Toxicity of single-walled carbon nanotube: How we were wrong?

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The first issue that we address and justify in this paper is the pejorative and provocative tone of the title; the contradictory data on the toxic effects of single-walled carbon nanotubes (SWCNT) make us believe that it is appropriate and necessary. Two of the first studies in toxicity of carbon nanotube were by Chiu Wing Lam et al.1 and David Warheit et al.2, who reported that carbon nanotube can damage lung tissue in mice. Their works were subsequently introduced in Science in 2003. Since then, toxicity of carbon nanotube has become a fresh research topic and a number of papers have been dedicated to this field. Fig. 1 plots the citations versus calendar year for the past five years to these two papers. A linear increasing number of publications citing Chiu Wing Lam et al. and David Warheit et al. have appeared. In 2001, Huczko A. et al.3,4 first discussed the fact that fullerene soot with a high content of single-walled carbon nanotubes did not show any signs of health hazard related to skin irritation and allergic risks and did not induce any abnormalities of pulmonary function or measurable inflammation in guinea pigs. Unfortunately, his publications have essentially been ignored (Sum of citations, Fig. 1).

Does carbon nanotube really cause toxic effects? We attempt here to briefly demonstrate why we should rationally understand this conception by taking HiPco® single-walled carbon nanotube (Carbon Nanotechnologies, Inc. Houston, TX) as an example. Though biocompatible behavior of functionalized HiPco® carbon nanotube has been researched by a number of authors, we intend to exclude them from the scope of this work.

Table 1 highlights some brief description (details can be found from the literatures) about the key points of investigation of toxicity of single-walled carbon nanotube. The similar experimental process includes:

1. Pretreatment of SWCNT (acid treatment to remove metal contaminant; suspension preparation in PBS, ethanol, DMF, serum, cell culture medium or any other medium, and sequential problems).
2. Cell culture (medium, concentration, time).
3. Exposure conditions (In vitro: mixed with SWCNT; In vivo: pharyngeal aspiration; food paste; intrapharyngeal instillation).

However, the conflicting results can be easily found from the comparison. So what is toxicity anyway? From Wikipedia, Toxicity is the degree to which a substance is able to damage an exposed cell (cytotoxicity) or a whole organism, such as an animal, bacterium, or plant, as well as organs (organotoxicity). Firstly, we want to emphasize that the important point is ‘Damage’. Some changes within cells, organs, or whole organisms cannot be referred to as such, namely, it is not real toxicity. This is because cells, organs, or whole organisms will make a natural response (some changes) to stimulation when carbon nanotube attaching or entering. Even flour powder can lead to pulmonary changes when taken in. Secondly, a central concept of toxicology is that effects are dose-dependent. Considering the micro/nano- properties, the toxicology of carbon nanotube is more complicated than that of common chemicals like CO gas. Besides dose-dependence, many factors should be considered in the study, such as impurities (catalyst, graphite, carbon powder, etc.), dimension.

and its distribution, crystal structure; aggregation degree, effect of cell culture medium, many other secondary chemicals, pH values, etc. After reviewing the literature, we noted that different pretreatment processes of SWCNT were used. No uniform criterion can be found in the studies. The same problems apply to exposure conditions (different cell culture medium, different amount of micronutrients, and different ratio between SWCNT and medium). The assay method can also be problematic (see Table 1 for different assay methods), as there were no comparisons and references for the same instruments of different companies, as well as for the test results by different techniques.

In view of the issues concerned above and based on the current developing status of carbon nanotube, we should rationally understand this conception of toxicity of SWCNT, as well as other nanomaterials. We suggest that the assessment of effect of carbon nanotube on the cells, organ, or whole organism should also be standardized.

