and cell traction force (CTF) microscopy, Tay et al.[267] were able to decouple the "cause–effect" relationship between NM-mediated cytoskeletal damage and the slowing down of cell migration, thus providing important nanospecific mechanistic insights (**Figure 9**). Internalized nano-TiO_2, hydroxyapatite, and SiO_2 were shown to upset the force balance between the MTs and F-actin by destabilizing the MT network.[267] According to the cellular tensegrity model,[270] the MT plays an important structural role to counteract the tension in the F-actin. Therefore, the net loss in the MT caused by the NMs forced the cells to be more tense, driving the adoption of a "sticky" phenotype, and limiting cell migration.[267] In another related study, loosely bound Au NMs on a glass surface generally decreased the migration of PC3 human prostate cancer cells and human dermal fibroblast (HDF) cells, which is dependent on both Au NM size and surface charge.[266] This seemingly innate ability to target the MTs with NMs may point to a potential therapeutic usage, similar to cytoskeletal targeting drugs such as colchicine[271] and paclitaxel[272] that are used for the treatment of gout and chemotherapy respectively.

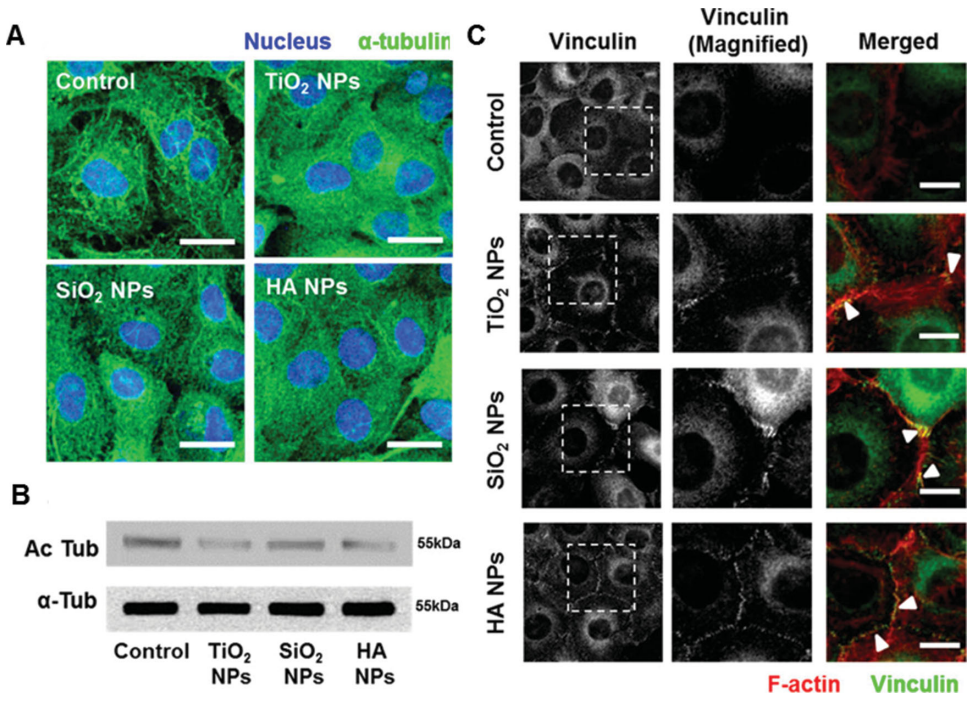

Figure 9. (A) Internalization of several types of NPs was shown to disrupt microtubule assembly by (B) destabilizing the structure, as indicated by a lower expression of acetylated tubulin at the lysine 40 site. (C) This NP-mediated cellular response causes the cells to increase expression of focal adhesions (white arrows) and forces the cells to adhere more strongly to the underlying substrate. Scale bar = 20 μm. Reproduced with permission.[267] Copyright 2014, American Chemical Society.

More than just an architectural support for the cells, the cytoskeleton is also heavily involved in the regulation of intracellular signalling pathways. Besides physically binding to the cytoskeletal apparatus, NMs can also indirectly target the cytoskeletal system by provoking certain cytoskeletal-associated signalling pathways. For instance, it was proposed that graphene oxide nanosheets can induce cytoskeletal damage by dampening ROCK activity,[190] a downstream effector of RhoA activity that is known to positively regulate F-actin formation and contractility. This resulted in the drastic attenuation of actin polymerization and caused macrophages to lose their phagocytic ability. Treatment with high concentrations of iron oxide NPs was also shown to destroy the cytoskeleton network and diminish focal adhesion kinase-mediated signalling.[260] Conversely, treatment of hMSCs with a nontoxic concentration of mesoporous silica nanoparticles (MSNs) can, in turn, upregulate GTP-RhoA activity in hMSCs with a concomitant increase in stress fiber formation.[235] Interestingly, MSN treatment alone was sufficient to induce transient upregulation of osteogenic gene expression, suggesting that MSNs may hold the potential to modulate stem cell lineage commitment.

4. Conclusions

Nanomedicine is on an upward trajectory with the rapid expansion of nanotechnology and nanofabrication techniques bolstered by a strong understanding of both the chemical and biological sciences. Since the receiving end of many of these nanomedicines is diseased cells or tissues, the biological design has always adopted a problem-centric approach. While it is clearly a great start to apply nanotechnology to medicine, it is hardly sustainable because it is usually the unwanted side effects that derail the paths to clinical applications. Therefore, to follow the disease problem-centric approach to design nanomedicine is inadequate. Much consideration needs to be refocussed on the generic properties of NMs and their physico-chemical nuances because it is these nuances that may bring about those unwanted side-effects. Having a deep understanding is likewise important because only with understanding can we engineer control over these effects. While there are unwanted side effects, the flip-side would be that with understanding of the biological mechanisms arising from NM interactions with cells, we could exploit these effects as novel strategies. From a strategic perspective, it is important to build a multidisciplinary network to focus on both the classical disease-centric approach and "expect the unexpected effects". In similar ways, we can learn from outer space exploration on these two issues as we further explore the inner space within us with nanotechnology.

Acknowledgements

C. Y. Tay and M. I. Setyawati contributed equally to this work. This work was funded by Ministry of Education, Singapore (R-279–000–350–112 to DTL and R-279–000–376–112 to CYT). Parts of this work were supported by HSFP (project RGP0052/2012 to WJP). WJP is grateful to Prof. Dr. Terry Tetley for helpful discussions.

Received: May 22, 2014
Revised: June 27, 2014
Published online: August 1, 2014

[1] R. P. Feynman, *J. Microelectromech. Syst.* **1992**, *1*, 60.
[2] M. S. Muthu, D. T. Leong, L. Mei, S. S. Feng, *Theranostics* **2014**, *4*, 660.
[3] Z. Zhang, L. Wang, J. Wang, X. Jiang, X. Li, Z. Hu, Y. Ji, X. Wu, C. Chen, *Adv. Mater.* **2012**, *24*, 1418.
[4] D. Shi, *Adv. Funct. Mater.* **2009**, *19*, 3356.
