Carbon-nanotube Biofibre Microelectrodes

Carol Lynam, Willo Grosse, Gordon G. Wallace*

ARC Centre of Excellence for Electromaterials Science,
Intelligent Polymer Research Institute, University of Wollongong,
Northfields Avenue, Wollongong, NSW 2522, Australia.

*Corresponding author

Abstract/Summary

Novel all-biocompatible carbon nanotube fibres were formed using wet-spinning. In this process the spinning solutions used are carbon nanotubes dispersed using biomolecules such as hyaluronic acid and chitosan. We compare the effect of a coagulation bath containing either a polymer binder e.g. polyethylene imine or simply a precipitating solvent system e.g. acetone; the electrical, mechanical and morphological properties of the resulting fibres were studied. Novel biocompatible electrode structures were generated suitable for a variety of biomedical applications, e.g. in biosensors or in systems where the application of an electrical field is advantageous (such as stimulation of electrically excitable cells such as nerve and muscle cells).

Keywords: carbon nanotube, biomolecule, conducting fibre, fibre spinning, coagulation.
**Introduction**

The unique electrical and mechanical properties of carbon nanotubes (CNTs) have attracted interest in their biological application both at the molecular and cellular level.\(^1\),\(^2\) The electrical properties of CNTs may permit them to be utilised to stimulate electrically excitable cells (nerve and muscle cells). Studies on interfacing CNTs with biomolecules have been of interest to function as platforms to support the growth of nerve cells.\(^3\) It has been shown that varying the mechanical\(^4\), electrical\(^5\) and chemical\(^6\) characteristics of surfaces influences neurite outgrowth rates and can even allow control of neuron shape.

The possibility of employing CNTs as substrates able to improve neural signal transfer has been demonstrated by Lovat et al.\(^7\). In another study, while neurons were found to extend neurites when grown on multiwalled carbon nanotubes (MWNTs)\(^3\) more elaborate neurite branching was observed when neurons were grown on MWNTs functionalised with polyethylene imine.\(^2\) PEI is a widely used permissive substrate for nerve cells; supporting attachment and growth of neurons. Other molecules of interest are chitosan (CHIT) and hyaluronic acid (HA). CHIT and HA have independently shown to be effective in supporting the growth of a range of mammalian cell lines including osteoblasts\(^8\),\(^9\) and nerve cells.\(^10\),\(^11\) CHIT has also been blended with HA to form composite biomaterials for cartilaginous tissue scaffolds.\(^12\) Recent, innovative approaches to tissue repair involving these biomolecules have been developed.\(^13\)

These studies imply that suitably functionalised CNTs could potentially be used in scaffolds to guide neurite outgrowth and highlights the opportunity available in combining CNTs with appropriate biomolecules. CNTs must be built into macroscopic
structures in order to exploit their remarkable properties. Stable CNT dispersions exhibit liquid crystalline behaviour similar to conventional rod-like polymers in solution\textsuperscript{[14]} (such as poly-paraphenylene terephthalamide) and so lend themselves to solution spinning processing. The steps involved in wet spinning of CNTs present an ideal opportunity to introduce CNTs and biomolecules into practically useful structures. Vigolo et al.\textsuperscript{[15]} dispersed CNTs in a surfactant containing solution and then assembled the CNTs into long ribbons and fibres in a polyvinyl alcohol (PVA) containing coagulation bath. Barisci et al.\textsuperscript{[16]} developed this fibre spinning process further by using DNA as the CNT dispersant and thereby adding bio-functionality to the fibre. The fibres produced however had electrical conductivity values several orders of magnitude lower than individual CNTs. Removing the polymer (PVA) binder in these fibres improved the electrical properties at great cost to the mechanical properties. Munoz et al.\textsuperscript{[17]} reported the use of surfactants as dispersants while employing a polyethylene-imine coagulation bath to produce more highly conducting carbon nanotube fibres. Here we report the novel combination of using biocompatible molecules as dispersants and spinning into coagulation baths including polyethylene-imine or acetone. This results in mechanically robust yet electrically conducting biomolecule containing fibres.
Results

