Fabrication and characterization of vertically aligned carbon nanofiber electrodes for biosensing applications

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Available online 19 September 2005

Abstract

We describe recent experiments aimed at using carbon nanofibers for biosensing applications. Vertically aligned carbon nanofibers are grown on molybdenum electrodes to provide electrical contact to the nanofibers. Upon exposure to electrolyte solutions, we find that short nanofibers of <1 μm length can be wet and dried without significant mechanical disruption. However, longer fibers are prone to clumping due to meniscus forces. Deposition of SiO2 adds mechanical strength and electrically insulates the nanofiber sidewalls, which can be further augmented by additional deposition of epoxy-based photoresist. Reactive-ion etching re-exposes the carbon core, localizing the electrical response to the nanofiber ends and leading to electrically active electrodes of ~40 nm radius. Measurements of the diffusion-limited current from ensembles of nanofibers are solutions containing redox agents are in excellent agreement with classical electrochemical theory. We also briefly describe the use of chemical and electrochemical methods to functionalize carbon nanofibers.

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Keywords: Defects; Optical properties; Characterization; Nanostructures; Impurities

1. Introduction

Vertically aligned carbon nanofibers (VACNFs) are emerging as a useful materials for applications such as chemical/biochemical sensing. The high surface area of such material may also be useful for applications such as energy storage. While many nanoscale materials exist, carbon-based materials are of especially great interest due to their intrinsic stability under a wide range of chemical and electrochemical conditions. Vertically aligned carbon nanofibers are of especially great interest because the vertical alignment of the fibers [1] may provide a very high degree of surface area that is easily accessible to molecules in the solution phase; this accessibility is important for sensing applications in which, for example, it may be important to detect molecules of a wide range of sizes. The ends and sidewalls of the nanofibers provide a very high surface area and consequently, a very high number of biological binding sites. This high density of binding sites may increase sensitivity in the same manner as in porous materials such as porous carbon and silicon [2–4]. The sidewalls of nanofibers can also be insulated, leaving exposed electrodes of extremely small size that have been used for highly sensitive detection of DNA [5] and glucose [6] via measurements of the electrochemically active molecules in solution. However, many aspects of the growth, functionalization, and electrical properties of carbon nanofibers are yet to be understood.

Most applications of carbon nanofibers have been based on “bare” fibers with little or no chemical modification of the surface. In many applications, however it is important to functionalize the electrodes with molecules of interest in order to provide them with specific chemical and/or electrical properties. For example, by linking a biomolecule of interest (such as DNA) to the nanofiber surface, it is possible to detect specific molecules in solution, such as DNA molecules with sequences complementary to those bonded to the surface. Here, we describe recent work aimed at fabrication and chemical functionalization of carbon nanofibers for applications in chemical and biological sensing.

2. Results and discussion

2.1. Growth of carbon nanofibers

Vertically aligned carbon nanofibers were grown using DC plasma-enhanced chemical vapor deposition (DC-PECVD) [7]...
in a custom-built chamber. Typical growth conditions used flow rates of 80 standard cubic centimeters per minute (sccm) ammonia and 30 sccm acetylene, with a chamber pressure of 4 Torr and a DC power of 360 Watts. While nanofibers can be grown on a variety of materials, in order to have a chemically and physically robust electrical connection to the nanofibers we found it necessary to use a multilayer structure underneath. This structure consisted of 50 nm Mo, followed by 20 nm Ti, and finally 20 nm Ni as the top layer. Fig. 1a and b show scanning electron microscope images of the resulting carbon nanofibers. SEM images show that the nanofibers are vertically aligned. By controlling the growth time, it is possible to control the length of the fibers. By analyzing the images, we find an average diameter of approximately 80 nm with a standard deviation of 24 nm.

Instead of growing complete nanofiber “forests”, it is also possible to grow smaller clusters and individual nanofibers. In order to do this, silicon wafers having a silicon nitride overlayer were coated with Mo and then a layer of resist. Electron-beam lithography was then used to produce small holes in the resist; evaporation of Ti, then Ni followed by removal of the resist (i.e., a standard “lift-off” process) was used to produce small spots of catalyst material. Growth of the nanofibers can then lead to formation of small clusters consisting of 3–5 individual nanofibers in Fig. 1c, or in some cases to isolated nanofibers as in Fig. 1d.