[5] H. Wu, S. Zhang, J. Zhang, G. Liu, J. Shi, L. Zhang, X. Cui, M. Ruan, Q. He, W. Bu, *Adv. Funct. Mater.* **2011**, *21*, 1850.
[6] Y. Lu, C. Shi, M.-J. Hu, Y.-J. Xu, L. Yu, L.-P. Wen, Y. Zhao, W.-P. Xu, S.-H. Yu, *Adv. Funct. Mater.* **2010**, *20*, 3701.
[7] C. Y. Tay, S. A. Irvine, F. Y. Boey, L. P. Tan, S. Venkatraman, *Small* **2011**, *7*, 1361.
[8] T. Dvir, B. P. Timko, D. S. Kohane, R. Langer, *Nat. Nanotechnol.* **2011**, *6*, 13.
[9] E. Roduner, *Chem. Soc. Rev.* **2006**, *35*, 583.
[10] Y. Yu, Q. Yao, Z. Luo, X. Yuan, J. Y. Lee, J. Xie, *Nanoscale* **2013**, *5*, 4606.
[11] R. Katzschmann, A. Rehfeld, R. Kranold, *Phys. Stat. Solidi A* **1977**, *40*, K161.
[12] H. Weller, H. M. Schmidt, U. Koch, A. Fojtik, S. Baral, A. Henglein, W. Kunath, K. Weiss, E. Dieman, *Chem. Phys. Lett.* **1986**, *124*, 557.
[13] A. I. Ekimov, A. L. Efros, A. A. Onushchenko, *Solid State Commun.* **1985**, *56*, 921.
[14] Y. Yu, Z. Luo, D. M. Chevrier, D. T. Leong, P. Zhang, D. E. Jiang, J. Xie, *J. Am. Chem. Soc.* **2014**, *136*, 1246.
[15] X. Yuan, Y. Tay, X. Dou, Z. Luo, D. T. Leong, J. Xie, *Anal. Chem.* **2013**, *85*, 1913.
[16] Z. Luo, X. Yuan, Y. Yu, Q. Zhang, D. T. Leong, J. Y. Lee, J. Xie, *J. Am. Chem. Soc.* **2012**, *134*, 16662.
[17] J. I. Gittleman, B. Abeles, S. Bozowski, *Phys. Rev. B: Condens. Matter Mater. Phys.* **1974**, *9*, 3891.
[18] M. Faraday, *Philos. Trans. R. Soc. London* **1857**, *147*, 145.
[19] J. Turkevich, P. C. Stevenson, J. Hillier, *Discuss. Faraday Soc.* **1951**, *11*, 55.
[20] I. Freestone, N. Meeks, M. Sax, C. Higgitt, *Gold Bull.* **2007**, *40*, 270.
[21] L. Hutson, *Mater. World* **2005**, *13*, 18.
[22] R. Weissleder, D. D. Stark, B. L. Engelstad, B. R. Bacon, C. C. Compton, D. L. White, P. Jacobs, J. Lewis, *AJR, Am. J. Roentgenol.* **1989**, *152*, 167.
[23] S. Saini, D. D. Stark, P. F. Hahn, J. Wittenberg, T. J. Brady, J. T. Ferrucci Jr., *Radiology* **1987**, *162*, 211.
[24] Z. Li, K. Dong, S. Huang, E. Ju, Z. Liu, M. Yin, J. Ren, X. Qu, *Adv. Funct. Mater.* **2014**, DOI 10.1002/adfm.201303662.
[25] A. Lund, A. L. Hellemann, F. Vartdal, *J. Clin. Microbiol.* **1988**, *26*, 2572.
[26] J. H. W. Leuvering, P. J. H. M. Thal, M. v. d. Waart, A. H. W. M. Schuurs, *Fresenius' Z. Anal. Chem.* **1980**, *301*, 132.
[27] J. H. Leuvering, P. J. Thal, M. Van der Waart, A. H. Schuurs, *J. Immunol. Methods* **1981**, *45*, 183.
[28] M. Moeremans, G. Daneels, A. Van Dijck, G. Langanger, J. De Mey, *J. Immunol. Methods* **1984**, *74*, 353.
[29] X. D. Zhang, Z. Luo, J. Chen, X. Shen, S. Song, Y. Sun, S. Fan, F. Fan, D. T. Leong, J. Xie, *Adv. Mater.* **2014**, *26*, 4565.
[30] X. D. Zhang, J. Chen, Z. Luo, D. Wu, X. Shen, S. S. Song, Y. M. Sun, P. X. Liu, J. Zhao, S. Huo, S. Fan, F. Fan, X. J. Liang, J. Xie, *Adv. Healthcare Mater.* **2014**, *3*, 133.
[31] D. Y. Joh, G. D. Kao, S. Murty, M. Stangl, L. Sun, A. Al Zaki, X. Xu, S. M. Hahn, A. Tsourkas, J. F. Dorsey, *Transl. Oncol.* **2013**, *6*, 722.
[32] N. S. Yang, J. Burkholder, B. Roberts, B. Martinell, D. McCabe, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9568.
[33] H. P. Erickson, *Biol. Proced. Online* **2009**, *11*, 32.
[34] P. Kanchanawong, G. Shtengel, A. M. Pasapera, E. B. Ramko, M. W. Davidson, H. F. Hess, C. M. Waterman, *Nature* **2010**, *468*, 580.
[35] D. T. Leong, K. W. Ng, *Adv. Drug Delivery Rev.* **2014**, DOI 10.1016/j.addr.2014.06.007.
[36] G. R. Tan, S. S. Feng, D. T. Leong, *Biomaterials* **2014**, *35*, 3044.
[37] F. Peng, Y. Su, Y. Zhong, C. Fan, S. T. Lee, Y. He, *Acc. Chem. Res.* **2014**, *47*, 612.
[38] D. Liu, E. Mäkilä, H. Zhang, B. Herranz, M. Kaasalainen, P. Kinnari, J. Salonen, J. Hirvonen, H. A. Santos, *Adv. Funct. Mater.* **2013**, *23*, 1893.
[39] Z. Luo, K. Zheng, J. Xie, *Chem. Commun.* **2014**, *50*, 5143.
[40] N. Sanvicens, M. P. Marco, *Trends Biotechnol.* **2008**, *26*, 425.
[41] G. Bao, S. Mitragotri, S. Tong, *Annu. Rev. Biomed. Eng.* **2013**, *15*, 253.
[42] Z.-G. Yue, W. Wei, Z.-X. You, Q.-Z. Yang, H. Yue, Z.-G. Su, G.-H. Ma, *Adv. Funct. Mater.* **2011**, *21*, 3446.
[43] Q. Zhang, F. Liu, K. T. Nguyen, X. Ma, X. Wang, B. Xing, Y. Zhao, *Adv. Funct. Mater.* **2012**, *22*, 5144.
[44] Z. Cheng, A. Al Zaki, J. Z. Hui, V. R. Muzykantov, A. Tsourkas, *Science* **2012**, *338*, 903.
