19 Carbon Nanotube Smart Materials for Biology and Medicine

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Abstract This chapter is an overview of potential applications of carbon nanotube smart materials in biology and medicine. Carbon nanotube arrays are forests of aligned nanotubes prepared on a substrate. The nanotubes have multifunctional properties that include high strength, sensing, actuation, and electronic properties. Several prototype smart material devices using nanotube arrays or nanotubes from the array are being developed by various groups that are co-authors of this chapter. The new devices include a biosensor, electrochemical actuator, nanotube probes, and a concept for a future in-body biosensor. Recently, aligned multi-wall carbon nanotube arrays over 1 cm tall were synthesized on large area substrates using a chemical vapor deposition process. The technique for growing nanotubes on large area substrates will open the door for low cost manufacturing of novel sensors, actuators, and devices for biology and medicine.

19.1 Introduction

Engineering materials have limitations in their physical or mechanical properties and biocompatibility for use in biomedical applications. Nanoengineering of

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smart materials is one approach to address the need for improved materials for biomedical applications. Nanoengineering can be described as the process of synthesizing (Louchev et al., 2002; Vinciguerra et al., 2003; Vajtai et al., 2004; Hata et al., 2004; Yarmolenko et al., 2005; Schulz et al., 2006; Tu et al., 2002, 2003;Shanov et al., 2006) unique almost defect-free multifunctional material systems and machines starting from the nanoscale up. Nanoengineering is a new frontier that mimics the chemical and evolutionary processes found in nature to develop new generations of smart materials and intelligent systems that can sense and respond to their environments. The research described in this chapter is based on recent advances in manufacturing high density multi-wall carbon nanotube (MWCNT) arrays of parallel nanotubes. The many potential applications for nanotubes have accelerated research into techniques for their synthesis and processing, and the fabrication of devices build using carbon nanotubes (CNT). CNT growth, for example, has recently been improved with the help of intensive research and material characterization (Hata et al., 2004).

CNT are an exciting and versatile material. Electrical conductance, high mechanical stiffness, light weight, electron-spin resonance, electrochemical actuation, transistor behavior, piezoresistance, contact resistance, coulomb drag power generation, thermal conductivity, luminescence, and the possibilities for functionalizing CNT to change their intrinsic properties are reasons for the excitement. Biomedical applications of nanotubes have an especially large and immediate potential. Three applications can be categorized as: (1) biomedical diagnostic techniques (e.g. a nanoelectrode to record electrical activities of neurons), (2) drug delivery, and (3) prostheses, implants (e.g. neuroprostheses), and scaffolds for cell culturing. Carbon nanotube arrays can be considered smart materials because nanotubes have high mechanical strength, electrical conductivity, and piezoresistive and electrochemical sensing and actuation properties. The small size of the nanotubes is also an advantage in many biological applications. Moreover, macroscale smart materials can be built using nanophase constituent materials. Different properties of nanotube arrays and intermediate forms of nanotubes, and their applications are discussed in this paper. The applications are all work in progress and are presented in hope of generating new ideas for processing and application of nanotubes that can benefit medicine. The basic properties of nanotubes are well covered in the literature and are not described here. Several near-term practical applications of nanotubes in biomedicine are also well covered in the literature. This chapter focuses more on advanced synthesis and potentially breakthrough and futuristic applications of nanotubes in medicine. Topics covered include synthesis of super long nanotubes and patterned nanotubes and large area arrays, hydrophobicity, surface tension-induced swelling behavior, actuation, and sensing properties, and beginning development of several devices based on nanotubes.

The benefit of all this nanotechnology in the end will be measured by its application. Development of smart materials based on nanotechnology is in the

beginning stages and has the potential to improve the way we generate and measure motion, and probe biomaterials from the nano to the macro scale. This chapter provides ideas how nanotubes can be put into applications. As you will see, nanotechnology is very interdisciplinary. Therefore, a large number of collaborators have contributed to this paper. It is felt that intersecting different technologies and investigating multiple applications has a synergistic effect and has enabled a lot of the progress in the field of nanomedicine. For example, a problem in developing nanoparticle contrast agents for magnetic resonance imaging is that nanotubes contain metal catalyst which must be removed. Removing the catalyst is a very difficult process. Recently carbon nanosphere chains are being developed for structural and electrical applications. The nanospheres are synthesized by a condensation process and are almost catalyst free. Thus, the nanospheres are a new material that may greatly simplify building nanoparticle contrast agents. As a second example, different substrates were being developed to grow nanotube arrays for medical applications. One substrate approach produced very long nanotubes, not useful for the intended medical application. However, the long nanotubes were ideal for structural reinforcement applications. Important is that in the end, applications come out.

19.2 Carbon Nanotube Array Synthesis

A brief overview of CNT synthesis is given here. Our group previously reported the synthesis of carbon nanotube arrays using water assisted chemical vapor deposition (Schulz et al., 2006; Shanov et al., 2006). Briefly, an electron-beam evaporator was used to deposit a 10-nm thick Al film on a Si/SiO_2 wafer, and the film was oxidized to produce Al_2O_3. Then, a composite catalyst (patent pending) was deposited on the $Si/SiO_2/Al_2O_3$ substrate. The nanotube array was then synthesized by thermal chemical vapor deposition (CVD) in a horizontal 2-in furnace (the EasyTube™ ET1000 by First Nano) furnace or a horizontal 3-in furnace (the EasyTube™ ET3000 by First Nano). Argon, ethylene, water, and hydrogen were used for deposition of the carbon nanotubes at a 750℃ growth temperature. The synthesized CNT arrays were characterized by environmental scanning electron microscopy (ESEM).

19.2.1 Array Synthesis

Nanotube arrays synthesized using an ET3000 furnace are shown in Figs. 19.1 and 19.2. The arrays were synthesized by First Nano Inc. using a substrate prepared at the University of Cincinnati. One-half of a 4 inch diameter substrate was used as shown in Fig. 19.1. The nanotube growth was about 11 mm. On a

smaller 5 mm square substrate shown in Fig. 19.2, using the same process conditions, the growth was 17 mm. Better gas diffusion may have caused the larger growth for the smaller substrate. These two examples demonstrate that nanotubes can be mass produced on large substrates and that long nanotubes can be grown. Furthermore, it is expected that both these results will be exceeded soon.

Figure 19.1 Nanotube large area (half of 4 inch dia wafer) array synthesized by First Nano Inc. using a UC prepared substrate and the EasyTube^{TM} ET3000 Nanofurnace produced by First Nano

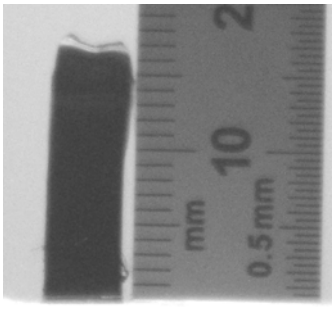


Figure 19.2 Nanotube long array synthesized by First Nano Inc. using a UC prepared substrate and the EasyTube^{TM} ET3000 nanofurnace produced by First Nano

19.2.2 Synthesis of Carbon Nanotube Towers

Carbon nanotube towers are more convenient than bulk arrays for use in making biosensors and actuators. Our group previously reported the synthesis of carbon nanotube arrays using water assisted chemical vapor deposition process (Schulz et al., 2006). Briefly, an electron-beam evaporator was used to deposit a 10-nm thick Al film on a Si/SiO_2 wafer, before the film was oxidized to produce Al_2O_3. Then, iron catalyst was deposited through a shadow mask on the $Si/SiO_2/Al_2O_3$ substrate (Fig. 19.3). The nanotube array was then synthesized by thermal chemical vapor deposition in a horizontal 2-in EasyTubeTM (ET1000, FirstNano) furnace. Argon, ethylene, water, and hydrogen were used for deposition of the carbon nanotubes. Our group (Shanov et al., 2006) reported the influence of the intermediate buffer layer, Al_2O_3(10 nm) on the growth of MWCNT arrays with Fe used as the active catalyst, which is an effective way to grow long high

density MWCNT arrays. SiO_2 on Si helps to prevent chemical reactions since pure Si can react with various catalysts, and the C in the catalyst might form silicates and C-silicate. Therefore, a chemically inert layer like SiO_2 helps to grow longer MWCNT arrays with high purity. The synthesized CNT arrays were characterized by environmental scanning electron microscopy.

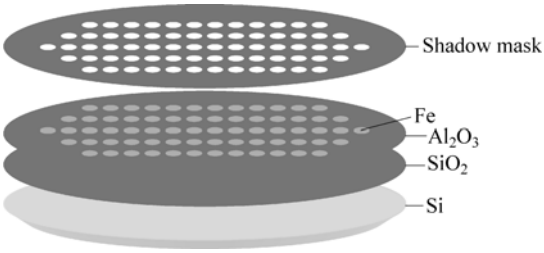


Figure 19.3 Patterned substrate preparation for nanotube array growth

Figures 19.4(a) – (c) show results of nanotube growth. Different magnifications are used in the images. Water vapor is used in the reaction to remove amorphous carbon. The $Fe/Al_2O_3/SiO_2/Si$ substrate is cut into 5 mm squares from one wafer and catalyst is patterned on 1 mm circles with 1 mm spacing between the circles. With the increase of growth time, the length of the CNT arrays increases for up to 10 hours. The goal is to grow nanotube towers in the mm length range. The mm-long nanotube posts are easy to handle to form devices.

