The market for microelectromechanical systems (MEMS) is worth ~$10bn per year, and it is difficult to avoid MEMS devices in everyday life — they are found in automobiles, mobile phones and video-game controllers to name just a few applications. The most difficult challenge that carbon nanotubes and graphene face in this field is that silicon reigns supreme, just as it does in electronics. It will not be easy to displace the silicon behemoth, but the superior mechanical properties of carbon nanotubes and graphene — they are the thinnest, stiffest, and strongest materials in the world — could be reason enough to bet on carbon. ❐

J. Scott Bunch is in the Department of Mechanical Engineering, University of Colorado, Boulder, Colorado 80309, USA.
e-mail: jbunch@colorado.edu

References

1. Eichler, A. *et al. Nature Nanotech.* **6,** 339–342 (2011).
2. Bunch, J. S. *et al. Nano Lett.* **8,** 2458–2462 (2008).
3. Lee, C. *et al. Science* **321,** 385–388 (2008).
4. Barton, R. A. *et al. Nano Lett.* **11,** 1232–1236 (2011).
5. Lifshitz, R. & Cross, M. C. in *Reviews of Nonlinear Dynamics and Complexity* Vol. 1 (ed. Schuster, H. G.) Ch. 8 (Wiley, 2008). Available at: http://www.tau.ac.il/~ronlif/pubs/RNDC1-1-2008-preprint.pdf
6. Bunch, J. S. *et al. Science* **315,** 490–493 (2007).
7. van der Zande, A. M. *et al. Nano Lett.* **10,** 4869–4873 (2010).
8. Shivaraman, S. *et al. Nano Lett.* **9,** 3100–3105 (2009).
9. Robinson, J. T. *et al. Nano Lett.* **8,** 3441–3445 (2008).
10. Poncharal, P. *et al. Science* **283,** 1513–1516 (1999).
11. Sazonova, V. *et al. Nature* **431,** 284–287 (2004).
12. Chen, C. *et al. Nature Nanotech.* **4,** 861–867 (2009).
13. Xu, Y. *et al. Appl. Phys. Lett.* **97,** 243111 (2010).

IN VITRO STUDIES

Ups and downs of cellular uptake

Experiments on the uptake of gold nanoparticles by cells grown in different cell culture configurations suggest that the influence of sedimentation should be taken into account when performing *in vitro* studies.

Dominique Lison and François Huaux

In vitro cell culture studies, which are commonly used in toxicological research to screen new compounds and to explore the mechanisms of toxicity, typically involve subjecting cells grown at the bottom of a culture well to a dose of test material, and measuring their response to determine the dose–effect relationship. Traditional *in vitro* assays have been primarily designed for testing soluble molecules. However, using *in vitro* assays to test nanoparticles and fibres has been problematic because solid objects do not behave the same as soluble molecules and, therefore, it has been difficult to define appropriate expressions for the dose.

Writing in *Nature Nanotechnology*, Eun Chul Cho, Qiang Zhang and Younan Xia[1] from Washington University in St. Louis report, based on experiments with upright and inverted cell cultures, that sedimentation of nanoparticles is an important determinant of cellular dose in *in vitro* cell studies. Gold nanoparticles of various shapes, sizes, surface coating, density and initial concentration were examined and those with faster sedimentation rates showed higher cellular uptake in the upright setup compared with the inverted one.

The concentration of test molecules in *in vitro* assays is normally expressed as the nominal mass dose, which is quoted in units of micrograms of chemical per millilitre of cell culture medium (μg chemical per ml). The relevant dose is more difficult to define for solids because the cellular response can be driven by various parameters, depending on the site and mechanism of action (Fig. 1a). When surface activity, such as the release of reactive oxygen species by crystalline silica particles[2], is involved, the surface area of the nanoparticle per millilitre of cell culture medium (cm^2 particle per ml) is considered to be the appropriate expression of dose.

