Potential uses of carbon nanotubes in the medical field: how worried should patients be?

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‘...the same novel properties that make CNTs interesting raise concerns about their potential adverse effects on biological systems, which could lead to health issues, particularly when thinking of their potential use in the medical field.’

Carbon nanotubes (CNTs) are cylinders of one or several coaxial graphite layer(s) (single-walled [SW]CNTs or multiwalled [MW]CNTs, respectively) with a catalytic material (iron, nickel or cobalt) often present inside and/or at their extremity, as well as variable amounts of inert synthesis support, depending on the synthesis method [1]. Their diameter is in the order of nanometers (depending on the number of walls) and they can reach several micrometers in length. Owing to their unique electrical properties, unusual strength and particular effectiveness in heat conduction [2], CNTs are particularly promising nanomaterials for industrial use (see [101] for inventory) and, therefore, one can easily imagine that their production will continue to increase in the future. However, the same novel properties that make CNTs interesting raise concerns about their potential adverse effects on biological systems, which could lead to health issues, particularly when thinking of their potential use in the medical field.

Potential uses of CNTs in the medical field

There are several applications for CNTs that could be of major interest in the medical field. Owing to their semiconducting properties, SWCNTs have been proposed as chemical sensors for gaseous molecules, such as NO₂ or NH₃ [3]. Functionalization of SWCNTs with polyethylene oxide chains not only overcomes nonspecific binding of proteins to CNTs but also further enables the binding of specific proteins of interest (by conjugation of their specific receptor to the functionalized SWCNTs), proteins that can be ultimately detected electronically without

the need for labeling [4]. The authors concluded that the potential application of this system could be for the detection of clinically important biomolecules, such as antibodies associated with human autoimmune diseases, therefore they proposed CNTs as diagnostic tools.

Another potential application of CNTs to nanomedicine is their use in the therapeutic field as vectors for drug delivery. For example, Pantarotto *et al.* demonstrated that functionalized CNTs (water-soluble SWCNTs modified with a fluorescent probe) are able to cross the membrane of fibroblasts *in vitro* and accumulate in the cytoplasm or reach the nucleus, without any associated toxicity [5]. Therefore, these systems could help to solve transport problems for pharmacologically relevant compounds that need to be internalized and, for that reason, could find potential therapeutic applications. Another area of application of CNTs in the therapeutic field is photothermal therapy for cancer. Indeed, although biological systems are transparent to 700–110-nm near-infrared (NIR) light, the intrinsic strong absorbance of SWCNTs in this window can be used for optical stimulation of CNTs inside living cells to afford various useful functions [6]. Kam *et al.* have shown that this singular property of SWCNTs can be used to destroy cancer cells selectively upon irradiation with NIR light. In this study, functionalization of SWCNTs with a folate moiety and selective internalization of SWCNTs inside cells labeled with the tumor marker folate receptor was followed by NIR-triggered cell death, without harming the receptor-free normal cells [6]. This selective cancer cell destruction by appropriately functionalized SWCNTs provides new opportunities in the area of cancer therapy.

There is an increasing list of potential applications of CNTs in the medical field, should it be as diagnostic or therapeutic tools. However, a major issue occurring with these exponential applications is the potential inherent toxicity of CNTs while in biological systems.

What is known about CNT toxicity?

Schematically, there are two situations in which people could be exposed to CNTs: accidental exposure, essentially to an aerosol in the context

of CNT production; and exposure as a result of CNT use for biomedical purposes. To date, studies on CNT toxicity have focused mainly on the effects of CNTs administered as a single dose by the intratracheal or pharyngeal route to animals, which mostly mimics accidental exposure by inhalation [7–12]. These studies show that CNTs can induce pulmonary inflammation (elevated inflammatory cell content and/or inflammatory cytokine production) as well as the development of interstitial fibrosis [7–9,11]. Some studies, however, nuance those findings, reporting that the pulmonary inflammatory response is only transient [10], or even absent [12]. Those discrepancies could be related to the evaluation of different types of CNTs (SW or MW), CNTs synthesized by different methods (e.g., high-pressure carbon monoxide proportionation process [HiPco] or chemical vapor deposition) and CNTs further treated or not (purification by acidic or basic treatment) before animal exposure. One *in vivo* study raised the possibility of systemic effects of CNTs after pulmonary exposure. Li *et al.* showed that pharyngeal aspiration of HiPco-produced SWCNTs in mice (10 and 40 µg/mouse) induces aortic mitochondrial DNA damage, glutathione depletion and increased formation of protein carbonyl groups, 7, 28 and 60 days after exposure. Moreover, administration of SWCNTs (20 µg/mouse, once every other week for 8 weeks) resulted in an accelerated plaque formation in a model of atherosclerosis in mice (apolipoprotein [Apo]E^{-/-} mice fed an atherogenic diet) [11]. This study clearly calls for more research on the potential systemic effects of CNTs after pulmonary exposure.

