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biological databases and competitors in CASP (Critical Assesment of Techniques for Protein Structure Prediction) can attest.

Unavoidably, some devices will be nearly one-off characterizations. Complex multifactor systems such as type III secretion, flagellar biosynthesis or photosynthetic systems will require very specialized measurements for their characterization[10]. The existence of specialized parts is prevalent in other engineering systems. But the work of Endy and colleagues[2] and others in the community gives hope that there will be basis sets of parts that make scalable, predictable, reliable design of certain functions a reality for biological systems.

No standard, however mature, is set in stone. It must evolve with the development of a field and its technology. Some engineering fields have more formal and less mutable standards than others owing to the nature of their substrate and the uncertainties that plague their manufacture and deployment. Standards can be quite contentious things, especially when the principles of design and the predictability of manufacture are still in their infancy. Synthetic biology is in early gestation, although it is developing quickly. BBa_F2620 is built, for example, to comply with BioBricks version alpha, in which cutting and pasting together of parts is accomplished by particular restriction enzymes and ligation protocols. New protocols for efficient and automated cloning and assembly of synthetic biological parts are being continually developed. The ability to simply synthesize very large pieces of DNA quickly, cheaply and without error is rapidly improving, as are methods for integrating these large constructs into organisms. Whole viral and bacterial genomes have been constructed in one or a few lengths of synthetic DNA[17,18]. Further, our ability to measure the circuit behavior in cells, even at single-molecule resolution, is rapidly advancing. Thus, what constitutes satisfying standards for manufacturing and characterization is changing quickly as well. In the words attributed to Ken Olsen, the founder of Digital Equipment Corporation: "The nicest thing about standards is that there are so many of them to choose from."

Those of us with pressing practical or commercial applications of synthetic biology will certainly use whatever means necessary to create and optimize our systems and may feel that it is too early and burdensome to develop standards. But it is in our interests to contribute to this mission both because we are familiar with the practical need for and limitations of different proposed approaches and because we have the most to gain if the effort is successful. With their work, Endy and colleagues[2] have enunciated a challenge. However difficult and imperfect our standards may be, let's push this idea to its limits and see where it will take us.

This view is shared by many in the field and is a central thrust within the Synthetic Biology Engineering Research Center (SynBERC, http://www.synberc.org/), to which the authors of this paper, and I, belong. There may also be an opportunity for journals to foster this activity during a period when only a few specialize in the field. For example, a newly launched journal, *Synthetic Biology*, will be accepting datasheets in the spirit of Figure 1 of the paper[2] (and the examples in **Box 1** above). Authors will be requested to store experimental constructs in a public repository. (In the spirit of full disclosure, I am Editor-in-Chief of this journal.)

Such community repositories will yield the most benefit when synthetic-biology designs scale to systems requiring many interacting parts, thereby limiting the utility of even inspired tinkering to optimize function. Our planes and computer processors are made possible by sophisticated engineering programs that model characterized parts that are designed and manufactured to work together predictably. Although we cannot quite yet imagine what synthetic biological applications might require the numbers and quality of elements on which these advanced technological systems rely, it is economically and socially important that we improve the efficiency, reliability and predictability of our biological designs. Engineering cells for production of chemicals in a fermentor remains a key technical and economic challenge[1]. But there also exist critical applications beyond the bioreactor—in the environment, in agriculture and in medicine—for which it would be at least soothing to know that they could be engineered for dependable and safe function. Setting the standards—high standards—is a clear prerequisite.

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The long and short of carbon nanotube toxicity

Kostas Kostarelos

Toxicological and pharmacological studies suggest guidelines for the safe use of carbon nanotubes in medicine.

The unique physical, chemical and electronic properties of carbon nanotubes (CNTs) have generated much interest in their potential medical applications. Although most studies have assessed the pharmacological efficacy, stability and toxicity of CNTs *in vitro*[1], two recent reports, in the *Journal of Toxicological Sciences*[2] and *Nature Nanotechnology*[3], explore their carcinogenic risk *in vivo*. Notably, these studies reveal that CNTs delivered to the abdominal cavity of mice can induce a response resembling that associated with exposure to certain asbestos fibers. What is the significance of these findings for efforts to develop CNTs as delivery vehicles for therapeutic and diagnostic agents?

Carbon nanotubes are seamless cylindrical structures comprising single or multiple concentric graphene sheets. Applications of both single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs) have long been haunted by fears of toxicity because of their

Kostas Kostarelos is at the Nanomedicine Lab, Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29-39 Brunswick Square, London, WC1N 1AX, UK.
e-mail: kostas.kostarelos@pharmacy.ac.uk

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nonbiodegradable nature and their resemblance to needle-like, carcinogenic asbestos fibers in size, shape and cellular persistence[4]. The most recent study, by Donaldson and colleagues[3], tested a structure-toxicity paradigm pioneered by the same group more than two decades ago. In their original study[5], they linked the structure of asbestos fibers to the development of mesothelioma (a cancer of the membranous lining that covers the outer surface of the chest and abdominal cavities) by demonstrating that carcinogenicity resulted from exposure to long (>20 μm), but not short (<5–10 μm), rigid asbestos fibers.