The most common means of obtaining relatively concentrated CNT dispersions consists of using low molecular weight surfactants as dispersants in water. Biomolecules such as CHIT and HA are excellent dispersants for CNTs, usually at lower concentrations than surfactants.\textsuperscript{[14]} We prepared SWNT-biomolecule dispersions by sonication of a given amount of SWNTs in an aqueous solution of biomolecule (CHIT or HA), to form highly stable biomolecule-SWNT suspensions. In the case of CHIT as dispersant, a homogenous dispersion was obtained with a concentration of 0.3 % by weight of SWNTs and employing a 2:1 ratio by weight of CHIT:SWNT. Dispersions differing from this concentration and ratio contained large clusters, between 50 and 100µm in size, of non-dispersed SWNTs. This is in contrast to dispersions made with HA.\textsuperscript{[14]} In the case of HA as dispersant, concentrations of up to 0.6 % by weight of SWNTs were possible. To obtain a homogenous dispersion, at least a 2:3 ratio by weight of biomolecule:SWNT was necessary.

The affinity for SWNTs and permissiveness as a substrate for neuronal growth led us to consider employing PEI as the coagulant in the fibre spinning process. A coagulation drop test indicated that acetone is also a suitable coagulant for the dispersions. Since acetone has a low boiling point and high vapour pressure, it is easily removed from the fibre resulting in a biocompatible structure. Processing these biomolecule-SWNT dispersions by injection into a flowing stream of polymer solution/solvent produces gel fibres containing SWNTs. As a result of diffusion of the PEI into the fibre during the coagulation process cylindrical fibres were formed. Ribbon-like fibres formed in the case of acetone as coagulant.
To achieve optimal spinning conditions, the influence of experimental variables: concentration of coagulant (between 5 and 40% w/v for PEI), time spent in coagulation bath (between 5 and 60 minutes) and the rate of injection into the coagulation bath (injection rate 100-250 ml/hr, coagulation bath rotation rate 20-40 rpm) were investigated. Employing injection rates and spinning speeds found to be most favourable (rotation speed of 25 rpm; injection rate of 250 ml/hr for PEI and 100 ml/hr for acetone), the SWNT-biomolecule dispersions were spun into a coagulation bath to form CNT-bio-fibres. The coagulation bath consisted of either polyethylene imine in methanol (5 wt. %) or acetone. Fibre lengths of up to several metres could be made using optimal conditions; however to avoid entanglement in the rotating coagulation bath typical fibre lengths were 10 cm.

We obtained uniform cylindrical HA-SWNT-PEI and CHIT-SWNT-PEI fibres with typical diameters of 80 µm. In contrast HA-SWNT and CHIT-SWNT fibres coagulated by acetone were ribbon-like and had typical widths of 70 µm and thicknesses of 50 µm. Biomolecules have been shown to affect the assembly of CNTs e.g. HA and denatured DNA are effective at stabilising CNTs leading to suspensions that are isotropic.\[14, 18\] Injecting the SWNT-dispersion in the flowing stream of polymer solution mimics the conditions in solutions of rigid polymers or anisotropic colloids.\[19\] As a result, both biomolecule assisted and flow induced alignment of the nanotubes is expected in the direction of the fluid velocity, which is parallel to the fibre axis. Scanning electron microscopy (SEM) images reveal that the CHIT-SWNT-PEI fibres have a corrugated surface with the axis of corrugation approximately aligned parallel to the fibre axis. These corrugations (typically 0.3-1 µm) are also observed on the HA-SWNT-PEI,
HA-SWNT and CHIT-SWNT fibres, with SEM images suggesting a rougher surface structure than for the CHIT-SWNT-PEI fibre. Images of fractured ends of the fibres indicate the presence of SWNT bundles coated with biomolecule dispersant and/or PEI and that a large majority of the SWNTs are aligned along the fibre axis.

