NANO LETTERS

2009
Vol. 9, No. 3
1007-1010

Carbon Nanotubes as Molecular Transporters for Walled Plant Cells

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Received October 11, 2008; Revised Manuscript Received January 6, 2009

ABSTRACT

We have investigated the capability of single-walled carbon nanotubes (SWNTs) to penetrate the cell wall and cell membrane of intact plant cells. Confocal fluorescence images revealed the cellular uptake of both SWNT/fluorescein isothiocyanate and SWNT/DNA conjugates, demonstrating that SWNTs also hold great promise as nanotransporters for walled plant cells. Moreover, the result suggested that SWNTs could deliver different cargoes into different plant cell organelles.

Recent years have seen increasing interest in the biological and biomedical applications of carbon nanotubes.[1,2] As carbon nanotubes have the unique ability to easily penetrate cell membranes while displaying low cytotoxicity,[3] one such promising application is using them as vectors to deliver biomolecules into living cells.[4−6] To date, however, most investigations have focused on interactions between carbon nanotubes and mammalian cells. Whether carbon nanotubes can be used as molecular transporters for plant cells is not yet clear.

Transportation of molecules into plant cells is more complicated due to the plant cell wall: an extra barrier in addition to the cell membrane. The cell wall, which is mostly made up of cross-linked polysaccharides, distinguishes plant cells from animal cells. The cell wall's complex architecture has limited the use of many mammalian cell applicable nanomaterials and molecules in plants.[7,8] Therefore, protoplasts, plant cells where the cell wall is removed enzymatically together with certain cell surface proteins, have previously been used to study the internalization of nanomaterials such as silicon nanoparticles[7] and polystyrene nanospheres.[8] However, there are uncertainties regarding how closely protoplasts may resemble intact cells. Recently, uptake of fluorescent quantum dots directly into the cytoplasm of walled plant cells was achieved after starving the cells for 24 h, yet the delivery of molecules was not attempted.[8]

In this work we investigated whether carbon nanotubes are able to penetrate intact plant cell walls and cell membranes to deliver molecular cargoes. Comparing to the existing delivery methods for walled plant cells such as the gene gun, electroporation, and microinjection,[7,9,10] the nanoparticle-based strategy is advantageous in easy operation, high efficiency, and the ability to deliver molecules other than nucleic acids (e.g., imaging agents and regulatory-active molecules). The development of carbon nanotubes as nanotransporters for intact plant cells is of practical and fundamental importance for plant intracellular labeling and imaging, genetic transformation, and for advancing of our knowledge of plant cell biology.

Water-soluble carbon nanotubes were first prepared using Hipco single-walled carbon nanotubes (SWNTs). The SWNTs were treated with a concentrated H_2SO_4/HNO_3 mixture to form a stable aqueous suspension containing individual or small bundles of oxidized SWNTs (o-SWNTs) less than 500 nm in length.[11] The o-SWNTs were then labeled noncovalently with fluorescein isothiocyanate (FITC) by sonicating the mixture of o-SWNT suspension and FITC solution. Hydrophobic interactions between the aromatic groups in FITC and hydrophobic regions of the SWNTs sidewalls resulted in the stable physical adsorption of FITC to SWNTs.[12,13] A typical atomic force microscopy (AFM) image of FITC-labeled SWNTs (SWNT/FITC) is shown in Figure 1A.

To test whether the SWNTs could enter into walled plant cells, Nicotiana tobacum L.cv. Bright Yellow (BY-2) cells, a popular plant cell model, was used. The cultured BY-2 suspension cells were incubated in the standard growth

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10.1021/nl803083u CCC: $40.75
Published on Web 02/03/2009

