

Carbon nanotube probes for single-cell experimentation and assays

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Integrating nanotechnology with experimental biology is paramount to advancing fundamental biological science and technology, and, therefore, of high current interest and importance. In this article, we report on a new possibility of utilizing carbon nanotube probes assembled by a modified dielectrophoretic based technique for single-cell experimentation and delivery. The modified approach permits highly reproducible construction of water-stable, highly-aligned, and electrically-conductive probes several hundred microns in length, which hold a great promise for enhancing previously developed molecular-scale intracellular experimental techniques. The results of this work, in particular, indicate that the minimally invasive nanotube probes could be advantageous for studies involving permeabilization and subsequent desorption of molecules into a cell's interior, thereby obviating permeabilization and diffusion across membranes. © 2005 American Institute of Physics. [DOI: [10.1063/1.2112183](https://doi.org/10.1063/1.2112183)]

Nanotechnology is likely to revolutionize our understanding of a living cell's function. Semiconductor quantum dots, with their excellent optical characteristics and stability, are now regularly used in advanced fluorescence-based biological studies and experimentation. Carbon nanotubes (CNTs),¹ on the other hand, with their molecular size, biocompatibility, ability to easily conduct electrical current, and reversible response to bio-chemical agents might become indispensable for a variety of bio-probing and detection applications. A possibility of interfacing of CNTs with electroactive proteins via establishing a molecular link might serve as a convenient vehicle for studying cell organization and functioning on a molecular level. If successfully developed, carbon nanotube bio-sensors may become superior in diagnosis of cell disease by providing early information of cell malfunctioning on a molecular level. On this front, many researchers have already demonstrated the possibility of electrical detection of a variety of biological molecules using carbon nanotube materials,²⁻⁴ which highly suggests the feasibility of bio-probing through a nano-technological approach.

Currently, there is an elevated interest in performing single-cell experimentation and manipulation including single-cell surgery, precise drug release, and spatially resolved cell function monitoring. Recently, Obataya⁵ has been able to successfully penetrate a cell membrane by using a ~200–300 nm diameter etched Si needles as a tip in atomic force microscopy (AFM), opening the door for high resolution cell manipulation and analysis. Carbon nanotubes, with their large Young's modulus, high strength, and small size would provide further advancement in the field. Until recently, using CNTs for studying biological systems remained difficult as precise positioning, alignment, and assembly of individual nanotubes reproducibly and en masse into probe like configurations was not feasible. Recently, several groups

have reported their success in engineering nanotube probes by a direct attachment of nanotubes to AFM tips,⁶ tip-specific catalyst patterning, and subsequent nanotube growth by chemical vapor deposition process.⁷ Moreover, a possibility of arranging single nanotubes into submicron diameter bundles utilizing dielectrophoretic forces has been recently reported in Ref. 8. In this work, the authors simply relied on different shape electrodes (planar substrate and needle-like electrode) to assemble very long nanotube bundles or probes that can be potentially used in biological experimentation and assays. By practicing similar approaches, however we, observed that the resultant bundles normally become highly unstable in liquid phases, and, therefore, they are not suitable for any application in biologically related experimentation and assays. The observed water instability is normally associated with inferior alignment and low integrity of constituent nanotubes as well as their poor attachment to support electrodes, which severely affects the bundle's mechanical and electrical characteristics.

Here, we provide details of highly reproducible and facile integration of carbon nanotubes into highly aligned and water stable probes by a modified dielectrophoretic based assembly employing a pair of needle-like and oppositely aligned electrodes instead. In this case, all constituent nanotubes can simultaneously align and position across the gap between two electrodes, therefore leading to highly improved overall alignment and integrity in the probes. Excellent mechanical stability and integrity of the resultant bundles in both air and liquid phases was experimentally verified, which makes them highly attractive candidates to augment current invasive cell and intracellular manipulation or an alternative to membrane permeabilization as a delivery technique. In contrast to glass micropipettes widely used for decades for patch-clamp electrophysiology, ionophoretic stimulation, and single-cell injections, carbon nanotube probes are less invasive (they have orders of magnitude reduced tip size), mechanically robust (glass tips are known to

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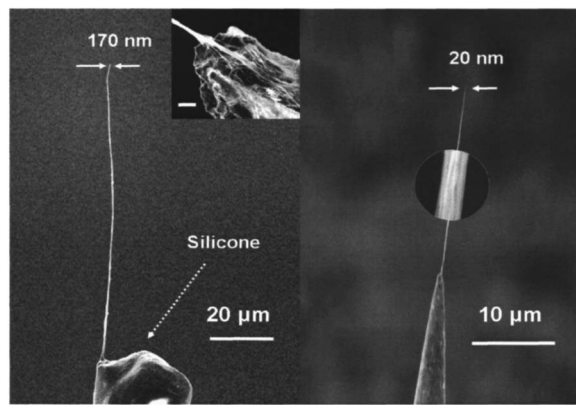


FIG. 1. SEM images of MWNT-bundle (left) coated with silicone as described in the text and SWNT-bundle assembled from 1.5 μm long and 2 nm diameter arc-produced nanotubes (right). The inset shows a detailed view of a typical MWNT bundle-electrode contact area with no coating applied, bar is 1 μm .

be highly fragile and easily break), bio-compatible, and chemically inert (consist of sp^2 hybridized carbon), highly conductive (single-walled nanotubes exhibit ballistic transport characteristics even at room temperatures)—the attributes, along with their reduced dimensions, make carbon nanotubes a highly attractive platform for future-generation ultra-sensitive minimally invasive bio-molecular probing, single-cell experimentation, and delivery.

A modified assembly configuration enables controlled field-alignment of CNTs while not relying on vacuum assembly or welding techniques. Using two standard Tungsten microelectrodes (Micromanipulator, 7A) as the base for assembly allows for highly controlled and reproducible assembly. The electrodes are set to align tip to tip and separated by a small tunable gap. An AC voltage bias is applied to align the CNTs suspended in a [9:1] ethanol/water solution. Probes of varying diameter and length are a result of controlling the bias, inter-electrode distance, and CNT concentration. Small diameter bundles consisting of several CNTs, for example, are fabricated in relatively low amplitude electric fields and dilute CNT concentrations (<0.01 pM). The nanotube probe bundles are extremely thin and rigid and can be extended from the Tungsten base over hundreds of microns. Probes with aspect ratios of $\sim 10^3$, while difficult to achieve with other micro/nano-probe fabrication methods, are easily produced (Fig. 1). Moreover, the assembly is not sensitive to the type of nanotubes used, i.e., multiwalled nanotubes (MWNTs) vs single-wall nanotubes (SWNTs). The dense bundling is attributed to a strong van der Waals interaction (known to lead to undesirable CNT clustering in solution⁹) and the parallel alignment of individual carbon nanotubes.

A typical probe is made in the following manner. A 50 ul 0.1 pM, 90% ethanol solution of CNTs are placed across support electrodes [Fig. 2(a)], in an applied AC electric field of $\sim 10^5$ V/m (peak-to-peak). The droplet's surface tension is used to bring CNTs into close proximity by edging the suspension towards a contact electrode as shown in Fig. 2(b). Once the CNTs are aligned [Fig. 2(c)], van der Waals forces keep the probe rigid and straight. The probes, while naturally rigid, flex and return to their original shape when pushed against an obstacle. SEM analysis performed after bending revealed no evident change in the bundle or its contact to the Tungsten base. Notably, the probes do not lose their rigidity or disintegrate when immersed in water based solutions suggesting the assembly process is irreversible [Fig. 2(d)]. The

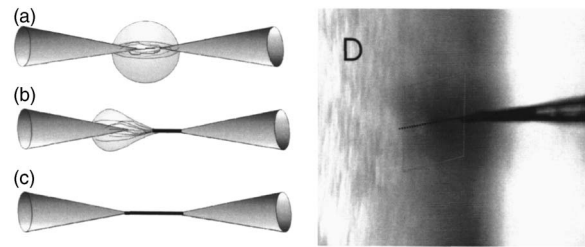


FIG. 2. On the left: schematics of the assembly mechanism displaying CNTs suspended in solution are aligned in an electric field (A), CNT bundle forms via strong surface water tension as the suspension is retracted (B), the resultant CNT rope is shown in (C), on the right (D): microscope image confirming excellent stability of CNT-probe upon immersion in a water droplet.

current-voltage characteristics measured on the bundles comprised of both MWNTs and SWNTs are linear and highly reproducible over an extended period of time (several weeks), suggesting that both nanotube-nanotube contacts and those between the CNTs and support electrodes are Ohmic and stable. Furthermore, we measured the resistance of a ~ 40 nanotube cross-section, ~ 80 μm long CNT bundle, comprised of monodispersed MWNTs prepared by Chemical Vapor Deposition growth to be $\sim 10^6$ Ω . In comparison, the resistance of a single ~ 8 μm long MWNT, was also $\sim 10^6$ Ω . The two configurations, upon scaling, exhibit the same order of resistivity. Since annealed Au-nanotube contacts (used for single CNT resistance measurements) are expected to be, in general, less resistive¹⁰ than contacts formed on Tungsten electrodes, and that nanotube-nanotube interconnects may result in a higher overall bundle resistance,¹¹ strong inter-tube electronic coupling could possibly provide additional enhancement of the overall bundle conductivity. Improved transport characteristics of a bundle with tight tube-tube coupling were predicted by first principle calculations suggesting the formation of “nearly free electron states” serving as additional conductance channels.¹² However, further research effort would be needed to verify the origin of the large electrical conductance observed, which is out of the focus of this work. In general, small diameter, high aspect ratio, and highly conductive carbon nanotube tips offer orders of magnitude improved resolution, sensitivity, and reduced artifact effects in nanoscale imaging and measurements.^{13–16}

