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Magnetically assembled carbon nanotube tipped pipettes

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The authors have developed a biological probe at the nanoscale with a magnetic carbon nanotube (mCNT) tip that has the ability to transfer fluids. Fabrication is performed by injection of mCNTs into micropipettes, which are then positioned as probe tips via magnetophoresis, and affixed with polymeric adhesive. In this letter the authors discuss the magnetic fabrication process and demonstrate the versatility of this probe. © *2007 American Institute of Physics*.
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Traditionally, glass micropipettes have been employed for cellular injection and recovery applications ranging from therapeutic cloning to pharmacological investigation. Difficulties encountered using these capillaries with particularly small cells include excessive membrane rupture, inaccurate transplant, and fatal deformation of crucial organelles. Attempts have been made to overcome these difficulties through the development of nanoscale biological probes for intracellular studies.[1–5] Current generations of sharpened tubules formed by quartz capillary pulling, dubbed "nanopipettes," are capable of being drawn to roughly 25 nm.[6] However, they bend and break too easily for biological probing. Carbon nanotube (CNT) tipped probes offer the advantages of mechanical robustness,[7] rigidity, electrical conductivity, and further scale reduction.

It has been established that carbon nanotube tipped probes can be used to dip into a chemical and deliver it into the cytosol of a cell by subsequently puncturing the cell membrane and allowing passive diffusion to occur.[1,4] In contrast to dip-pen methods [8–11] this technique does not rely on differential surface tension for fluid transfer, and undesired diffusion of molecules into the extracellular and intracellular fluids prior to reaching a targeted organelle limits its reliability. In addition, the quantity of molecules which will adhere to the nanotube tip is also limited, potentially necessitating multiple penetrations.

We have developed a carbon nanotube tipped probe by magnetic fabrication methods, which provides a unique set of properties vital to single cell investigations and surgeries. One objective of our probe is to permit molecules of interest to pass into and out of an individual cell, nuclei, or organelle through the conjoined nanotube and pipette device. This would allow for controlled substance delivery and quantitative sampling.

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Magnetic carbon nanotubes (mCNTs) were used in this work,[12] which facilitate the manufacturing process and provide means for their magnetic manipulation within cells in the future. To date, magnetic manipulations in biological applications have included cell sorting, cell rheology studies, and magnetic tweezing.[13–16] Magnetic manipulation of magnetic and nonmagnetic particles has also been demonstrated.[17,18] The probe we have developed builds further upon these techniques by combining the magnetic manipulative component with the ability to deliver molecules to, or transfer sample molecules from, the intracellular regions of interest. The nanotubes (~200 nm diameter) used in this investigation demonstrate the feasibility of this technique in an optically verifiable regime; however, since the size-limiting factor is the magnetic particles (~10 nm) lining the tubes, the technique can be scaled down to smaller CNTs (20–40 nm).

The tubes employed were synthesized by noncatalytic chemical vapor deposition (CVD) in a porous alumina membrane, which produces open-ended CNTs. The pore size and alumina membrane thickness were chosen to provide 200 nm diameter nanotubes of lengths up to 60 μm.[19] Subsequent to growth, a commercially available ferrofluid (aqueous EMG 508 or organic based EMG 911, iron oxide particle size of ~10 nm) was deposited atop the membrane where capillary action pulled it into the open-ended nanotubes. Finally, the alumina membrane was dissolved with 4 *M* NaOH to disperse the individual mCNTs, and they are then suspended in isopropanol for further use.[12] The treatment in NaOH makes the nanotubes hydrophilic, enhancing the compatibility with the intracellular environment.[19] The tubes produced show a homogeneous dispersion of iron oxide nanoparticles [Fig. 1(a)] adhering to the inner walls of the mCNTs [Fig. 1(b)].

