

DNA-Associated Single-walled Carbon Nanotubes as a Platform for Drug Delivery

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ABSTRACT

Conjugation of DNA to single-walled carbon nanotubes (SWCNTs) represents an important link between nanoscience and biology. We show here that when in the presence of sonication, DNA forms a covalent cross-link to carbon nanotubes. The attached sequence is available to effectively hybridize to a complementary strand as verified using fluorescence spectroscopy and scanning probe microscopy (SPM). If the attached strand is extended, it can be hybridized to multiple short DNA strands. The DNA:SWCNTs can effectively carry two different DNA strands (each with a different fluorophore). Microscopy imaging shows the dual-fluorophore labeled can then cross the cell membrane of MCF-7 cells.

Keywords: carbon nanotubes, DNA, delivery, radicals, biological

1 BACKGROUND

Single-walled carbon nanotubes (SWCNTs) have shown potential in biological applications. [1-3] Studies have shown the ability of SWCNTs functionalized with various biomolecules to translocate across the cell membrane with little cytotoxicity. [1, 3-5] Although the mechanism of translocation is still being debated, it seems the high aspect ratio and hydrophobicity of the SWCNTs contribute to their ability to readily cross the cell membrane.

The association of nucleic acids (DNA, RNA) to SWCNTs is of particular interest to our research group. The dispersion of SWCNTs by DNA via sonication has been described as a non-covalent “wrapping” of the DNA about the nanotube due to π -stacking interactions and hydrophobic forces. [6, 7] The idea of the “wrapping” has been derived through theoretical studies, however there is a limited amount of experimental data to verify these interactions. [6, 8-10] Here we report a systematic experimental study to support the hypothesis that free radicals generated by the sonication used to prepare the conjugates lead to a covalent linkage between DNA and SWCNTs (DNA:SWCNTs).

2 COVALENT CROSS-LINKING

This study was carried out by sonication of SWCNTs with DNA as previously described. [11] The addition of free radical inhibitors ascorbic acid (vitamin C) and Trolox (a water-soluble vitamin E derivative) was used to observe the role of radical intermediates in the process of association.

2.1 Free Radical Inhibitors

The presence of either ascorbic acid or Trolox prevented the conjugation of DNA to SWCNTs (less than 1% or 5%, respectively when compared to solutions prepared in the absence of the free radical inhibitors as observed by ultraviolet-visible spectroscopy). The results show that conjugation is affected by free radical inhibitors for both polyT and poly A homomer sequences. The same effect is also observed with mixed sequences. In order to verify the role of free radical inhibitors as opposed to competitive binding, (S)- Trolox methyl ether (a Trolox-derivative lacking free radical scavenging activity) was used as a control. S-Trolox methyl ether did not prevent the conjugation as seen with ascorbic acid and Trolox. The absence of conjugation when free radicals are scavenged suggests that a covalent “anchor” is formed between the DNA and the carbon nanotube.

2.2 Radical Damage by Sonication

It is important to know the extent of damage or alteration in respect to each moiety, DNA and carbon nanotubes. To probe possible damage to DNA, a Cy5-labeled polyT (Cy5-T30) was sonicated in aqueous buffer in the absence of carbon nanotubes. The complementary DNA (A30) was added as a template to hybridize the sonicated strand forming double-stranded DNA (dsDNA). The dsDNA was then treated with endonuclease III (EndoIII), an enzyme that recognizes oxidative damage to thymidine bases and cleaves dsDNA at the point of damage. The extent of cleavage was studied using capillary electrophoresis (CAP). Unexpectedly, little damage was present on the DNA. This suggests radicals occur more readily in solution on the carbon nanotubes. We hypothesize that radical cations are being formed on the SWCNTs, which then react with the DNA.

