Expert Opinion



# Utilization of solid nanomaterials for drug delivery

Miles F Beaux II, David N McIlroy† & Kurt E Gustin
†*University of Idaho, Department of Physics, Engineering and Physics Building, Moscow, ID 83844-0903, USA*

*Background*: Solid nanostructures are versatile platforms for constructing hybrid drug delivery systems that have tremendous potential for improving disease prevention and treatment. The rationale and application of solid nanostructures in the context of drug delivery are explored in this article. *Objective*: The purpose of this paper is to provide a concise review of the major attributes of solid nanostructures as they relate to drug delivery and to describe the outstanding issues that need to be addressed in order to develop these materials into clinically useful reagents. *Methods*: The scope of this opinion has been restricted to solid nanostructures, where solid nanostructures are defined as those that are not biodegradable. The opinion has been further limited to the three primary types of nanostructures: nanoparticles, nanowires and nanotubes. *Results/conclusion*: There is a need for cross-disciplinary training and standardized protocols for developing and evaluating the efficacy of solid nanomaterials.

**Keywords:** nanomaterials, nanoparticles, nanotoxicology, nanowires

*Expert Opin. Drug Deliv. (2008) 5(7):725-735*

## 1. Introduction

Why use nanomaterials for drug delivery? The first and most important reason is that their size is ideally suited for internalization into cells. On average, a cell is 7 μm across and contains a nucleus with a 2 – 3 μm diameter. Nanomaterials fall into a similar size range, as do proteins and other macromolecular structures found inside cells, and are thus poised to take advantage of existing cellular machinery to facilitate the delivery of drugs. In addition, the vast majority of atoms in a nanostructure reside at the surface [1], which maximizes their ability to be loaded with and to deliver cargo, such as therapeutic drugs, to cells and tissues. In some instances these characteristics provide nanomaterials with the capability to pass through physiological barriers and penetrate tumors to deliver a cargo of drugs directly to target cells [2-6]. High efficiency drug delivery using nanomaterials could potentially reduce the drug dose needed to achieve therapeutic benefit, which in turn would save money and reduce the side effects associated with particular drugs.

Nanomaterials are considered a unique class of materials, where at least one dimension of the structure must be less than 100 nm (1 nm = $1 \times 10^{-9}$ m). This is a rule of thumb in the materials science community, not an exact criteria. Given the endless types of nanomaterials that can be utilized for drug delivery, this opinion will focus on solid-phase nanomaterials, which excludes 'softer' nanocarrier colloidal systems consisting of polymers, macromolecules, lipid structures, as well as other chemical structures that do not fall under the definition of a solid. While polymers do form solid structures, the types most attractive for drug delivery are those that dissolve in the body and therefore should be distinguished from solid-state materials that must be filtered out of the body by the kidney. Furthermore, the subjects of lipid and polymer systems for drug

informa
healthcare

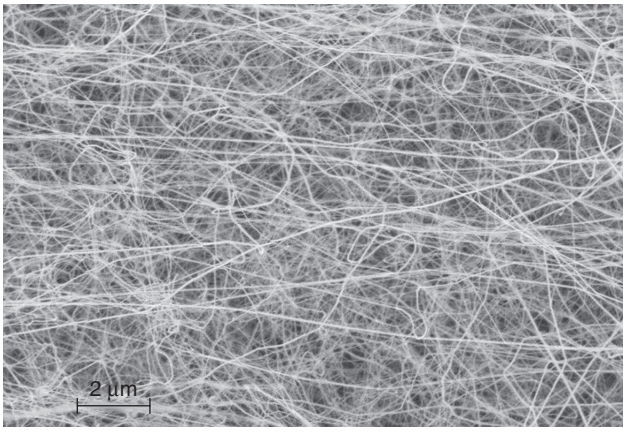


**Figure 1. A scanning electron microscope image of a mat of silica nanowires.**

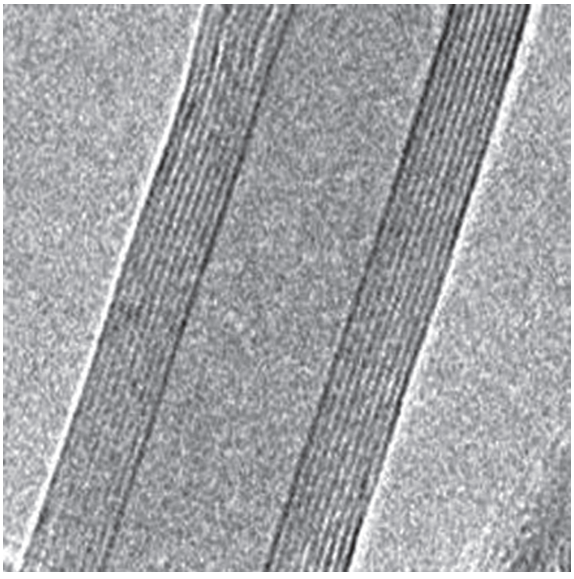

**Figure 2. A transmission electron microscope image of a multi-walled carbon nanotube.**
Courtesy of Nano-Lab [66].

delivery are vast enough to justify separate reviews. Solid nanomaterials, lipids and polymers all have advantages and disadvantages. Because solid nanomaterials do not dissolve, they can exist in the body as long as they do not reach the kidney, while dissolvable polymers cannot. Conversely, polymers will not build up in the liver if not filtered by the kidney, which will not always be the case with solid nanostructures. In the case of lipids, specificity, that is targeting, can be problematic. This is less of a problem with solid nanomaterials and polymers. In the end, there are pros and cons for all types of nanomaterials that must be weighed with the application and the desired outcome.

There are three distinct classes of solid nanomaterials. The first class is thin films, or two-dimensional nanostructures. Two-dimensional nanostructures have been utilized for over 40 years. The next class is one-dimensional nanostructures. In this case, two of the dimensions of the nanostructure must be less than 100 nm. One-dimensional nanostructures are generally referred to as nanowires (**Figure 1**), which have a cylindrical cross-section and can be hundreds of microns long. Nanowires come in a variety of forms such as carbon nanotubes (**Figure 2**), which are formed by rolling a sheet of graphene into a cylinder, and consequently are hollow. **Figure 2** shows a multi-walled nanotube where the lines are the individual graphene sheets forming the multiple walls of the hollow nanotube. The other types of nanowires are solid and are formed from virtually any type of material. Because carbon nanotubes are hollow and the other types of nanowires are solid, the nanoscience community considers carbon nanotubes to be a subclass of nanowires, or in some cases a category of its own. In order to eliminate confusion, carbon nanotubes will be referred to as nanotubes and solid nanowires as nanowires. The final class of nanomaterials that will be discussed here are nanoparticles, or zero-dimension nanostructures (**Figure 3**). The term 'nanoparticle' encompasses two classes of nanoparticles. The first class is quantum dots (QDs), which are nanoparticles made with semiconductor materials, and are typically more optically active in relation to other types of nanoparticles. The second class is quite broad and essentially consists of every type of material that is not a semiconductor. For simplicity, 'nanoparticles' will be used throughout this opinion to include all types of zero-dimensional nanostructures.