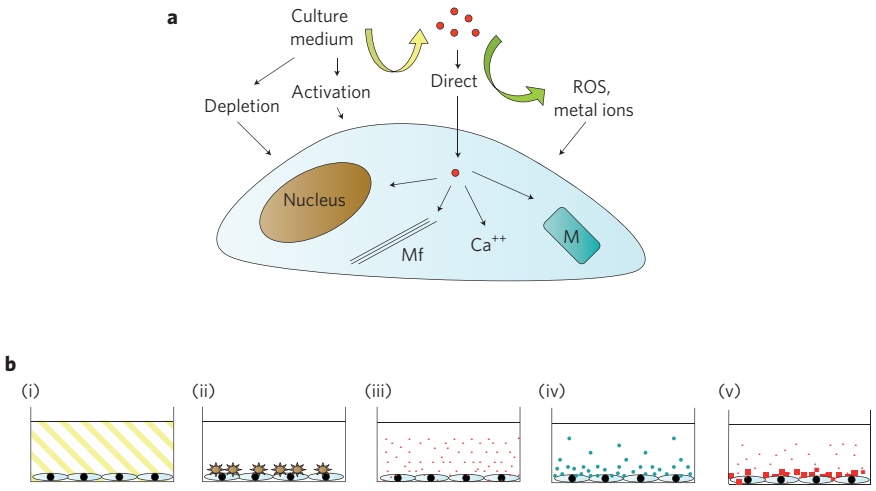

Figure 1 | The variety of ways in which solid nanoparticles interact with cells (**a**) and behave in culture medium (**b**) make it difficult to define the relevant dose for nanotoxicology studies. **a**, Nanoparticles (red circles) can act directly on targets inside cells (including mitochondria (M), calcium stores (Ca^{++}), microfilaments (Mf) or the nucleus), or indirectly by releasing compounds (such as reactive oxygen species (ROS) or metal ions) that damage the cells from outside, or by changing the cell culture medium in ways that influence the cellular response (by, for example, activating or depleting various constituents in the medium). **b**, When cells are exposed to toxic species in the form of soluble molecules (i), the relevant dose is the concentration of the molecules in the culture medium. However, the situation is more complex for solid particles that are not soluble. Microparticles (ii) generally sediment and rapidly come in contact with the cells. Small nanoparticles (iii) sediment less and their contact with cells is determined by diffusion and convection forces. However, larger nanoparticles (iv) settle more rapidly because of the additional influence of sedimentation forces. In most cases, nanoparticles form aggregates (v) in the culture medium, so cells are exposed to a mixture of single and aggregated nanoparticles that settle in different ways.

However, when toxicity is mediated by ions released from the solids[3], the relevant dose should be measured in units of mass of ions per millilitre of cell culture medium

(μg ions per ml). Some solids also exert toxicity indirectly because they adsorb essential components and deplete the cell culture medium[4] or activate extracellular proteins[5]; in such cases, the nominal surface area dose (cm^2 particle per ml) might again be the most appropriate metric.

Furthermore, because toxic phenomena may require contact between the solids and the cells, the fraction of particles that reach the cells at the bottom of the culture well needs to be considered (Fig. 1b). This is not a serious issue for microparticles (except for very low density materials) because most particles are assumed to rapidly sediment by gravitation and contact the target cells for possible uptake. This assumption is reflected by the tendency to normalize the dose to the surface area of adherent cells (μg particles per cm^2 cells)[6].

For nanoparticles, the same considerations apply but the role of surface reactivity is generally amplified and sedimentation of nanoparticles is more complex as diffusion forces become significant for particles smaller than 50 nm (ref. 7). Teeguarden and co-workers[8] proposed that relying on nominal dose may be misleading because only a fraction of the suspended nanoparticles may actually reach the cell surface; their calculations showed that for a 50-nm spherical silica nanoparticle to travel a distance of 1 mm based on gravitation or diffusion forces, it would take 17 days or 13 hours, respectively. Most *in vitro* studies are done in 1–24 hours. Importantly, because the fractional deposition rate varies with particle size and density, it was suggested that the appropriate way to compare the toxic effects of different types of nanoparticle is by measuring the cellular dose of the nanoparticles. Because analytical methods are not always available to measure cellular dose, computational approaches have been developed to predict fractional deposition rates[9]. However, these calculations assume that the nanoparticles are not charged, that they do not interact, and that they are monodisperse. One of us (D.L.) and co-workers have suggested that convection forces, which are always present in sols, also contribute to the contact of nanoparticles with adherent cells[10].

