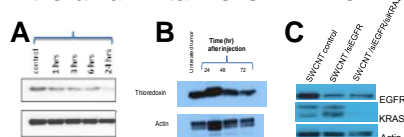


## Abstract

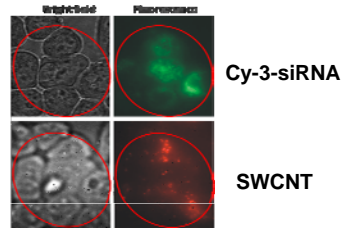
Carbon nanotubes have unique physical and chemical properties that are being widely explored for applications in biomedicine, especially as transporters of drugs, proteins, DNA and RNA. We have shown that siRNA/SWCNT complexes can be delivered safely to animals at high doses over an extended period with no weight loss or changes in blood hematology or chemistry, and produce excellent biological activity including target knockdown and antitumor activity. The current study aimed to prepare stable, optimized SWCNT solutions with consistent siRNA compositions and physical properties and explore cellular uptake. Atomic force microscopy revealed siRNA covers the SWCNT on a mass ratio basis and provided length distributions of the samples. Quantitation of siRNA payload on the SWCNT was achieved using electrophoretic separation of the siRNA from the SWCNT. The stability of siRNA/SWCNT was explored in ribonuclease at 37° C over time and found that siRNA in the complex was stable in 37° C with 80% remaining after 1 hr and 40% after 6 hr versus free siRNA where none remained in solution at 1 hr. Labeled Cy-3-siRNA whose fluorescence is quenched when complexed with SWCNT was used to determine whether the siRNA payload is released intracellularly from the SWCNT following delivery into cells. MiaPaCa pancreatic carcinoma and H2122 non-small cell lung cancer cells were exposed to Cy-3 siRNA/SWCNT for 30 min to 6 hrs. The cells were examined microscopically using near infrared fluorescence detection to observe intracellular SWCNT and visible fluorescence detection for intracellular Cy-3-siRNA. The siRNA/SWCNT complexes readily enter cells within 1 hr and the amount of intracellular SWCNT increase over time to 6 hr. Cy-3-siRNA was released within 1 hr and the siRNA is widely dispersed throughout the cytoplasm. Control cells showed no fluorescence under either condition and intact Cy-3-siRNA/SWCNT complex was not fluorescent due to quenching. We also used Cy-3 labeled siRNA to compare the transfection efficiency of siRNA/SWCNT to that of liposomal delivery. Using equivalent amounts of siRNA, SWCNT/siRNA complexes delivered and released siRNA intracellularly more quickly and distributed the siRNA more evenly within a 1 hr time frame as compared to liposomal delivery. Finally, *in vivo* delivery of Cy-3-siRNA/SWCNT appears to demonstrate a more prolonged and pronounced distribution of siRNA than that of free Cy-3 siRNA. The studies reported here provide data showing the stability of the SWCNT/siRNA complexes *in vitro* and *in vivo* and illustrate their cell penetrating ability with release of the payload intracellularly. We believe the low toxicity, the excellent membrane penetration ability, the protection afforded against blood breakdown of the siRNA payload and the good biological activity seen *in vivo* will allow SWCNTs to become universal transfection vehicles for siRNA and other RNAs for therapeutic applications.

## SWCNT/siRNA complexes produce time dependent target knockdown *in vitro* and *in tumors in vivo*.



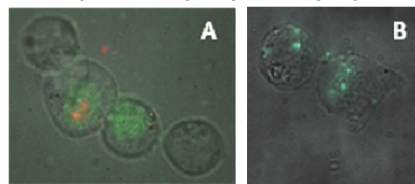
**Figure 1 A:** MiaPaCa cells *in vitro* exposed to up to 36 nM siTx at times shown. **B:** *in vivo* mice bearing MiaPaCa tumors treated iv with siTx/SWCNT 0.8 mg/kg siRNA. **C:** 96 hr following 4<sup>th</sup> week of *iv* treatment with single or dual payload (18 µg siRNA).

## SWCNT delivers siRNA into cells



**Figure 2:** H2122 NSCLC cells exposed to 1µg Cy-3-siRNA/SWCNT for 6 h were examined by bright field and fluorescent microscopy. **Top views** left: the H2122 cells (10.6 pix/mm); right: intracellular Cy-3-siRNA by visible fluorescence (10.6 pix/mm); and **bottom views** left: the same H2122 cells (2 pix/mm); right: the intracellular SWCNT by NIR fluorescence (2 pix/mm) in the same cells. Control cells had no fluorescence. Cy-3-siRNA/ SWCNT is not fluorescent due to quenching by SWCNT.