In their latest study[3], the authors dispersed 50 μg of nonfunctionalized MWNTs of different lengths in saline using bovine serum albumin and injected it into normal mice intraperitoneally. The accumulation of MWNTs in the diaphragmatic mesothelium and the subsequent degree of granuloma lesion formation were significantly higher after injection of rigid MWNTs longer than 20 μm compared with low-aspect-ratio, tangled nanotube aggregates or a negative-control carbon-containing compound that is not needle shaped. This suggests increased risk associated with lung exposure to long and rigid CNTs, presumably because macrophages cannot completely engulf longer fibers (**Fig. 1a**). Although the study did not assess whether the granuloma lesions progressed to tumor formation, the symptoms arising from exposure to long MWNTs resembled those of animals injected with amosite, the type of asbestos used as a positive control.

The second study, published earlier this year by Kanno and co-workers[2], used mice heterozygous for a mutation in p53, which are more susceptible to developing mesothelioma rapidly after exposure to asbestos. In that study, a 60-fold higher dose (3 mg) of nonfunctionalized MWNTs was dispersed in a surfactant (Tween 80)-containing methyl cellulose solution and administered intraperitoneally. Animal morbidity and histological examination of the mesothelium after 25 weeks showed the carcinogenic effect of exposure to either the MWNT suspension or to the positive control, blue asbestos (crocodilite). However, based on the electron microscopy images in this study, the quality of the MWNT dispersions injected seems poor, probably due to the very high doses used. Moreover, although Donaldson and colleagues[3] dismissed the possibility that metals present in the longer MWNT preparation might explain its greater toxicity, Kanno and colleagues[2] acknowledged that metal impurities in their preparations may have contributed to carcinogenicity.

