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Antiseptic single wall carbon nanotube bandages

T.J. Simmons^{a,b,c}, S.-H. Lee^d, T.-J. Park^d, D.P. Hashim^a, P.M. Ajayan^{a,c,g}, R.J. Linhardt^{b,c,d,e,f,*}

^aDepartment of Material Science and Engineering, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180, USA

^bDepartment of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180, USA

^cRensselaer Nanotechnology Center, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180, USA

^dDepartment of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180, USA

^eCenter for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180, USA

^fDepartment of Biology, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180, USA

^gDepartment of Mechanical Engineering and Materials Science, Rice University, 6100 Main Street, Houston, TX, 77005, USA

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ABSTRACT

Single wall carbon nanotubes (SWCNTs) are coated with polyvinylpyrrolidone-iodine (povidone-iodine or PVPI) in water. This solution of SWCNT and PVPI is deposited as a composite film, composed of individual and bundled SWCNTs with a PVPI coating. This material acts as a conductive nanotextured bandage with high flexibility and self contained slow-release antiseptic iodine. Antibacterial properties were tested on *Escherichia coli*, showing high efficacy over 48 h. Four-probe resistance tests showed a sheet resistance of approximately 10 k Ω/\square . This material show promise for wound healing applications where regeneration of nervous tissue connections is desired, as it will act to prevent infection, allow oxygen to the wound site through micron sized pores, provide a nanotextured substrate material for nervous and tissue growth, and stimulate reconnection of nerve cells by electrical pulsing.

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1. Introduction

Carbon nanotubes are a unique quasi one-dimensional material with an ever expanding range of applications. Carbon nanotubes (CNTs) are increasingly being considered for biomedical applications, despite concerns over the toxicity of these materials. Although, there are valid concerns about the safety of nanomaterials, their prudent use can result in remarkable improvements to existing technologies, and such possibilities cannot be ignored. Increasing attention has been focused on the impact of CNTs on cell growth, with results of some studies showing toxicity and others enhanced cell growth. These seemingly contradictory results can be rationalized by the various compositions, lengths, diameters, and

levels of CNT of purity, all of which can have a significant impact on their toxicity. Toxicity has been mainly attributed to the presence of metallic impurities, and to the presence of very small CNT fragments [1,2]. The work presented here uses high purity CNT material and filtration allows for the reduction of metallic impurities and small CNT fragments, to create a novel conductive antiseptic bandage material. This material may enable the enhanced recovery of nervous and muscle tissue damage resulting from injury while preventing infection. Studies have shown that CNTs can be used effectively as scaffolds for the enhanced growth of mammalian cells such as neurons, stem cells, smooth muscle cells, and epithelial cells [3–6]. These advances use CNTs for cell growth, with no apparent toxicity, and provide motivation for this work.

* Corresponding author: Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 Eighth Street, Troy, NY 12180, USA. Fax: +1 518 276 3405.

E-mail address: linhar@rpi.edu (R.J. Linhardt).

URL: <http://www-heparin.rpi.edu> (R.J. Linhardt).

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2. Results and discussion

Presented here is a method is described combining single wall carbon nanotubes (SWCNTs) with a water-based povidone-iodine (PVPI) complex (Fig. 1) as an aqueous suspension, which is then deposited as a film on a polytetrafluoroethylene (PTFE) filter membrane to form a three-dimensional nanocomposite network.

CNTs are routinely solubilized with povidone (polyvinylpyrrolidone or PVP) by relying on a polymer wrapping mechanism that essentially encases the SWCNT in a polymer monolayer with a helical coil conformation, which is the proposed structure of the povidone-iodine complex in water [7–11].

The povidone-iodine complex (PVPI) has well-known anti-septic properties and is effective against a wide spectrum of pathogens, including *Escherichia coli* (*E. coli*) [12]. Aqueous PVPI has been used as a topical antiseptic and surgical scrub for more than 40 years and microbial resistance has not yet been reported [11]. Combining PVPI with SWCNTs in water can allow for a stable water-based dispersion of SWCNTs with iodine non-covalently bound to the surface. This film is micro-porous, several microns thick, and has micron-length SWCNTs randomly arranged within a polymer (povidone) coating. The CNT-PVPI film (Fig. 2) is highly flexible and remains bound to the PTFE membrane unless removed with a strong adhesive tape.

