Chapter 7

Application of Carbon Nanotubes to Wound Healing Biotechnology

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Carbon nanotubes have remained at the center of nanotechnology research for the past two decades, and have been increasingly present in the areas of biology and biotechnology. While questions still remain about the toxicity of these materials, there is great interest in exploiting their unique properties to create innovative biotechnology applications. The application of carbon nanotubes to wound healing offers the possibility of dressings with enhanced functionality, controlled delivery of antiseptics, and real-time monitoring of healing events. By briefly examining the development of wound healing biotechnology, a context for

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the use of carbon nanotubes in modern medical practices can be established. Several applications have been evaluated by preliminary studies herein to provide a proof-of-concept demonstration of the potential of carbon nanotubes to be incorporated into wound healing biotechnology.

Introduction

Carbon nanotubes (CNTs) are a quasi one-dimensional allotrope of carbon with an ever expanding range of applications. There are both single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs), and these nanotubes can be metallic, semi-metallic, or semiconducting depending on their chirality. Typically nanotubes have diameters of 1-50 nm depending on the number of sp^2-hybridized carbon walls, and lengths of several microns, giving them very high aspect ratios. These materials are exceedingly strong and flexible, showing superior mechanical properties compared to many conventional materials. Most applications of CNTs to date have been in materials science. CNTs are increasingly being considered for biomedical applications, although there are serious concerns over the toxicity of these materials. The medical application of CNTs been slowed by the complexity of the nano-bio interface and a common perception that such nanomaterials should be inherently toxic. This perception is based in part on lessons learned over the last century about the harmful effects of microparticulate and biopersistent materials such as asbestos. Despite these justifiable concerns, the application of CNTs to medicine, particularly as stable composites, offers potential benefits that require careful exploration. This chapter focuses on the application of CNTs to a model system, wound healing biotechnology.

Wound Healing Biotechnology

The primary goal of wound care is to allow for rapid wound closure, prevent infection, and to improve the aesthetic appearance and maximize the functionality of the resulting scar tissue. The natural process of wound healing consists of three overlapping phases – inflammation, tissue formation, and tissue remodeling. For the sake of this study, we will focus on the first two stages of wound healing that are the primary foci of wound care strategies.

Early Stages of Wound Healing

When a wound is sustained, typically there will be a disruption of blood vessels and surrounding tissues such as skin. Inflammation occurs in the early stage of wound healing, just after a clotting cascade where fibrin begins clotting to reestablish hemostasis while providing an extracellular matrix to

enable cell migration. Platelets create a hemostatic plug while simultaneously releasing several wound healing mediators such as platelet-derived growth factor that both attracts and stimulates fibroblasts and macrophages. In the absence of hemorrhage, platelets are not believed to be essential to wound healing. Inflammatory leukocytes begin to work at the wound site while neutrophils cleanse the site of foreign matter and bacteria, with some undergoing phagocytosis by macrophages. Monocytes arrive at the wound site and become activated macrophages, which initiate the formation of granulation tissue. The macrophages continue phagocytosis to remove damaged tissues, foreign matter, and microbes. Inflammatory cytokines expressed at the wound site have effects such as the stimulation monocytes to become macrophages, promotion of colony formation, and the attraction of fibroblasts. Growth factors released by the monocytes and macrophages are also important, as reflected by defective wound repair in macrophage-depleted animals (*1*).

Tissue formation in the form of re-epithelialization begins to take place just hours from the initial injury. Epidermal cells from skin appendages such as hair follicles and sweat glands remove damaged stroma and clotted blood from the wound while undergoing phenotypic alterations. The epidermal and dermal cells no longer adhere to one another, as their hemidesmosomal links are dissolved. This allows for the lateral movement of the epidermal cells toward the center of the wound by following an ionic current known as the "current of injury" (*2*).The migrating epidermal cells dissect the wound, separating dried eschar from the viable tissues. The extracellular matrix begins to remodel as macrophages and fibroblasts use matrix metalloproteases to cut their way through the matrix and allow the epidermal cells to migrate between the collagenous dermis and the fibrin eschar. One to two days after the after the initial injury, the epidermal cells at the wound margin proliferate behind the migrating epidermal cells. The mechanism of cell migration is not clear, and several factors coordinate this activity (*3*–*5*). The epidermal cells eventually return to their normal phenotype during the remodeling phase, reattaching to the underlying dermis. The remodeling phase then continues for months to years, and the resultant scar tissue has reduced strength and functionality.