The MWCNT array has high density without many impurities, which is ideal for further application development. Adhesion to the substrate is weak. One bundle of a MWCNT array block can be easily removed from the array using tweezers without damage. Figure 19.4(d) shows an 8 mm tall MWCNT patterned array on a Si wafer. Each tower (1 mm × 1 mm) of the patterned array contains around 1 billion nanotubes. The nanotube average diameter is 20 nm. The length to diameter aspect ratio is 200,000∶1. The surface area of each tower (for an average 20 nm diameter, 4 mm length nanotube) is 2500 mm^2. In order to measure the resistivity of nanotube tower, epoxy was case into a nanotube tower. Both ends of the nanocomposite tower were polished and wired using conductive epoxy. The volume resistivity of the tower was $0.11\,\Omega \cdot cm$. The resistivity could be reduced by reducing the contact resistance of the wire to nanotube connection.

19.2.3 CNT Array Nanoskin and Nanostrands

Nanoskin and nanostrand fibers shown in Fig. 19.5(a),(b), respectively, are two other geometries of nanotubes that can be patterned on substrates. The nanoskin and nanostrands can improve the properties of composites that might be used in

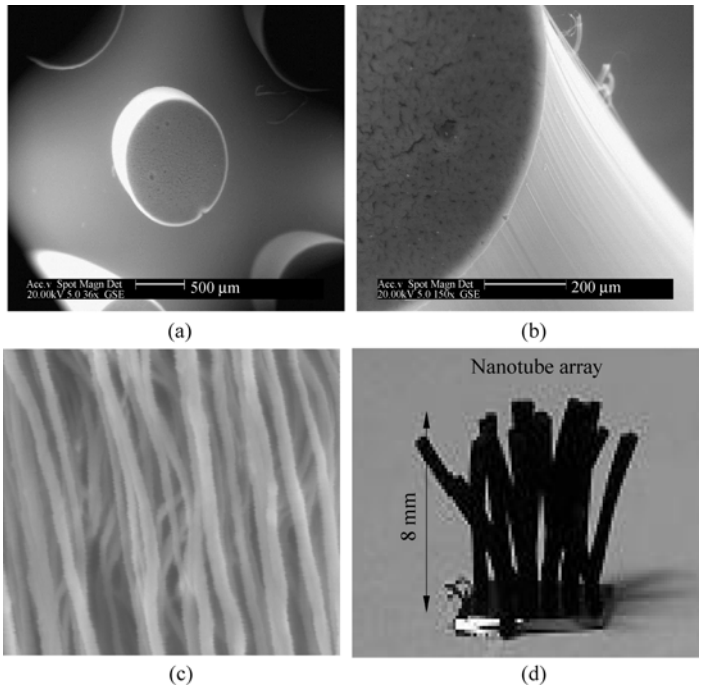
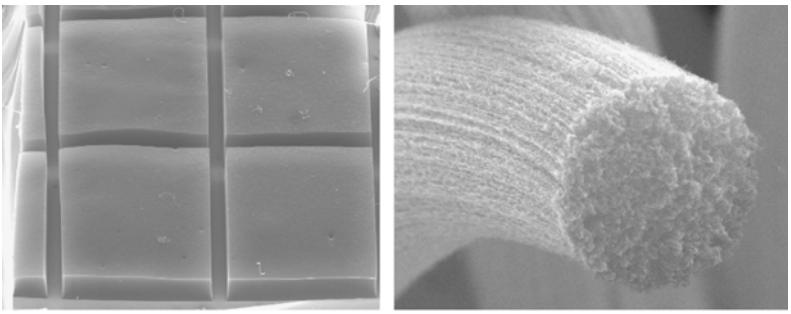


Figure 19.4 ESEM images of aligned multi-wall carbon nanotube patterned arrays: (a) one bundle of nanotubes called a tower. (b) top view of a nanotube tower. (c) high resolution side view of the nanotube tower. (d) picture of 8 mm tall MWCNT towers patterned on a Si wafer

biological applications, such as in prosthetics. CNT nanoskin can be strong, lightweight, absorb energy, reduce impact damage, and it can have electrical and thermal conductivity, and EMI shielding. The nanoskin can also be used for material health monitoring by sensing the electrochemical impedance of the material. The nanoskin can be filled with different polymer and elastomeric matrices, and the size of the nanotubes can be adjusted for use as a highly-compressible energy absorber. Tiles of nanotube arrays (Fig. 19.5(a)) can be formed into nanoskin for use on complex shapes.

CNT nanostrands (Fig. 19.5(b)) are micron diameter strands composed of millions of continuous multi-wall carbon nanotubes grown in a loose bundle by the chemical vapor deposition process on a silicon substrate. The nanostrands are similar to the nanotube towers but the strands are curved and longer than then towers. The nanostrands bend when as the length increases. The MWCNT forming the CNT nanostrands are 20 nm in outer diameter, 7 nm inner diameter, with about 15 concentric shells where the shells are separated by 0.34 nm. Potential applications of CNT nanostrands include polymer reinforcement, fibers for composite biomaterials, lightweight electrical wires, piezoresistive sensors for material health monitoring, sensors for biologic and haptic perception, and

electrochemical biosensors. The microfibers can be filled with polymers, elastomers, conductive polymers, or ceramics to form nanocomposite biomaterials.



(a) (b)

Figure 19.5 Nanotube materials: (a) 1 mm square tiles of arrays. (b) 7 micron diameter nanostrand containing millions of MWCNT's

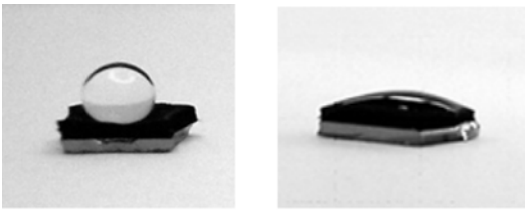
19.3 Properties of Carbon Nanotube Arrays

Different properties of CNT arrays that we consider to be smart material properties are discussed in this section. Smart materials possess multi-functional properties that can be used to sense or respond to their environment. Experimental studies of super-hydrophobicity and electro-wetting of nanotube carpet are important for processing the as-grown nanotubes, and to densify the carpet for some applications. The electro-wetting may also be used as an actuation property.

19.3.1 Hydrophobic Property

In Fig .19.6(a), a 10 μL drop of water is placed on the surface of a 1 mm long as-grown CNT array. The super-hydrophobic surface behavior is shown with an experimentally determined ($150^\circ \pm 5^\circ$) contact angle. The sliding angle was estimated as $20^\circ \pm 5^\circ$. The 1 mm long CNT carpet with about 10^8 CNT/cm^2 creates a large roughness resulting in the high contact angle. Shorter length CNT arrays had a lower contact angle. The CNT array can be chemically functionalized to make it more hydrophilic. It is first annealed at 500℃ for 5 h and immersed in concentrated 3∶1 H_2SO_4/70% HNO_3 for acid treatment. HCl is added to the acid mixture to facilitate the termination of the opened ends and defects of the nanotube array with carboxylic acid groups. Then nanotube array is rinsed with NaOH solution to neutralize the sample. The final sample is washed with distilled water. Figure 19.6(b) shows the water drop wetting the functionalized

array which has become hydrophilic. The hydrophobic property may be used to provide a synthetic material with selective interaction with biological materials.

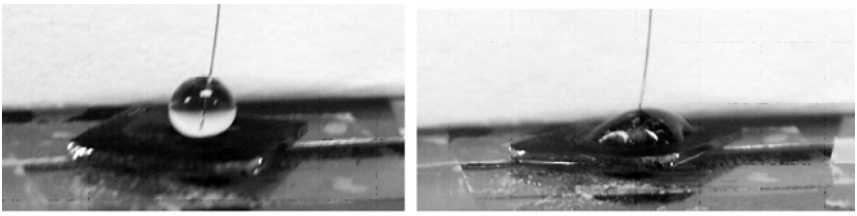


(a) (b)

Figure 19.6 Photo images of aligned multi-wall CNT array: (a) photo of a water droplet on a 1 mm long nanotube as grown array. (b) array after functionalization showing wetting of the surface

19.3.2 Electrowetting Property

Electrowetting is used for manipulating liquids on surfaces by applying small voltages. The electrostatic forces in electrowetting can reduce the contact angle and produce linear and oscillatory motion of entire droplets. It is demonstrated here that electrowetting can wet a CNT array that is hydrophobic. Figure 19.7 shows that a liquid drop, 10 μL of 1 mol/L NaCl, on a hydrophobic CNT array was made to wet the array by applying a small voltage (2 V) to the drop using a platinum wire. This experiment shows the potential to control the electrowetting property of the surface of a carbon nanotube array by connecting electrodes to the bottom of the nanotube array and applying a voltage. In this case, the nanotube electrode becomes permanently hydrophilic because the voltage caused the nanotube tips to be functionalized with hydroxyl or carboxyl groups. Reversing the electrowetting to re-form the droplet might be achieved by coating the carpet with parylene. Electrowetting gives freedom of water shedding design. This might be useful for example in biological implants and for body surfaces where control of the motion of fluid films is critical.



(a) (b)

Figure 19.7 Electrowetting on a CNT array: (a) platinum wire in the water drop at zero V. (b) with 2 V applied

19.3.3 Capillarity Property

Figures 19.8(a) – (c) show side-walls of an aligned nanotube array peeled off the Si wafer with 1 mm × 1 mm × 1 mm dimension. Even though it looks like the nanotube array is highly aligned at low magnification shown in Fig. 19.8(a), high resolution images in Figs. 19.8(b), (c) show the array has loosely oriented nanotubes with low density. In particular, the 1 mm long nanotube array behaves like a porous structure such as a sponge. Figures 19.8(d) – (f) show the dense structure obtained by infiltrating water into the array and evaporating the water. Since there is no holding force, the freestanding nanotube array shrinks freely without the generation of a crack when the water evaporates. The narrow spacing between the individual nanotubes creates a large surface tension. The huge surface area causes a large force and the array shrinks. Using acid instead of water and evaporating the liquid also causes the array to shrink.