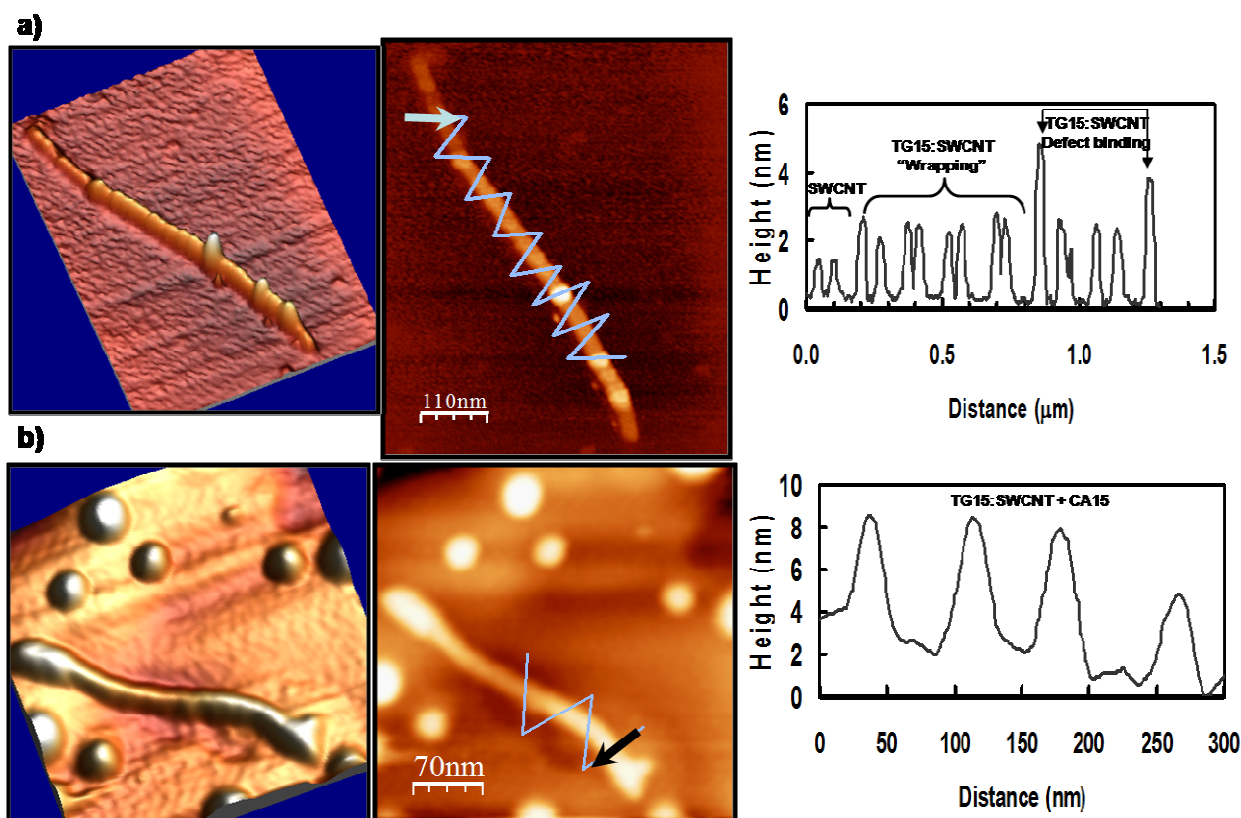


Figure 1 SPM imaging and height analysis of TG15:SWCNT hybridization. a) TG15:SWCNT. b) TG15:SWCNT hybridized to CA15.

SWCNTs were sonicated in aqueous buffer in the absence of DNA to study possible damage to the carbon nanotubes by sonication. X-ray photoelectron spectroscopy (XPS) showed a decrease in the C:O ratio after sonication of the SWCNTs.

3 HYBRIDIZATION OF DNA:SWCNTS

Although one stated advantage of the supposed non-covalent linkage allowed for “release” of the DNA from the carbon nanotube, the covalent anchoring offers advantages as a platform for drug delivery. Specific base-pairing in DNA allows for controlled assembly. If the associated DNA is covalently cross-linked at one or few points and has little damage, then hybridization of a complementary sequence should be effective.

A fluorescein-labeled polyA (Flu-A30) was added to the complementary T30:SWCNTs. Hybridization was observed using fluorescence spectroscopy. Conversely, with fluorescein-labeled polyT (Flu-T30), the fluorescence intensity was significantly decreased. This supports the notion of a covalent cross-linking, as a general “wrapping” mechanism would be non-specific. However, the presence of complementary sequences facilitates the increase in fluorescence intensity.

The hybridization was also observed using scanning probe microscopy (SPM). As seen in Figure 1, TG15:SWCNTs (with DNA alternating thymidine and quanosine nucleotides) were imaged showing features with heights approximately 3 nm, which is consistent of DNA-associated SWCNTs. After hybridization to the complementary sequence (CA15), the heights increase to ~8 nm. The increase in height suggests hybridization primarily occurs on the associated DNA, not directly on the nanotube.

4 CELL MEMBRANE TRANSLOCATION

In order to establish that carbon nanotubes can be effective as drug delivery systems, the interaction of the nanotubes with cells must be studied. Several reports have shown that SWCNTs translocate across the cell membrane. [1-3] To our knowledge there are no reports of the ability of DNA hybridized to a complementary strand covalently anchored to SWCNTs. Furthermore, there are no reports showing the ability of SWCNTs containing multiple DNA strands to translocate across the cell membrane. Here we show the hybridization of two different fluorophore-labeled DNA sequences to DNA:SWCNTs and show their ability to enter cells.