Solid nanomaterials, be they thin films, nanotubes, nanowires or nanoparticles, come in essentially every type of material known to mankind. Consequently, nanomaterials can be engineered to meet virtually any application; in the present case, drug delivery. However, all nanomaterials have one thing in common; they have extremely large surface to volume ratios, so that most of the atoms of a nanoparticle, or nanowire, reside at, or near, the surface. Different nanostructures will have properties better suited for specific drug delivery applications. Where extremely small structures are needed in order to penetrate a specific region of the cell, or move through a transport channel that will only accommodate a structure a few nanometers in diameter, the obvious choice is nanoparticles. If, however, a large amount of drugs need to be delivered but with minimal cellular disruption, a nanowire might be a better choice. For example, a single 10 by 500 nm nanowire has a surface area equivalent to that of 139 6 nm diameter nanoparticles. Once again, different applications will call for different types of nanomaterials.

Nanomaterials allow for a greater range and ease of pharmacological design. In principle, one designs a nanomaterial to exhibit specific properties that will enhance

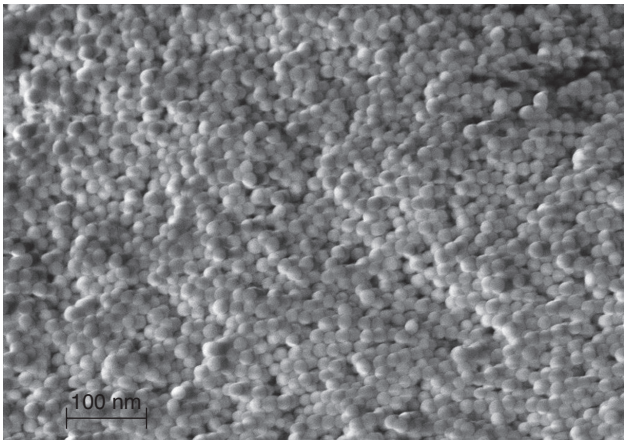


**Figure 3. A scanning electron microscope image of silica nanoparticles.**

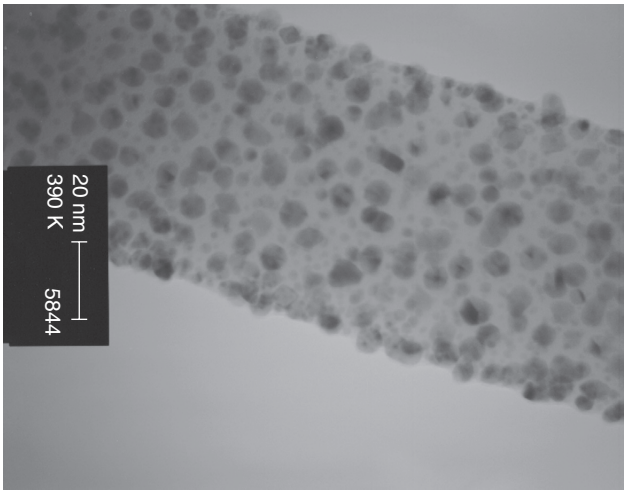


**Figure 4. A transmission electron microscope image of a silica nanowire decorated with gold nanoparticles.**

drug delivery or targeting. For example, consider a silica nanowire coated with gold nanoparticles (**Figure 4**), which is referred to as a hybrid nanostructure. The advantage of a hybrid nanostructure such as this is the following. Silane chemistry is used to attach chemicals or drugs to the silica nanowire, while thiol chemistry is used to attach drugs to the gold nanoparticles. For example, silane chemistry can be utilized to attach proteins for cell targeting, or endocytosis of the silica nanowire, and thiol chemistry can be used to attach the drug in question to the gold nanoparticles. This eliminates the need for complicated chemistry that integrates the therapeutic drug with a protein or antibody that targets a specific cell. Finally, the use of separate and distinctly different chemistries for the drug and the targeting material eliminates the potential for altering the properties of the drug in question. This is a rather simplistic picture, but it effectively captures the basic premise for using nanomaterials for targeted drug delivery.

This opinion will focus on the prevalent factors affecting the properties of solid nanomaterials, their toxicology and the advantages and disadvantages of the different types of solid nanomaterials that have been proposed or demonstrated as drug carriers for drug delivery. The article will conclude with an opinion that includes guidelines for nanomaterials characterization, evaluating their toxicity and the future of solid nanomaterials for drug delivery.

## 2. Nanomaterials and their applications

### 2.1 Significance of the surface and morphology of nanomaterials

In order to understand, predict and control the interactions of nanomaterials with biological systems, one must understand the factors responsible for their unique properties. The most well-known factor is the high surface area to volume ratio of nanomaterials. In order to illustrate this point, consider a solid rectangular block of material (**Figure 5**). The surface area of the block is the sum of the six faces. When this block is cut in half, the volume of the block does not change, but two new surfaces are formed on either side of the cut. Thus, cutting the single block into two blocks increases the total surface area while maintaining a constant volume. The more cuts made, the greater the surface area, as well as the surface area to volume ratio. An example more relevant to nanomaterials is to consider a sphere with volume of 1/3 $cm^3$. If this sphere is dissected into increasingly smaller spheres, the surface area increases dramatically. The increase in surface area is summarized in **Table 1**. Each decrease in radius by a factor of 10 increases the surface area by a factor of 10. Thus, 1 $cm^3$ of material in the form of 10 nm radius spheres yields 300 $m^2$ of surface area. If one assumes that the drug dose is directly proportional to the surface area, then it is clear that a small volume of nanomaterial can potentially deliver a significant dose. The astoundingly large surface area to volume ratios of nanomaterials is more than just mind candy for physical scientists; it is the key to opening the door to our understanding of the unique physical and chemical properties of nanomaterials. In the case of drug delivery, the surface tells the entire story. Consider, for example, the process of iron rusting, that is oxidation. Iron does not rust from the inside out but from the outside in. Consequently, if one modifies the surface of iron so that oxygen and moisture does not contact the surface, the iron will not rust. Now let us assume that the rate at which iron will rust is proportional to the surface area to volume ratio. If we return to **Table 1** we see that our assumption will predict that the smaller the size of iron, the more material exposed to the surface and therefore the faster it will oxidize (rust). Of course this is an oversimplified view of oxidation. An exposed surface is not the only requirement for oxidation. Oxidation takes place at