Raman spectroscopy (Figure 2) has confirmed the presence of CNTs in the fibres. High resolution Raman spectroscopy measurements of the radial breathing modes (RBM) of SWNTs was carried out on the raw SWNT powder used to make the dispersions, SWNT-biomolecule dispersions and the fibres produced. The spectra (representative spectra are shown in Figure 2) indicate that a significant interaction takes place between the nanotubes and each biomolecule used as a dispersant. An upshift in the RBM peaks of between 3 and 5 cm\(^{-1}\) has been observed for the SWNT-biomolecule dispersions and between 5 and 7 cm\(^{-1}\) for the SWNT-biofibres. Positions of the D and G bands for raw nanotubes, dispersions, and fibres are within 4 cm\(^{-1}\) variation, which is not significant enough to imply doping of the nanotubes by the biomolecules.\(^{20}\) These observations are consistent with de-bundling as previously reported.\(^{21}\)

Table 1 compares mechanical properties, conductivity, and capacitance values for fibres coagulated under different conditions. Mechanical testing determined that the average tensile strength of the HA-SWNT-PEI fibre was 120 MPa while for the CHIT-SWNT-PEI fibre it was measured as 40 MPa (Table 1). The positively charged amino groups of CHIT may repel any cross-linking interaction with the amino groups of PEI in the fibre resulting in a lower tensile strength. A more favourable cross-linking interaction of the PEI amino groups with the negative acidic residues of the glycosaminoglycan chains of HA is possible which is supported by higher tensile strength values. The
average tensile strength of the HA-SWNT fibre was 51 MPa, while for the CHIT-SWNT fibre it was measured as 61 MPa. We attribute these lower tensile strength values in the acetone coagulated fibres to the absence of a supporting/cross-linking polymer matrix in the coagulation medium e.g. PEI. The average tensile strength of the HA-SWNT-PEI fibre is slightly higher than that of the DNA-SWNT-PVA fibres previously reported. A skin–core fibre microstructure was not observed for the fibres reported here unlike CNT-PVA fibres with CNT-enriched inner core and PVA-enriched outer skin. Homogenous distribution of the biomolecule and CNTs was observed on the fibre cross section (Figure 3). Although the mechanical properties of these fibres do not compare favourably with commercial high-strength fibres used in structural composites, they possess mechanical properties sufficient for use as biosensor electrodes or for the fabrication of substrates for nerve and muscle repair.

The electrical conductivity of previously formed DNA-SWNT-PVA fibres was reported as 0.04 S cm$^{-1}$.Annealed fibres have been reported with conductivities as high as 167 S cm$^{-1}$, however the increase in electrical properties is accompanied with severe losses in mechanical properties. Conductivities of 0.2 and 8.4 S cm$^{-1}$ were measured for the CHIT-SWNT-PEI and HA-SWNT-PEI fibres produced here respectively (Table 1), which is five times higher and over two orders of magnitude higher respectively than for the DNA-SWNT-PVA fibres previously reported. It was found that the length of time spent in the coagulation bath did not significantly affect the conductivity of the fibres; however in the case of PEI a longer rinsing time proved beneficial (e.g. soaking for 3 minutes resulted in a fibre conductivity of 0.04 S cm$^{-1}$, compared to conductivities of $>1.0$ S cm$^{-1}$ for 30 min rinses). This is presumably due to a
lower insulating PEI content within the fibre resulting in more electrical connections between CNTs. It was difficult to accurately measure the CNT content of the fibres. Overlap in the decomposition temperature of each component (CNTs and biomolecules) of the composite fibre was observed during thermogravimetric analysis (results not shown).

Conductivities of 23 and 186 S cm\(^{-1}\) were measured for CHIT-SWNT and HA-SWNT fibres respectively; the fibres produced from acetone. This is nearly four orders of magnitude higher in the case of the HA-SWNT fibre than for the DNA-SWNT-PVA fibres previously reported, while still retaining bio-functionality. We attribute the high conductivities in the acetone coagulated fibres to the absence of a supporting polymer matrix in the coagulation medium e.g. PEI. It appears that the amount of biomolecule material remaining in the fibre was sufficient to provide mechanical support but low enough to allow electrical conductivity via intimate contact between CNT junctions. In support of this, CHIT-SWNT fibres had conductivities up to 8 times lower than that of HA-SWNT fibres, presumably because of the higher biomolecule concentration required to achieve a homogeneous spinning dispersion (0.6 wt % CHIT for 0.3 wt % CNTs). Assuming no CNT and biomolecule loss during fibre formation, the CNT composition of the acetone coagulated fibres could be estimated based on the concentration of the CNT dispersions. This translates to 60 wt % and 33 wt % CNT content for the HA-SWNT, and CHIT-SWNT fibres, respectively.