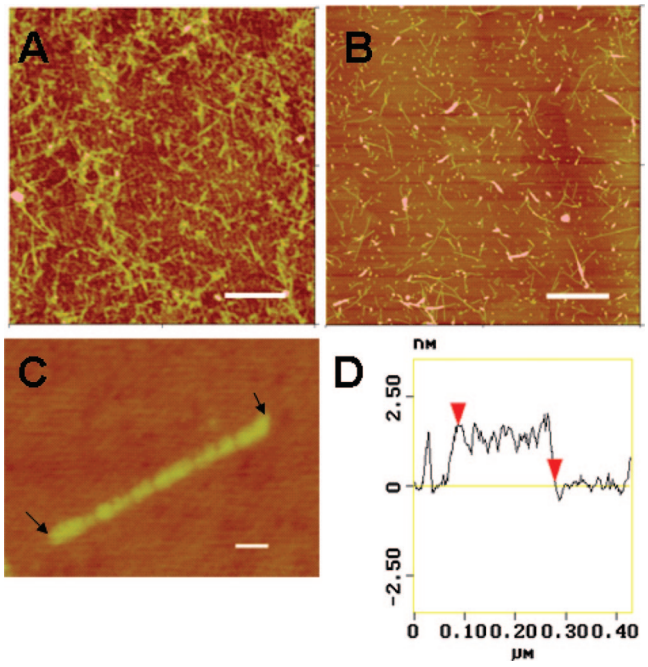

Figure 1. AFM images of SWNT/FITC (A) and SWNT/DNA (B, C). Height analysis of the SWNT/DNA in (C) is shown in (D). Scale bars are 1 μm (A, B) and 25 nm (C).

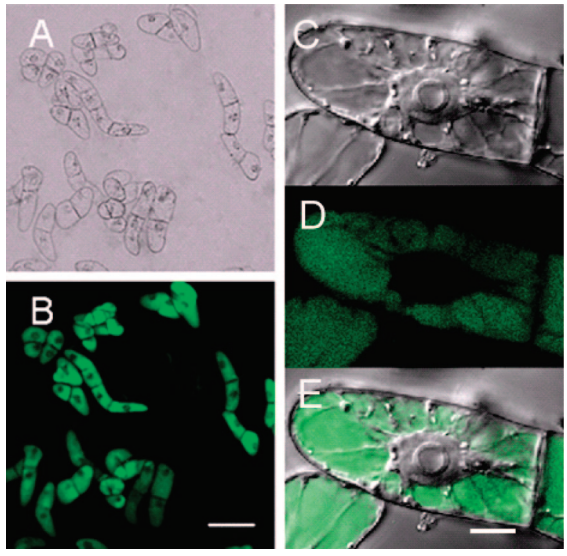

Figure 2. Confocal microscopy images of BY-2 incubated with SWNT/FITC: (A) bright field image; (B) fluorescence image; (C) DIC image under high magnification; (D) fluorescence image under high magnification; (E) overlay of C and D. Scale bars are 100 μm for (A) and (B) and 10 μm for (C−E).

medium added with SWNT/FITC for 2 h at 26 °C. After being washed with the growth medium, cells were imaged by confocal microscopy.

As shown in Figure 2, an intense fluorescence signal can be seen inside almost all the cells incubated with SWNT/FITC. The fluorescence was caused by FITC dye attached to the o-SWNTs. As a control, cells were incubated in growth medium containing the same amount of FITC without o-SWNTs. The fluorescence signal was minimal, comparable instead to the background fluorescence of just plain cells (Figure S1A in Supporting Information), showing FITC by

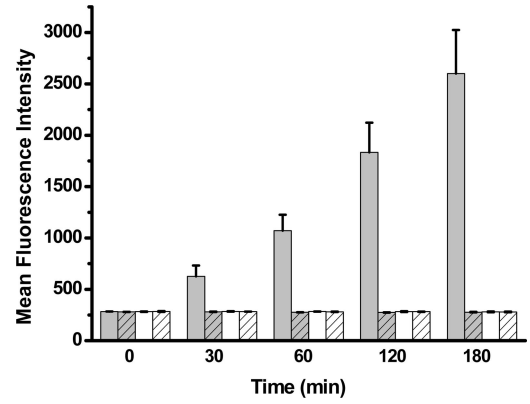

Figure 3. Mean fluorescence intensity of BY-2 cells incubated in SWNT/FITC with different incubation times and temperatures. Cells incubated with SWNT/FITC at 26 °C (gray bar) and 4 °C (dashed gray bar) and cells incubated with FITC at 26 °C (white bar) and 4 °C (dashed white bar) are compared.

itself was hardly taken up by the walled BY-2 cells. The results indicate SWNT/FITC is effectively translocated into the intact cells.