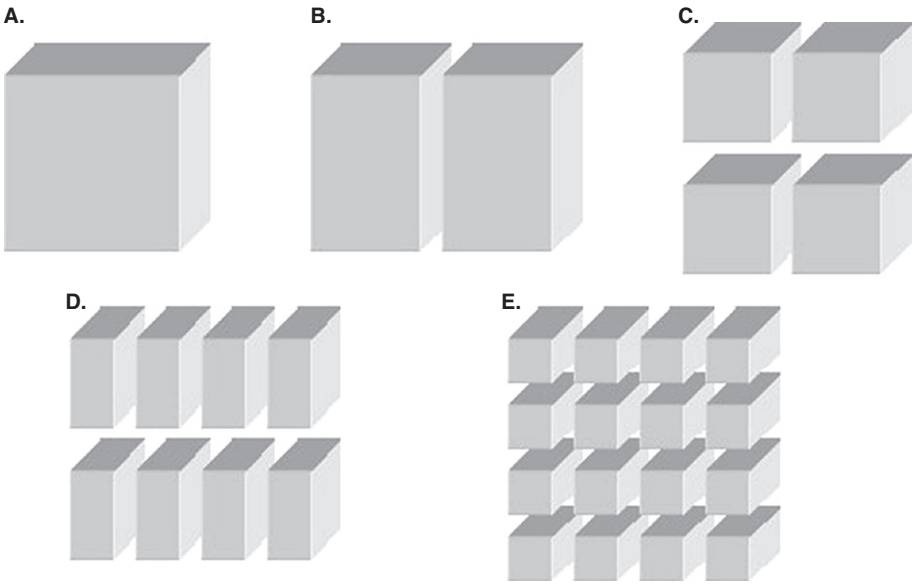


**Figure 5. An illustration of how the surface area increases as a cube is fragmented into smaller cubes, while maintaining a constant volume.**

**Table 1. The dependence of the surface area as a function of the spherical particles' radii used to form a volume equivalent to 1/3 cm$^3$.**

| Radius | Surface area [m$^2$] |
|---|---|
| 1 cm | 0.0001 |
| 1 mm | 0.001 |
| 100 µm | 0.01 |
| 10 µm | 0.1 |
| 1 µm | 1 |
| 100 nm | 10 |
| 10 nm | 100 |
| 1 nm | 1000 |

surfaces because atoms at the surface have a chemical environment that differs from atoms deep in the material. In fact, the surface chemistry of the iron will evolve as the particles become smaller and smaller. Subsequently, when evaluating the properties of a nanostructure, the size – surface area – of the nanostructure must be considered.

While the oxidation of iron is an excellent illustration of the effects of nanostructure size on chemical reactivity, a more dramatic example is that of gold. Gold is considered to be immune to oxidation. However, in the late 1990s Haruta *et al.* [7] showed that nanoparticles of gold are highly reactive for a variety of gases. Their investigation went on to show that the most reactive gold nanoparticles have a diameter of less than 5 nm. They proved that the change in the chemical properties of gold nanoparticles as a function of size is due to changes in the geometry of the nanoparticles, that is the types and numbers of facets, vertices, apexes, etc. Another example of how simple morphology (shape) of a nanostructure can affect its physical properties is the contrast in properties of silica nanowires (NWs) (**Figure 1**) relative to silica nanosprings (**Figure 6**) [8]. Mats of silica nanosprings can be extremely hydrophobic, and when a bead of water is placed on the mat it immediately rolls off. In contrast, silica nanowires are hydrophilic and will readily absorb water. Even though the nanosprings and nanowires are compositionally identical, the nanosprings possess a hydrophobic property while the nanowires do not. While any one morphology is not intrinsically better or worse than another, specific drug delivery applications may find it advantageous to utilize one morphology over another.

What one gleans from the proceeding example and the work by Haruta *et al.* [7] is that the surface properties of a nanostructure must be thoroughly investigated in order to understand their toxicity and how drugs will interact with their surface. Furthermore, as nanomaterial toxicology will depend on the geometry and size of the nanostructures–nanoparticle, nanowire, nanospring, etc. (see below) – one cannot simply use any size of nanomaterial because it has been shown for one specific size that it is non-toxic.

### 2.2 Particles

The most thoroughly studied family of nanomaterials is zero-dimensional nanoparticles. This is largely due to the ease and efficiency of their production [9,10]. Essentially, nanoparticles of virtually any material can be produced; however, the utility of many of these materials is limited due to biocompatibility issues. Nanoparticle production is a very straightforward process that allows their size (diameter) to

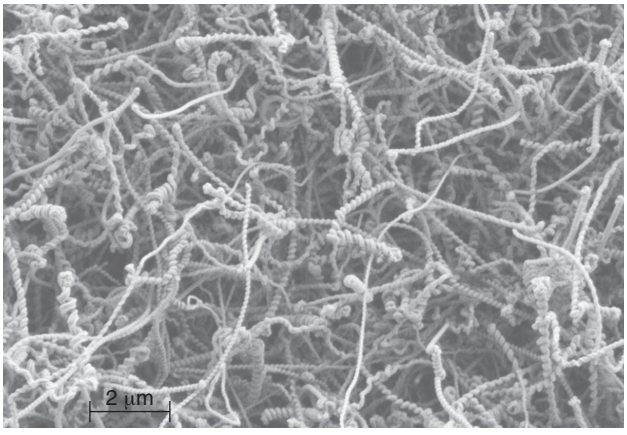


**Figure 6. A field emission scanning electron microscope image of a mat of nanosprings.**

be controlled as well as the size distribution, that is the spread in the diameter of particles within a batch is fairly narrow relative to one-dimensional structures. In addition, their spherical symmetry and the ability to tune their size makes them better suited for removal from the bloodstream by the kidney, thereby eliminating the build up of nanomaterials in the liver. On the flipside, however, nanoparticles have a tendency to agglomerate, thus reducing their effective concentration and the number of cells that can be treated with a given amount of nanoparticles [11-15]. To overcome this problem nanoparticles are often treated with surface surfactants that resist agglomeration [14,16], or de-agglomerated by sonication [15]. A surfactant layer may, or may not, add a level of complexity to the chemistry needed to functionalize with the desired complexes, and therefore should be avoided if at all possible.