Cyclic voltammetry, (performed in the physiological medium 0.2M PBS, pH=7.4), gave responses that indicate capacitive behaviour predominates, irrespective of the coagulation system used. A linear dependence of the current flows on scan rate was
observed and specific capacitance values of 34 F/g, 9 F/g and 2.4 F/g for HA-SWNT, CHIT-SWNT and HA-SWNT-PEI fibres respectively were obtained. However, more resistive CV measurements were observed for CHIT-SWNT-PEI fibres because of their reduced electrical conductivity (giving a specific capacitance of 6 mF/g).

A series of preliminary measurements using 1mM potassium ferricyanide were carried out to characterise the electrochemical response of these novel CNT-fibre electrodes. Measurement of the ferricyanide faradaic current as a function of the scan rate was performed on two of the fibres with the highest electrical conductivity (HA-SWNT and CHIT-SWNT) to establish whether the electrochemical reaction was diffusion-controlled. Figure 4b shows voltammograms of the fibres HA-SWNT and CHIT-SWNT and a glassy carbon electrode recorded at a scan rate of 100 mV/s. Analysis of the faradaic current obtained resulted in linear plots of Ip vs. $v^{1/2}$ plot over range studied (5-200mV/s), indicating that the current is controlled by semi-infinite linear diffusion in the case of this redox couple. Similarly, diffusion-controlled electron transfer at these CNT-biofibre microelectrodes was also found for ferrocene monocarboxylic acid and ruthenium hexamine chloride (data not shown). The potential differences ($\Delta E_p$) and the ratios of the anodic peak current to the cathodic peak current ($I_{pa}/I_{pc}$ of ferricyanide) are compared in Figure 4c. As expected, as the conductivity of the fibres increases, the peak potential difference as well as $I_{pa}/I_{pc}$ decreases and the $I_{pa}/I_{pc}$ is closer to 1 with an improvement in reversibility of the couple. Overall, the results presented here demonstrate that acetone coagulated CNT-biofibre microelectrodes possess sufficient electrical conductivity and a wide working potential range making them useful for many analytical applications.
While CHIT as dispersant and PEI as coagulant produced fibres with higher electrical conductivity than the DNA-SWNT-PVA fibres, it did so at the cost of mechanical properties. On the other hand, HA as dispersant and PEI as coagulant provided novel fibres with the same mechanical properties as the DNA-SWNT-PVA fibres but with a two-order of magnitude increase in electrical conductivity. Further increases in conductivity were obtained when acetone was used as the coagulant, but unfortunately to the detriment of mechanical properties.

In summary we have successfully prepared novel carbon nanotube fibre microelectrodes from all biocompatible polymers. While incorporating bio-functionality, the HA-SWNT-PEI fibres compromise little in terms of mechanical properties with a two order of magnitude increase in electronic conductivity compared to DNA-SWNT-PVA fibres. With adequate mechanical properties for the intended applications the HA-SWNT acetone coagulated fibres have electrical conductivities four orders of magnitude higher than the DNA-SWNT-PVA fibres. These fibres may prove useful as biosensor microelectrodes and substrates which support and allow the electrical stimulation of mammalian cells – studies currently under way in our laboratories.

Acknowledgements

The authors gratefully acknowledge the financial support of the Australian Research Council.
Table 1: Mechanical and electrical properties of SWNT-fibres. * Data taken from reference [16].

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<tr>
<th>Dispersant</th>
<th>HA</th>
<th>CHIT</th>
<th>DNA*</th>
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<tr>
<td>Coagulant</td>
<td>PEI</td>
<td>Acetone</td>
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<tr>
<td>CNT: dispersant ratio</td>
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<tr>
<td>Conductivity (S/cm)</td>
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<tr>
<td>Capacitance (F/g)</td>
<td>4</td>
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Figure 1: Structures of biomolecules used as SWNT dispersant; (a) Chitosan and (b) Hyaluronic Acid.