2.2. Wetting of nanofiber electrodes

For application in wet-chemical environments, the poor mechanical strength of long nanofiber electrodes can lead to aggregation of the nanofibers. Fig. 2a shows a scanning electron microscopy of relatively short (≈ 0.6 μm) nanofiber electrodes after exposure to aqueous KCl and then drying. For short nanofiber electrodes such as these, wetting and drying do not appear to have any significant effect on the electrodes. However, for longer nanofibers like those shown in Fig. 2b and c (here, approximately 2 μm long), there is significant clumping of the nanofibers. In general, this appears to be significant only at the very ends of the nanofibers, and it appears that most of the microscopic surface area remains accessible. However, it is clear that the forces associated with the water meniscus can significantly disrupt the physical structure of the nanofibers. Thus, to fully realize the high surface area that nanofibers can provide, it is necessary to mechanically stabilize them.

2.3. Fabrication and electrochemical characterization of sidewall-insulated nanofiber electrodes

While for many applications the nanofibers can be used as electrodes without further modification, in many instances it is necessary to protect the underlying metal contacts and possibly to insulate the sidewalls of the nanofibers. We investigated a number of possible methods for achieving this, including the use of photoresist as an organic protecting layer. However, the most successful method involved deposition of silicon dioxide via plasma-enhanced chemical vapor deposition. This deposition leads to a conformal coating that covers both the underlying electrodes and the nanofibers. However, by following this process with a reactive ion etch, it is possible to selectively re-open the ends of the nanofiber electrodes [8]. Fig. 3 shows SEM images of nanofiber electrodes that were insulated with SiO2 and then etched using CHF3/O2 RIE at a power of 300 W to re-expose the ends. Fig. 3a and b show carbon nanofibers with a (≈ 250 nm) SiO2 coating. In the SEM, the exposed carbon core is easily identified because the yield of
secondary electrons from carbon regions is much higher than from the surrounding SiO₂ regions. The 250 nm SiO₂ layer provides good insulation over most of the substrate, and provides some additional mechanical support for the nanofibers. Further electrical insulation and mechanical support can be provided by spin-coating an epoxy-based photoresist such as Shipley SU-8 onto the regions to form an additional protective barrier. Deposition of an initial SiO₂ layer is important as the strength provided by the SiO₂ layer keeps the nanofibers from being mechanically disrupted by the SU-8 during spin-coating. Fig. 3c shows a SEM image of a nanofiber after modification with SiO₂ and then SU-8. The nanofibers now appear shorter than those in Fig. 3a and b because of the significant thickness of the SU-8 resist. These nanofibers are extremely robust.

The effectiveness of this insulation process can be demonstrated through measurements of the electrochemical properties of the electrodes. Fig. 4a shows a set of molybdenum electrodes that were patterned with nanofiber catalyst. Using electron-beam lithography, two of the electrodes (labeled E1 and E3) were then patterned with 20 nm Ti, then 20 nm nickel catalyst, while electrode E2, midway between them, was not. After growth of carbon nanofibers, the SEM image in Fig. 4a shows that the two electrodes that were specifically patterned with nickel show rectangular regions of carbon nanofibers, while the non-patterned regions have no fibers grown on them. After deposition of SiO₂ around the nanofibers an additional layer of SU-8 was used to block all conductivity from the underlying Mo contacts; the fibers were then exposed to reactive ion etch processes as follows: After protecting the contact regions on the
chip with a thick layer of photoresist, an O₂ RIE was applied to the substrate using a Unaxis 790 instrument at a power of 100 W in order to etch the SU-8 and expose the underlying SiO₂ layer. Next, a CHF₃ RIE was used to etch the SiO₂ covering nanofiber tips as described above. The thickness of the SU-8 layers are typically on the order of 1–2 μm in thickness; consequently, the SU-8 buries many of the shorter fibers, leaving exposed a smaller number of longer nanofibers. Fig. 4b, for example, shows a SEM image of the same sample from Fig. 4a. In this case, electrode E3 has approximately 190 nanofibers protruding from the electrode with their ends exposed.