Ancient Wound Care Techniques

Wound care may be one of the earliest forms of biotechnology. It is an animal instinct to lick wounds in an attempt to clean them and promote healing, and this can be observed in mammals such as dogs, cats, rodents, apes, and even humans. Prehistoric man should have noticed the connection between wound hygiene and recovery, and based on this likely began devising techniques to promote healing. Early hunter gatherers would surely have experimented with herbal and other natural remedies to aid wound healing and stop bleeding. One of the earliest wound care strategies is outlined in the Ebers Papyrus (*6*) ca. 1500 B.C., where a combination of grease from animal fat, cotton fibers, and honey was used for wound care. It is likely that this treatment was the result of trial and error and that the underlying mechanisms behind the success of the treatment were not completely understood at the time. The animal fats helped provide protection

from the environment and kept the wound moist, the cotton fibers provided a fibrous matrix for clotting, and the honey was added as an antiseptic as well as perhaps a binder. Honey was one of the early antiseptic compounds placed on wounds, and while debate exists over its effectiveness (*7*), it continues to be used in modern medicine.

Wound Care in the 19th and 20th Centuries

While wound care saw steady advances dating from prehistory, it was not until the 19th century that serious advances in medical science allowed for a revolution in wound care. One of the pioneers of medical hygiene was the American physician Oliver Wendell Holmes, an outspoken critic of medical practices of the 19th Century. He was criticized for his theories regarding the spread of puerperal fever, known today as septicemia, from doctor to patient. One critic, Charles D. Meigs, wrote (*8*) that doctors are gentlemen and that "…gentleman's hands are clean." The Hungarian obstetrician Ignaz Schemmelweis also supported the benefits of hand washing in maternal survival several years later in Vienna, and began using an analog of modern bleach as prophylaxis in 1847 to cleanse away "cadaverous particles" (*9*). This work was further developed in the 1860's by the British surgeon Joseph Lister, also known as the "Father of Modern Antisepsis", who developed several procedures and practices involving carbolic acid (phenol) solutions, non-porous materials in surgery, and the use of clean gloves (*10*). It was the early work of the French chemist Louis Pasteur that had motivated Joseph Lister, and this along with the work of Holmes and Schemmelweis helped establish the germ theory of disease, arguably the most significant advance in modern medicine. The German surgeons Ernst von Bergmann (*11*) and Paul Leopold Friedrich (*12*), would later introduce the heat sterilization of surgical instruments in 1886 and the excision of wound sites in 1898 respectively.

The 20th Century saw advances in synthetic polymers such as nylon, polyolefins, and polyvinyls. This provided a wide variety of new materials for the development of dressings and other wound care materials. During the mid-20th Century it was the work of the British doctor George D. Winter (*13*) that rediscovered the importance of moist wound healing, something noted by the Greek surgeon Galen of Pergamum ca. 120-200 A.D. (*14*). This is considered by many to be the dawn of modern wound care, with a significant enhancement of re-epithialization resulting in superior wound healing.

Antiseptics as a Double-Edged Sword

The contributions of Holmes, Lister, and Schemmelweis involved the use of antiseptic chemicals to create a sterile environment in and around open wounds. While the development of such techniques has undoubtedly saved countless lives, it has been recognized that such antiseptics also pose the risk of causing undesirable effects as well. Bleach is an excellent disinfectant for surgical equipment, but is not recommended for cleaning wounds, as it has a deleterious affect on mammalian cells. The use of iodine tinctures has long been established

as an effective topical antiseptic, and is generally considered non-toxic to humans. In fact some surgeries involve irrigation of the thoracic cavity with large quantities of povidone-iodine solution during surgery. Nevertheless, there have been reports of acute sensitivity in some patients causing serious complications (*15*). This suggests that to determine the suitability of an antiseptic, both the microbicidal activity and the cytotoxicity must be taken into consideration.