Figure 2: Raman Spectra of (a) raw SWNT, (b) HA-SWNT dispersion and (c) HA-SWNT-PEI fibre confirming presence of SWNTs.
**Figure 3:** High Resolution SEM images of SWNT-Fibres and of fractured fibre ends, showing differences in fibre morphology, (a) HA-SWNT-PEI, (b) CHIT-SWNT-PEI, (c) HA-SWNT and (d) CHIT-SWNT fibres.

**Figure 4:** Electrochemical behaviour of CNT fibres HA-SWNT (solid line), CHIT-SWNT (dashed line), HA-SWNT-PEI (dotted line) and CHIT-SWNT-PEI (solid grey line). (a) Plot of current versus scan rate of fibres, (b) cyclic voltammograms in 1 mM potassium ferricyanide at 100mV s⁻¹ and (c) effect of fibre composition on ratios of the anodic peak current to the cathodic peak current ($I_{pa}/I_{pc}$) and the peak potential separation, $\Delta E_p$. 
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(a) The graph shows the current (mA) as a function of the scan rate (mV/s) for different samples: HA-SWNT, CHIT-SWNT, HA-SWNT-PEI, and CHIT-SWNT-PEI.

(b) The cyclic voltammogram compares the current (A) at different potentials vs Ag/AgCl (V) for HA-SWNT, CHIT-SWNT, and GCE.
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Notes and References:

Materials: All chemicals, Single Wall Carbon Nanotubes (HiPCo produced from CNI), chitosan (Jakwang Co. Ltd.), hyaluronic acid (Sigma), polyethylene imine (Aldrich), acetone (Univar, Ajax Finechem), methanol (Univar, Ajax Finechem), potassium ferricyanide (Sigma), ferrocene monocarboxylic acid (Sigma), and ruthenium hexamine chloride (Sigma), were used as received.

Instrumentation: SEM images were acquired using a Hitachi S-900 field-emission scanning electron microscope (FESEM). Samples for FESEM were sputter coated with chromium prior to analysis. Raman spectroscopy measurements were performed using a Jobin Yvon Horiba HR800 Spectrometer equipped with a He:Ne laser (λ = 632.8 nm) utilizing a 1800-line grating Electrical conductivity measurements were carried out using the conventional four-point probe method at room temperature. Electrochemical capacitance was calculated from the slope of anodic current amplitude when graphed against the scan rate, obtained from cyclic voltammetry at different potential scan rates, in phosphate buffered saline solution (PBS - 0.2M pH 7.4) with Ag/AgCl reference electrode. Cyclic Voltammetry were performed using an eDAQ e-corder (401) and potentiostat/galvanostat (EA 160) with Chart v5.1.2/EChem v 2.0.2 software (ADInstruments), and a PC computer. Mechanical testing was carried out using a Dynamic Mechanical Analyser Q800 (TA Instruments). Thermogravimetric analysis (TGA) was carried out using a Thermo Gravimetric Analyser TGA Q500 (TA Instruments) at a heating rate of 10 °C min⁻¹ and an air flow rate of 60 cm³ min⁻¹.
Procedure: HA-SWNT dispersions were prepared from an aqueous solution of HA (0.4 wt %) containing SWNT in a ratio of 1:1, which was ultra-sonicated using pulse (2s on, 1s off) for 30 minutes using a high power sonic tip (500W, 30% amplitude). HA-SWNT-PEI composite fibres were prepared from a HA-SWNT dispersion, 1:1 (0.4 wt %), utilising a rotating PEI coagulant solution (5 wt % in methanol). Following coagulation, fibres were washed with methanol prior to drying in ambient conditions. Chitosan-SWNT-PEI fibres were produced from a Chitosan-SWNT dispersion, 2:1 (0.3 wt %) and PEI coagulant solution in a similar manner to the HA-SWNT-PEI fibres. HA-SWNT and CHIT-SWNT fibres were produced by injecting the respective dispersions into a rotating coagulant solution of acetone. Following coagulation, fibres were dried in ambient conditions.

References:


