team can be readily extended to many other hybrid systems such as calcium phosphate and Ca^{2+}-assisted enzymes like α-amylase and calpain. The use of biocompatible inorganic materials such as hydroxyapatite will also enable the protein-incorporated nanostructures to be used for drug delivery and tissue engineering.

However, there are a number of issues that need to be addressed in future work. First, a better understanding of the interaction between the inorganic phase and protein is required. At present, it remains unclear how and at what density and orientation the protein molecules bind to the surface of an inorganic particle. Furthermore, it is difficult to determine where the immobilized protein molecules are located and whether their conformation is retained or altered during an immobilization process. Second, if the nucleation event of an inorganic phase in the presence of protein molecules at different concentrations can be described quantitatively, it is possible to develop more effective strategies for immobilizing proteins and for controlling the shape of the inorganic nanocrystals that form[7]. It is known that capping agents (such as ionic species, small molecules or macromolecules) are important in the nucleation, growth and shape evolution of nanocrystals. ❐

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NANOTOXICOLOGY

No signs of illness

Quantum dots that contain cadmium, selenium and zinc are not toxic to monkeys for periods of up to 90 days, but longer-term studies are needed to determine the ultimate fate of the heavy metals that accumulate in the organs.

Leo Y. T. Chou and Warren C. W. Chan

Semiconductor nanocrystals — also known as quantum dots — have significant potential to be used as fluorescent probes for medical imaging, image-guided surgery and drug delivery, but clinical applications have been delayed because some studies have shown that metals released from the degradation of quantum dots can kill cells that are grown in culture, as can reactive oxygen species generated by the transfer of energy from quantum dots to nearby oxygen molecules[1,2] (Table 1). There is, however, one rodent study that showed that cadmium selenide (CdSe) quantum dots capped with a zinc sulphide (ZnS) shell did not have any apparent toxic effects at a concentration suited for guiding tumour resection despite the breakdown of the quantum dots[3]. Although the results from the rodent study are difficult to extrapolate to humans, researchers from China, Singapore and the US now report in *Nature Nanotechnology* that quantum dots with a CdSe core, a CdS–ZnS shell and a phospholipid coating do not exhibit acute toxicity in rhesus monkeys[4], which is a primate model that closely resembles humans.

The team — led by Ling Ye of the Chinese PLA General Hospital in Beijing, Ken-Tye Yong of Nanyang Technological University in Singapore and Paras Prasad of the State University of New York in Buffalo — investigated the biodistribution and toxicity of 50-nm-diameter phospholipid-coated CdSe–CdS–ZnS quantum dots by intravenously injecting the particles into monkeys and measuring the concentrations of cadmium, selenium and zinc in the blood and various tissues of the animals at various timepoints using inductively coupled plasma mass spectroscopy. The quantum-dot clusters were cleared from the blood of the monkeys and distributed to various organs within six hours of intravenous injection. After 90 days, more than 90% of the cadmium from the injected quantum dots remained in the body with most of it accumulating in the kidneys, liver and spleen. The relative abundance of the injected elemental cadmium, zinc and selenium in each organ was different at the end of the study. These results suggest that these quantum dots slowly degrade *in vivo* and the free metal ions redistribute to various organs over time.

To evaluate acute toxicity, the team measured the number of white blood cells and the amount of different proteins in the serum. Levels of all the measured biomarkers — which are indicators of organ function and inflammation — were within normal physiological ranges, and the subjects did not display behavioural abnormality or weight loss. This suggests that these types of quantum dots were well tolerated by the animals. Histology of the tissues collected from four of the six treated animals at the end of the 90-day period did not show any major changes in the tissue structure; there were no signs of cell death or inflammatory response. All of these results suggest that the injected quantum dots did not cause any toxic damage to organs in which the particles accumulated.