[45] S. Bhattacharyya, R. Bhattacharya, S. Curley, M. A. McNiven, P. Mukherjee, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 14541.
[46] E. P. Ivanova, J. Hasan, H. K. Webb, G. Gervinskas, S. Juodkazis, V. K. Truong, A. H. Wu, R. N. Lamb, V. A. Baulin, G. S. Watson, J. A. Watson, D. E. Mainwaring, R. J. Crawford, *Nat. Commun.* **2013**, *4*, 2838.
[47] M. I. Setyawati, C. Y. Tay, D. T. Leong, *Nanomedicine* **2014**, *9*, 369.
[48] F. Tourinho, R. Franck, R. Massart, *J. Mater. Sci.* **1990**, *25*, 3249.
[49] A. Henglein, *Ber. Bunsenges. Phys. Chem.* **1982**, *86*, 301.
[50] C. Ducamp-Sanguesa, R. Herrera-Urbina, M. Figlarz, *J. Solid State Chem.* **1992**, *100*, 272.
[51] E. Hückel, in *Ergebnisse der exakten naturwissenschaften*, Vol. *3*, Springer, Berlin Heidelberg **1924**, p. 199.
[52] C. G. Harford, A. Hamlin, E. Parker, *J. Biophys. Biochem. Cytol.* **1957**, *3*, 749.
[53] P. Gehr, J. D. Brain, S. B. Bloom, P. A. Valberg, *Nature* **1983**, *302*, 336.
[54] A. Albanese, P. S. Tang, W. C. Chan, *Annu. Rev. Biomed. Eng.* **2012**, *14*, 1.
[55] P. Rivera Gil, G. Oberdorster, A. Elder, V. Puntes, W. J. Parak, *ACS Nano* **2010**, *4*, 5527.
[56] V. Merk, C. Rehbock, F. Becker, U. Hagemann, H. Nienhaus, S. Barcikowski, *Langmuir* **2014**, *30*, 4213.
[57] N. Barsch, J. Jakobi, S. Weiler, S. Barcikowski, *Nanotechnology* **2009**, *20*, 445603.
[58] T. Pellegrino, S. Kudera, T. Liedl, A. Munoz Javier, L. Manna, W. J. Parak, *Small* **2005**, *1*, 48.
[59] G. Fritz, V. Schädler, N. Willenbacher, N. J. Wagner, *Langmuir* **2002**, *18*, 6381.
[60] F. Zhang, Z. Ali, F. Amin, A. Feltz, M. Oheim, W. J. Parak, *Chemphyschem* **2010**, *11*, 730.
[61] A. Riedinger, F. Zhang, F. Dommershausen, C. Rocker, S. Brandholt, G. U. Nienhaus, U. Koert, W. J. Parak, *Small* **2010**, *6*, 2590.
[62] T. Cedervall, I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson, K. A. Dawson, S. Linse, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2050.
[63] C. Carrillo-Carrión, M. Nazarenus, S. S. Paradinas, S. Carregal-Romero, M. J. Almendral, M. Fuentes, B. Pelaz, P. del Pino, I. Hussain, M. J. D. Clift, B. Rothen-Rutishauser, X.-J. Liang, W. J. Parak, *Curr. Opin. Chem. Eng.* **2014**, *4*, 88.
[64] P. Rivera-Gil, D. Jimenez de Aberasturi, V. Wulf, B. Pelaz, P. del Pino, Y. Zhao, J. M. de la Fuente, I. Ruiz de Larramendi, T. Rojo, X. J. Liang, W. J. Parak, *Acc. Chem. Res.* **2013**, *46*, 743.

[65] B. D. Chithrani, A. A. Ghazani, W. C. Chan, *Nano Lett.* **2006**, *6*, 662.
[66] D. Huhn, K. Kantner, C. Geidel, S. Brandholt, I. De Cock, S. J. Soenen, P. Rivera Gil, J. M. Montenegro, K. Braeckmans, K. Mullen, G. U. Nienhaus, M. Klapper, W. J. Parak, *ACS Nano* **2013**, *7*, 3253.
[67] K. M. Krueger, A. M. Al-Somali, M. Mejia, V. L. Colvin, *Nanotechnology* **2007**, *18*, 475709.
[68] R. A. Sperling, T. Liedl, S. Duhr, S. Kudera, M. Zanella, C. A. J. Lin, W. H. Chang, D. Braun, W. J. Parak, *J. Phys. Chem. C* **2007**, *111*, 11552.
[69] G. Roebben, S. Ramirez-Garcia, V. A. Hackley, M. Roesslein, F. Klaessig, V. Kestens, I. Lynch, C. M. Garner, A. Rawle, A. Elder, V. L. Colvin, W. Kreyling, H. F. Krug, Z. A. Lewicka, S. McNeil, A. Nel, A. Patri, P. Wick, M. Wiesner, T. Xia, G. Oberdörster, K. A. Dawson, *J. Nanopart. Res.* **2011**, *13*, 2675.
[70] P. Hole, K. Sillence, C. Hannell, C. M. Maguire, M. Roesslein, G. Suarez, S. Capracotta, Z. Magdolenova, L. Horev-Azaria, A. Dybowska, L. Cooke, A. Haase, S. Contal, S. Mano, A. Vennemann, J. J. Sauvain, K. C. Staunton, S. Anguissola, A. Luch, M. Dusinska, R. Korenstein, A. C. Gutleb, M. Wiemann, A. Prina-Mello, M. Riediker, P. Wick, *J. Nanopart. Res.* **2013**, *15*, 2101.
[71] A. Einstein, *Ann. Phys.* **1905**, *17*, 549.
[72] B. D. Chithrani, W. C. Chan, *Nano Lett.* **2007**, *7*, 1542.
[73] L. C. Stoehr, E. Gonzalez, A. Stampfl, E. Casals, A. Duschl, V. Puntes, G. J. Oostingh, *Part. Fibre Toxicol.* **2011**, *8*, 36.
[74] B. Fadeel, N. Feliu, C. Vogt, A. M. Abdelmonem, W. J. Parak, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2013**, *5*, 111.
[75] C. Pfeiffer, C. Rehbock, D. Hühn, C. Carrillo-Carrion, D. J. de Aberasturi, V. Merk, S. Barcikowski, W. J. Parak, *J. R. Soc., Interface* **2014**, *11*, 20130931.
[76] D. L. Harame, L. J. Bousse, J. D. Shott, J. D. Meindl, *IEEE Trans. Electron Devices* **1987**, *34*, 1700.
[77] L. Bousse, N. F. d. Rooij, P. Bergveld, *Surf. Sci.* **1983**, *135*, 479.
[78] H. Helmholtz, *Wiedemanns Annalen der Physik und Chemie* **1879**, *7*, 337.
[79] D. L. Chapman, *Philos. Mag.* **1913**, *25*, 475.
[80] M. Gouy, *J. Phys. Theor. Appl.* **1910**, *9*, 457.
[81] P. Debye, E. Hückel, *Phys. Z.* **1923**, *24*, 185.