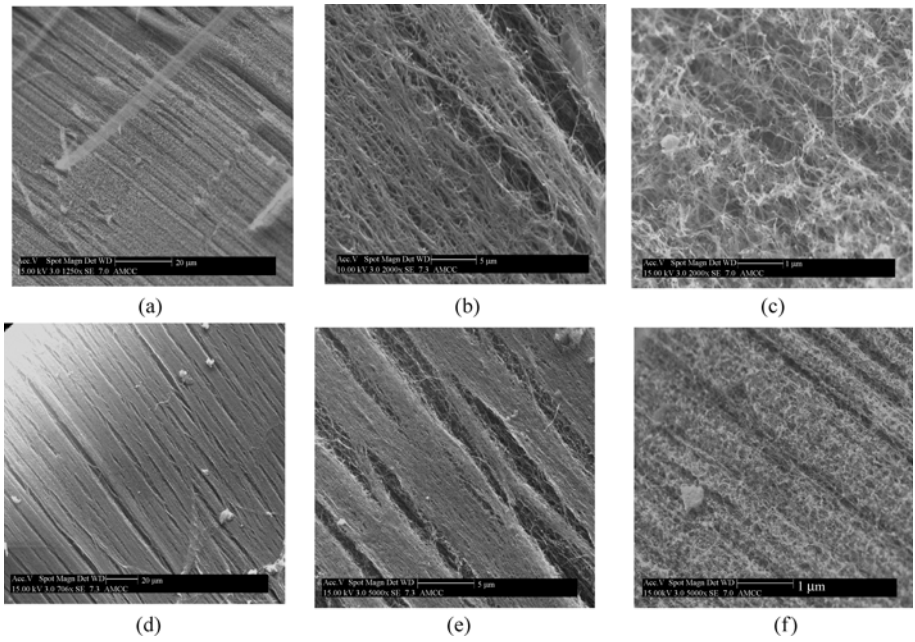


Figure 19.8 ESEM images of the side view of a CNT array as grown: (a) low magnification to (b),(c) high magnification. Filling the array with water or acid and evaporating the liquid causes the array to shrink. (d) low magnification to (f) high magnification

Figure 19.9 shows other views of the shrinking behavior of nanotube array. Even though adhesion between nanotube array and substrate creates a resistance to shrinking, overall, the capillarity force causes shrinking and creates a highly

aligned nanotube array with high density. Compared to previous short nanotube arrays, the long nanotube array creates a large surface tension resulting in a highly dense nanotube array. These results are very reversible for more than 20 times from the wet to the dry state. This self-assembly method is one approach to improve the alignment and increase the density of the CNT array. The expansion property based on the re-wetting mechanism may be explored to develop a new volume expansion-type of actuator that might be used in the body.

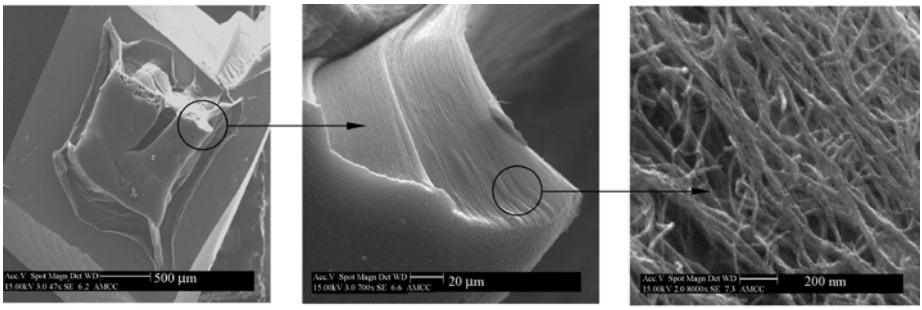


Figure 19.9 Dense CNT array on a Si wafer: (a) low magnification showing the shrinking of the array on the substrate, (b) structure of the array, and (c) high magnification showing individual nanotubes in the array

19.3.4 Nanotube Array Actuator

Based on the CNT towers which were synthesized, Fig. 19.10(a) shows the fabrication steps to make a nanotube tower electrode. First, the CNT tower is peeled off from the Si substrate by tweezers. One drop of preheated conductive epoxy is deposited on the glass substrate, and the nanotube tower is placed on the glass. A copper wire is connected to the conducting epoxy and cured in an oven at 80℃ for 1 h, as shown in Fig .19.10(b). The exposed conducting epoxy is sealed by Epon Resin 862 and EPICURE curing agent W at 120℃ for 4 h. Glass bead tape is pasted on the top of nanotube tower to reflect the laser displacement sensor optical signal.

The displacement of the nanotube tower actuator with applied voltage was measured using a laser displacement sensor (Keyence, LC-2400 Series) and a specially built test cell for characterizing the electrochemical properties of nanotubes, as shown in Fig. 19.10(c). Square wave potentials were applied between the working and counter electrodes using a National Instruments PCI board through a custom designed operational amplifier. Various square wave amplitudes are applied with frequencies ranging from 0.2 to 20 Hz. In order to supply enough power from the NI board, a voltage follower using a noninverting amplifier was designed using an operational amplifier with a gain of 10∶1.

An electrochemical impedance spectroscopy (EIS) analysis was performed using a three-electrode cell, with the nanotube tower actuator as the working electrode, a Ag/AgCl used as the reference electrode, and a platinum plate as the counter electrode. EIS measurements were performed using a Gamry Potentiostat (Model: PCI4/750) coupled with the EIS (Gamry, EIS300) software. The cell was equilibrated for several hours after each step. A 2 mol/L NaCl electrolyte solution is used for the experiments.

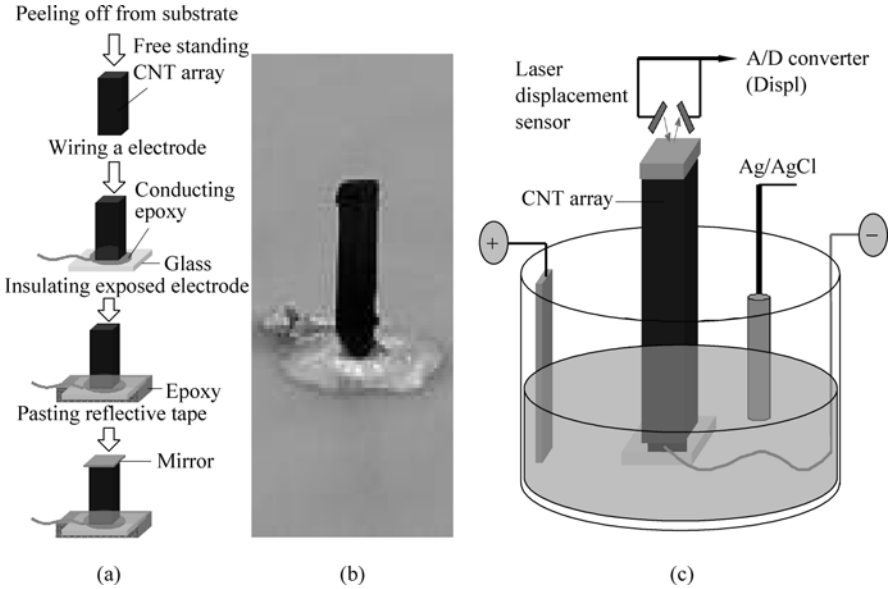


Figure 19.10 Testing an MWCNT tower actuator: (a) fabrication steps for the nanotube tower actuator. (b) the final fabricated actuator. and (c) the electrochemical analysis setup for testing the nanotube tower actuator, consisting of one nanotube tower which is fixed on a glass substrate, an Ag/AgCl reference electrode, and a Pt counter electrode

Figures 19.11(a),(b) show the relationship between the strain and the voltage applied to the nanotube tower actuator. As shown in Fig. 19.11(a), there is a non-uniform strain response with the square wave input. This problem would be solved by depositing an electrode layer on the top of the nanotube tower to bind the CNT electrodes to provide a higher voltage and uniform displacement. Strain of the nanotube tower closely follows the applied square wave potential of ± 2 V. With the increase of frequency, strain of the nanotube array decreases as shown in Fig. 19.11(b). The exact actuation behavior of the MWCNT needs further study to determine if the strain is uniform along the length and if only the outer surface of the nanotubes are expanding and actuating. If all the shells of the MWCNT could be made to actuate, the force would be the greatest possible. The results herein verified the actuation effect and that increasing the magnitude of

the applied potential increases the strain. Probably the higher potential increases the charge accumulation at the nanotube tower/electrolyte interface and causes the faster response. However, too high of voltage would cause electrolysis of water and generate the bubbles on the surface of nanotube tower. This would decrease the lifetime of the actuator. Therefore, there is some limitation to increasing voltage to achieve high strain. A maximum strain of 2% has been predicted (Baughman et al., 1999) based on basal plane theory, but actual strains are up to 0.1% based on film-type single wall carbon nanotube actuators. The nanotube tower actuator shows strain up to 0.15%. Probably the straight aligned nanotube tower is the reason for higher strain comparing to entangled nanotube-film actuators. Excellent mechanical properties and good strain generation of the nanotube tower actuator might provide a solution for a new actuator material. Compared to the best-known ferroelectric, electrostrictive, and magnetostrictive materials, the low driving voltage of the CNT tower actuator is an advantage for various future applications such as smart structures, multi-link active catheters, artificial muscle, micro-pumps, molecular motors, or nano-robots. Another advantage is direct conversion of electrical energy to mechanical energy resulting in high strain generation. The high strength and high elastic modulus of nanotubes potentially might generate large forces during actuation. Since faradaic actuators basically come from the charge-discharge-charge method like a battery, it might be possible to design a self-powered actuator which means this device can actuate motion by storing capacitive charge in the nanotube structure. Power harvesting and strain sensing are other possible applications of the nanotube electrochemical tower. One other consideration when making the actuator is that the individual nanotubes are very long and winding and would buckle under a small load. Stabilizing nanotubes against buckling while still allowing access to the electrolyte is an area of further research. Smart materials using nanoscale particles are discussed.