Wound Care in the Modern Era

The rediscovery of moist wound healing by George D. Winter and Howard Maibach in the 1960's led researchers to design wound care strategies that more closely mimicked the human body. Superabsorbent polymers known as hydrogels were incorporated into treatments for burns and other wounds throughout the 1990's. Recent developments in tissue engineering have allowed for "living skin equivalents" to be created from cultured epithelial cells in a matrix such as a hydrogel or biopolymer. While these materials lack many of the components of whole living skin, they provide the potential for novel skin grafting techniques for extensive wounds.

Use of Carbon Nanotubes in Biotechnology Applications

Since the emergence of carbon nanotubes in the early 1990's, there has been a steadily increasing interest regarding the application of these materials to existing technologies that had employed other carbonaceous materials such as graphite and charcoal, as well as to develop novel applications that would exploit the unique characteristics of these structures. While the actual number of carbon nanotube applications to biotechnology and other biologically centered fields of study is vast, they generally fit into one of several categories. The most commonly discussed categories are composites (*16*), interfaces (*17*), sensors (*18*), biomimetic actuators (*19*), drug delivery (*20*), and therapeutic agents (*21*). The present work encompasses several of these categories, namely composites, interfaces, sensors, and drug delivery.

Elephant in the Room: Nanotoxicity

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 (*22*). Increasing attention has been focused on the impact of CNTs on cell growth, with results of some studies showing toxicity and others showing enhanced cell growth. These seemingly contradictory results can be rationalized by the various compositions, lengths, diameters, and levels of CNT 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 (*23*, *24*). The work presented here uses high purity CNT material (filtration allows for further

reduction of amorphous carbon 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 (*25*, *26*). These pre ious studies employed CNTs for cell growth substrates with no apparent toxicity, and provide motivation for the work presented herein.

Carbon Nanotube-Based Antiseptic Bandages

Single wall carbon nanotubes (SWNTs) were combined with a water-based povidone-iodine (PVPI) complex as an aqueous suspension, which was then deposited as a film on a polytetrafluoroethylene (PTFE) filter membrane to form a three-dimensional nanocomposite network supported on PTFE. CNTs are routinely solubilized with povidone (also referred to as polyvinylpyrrolidone or PVP) by relying on a polymer wrapping mechanism that essentially encases the SWNT in a polymer monolayer (*27*) with a helical coil conformation, which is also the proposed structure of the povidone-iodine complex in water (*28*, *29*). The povidone-iodine complex (PVPI) has well-known antiseptic properties and is effective against a wide spectrum of pathogens, including *Escherichia coli* (*E. coli*) (*30*). 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 (*29*). Combining PVPI with SWNTs in water can allow for a stable water-based dispersion of SWNTs with iodine non-covalently bound to the surface. The film resulting from suction filtration onto a PTFE membrane is micro-porous, several microns thick, and has micron-length SWNTs randomly arranged within a polymer (povidone) coating. The SWNT–PVPI film (*31*) is highly flexible and remains bound to the PTFE membrane unless removed with an adhesive tape or similar adhesive material.

Synthesis of Antiseptic Bandage Material

Comprehensive details (*31*) of the material synthesis and analysis are provided elsewhere, however selected procedures are restated here for clarity. Purified SWNTs, obtained from Swan Chemical, Inc., have less than 3.7% wt. ash content and less than 1.7% wt. iron content (the majority of which is encapsulated) 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 SWNTs were solubilized in water by adding 10 mg SWNT to an aqueous solution that contained 1.5 mL PVPI solution (10% PVPI) and 18.5 mL deionized (DI) water, which is a ratio of approximately 150 mg PVPI to 10 mg SWNTs in 20 mL DI water, with a minority of stabilizing components present in Betadine® brand PVPI solution. The mixture is bath sonicated for 30 min to aid the suspension of SWNTs. The solution is then deposited onto a PTFE membrane with 1 μm pores (Millipore Omnipore JAWP-47mm) using vacuum

