Systemic Effect of Different Carbon Nanoparticles in Mice

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Statement of Purpose: In an effort to overcome the many shortcomings of carbon nanotubes (CNTs) including aggregation[1], clumping[1], causing organ accumulation[1], and inducing chronic inflammation and platelet aggregation[2], carbon nanocapsules (CNCs) have been investigated as an alternative for biomedical applications[3]. Here we tested if CNCs have lower toxicity as well as retain and aggregate less in organs after systemic injection in mice. We compared the *in vitro* and *in vivo* toxicity effects of CNCs synthesized by carbon arc discharge with single-wall CNTs (swCNTs), multi-wall CNTs (mwCNTs), and C60. Furthermore, the systemic inflammatory response of each treatment was investigated to reveal the *in vivo* biocompatibility of CNCs.

Methods: *Carbon nanocapsule synthesis:* The carbon nanocapsule (CNC) was prepared by introducing an inert gas into an arc chamber containing a graphitic cathode and anode. The pulse current used was 50 ~ 500 A at 50 ~ 70 Hz and the deposit on the cathode was collected and passed through a 0.22 μm filter. *In vitro toxicity study:* Different concentrations of carbon nanoparticles were added to HeLa and 3T3 cells. Cell viability was assayed by MTT while cell apoptosis was measured by flow cytometry using PI staining. *In vivo survival study:* All animal experiments were approved by the Institutional Animal Care and Use committee at NCKU. Different treatments of carbon nanoparticles were injected via tail vein into FVB mice 8-12 weeks old at 25 μg/g body weight. Animals were observed after treatments and times of deaths were recorded. *Ex vivo cytokine analysis:* FVB mice were injected with carbon nanoparticles at a lower dose to avoid any immediate deaths to induce cytokines. Blood was collected and centrifuged. The serum was then collected for ELISA (IL-1β, IL-6, and TNF-α, Assayro, MO, USA).

Results: The CNCs synthesized by carbon arc discharge showed similar spherical geometry to C_{60}, revealed by TEM (Fig. 1). Unlike CNTs that aggregated up to 3 microns into irregular shapes, CNCs were smaller in diameter at around 50 nm and did not aggregate to the same extent.

When treated to HeLa and 3T3 cells, viability assayed by MTT showed no significant difference compared to swCNTs, mwCNTs, and C60 (data not shown). PI staining revealed that CNCs induced least apoptosis in both cell lines compared to swCNTs, mwCNTs, and C60 (data not shown, $p<0.01$ in all).

Results from the *in vivo* survival studies concurred with *in vitro* results. CNCs did not induce immediate death as severely as swCNTs and swCNTs (Fig. 2).

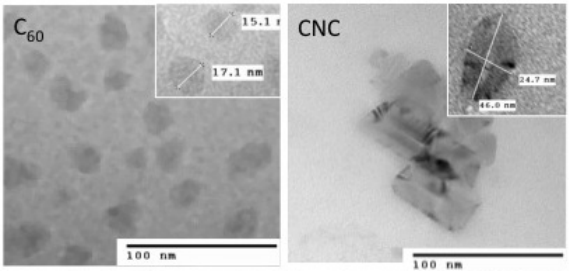

Fig. 1 – TEM images of commercial C_{60} (left) and CNCs developed by carbon arc discharge (right). CNCs are measured to be around 50 nm.

The survival rate of CNC treated mice was close to both PBS and PVA treated mice. As expected, most of the CNTs were found to aggregate in the lungs, leading to immediate death of the animals (data not shown). Cytokine studies also revealed higher inflammatory response in CNT treated mice (data not shown).

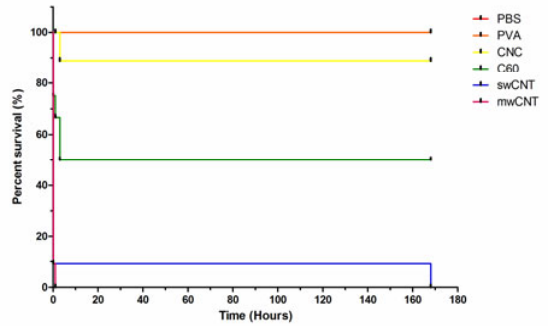

Figure 2 – Survival study showing *in vivo* toxicity of different carbon nanoparticles. Survival rate of CNCs are at 90% while C_{60} survival rates are at 50%. CNT treated mice all died within 168 hours post-injection.

Conclusions: Our *in vitro* studies showed high biocompatibility of CNCs compared to swCNTs, mwCNTs, and C60. Nearly all CNC treated mice survived while only half of C_{60} treated mice survived and no mice treated with swCNTs or mwCNTs survived 168 hours post-treatment. Initial systemic inflammatory response studies showed higher inflammatory cytokine release from CNT treated mice. Future studies will include functionalizing CNCs to increase biocompatibility and conjugating drugs for therapeutic purposes.

References:

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3. Uo M. Small. 2005;1:816-819