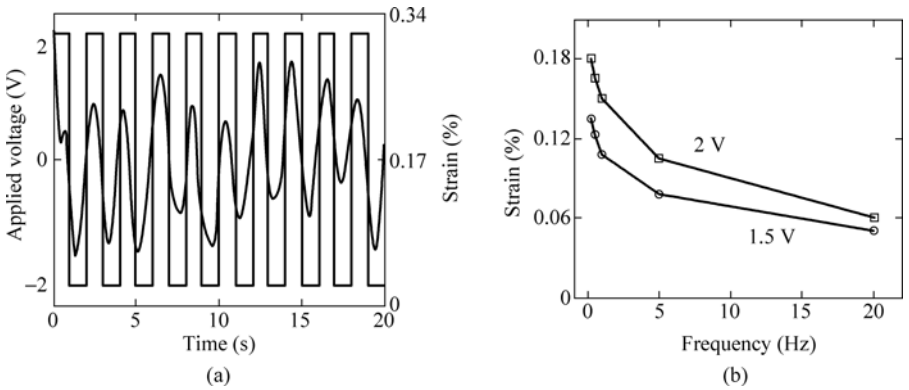


Figure 19.11 Strain of the nanotube tower: (a) at excitation frequency of 0.5 Hz, and (b) strain as a function of frequency and applied square wave voltage

19.4 Potential Applications of Nanotube Arrays In Biology and Medicine

The morphology and some of the properties of nanotubes were described. Now areas where CNT arrays and CNT can be put into applications in biology and medicine are described. An area of great interest across the world is nanoengineering of electronic devices that can detect disease in vitro using samples of blood, serum, or tissue. A more futurist area is to develop devices that can go inside the body to repair, monitor, and control biological systems. Overall, there is great opportunity to develop nanoscale smart materials for biology and medicine because of the many possibilities to engineer devices that work at the scale of biological molecules (the nanoscale) or cells (the micro-scale). Smart nanoscale materials and their devices would need some of the following capabilities depending on their specific application and whether the application is in vivo or in vitro: (1) a safe biocompatible material used for tissue scaffolding, bone repair; (2) power and communication by RF, or magnetically, or by light; (3) propulsion by shape control or vibration or magnetism; (4) illumination and vision; (5) drug release; (6) a piezoelectric shape changing particle; (7) power harvesting in ionic fluid; (8) materials to repair bone and cells; (9) a device to sample fluids in the body and return to a docking station in the body; (10) a contrast agent material; (11) stents; (12) magnetic collection; (13) an electrode to measure potentials; (14) a voltage source to control calcium channel activation and membrane potential; (15) surgical tools; (16) biosensing to detect disease; (17) ultrasound to break up blood clots while circulating in the arteries; (18) RF hypethermia to ablate and kill cancer cells; (19) strong wear resistant composite prostheses, and there are other properties that can be thought of.

Surprisingly, significant progress has already been made toward developing several of these capabilities. Advances include nanotube impedance biosensors and nanoparticles dispersed in polymers or electrolytes to provide electrical properties that can be interrogated to detect disease, and concepts and initial design for in body biosensors. In the literature, testing of nanotube biosensors with high sensitivity (Yun et al., 2007a, 2007b, 2006, in press, accepted; Li et al., 2003; Koehne et al., 2004; Schulz et al., 2007), and concept designs for nanobots and devices are reported. Future sensing applications will require specialized nano-electro-mechanical-systems (NEMS) such as sensor-transponders that can detect growth of cancer cells or overexpression of cytokines in the human body. Smart materials may also harvest power, actuate, and communicate with computers remotely. Design of nanostructured smart materials, devices, and interfaces will also provide tools for making advances in synthetic biology and systems biology. Nanoscale electrodes and biosensors may provide dynamic responses of cell signaling than can help model and understand systems biology. The following sections outline some early applications of nanoscale smart materials.

19.4.1 Electronic Biosensors

There are an increasing number of studies to develop biosensors using CNT. There are also a few studies using EIS and CNT to develop a biosensor. This approach is promising because the small electrode is coupled with an impedance analysis which is more sensitive than typical cyclic voltammetry (CV) analyses. This section reports fabrication of a nanotube array electrode and the immobilization of anti-mouse IgG on the open-end of the nanotube. The EIS of the nanotube-based biosensor was then tested under different concentrations of mouse-IgG. Based on a simple equivalent circuit, the relationship between electron transfer resistance and concentration of IgG was studied.

Figure 19.12 shows the fabrication steps to construct the nanotube tower electrode. First, the CNT tower was peeled off the Si substrate by a pair of tweezers and the array was cast in epoxy. The bottom section of a CNT tower was then polished and conducting epoxy was used to connect a Cu wire to the CNT tower. Then, the conducting portion of the nanotube was insulated using nonconductive epoxy. The top section of the array was polished to expose the nano-electrode.

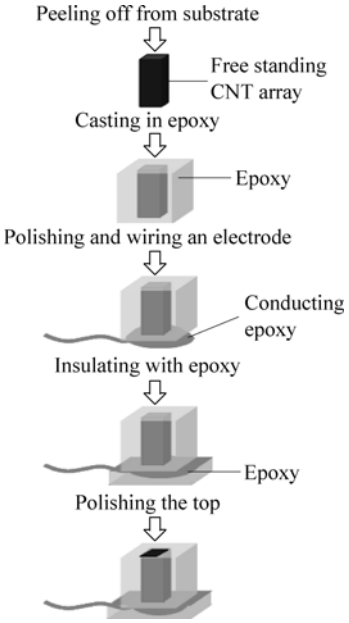


Figure 19.12 Tower electrode fabrication

Functionalization of the nanotube electrode is to attach special chemical groups to open-ended nanotubes that can act as receptors for other molecules. Electrochemical treatment at 1.5 V (versus Ag/AgCl) in 1.0 mol/L NaOH for 10 – 30 s was used to create carboxylic groups on the end of nanotube array. After functionalizing the nanotube electrodes with carboxylic groups, 1-Ethyl-3-

[3-dimethyalminopropyl] carbodimide hydrochloride (EDC) with sulfo-NHS was used to immobilize antibody to nanotube array. The electrode was immediately incubated for approximately 20 min with 10 mg EDC in 500 mL 2-(N-morpholio) ethanesulfonic acid (MES) buffer. Then the electrode was removed from EDC solution and immediately put into NHS solution (5 mg N-hydroxysuccinimide NHS in 500 μL MES buffer) for 20 min. The nanotube electrode was then immersed in 1% ethanol amine solution. Finally, the nanotube electrode was immersed in anti-mouse IgG solution (20 μL of donkey anti-mouse IgG in 1 mL PBS, pH 7.0) and incubated for 4 h. After rinsing with cold water, the sensor electrode was immersed into different concentrations of mouse IgG solution. Fig. 19.13(a) shows the final structure of the nanotube immunosensor. The EIS was performed using a three-electrode cell, with the nanotube electrode as the working electrode, an Ag/AgCl electrode used as the reference electrode, and a platinum wire as the counter electrode, as shown in Fig. 19.13(b). EIS measurements were performed using a Gamry Potentiostat (Model: PCI4/750) coupled with the EIS (Gamry, EIS300) software, Fig. 19.13(b). All testing was done at 0 V DC and 0.1 Hz to 300 kHz, and the sinusoidal potential magnitude is ± 20 mV in the redox probe 5 mmol/L $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$ with PBS (pH 7.0).

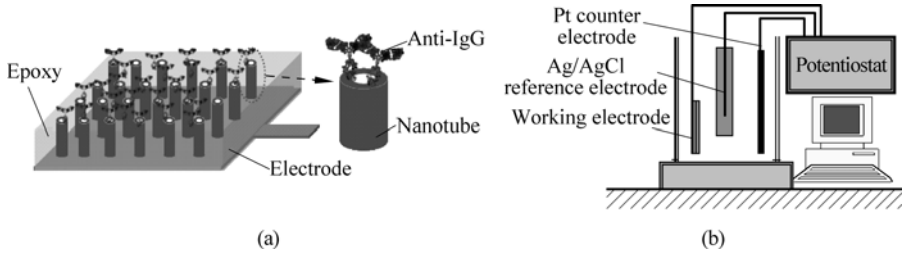


Figure 19.13 Nanotube array biosensor: (a) final structure of the nanotube immunosensor, and (b) test setup

Electron transfer of the redox couple is hindered by immobilization of the antibody and binding of the antigen. At the same time, the electron transfer resistance can be computed based on EIS measurements. Modeling the EIS response can be done using Randle's circuit. Randles circuit is an equivalent circuit representing each component at the interface and in the solution during an electrochemical reaction for comparison with the physical components; C_{dl}, the double layer capacitor; R_{et}, the electron transfer or polarization resistance; and R_s, the solution resistance. Randles circuit can be expressed as

$$Z(\omega) = R_{\mathrm{et}} + \frac{R_{\mathrm{et}}}{1+\omega^2 R_{\mathrm{et}}^2 C_{\mathrm{dl}}^2} - \frac{\mathrm{j}\omega R_{\mathrm{et}}^2 C_{\mathrm{dl}}}{1+\omega^2 R_{\mathrm{et}}^2 C_{\mathrm{dl}}^2} = Z_{\mathrm{real}} + \mathrm{j}Z_{\mathrm{imag}} \tag{19.1}$$

The EIS response of Randle circuit is illustrated in Fig. 19.14(a). The diameter of the semicircle in the EIS Nyquist plot is called the electron transfer resistance,

R_{et}. The electron transfer resistance shows the electron transfer kinetics of the redox probe at the electrode diffusion layer. As shown in Fig. 19. 14(b), the electron transfer resistance, e.g., the diameter of semicircle, kept increasing after immobilization of the antibody and then the binding with the antigen. The EIS results show the typical Randles circuit response (Yun et al., 2007a, 2007b, 2006, in press, accepted; Li et al., 2003; Koehne et al., 2004; Schulz et al., 2007).

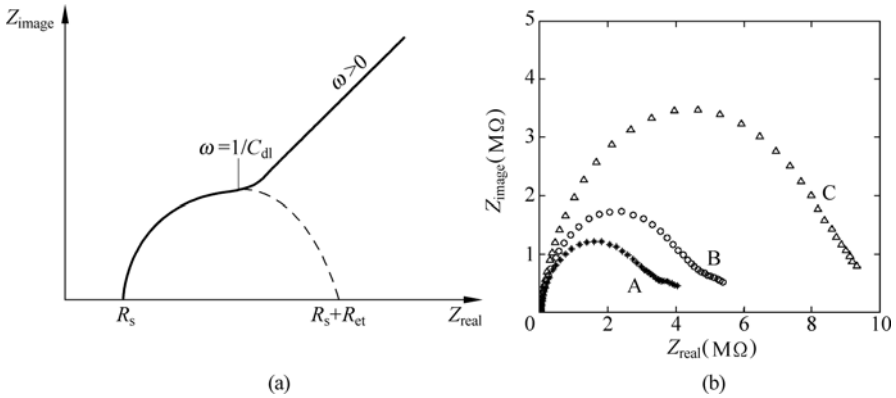


Figure 19.14 EIS theory and experimental result: (a) EIS Nyquist plot based on the theoretical Randles circuit; and (b) EIS for the nanotube electrode, after (a) functionalized nanotube array; (b) immobilized Donkey anti-mouse IgG; and (c) binding Mouse IgG. All experimental results are at a DC potential of 0 V, frequencies between 0.1 Hz and 300 kHz., and a sinusoidal potential magnitude of ± 20 mV in 5 mmol/L $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$ with PBS (pH 7.0)

Using the equivalent circuit model, the values of the curve fit parameters based on the experimental results in Fig. 19.14(b) are shown in Table 19. 1. Since the solution resistance represents the bulk properties of the electrolyte solution and diffusion of the applied redox probe, $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$, they are not affected much by the nanotube electrode chemical transformation. On the other hand, the double layer capacitance and electron transfer resistance represent the interface property of the electrode/electrolyte and will change due to insulating or coating the nanotube surface. As shown in Table 19.1, the double layer capacitance change is not as sensitive as the electron transfer resistance. The change in electron transfer resistance before and after adding the donkey anti-mouse IgG and the mouse IgG reaction is dramatically increased to 3500 kΩ. Therefore, the electron transfer resistance change, ΔR_{et} is chosen as the most sensitive parameter to indicate the detection of antibodies using the immunosensor. Future work is aimed at reducing the size of the electrode and to improve the sensitivity. The advantage of the electronic biosensor is that a fast result is obtained from a relatively simple test. This type of sensor may be useful to save time and reduce costs in clinical testing, for use as a portable at home test, and for use in remote regions such as Africa to check for disease.

Table 19.1 Estimated parameters for Randles equivalent circuit model

	R_{et} (MΩ)	C_{dl} (nF)	R_s (kΩ)
Bare electrode	2.9	2.5	6.4
Ab	4.0	2.0	7.2
Ab-Ag	7.5	2.7	7.7

19.4.2 Nanotube Electrodes for Biovoltage and Chemical Sensing

MWCNT arrays can be used as probes and needles. Figure 19.15(a) shows the experimental setup for welding nanotubes on a tungsten needle. An electronic micrometer was modified to hold two electrodes with one electrode fixed to a tungsten needle (Earnest F Fullam Inc.) for welding to nanotubes. The nanotube array is attached to the other electrode using carbon tape for holding scanning electron microscope samples. The nanotube welding is done by moving the needle toward the nanotube as shown in Fig. 19.15(b) and applying a voltage. High current makes the nanotube weld to the needle. Figure 9.15(c) shows welded nanotubes on tungsten tips. The nanotube tip diameters are about 200 nm. The welding is done in an inert atmosphere such as argon or nitrogen in a plastic glove box. Welding nanotubes to nanotubes is also being investigated. The nanotube probes can be used as a smaller biosensor to increase sensitivity for cancer detection, as electrodes for probing the neuronal response of cells in electrophysiology, for locating centers of epilepsy in the field of neurology, and for detecting chemical such as neurotransmitters. A variation on the nanotube electrode is do disperse nanotubes into eposy and for a needle. A nanotube composite microelectrode for monitoring dopamine levels using cyclic voltammetry and differential pulse voltammetry is discussed (Yun et al., in press).

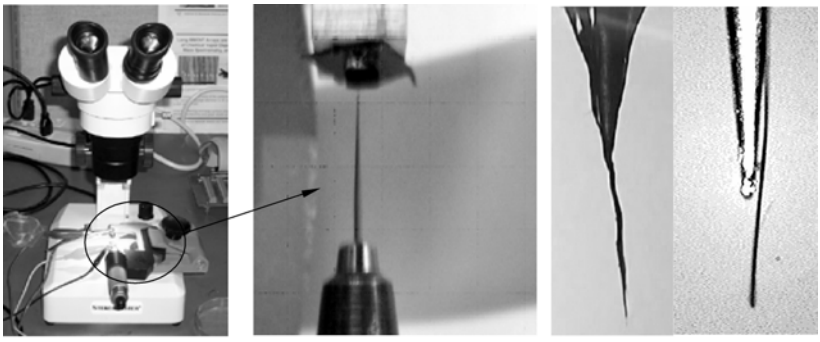


Figure 19.15 Experimental setup for welding CNT on a tungsten needle: (a) an optical stereo microscope. (b) probe tip positioned for welding to a nanotube array. (c) welded CNT bundles on tips of tungsten probes

19.4.3 Carbon Nanotube Sensor Film for Environmental Monitoring

Monitoring the environment for chemicals or biological agents may be done based on the biosensor using the EIS method. In this approach CNT film sensors are highly distributed on the surface of clothing to detect chemicals or biocides. An example of nanotube film (Kang et al., 2006a, 2006b) on a simple panel is shown in Fig. 19.16. CNT thread could be used in place of the film.

Figure 19.16 Spray-on carbon nanotube neurons in a grid pattern for environmental monitoring

The EIS system is a highly distributed network of CNT continuous film sensors that sense along their entire length and have a biomimetic architecture that allows coverage of large areas (Kang et al., 2006a). A 2 row and 2 column neuron prototype of the sensor is shown as a grid pattern in Fig. 19.16. The rows overlap the column neurons and are electrically insulated. The films can be independently connected or connected in series or parallel, or a combination of series and parallel depending on the sensitivity and coverage area desired. Chemicals or a liquid electrolyte can be detected by the film. The electrical impedance increases with concentration of the analyte. An example of the change in capacitance as electrolyte is put on a single sensor film is shown in Fig. 17. Up to a factor of 50 increase in capacitance occurs because of the double layer supercapacitance property of nanofibers. The change in resistance of the film due to the electrolyte is 6%. The electrical impedance properties of the sensor film can be used to characterize environmental contamination. Each contaminant may have a different electrical impedance spectrum. A table look up can be used to identify the contaminant. To improve selectivity, the nanotube film can be functionalized (chemically modified) to react with specific analytes, gases or liquids. The huge effective surface area of the film is an advantage for a gas sensor.

Using the electrical impedance (EI) analysis, long continuous sensors using spray-on nanotube film or embedded thread can be developed that significantly

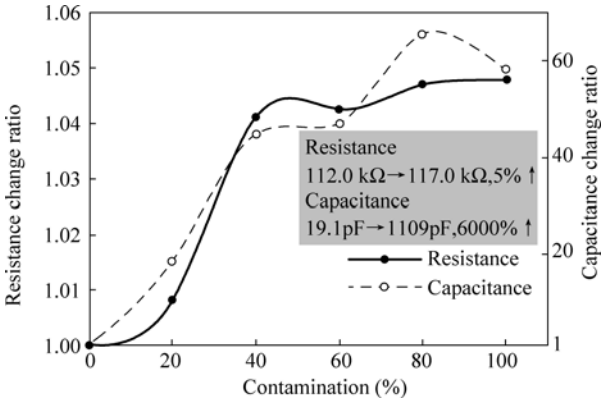


Figure 19.17 Electrolyte changes capacitance of the nanotube film, but resistance is almost constant

reduce the complexity and cost of environmental monitoring. A continuous sensor is a single long sensor that has one output signal. The electrochemical impedance of the thread is monitored continuously. An anomalous response from any section of the long sensor can be detected. Thus, a continuous sensor has only one output signal and can replace many individual sensors and still detect abnormal events. The thread will sense an electrolyte or gas. EIS based analysis at high frequency will be used to monitor the thread and to identify chemicals and ions in the environment or biological fluids. A Gamry Potentiostat is used for electrochemistry and impedance monitoring, as discussed in the sections on biosensing and actuation. The sensor can be modeled to represent EIS measurements using the Randles-Warburg model. Randles circuit is an equivalent circuit representing each component at the interface and in the thread during an electrochemical reaction for comparison with physical components. Randles Warburg parameters will be related to contamination in the environment.