filtration, after which the film is dried in an oven (60 °C) for several hours *in vacuo*. Analysis of the deposited material with scanning electron microscopy (SEM) revealed a tangled mat of CNT-PVPI fibers (Figure 1). The presence of iodine in the finished bandage material was confirmed using energy-dispersive X-ray spectroscopy (EDX) performed during the SEM imaging, which shows two substantial iodine peaks (Figure 2). Additional peaks from the polymer and the PTFE filter membrane were also observed. No peaks for iron catalyst impurities were observed, indirectly confirming the high purity of the material as claimed by the manufacturer.

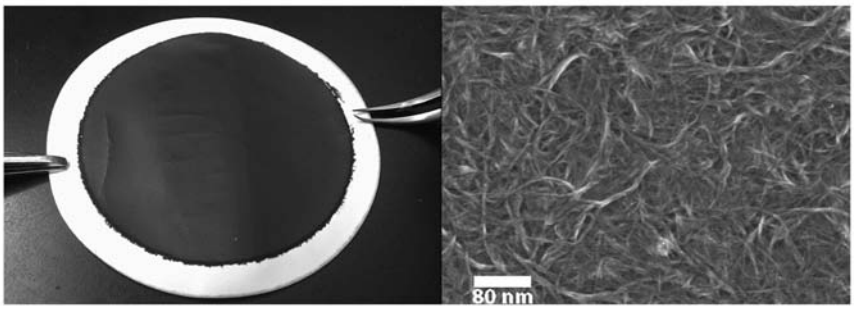

Figure 1. Digital photograph (left) and field emission-scanning electron microscopy images of CNT–PVPI bandage material. The SWNTs in the bandage films are covered by a layer of PVPI, scale bar approximately 80 nm. Image (left) reproduced with permission from ref. (31). Copyright 2009 Elsevier.

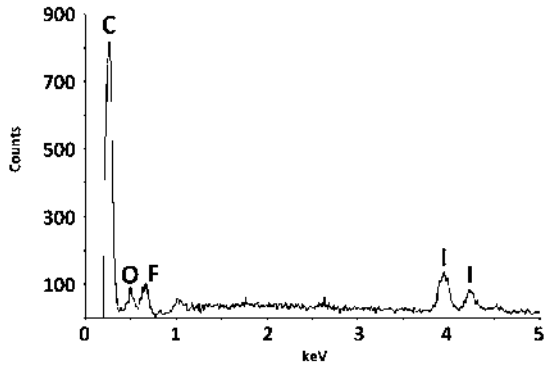

Figure 2. Energy-dispersive X-ray spectroscopy data obtained during imaging in the electron microscope show peaks indicative of carbon, oxygen, fluorine, and iodine. Data reproduced with permission from ref. (31). Copyright 2009 Elsevier.

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.

Upper images are bacterial cell cultures with the CNT material removed from the agar plates to reveal the amount of *E. coli* growth, with 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 (Figure 3 & 4).

This material would be useful as a bandage for wounds where there is a significant 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 have relatively low-toxicity to mammalian cells, although studies performed in our laboratories seem to contradict several studies that suggest PVPI will not slow wound healing or cell growth (*32–34*), and this point will be discussed in the following section of this chapter.

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 excess PVPI solution (*35*). The advantage of the CNT–PVPI bandage is that it is embedded with dry PVPI supported by SWNTs, and would be ready for immediate use, with no risk of burns from excess PVPI solution being trapped against the skin. The iodine is slowly released from the PVPI complex wrapping SWNTs (Figure 5), leaving behind PVP wrapped SWNTs in the bandage material, as they are strongly bound in the woven network of the bandage material.

The iodine is released from the SWNT-PVPI 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. When cellular adherence is desired, this can be controlled by the addition of a polymer such as poly-L-lysine (PLL). SWNTs 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 significant amout of direct contact between CNTs and the cells at the wound site.