This material has potential application as a flexible anti-septic bandage that is both nanotextured and electroactive. Antibacterial efficacy of the bandages was determined by visually examining *E. coli*, which was designed to produce a green fluorescent protein (GFP) that is visible under UVA illumination (Figs. 3 and 4).

Upper images are bacterial cell cultures with the CNT material removed to reveal the amount of *E. coli* growth, with

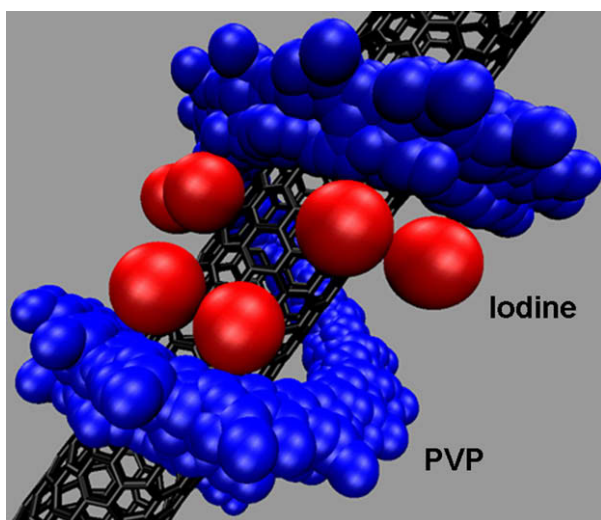


Fig. 1 – Schematic representation of proposed SWCNT-PVPI wrapping. PVP (blue) chain complexed with iodine (red) wrapping a SWCNT (black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

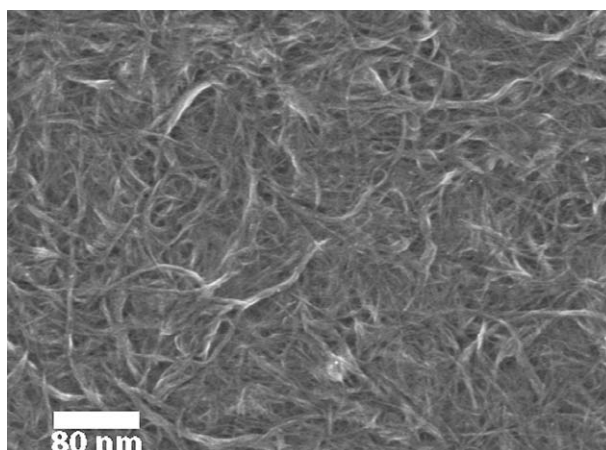


Fig. 2 – Field emission-scanning electron microscopy images of CNT-PVPI bandage material. Scale bar approximately 80 nm.

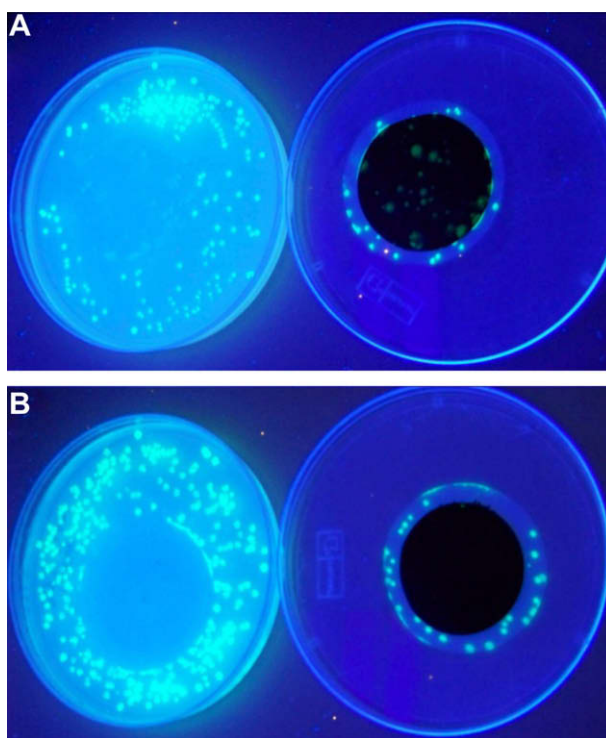


Fig. 3 – Photographs of bacterial growth media (left) and bandage material (right). CNT-PVP control (A) and CNT-PVPI (B) on 47 mm PTFE filter membranes, under UVA illumination.