We then studied the internalization of SWNTs under different conditions. We varied the concentration of SWNT/FITC in the cell medium and observed an increase in cellular fluorescence upon increasing the concentration (Figure S1B in Supporting Information). Moreover, the uptake of SWNT/FITC was found to be time- and temperature-dependent as illustrated in Figure 3. Longer incubation time at 26 °C resulted in a fluorescence increase, while little cellular fluorescence was detected at 4 °C, even with a high concentration and long incubation time. Up to now, the uptake of carbon nanotubes into mammalian cells has been inconsistently reported to be via endocytosis or insertion/diffusion.[5,6] Endocytosis of extracellular molecules has also been proposed for plant cells, but the mechanism is far from understood as in mammalian cells.[8,14,15] In our study, the temperature-dependent uptake suggested the involvement of endocytosis for SWNTs, since it has been found that endocytosis in the plant cell was blocked at low temperature.[15] In addition, when we pretreated the cells with wortmanin, the inhibitor of plant cell endocytosis,[15] the cellular fluorescence of SWNT/FITC decreased significantly (about 64% decrease, Figure S2 in Supporting Information). Therefore, we proposed that SWNT/FITC was taken up by fluidic-phase endocytosis.

We have demonstrated that SWNTs are able to penetrate the walled plant cell from the cellular fluorescence of SWNT/FITC. In this experiment, FITC serves not only as a fluorescent label to indicate SWNTs' location but also as a representative small molecule which could be physically adsorbed on SWNTs and transported to the cells. We further evaluated the ability of nanotubes to carry other macromolecules, such as single-stranded DNA (ssDNA), into intact plant cells.

As FITC-labeled ssDNA was hardly absorbed onto the negative charged o-SWNTs, SWNT/DNA conjugates were prepared by ultrasonicating pristine Hipco SWNTs and the

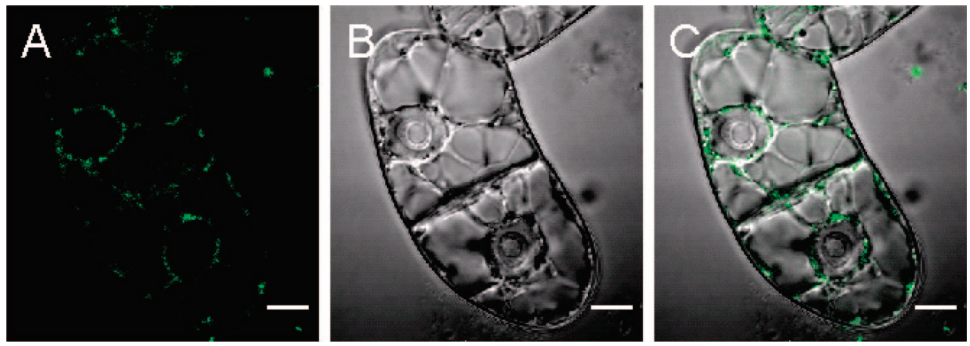

Figure 4. Confocal microscopy images of BY-2 cells incubated with SWNT/DNA: (A) fluorescence image; (B) bright field image; (C) overlay of A and B. Scale bars are 10 μm.

ssDNA−FITC together in aqueous solution, followed by centrifugation and dialysis to obtain stably suspended SWNT/DNA conjugates.[16,17] It has been reported that the noncovalent binding of ssDNA to SWNT is accomplished due to the interaction of aromatic nucleotide of DNA with hydrophobic SWNT surface via π-stacking, and the anionic DNA backbone confers water solubility.[18,19] In our experiment, AFM images reveal the well-dispersed SWNT/DNA conjugates which are less than 500 nm long, similar to that of SWNT/FITC. The regular pattern on the SWNT/DNA surface consisting of peaks and valleys in height is consistent with that observed for the other reported SWNT conjugated with unlabeled ssDNA.[17−19] This demonstrated the wrapping of ssDNA−FITC onto SWNTs (Figure 1).