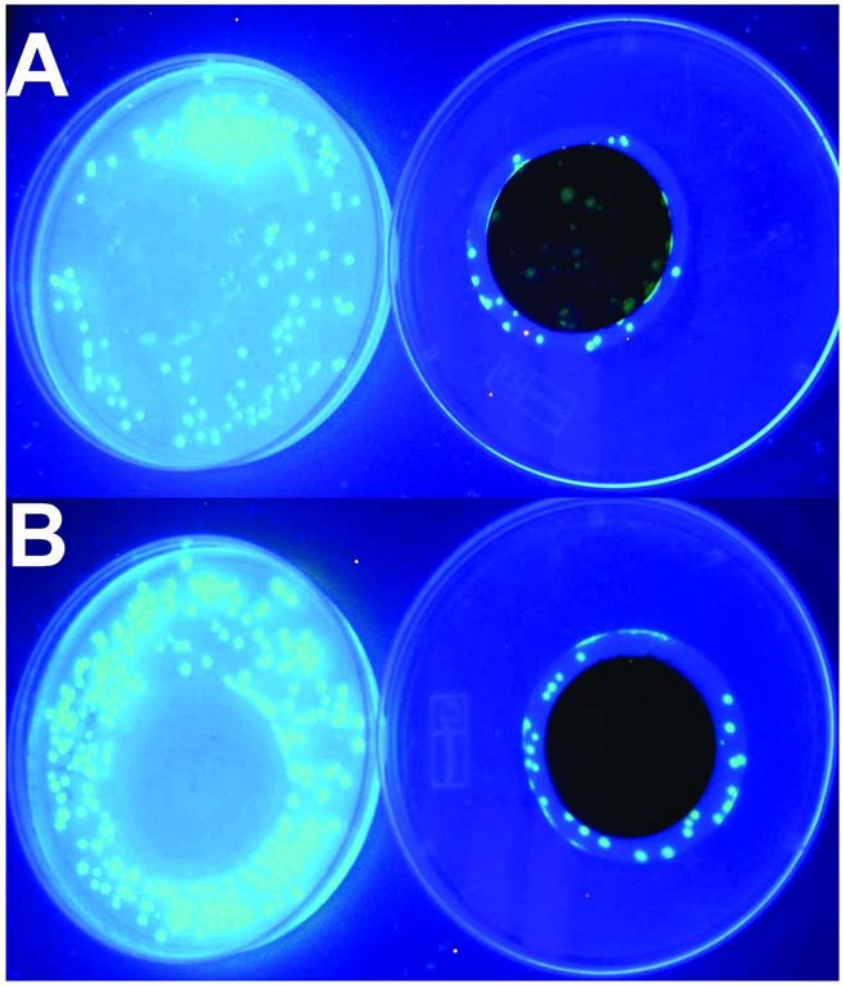

Figure 3. Photographs of bacterial growth media (left) and bandage material (right). CNT–PVP control (A) and CNT–PVPI (B) on 47mm PTFE filter membranes, under UVA illumination. Images reproduced with permission from ref. (31). Copyright 2009 Elsevier. (see color insert)

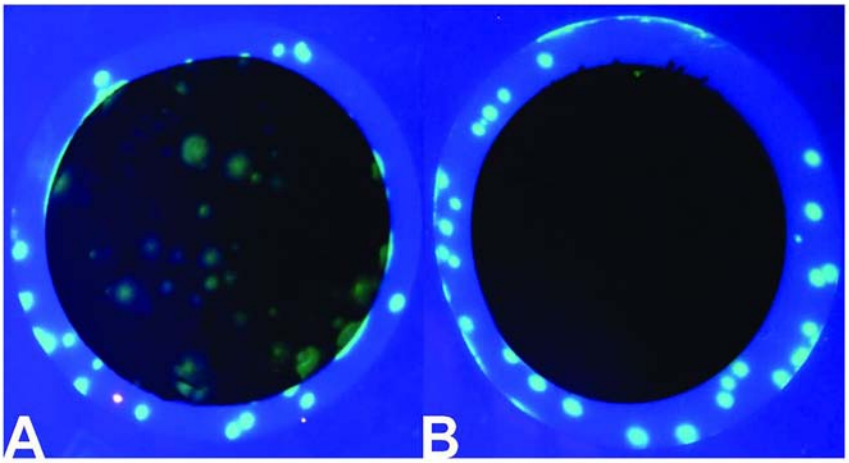

Figure 4. Photograph showing the control (A) sample showing significant E. coli growth, while the iodine containing sample (B) shows virtually no E. coli growth. Images reproduced with permission from ref. (31). Copyright 2009 Elsevier. (see color insert)