Xia and co-workers developed a clever experiment to assess the effects of sedimentation on cellular uptake. They compared the nanoparticle uptake by cells cultured as usual at the bottom of a well (upright) with those cultured on a coverslip but suspended into the medium from above (inverted). They reasoned that nanoparticles can be transported to the cells only by diffusion in the inverted setup whereas particles in the upright configuration can reach cells by diffusion and sedimentation. All the six different gold nanoparticles (spheres, rods and cages ranging from 15 to 118 nm in hydrodynamic diameter) examined showed greater uptake in the upright arrangement but differences in uptake between the two setups were more prominent for larger particles. The smaller 15-nm particles, which are thought to experience mainly diffusion forces, displayed similar uptake profiles in both setups, whereas the larger 118-nm nanoparticles that were subjected to sedimentation forces showed greater uptake in the upright arrangement than in the inverted one. Nanoparticles that were subjected to both sedimentation and diffusion forces showed intermediate responses.

This work demonstrates the influence of nanoparticle sedimentation on the dose delivered to cells in *in vitro* assays and the results imply that for large and/or dense nanoparticles (Xia and co-workers propose a minimal threshold of 40 nm in hydrodynamic diameter), the toxicologically relevant dose should consider sedimentation effects. These conclusions are valid for monodisperse, non-aggregated insoluble nanoparticles and assume that cellular uptake is not a selective and/or limiting step in the interaction between nanoparticles and cells, which is an oversimplication. The possible contribution of convection forces is not addressed by this work.

Whether cellular responses (for example, cytotoxicity, genotoxicity or oxidative stress) are also influenced by nanoparticle sedimentation will be an interesting study in the future. A practical consequence of the findings is that researchers will now need to systematically assess whether their results can be affected by the issue of fractional deposition. If relevant, an analytical or computational assessment of the cellular dose *in vitro* will be required. ❐

Dominique Lison and François Huaux are in the Louvain Centre for Toxicology and Applied Pharmacology, Avenue E. Mounier, 53.02, 1200 Brussels, Belgium.
e-mail: dominique.lison@uclouvain.be; francois.huaux@uclouvain.be

References

1. Cho, E. C., Zhang, Q. & Xia, Y. *Nature Nanotech.* **6,** 385–391 (2011).
2. Fubini, B. *Environ. Health Perspect.* **105** (suppl 5), 1013–1020 (1997).
3. Prahalad, A. K. *et al. Chem. Res. Toxicol.* **14,** 879–887 (2001).
4. Casey, A. *et al. Toxicol. Lett.* **179,** 78–84 (2008).
5. Deng, Z. J., Liang, M., Monteiro, M., Toth, I. & Minchin, R. F. *Nature Nanotech.* **6,** 39–44 (2011).
6. Geys, J., Nemery, B. & Hoet, P. H. *Toxicol. In Vitro* **24,** 620–629 (2010).
7. Limbach, L. K. *et al. Environ. Sci. Technol.* **39,** 9370–9376 (2005).
8. Teeguarden, J. G., Hinderliter, P. M., Orr, G., Thrall, B. D. & Pounds, J. G. *Toxicol. Sci.* **95,** 300–312 (2007).
9. Hinderliter, P. M. *et al. Part. Fibre Toxicol.* **7,** 36 (2010).
10. Lison, D. *et al. Toxicol. Sci.* **104,** 155–162 (2008).

COVALENT ORGANIC FRAMEWORKS

Growing honeycombs on graphene

Layered films of two-dimensional covalent organic frameworks with accessible and aligned pores can be created on graphene surfaces using a solvothermal condensation reaction.

Mirjam Dogru and Thomas Bein

Covalent organic frameworks (COFs) are a class of highly porous, purely organic crystalline materials that are held together by covalent bonds between boronic acids and polyalcohols. COFs can exhibit high thermal stability and the size of their pores can be precisely tuned — properties that make them promising candidates for gas storage, separation and catalysis[1]. One of the most exciting features of some COFs is a framework made up of π-stacked aromatic building blocks that creates porous networks with electronically coupled 'walls'. This property has recently inspired researchers to create the first semiconducting and photoconducting COFs using pyrene (a flat hydrocarbon made up of four fused benzene rings) building blocks[2]. COFs containing phthalocyanines[3] (large, planar macrocycles) and metallophthalocyanines[4] have also