[82] O. Stern, *Z. Elektrochem. Angew. P.* **1924**, *30*, 508.
[83] J. O. M. Bockris, S. U. M. Khan, *Surface Electrochemistry – A Molecular Level Approach*, Plenum Press, New York, USA **1993**.
[84] S. Jailani, G. V. Franks, T. W. Healy, *J. Am. Ceram. Soc.* **2008**, *91*, 1141.
[85] T. L. Doane, C. H. Chuang, R. J. Hill, C. Burda, *Acc. Chem. Res.* **2012**, *45*, 317.
[86] Y. Zhang, M. Yang, N. G. Portney, D. Cui, G. Budak, E. Ozbay, M. Ozkan, C. S. Ozkan, *Biomed. Microdevices* **2008**, *10*, 321.
[87] G. M. Dougherty, K. A. Rose, J. B. Tok, S. S. Pannu, F. Y. Chuang, M. Y. Sha, G. Chakarova, S. G. Penn, *Electrophoresis* **2008**, *29*, 1131.
[88] M. M. Smoluchowski, *Bull. Int. Acad. Sci. Cracovie, Cl. Sci. Math.* **1903**, 182.
[89] E. Hückel, *Phys. Z.* **1924**, *25*, 204.
[90] S. Park, N. Sinha, K. Hamad-Schifferli, *Langmuir* **2010**, *26*, 13071.
[91] A. Morgan, *Environ. Res.* **1974**, *7*, 330.
[92] F. Valerio, D. Balducci, L. Scarabelli, *Environ. Res.* **1986**, *41*, 432.
[93] R. Desai, R. J. Richards, *Environ. Res.* **1978**, *16*, 449.
[94] R. Desai, P. Hext, R. Richards, *Life Sci.* **1975**, *16*, 1931.
[95] A. Munoz Javier, O. Kreft, A. Piera Alberola, C. Kirchner, B. Zebli, A. S. Susha, E. Horn, S. Kempter, A. G. Skirtach, A. L. Rogach, J. Radler, G. B. Sukhorukov, M. Benoit, W. J. Parak, *Small* **2006**, *2*, 394.
[96] I. Lynch, T. Cedervall, M. Lundqvist, C. Cabaleiro-Lago, S. Linse, K. A. Dawson, *Adv. Colloid Interface Sci.* **2007**, *134–135*, 167.
[97] S. Tenzer, D. Docter, S. Rosfa, A. Wlodarski, J. Kuharev, A. Rekik, S. K. Knauer, C. Bantz, T. Nawroth, C. Bier, J. Sirirattanapan, W. Mann, L. Treuel, R. Zellner, M. Maskos, H. Schild, R. H. Stauber, *ACS Nano* **2011**, *5*, 7155.
[98] L. Treuel, S. Brandholt, P. Maffre, S. Wiegele, L. Shang, G. U. Nienhaus, *ACS Nano* **2014**, *8*, 503.
[99] C. Röcker, M. Pötzl, F. Zhang, W. J. Parak, G. U. Nienhaus, *Nat. Nanotechnol.* **2009**, *4*, 577.
[100] G. U. Nienhaus, J. Heinzl, E. Huenges, F. Parak, *Nature* **1989**, *338*, 665.
[101] C. Rehbock, V. Merk, L. Gamrad, R. Streubel, S. Barcikowski, *Phys. Chem. Chem. Phys.* **2013**, *15*, 3057.
[102] M. H. Ali, B. Imperiali, *Bioorg. Med. Chem.* **2005**, *13*, 5013.
[103] V. Levi, F. L. Gonzalez Flecha, *Biochim. Biophys. Acta* **2002**, *1599*, 141.
[104] R. F. Atmeh, I. M. Arafa, M. Al-Khateeb, *Jordan J. Chem.* **2007**, *2*, 169.
[105] P. Maffre, K. Nienhaus, F. Amin, W. J. Parak, G. U. Nienhaus, *Beilstein J. Nanotechnol.* **2011**, *2*, 374.
[106] S. Goy-Lopez, J. Juarez, M. Alatorre-Meda, E. Casals, V. F. Puntes, P. Taboada, V. Mosquera, *Langmuir* **2012**, *28*, 9113.
[107] A. Sukhanova, S. Poly, A. Shemetov, I. Nabiev, *Proc. SPIE* **2012**, 8548.
[108] S. Milani, F. B. Bombelli, A. S. Pitek, K. A. Dawson, J. Radler, *ACS Nano* **2012**, *6*, 2532.
[109] P. d. Pino, B. Pelaz, Q. Zhang, P. Maffre, G. U. Nienhaus, W. J. Parak, *Mater. Horiz.* **2014**, *1*, 301.
[110] S. Tenzer, D. Docter, J. Kuharev, A. Musyanovych, V. Fetz, R. Hecht, F. Schlenk, D. Fischer, K. Kiouptsi, C. Reinhardt, K. Landfester, H. Schild, M. Maskos, S. K. Knauer, R. H. Stauber, *Nat. Nanotechnol.* **2013**, *8*, 772.
[111] E. Casals, T. Pfaller, A. Duschl, G. J. Oostingh, V. Puntes, *ACS Nano* **2010**, *4*, 3623.
[112] L. Vroman, *Nature* **1962**, *196*, 476.
[113] J. E. Riviere, *Nanomedicine* **2013**, *8*, 1357.
[114] M. Cavey, T. Lecuit, *Cold Spring Harb Perspect Biol* **2009**, *1*, a002998.
[115] G. Bazzoni, E. Dejana, *Physiol. Rev.* **2004**, *84*, 869.
[116] E. Dejana, *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 261.
[117] P. L. Apopa, Y. Qian, R. Shao, N. L. Guo, D. Schwegler-Berry, M. Pacurari, D. Porter, X. Shi, V. Vallyathan, V. Castranova, D. C. Flynn, *Part. Fibre Toxicol.* **2009**, *6*, 1.
[118] M. I. Setyawati, C. Y. Tay, S. L. Chia, S. L. Goh, W. Fang, M. J. Neo, H. C. Chong, S. M. Tan, S. C. Loo, K. W. Ng, J. P. Xie, C. N. Ong, N. S. Tan, D. T. Leong, *Nat. Commun.* **2013**, *4*, 1673.
[119] E. Paszek, J. Czyz, O. Woznicka, D. Jakubiak, J. Wojnarowicz, W. Lojkowski, E. Stepien, *J. Biomed. Nanotechnol.* **2012**, *8*, 957.
[120] L. R. Farcal, C. Uboldi, D. Mehn, G. Giudetti, P. Nativo, J. Ponti, D. Gilliland, F. Rossi, A. Bal-Price, *Nanotoxicology* **2013**, *7*, 1095.
[121] B. N. Snyder-Talkington, D. Schwegler-Berry, V. Castranova, Y. Qian, N. L. Guo, *Part. Fiber Toxicol.* **2013**, *10*, 35.