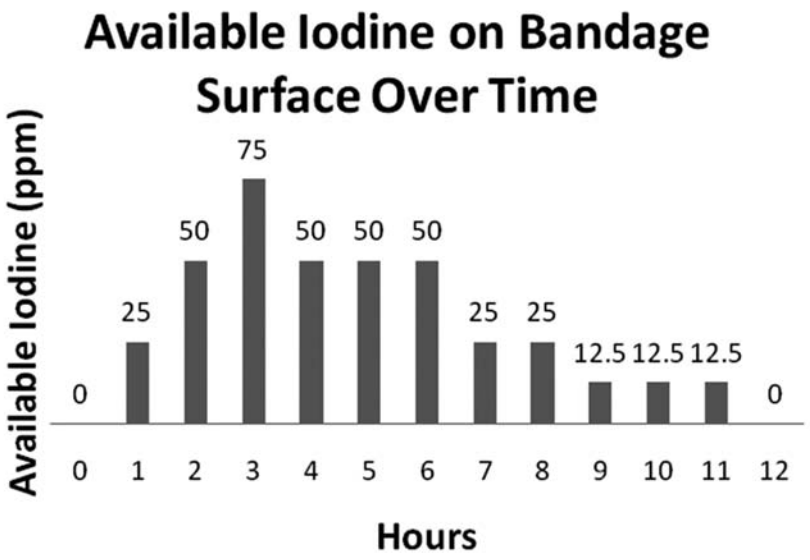

Figure 5. Measurement of available iodine at the bandage surface using iodine test paper containing starch and potassium iodide. A bandage sample 1 cm² was soaked in 2 mL phosphate buffered saline (PBS) solution at 37° C, with the solution being changed after each measurement. Data reproduced with permission from ref. (31). Copyright 2009 Elsevier.

Keratinocyte Growth Study

Although there are numerous studies which suggest that PVPI is of low-toxicity to human keratinocytes and other cells (*32–34*), other studies have suggested the contrary. It has been shown (*36*) that even dilute PVPI solutions will inhibit the growth of human fibroblast cells. In light of these conflicting bodies of work, a study was carried out to evaluate the effect of PVPI containing CNT films on human keratinocyte growth. CNT-PVPI films were created as previously described, and one set of films was washed thoroughly with deionized water to remove excess PVPI and to reduce the overall iodine content of the film. Another set of films was prepared by substituting PVP (10 kDa, Aldrich) solution for the PVPI solution (equivalent concentration), to provide a control with no iodine content.

Human keratinocytes (HaCaT cell line) were generously donated by Dr. Torsten Wittmann at the University of California at San Francisco. These cells were cultured at 37°C in 5% CO_2 in Dulbecco's modification of Eagle's Medium (DMEM; Mediatech). It was additionally supplemented with 15% Fetal Bovine Serum (FBS; Thermo Scientific) and 1% penicillin/streptomycin (Mediatech). The culture media was changed every three days, and the cells were routinely passaged in 75 cm^2 tissue culture flasks, discarding after 20 passages. A solution of 0.25% Trypsin/ethylene diaminetetraacetic acid (EDTA) (Mediatech) was used to harvest the cells. These human keratinocytes were added onto the CNT-PVPI and CNT-PVP films at a density of 25,000 cells in a volume of 100 μL in wells of a 96-well plate. Experiments were carried out in duplicate. The cells were incubated for 24-48 h and then evaluated by confocal fluorescence microscopy. The positive and negative controls were seeded with identical cell concentrations and incubated under identical conditions. Bovine serum albumin (BSA) inhibits cell attachment to growth surface while PLL promotes cell attachment, and therefore these materials were chosen for the negative and positive controls repectively. The polystyrene well plates of the negative and positive controls were exposed to a 5g/100 mL PBS solution of BSA and PLL respectively for 3 hours, then rinsed with fresh PBS solution and seeded.