Silica (glass) nanoparticles are an extremely popular type of nanomaterial due to their biocompatibility [17-20]. A recent study by Adili *et al.* [17] showed that HEp-2 and HeLa cells remain viable when exposed to a silica nanoparticle concentration of 190 µg/ml. Chen and von Mikecz [21] also observed that cell viability was not compromised by exposure to silica nanoparticles, although a significant reduction in the synthesis of RNA and the replication of DNA was noted. So while silica nanoparticles are non-toxic at fairly high concentrations, they can interfere with cell functions. The biocompatibility of silica nanoparticles makes them an excellent candidate for drug delivery. Furthermore, the maturity of silane chemistry will facilitate the crosslinking of drugs to silica nanoparticles [22,23]. Silica nanoparticles have been used to deliver anticancer drugs to tumor cells, erythropoietin to the small intestines of rats and other drugs and membrane impermeable proteins to various cell types [4-6,18-20]. They have also shown great promise for slow drug release applications [24].

Noble metal nanoparticles of silver and gold have been shown to be non-toxic, as have their respective conjugates with silicon [25-27]. Gold nanoparticles have been proposed as drug carriers due to their physical and chemical properties that allow them to be readily functionalized using thiol chemistry for target delivery [28]. Gold nanoparticles have been shown to penetrate into the nucleus of HepG2 cells [3], to enhance gene expression in yeast cells [27] and to deliver oligonucleotides into mouse endothelial cells for the purpose of regulating gene expression [26].

Magnetic nanoparticles have been proposed as drug carriers with a push towards clinical trials [29,30]. One advantage of using magnetic nanoparticles is the ability to heat the particles after internalization, referred to as the hyperthermia effect. In thermoresponsive drug delivery the magnetic nanoparticles are heated to release a drug upon internalization into target cells [31-33]. Another proposed application of magnetic nanoparticles for drug delivery involves 'ferrosponges,' or magnetic nanoparticles suspended in gelatin. The manipulation of the magnetic nanoparticles with an external magnetic field enables a degree of control over the shape of the gelatin, such as expansion and contraction. With the addition of a drug to the gelatin, it has been proposed that a manipulation of the ferrosponge can induce the release of the drug [34].

While not directly related to drug delivery, metallic and magnetic nanoparticles have biomedical applications worth mentioning. For example, it is well known that materials coated with silver nanoparticles form antibacterial surfaces [35] and bandages coated with silver nanoparticles have been very effective at staving off infections on burn victims [36,37]. One can imagine coating surgical tools and sutures with silver nanoparticles in order to more effectively reduce the chance of post-surgical infection.

### 2.3 Carbon-based nanomaterials

Carbon-based nanomaterials that have been proposed for drug delivery applications include carbon nanoparticles such as fullerenes or buckyballs [38] and carbon nanotubes (CNTs) [39-43]. The vast majority of studies of carbon-based nanomaterials for drug delivery deal with carbon nanotubes. Single-walled carbon nanotubes (SWCNTs) have been used for tumor therapy and drug delivery. For example, delivery of antisense myc to HL-60 cells has been shown to be effective in regulating intracellular gene expression [39]. Multi-walled carbon nanotubes (MWCNTs) have been internalized by microglia, where the application is as a non-toxic, biodegradable nano-vehicle for targeted therapy in brain cancer [40]. In addition to the internalization of CNTs, a technique called nanotube spearing has been developed, where nickel-embedded CNTs are driven into cell membranes by a magnetic field as a type of nano-syringe [41]. With this technique researchers were able to deliver DNA plasmids into target cells with high transfection efficiency. Comparative studies between CNTs and alternative nanomaterials and their effectiveness for adsorbing drugs have been performed [43,44]. Of the nanomaterials studied,

which included silica nanoparticles, fullerene, CNTs and other adsorbates, CNTs showed the greatest level of drug adsorption [43].

The primary drawback to carbon-based nanomaterials appears to be their toxicity. MWCNTs and carbon nanofibers have been found to be significantly toxic to human lung tumor cells as early as 24 h after exposure [45]. SWCNTs have also been found to be toxic in some systems [46,47], although a recent report examining SWCNTs in mice revealed no apparent toxicity over a four-month period [48]. Due to these and other conflicting reports on the toxicity of CNTs [48,49], studies involving the application of CNTs for drug delivery are still being conducted [39,40]. In order to further the use of CNTs for drug delivery, researchers have functionalized the surface of CNTs, rendering them benign [49]. Unfortunately, concerns that functionalized CNTs may revert back to a toxic state if the functional group detaches has limited the pursuit of the biomedical applications of these modified CNTs.

The toxicity of other forms of nanocarbon has also been reported [38,45,50]. In one study juvenile largemouth bass were exposed to C60 fullerenes (buckyballs) [50]. Within 48 h of exposure to a 0.5 ppm aqueous suspended colloid, bass were found with significant lipid peroxidation in the brains, as well as marginal depletion of glutathione in the gills. Another study of human lung tumor cells showed that carbon nanoparticles are even more toxic than MWCNTs and carbon nanofibers [45]. In yet another study, it was observed that carbon nanoparticles accumulate adjacent to the nuclear membrane and within the nucleus when internalized into human monocyte macrophages and possibly lead to DNA damage [38]. Given the mounting evidence demonstrating the toxicity of carbon nanoparticles, efforts to develop carbon nanoparticles for drug delivery have dropped off significantly in recent years.