closer views of the CNT bandage material in the lower images. The control (A) shows a large number of *E. coli* colonies on the bandage material, while the CNT-PVPI sample (B) shows almost no colony formation on the bandage material after 48 h. The CNT-PVPI material significantly inhibited the growth of bacterial colonies, despite the growth medium containing a larger number of colonies than the control sample.

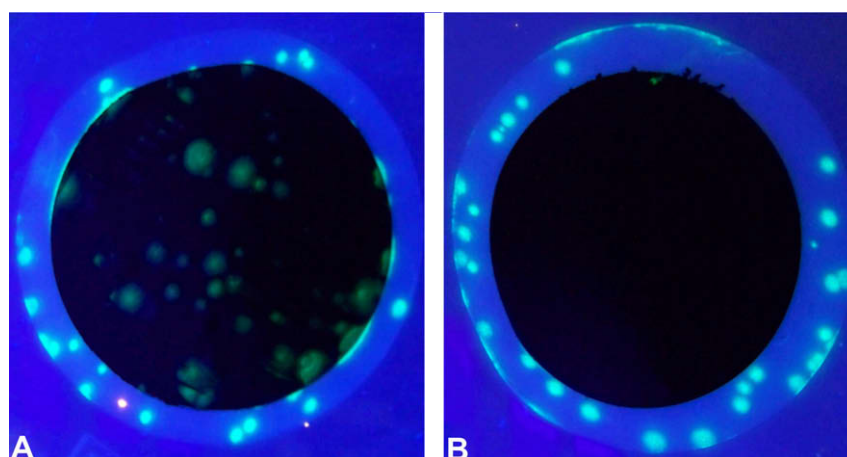


Fig. 4 – Photograph showing the control (A) sample showing significant *E. coli* growth, while the iodine containing sample (B) shows virtually no *E. coli* growth.

This material would be useful as a bandage for wounds where there is a risk of infection. PVPI solutions are routinely administered to gauze or other absorbent materials used as bandages and dressings, and placed on the wound site. This material is similar to conventional wound dressings that are both flexible and breathable, but unlike conventional dressings it is nanotextured and has a self contained slow-release antiseptic with no known bacterial resistance. PVPI has been shown by numerous studies to be of very low-toxicity to mammalian cells, and has not been shown to slow wound healing or cell growth [13–15]. The most common method for the application of PVPI to wounds is to presoak standard bandage material prior to application. This method presents the possible hazard of causing burns to the skin from irritation caused by the liquid PVPI solution [16]. The advantage of the CNT–PVPI bandage is that it is embedded with dry PVPI supported by SWCNTs, and would be ready for immediate use, with no risk of burns from wet PVPI solution being trapped against the skin. The iodine is slowly released from the PVPI complex wrapping SWCNTs (see Supplemental information), leaving behind PVP wrapped SWCNTs in the bandage material, as they are strongly bound in the woven network of the bandage material. The iodine is released into the fluids at the wound site, as well as any unbound PVPI in the bandage material. PVP is a water soluble polymeric surfactant, and therefore there will not be any significant binding of tissue to the bandage material which is completely coated by the PVPI. SWCNTs which are not completely wrapped by the PVPI in solution are removed during the solution processing, and therefore all of the bandage material is covered with a layer of PVPI, and there is no direct contact between CNTs and the cells at the wound site.

The presence of iodine in the finished bandage material was confirmed using energy dispersive X-ray spectroscopy performed during FE-SEM imaging, which shows two substantial iodine peaks (see Supplemental information). Additional peaks from the polymer and the PTFE filter membrane were also observed. No peaks for metallic impurities were observed, confirming the high purity of the material.