Assembly of the probes is achieved by the magnetic transport of mCNTs through fluid within a pipette. Micropipettes pulled down to submicron tips (0.4–0.9 μm) are po-

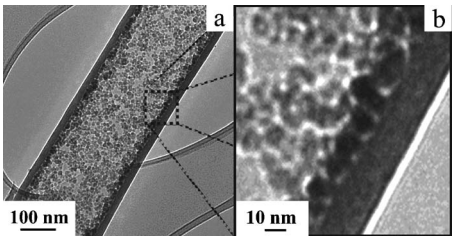

FIG. 1. (a) Transmission electron microscopy image of a CVD grown CNT filled with iron oxide nanoparticles (mCNTs). (b) Illustration of the homogeneity of particular dispersion.

sitioned horizontally and injected with a mCNT solution (5 μl 0.1% mCNT, 99.9% isopropanol) with a syringe (30 g) [Fig. 2(a)]. Juxtaposed to the pipette is a magnetizable (NiFe of 60 μm diameter) wire, the other end of which is held magnetically by an electromagnet's core (~10 V dc, 5A). At the tip of this wire, a thin substrate (~18 μm) of glass is adhered to induce capillary action and stop the nanotubes from protruding too far from the glass pipette when they reach the end [Fig. 2(c)].

The tip of the micropipette is brought in contact with the glass surface backed by the magnetic wire. The electromagnet produces a field that aligns the mCNTs along the axis of the micropipette, while the wire serves to create a large magnetic field gradient that produces a notable movement of nanotubes toward the micropipette tip [Fig. 2(b)]. The mCNTs moving through the micropipette tend to accelerate as they approach the glass substrate where the field gradient increases. When the micropipette contacts the glass, the mCNT solvent contained within wets the substrate forming a small drop there and allowing a mCNT to emerge from the tip. Retraction of the pipette results in drawing the nanotube from the pipette tip. For stability, the mCNT is only drawn out to a fraction (~60%) of its length. Subsequently, the solvent is allowed to evaporate and the magnetic field is turned off, after which the attractive forces between mCNT and inner pipette surface are sufficiently strong to hold the nanotube as the pipette and nanotube are separated from the substrate forming a probe, as shown in Fig. 3.

While in principle, both magnetic forces and fluid flow could contribute to the motoin of mCNTs through the pipette, in practice it is evident that fluid flow is minimal and mag-

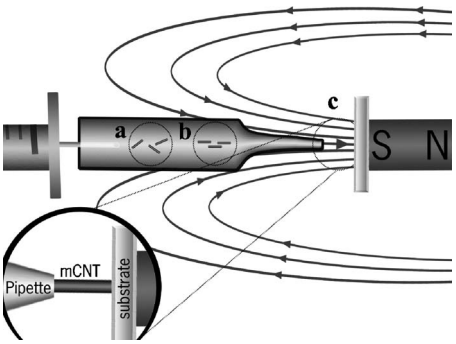

FIG. 2. (a) 5 μl of magnetic CNT solution are injected into a pipette using a 30 gauge syringe. (b) As the CNTs approach the magnetic field created by the magnetized wire (right) they align themselves perpendicularly. (c) A thin hydrophilic substrate is placed between the pipette and a powerful magnet. When the pipette is moved within a few microns of the substrate, capillary action pulls the fluid from the pipette until a CNT or CNT bundle is drawn out.

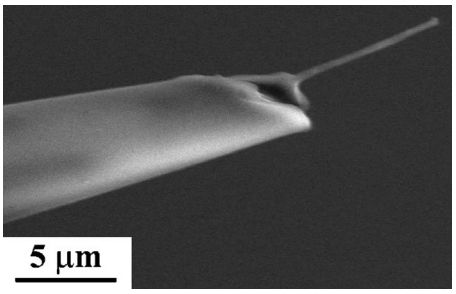

FIG. 3. SEM image of an aluminosilicate pipette with as-grown magnetic carbon nanotube tip protruding from the end. The agglomerate visible around the base of the nanotube is optical adhesive, which was used to seal the nanotube/pipette junction.

netic forces dominate this process. This is due to the fact that the small tip diameter creates a large hydraulic resistance in the pipette, resulting in negligible flow. The dominance of magnetic forces is apparent experimentally; in the absence of a field gradient, little or no observable mCNT motion occurs.

An ultraviolet polymerizable adhesive (NOA74 from Norland, Inc.) of low viscosity is used to fix the mCNTs to the tips of the glass pipettes. The adhesive is placed in small quantity at the pipette tip prior to the injection of nanotubes. The isopropanol solvent in which the nanotubes are suspended probably disperses this adhesive somewhat throughout the process. The front end of the nanotube likely is lightly coated in this glue while passing through the micropipette tip area, however, the curing is achieved locally at the nanotube/pipette junction by focusing the microscope objective at that point and through application of UV radiation. The remaining uncured glue at the nanotube tip disperses in the solvent. Although, for reasons yet unclear, some nanotubes do not appear to pass fluid in subsequent testing, the high percentage yield of probes achieved by this technique (~55%) is promising and may, with careful parameter selection, lend itself toward the automation of this process and allow entire arrays of these nanoprobes to be fabricated at once.