### 2.4 Non-carbon based nanowires

An alternative to nanoparticles and carbon-based nanomaterials are the one-dimensional nanomaterials referred to as nanowires (NWs). In those cases where the aspect ratio of length to diameter is not large (e.g., approximately 10:1), these nanomaterials have sometimes been termed nanorods. Nanowires (and rods) can be formed from virtually any material, but are most commonly formed from oxides such as zinc oxide, and semiconductors such as silicon. Nanowires differ from nanoparticles in several respects, even when prepared from the same materials. A striking example of this was provided by a recent study that examined the toxicity of nanowires and nanoparticles made from silica on human tissue culture cells. These authors found that at concentrations below 190 μg/ml neither nanowires nor nanoparticles induced a toxic effect when added to cells [17]. Surprisingly, nanowires but not nanoparticles exhibited significant toxicity at concentrations greater than 190 μg/ml [17]. These results indicate that despite their chemical composition being identical, silica nanowires and nanoparticles interact with cells in very different ways with very different outcomes that are most likely due to changes in surface chemistries resulting from the different forms taken by these materials. In addition, these findings illustrate that changing the shape of the nanomaterial can have unexpected consequences on biological systems and point to a need for carefully conducted toxicological studies for new nanomaterial formulations as they are developed. Nanowires do offer potential advantages for drug delivery when compared to nanoparticles. For example, nanowires are much less prone to agglomeration when suspended in a buffer than are nanoparticles. Consequently, unlike nanoparticles, nanowires do not need to be coated with a surfactant, which can simplify the loading of proteins and drugs onto the nanomaterial. Another potential advantage of nanowires is provided by their larger surface area compared to nanoparticles of similar diameter. The greater available surface area translates into a larger payload capacity per unit nanomaterial. This in turn may allow for the delivery of higher effective drug doses to desired locations in the patient, while at the same time using less nanomaterial and reducing the risk of off-target effects caused by the nanomaterial and/or the payload. Another benefit presented by the large surface area of nanowires is the ability to attach nanoparticles to their surface [51]. Such tethering would allow the engineering of NWs with the functionalization capabilities and benefits of a variety of different nanoparticles.

Despite these potential benefits, published reports developing NWs for drug delivery applications are scarce. The scarcity of such investigations is likely due to the difficulties associated with preparing sufficient quantities of nanowires for biological applications. Of the studies that have been performed, one examined the use of titania NWs to enhance drug delivery in a rat model for spinal injury [52]. This study found that topical administration of drugs complexed with $TiO_2$ nanowires enhanced spinal chord function and increased the duration of beneficial effects, as compared to administrations of drugs without nanowires [52]. Studies using silica NWs have shown that coating these wires with fibronectin enhances their uptake into cells in tissue culture [53]. Building on this observation, Kwon *et al.* demonstrated that fibronectin-coated silica nanowires could be used to deliver the cytotoxic shiga toxin A subunit to the inside of bovine and human epithelial cells, resulting in the death of these cells [2]. Although these exciting results clearly indicate that it may be possible to use silica nanowires for direct delivery of drugs to the inside of cells, several hurdles still need to be overcome before this material can be developed for clinical use. For example, the use of fibronectin for internalization, while effective, does not provide the level of specificity needed to target individual cell types. Perhaps this problem may be overcome by conjugating cell type specific antibodies or RNA aptamers to the nanowires that

would target the nanomaterial along with its drug payload to the desired cell type. In addition, in order to more precisely control targeting and delivery of specific drugs to the desired tissues in the patient, methods that allow for precise control over the attachment of cargos to the nanowires need to be developed.

## 3. Conclusion

In summary, solid nanomaterials have incredible potential for improving drug delivery and therapy. Optimizing the integration of nanomaterials into drug delivery or therapy will require standardized metrics for their classification, as well as protocols for their handling. This in turn will result in a better understanding of the interactions of nanomaterials with biological systems, which in turn will facilitate better engineering of their properties specific to biomedical applications. Promises of miracle pill cure-alls have never been brought to fruition. Likewise, the promise of a nanomaterial drug carrier that is perfect for all drug delivery applications is unrealistic. Rather, specific nanomaterials should be engineered for specific drug delivery applications. The development of such drug carriers with properties suitable for specific applications will require a greater understanding of both the surface chemistry of nanomaterials and the interaction chemistry of these nanomaterials with biological systems. This can only be achieved through collaborative efforts between the physical and biological sciences.

## 4. Expert opinion

Nanomaterials clearly have incredible potential for drug delivery, as well as many other types of therapy that are beyond the scope of this opinion. Unfortunately, the scientific community is dysfunctional, for lack of a better choice of a word. This is not to say that researchers are not conducting excellent research, but that the community as a whole is not well organized. As an example, consider the above discussion on the toxicity of silica. Research groups studying silica nanoparticles have arrived at conflicting conclusions. Is this to say that one is correct and the other incorrect? No. They are likely all correct. The problem lies in the lack of understanding of the properties of the nanomaterials being utilized by the researchers. In other words, many researchers are failing to appreciate that the properties of the nanomaterials are dictated by their surface. Without careful characterization of the surface properties of nanomaterials prior to their use in biological studies, the conclusions of any study will be suspect. Is this a fault of the biological scientists? No. They are often led to believe that the properties of their nanomaterials are always the same from batch to batch. Unfortunately, this is not always the case. So does the blame fall on the shoulders of the material scientists supplying the nanomaterials? No. Once again, it is the lack of appreciation of the sensitivity of biological systems to the surface properties of nanomaterials. In the end it is the mutual lack of understanding on the part of the biomedical researchers and the material scientists of the other's needs and capabilities. The solution is the cross-training of both parties. The biological/medical researchers need to learn some surface science and the material scientists some biology. Only through in-depth conversations between the two sides of the fence can the complex interactions of biological systems with nanomaterials be understood to the point that nanomaterials will gain FDA approval. For the fields of bionanoscience and bionanotechnology to reach their potential, the next generation of students will need to be cross-trained to speak both languages. Furthermore, future researchers, especially those that are not working in a specific medical field such as oncology, must be educated on the expectations of nanomaterials-based drug delivery or therapy. In the case of oncology, while the injection of nanomaterials into solid tumors may provide some practical benefit, a nanomaterial-based delivery platform that will home to and destroy individual cancer cells would be far superior. The same argument holds true for brain tumors. Nanomaterials will need to cross the blood–brain barrier in order to be effective. Unfortunately, too many medical applications are proposed by non-medical researchers that utilize nanomaterials without a proper understanding of the expectations of the medical community. Once again, better cross training would produce better proposals with a greater likelihood of success.

