NANOTOXICOLOGY

Nanoparticles reconstruct lipids

Charged nanoparticles can alter the local physical properties of lipid membranes, which could shed new light on the interactions between living cells and nanomaterials.

Kenneth A. Dawson, Anna Salvati and Iseult Lynch

An emerging 'rule of thumb' suggests that nanoparticles less than 100 nm in diameter can enter cells, those with diameters below 40 nm can enter the cell nucleus and those that are smaller than 35 nm can pass through the blood–brain barrier and enter the brain. Understanding the way nanoparticles interact with living matter will open up fundamentally new opportunities in medicine and diagnostics. This knowledge equally imposes on us the necessity for consideration, without excessive and unscientific alarm, of key safety issues in implementing nanoscience. It is early days in this field and much is still unknown.

Writing in the *Proceedings of the National Academy of Sciences USA*, Steve Granick and colleagues[1] at the University of Illinois in Urbana report that nanoparticles can actively modulate the phase structure of lipid membranes so that the stiffness differs from spot-to-spot. This variation in stiffness is functionally important for material and sensor applications, but the findings could also have broader implications for understanding nanoparticle–cell interactions and their safety issues.

Granick and co-workers mixed positively or negatively charged polystyrene nanoparticles (~20 nm in diameter) with different suspensions of liposomes — spherical lipid bilayer membranes that contain aqueous compartments — and measured the state of the membrane phases using fluorescence and calorimetry. A charge-dependent reconstruction of the membrane surface was observed at the local spots where the nanoparticles bound; negatively charged nanoparticles bound to a fluid area of the membrane induced gelation, whereas positively charged nanoparticles turned gelled areas into a fluid state.

Experiments with liposomes made from different types of lipids showed that the local phase-change did not depend on the choice of lipids, the size of the liposomes or the size of the nanoparticles. Rather, it seemed to depend on the density and placement of charges on the surface of the nanoparticles; nanoparticles with a higher density of surface charge resulted in a greater degree of membrane gelation, whereas DNA, which is a flexible rod-like molecule, did not induce this effect. The Illinois team suggests that the rigid placement of charges on the surface enables the nanoparticles to induce structural reorganization of the lipids and change their local state. Although these studies relate to a very simple model system of a single component, the clarity of the conclusions prompt broader questions.

It is now appreciated that, below a certain size, a broad range of nanoparticle materials enter a variety of cells by different processes — the details of which remain to be clarified (Fig. 1). Moreover, positively charged particles (seemingly irrespective of the material type, including liposomes) induce cell death[2], also through mechanisms not yet fully understood[3]. No clear consensus has yet been reached on the origins of these effects; it is possible there are multiple entry pathways for nanoparticles, which might affect the function of cells differently[3,4].

There is, however, an immediate presumption on the part of the scientific community that nanoparticle–cell interactions are mediated by classical

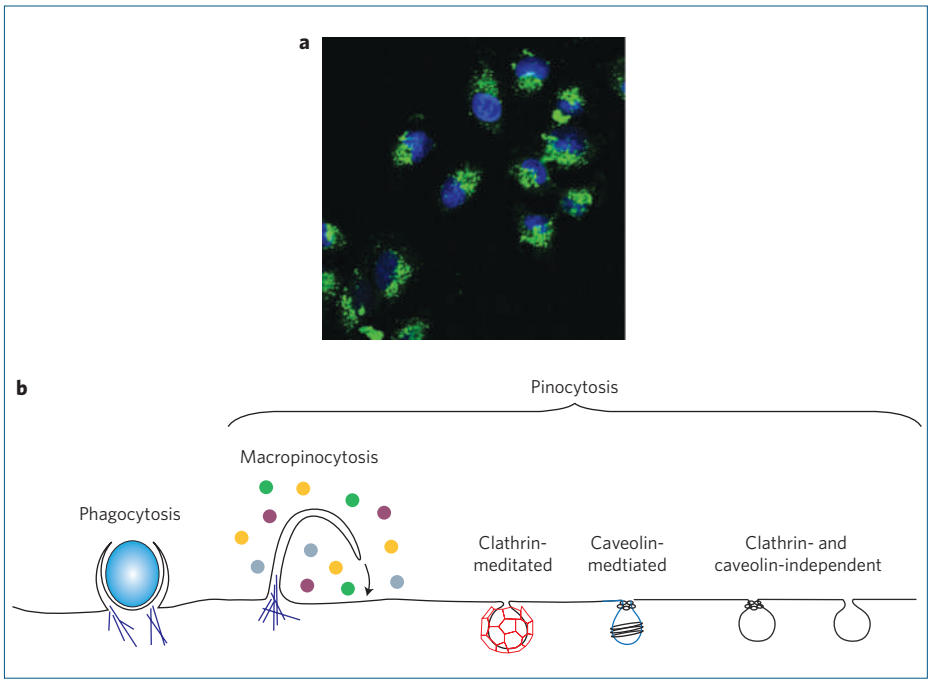

Figure 1 | Nanoparticles may enter cells in various ways by known mechanisms. **a**, Confocal microscopy image showing the active uptake of 50-nm negatively charged polystyrene nanoparticles (green) by epithelial cells. The cell nucleus is stained with DAPI (blue). **b**, Schematic showing the different ways that nutrients and signals can be taken-up by cells: entry of nanoparticles could follow similar mechanisms Reprinted from ref. 10 (© 2003 NPG). In phagocytosis — which is mainly relevant to specialized macrophage cells — micro-sized particles are transported in vesicles that pinch off as invaginations of the cell membrane. Pinocytosis is phagocytosis of fluid-filled vesicles. Macropinocytosis traps large droplets of fluid under the extensions of the cell surface and occurs in many different types of cells. Processes mediated by proteins such as clathrin and caveolin, transport sub-100-nm proteins and protein-like species into cells. Other less well understood processes that are independent of clathrin or caveolin are probably also involved with nanoparticle uptake.