[122] J. Sun, S. Wang, D. Zhao, F. H. Hun, L. Weng, H. Liu, *Cell Biol. Toxicol.* **2011**, *27*, 333.
[123] L. Chen, R. A. Yokel, B. Hennig, M. Toborek, *J. Neuroimmune Pharmacol.* **2008**, *3*, 286.
[124] H. Lum, K. A. Roebuck, *Am. J. Physiol. Cell Physiol.* **2001**, *280*, C719.
[125] E. Chau, J. F. Galloway, A. Nelson, P. N. Breysse, D. Wirtz, P. C. Searson, V. K. Sidhaye, *Nanotoxicology* **2013**, *7*, 1143.
[126] J. A. Tunggal, I. Helfrich, A. Schmitz, H. Schwarz, D. Gunzel, M. Fromm, R. Kemler, T. Krieg, C. M. Niessen, *EMBO J.* **2005**, *24*, 1146.

[127] A. D. Lehmann, F. Blank, O. Baum, P. Gehr, B. M. Rothen-Rutishauser, *Part. Fibre Toxicol.* **2009**, *6*, 26.
[128] B. M. Rotoli, O. Bussolati, M. G. Bianchi, A. Barilli, C. Balasubramanian, S. Bellucci, E. Bergamaschi, *Toxicol. Lett.* **2008**, *178*, 95.
[129] B. Rothen-Rutishauser, R. N. Grass, F. Blank, L. K. Limbach, C. Muhlfeld, C. Brandenberger, D. O. Raemy, P. Gehr, W. J. Stark, *Environ. Sci. Technol.* **2009**, *43*, 2634.
[130] J. C. Coyuco, Y. Liu, B. J. Tan, G. N. Chiu, *Int. J. Nanomed.* **2011**, *6*, 2253.
[131] M. Tarantola, A. Pietuch, D. Schneider, J. Rother, E. Sunnick, C. Rosman, S. Pierrat, C. Sonnichsen, J. Wegener, A. Janshoff, *Nanotoxicology* **2011**, *5*, 254.
[132] C. R. Lam, C. Tan, Z. Teo, C. Y. Tay, T. Phua, Y. L. Wu, P. Q. Cai, L. P. Tan, X. Chen, P. Zhu, N. S. Tan, *Cell Death Dis.* **2013**, *4*, e848.
[133] T. T. Onder, P. B. Gupta, S. A. Mani, J. Yang, E. S. Lander, R. A. Weinberg, *Cancer Res.* **2008**, *68*, 3645.
[134] T. Chen, H. Nie, X. Gao, J. Yang, J. Pu, Z. Chen, X. Cui, Y. Wang, H. Wang, G. Jia, *Toxicol. Lett.* **2014**, *226*, 150.
[135] R. R. Arvizo, S. Saha, E. Wang, J. D. Robertson, R. Bhattacharya, P. Mukherjee, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6700.
[136] B. Erickson, M. Fang, J. M. Wallace, B. G. Orr, C. M. Les, M. M. Banaszak Holl, *Biotechnol. J.* **2013**, *8*, 117.
[137] J. Takagi, B. M. Petre, T. Walz, T. A. Springer, *Cell* **2002**, *110*, 599.
[138] C. Y. Tay, C. G. Koh, N. S. Tan, D. T. Leong, L. P. Tan, *Nanomedicine* **2013**, *8*, 623.
[139] L. E. McNamara, R. Burchmore, M. O. Riehle, P. Herzyk, M. J. Biggs, C. D. Wilkinson, A. S. Curtis, M. J. Dalby, *Biomaterials* **2012**, *33*, 2835.
[140] M. J. Biggs, R. G. Richards, M. J. Dalby, *Nanomedicine* **2010**, *6*, 619.
[141] D. Pallarola, A. Bochen, H. Boehm, F. Rechenmacher, T. R. Sobahi, J. P. Spatz, H. Kessler, *Adv. Funct. Mater.* **2014**, *24*, 943.
[142] E. A. Cavalcanti-Adam, T. Volberg, A. Micoulet, H. Kessler, B. Geiger, J. P. Spatz, *Biophys. J.* **2007**, *92*, 2964.
[143] E. A. Cavalcanti-Adam, A. Micoulet, J. Blummel, J. Auernheimer, H. Kessler, J. P. Spatz, *Eur. J. Cell Biol.* **2006**, *85*, 219.
[144] J. Park, S. Bauer, K. von der Mark, P. Schmuki, *Nano Lett.* **2007**, *7*, 1686.
[145] M. J. Dalby, N. Gadegaard, M. O. Riehle, C. D. Wilkinson, A. S. Curtis, *Int. J. Biochem. Cell Biol.* **2004**, *36*, 2005.
[146] P. Weiss, *Yale J. Biol. Med.* **1947**, *19*, 235.
[147] R. G. Harrison, *Anat. Rec.* **1912**, *6*, 181.
[148] E. V. Skorb, D. V. Andreeva, *Adv. Funct. Mater.* **2013**, *23*, 4483.
[149] Z. Shi, K. G. Neoh, E. T. Kang, C. K. Poh, W. Wang, *J. Tissue Eng. Regener. Med.* **2014**, *8*, 50.
[150] D. H. Kim, E. A. Lipke, P. Kim, R. Cheong, S. Thompson, M. Delannoy, K. Y. Suh, L. Tung, A. Levchenko, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 565.
[151] M. Abdul Kafi, W. A. El-Said, T. H. Kim, J. W. Choi, *Biomaterials* **2012**, *33*, 731.
[152] L. Hanson, Z. C. Lin, C. Xie, Y. Cui, B. Cui, *Nano Lett.* **2012**, *12*, 5815.
[153] L. E. McNamara, T. Sjostrom, K. E. Burgess, J. J. Kim, E. Liu, S. Gordonov, P. V. Moghe, R. M. Meek, R. O. Oreffo, B. Su, M. J. Dalby, *Biomaterials* **2011**, *32*, 7403.
[154] M. Mahmoudi, S. Bonakdar, M. A. Shokrgozar, H. Aghaverdi, R. Hartmann, A. Pick, G. Witte, W. J. Parak, *ACS Nano* **2013**, *7*, 8379.
[155] I. Tonazzini, S. Meucci, P. Faraci, F. Beltram, M. Cecchini, *Biomaterials* **2013**, *34*, 6027.
[156] C. Y. Tay, M. Pal, H. Yu, W. S. Leong, N. S. Tan, K. W. Ng, S. Venkatraman, F. Boey, D. T. Leong, L. P. Tan, *Small* **2011**, *7*, 1416.
[157] C. Y. Tay, H. Yu, M. Pal, W. S. Leong, N. S. Tan, K. W. Ng, D. T. Leong, L. P. Tan, *Exp. Cell Res.* **2010**, *316*, 1159.
[158] G. M. Harris, M. E. Piroli, E. Jabbarzadeh, *Adv. Funct. Mater.* **2014**, *24*, 2396.
[159] L. Gao, R. McBeath, C. S. Chen, *Stem Cells* **2010**, *28*, 564.
[160] J. S. Park, J. S. Chu, A. D. Tsou, R. Diop, Z. Tang, A. Wang, S. Li, *Biomaterials* **2011**, *32*, 3921.
[161] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677.
[162] W. S. Leong, C. Y. Tay, H. Yu, A. Li, S. C. Wu, D. H. Duc, C. T. Lim, L. P. Tan, *Biochem. Biophys. Res. Commun.* **2010**, *401*, 287.
[163] M. J. Dalby, N. Gadegaard, R. Tare, A. Andar, M. O. Riehle, P. Herzyk, C. D. Wilkinson, R. O. Oreffo, *Nat. Mater.* **2007**, *6*, 997.
[164] R. J. McMurray, N. Gadegaard, P. M. Tsimbouri, K. V. Burgess, L. E. McNamara, R. Tare, K. Murawski, E. Kingham, R. O. Oreffo, M. J. Dalby, *Nat. Mater.* **2011**, *10*, 637.
[165] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, Garland Science, New York, USA **2007**.
[166] P. K. Stoimenov, R. L. Klinger, G. L. Marchin, K. J. Klabunde, *Langmuir* **2002**, *18*, 6679.
[167] K. L. Chen, G. D. Bothun, *Environ. Sci. Technol.* **2013**, *48*, 873.
[168] X. Yuan, M. I. Setyawati, D. T. Leong, J. Xie, *Nano Res.* **2014**, *7*, 301.
[169] X. Yuan, M. I. Setyawati, A. S. Tan, C. N. Ong, D. T. Leong, J. Xie, *NPG Asia Mater.* **2013**, *5*, e39.
[170] K. Feris, C. Otto, J. Tinker, D. Wingett, A. Punnoose, A. Thurber, M. Kongara, M. Sabetian, B. Quinn, C. Hanna, D. Pink, *Langmuir* **2009**, *26*, 4429.
[171] V. B. Schwartz, F. Thétiot, S. Ritz, S. Pütz, L. Choritz, A. Lappas, R. Förch, K. Landfester, U. Jonas, *Adv. Funct. Mater.* **2012**, *22*, 2376.
[172] G. Rusciano, A. De Luca, G. Pesce, A. Sasso, *Carbon* **2009**, *47*, 2950.
[173] Y. H. Leung, A. M. Ng, X. Xu, Z. Shen, L. A. Gethings, M. T. Wong, C. M. Chan, M. Y. Guo, Y. H. Ng, A. B. Djurisic, P. K. Lee, W. K. Chan, L. H. Yu, D. L. Phillips, A. P. Ma, F. C. Leung, *Small* **2014**, *10*, 1171.
[174] O. Akhavan, E. Ghaderi, *ACS Nano* **2010**, *4*, 5731.
[175] Y. Tu, M. Lv, P. Xiu, T. Huynh, M. Zhang, M. Castelli, Z. Liu, Q. Huang, C. Fan, H. Fang, R. Zhou, *Nat. Nanotechnol.* **2013**, *8*, 594.
[176] N. A. Amro, L. P. Kotra, K. Wadu-Mesthrige, A. Bulychev, S. Mobashery, G.-Y. Liu, *Langmuir* **2000**, *16*, 2789.
[177] I. Sondi, B. Salopek-Sondi, *J. Colloid Interface Sci.* **2004**, *275*, 177.
[178] M. I. Setyawati, W. Fang, S. L. Chia, D. T. Leong, *Asia-Pac. J. Chem. Eng.* **2013**, *8*, 205.
[179] N. Wang, J. P. Butler, D. E. Ingber, *Science* **1993**, *260*, 1124.
[180] J. Dobson, *Nat. Nanotechnol.* **2008**, *3*, 139.
[181] J. H. Lee, E. S. Kim, M. H. Cho, M. Son, S. I. Yeon, J. S. Shin, J. Cheon, *Angew. Chem., Int. Ed. Engl.* **2010**, *49*, 5698.
[182] J. M. Kanczler, H. S. Sura, J. Magnay, D. Green, R. O. Oreffo, J. P. Dobson, A. J. El Haj, *Tissue Eng., Part A* **2010**, *16*, 3241.
[183] W. Tai, R. Mahato, K. Cheng, *J. Controlled Release* **2010**, *146*, 264.
[184] K. Unfried, U. Sydlik, K. Bierhals, A. Weissenberg, J. Abel, *Am. J. Physiol.: Lung Cell. Mol. Physiol.* **2008**, *294*, L358.
[185] M. A. Ramirez-Lee, H. Rosas-Hernandez, S. Salazar-Garcia, J. M. Gutierrez-Hernandez, R. Espinosa-Tanguma, F. J. Gonzalez, S. F. Ali, C. Gonzalez, *Toxicol. Lett.* **2014**, *224*, 246.
[186] Z. Chen, Y. Liu, B. Sun, H. Li, J. Dong, L. Zhang, L. Wang, P. Wang, Y. Zhao, C. Chen, *Small* **2014**, DOI 10.1002/smll.201302825.
[187] H. Zhou, K. Zhao, W. Li, N. Yang, Y. Liu, C. Chen, T. Wei, *Biomaterials* **2012**, *33*, 6933.
[188] A. S. Yazdi, G. Guarda, N. Riteau, S. K. Drexler, A. Tardivel, I. Couillin, J. Tschopp, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19449.

[189] J. Rauch, W. Kolch, S. Laurent, M. Mahmoudi, *Chem. Rev.* **2013**, *113*, 3391.
[190] G. Qu, S. Liu, S. Zhang, L. Wang, X. Wang, B. Sun, N. Yin, X. Gao, T. Xia, J. J. Chen, G. B. Jiang, *ACS Nano* **2013**, *7*, 5732.
[191] Z. J. Deng, M. Liang, M. Monteiro, I. Toth, R. F. Minchin, *Nat. Nanotechnol.* **2011**, *6*, 39.
[192] J. Rauch, W. Kolch, M. Mahmoudi, *Sci. Rep.* **2012**, *2*, 868.
[193] S. Xu, B. Z. Olenyuk, C. T. Okamoto, S. F. Hamm-Alvarez, *Adv. Drug Delivery Rev.* **2013**, *65*, 121.
[194] L. Yang, X. Zhang, M. Ye, J. Jiang, R. Yang, T. Fu, Y. Chen, K. Wang, C. Liu, W. Tan, *Adv. Drug Delivery Rev.* **2011**, *63*, 1361.
[195] N. K. Mehra, V. Mishra, N. K. Jain, *Ther. Delivery* **2013**, *4*, 369.
[196] Z. Krpetic, F. Porta, E. Caneva, V. Dal Santo, G. Scari, *Langmuir* **2010**, *26*, 14799.
[197] A. Franca, P. Aggarwal, E. V. Barsov, S. V. Kozlov, M. A. Dobrovolskaia, A. Gonzalez-Fernandez, *Nanomedicine* **2011**, *6*, 1175.
[198] J. Park, D. H. Lim, H. J. Lim, T. Kwon, J. S. Choi, S. Jeong, I. H. Choi, J. Cheon, *Chem. Commun.* **2011**, *47*, 4382.
[199] H. C. Fischer, T. S. Hauck, A. Gomez-Aristizabal, W. C. Chan, *Adv. Mater.* **2010**, *22*, 2520.
[200] L. Kastl, D. Sasse, V. Wulf, R. Hartmann, J. Mircheski, C. Ranke, S. Carregal-Romero, J. A. Martínez-López, R. Fernández-Chacón, W. J. Parak, H. P. Elsasser, P. Rivera Gil, *ACS Nano* **2013**, *7*, 6605.
[201] F. Corsi, C. De Palma, M. Colombo, R. Allevi, M. Nebuloni, S. Ronchi, G. Rizzi, A. Tosoni, E. Trabucchi, E. Clementi, D. Prosperi, *Small* **2009**, *5*, 2555.
[202] K. T. Thurn, H. Arora, T. Paunesku, A. Wu, E. M. Brown, C. Doty, J. Kremer, G. Woloschak, *Nanomedicine* **2011**, *7*, 123.
[203] C. Y. Tay, W. Fang, M. I. Setyawati, S. L. Chia, K. S. Tan, C. H. Hong, D. T. Leong, *ACS Appl. Mater. Interfaces* **2014**, *6*, 6248.
[204] H. Herd, N. Daum, A. T. Jones, H. Huwer, H. Ghandehari, C. M. Lehr, *ACS Nano* **2013**, *7*, 1961.
[205] M. Zhu, G. Nie, H. Meng, T. Xia, A. Nel, Y. Zhao, *Acc. Chem. Res.* **2013**, *46*, 622.
[206] S. Zhang, J. Li, G. Lykotrafitis, G. Bao, S. Suresh, *Adv. Mater.* **2009**, *21*, 419.
[207] H. Gao, W. Shi, L. B. Freund, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 9469.
[208] T. Nakai, T. Kanamori, S. Sando, Y. Aoyama, *J. Am. Chem. Soc.* **2003**, *125*, 8465.
[209] W. Jiang, B. Y. Kim, J. T. Rutka, W. C. Chan, *Nat. Nanotechnol.* **2008**, *3*, 145.
[210] X. Huang, X. Teng, D. Chen, F. Tang, J. He, *Biomaterials* **2010**, *31*, 438.
[211] H. Jin, D. A. Heller, R. Sharma, M. S. Strano, *ACS Nano* **2009**, *3*, 149.
[212] P. Decuzzi, M. Ferrari, *Biophys. J.* **2008**, *94*, 3790.
[213] K. Donaldson, F. A. Murphy, R. Duffin, C. A. Poland, *Part. Fiber Toxicol.* **2010**, *7*, 5.
[214] X. Zhao, B. C. Heng, S. Xiong, J. Guo, T. T. Tan, F. Y. Boey, K. W. Ng, J. S. Loo, *Nanotoxicology* **2011**, *5*, 182.
[215] S. Nangia, R. Sureshkumar, *Langmuir* **2012**, *28*, 17666.
[216] N. Hao, L. Li, Q. Zhang, X. Huang, X. Meng, Y. Zhang, D. Chen, F. Tang, L. Li, *Microporous Mesoporous Mater.* **2012**, *162*, 14.
[217] E. C. Cho, J. Xie, P. A. Wurm, Y. Xia, *Nano Lett.* **2009**, *9*, 1080.
[218] R. R. Arvizo, O. R. Miranda, M. A. Thompson, C. M. Pabelick, R. Bhattacharya, J. D. Robertson, V. M. Rotello, Y. S. Prakash, P. Mukherjee, *Nano Lett.* **2010**, *10*, 2543.
[219] K. W. Ng, S. P. K. Khoo, B. C. Heng, M. I. Setyawati, E. C. Tan, X. Zhao, S. Xiong, W. Fang, D. T. Leong, J. S. C. Loo, *Biomaterials* **2011**, *32*, 8218.
[220] M. I. Setyawati, C. Y. Tay, D. T. Leong, *Biomaterials* **2013**, *34*, 10133.
[221] K. A. Dawson, A. Salvati, I. Lynch, *Nat. Nanotechnol.* **2009**, *4*, 84.
[222] M. I. Setyawati, P. K. S. Khoo, B. H. Eng, S. Xiong, X. Zhao, G. K. Das, T. T. -Y. Tan, J. S. C. Loo, D. T. Leong, K. W. Ng, *J. Biomed. Mater. Res., Part A* **2013**, *101A*, 633.
[223] B. C. Heng, X. Zhao, S. Xiong, K. W. Ng, F. Y. Boey, J. S. Loo, *Arch. Toxicol.* **2011**, *85*, 695.
[224] C. Y. Tay, Y. Yu, M. I. Setyawati, J. Xie, D. T. Leong, *Nano Res.* **2014**, *7*, 805.
[225] Z. J. Deng, G. Mortimer, T. Schiller, A. Musumeci, D. Martin, R. F. Minchin, *Nanotechnology* **2009**, *20*, 455101.
[226] M. Lundqvist, J. Stigler, G. Elia, I. Lynch, T. Cedervall, K. A. Dawson, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14265.
[227] C. D. Walkey, J. B. Olsen, F. Song, R. Liu, H. Guo, D. W. Olsen, Y. Cohen, A. Emili, W. C. Chan, *ACS Nano* **2014**, *8*, 2439.
[228] J. H. Shannahan, J. M. Brown, R. Chen, P. C. Ke, X. Lai, S. Mitra, F. A. Witzmann, *Small* **2013**, *9*, 2171.
[229] A. Lesniak, F. Fenaroli, M. P. Monopoli, C. Aberg, K. A. Dawson, A. Salvati, *ACS Nano* **2012**, *6*, 5845.
[230] A. Lesniak, A. Campbell, M. P. Monopoli, I. Lynch, A. Salvati, K. A. Dawson, *Biomaterials* **2010**, *31*, 9511.
[231] D. E. Owens 3rd, N. A. Peppas, *Int. J. Pharm.* **2006**, *307*, 93.
[232] A. Zensi, D. Begley, C. Pontikis, C. Legros, L. Mihoreanu, S. Wagner, C. Buchel, H. von Briesen, J. Kreuter, *J. Controlled Release* **2009**, *137*, 78.
[233] J. Meng, L. Song, J. Meng, H. Kong, G. Zhu, C. Wang, L. Xu, S. Xie, H. Xu, *J. Biomed. Mater. Res., Part A* **2006**, *79A*, 298.
[234] J. Meng, M. Yang, L. Song, H. Kong, C. Y. Wang, R. Wang, C. Wang, S. S. Xie, H. Y. Xu, *Colloids Surf., B* **2009**, *71*, 148.
[235] D. M. Huang, T. H. Chung, Y. Hung, F. Lu, S. H. Wu, C. Y. Mou, M. Yao, Y. C. Chen, *Toxicol. Appl. Pharmacol.* **2008**, *231*, 208.