Verification of the adhesion was performed both by optically investigating the glued sample in the scanning electron microscope (SEM) (FEI XL30) using low vacuum mode (0.9 Torr) and by mechanical insertion experiments. It was found that unglued tubes held in place by van der Waals forces alone would be abstracted by submerging the probe in highly viscous materials. Glued tubes readily endure submersion into droplets of fluid, such as glycerin, puncturing Madin-Darby Canine Kidney (MDCK) (canine kidney epithelial) cellular membranes, and submersion in dyes which fail to enter the gap between CNT and pipette even when a negative pressure is applied.

The overall objective of developing decreasingly dimensioned probes is to refine our ability to interact with fundamental biological mechanisms within a single cell. To evaluate the potential of the probe for intracellular investigations, MDCK cells were injected to ascertain the probe's ability to pierce the plasma membrane. It was observed that negligible deformation of the cells occurred, and that a very tight membrane seal formed about the insertion point—evidenced by the tight adherence of the cellular membrane to the probe and the strong adherence of cells to the probe after insertion. An assay of probing stained H9C2 cells (Celltracker Orange 34551) was conducted to visibly ascertain the results of cel-

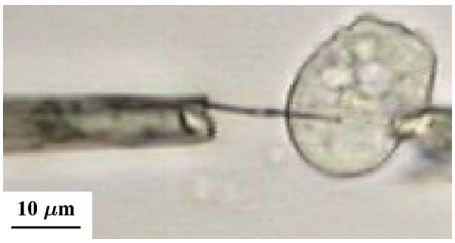

FIG. 4. Optical image of a nanotube tipped pipette (left) is injected into a MDCK cell held in place by negative pressure on a patch pipette (right). Negligible deformation of the cell occurs.

lular perforation. The sample cells were prepared by the standard staining procedure (issued by Celltracker), spread over collagen coated microscope slides, and fixed with formalin 20 min after passage. During these 20 min they were incubated at 37 °C, with 5% CO_2 levels. Upon withdrawal of probes, no fluorescent leakage of the cell tracker dye visible into the surrounding buffer solution was observed. Previously conducted studies in eukaryotic cells indicate that cellular viability should be retained for up to 1 h of probe insertion with probe tips <400 nm.[4] Figure 4 shows a patch pipette that was used to hold a cell in place with negative pressure, as a means to offer resistance against the insertion of the probe.

To demonstrate fluidic transport we submerged the tips of the nanotubes into a droplet of fluorescent dye (Duke Scientific 3100A) consisting of 100 nm polystyrene beads (Fig. 5), where they are dispersed by capillary action and no longer optically detectable. After several minutes, the individual fluorescing beads can be seen clearly to progress individually through the nanotube into the pipette, before diffusing into an indistinguishable glow. High resolution

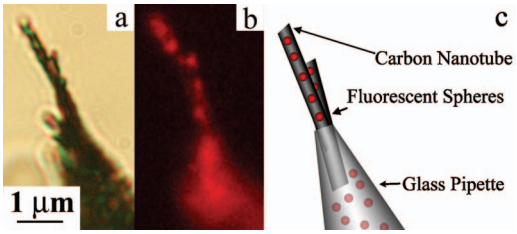

FIG. 5. (Color) (a) Optical microscopy of mCNT tipped probe. (b) Fluidic transport is shown in same probe using dye consisting of 100 nm fluorescing polystyrene beads which visibly align within the tube. (c) A sketch helps to illustrate the process.

imaging is difficult, as a fraction of mCNTs are occluded by excess iron oxide nanoparticles.

In this work, we have demonstrated a novel fabrication technique to assemble a nanoprobe with attractive features for biological investigation. This technique employs magnetic field gradients to position magnetic carbon nanotubes at the small end of glass pipettes, where they are affixed with polymer. We have shown that the physical robustness of our probe is sufficient for the penetration of cells through insertion assays. The capacity for fluidic transport was exhibited with fluorescent particle transfer. We presently report the fabrication of this probe as a proof of concept, which can be scaled to even smaller dimensions.

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