19.4.4 Nanocomposite Materials for Biological Applications

Engineers face broad challenges in developing materials for biological applications like prosthetics. One attribute of smart materials is that they should be self-reliant which means they should be capable of resisting damage and continuously monitoring their condition. In some cases, the system or structure should also be capable of responding to its environment to improve performance or prevent degradation including self-repair of small damage. Materials with nanoscale features are important because almost all solid matter with nanoscale size has new or improved properties compared to bulk materials. These properties depend on the composition, size, and shape of the material, and include high specific strength and modulus, high electrical and thermal conductivity, a large surface

area to volume ratio, nearly defect-free structure, and sensing and actuation properties. Carbon nanotube materials can form new composite materials (He et al., accepted; Shi et al., 2006; Kang et al., in press; Gao et al., 2006, 2007) that are strong and smart and thus may meet the requirements for biological applications. Composite materials for use in the body may consist of a nanoscale constituent material processed to an intermediate stage or combined with a matrix material to produce a durable macro-scale material that has multifunctional properties including sensing or actuation. Available nanoscale constituent materials include nanotubes, nanobelts, nanowires, and nanocoils made of carbon, silicon, nickel and other materials. The main advantage of nanostructured smart materials is that their mechanical, electronic, magnetic, optical, and thermal properties can be tailored to a specific bio application. Developing nanostructured smart materials for biology is a new area of research. To develop this area, three steps are suggested; (1) study the properties of nanoscale smart materials and develop a data base of the material properties and commercial availability of the materials, (2) use long nanotube materials to develop new intermediate forms of macro-scale smart materials such as thread, and (3) outline advantages that nanostructured smart materials would have in specific biological and medical applications.

Overall, this section proposes techniques for integrating nanoscale materials or intermediate components into polymers for use in advanced biological applications that can self-test and monitor their condition to provide enduring performance and safety. Materials that will be considered include super long carbon nanotubes, magnetic nickel nanowires (NW), and catalyst-free electrically conductive carbon nanosphere chains (CNSC). A take-home message from this chapter is that 'Nanoizing' materials is becoming a new technological science that should be put into widespread application by the bioengineering community.

19.4.4.1 Tailored Composites Using Carbon Nanotube Thread

A breakthrough application of long nanotubes is to spin thread. Long carbon nanotubes (CNT) called 'Black Cotton^{TM}' are being used to spin thread to provide reinforcement, sensing, and actuation simultaneously. An application example is using carbon nanotube thread/cloth to develop prosthetics with the desired properties in each direction. These structures will also evaluate and monitor their own integrity. Thread or rope/cables made from thread are the most basic structural elements that can be used alone as cables or muscle, or embedded in composite materials. Commercial scale quantities of the thread using the long carbon nanotubes grown in arrays may be available in 2008. The thread may provide the following properties for biological applications depending on the application: (1) reinforcement; (2) an antenna; (3) harvest a small amount of energy from ionic flow; (4) as a wet actuator; (5) chemical and biosensors; (6) a partial self-repair material; and (7) the nanotube thread can be used to reinforce polymers, elastomers, and possibly ceramics and powdered metals.

19.4.4.2 Smart Elastomer

New types of smart materials will be developed by loading CNT (Kang et al., 2006a, 2006c; Smart Materials Naon Lab at UC), CNT thread, CNSC, nickel nanowires or other nanoparticles into an elastomer such as polyurethane (PU) and forming a strain or magnetic sensor, actuator (to morph the material), or power harvester. A variation of this approach is to fill the grown long CNT arrays with PU. The smart elastomer will have electrical and thermal conduction, piezoresistive and piezomagnetic properties, and power harvesting properties. Initial experiments successfully integrated CNSC into PU. One application is to develop a sensor material that can be used in biomechanics studies. Forces generated in the material can be used to diagnose and predict several gait and safety problems, or to measure forces inside the body. The working principle of the smart material sensor considers forces generated in the material which are the normal force and shear force. Nanotube thread sensors are embedded in two directions in the elastomer, as shown in Fig. 19.18. The resistance of the thread changes with varying load. An analysis of the strains with two sensors embedded was performed. Red is the compressive strain sensor (vertical) and green is the shear strain sensor (at 45°). Stresses and forces can be computed knowing the strains. The normal compressive stress is $\sigma = E\varepsilon,$ where E is the elastic modulus and ε is the normal strain. The shear stress is $\tau = G\gamma$, where G is the shear modulus and γ is the shear strain. The normal force is $N = \sigma A,$ where A is the cross-sectional area. The horizontal shear force is $H = \tau A.$ The coefficient of friction can also be computed as $\mu = H / N.$ By measuring the normal and shear strains, the normal and shear forces can be determined and the minimum coefficient of friction computed. Slipping can be determined by monitoring the H and N forces and friction coefficient. Relationships between strains at the sensors and the x, y, and shear strains are

$$\varepsilon_{\theta_1} = \varepsilon_x \cos^2\theta_1 + \varepsilon_y \sin^2\theta_1 + \gamma_{xy} \sin\theta_1 \cos\theta_1,$$
$$\varepsilon_{\theta_2} = \varepsilon_x \cos^2\theta_2 + \varepsilon_y \sin^2\theta_2 + \gamma_{xy} \sin\theta_2 \cos\theta_2,$$
$$\varepsilon_{\theta_3} = \varepsilon_x \cos^2\theta_3 + \varepsilon_y \sin^2\theta_3 + \gamma_{xy} \sin\theta_3 \cos\theta_3.$$

These equations can be used to compute strains at any angle where the 'strain gage' threads are located. In the future, we envision adding an active friction modulator to the elastomer controlled by the friction sensor. Therefore, polymers and elastomers can be formed with CNT thread which reinforces the material and also acts as a sensor. Moreover, if the CNT thread is used in an electrolyte, it will have an actuation property. The effectiveness of the actuation is to be determined and is a good future research project.

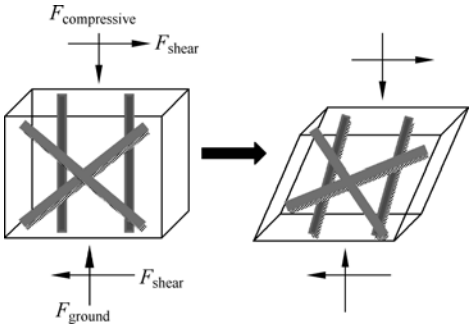


Figure 19.18 Nanotube thread sensors embedded in two directions in an elastomer

19.4.5 In-Body Biosensors: Optimistic Hopes and Wildest Outlook

This section gives ideas about producing biosensors that can go inside the body. This technology is futuristic but is becoming more feasible because of nanoscale materials. There are many compelling reasons for wanting to implant or temporarily put medical devices inside the body. Thus far, technologically, we have only been able to develop simple prosthetics that can go inside the body. But this is changing. Nanoscale materials are opening up the possibility to build revolutionary devices and tiny machines that can go inside the body and do what we want them to. This section describes a concept for an active biosensor that can go inside the body and detect disease early. Active biosensors perform electrochemical measurements and produce an electronic signal that is used to identify specific proteins, cells, ions or chemical species in body solutions. The active biosensor also has a feedback mechanism to increase its sensitivity or to activate a control function. The active biosensor is being designed and will be built using larger electronic components first, and finally pushed down in size using nanoscale materials.

Building small sensors and communicating with them is still an enormous technological problem. To attack this problem, nanoscale smart materials including nanotubes, nanowires, and nanobelts will be used to form nanostructured electronic components such as capacitors, inductors, solenoids, antennas, transistors, piezoresistive sensors, and electrochemical actuators. A four-arm nanomanipulator will be used for making prototype devices. The nanomanipulator operates under an environmental scanning electron microscope and can be used to assemble components into prototype biosensor devices. Bio-fouling and antenna design have been two long-standing obstacles to in-body sensor development and must be solved. Development of the In-Body Biosensor (IBB) will lay the framework to help to solve one of the grand challenges of medicine—putting medical devices in the body. The many possible applications for in-body sensors and medical devices include monitoring neurotransmitter signaling, wireless release of drugs in

the sensor to kill cancer, sensor heating to kill cancer remotely, monitoring ions related to toxic metals, measuring temperature, pH, vibration due to blood clotting, and axonal responses. Many states in the body can be measured without using antibodies. But we may also consider using antibodies, aptamers, lectins, etc. The antibody has a limited lifetime and is not reusable, and this is a fundamental limitation for use with IBB.

19.4.5.1 Prior Art

Medical device manufacturers and researchers have been trying to put biosensors in the body and have prototype glucose sensors that have not been very accurate. Biofouling is the major obstacle. The sensor becomes covered with proteins and the sensitivity goes down. It is likely that the biofouling problem will require a solution other than through biochemistry, e.g. by poly-ethylene-glycol (PEG) or other coatings. An active biosensor is needed to mechanically overcome biofouling. Active biosensors are electromechanical devices that produce an electronic signal related to the concentration of analytes in body solutions. The active biosensor also uses feedback to improve sensitivity or activate a mechanical device that affects the analyte.