[236] M. I. Setyawati, X. Yuan, J. Xie, D. T. Leong, *Biomaterials* **2014**, *35*, 6707.
[237] Y. Zhao, J. L. Howe, Z. Yu, D. T. Leong, J. J. Chu, J. S. Loo, K. W. Ng, *Small* **2013**, *9*, 387.
[238] Y. Miao, J. Xu, Y. Shen, L. Chen, Y. Bian, Y. Hu, W. Zhou, F. Zheng, N. Man, Y. Shen, Y. Zhang, M. Wang, L. Wen, *ACS Nano* **2014**, *8*, 6131.
[239] G. Zuo, S. G. Kang, P. Xiu, Y. Zhao, R. Zhou, *Small* **2013**, *9*, 1546.
[240] Z. Wang, S. Liu, J. Ma, G. Qu, X. Wang, S. Yu, J. He, J. Liu, T. Xia, G. B. Jiang, *ACS Nano* **2013**, *7*, 4171.
[241] R. R. Zhu, W. R. Wang, X. Y. Sun, H. Liu, S. L. Wang, *Toxicol. In vitro* **2010**, *24*, 1639.
[242] W. Gao, K. Xu, L. Ji, B. Tang, *Toxicol. Lett.* **2011**, *205*, 86.
[243] Z. J. Deng, N. J. Butcher, G. M. Mortimer, Z. Jia, M. J. Monteiro, D. J. Martin, R. F. Minchin, *Drug. Metab. Dispos.* **2014**, *42*, 377.
[244] S. T. Stern, P. P. Adiseshaiah, R. M. Crist, *Part. Fibre Toxicol.* **2012**, *9*, 20.
[245] K. Peynshaert, B. B. Manshian, F. Joris, K. Braeckmans, S. C. De Smedt, J. Demeester, S. J. Soenen, *Chem. Rev.* **2014**, DOI: 10.1021/cr400372p.
[246] Y. N. Wu, L. X. Yang, X. Y. Shi, I. C. Li, J. M. Biazik, K. R. Ratinac, D. H. Chen, P. Thordarson, D. B. Shieh, F. Braet, *Biomaterials* **2011**, *32*, 4565.
[247] Y. Zhang, F. Zheng, T. Yang, W. Zhou, Y. Liu, N. Man, L. Zhang, N. Jin, Q. Dou, Y. Zhang, Z. Li, L. P. Wen, *Nat. Mater.* **2012**, *11*, 817.
[248] L. Calzolai, F. Franchini, D. Gilliland, F. Rossi, *Nano Lett.* **2010**, *10*, 3101.
[249] H. Li, Y. Li, J. Jiao, H. M. Hu, *Nat. Nanotechnol.* **2011**, *6*, 645.
[250] T. Yokoyama, J. Tam, S. Kuroda, A. W. Scott, J. Aaron, T. Larson, M. Shanker, A. M. Correa, S. Kondo, J. A. Roth, K. Sokolov, R. Ramesh, *PLoS One* **2011**, *6*, e25507.
[251] S. T. Stern, B. S. Zolnik, C. B. McLeland, J. Clogston, J. Zheng, S. E. McNeil, *Toxicol. Sci.* **2008**, *106*, 140.

[252] C. Y. Tay, W. Fang, M. I. Setyawati, C. P. Sum, J. Xie, K. W. Ng, X. Chen, C. H. L. Hong, D. T. Leong, *Part. Part. Syst. Charact.* **2013**, *30*, 784.
[253] M. Zheng, A. Jagota, E. D. Semke, B. A. Diner, R. S. McLean, S. R. Lustig, R. E. Richardson, N. G. Tassi, *Nat. Mater.* **2003**, *2*, 338.
[254] P. Thongkumkoon, K. Sangwijit, C. Chaiwong, S. Thongtem, P. Singjai, L. D. Yu, *Toxicol. Lett.* **2014**, *226*, 90.
[255] H. An, Q. Liu, Q. Ji, B. Jin, *Biochem. Biophys. Res. Commun.* **2010**, *393*, 571.
[256] W. Yang, C. Shen, Q. Ji, H. An, J. Wang, Q. Liu, Z. Zhang, *Nanotechnology* **2009**, *20*, 085102.
[257] H. Gehrke, J. Pelka, C. G. Hartinger, H. Blank, F. Bleimund, R. Schneider, D. Gerthsen, S. Brase, M. Crone, M. Turk, D. Marko, *Arch. Toxicol.* **2011**, *85*, 799.
[258] C. F. Chin, Q. Tian, M. I. Setyawati, W. Fang, E. S. Tan, D. T. Leong, W. H. Ang, *J. Med. Chem.* **2012**, *55*, 7571.
[259] G. Ruan, A. Agrawal, A. I. Marcus, S. Nie, *J. Am. Chem. Soc.* **2007**, *129*, 14759.
[260] S. J. Soenen, N. Nuytten, S. F. De Meyer, S. C. De Smedt, M. De Cuyper, *Small* **2010**, *6*, 832.
[261] Z. N. Gheshlaghi, G. H. Riazi, S. Ahmadian, M. Ghafari, R. Mahinpour, *Acta Biochim. Biophys. Sin.* **2008**, *40*, 777.
[262] A. Dadras, G. H. Riazi, A. Afrasiabi, A. Naghshineh, B. Ghalandari, F. Mokhtari, *JBIC, J. Biol. Inorg. Chem.* **2013**, *18*, 357.
[263] D. Choudhury, P. L. Xavier, K. Chaudhari, R. John, A. K. Dasgupta, T. Pradeep, G. Chakrabarti, *Nanoscale* **2013**, *5*, 4476.
[264] T. A. Ratnikova, P. N. Govindan, E. Salonen, P. C. Ke, *ACS Nano* **2011**, *5*, 6306.
[265] S. J. Soenen, B. Manshian, J. M. Montenegro, F. Amin, B. Meermann, T. Thiron, M. Cornelissen, F. Vanhaecke, S. Doak, W. J. Parak, S. De Smedt, K. Braeckmans, *ACS Nano* **2012**, *6*, 5767.
[266] J. A. Yang, H. T. Phan, S. Vaidya, C. J. Murphy, *Nano Lett.* **2013**, *13*, 2295.
[267] C. Y. Tay, P. Q. Cai, M. I. Setyawati, W. Fang, L. P. Tan, C. H. L. Hong, X. Chen, D. T. Leong, *Nano Lett.* **2014**, *14*, 83.
[268] J. X. Yang, W. L. Tang, X. X. Wang, *Cytotherapy* **2010**, *12*, 251.
[269] J. Wang, F. Gu, T. Ding, X. Liu, G. Xing, Y. Zhao, N. Zhang, Y. Ma, *Oncol. Lett.* **2010**, *1*, 771.
[270] D. E. Ingber, *Annu. Rev. Physiol.* **1997**, *59*, 575.
[271] G. Nuki, *Curr. Rheumatol. Rep.* **2008**, *10*, 218.
[272] S. B. Horwitz, *Ann. Oncol.* **1994**, *6*, S3.