Furthermore, the assembly technique can be extended to produce sidewall insulated probes for high spatial resolution electrical and chemical sensing via self-coating procedure. For this, CNTs are first deposited on top of both electrodes, after which silicone coating (silicone windshield and glass seal, Permatex®) is applied. Next, an ethanol droplet is placed across the electrodes, and upon application of an electric field the CNTs are pulled through the silicone, coated, and arranged into highly aligned bundles between the electrodes (left). A high electric current or focused laser beam can be used to break a bundle in two exposing an electroactive tip.

Nanoscale dimensions, high aspect ratio, and the excellent mechanical properties of carbon nanotubes suggest high potential for nanotube probes in many advanced applications including minimally invasive biological manipulation or delivery through specific or nonspecific loading. Covalent linking of biological molecules which normally offers better control over loading and releasing suffers from low payloads as the number of sites available for binding of molecules is

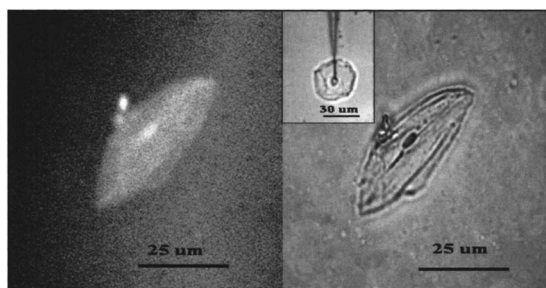


FIG. 3. Fluorescent (left) and phase-contrast images of the cell after penetration with a CNT probe onto the tip of which Alexa 1363 (fluoresceinyl glycine amide) was adsorbed. The inset shows a microscope image of CNT-probe penetrating the membrane of another epithelial cell and contacting the nuclear membrane.

limited. Noncovalent attachment is higher for unprocessed nanotubes because of their highly nonpolar sidewalls (facilitating hydrophobic–hydrophobic interaction) and preferred when larger payloads are required. In general, to improve the resolution and precision of delivery, tip specific loading can be effected via electrophoretic attachment of bio-molecules.¹⁷

Figure 3 (inset) demonstrates the ability of CNT probes, assembled in the manner described above, to easily penetrate the cell membrane of a human oral epithelial cell; this ability is unique to this configuration of CNTs as an individual CNT in solution would lack the requisite structural strength.^{18,19} The two panels show, respectively, fluorescent and phase-contrast images of the epithelial cell after penetration with a CNT probe onto the tip of which Alexa 1363 (fluoresceinyl glycine amide) was allowed to adsorb (non-specific attachment). The bright spot in the left fluorescent image that corresponds to the irregularity on the upper side of the cell membrane in the phase contrast image is excess Alexa 1363 dye which accumulated on the exterior of the cell during the process of probe insertion. The fluorescence observed in the bulk of the cell represents the Alexa dye which successfully diffused from the tip of the probe into the volume of the cell interior. Many intracellular assays require the prior permeabilization of the membrane which often times leads to undesirable side effects such as apoptosis or even whole-scale necrosis.^{20,21} When permeabilization is successful, the molecules-of-interest must then diffuse in sufficient quantity in order to realize an effective assay or transfection system. This diffusion-driven process significantly complicates as well as presents a bottleneck in many techniques found in biological experimentation.^{22,23} Such limitations have already been recognized and addressed in other literature²⁴ in which systems are developed to deliver transfectants in a manner which obviates the need for permeabilization and diffusion. In the demonstration above we employ the Alexa dye merely as a convenient proxy for any number of possible molecules such as DNA (as in Ref. 24), proteins, dyes, or other labels. Unlike Ref. 24 which relies on a stochastic process, one can easily select a specific cell using our high-aspect ratio, minimally invasive and bio-compatible probes.

Notably, during the aforementioned experimentations, we noticed that the bundles could easily form a tight seal with the cell membrane, which is highly important as it helps to preserve overall cell integrity and raise its viability. CNTs exhibit mostly nonpolar properties the degree of which is related to surface defects and bound functional group concentration. It is likely that this nonpolarity enables the coupling of membrane-constituent lipids to the surface of the

CNT probes which results in tight seals formed by cell membranes around a probe. Viability of cells was verified through trypan blue exclusion and is in agreement with other studies to have shown cells viability after penetration by larger CNT-based structures.