biological processes. For example, the uptake of materials by cells has been reported to occur via receptors, mediated by proteins such as clathrin that coat membrane vesicles at the surface of the cell during entry[5]. Furthermore, the protein coating or 'corona' on the surface of the nanoparticles may assist in the nanoparticle–cell interaction[6]. However, it is worth taking a step back and recognizing just how different, and perhaps how varied, the particle–cell interface could be, and almost certainly some of what we see arises from physical interactions such as those reported by Granick and co-workers.

Indeed, there have already been suggestions that lipid rafts — specific areas in the cell membrane that are enriched with lipids — might represent a new route for the entry of viruses into cells[7]. Clearly the finding that nanoparticles can induce local phase-changes in the lipids points to the potential disturbance or creation of similar lipid rafts in the cell membrane. It is, for example, noteworthy that positively charged nanoparticles induce gelled areas of the membrane to become fluid, and one wonders if this local phase-change can modulate or disrupt the functioning of proteins, receptors and ion channels in the membrane[8]. Furthermore, other local changes in the membrane structure may well have the potential to affect the normal signalling of cells[9]. The fact that nanoparticles can re-structure the local lipid-organization and overall stiffness of lipid membranes is sufficient to suspect that the biology will be affected.

In studying the interactions of nanomaterials with living systems, one is confronted with the tension between society's urgent need for information and the complexity of biological processes. It is tempting to reach for well-established biological paradigms that explain the uptake and processing mechanisms of naturally occurring 'nanoparticles' such as proteins (Fig. 1; ref. 10). However, studies such as the one by Granick and colleagues[1] remind us that another scientific direction based on direct physical interactions could also be important. It is too early to over-commit to any single view of the field, and significant fundamental research is still needed. ❐

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BIOSENSORS

Nanotubes light up cells

By measuring changes in the photoluminescence of single-walled carbon nanotubes caused by the presence of molecules that damage DNA, it could be possible to build a biosensor that can identify multiple analytes in real time.

Todd D. Krauss

The development of optically based biological sensors that can detect multiple analytes has revolutionized molecular biology. In addition to greatly aiding basic research, these devices have led to pioneering applications in gene expression, detection of biowarfare agents, medical diagnostics, drug discovery and forensics. However, most optically based biosensors, like the well-known DNA chip[1], have limitations such as a slow response or unsuitability for *in vivo* use. On page 114 of this issue, Michael Strano and co-workers demonstrate that the near-infrared photoluminescence from single-walled carbon nanotubes can be used to detect multiple toxic agents inside a living cell in real time[2]. This breakthrough could lead to the development of optical sensors that can identify multiple biological processes inside living organisms as they occur.

Carbon nanotubes display exceptional and unusual mechanical, electrical and optical properties as a result of their one-dimensional structure[3]. A single-walled nanotube can be pictured as a sheet of carbon atoms rolled into a hollow cylinder, and it is characterized by two integers, n and m, that determine, among other things, its diameter and electronic properties. In particular, two-thirds of all single-walled nanotubes are semiconducting and are therefore photoluminescent, which means they can absorb radiation and then re-emit photons at specific wavelengths dependent on n and m (ref. 4).

Semiconducting nanotubes have many properties that are desirable for a potential *in vivo* optical biosensor. First, the variation of their emission wavelength with n and m can be exploited to detect different biological analytes at the same time (Fig. 1). The absorption and emission maxima are in the near-infrared region of the spectrum where biological tissue is highly transparent[5] and background cellular fluorescence is low. Nanotubes can also easily enter cells and have no obvious short-term toxicity[6]. Finally, nanotube photoluminescence can be detected down to the single-molecule level and is more photostable than other common fluorophores such as organic dye molecules and semiconductor quantum dots[7].

Despite these advantages, the development of carbon nanotubes as optical biosensors will require several problems to be solved. Nanotubes are synthesized as mixtures containing structures with different values of n and m, which leads to the emission and absorption lines of different nanotubes interfering with each other. Worse, nanotubes aggregate into bundles when they are synthesized — which leads to the fluorescence being quenched by metallic nanotubes in the bundles — so it is necessary to isolate the individual nanotubes from each other to allow them to emit[8]. However, this isolation process typically involves strong sonication — which can damage the nanotubes — followed by a step where a surfactant is wrapped around the nanotube, limiting the usefulness of this approach. Moreover, once isolated, the photoluminescence efficiency of the nanotubes is also very poor, typically less