In addition to the antibacterial properties of this material, it is also a conductive material due to the SWCNTs, showing a

sheet resistance of approximately $10 \text{ k}\Omega/\square$. This was determined by both 2-point and 4-point probe measurements; with an inter-probe distance being 1 mm (see Supplemental information). The resistance is low enough that a significant electrical current can be passed through the material. This electrical property makes it possible to explore enhanced cell growth through electrical stimulation. Liopo et al. showed that electrical stimulation through SWCNT networks can help carry ionic currents that aide in the extension of neurites, and ultimately in building networks between nerve cells [17]. Patterned networks of neurons have been grown on SWCNTs, and establishing connections between nerve cells is apparently aided by randomly oriented SWCNT material [18]. Since none of these studies has examined neuronal growth on PVPI wrapped SWCNTs, further studies will be needed in order to determine the viability of the material for such purposes. If this material is shown to be a suitable material for neuronal growth, it may be possible to place transplanted nerve cells (such as an autograph) on the SWCNT bandage material and then apply to a wound where re-growth of nervous tissue would be desirable, such as in an injury like a severe burn.

In summary, we have created a novel nanocomposite material from the combination of SWCNTs with PVPI in water. The filtration of this aqueous suspension creates a high purity micro-porous film that has antiseptic iodine available on the surface of a network of SWCNT wrapped in polymer. This material is strongly antiseptic and control samples lacking iodine had no noticeable microbicidal activity towards *E. coli*, which further supports the suitability of this material for use as a non-toxic antiseptic bandage, since PVPI is has been determined to have low-toxicity towards mammalian cells. Electrical pulses sent through this SWCNT composite material may allow for enhanced cell growth as in several previous studies, and possibly enable faster reconnection of damaged neuronal networks. When carefully employed, CNTs can be non-toxic to mammalian cells and therefore an extremely valuable addition to medicine, biotechnology, and therapeutics. Further studies will be needed to fully determine the efficacy of this bandage with regard to wound healing, and the effects of electrical stimulation on neuronal growth.

3. Experimental

Purified SWCNTs, obtained from Swan Chemical, Inc., have less than 3.7% wt. ash content and less than 1.7% wt. iron content, according to the certificate of analysis from the manufacturer. An aqueous suspension of PVPI was obtained from Purdue L.P., as the product Betadine®. The SWCNTs were solubilized in water by adding 5 mg SWCNT powder to an aqueous solution that contained 1.5 mL PVPI concentrated solution and 18.5 mL deionized (DI) water, which is a ratio of approximately 150 mg PVPI to 5 mg SWCNTs in 20 mL DI water. The total amount of PVPI which complexes to the SWCNTs is approximately 1:1, meaning each circular bandage which is about 10 cm² has 15 mg SWCNT and 15 mg of PVPI total. The mixture is bath sonicated for 30 min to aide the suspension of SWCNTs. The solution is then deposited onto a Millipore PTFE membrane with 5 μm pores via vacuum filtration, after which the film is dried in an oven (40 °C) for several hours. Filtration using membranes with such large pores sizes allows some of the sub-micron sized CNTs as well as metallic impurities to be removed from the solution and not be incorporated into the bandage material.

The antiseptic properties of the bandage were confirmed by applying the film to a bacterial culture for 48 h. *E. coli* BL-21 was transformed with pGFPuv (Clontech, CA, USA) by the standard calcium chloride method. The pGFPuv expresses β-galactosidase-GFPuv fusion protein that can be induced by isopropyl-β-D-1-thiogalactopyranoside (IPTG) and includes an ampicillin (amp) resistance gene [19]. Transformed *E. coli* was transferred on to the surface of Luria Broth(LB)/amp/IPTG agar and incubated at 37 °C for 24 h. Illuminating the agar surface with a UVA lamp, isolated green fluorescent colonies were picked and incubated in 10 mL LB/amp broth at 37 °C for 12 h. After centrifugation (5000 rpm, 10 min), the recombinant cells were washed with and resuspended in distilled water at a concentration of 10⁴ CFU per mL. Then 0.1 ml of the cell suspension was spread on a LB/amp/IPTG agar plate. After drying for 30 min, CNT/PVP control and CNT/PVPI bandages were placed on the surface of separate agar plates and incubated at 37 °C for 48 h.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.carbon.2009.02.005](https://doi.org/10.1016/j.carbon.2009.02.005).

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