Cellular viability was assessed using calcein-AM, a fluorescent green dye that becomes activated upon cleavage by intracellular esterases in live cells, and propidium iodide which is a red nuclear stain that is membrane permeable only for dead cells. Cells were first stained with propidium iodide in PBS (2μg/mL) for 30 minutes and washed twice with fresh PBS. Cells were then stained with a solution of calcein-AM in PBS (2μg/mL) for 15 minutes, washed twice with fresh PBS and imaged using an Olympus DSU fluorescence microscope. All samples were imaged at 4x magnification using constant exposure times and electronic gain settings.

It was immediately clear that the iodine containing films, even the washed CNT-PVPI films, showed very few live cells (Figure 6). The control CNT-PVP films showed growth of living cells at 24h and substantial clusters of live cells after 48 h. The cellular population of PVPI containing films do seem to corroborate the studies that claim PVPI inhibits the growth of mammalian cells (*36*). The slow increase of live cell populations of PVPI containing films over 48 h seems

to confirm that the iodine inhibits growth but is not acutely toxic, suggesting that PVPI does exhibit a low-toxicity to mammalian cells (*32–34*). The presence of polymer coated CNTs were not noticeably toxic to the cells, and this has also been reported in previous studies (*31*, *37*, *38*). The CNT-PVP film containing PLL showed results similar to the positive control (PLL coated cover glass). These findings suggest that the use of iodine in such CNT-PVP films should be limited in cases where the rapid establishment of keratinocytes and other mammalian cells is critical. If infection of the wound site by bacteria, fungi, or viruses is the key concern, then the use of iodine in such CNT-PVP films is acceptable. CNT-PVPI films would prevent the establishment of such infections, and eventually allow for the growth of keratinocytes and similar cells after a period of time. If the use of iodine-based antiseptics is indicated in a particular case, this study suggests that it would be acceptable to employ the CNT-PVPI bandage films to the wound site.

Electrical Stimulation To Promote Wound Healing

Humans and other animals have bioelectric systems that help to coordinate wound healing. One example of this is in the red spotted newt (*2*), where a steady polarized ionic current is produced when a limb is amputated, and continues until the limb has regrown. This "current of injury" was discussed previously in the outline of early wound healing. Keratinocytes migrate toward the center of a wound by following the current of injury along with other cues, and for this reason it is possible to increase the cell mobility at a wound site with electrical stimulation. Directed movement of cells by electric currents is known as galvanotaxis, and has been shown to occur within 10-20 minutes of direct current (DC) stimulation (*39*).

Electrical Stimulation of Keratinocytes with Carbon Nanotube Films

In addition to the antibacterial properties of this CNT-PVPI material, it is also a conductive material, showing a sheet resistance of approximately 10 kΩ/sq. Conductivity was determined by both 2-point and 4-point probe measurements, with an inter-probe distance of 1mm. The resistance determined was low enough to allow a significant electrical current to be passed through the material. This desirable electrical property makes it possible to explore enhanced cell growth through electrical stimulation, as electrical currents can enhance growth of cells such as neurons, keratinocytes, and fibroblasts (*40*). Liopo et al. showed that electrical stimulation through SWNT networks can help carry ionic currents that aid in the extension of neurites, and ultimately in building networks between nerve cells (*41*). Unfortunately it is contraindicated to use electrical currents in the presence of metals and certain elements, and this is especially important when antimicrobial metals like silver and elemental iodine may be present. It is for this reason that the electrical stimulation of cells should be performed only with CNT-PVP materials, and not CNT-PVPI materials. Therefore it will be necessary to develop alternative antiseptic agents if they are to be included in an electrical stimulation application.