Biosensing has characteristically relied on standard sandwich bioaffinity assays (antibody-antigen) in connection with enzyme, fluorophore, or nanoparticle labels to detect disease-related proteins and toxins. The electronic biosensor proposed ideally would be non-functionalized (no antibody or binding reaction is used) and the sensor would respond directly to the analyte, which is required for long term in-body use. This type of biosensor for protein detection will be less sensitive and selective than conventional immunosensors. However, the in-body biosensor may detect ions, chemicals, and proteins in high concentrations. In some applications a low detection limit is not as important as detecting protein over-expression when it first occurs. In certain applications the biosensor will measure membrane potentials or nerve responses. The biosensor should be developed in stages; (1) first for in vitro use, (2) then for in vivo use. The active biosensor will be biocompatible and responsive to environmental and external stimuli, and will use electrochemical impedance (EI) (Yun et al., 2007) to monitor the concentration of proteins, chemicals, and ions in the body, including Mg^{2+} and Ca^{2+}. A test bed should be set up to evaluate and compare biosensors to the gold standard Enzyme-linked Immunosorbent Assay (ELISA) method.

19.4.5.2 Sensor Initial Design

Impedance based biosensors are useful to identify chemicals and ions in biological fluids. Requirements for the sensors are high sensitivity, high selectivity, and fast response. The basic sensor platform we have been developing uses carbon nanotubes. Advantages of CNT electrodes for biosensors include high electrical

conductivity, chemically inert, high mechanical modulus and strength, continuous measurements, and nano-scale size. A nanogap electrode is under development and will be used to obtain a high frequency impedance signature of ions in solution. A Gamry Potentiostat will be used for electrochemistry. LABVIEW will be use to develop the wireless control method.

The concept for an initial sensor design is briefly discussed. The sensor may be cylindrical with a coil antenna on the outside. The sensor receives an Radio frequency (RF) signal. Nanowires rectify the signal to charge nanotube capacitors. The circuit receives a high frequency signal and reflects it back. The frequency of the reflected signal depends on the impedance of the analyte thus detecting ion or protein concentrations. The sensor could also be used to measure axon potential, membrane potential, apply voltage or heat cells, deliver drugs, sample fluids, and do other tasks. The biosensor can go inside a living organism (plant and animal) and in particular the human body to detect disease and to understand cellular processes. The plan to develop the IBB has the following points: (1) Modeling. Develop a full electrical model of the sensor; (2) Large scale prototype. Build a cm size prototype of the sensor to demonstrate the principle**;** (3) Preliminary analyses for long-term development paths. This constitutes preliminary work for the eventual use of sensors in the body and includes: (a) test remote electrodes on the skin; (b) propagation of a biosignal through blood plasma; (c) nanocircuit analysis; (d) EIS modeling; (e) high frequency impedance; and (f) electrodes to prevent biofouling for long term in-body use; (4) Synthesis of nanoscale materials. Different appropriate processing techniques will be used for synthesis of the required nanoscale materials; (5) Nanostructured sensor design and fabrication. Nanoscale smart materials will be used to make a small sensor that will be tested in vitro.

This work will lay the groundwork for pushing down in size so the sensor could be used in the body (Schulz et al., 2007). A sensor may also be heated and used for destroying cancerous tissues. Doctors will be cautious about allowing nanosensors to float freely through the body. If sensors blocked a blood vessel it could cause a clot. Initially the sensors would be stationary in the body. Later they could circulate in blood when the size is small enough. Current within the biosensor would be induced much in the same way that a radio-frequency identification (RFID) chip works. Building small sensors and developing a way to communicate with these sensors is a large technological challenge. Approaches are also being considered to mass-produce the devices. A nanomanipulator (Fig. 19.19) can be used to develop a biosensor-transponder sensor. The sensor may also detect the growth of cancer cells or overexpression of cytokines in the human body. The sensor may also harvest power and communicate with a personal digital assistant (PDA) wirelessly.

It may be worthwhile to also consider the possibility of interfacing electronic devices with the human power and electrical system (muscles and neural system). Converting power in the body to electrical power may be more feasible than

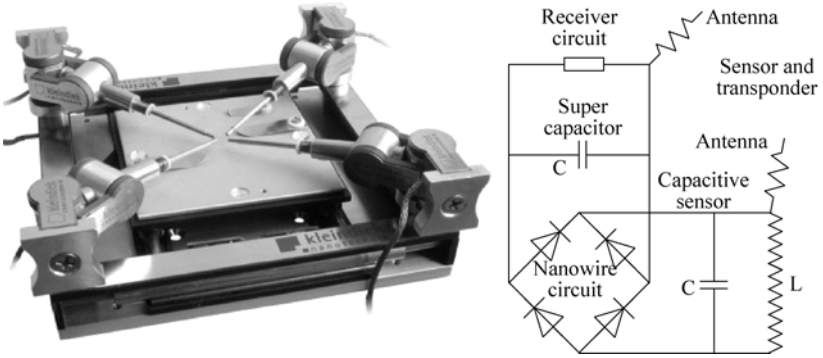


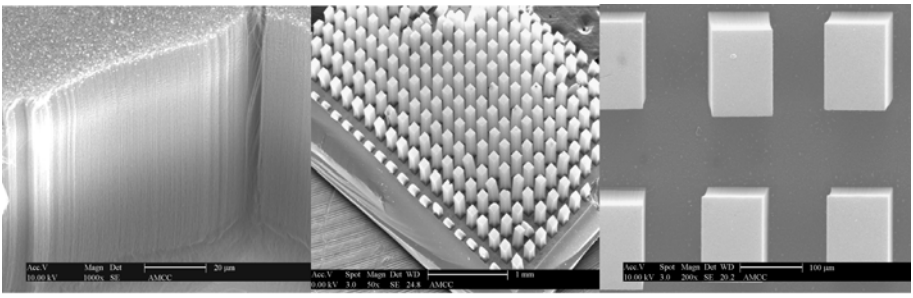
Figure 19.19 Nano-manipulator and initial sensor design

using RF or magnetic coupling of power to the biosensor. In the future, biosensors may interface with the biological neural system for in-messaging (control) and out-messaging (feedback). Use of biological material as a sensor to avoid the biocompatibility problem and to interface with the human neural system may also be considered.

19.4.6 Investigating Neuronal Activity and Function Using Nanotubes

Neurological disorders are thought to result from disruption of either normal neuronal function, which involves neuronal electrical activity, disruption of neuron or neuronal process migration that is necessary for regeneration after nerve injury, or disruption of developmental processes that occur during embryonic formation of the brain and spinal cord. To understand the mechanisms underlying these disruptions, there is an urgent need to better understand the biology of neurons. The unique properties of carbon nanotubes can be utilized to study neuronal function. First, the fact that the nanotubes conduct electricity can be exploited to; (1) detect and study neuronal electrical activity; and (2) to allow cross-talk between neurons when they are grown in vitro, thus duplicating the natural environment observed within the body. Second, the fact that the nanotubes are long, very thin structures can be used to duplicate the environment of migrating embryonic neurons or the outgrowth of regenerating neuronal processes. During development, neurons migrate along long, thin, radial glia to reach their final positions within the brain and spinal cord. When neuronal migration is disrupted during embryonic formation of the brain, the symptoms include mental retardation or epilepsy. Disorders that are thought to involve disruption of brain development (possibly neuronal migration) include autism and schizophrenia. Neuronal migration also occurs after tissue damage that results in cut nerves. The best substrates in normal tissue for successful nerve regeneration are long thin tubes or strings of

glial cells. Thus, nanotubes are a novel substrate that strongly resembles natural substrates for neuronal migration. Nanotube arrays of different sizes and shapes and with different types of functionalization can be used to study key problems in neurobiology. Figure 19.20 illustrates the control over nanotube synthesis achieved by selectively growing aligned blocks of MWCNT on patterned silicon wafers. An e-beam writer is used to pattern the arrays.



(a) (b) (c)

Figure 19.20 UC patterned nanotube arrays grown on Si wafers: (a) close-up view of side of array, (b) array of towers ~100 micron width, and (c) top view of towers each containing ~10^6 nanotubes

19.4.6.1 Goals of Neuron Research

The primary goal of this research is to design ways in which nanotubes or nanotube arrays can be used to study neuronal cell biology and function. Nanotube arrays can be developed that will: (1) allow the electrical recording of multiple neuron assemblies; and (2) promote the migration of neurons in a cell culture. We expect this work will encourage others in the neuroscience community to become interested in the use of nanotube technologies to study neuronal function. Conventional bioelectronic multi-electrode arrays (MEA) are relatively macroscale in size and are based on micro-electro-mechanical system (MEMS) technology. The sensitivity of the MEA is affected by global variables such as temperature, electrical noise, non-specific absorption, and the averaging of properties in the analyte. Our approach is to synthesize carbon nanotube arrays of the size scale of the cells, and to use a massively parallel array of millions of nanotube pairs to detect small electrical signals in the neural network. The nanoelectrode is actually becoming a key device in the field of electrophysiology and is expected to apply to neural diagnostics such as epilepsy and common neural-related diseases.