Literature regarding *in vitro* studies on the biological effects of CNTs is more abundant and gives some mechanistic insights into the key factors that influence toxicity. These studies highlight that CNTs can be toxic for macrophages [13–15], lymphocytes [16], keratinocytes [17–19], type II alveolar epithelial cells [13,14,20], mesothelial cells [21], aortic smooth muscle cells [22], skin fibroblasts [23–25] and embryo kidney cells [26]. In terms of the intracellular mechanism, oxidative stress is proposed frequently as a key mechanism of CNT-induced toxicity, usually linked to the metallic impurities of CNTs [14,27,28]. Induction of apoptosis and/or of an inflammatory response has been described in some studies [15,18,23,26,29] but not in others [14,30].

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What care should be taken in interpreting CNT toxicity studies?

Similar to other nanomaterials, the intrinsic danger level of CNTs depends on their physicochemical characteristics. This is a complex issue because, coming back to initial CNT synthesis, there are a lot of possible end products with different physicochemical characteristics given the combination of the different synthesis methods (e.g., arc-discharge or chemical vapor deposition HiPco), cleaning processes, number of walls (SWCNTs or MWCNTs), metal catalyst (e.g., iron, nickel or cobalt), size (internal/external diameter or length) and surface modifications (acidic treatment or functionalization). Although some information is available concerning the structure–toxicity relationship of CNTs, some key physicochemical characteristics of CNTs that could be a determining factor are beginning to arise [31]. For example, the acidic treatment of CNTs, often used to diminish their catalyst content, is associated with increased toxicity [16,24,32]. Another point is the status of CNT dispersion in solution because CNTs are highly hydrophobic [33]. Exposure of mice fibroblasts, rat aortic smooth muscle cells or human mesothelial cells to well-dispersed SWCNTs is associated with lesser cytotoxicity compared with the same SWCNTs present in an agglomerated form [21,22,34]. This high hydrophobicity of pristine CNTs has induced the need for researchers to modify the surface chemistry of CNTs, namely the ‘functionalization’ process, to improve their aqueous solubility, which is a very important point in terms of their subsequent potential toxicity. Indeed, Sayes *et al.* demonstrated that the toxicity of SWCNTs functionalized with COOH or SO₃H groups is decreased as the degree of functionalization increases (together with their solubility) [25]. Moreover, Dumortier *et al.* demonstrated that NH₃-functionalized SWCNTs did not induce any toxicity after their exposure to mouse B and T lymphocytes and macrophages *in vitro* [35]. Only one *in vivo* study has assessed the compatibility of such functionalized CNTs within the biological milieu. Singh *et al.* showed that indium-labeled functionalized SWCNTs administered intravenously to mice followed a rapid clearance from

the blood compartment through the renal excretion route, without any adverse side effects (absence of renal or other severe acute toxicity and mortality) [36]. These results are of great interest when considering the use of functionalized (as opposed to pristine) CNTs as novel medical tools.

Another critical issue in considering the interpretation of toxicity data is the potential interaction of CNTs with well-described and well-used viability assays [13]. For example, Wörle-Knirsch *et al.* demonstrated recently that SWCNTs can interact physically with the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT]), resulting in a false diminution of MTT reduction, which leads to the misleading conclusion that the SWCNTs were cytotoxic to human lung alveolar epithelial cells [13]. Such interference of CNTs has also been described with other widely used cytotoxicity assays (i.e., neutral red incorporation, lactate dehydrogenase or adenylate kinase release) [20,37]. This could explain the differences observed in some studies when assessing the toxicity of a single CNT with different tests [13,14,20]. Moreover, CNTs can interact directly with culture medium and its additives, such as serum [38], and also with proteins [39–41], and can therefore interfere with techniques, including enzyme-linked immunosorbent assay (ELISA) dosages [20]. These technical issues have highlighted the potential need for the establishment of a new specific discipline, nanotoxicology, stressing the necessity to reposition toxicology [42,43].

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Conclusion

In conclusion, evidence from the literature shows that accidental exposure to pristine CNTs, mainly as an aerosol, might have adverse consequences to health, even if the physicochemical determinants of these effects are not yet identified clearly. Importantly, although the available data are very limited, these adverse effects appear to be less important in the case of CNTs modified for biomedical use.

There is an obvious urgent need for biologists to work together with physicists and chemists to obtain relevant information about the physicochemical properties of CNTs. This could help to provide an improved comprehensive understanding of biological studies, lead to improved relevant knowledge on CNT potential toxicity and, ultimately, enable the safe(r) use of CNTs as novel medical tools.

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