The prospect of using nanomaterials for biomedical applications, as well as the introduction of hundreds of new products containing nanomaterials, such as sunscreens containing zinc oxide nanoparticles and toothpastes containing silica nanoparticles (**Figure 3**), has given rise to concerns about the safety of these materials [54-64]. Such concerns have led to numerous investigations of the potential health risks of nanomaterials [56-59,65], or what has been termed nanotoxicology [56]. Many of these preliminary investigations have resulted in conflicting conclusions about the toxicology of materials [56,62]. Nevertheless, these studies have led to speculation that nanomaterials may contribute to the formation of free radicals [65], damage brain cells [57] and possibly undesirably penetrate through the epidermis or other physiological barriers into areas of the body more susceptible to toxic effects [56]. The conflicting conclusions as to the toxicity of specific nanomaterials makes one question the assertion by the cosmetic industry that nanomaterials used in cosmetics do not penetrate the epidermal layer [58,59]. This debate has fuelled the question: do the dangers of nanomaterials outweigh their advantages [58-60]? Although the evidence against epidermal penetration is encouraging, the effects of internalization of nanomaterials is not well documented or understood and thus their impact on human health remains an open question [56]. One of the most pressing issues of nanomaterials

toxicity is their long-term effects. Most of the reported studies are *in vitro* and therefore short-term at best. One would hope that the benefits of using nanomaterials for drug delivery outweigh the possible health risks, however significantly more data are needed in order to determine their potential health risks unequivocally.

Recently, both the National Science and Technology Council in the USA along with the Royal Society and the Royal Academy of Engineering in the UK have recommended a significant investment in research devoted to understanding nanomaterial toxicology. Given the interest and potential impact that nanomaterials may have on the medical field, a national database of toxicology of nanomaterials is in order. This database will be useful within the medical research community that is trying to utilize nanomaterials for drug delivery or therapy by helping to increase consistency and reduce redundancy of experimental effort. The database should include toxicity as a function of material, that is silica, gold, etc.; nanomaterial shape, that is particle, nanowire etc.; nanomaterial size; cell type or animal, the duration of exposure and the methods used to assay toxicity; and lastly, storage and handling protocol. Without such a database the translation of biomedical nanotechnology from the laboratory to the general public will be significantly hampered.

The next hurdle for realizing the medicinal use of nanomaterials is materials handling protocols. Because of the extremely active nature of the surface of solid nanomaterials, their storage needs have to be thoroughly evaluated. Preliminary studies at the University of Idaho suggest that the surface chemistry of nanomaterials may be much more sensitive to change than ever anticipated, and therefore very subtle changes in the handling of nanomaterials may result in extreme changes in their properties and interactions with biological materials, that is toxicity. If this hypothesis is correct, which the authors believe to be true, it is not surprising that nanotoxicology investigations have resulted in conflicting results, due to differences in the handling and storage procedures used by different groups. The assumption that a nanomaterial's interactions with biological systems only depends on its composition must be rejected. Verification of this hypothesis would call for greater attention to be placed on the handling and storage procedures of nanomaterials. If changes are being made to the surface chemistry of nanomaterials due to seemingly insignificant variations in handling, more rigorous surface analysis of nanomaterials would be needed to identify these changes and their causes. To the best of the author's knowledge, no one has conducted a thorough study of the effects of nanomaterials storage on their toxicity. The study should include different geometries, such as silica nanoparticles and nanowires which, as described above, have already been shown to exhibit differential toxicity on human cells [29]. In addition, studies would need to be performed on a variety of cell types and ultimately in animal models. While addressing nanomaterials safety, these types of studies would also provide valuable information on the nature of nanomaterial interactions at the cellular and organismal level.

Another important issue facing biomedical nanotechnology, in particular nanomaterials drug delivery, are the issues arising from the inherent challenges of producing nanomaterials. Most biomedical researchers are more experienced with drugs and chemicals of extremely high purity and reproducibility from batch to batch. Unfortunately, nanomaterials cannot be as precisely controlled; the nanoscale falls in the valley of death between microscopic and molecular. Consequently, nanomaterials synthesis is much less precise and the best one can expect is a narrow distribution in size, aspect ratio, etc. One obvious solution to this dilemma is to improve the synthesis process. While efforts to improve synthesis should certainly continue, it is not clear if this will lead to substantial or economically feasible solutions to these issues. The authors therefore propose that the biomedical community rethink the level of control needed when working with nanomaterials. Rather than requiring perfect control of the physical dimensions of nanomaterials, a statistical approach should be adopted: a size distribution, or aspect ratio distribution, with a well-defined average size and standard deviation. The adoption of such a policy would help to establish a metric for classifying nanomaterials by material, average size, aspect ratio, etc. and standard deviation. This would fit well with the formation of a toxicology database, since it is unrealistic to establish the toxicology of every size or aspect ratio of nanomaterial.

In order for these bionanotechnologies to progress toward human applications, a better understanding of the fate of nanomaterials in the body is needed; such as whether nanomaterials clear the body, biodegrade within the body, or are permanently retained within specific tissues of the body. If nanomaterials are retained within specific tissues of the body, the long-term effects of the presence of these nanomaterials need to be investigated in appropriate animal models *in vivo*. Such investigations are well within current capabilities of being performed and are most likely on the not too distant horizon. While the biological and medical communities appreciate that toxicity studies done in tissue culture may not accurately reflect the toxicity seen *in vivo*, many nanomaterials researchers are unaware of these issues or their importance in nanomaterials development. Consequently, applications are proposed that fail to appreciate the problem as a whole.

The last issue to consider is the pros and cons of one type of nanomaterial over another. While nanoparticles are easy to work with, they have limitations such as those described above that are associated with the need for a surfactant to reduce agglomeration. Nanowires, however, do not require a surfactant, which simplifies the process of tailoring their properties for a particular application, but these materials may exhibit greater toxicity at high concentrations. By necessity, compromises will have to be made when deciding on the best nanomaterial for a given

application. Consider carbon nanotubes. They can be rendered non-toxic via modification of their surface, but is this a safe and sensible approach? How long will it be before the nanotubes revert back to their toxic state? As researchers, we need to be pragmatic when pursuing nanomaterials for biomedical applications. Just because we can render carbon nanotubes non-toxic, does not mean this is suitable for biomedical applications. One must also consider the effects of the ageing nanomaterials that remain in the body. The earlier analogy of rust formation, while simplistic, illustrates how materials, especially high surface area nanomaterials, can evolve over time. The importance of nanomaterials ageing is illustrated by the aforementioned studies of the effects of nanomaterials storage on their toxicity. This is an example of the many areas of study that are needed before solid nanostructures will find their way into prescribed drugs.