In conclusion, we have developed and employed a modified technique for assembling individual carbon nanotubes into highly aligned, nano-scale probes that are electrically and mechanically interfaced to micro-probe bases. In all cases, probes produced in this fashion are electrically conductive and resilient to mechanical and biomolecule loading. Due to their high aspect ratio and demonstrated ability to easily penetrate cells in a manner that keeps them viable, nanotube probes represent an important tool with which life scientists may use to deliver payloads quickly without needing to rely on current standard permeabilization protocols and at the same time to locally probe cell function *in vivo*. In general, the nano-probing techniques in this study might serve as a new platform for high accuracy single-cell delivery, manipulation, or surgery.

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¹S. Iijima, *Nature* (London) **354**, 56 (1991).

²P. Britto, K. Santhanam, and P. Ajayan, *Bioelectrochem. Bioenerg.* **41**, 121 (1996).

³S. Fei, J. Chen, S. Yao, G. Deng, D. He, and Y. Kuang, *Anal. Biochem.* **339**, 29 (2005).

⁴R. Chen, S. Bangsaruntip, K. Drouvalakis, N. Wong, S. Kam, M. Shim, Y. Li, W. Kim, P. Utz, and H. Dai, *PNAS* **100**, 4984 (2003).

⁵I. Obataya, C. Nakamura, S. Han, N. Nakamura, and J. Miyake, *Nano Lett.* **5**, 27 (2005).

⁶J. H. Hafner, C. Cheung, T. H. Oosterkamp, and C. Lieber, *J. Phys. Chem. B* **105**, 743 (2001).

⁷E. Yenilmez, Q. Wang, R. Chen, D. Wang, and H. Dai, *Appl. Phys. Lett.* **80**, 2225 (2002).

⁸J. Zhang, J. Tang, G. Yang, Q. Qui, L. Quin, and O. Zhou, *Adv. Mater. (Weinheim, Ger.)* **16**, 1219 (2004).

⁹R. Bandyopadhyay, E. Nativ-Roth, O. Regev, and R. Yerushalmi-Rozen, *Nano Lett.* **2**, 25 (2002).

¹⁰P. McEuen, M. Fuhrer, and H. Park, *IEEE Trans. Nanotechnol.* **1**, 78 (2002).

¹¹While carbon nanotube intermolecular junctions demonstrate excellent conductance characteristics for “in-registry” junction configurations according to studies of A. Buldum and J. Lu, published in *Phys. Rev. B* **63**, 161403 (2001), there is no *a priori* reason to believe that all junction should be “in-registry,” suggesting that the bundle resistance should be normally higher than that of an equivalent constituent single CNT.

¹²S. Okada, A. Oshiyama, and S. Saito, *Phys. Rev. B* **62**, 7634 (2000).

¹³S. Tans, A. Verschueren, and C. Dekker, *Nature* (London) **393**, 49 (1998).

¹⁴E. S. Snow, P. M. Campbell, and J. P. Novak, *Appl. Phys. Lett.* **80**, 202 (2002).

¹⁵M. Yasutake, Y. Shirakawabe, T. Okawa, S. Mizooka, and Y. Nakayama, *J. Phys. D* **32**, 1044 (1999).

¹⁶N. Jonge, Y. Lamy, K. Schoots, and T. Oosterkamp, *Nature* (London) **420**, 393 (2002).

¹⁷N. Kouklin (to be published).

¹⁸See, for example, *Physical Review Focus*: <http://focus.aps.org/story/v13/st7>

¹⁹V. Tsukruk, H. Ko, and S. Peleshanko, *Phys. Rev. Lett.* **92**, 065502 (2004).

²⁰A. Hameed, K. J. Olsen, M. Lee, M. G. Lichtenheld, and E. R. Podack, *J. Exp. Med.* **169**, 765 (1989).

²¹D. Jonas, I. Walev, T. Berger, M. Liebetau, M. Palmer, and S. Bhakdi, *Infect. Immun.* **62**, 1304 (1994).

²²K. Jain, Cambridge Healthtech Institute’s Third Annual Conference on Lab-on-a-Chip and Microarrays Pharmacogenomics, Vol. 2 (Ashley, 2001), pp. 73–77.

²³A. You, R. Jackman, G. Whitesides, and S. Schreiber, *Chem. Biol.* **4**, 969 (1997).

²⁴T. McKnight, *Nanotechnology* **14**, 551 (2003).