The fluorescence image of BY-2 cells after incubation with the SWNT/DNA conjugates showed obvious intracellular fluorescence (Figure 4). The control by contrast, where only FITC-labeled DNA was added to the growth medium, had no fluorescence, indicating ssDNA−FITC was unable to penetrate the cell wall without SWNTs (Figure S3 in Supporting Information). As the intracellular fluorescence was observed for more than 80% of the cells that incubated with SWNT/DNA, our results clearly demonstrate that SWNTs can effectively carry macromolecule DNA into intact plant cells with high efficiency.

It is interesting to observe the different fluorescent signal distributions of SWNT conjugates in the BY-2 cell. Although both were not present in the cell nucleus, the DIC images with high magnification of cells incubated with SWNT/FITC (Figure 2) or SWNT/DNA (Figure 4) showed obvious difference in the distribution of cellular fluorescence. For the cells incubated with SWNT/FITC, an intense fluorescence was localized just at the vacuoles, while the fluorescence of SWNT/DNA was seen in the cytoplasmic strands. Time lapse fluorescence images showed many fluorescence spots of SWNT/DNA moving around quickly in the interspaces of vacuoles (see the movie in the Supporting Information).

The mechanism for different distribution of these two SWNT conjugates is not clear yet. In the plant cell, the vacuoles occupy most of the cell volume and one of their important functions is molecular degradation and storage. According to previous reports, the vacuoles were the destination of most endocytic solutes uptake in plant cells, such as membrane dye FM 1-43,[15] cationized ferritin,[20] and artificial polystyrene beads.[8] However, it was recently found that the internalized CdSe/ZnS quantum dots were not delivered to vacuoles but sequestrated into other cytoplasmic compartments.[8] It is expected that a mechanism of recognition, separation, and redistribution occurring in the plant cell endocytic process results in different cellular distribution.[8] It is worth noting that for the two SWNT conjugates SWNT/FITC and SWNT/DNA, not only the molecules attached to SWNT were different but also the property of the SWNT carriers was different; the former was oxidized in acidic solution, whereas the later was pristine SWNT. Although the reason for their different destinations needs further investigation, our results indicate that using SWNTs to deliver different cargoes into different compartments in plant cells is possible.

We also examined the potential toxicity of SWNTs to the plant cells. After BY-2 cells were incubated with SWNT/FITC or SWNT/DNA for 24 h, no apparent cell death was observed and the cells preserved a normal morphology and cytoplasmic fluidity. The proliferation rates were about the same as the control without SWNTs (Figure S4 in Supporting Information). Furthermore, a cell viability experiment was performed by staining the cells with propidium iodide (PI) dye. PI is known to be excluded by intact cell membranes but enter into the cells which have lost their membrane integrity and stain the nucleus with red fluorescence.[21] We used Pl dye to stain the cells that incubated with SWNT/DNA or SWNT/FITC. No PI signal from the cell nucleus was found in the cells displaying FITC fluorescence, suggesting the viability of cells is normal (Figure S5 in Supporting Information). Therefore, SWNTs exhibit little toxicity to BY-2 cells.

In conclusion, we have shown, for the first time, the capacity of carbon nanotubes to traverse across both the plant cell wall and cell membrane. This property can be used to effectively deliver DNA and small dye molecules into intact plant cells. Our results demonstrate that SWNTs hold great promise as nanotransporters for plant cells in addition to their previously reported applications in mammalian cells. Interestingly, it was found that different SWNT conjugates were delivered to different intracellular organelles. The study of internalization mechanism of SWNTs in walled cells, and the exploration of their widespread use in plant cell biology deserve further investigation.

Acknowledgment. This work was supported by National Natural Science Foundation of China (No 90713024, 20821003), the National Basic Research Program of China (2007CB935601), and the Chinese Academy of Sciences. The authors thank Prof. Xueyun Gao (Institute of High Energy Physics at CAS) and Dr. Maozhong Zheng (Institute of Botany at CAS) for the helpful discussions.

Supporting Information Available: Experimental procedures and additional data (Figures S1−S5 and a movie). This material is available free of charge via the Internet at http://pubs.acs.org.

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NL803083U