A carbon nanotube array-based sensor can effectively use the electrochemical properties of nanotubes as an electrophysiological analysis system. The nanotube-based array avoids the drawbacks of other electrode materials that include short life, unstable mechanical strength and low reliability. For electrophysiological measurement applications, carbon nanotubes have the advantages of small size, large surface area, sensitivity to electrolytes, fast response, and good reversibility

at room temperature. At the same time, a carbon nanotube electrode system can be integrated with microelectronics and microfluids systems to gain advantages in miniaturization, multiplexing and automation. Multi-wall carbon nanotube arrays functionalized with special chemical groups can be used for enzyme immobilization and to form stabilizers and mediators used in cell culture research.

Carbon nanotubes whose length is on a multi-cellular scale will allow neuronal migration studies in cell culture. It is also possible to test the effects of coating nanotubes with proteins known to affect neuronal migration in vivo. If nanotubes promote neuronal migration in vitro, then their effects as implants can be explored to promote nerve regeneration in the intact animal.

19.4.6.2 Synthesis and Fabrication of Carbon Nanotube Array Electrodes

Nanotubes will be synthesized using patterned array growth to form electrodes. The design of the nanotube array will determine the sensitivity of the sensor. Figure 19.21 illustrates the sequential steps in the fabrication process, which will result in producing the nanotube array. Step 10 shows the array to be used as the neural sensor.

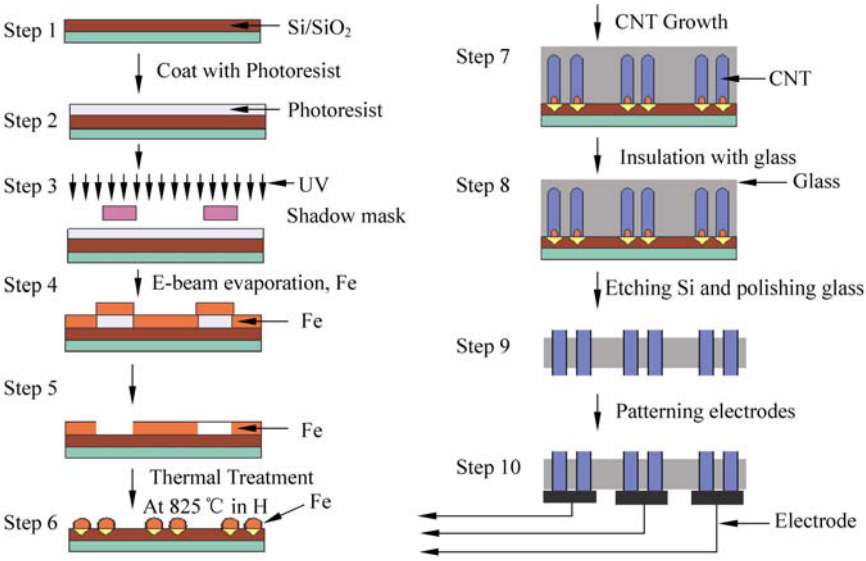


Figure 19.21 Fabrication steps for the vertically grown nanotube array (Color Fig. 26)

19.4.6.3 Culturing Cells on Nanotubes

Initial results growing cultured cells on carbon nanofibers (CNF) and nanotubes are shown in Fig. 19.22. The carbon nanofibers are a low cost lower performance analog to the multi-wall carbon nanotubes which will be used in later stages of testing. A detailed procedure for preparing nanotubes as a substrate for growing neurons is being developed. Experience gained has provided confidence that

cells can be grown on nanotubes. We are at the stage of preparing different types of electrodes for cell growth and monitoring neuronal activity. Nanotubes will be spun into thread to make long conductors.

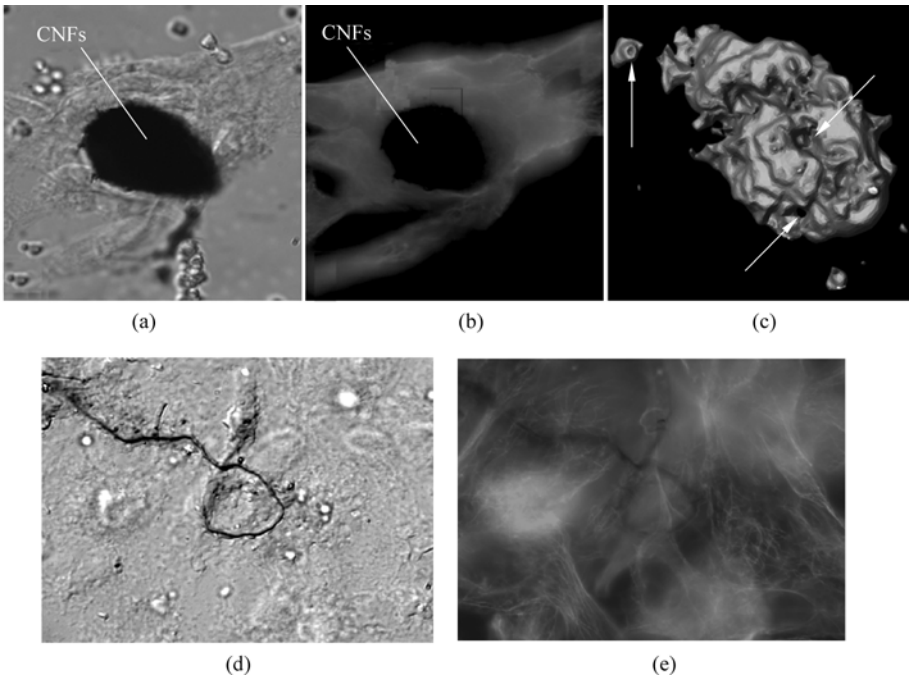


Figure 19.22 Cells cultured on nanofibers/nanotubes: (a) DIC image and (b) immunostaining of microtubules in NIH 3T3 fibroblasts encircling CNF grown on a glass coverslip (olfactory cells grew similarly on CNFs, not shown). (c) Brain cell grown on top of a CNF array, on the vertical tips of CNFs, labeled with Cell Trakker Green, imaged on a confocal microscope, and viewed as a 3-D reconstruction. Arrows show indentations and holes made by tips of CNFs. (d) Fibroblast cell behavior with nanotubes—3T3 Cells and stained with α, β tubulin (microtubule)

19.4.6.4 Signal Analysis of a Neural Network

Neuronal signals can be measured using intracellular or extracellular recordings. An intracellular signal is known as a membrane potential, since the voltage signal is due to the electrical imbalance across the biological membrane. However, an extracellular signal arises from the electrical charge imbalance near the outside of the biological membrane. The extracellular potential is typically in the microvolt range, while the intracellular potential is measured in millivolts. The cell potential can be measured using a single channel recording device, like a patch-clamp electrode, which is now a standard and essential tool in any neuroscience research. However, to obtain a more complete picture of the neuronal network behavior, an experimental method to simultaneously record neuronal activity from many

neurons is needed. A multi-electrode array in conjunction with multiple neurons in cell culture is a recent method to simultaneously record multi-neuronal activity and is based on MEMS technology. However, the MEMS Si-based micro size electrode arrays cannot provide electrochemical stability, mechanical strength, and long term multi-channel monitoring ability of spontaneous or evoked electrophysiological activity. The nanotube array is expected to overcome most of the problems of using Si.

From an experimental viewpoint, there are two approaches to the measurement of neuronal activity. The first is to grow dissociated neurons in vitro on the nanotube arrays and test them in vitro. Cells can be used either from established neuronal cell lines or prepared by dissociating neural tissue from a living system into individual cells (brain or olfactory sensory neurons). The second approach for neuronal sensing is to place a brain or olfactory tissue slice on the nanotube array. Both of these approaches should be evaluated. In both cases, to understand spatiotemporally coordinated activity in neural networks and the interaction between different areas or layers in tissue or cells, simultaneous multi-site nanoscale recording is a prerequisite. Thus, a nanoelectrode array (NEA) should be developed to provide ultrasensitive measurements of neuronal activity. A concept NEA is shown in Fig. 19.23. The following work is underway to develop a NEA: (1) design of a NEA to allow electrical recording from multiple neuron assemblies; (2) perform in vitro testing using the NEA. Using a tissue slice, the NEA will be used to monitor spikes and local field potentials and growth of the neurons. A goal would be to report for the first time recordings of single-unit spike activity with NEAs in acute slice preparations of neural tissues; (3) analyze neuronal activity and function. Analyze the neuronal activity and function, and the neuronal response to growth factors and electrical monitoring; (4) analyze neuronal migration. Using morphological measurements, analyze neuronal migration on NEAs or along the length of nanotubes coated with proteins known to affect neuronal migration in vivo; and (5) plan new medicine. Based on the analysis, propose new approaches to stimulate neuron growth and repair, and develop new concepts to treat neurological disorders.

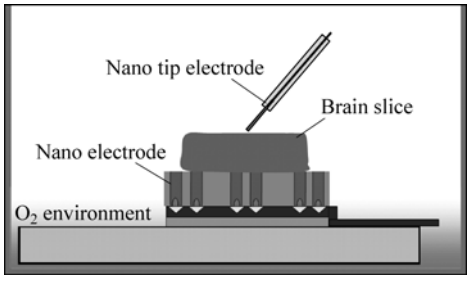


Figure 19.23 Concept nanotube electrode array

19.5 Conclusions

Multi-wall carbon nanotube arrays up to cm in length and with precise patterns were synthesized and used to develop several prototype smart materials devices. Overall, synthesis of the long multi-wall carbon nanotube arrays is opening the door for the development of new sensors, actuators, and multifunctional materials. Excellent mechanical properties, high electrochemical sensitivity, high strain generation of the nanotubes and electrically conductive probes will provide benefits for many applications in the area of nanomedicine. Several concepts were presented in the chapter to spur further thinking and research to use smart nanomaterials to benefit medicine.

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