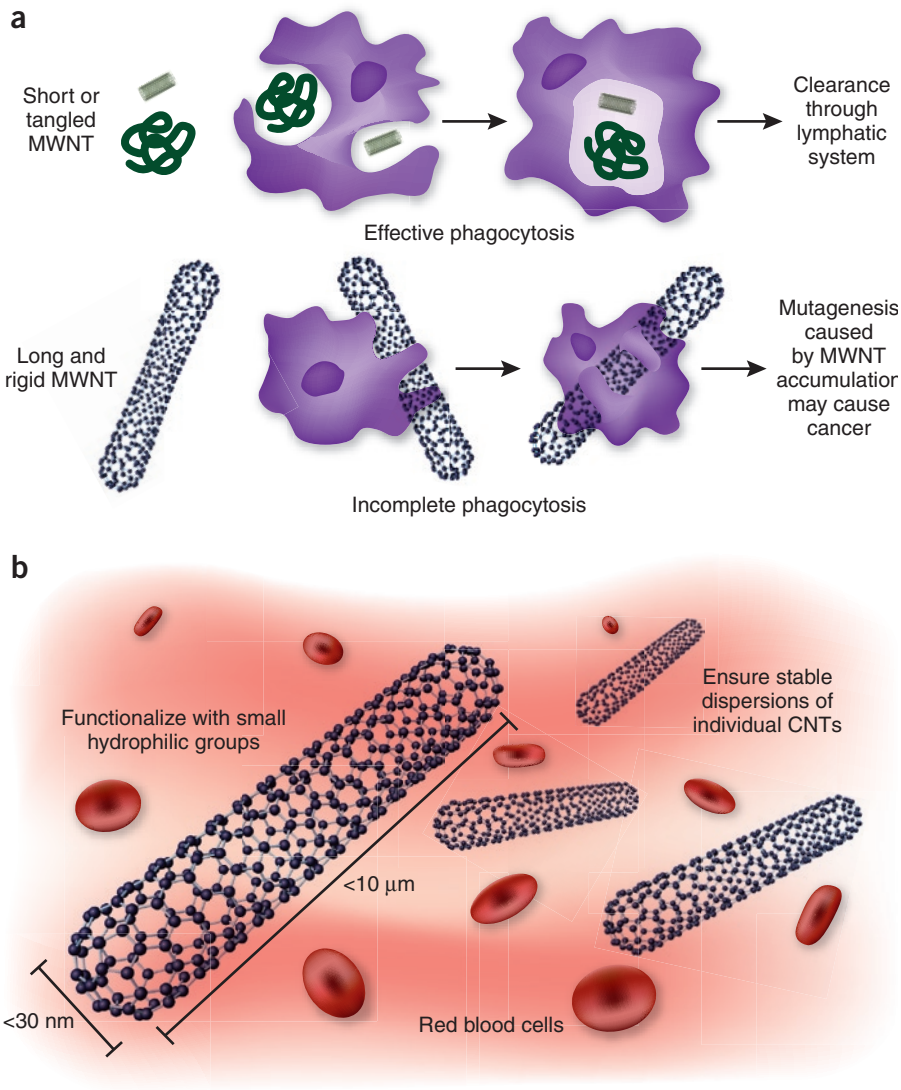

Figure 1 Factors influencing the safety of CNTs *in vivo*. (**a**) The effect of CNT structure on phagocytosis by macrophages and clearing from tissues. Whereas macrophages can engulf MWNTs with a low aspect ratio (ratio of length to width) before their clearance by draining lymph vessels, MWNTs with a high aspect ratio cannot be cleared and accumulate in tissues, where they promote carcinogenesis. (**b**) In addition to their dimensions, other considerations relevant to the safety of CNTs include increasing their solubility and preventing their aggregation, to facilitate urinary excretion and thereby prevent tissue accumulation.

Despite differences in the experimental design, materials and dosages, both studies point to increased risks of cancer from exposure to long, rigid MWNTs. It should be noted that the dose used by Kanno and colleagues[2], 100 mg/kg, is excessive relative to potential clinical doses, whereas the study by Donaldson and colleagues[3] used a lower dose, 1.7 mg/kg, that is more clinically relevant. However, as stated in both papers, the results of these studies are preliminary and should not be considered conclusive proof that MWNTs are carcinogenic because of the direct exposure of the mesothelium to nanotubes through intraperitoneal injection. More experimental work is necessary to assess the persistence and toxicity of MWNTs after administration through intravenous, pulmonary and other routes. An earlier study[6] reported that intratracheal instillation of 0.5–5 mg, nonfunctionalized, Tween 80–coated, long (6 μm) and short (0.7 μm) MWNTs led to long persistence, inflammation and fibrosis of lung tissue, without significant length-dependent differences. Further studies should also consider the effects of different types of CNTs, the agents used to disperse CNTs and the capacity of CNTs to migrate to and accumulate in tissues prone to malignancy, such as the mesothelium.

Table 1 Recent studies of the toxicology and pharmacology of CNTs in mice

Class of CNT	Length	Diameter	Dose	Route	Dispersion	Outcome	Refs.
MWNT	1–20 μm	50–150 nm	3 mg	i.p.	Tween-80 in methyl cellulose	Long MWNTs (short not included) induce mesothelioma in p53+/– mice	2
MWNT	15–20 μm or longer	long: 50–150 nm tangled: 10–15 nm	50 μg	i.p	BSA-coated in saline before injection	Long MWNTs interact with mesothelium, causing inflammation and granulomas in normal mice; short (<10 μm) MWNTs do not interact with mesothelium	3
f-MWNT	<5 μm	20–30 nm	50–400 μg	i.v.	No dispersing agent required	Highly functionalized MWNTs predominantly excreted in urine without any apparent physiological abnormalities in normal mice	7–9

i.p., intraperitoneally; i.v., intravenously.

As with any therapeutic or diagnostic agent, the risk of toxic side effects must be evaluated in relation to potential benefits. Needle-like CNTs have possible advantages for drug delivery, including an enhanced capacity to penetrate cellular membranes, the potential to carry multiple moieties at high density, superior flow dynamics compared with spherical nanoparticles, and unique electronic and semiconducting properties. **Table 1** combines the toxicological data described above with data from recent studies aimed at developing therapeutic, intravenously administered, short (<5 μm) and chemically functionalized MWNTs (*f*-MWNTs)[7–9]. These latter studies indicated the importance of ensuring sufficient chemical functionalization to achieve a stable dispersion of individual *f*-MWNTs in physiological media and conditions[9] and revealed that urinary excretion rates are higher when *f*-MWNTs are individualized rather than aggregated in the bloodstream[7,8]. High rates of urinary excretion have been considered optimal for the biomedical development of other types of potentially toxic nanoparticles, such as quantum dots[10].

Experimental evidence to date clearly indicates that long, rigid CNTs should be avoided for *in vivo* applications and that chemical functionalization should be optimized to ensure adequate dispersibility, individualization and excretion rates sufficient to prevent tissue accumulation. Some suggestions that emerge from the recent investigations of CNT toxicity[2,3] and our own efforts to develop CNTs for medical applications[7–9] are summarized in **Figure 1b** in an initial attempt to guide development of safe CNTs. If the unique clinical potential of CNTs is to be exploited, toxicological studies and pharmacological development must continue in parallel, before eventually converging to provide a clear framework acceptable to regulatory authorities and the public.

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