This first set of non-human primate data serves to dampen some of the fears over the toxicity of quantum dots intended for applications in humans. However, much work remains to be done because current toxicological and pharmacokinetic profiles of a particular quantum dot formulation has offered little predictive power over the behaviour of another. And this has generally been the difficulty in assessing the safety of engineered nanomaterials and in interpreting published results across the literature. The term 'quantum dot' does not refer to a specific nanomaterial, but rather to a large body of semiconductor nanocrystals that vary in their geometry, composition and surface chemistry. These parameters determine the biological fate and toxicity of the engineered nanomaterial[5]. Furthermore, the *in vivo* behaviour of quantum dots also depends on their dose and routes of administration. Because there are currently no standardized metrics and experimental conditions to

Table 1 | Brief summary of the toxicity of quantum dots in different biological models. The table summarizes the results of current *in vitro* and *in vivo* toxicity studies of a small list of different formulations of quantum dots available for clinical applications. The last two rows represent examples of near-infrared-emitting quantum-dot formulations that are potentially useful for medical imaging but where full toxicity analysis has not been conducted.





Quantum dot type	Cells	Rodents	Non-human primates	Humans
CdSe	T1 (ref. 1)	?	?	?
CdSe–CdS	T1 (ref. 7)	?	?	?
CdSe–ZnS	T2 (ref. 1)	NT3 (ref. 3)	?	?
CdSe–CdS–ZnS	?	?	NT3 (ref. 4)	?
CdTe	T1 (ref. 2)	?	?	?
$CuInS_2$–ZnS	?	?	?	?
InAs(ZnCdS)	?	?	?	?

The numbering refers to either the lowest concentration identified as toxic (T), or highest concentration reported as non-toxic (NT). 1 = < 10 nM; 2 = 10 nM–1 μM; 3 = > 1 μM. The question mark means that there are currently no systematic toxicity data for the specified experimental model and quantum dot formulation.

examine nanoparticle toxicity, many of these results are often difficult to interpret and compare[6].

Moreover, complete risk assessment can be a challenging endeavour even for a single quantum-dot-formulation. Although current *in vitro* characterization techniques are fast and relatively simple, they poorly recapitulate the complexity of the *in vivo* environment. Animal studies provide the best strategy to assess the toxicity of a nanoparticle but due to the large number of different designs of a single family of nanoparticles, animal testing would be slow and expensive, and could raise significant ethical concerns. It will be important to develop high-throughput molecular and cellular assays that can identify potentially toxic nanoparticle formulations for further animal testing.

Together with earlier *in vivo* studies in rodents, the new results encourage us to think about possible clinical applications of quantum dots, where their immediate benefits may outweigh their yet unknown, long-term biological effects. The diversity of quantum dot formulations means that any clinical approval will probably be on a case-by-case basis, where the design of the nanomaterial, its dosing regime and routes of administration are rationally validated for its intended application. Only by demonstrating benefits in the proposed application can sufficient context be provided for examining the toxicological and pharmacological profiles of the nanomaterial, justify the need for a complete *in vivo* risk assessment and perform thorough cost-to-benefit analysis of the proven benefits over its potential hazards. ❐

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COMPOSITE MATERIALS

Taking a leaf from nature's book

Amyloid protein fibrils and graphene sheets can be combined to make a material that is biodegradable and has useful shape-memory and enzyme-sensing properties.

Max I. Solar and Markus J. Buehler

Most biological materials are constructed from simple building blocks, yet they exhibit extraordinary properties. High-performance organic protein materials such as silk or collagen, for example, are usually composed of only a few distinct amino acids, but they combine to form structures that display a very wide range of material properties and perform many different biological functions[1–5]. And the addition of other types of building blocks leads to even better performance: for example, bone is strong and tough because the collagen matrix also contains hydroxyapatite mineral platelets. What's intriguing is that the macroscopic properties of the final material are much more than the sum of the parts, and that these properties can change in response to the demands being placed on them (a property referred to as tunability)[2–5].