We performed tests of electrical properties of the electrodes in Fig. 4 by placing the sidewall-insulated nanofiber electrodes into a microfluidic cell containing 0.1 M KCl with Ru(NH₃)₆³⁺ and Ru(NH₃)₆²⁺ at two different concentrations. The Ru(NH₃)₆²⁺/³⁺ solution provides a pathway for rapid electron exchange at exposed areas of the surface. Fig. 4c shows cyclic voltammograms of the nanofiber-coated electrode E3 at the two different concentrations and the intermediate buried electrode E2 as a control; these data were obtained at a sweep rate of 10 mV/s. At both concentrations, the exposed nanofibers give rise to a sigmoidal curve. At the most positive potentials the current levels off at a value that is directly proportional to the concentration of the reduced form (Ru(NH₃)₆²⁺). At negative potentials the current again becomes diffusion-limited in direct proportion to the concentration of the oxidized form (Ru(NH₃)₆³⁺). Thus, we can conclude that the overall current in both directions is limited by the reactants in solution, and not by the electrical contacts associated with metals or metal-nanowire contact. We also note that the buried electrode E2 shows only negligible current with a root-mean-square value of less than 10 pA. This control is important because it shows that even though the Mo electrodes have a large amount of surface area, they can be effectively insulated from the solution so that the total current is dominated by the exposed carbon nanofibers rather than the underlying electrode.

The diffusion-limited currents observed in Fig. 4 can be compared with those expected from classical electrochemical theory. The diffusion-limited reduction current at a planar electrode is given by

\[ i_d = 4FDCr \]

while that at a hemispherical electrode is

\[ i_d = 2\pi nFDCr \]

where \( F \) is the Faraday constant (96,485 C/mol), \( D \) is the diffusion coefficient (5.48 \times 10^{-6} \text{ cm}^2/\text{s} for Ru(NH₃)₆³⁺) [10], \( C \) is the concentration of Ru(NH₃)₆³⁺ in solution, and \( r \) is the radius of the electrode [9]. We measured the diameter of a large number of nanofibers using SEM and obtained an average radius of 40 nm. Using, for example, the concentration of 8 mM Ru(NH₃)₆³⁺, Fig. 4c shows that at positive potentials the current reaches a value of approximately 20 nA. For nanofibers with this radius, the hemispherical equation yields a diffusion-limited current of approximately 106 pA per fiber. For an ensemble of \(~190\) independent fibers this would yield a current of 20 nA, which agrees almost exactly with our experimental value of 20 nA visible in Fig. 4c.

The electrical stability of nanofiber electrodes was assessed from cyclic voltammetry measurements on bare (non-insulated) nanofiber electrodes. In 1 M KCl solution, nanofibers show virtually no current at potentials between +1.2 V and –1.0 V vs.
Ag/AgCl reference electrode. At higher potentials the current increases sharply due to water electrolysis. As long as the potential is maintained between approximately \(-1.5\) V and \(+1.5\) V (vs. Ag/AgCl reference with 1 M KCl) the current is highly reversible. At higher potentials, the electrical properties become irreversible, with reduced current at both anodic and cathodic sweep. Visual inspection shows under these conditions there is partial delamination of the underlying electrodes. We attribute this irreversibility at high potentials to the formation of bubbles from electrolysis of water, which delaminates the underlying metal electrodes. However, in some non-aqueous media we have found the nanofiber electrodes to be stable at potentials as large as 10 V. This suggests that efforts to mechanically stabilize and protect the underlying metal contacts may also widen the range of accessible potentials in aqueous media.

These measurements establish that it is possible to measure the electrical response from relatively small ensembles of carbon nanofibers. To be useful for chemical sensing applications, however, it is also necessary to provide the fibers with chemical selectivity. This is accomplished through the development of chemical functionalization strategies for carbon nanofibers.

2.4. Chemical functionalization of carbon nanofibers

For applications such as biosensing, it is necessary to provide the nanofiber electrodes with biomolecular recognition capability. This requires the development of methods for chemically functionalizing the nanofibers. We have developed two different methods to functionalize carbon nanofibers. One method is based upon earlier results from our group demonstrating that molecules containing at least one alkene (C=\(\text{C}\)) group will react with hydrogen-terminated surfaces of diamond [11,12] and glassy carbon [13]. A second is based on recent work showing that 4-nitrobenzene diazonium tetrafluoroborate salts will chemically react with carbon nanotubes or nanofiber surfaces, and that the nitro groups on the surface can be reduced to primary amines and then linked to molecules such as DNA [14,15]. These two methods are similar in many respects, but differ in some potentially important aspects, especially the presence/absence of conjugated groups within the molecular layer. Since conjugated groups strongly affect the electrical properties of molecular films, these two methods provide flexibility in the electrical properties of the molecular layer on the surface.