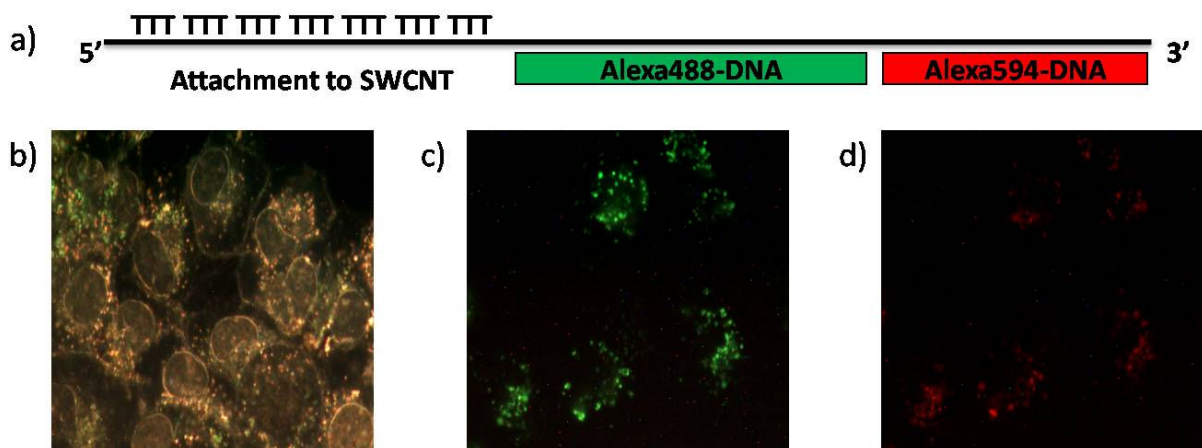


Figure 2: Bivalent DNA:SWCNTs translocate across the cell membrane of MCF-7 cells. a) Design of AttDNA, b) MCF-7 cells treated with 10 μ M dual-fluorophore labeled SWCNTs for 6 h and visualized under dark-field microscopy simultaneously with DMF, and MCF-7 cells treated with 10 μ M dual-fluorophore labeled SWCNTs for 24 hr and visualized with c) FITC excitation filter and d) Texas Red excitation filter.

4.1 Design of Bivalent Platform

The design of the bivalent nanotube:DNA platform began with a DNA attachment sequence (AttDNA) containing three parts (Figure 2a): a) poly-thymidine section included to optimize the probability of attachment at this site, b) sequence complementary to Alexa488-conjugated DNA (Alexa488-DNA), and c) sequence complementary to Alexa594-conjugated DNA (Alexa594-DNA). The AttDNA was conjugated to the SWCNTs as before [11] (AttDNA:SWCNT).

To maximize hybridization to the AttDNA, the sequences of the fluorophore-conjugated DNA were designed as to have little complementation between the two as well as minimal self-complementation. Alexa488-DNA and Alexa594-DNA were hybridized to AttDNA:SWCNT at 60 $^{\circ}$ C and excess DNA was removed by filtration. The hybridization process creates DNA:SWCNTs that contain two different DNA sequences each with a different fluorophore. The nanotubes are herein bivalent.

4.2 Cellular Delivery of Bivalent Nanotubes

The bivalent DNA:SWCNTs were used to detect the delivery of the nanotubes into cells. MCF-7 breast cancer cells were cultured on glass coverslips. The cells were treated with the bivalent, dual-fluorophore labeled (Alexa488 and Alexa594) DNA:SWCNTs for different time intervals.

The cells were visualized using a CytoViva system (Auburn, AL) equipped with a dual-mode fluorescence (DMF) module, allowing the excitation of two wavelengths simultaneously as well as dark-field microscopy. As seen in Figure 2b, after 6 hours of treatment, the MCF-7 cells exhibit fluorescence when excited simultaneously by excitation filters for FITC and Texas Red (corresponding

fluorophores to Alexa488 and Alexa594, respectively) and dark-field imaging. Figures 2c and 2d show fluorescence in cells by single excitation for Alexa488 (FITC excitation filter) and Alexa594 (Texas Red excitation filter, respectively). These images, collectively suggest there is colocalization of each of the fluorophores.

The mechanism of uptake of SWCNTs into cells is a highly debated subject in the literature. Thus far, our imaging suggests that DNA:SWCNTs are encapsulated, supporting the hypothesis that particular nanotubes are likely taken into cells via an endocytic mechanism. This observation supports some previous reports [12] and refutes others. [13]

5 SUMMARY

In this study, we have shown that DNA:SWCNT conjugates prepared by sonication are held in solution by a covalent crosslink. This covalent anchorage allows for hybridization of the attached sequence to complementary sequences, suggesting these DNA:SWCNT conjugates can act as platforms to add multiple function complementary DNA sequences. The platform was studied using hybridization to add two fluorophores to the carbon nanotube. The bivalent DNA:SWCNT effectively translocated across the cell membrane allowing visualization of both fluorophores within MCF-7 cells.

These results have great implications in drug delivery, in particular therapies using oligonucleotides such as antisense, siRNA, and CpG oligonucleotides. Current work is being done to investigate siRNA delivery by DNA:SWCNTs.

6 ACKNOWLEDGEMENTS

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