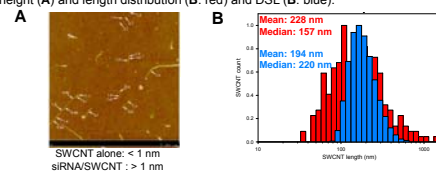
## SWCNT delivers siRNA into cells and disperses rapidly into cytoplasm



**Figure 3:** MiaCaPa cells exposed to: **A)** SWCNT/Cy-3-siRNA for 1 hr. SWCNT NIR fluorescence in red and Cy-3-siRNA fluorescence in green showing siRNA distribution throughout the cells. **B)** Cy-3-siRNA using liposomal delivery for 1 hr with same amount siRNA as in A. Green fluorescence showing the focused lipid delivery of siRNA even after 1 hr.

## Methods

**SWCNT Prep evaluation:** siRNA/SWCNT sample was assessed using AFM analyses of height (A) and length distribution (B: red) and DSL (B: blue).

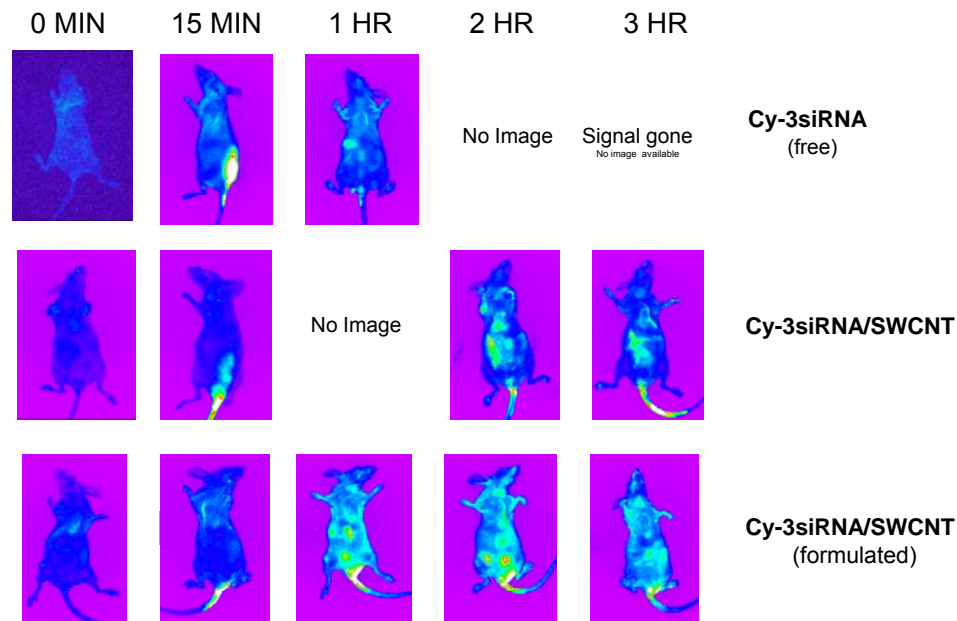


**In vitro:** H2122 cells were grown on coverslips and exposed to Cy-3 siRNA/SWCNT or Cy-3 siRNA/Dharmafect2 for up to 6 hours and examined using fluorescent and NIR microscopy.

**Animal studies:** Female nude (nu/nu) mice were administered Cy-3-siRNA or Cy-3-siRNA/SWCNT *iv*, delivering 20 µg siRNA. Imaging was performed using Carestream MS FX Pro imager over time to observe siRNA distribution and release from SWCNT.

## Results

## SWCNT delivery of siRNA *in vivo* protects siRNA from degradation and increases siRNA circulation time



**Figure 4:** Imaging of mice administered *iv* Cy-3-siRNA, Cy-3-siRNA/SWCNT or Cy-3-siRNA/SWCNT formulated complex. Solutions delivering 20 µg siRNA were injected via tail vein of nu/nu mice and animals were imaged over time using Carestream MS FX Pro imager. Cy-3-siRNA complexed to SWCNT is non-fluorescent due to quenching of signal by SWCNT.

## Conclusions

- SWCNT readily transport siRNA *in vitro* within 30 min of exposure and release the payload intracellularly by 1 hr with increased uptake and release over time.
- siRNA delivered intracellularly by SWCNT provide distribution throughout cytoplasm at 1 hr. Liposomal delivery less well distributed at same time point.
- SWCNT delivery of siRNA provides tumor knockdown of single and multiple targets following *iv* administration.
- Intravenous *biweekly* administration has been shown to be well tolerated over a period of 4 weeks.