Table 1 Toxicity studies on HiPco® SWCNT

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CNT treatment</th>
<th>Exposure conditions</th>
<th>Test</th>
<th>Conclusions</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549 cells (ATCC, CCL-185) a human lung carcinoma epithelial cell line</td>
<td>No acid treatment; SWCNT mix with serum medium.</td>
<td>In vitro</td>
<td>AB, NR, CB, and MTT assay</td>
<td>Very low acute toxicity; Greater toxicity in the absence of serum; Adsorption of SWCNT in medium resulted in an adverse effect on cellular proliferative capacity</td>
<td>5-8</td>
</tr>
<tr>
<td>Human hepatoma (HepG2, ATCC HB 8065) cell line</td>
<td>Functionalized with aryl sulfonate groups; Dispersed in RPMI1640-11875 cell culture medium</td>
<td>In vitro</td>
<td>MTS assay</td>
<td>Adsorption of essential micronutrients from cell culture medium results in the toxicity (Cell viability, DNA damage and apoptosis).</td>
<td>9</td>
</tr>
<tr>
<td>Human osteoblast-like (SAOS-2) cell line</td>
<td>No acid treatment; Suspended in ethanol</td>
<td>In vitro</td>
<td>Fluorescence Metabolic activity</td>
<td>SWCNTs films are not toxic for human osteoblasts and could be used for biomedical applications.</td>
<td>10</td>
</tr>
<tr>
<td>Mouse peritoneal macrophage-like (J774.1) cell lines</td>
<td>80 μg/mL of SWCNT suspended in 1 wt.% Pluronic F108</td>
<td>In vitro</td>
<td>Near-IR fluorescence</td>
<td>Macrophage cells can actively ingest significant quantities of SWCNT without showing toxic effects</td>
<td>11</td>
</tr>
<tr>
<td>Drosophila melanogaster (fruit flies)</td>
<td>No acid treatment SWCNT suspensions: raw SWCNT/bovine serum albumin/PBS</td>
<td>In vivo</td>
<td>Near-IR fluorescence</td>
<td>No short-term toxicity or impaired growth or viability or fertility; Negligible physiological impact.</td>
<td>12</td>
</tr>
<tr>
<td>Rat heart cell line (H9c2 (2-1), Cardiac muscle cells)</td>
<td>Highly purified SWCNTs were suspended in Dulbecco’s modified Eagle’s medium</td>
<td>In vitro</td>
<td>Fluorescence</td>
<td>No evident short-term toxicity; Long-term negative effects are probably due to physical interactions.</td>
<td>13</td>
</tr>
<tr>
<td>Human embryo kidney cells (HEK293 cells)</td>
<td>No pretreatment description</td>
<td>In vitro</td>
<td>MTT; Spectrophotometry; Flow cytometry; SDS-PAGE and Western blot</td>
<td>Inhibit cells growth; death of cells within 24h (250 μg/ml) only slight influence (less than 1 μg/ml SWCNTs in the medium)</td>
<td>14</td>
</tr>
<tr>
<td>Rat aortic smooth muscle cells (SMC)</td>
<td>Acid-treatment SWCNT mixed with DMEM/F-12, FBS, L-glutamine, penicillin, streptomycin</td>
<td>In vitro</td>
<td>Fluorescence image</td>
<td>Inhibit cells growth</td>
<td>15</td>
</tr>
</tbody>
</table>
**Table 1 continued...**

<table>
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<tr>
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</tr>
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<tbody>
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<td>Male FVB/N-TgN (Ho1-luc) Xen mice; Male C57BL/6j mice; B6.129P2-Apoetm1Unc (ApoE−/−) mice</td>
<td>Acid treatment</td>
<td>Suspension of CNT was prepared in PBS by sonification</td>
<td>QPCR; Lowry; Bioxytech GSH/GSSG-412 Colorimetric; en face method</td>
<td>Aortic mtDNA damage at 7, 28, and 60 days (C57BL/6 mice, 10 and 40 μg/mouse); Stimulates the progression of atherosclerosis in ApoE−/− transgenic mice.</td>
<td>16</td>
</tr>
<tr>
<td>Epidermal JB6 P+ cells; SKH-1 Hairless mice (3–4 weeks; 16–18 g body weight)</td>
<td>Acid treatment</td>
<td>In vitro</td>
<td>ESR spin trapping; AB; Luminometer; Flow cytometry; ELISA immunoassay</td>
<td>Unpurified SWCNT can cause dermal toxicity associated with free radical generation, oxidative stress, and inflammation</td>
<td>17</td>
</tr>
<tr>
<td>Human dermis fibroblasts cells</td>
<td>Refluxed at 120ºC in 4 M HCl for 19h</td>
<td>In vitro</td>
<td>MTT; Immunocytochemical analysis; Western blot</td>
<td>Refined SWCNTs are more toxic than its unrefined counterpart.</td>
<td>18</td>
</tr>
<tr>
<td>Human epidermal keratinocytes (HaCaT)</td>
<td>No acid treatment</td>
<td>SWCNT mix with KGM basal medium</td>
<td>ESR spin trapping; SEM; TEM; HPLC; Microphotograph; AB; Chemiluminescence; Fluorescence; Bradford</td>
<td>Cytotoxicity is associated with iron catalytic effects; Unrefined SWCNT can result in accelerated oxidative stress and may produce dermal toxicity</td>
<td>19</td>
</tr>
<tr>
<td>Specific-pathogen-free adult female C57BL/6 mice (7-8 week)</td>
<td>Acid treatment</td>
<td>In vitro</td>
<td>Immunofluorescence Bradford assay Spectrophotometry fluorescence luminescence</td>
<td>SWCNT aggregates induces a robust acute inflammatory reaction and forms granulomas; Pulmonary exposure to SWCNT caused persistent changes in pulmonary functions and decreased bacterial clearance</td>
<td>20</td>
</tr>
</tbody>
</table>

**Abbreviation:**
AB: Alamar blue
NR: Neutral red
CB: Coomassie blue
MTT: Ttetrazolium salt assay
SDS-PAGE: Sodium dodecylsulfatepolyacrylamide gel electrophoresis
QPCR: Quantitative polymerase chain reaction
GSH/GSSG: Mitochondrial reduced glutathione/oxidized glutathione
ELISA: Enzyme linked immunosorbent assay
IR: Infrared
ESR: Electron spin resonance
SEM: Scanning electron microscopy
TEM: Transmission electron microscopy

**REFERENCES:**