## Acknowledgements

The authors would like to acknowledge the support of the University of Idaho's Biological Applications of Nanotechnology (BANTech) Presidential Initiative and the National Center for Research Resources INBRE (P20 RR016454), the NSF (EPS0132626) and the WM Keck Foundation to DNM, the American Cancer Society (RSG 109705) and the NIH (AI 059467 and AI 064432) and the National Center for Research Resources COBRE (P20 RR016454) to KEG.



## Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Arzt E, Gumbsch P. 5.5 Small-Scale materials and structures. M Rühle, H Dosch, EJ Mittemeijer, MH Van de Voorde, editors, European white book on fundamental research in materials science. Max-Planck-Institut für Metallforschung; 2001
2. Kwon N, Beaux M, McIlroy D, et al. Nanowire-based delivery of Escherichia coli O157 shiga toxin 1 A Subunit into human and bovine cells. Nanoletters 2007;7:2718-23
•• **Demonstrates a practical application of nanomaterials for drug delivery.**
3. Tkachenko AG, Xie H, Coleman D, et al. Multifunction gold nanoparticle–peptide complexes for nuclear targeting. J Am Chem Soc 2003;125:4700-1
4. Roy I, Ohulchanskyy TY, Pudavar HE, et al. Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: a novel drug-carrier system for photodynamic therapy. J Am Chem Soc 2003;125:7860-5
5. Narbe C, Bartlett J, Kong L, et al. Silica particles, a novel drug-delivery system. Adv Mater 2004;16:1959-66
6. Li Z, Wen L, Shao L, J Chen. Fabrication of porous hollow silica nanoparticles and their applications in drug release control. J Control Release 2004;98:245-54
7. Haruta M. Size- and support-dependency in the catalysis of gold. Catal Today 1997;36:153-66
•• **Provides evidence that scaling down alters the chemical properties of materials.**
8. McIlroy DN, Alkhateeb A, Aston D, et al. Nanospring formation – unexpected catalyst mediated growth. J Phys Condens Matter 2004;16:R415-40, 2004
9. Gao L, Zhuang J, Nie L, et al. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. Nat Nanotechnol 2007;2:577-83
10. Chang J, Chang B, Hwang D, et al. In vitro cytotoxicity of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. Environ Sci Technol 2007;41:2064-8
11. Weng KC, Noble CO, Papahadjopoulos-Sternberg B, et al. Optimizing quantum-dot conjugated immunoliposomes for cancer diagnostics and targeted therapeutics. Nanotechnology 2007;2:776-9
12. Limbch LK, Li Y, Grass RN, et al. Oxide nanoparticle uptake in human lung fibroblasts: effects of particle size, agglomeration, and diffusion at low concentrations. Environ Sci Technol 2005;39:9370-6
13. Murdock RC, Braydich-Stolle L, Schrand AM, et al. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci 2008;101:239-53
14. Farah AA, Alverez-Puebla RA, Fenniri H. Chemically stable silver nanoparticle-crosslinked polymer microspheres. J Colloid Interface Sci 2007;319:572-6
15. Sauter C, Emin MA, Schuchmann HP, Tavman S. Influence of hydrostatic pressure and sound amplitude on the ultrasound induced dispersion and de-agglomeration of nanoparticles. Ultrason Sonochem 2008;15:517-23
16. Cheng X, Kuhn L. Chemotherapy drug delivery from calcium phosphate nanoparticles. Int J Nanomed 2007;2:667-74
17. Adili A, Crowe S, Beaux M, et al. Differential cytotoxicity exhibited by silica nanowires and nanoparticles. Nanotoxicology 2008;2:1-8
•• **Provides evidence that differences in morphology affect the toxicity of nanomaterials.**
18. Bottini M, D'Annibale F, Magrini A, et al. Quantum dot-doped silica nanoparticles as probes for targeting of T-lymphocytes. Int J Nanomed 2007;2:227-33
19. Ohulchanskyy TY, Roy I, Goswami LN, et al. Organically modified silica nanoparticles with covalently incorporated photosensitizer for photodynamic therapy of cancer. Nano Lett 2007;7:2835-42
20. Slowing II, Trewyn BG, Lin VS. Mesoporous silica nanoparticles for intracellular delivery of membrane-impermeable proteins. J Am Chem Soc 2007;129:8845-49
21. Chen M, von Mikecz A. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO2 nanoparticles. Exp Cell Res 2005;305:51-62
22. Bhatia SK, Shriver-Lake LC, Prior KC, et al. Use of thiol-terminal silanes and heterobifunctional crosslinkers for immobilization of antibodies on silica surfaces. Anal Biochem 1989;178:408-13
23. Lindoy, Leonard F. AU. Eaglen, Peter AU. Ion complexation by silica-immobilized polyethyleneimines. US5190660; 1993
24. Venkatesan N, Yoshimitsu J, Ito Y, et al. Liquid filled nanoparticles as a drug delivery

