# Photodynamic and Photothermal Effects of Semiconducting and Metallic-Enriched Single-Walled Carbon Nanotubes for Cancer Cell Killing

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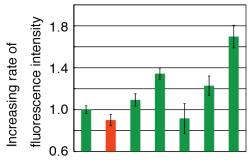
Single-walled carbon nanotubes (SWNTs) are known to be classified into two types, metallic and semiconducting ones, on the basis of the electronic properties. SWNTs reveal photothermal effect (PTE) and photodynamic effect (PDE), which result from photoinduced generation of heat and reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) and superoxide anion ($O_2^{\bullet-}$), respectively. While PTE of SWNTs has received much attention for cancer therapy, a recent report suggests that PDE of carbon nanotubes can be used for protein inactivation through photo-generation of $O_2^{\bullet-}$. Here we report on the first comparison of the PTE and PDE of metallic- and semiconducting-enriched SWNTs (m-SWNTs and s-SWNTs) in details, demonstrating that PDE of s-SWNTs can be an additional tool for photo killing of cancer cells [1].

Enrichment of each type of the nanotubes was performed according to the method reported by Kataura et al. [2]. In this method, s-SWNTs were adsorbed selectively to agarose gel beads. Based on Raman spectroscopy analysis, the ratio of m-SWNT : s-SWNT in the flow-through fraction and that in the adsorbed fraction was 55:45 and 14: 86, respectively. The structures of m- and s-SWNTs were characterized by atomic force microscopy (AFM). Their AFM images showed that vast majority of s-SWNTs were individually isolated, while m-SWNTs were significantly bundled.

The PTE and PDE of s- and m-SWNTs were compared under near-infrared laser irradiation at 808 nm. The temperature of m-SWNT dispersion was elevated higher than that of s-SWNT dispersion. This result indicated that m-SWNTs had higher PTE than s-SWNTs. The PDE was evaluated by fluorescently detecting the photo-generation of ROS. In Figure 1, photoinduced generation of $^1O_2$ and $O_2^{\bullet-}$ was obvious only for s-SWNTs. This result suggested that s-SWNTs had higher PDE than m-SWNTs.

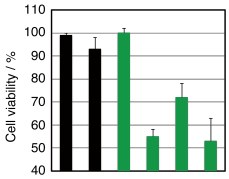



| Laser power (mW) | 0 | 50 | 50 | 100 | 100 | 100 | 100 |
|---|---|---|---|---|---|---|---|
| m-SWNT | | + | | | | | |
| s-SWNT | + | | + | + | + | + | + |
| Sodium azide | | | | | + | | |
| Mannitol | | | | | | + | |
| $O_2$ flow | | | | | | | + |

**Figure 1.** Photodynamic effect (PDE) of s- and m-SWNTs

Although m-SWNTs used in this study contained a significant amount of semiconducting components, PDE of m-SWNTs was not detected. AFM measurements revealed that more than half of m-SWNTs were bundled. From this data, aggregation quenching of semiconducting components as well as energy transfer quenching of semiconducting components by metallic ones in m-SWNTs was cited as a possible cause of the low PDE of m-SWNTs.

Finally, the photo killing acitivity of s-SWNTs against cancer cells was examined upon near-infrared laser irradiation at 808 nm for 10 min. To reduce the cytotoxicity of deoxycholate involved in the aqueous dispersion of s-SWNTs, s-SWNTs were dialyzed against $H_2O$ after stabilized with high-density lipoprotein (HDL). As shown in Figre 2, HDL-stabilized s-SWNTs (HDL-s-SWNTs) markedly decreased the cell viability by 45 %. Without the irradiation, HDL-s-SWNT did not affect the viability at all. These results clearly demonstrated that HDL-s-SWNTs had photo killing activity.




| Laser irradiation | | + | | + | + | + |
|---|---|---|---|---|---|---|
| s-SWNT | | | + | + | + | + |
| Sodium azide | | | | | + | |
| Mannitol | | | | | | + |

**Figure 2.** Photo killing of cancer cells by HDL-s-SWNTs

To gain insight into the mechanism responsible for the photo killing by HDL-s-SWNTs, the cells were irradiated in the presence of ROS quenchers, sodium azide (for $^1O_2$) or mannnitol (for $O_2^{\bullet-}$ and hydroxyl radical and the other free radicals). As shown in Figure 2, only sodium azide weakened the photo killing activity of s-SWNT. These results suggested that $^1O_2$ generated through PDE of s-SWNTs were responsible for the photo killing.

In conclusion, PDE and PTE of SWNTs have been evaluated extensively by using s- and m-SWNTs for the first time. s- and m-SWNTs had higher PDE and PTE, respectively. Another important finding in this study was that s-SWNTs, stabilized with HDL, caused photo killing of cancer cells through $^1O_2$ generation like the other photosensitizers. Our study provides fundamental insights for dveloping SWNTs-based cancer therapies.

[1] Murakami, T.; Nakatsuji, H.; Inada, M.; Matoba, Y.; Umeyama, T.; Tsujimoto, M.; Isoda, S.; Hashida, M.; Imahori, H.; *J. Am. Chem. Soc.*, **2012**, *134*, 17862-17865.

[2] Tanaka, T.; Urabe, Y.; Nishide, D.; Kataura, H. *Appl. Phys. Express* **2009**, *2*, 125002.