The grafting of aryldiazonium salts onto a variety of carbon surfaces has been investigated extensively [16–19]. The chemical reaction of nanofibers with aryldiazonium salts having a nitro group is especially useful because the grafted nitro group can be selectively electrochemically reduced to a primary amine. Primary amines are a very useful intermediate for selectively modifying specific electrodes with biomolecules such as DNA. Fig. 5a [14] depicts an electrically

![Fig. 5. Electrochemically addressable functionalization of nanofibers with DNA. (a) depicts the overall reaction scheme. (b) shows experimental results; the white light image at left shows dark patches corresponding to nanofiber bundles grown on Mo electrodes. After the entire chip was reacted with the benzenediazonium salt, electrodes 2, 4, and 6 were reduced to amines and used to link DNA to the surface; hybridization with complementary DNA shows three bright spots corresponding to these nanofiber bundles.](image-url)
controlled functionalization procedure in which entire surface is first reacted with the nitrobenzene diazonium salt, producing nanofibers that are linked to nitro groups. Applying a potential of $\sim -1.5$ V to a specific electrode will then reduce the nitro groups of the nanofibers on this electrode to primary amines, leaving the nanofibers on the other electrodes unaltered. The primary amines can then be reacted with a linker molecule such as sulfo-succinimidyl 4-((N-maleimido-methyl))cyclohexan-1-carboxylate ((SSMCC, depicted in Fig. 5) to covalently link them to thiol-modified DNA molecules [14,15].

Fig. 5b shows an example where electron-beam lithography was used to fabricate small bundles of $\sim 8–10$ nanofibers originating in a region of $\sim 500$ nm diameter on electrodes. After reacting the entire sample with the diazonium salt, the nanofibers on electrodes 2, 4, and 6 were selectively reduced. The entire chip was then exposed to the linker and the thiol-terminated DNA; the efficacy of this process was checked by exposing the entire chip to fluorescently labeled DNA that had a sequence complementary to that of the DNA molecules on the nanofibers. When imaged using fluorescence microscopy, Fig. 5b shows that fluorescence is observed from the nanofiber bundles on electrodes 2, 4, 6, with no significant fluorescence from the other bundles. This confirms that the combination of chemical functionalization followed by selective electrochemical reduction can be used to produce electrically addressable arrays on length scales smaller than possible by optical means. This result is important because many applications of carbon nanofibers are fundamentally based on the fabrication of very high density arrays in which many nanofiber-based sensors might be located in a small spatial region. On such small length scales it is very difficult to functionalize materials using microfluidics or other more conventional fluid-handling, and the ability to control chemistry in small spatial regions under electrical control becomes especially attractive. This method can also be applied to other carbon-based materials such as conductive diamond [20].

A second method for functionalizing carbon nanofibers involves the photochemical reaction of carbon nanofibers with molecules containing a carbon–carbon double bond [15]. While in principle it might be possible to use molecules containing an aromatic group (i.e., a benzene ring), in practice care must be taken that the molecules do not themselves absorb light at the wavelength of excitation. For example, we have found the photochemical process on molecules containing any aromatic groups, such as styrene ($H_2C=CH-C_6H_6$) leads to photopolymerization, due to the absorption of the aromatic group at 254 nm. In contrast, molecules containing a single $C=C$ bond are almost completely transparent at 254 nm, so that the ultraviolet excitation penetrates to the solid but does not directly induce any chemical changes in the liquid phase. Consequently, the photochemical method is applicable to molecules containing alkyl chains but not to those containing aromatic groups. Since alkyl chains are highly insulating while conjugated electron systems like those in aromatic groups typically confer electrical conductivity, the diazonium method provides a pathway toward electrically conductive molecular layers, while the photochemical method is more suitable for fabrication of insulating layers.

3. Conclusions

Vertically-aligned carbon nanofibers represent an attractive material for applications in chemical and biological sensing. The high degree of chemical stability provided by carbon-based materials such as diamond and carbon nanofibers is important for the construction of practical nanoscale sensing devices and systems. Although long fibers may be distorted by mechanical forces (in particular, those associated with the water meniscus), short (<1 μm) nanofibers possess sufficient mechanical strength to be used directly in aqueous media. In applications where it is desirable to expose only the ends of the nanofibers, the use of SiO$_2$, especially when combined with additional photosresist, provides additional mechanical strength and insulates the sidewalls, providing nanofibers with only the ends accessible. Electrochemical measurements on electrodes prepared in this manner yield diffusion-limited currents in excellent agreement with the values predicted from classical electrochemical theory. Since most sensing applications require functionalization of the nanofibers with chemical or biological molecules of interest, the use of electrochemical functionalization provides a pathway toward very high-density biosensing systems. These represent many of the most important issues needed for the practical use of nanofibers for applications in chemical and biological sensing.

Acknowledgments

This manuscript is based on work supported by the National Science Foundation grant DMR-0210806.

References