tool for protein therapeutics. Biomaterials 2005;26:7154-63

25. Hainfeld JF, Slatkin DN, Smilowitz HM. The use of gold nanoparticles to enhance radiotherapy in mice. Phys Med Biol 2004;49:N309-315
26. Rosi NL, Giljohann DA, Thaxton CS, et al. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. Science 2006;312:1027-30
27. Chien SF, Chen SH, Lin CT. Galactosidase gene expression in yeast cells enhanced by nanoparticles. Nanotechnology 2007;2:382-5
28. Han G, Ghosh P. Rotello VM. Multi-functional gold nanoparticles for drug delivery. Adv Exp Med Biol 2007;620:48-56
29. Dobson J. Magnetic mico- and nano-particle-based targeting for drug and gene delivery. Nanomedicine 2006;1:31-7
30. Leuschner C, Kumar C, Hansel W, et al. Hormone peptide conjugated ironoxide nanoparticles for detection and treatment of metastases. Nanotechnology 2007;2:429-30
31. Ankareddi I, Hampel ML, Sewell MK, Kim D-H. Development of a magnetically triggered drug delivery system using thermoresponsive grafted polymer networks with magnetic nanoparticles. Nanotechnology 2007;2:431-4
32. Lin CY, Ho K-C. Synthesis of superparamagnetic magnetite nanoparticles for thermoresponsive drug delivery. Nanotechnology 2007;2:405-8
33. Lin CY, Ho K-C. Hyperthermia effect of surface-modified magnetite nanoparticles in a microfluid system. Nanotechnology 2007;2:425-8
34. Hu SH, Liu TY, Liu D-M, Chen S-Y. Nano-ferrosponges for controlled drug release. J Control Release 2007;121:181-9
35. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 2004;275:177-82
36. DV Parikh, T Fink, K Rajasekharan, et al. Antimicrobial silver/sodium carboxymethyl cotton dressings for burn wounds. Textile Res J 2005;75:134-8
37. Pakinar, Kishore Madhukar (Pune, IN) Anti-microbial activity of biologically stabilized silver nano particles. US20070218555; 2007
38. Porter AE, Muller K, Skepper J, et al. Uptake of C60 by human monocyte macrophages, its localization and implications for toxicity: studies by high resolution electron microscopy and electron tomography. Acta Biomater 2006;2:409-19
39. Cui D, Tian F, Coyer SR, et al. Effects of antisense-myc-conjugated single-walled carbon nanotubes on HL-60 cells. J Nanosci Nanotechnol 2007;7:1639-46
40. Kateb B, Van Handel M, Zhang L, et al. Internalization of MWCNTs by microglia: possible application in immunotherapy of brain tumors. Neuroimage 2007;37(Suppl 1):S9-17
41. Cai D, Mataraza JM, Qin ZH, et al. Highly efficient molecular delivery into mammalian cells using carbon nanotube spearing. Nat Methods 2005;2:449-54
42. Zhang M, Yudasaka M, Iijima S. Dissociation of electrolytes in a nano-aqueous system within single-wall carbon nanotubes. J Phys Chem B 2005;109:6037-9
43. Venkatesan N, Yoshimitsu J, Ito Y, et al. Liquid filled nanoparticles as a drug delivery tool for protein therapeutics. Biomaterials 2005;26:7154-63
44. Striolo A. The mechanism of water diffusion in narrow carbon nanotubes. Nano Lett 2006;6:633-9
45. Magres A, Kasas S, Salicio V, et al. Cellular Toxicity of Carbon-Based Nanomaterials. Nano Lett 2006;6:1121-5
46. Lam C-W, James JT, McCluskey R, et al. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. Crit Rev Toxicol 2006;36:189-217
47. Manna SK, Sarkar S, Barr J, et al. Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-κB in human keratinocytes. Nano Lett 2005;5:1676-84
48. Schipper M, Nakayama-Ratchford N, Gambhir S, et al. A pilot toxicology study of single-walledcarbon nanotubes in a small sampleof mice. Nat Nanotech 2008;3:216
49. Dumortier H, Lacotte S, Astorin G, et al. Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells. Nano Lett 2006;6:1522-8
50. Oberdörster E. Manufactured nanomaterials (fullerenes, C60) induced oxidative stress in the brain of juvenile largemouth bass. Environ Health Perspect 2004;112:1058-62
51. Dobrokhotov V, McIlroy D, Berven C, et al. Principles and mechanisms of gas sensing by GaN nanowires functionalized with gold nanoparticles. J Appl Phys 2006;99:10
52. Sharma HS, Ali SF, Dong W, et al. Drug delivery to the spinal cord tagged with nanowire enhances neuroprotective efficacy and functional recovery following trauma to the rat spinal cord. Ann NY Acad Sci 2007;1122:197-218
53. Beaux M, Kwon N, McIlroy D, et al. Fibronectin bonding to nanowires and their internalization by epithelial cells. J Biomed Nanotechnol 2006;2:1-6
54. Nano sunblock safety under scrutiny. Anna Salleh, 2004. Available from: http://www.abc.net.au/science/news/stories/s1165709.htm [Last accessed 14 April 2008]
55. Study shows nanoparticles in sun cream may affect mice brain cells. Simon Pitman, 2004. Available from: http://www.cosmeticsdesign.com/news/ng.asp?n=68585-study-shows-nanoparticles [Last accessed 14 April 2008]
56. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113:823-39
57. Thrall L. Study links TiO2 nanoparticles with potential for brain-cell damage. Environ Sci Technol 2006;40:4326-7
58. A review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens. Australian Government Dept. of TGA, 2006. Available from: http://www.tga.gov.au/npmeds/sunscreen-zotd.pdf [Last accessed 14 April 2008]
59. Cross SE, Innes B, Roberts MS, et al. Human skin penetration of sunscreen nanoparticles: in-vitro assessment of a novel micronized zinc oxide formulation. Skin Pharmacol Physiol 2007;20:148-54
60. Do nanoparticles and sunscreen mix? David Biello, 2007. Available from: http://www.sciam.com/article.cfm?articleID=84355695-E7F2-

99DF-32AE048E858F385C
[Last accessed 14 April 2008]

61. Nanotechnology – in my toothpaste …? Michael Berger, 2006. Available from: http://www.nanowerk.com/spotlight/spotid=1091.php [Last accessed 14 April 2008]
62. Nanoparticle penetration of human skin – a double-edged sword. Michael Berger, 2007. Available from: http://www.nanowerk.com/spotlight/spotid=1820.php [Last accessed 14 April 2008]
63. Nanomaterials, sunscreens and cosmetics: small ingredients big risks. Friends of the Earth, 2006. Available from: http://nano.foe.org.au/filestore2/download/125/FoEA%20nano%20cosmetics%20report%20web.pdf [Last accessed 10 October 2007]
64. Nanoparticles and the skin – a health risk for the consumer? Gerhard J. Nohynek, 2006. Available from: http://www.bfr.bund.de/cm/232/nanoparticles_and_the_scin.pdf [Last accessed 10 October 2007]
65. Shvedova A, Castranova V, Kisin E, et al. Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. J Toxicol Environ Health A 2003;66:1909-26
66. Available from: http://www.nano-lab.com/nanotube-image3.html

## Affiliation

Miles F Beaux II[1], David N McIlroy[†2] & Kurt E Gustin[3]
[†]Author for correspondence
[1]Research Assistant, University of Idaho, Department of Physics, Engineering and Physics Building, Moscow, ID 83844-0903, USA
[2]Professor, University of Idaho, Department of Physics, Engineering and Physics Building, Moscow, ID 83844-0903, USA
Tel: +1 208 885 6809; Fax: +1 208 885 4055; E-mail: dmcilroy@uidaho.edu
[3]Professor, University of Idaho, Department of Microbiology, Molecular Biology and Biochemistry, Moscow, ID 83844, USA