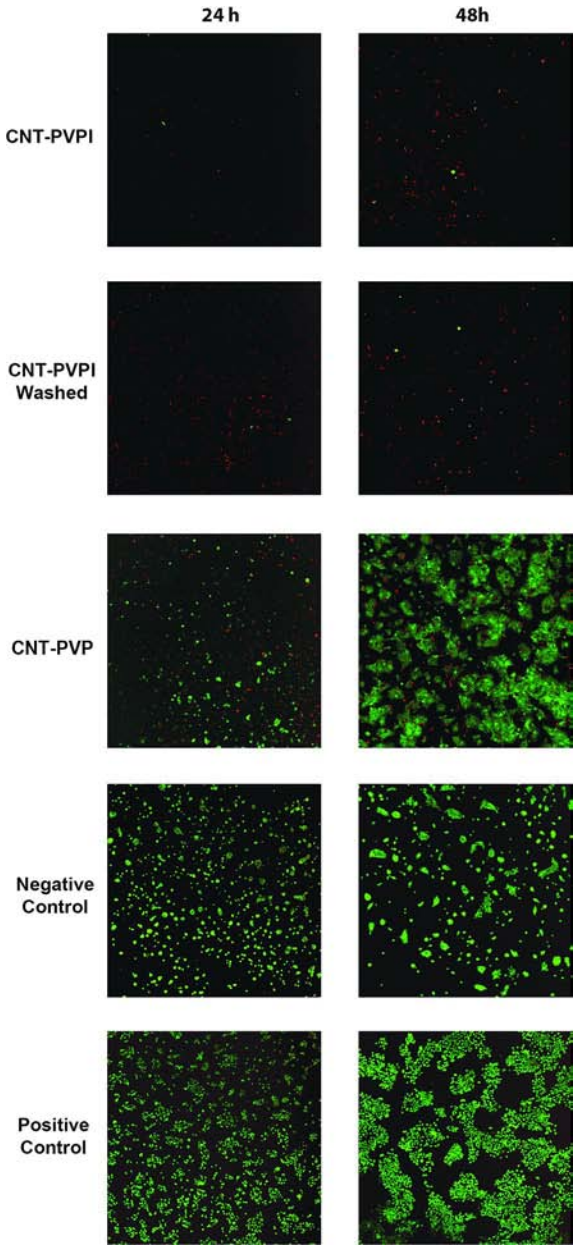

Figure 6. Confocal fluorescence microscopy images of live (green) and dead (red) cells after 24 (left) and 48 (right) hours respectively. All samples showed live cells after 24 h and an increase in live cells after 48 h, but the iodine containing samples (top and middle sets) showed significantly less. The negative control was grown on a BSA coated polystyrene well, and the positive control was grown on a PLL coated polystyrene well. (see color insert)

CNT-PVP films were evaluated as an electrically stimulating bandage for wound healing applications. Human keratinocytes cultured under conditions identical to those previously discussed in the cell viability study were seeded onto the carbon nanotube films at a density of 25,000 cells in a volume of 40 μL, creating a circular droplet. They were allowed to attach for a period of 3 h in a cell incubator. After 3 h of attachment, one of the carbon nanotube films was connected to a MPJA-14602PS DC power source with two gold-coated glass electrodes. The DC voltage supplied was set at 200 μV/mm at approximately 30 mA, which was calibrated to deliver approximately 50-100 μV/mm to the culture media containing the keratinocytes. The voltage was maintained between the electrodes with the keratinocytes in culture media in the middle of the carbon nanotube film avoiding direct contact with the electrodes (Figure 7).

The voltage was maintained for 1 h, with a drop in current from 30 mA to 20 mA over that time. During this time, an identical control sample, which received no electrical stimulation, was incubated in identical conditions. After the 1 h electrical stimulation, the cells on both the carbon nanotube films were then incubated for an additional 24 h before imaging. The same experiment was repeated with same cell seeding (25,000 cells), but in a volume of 100 μL, creating a relatively large circular droplet approximately 1 cm in diameter. These 100 μL cultures were allowed 3 h of attachment, 1 h of electrical stimulation, and 3 more h of growth. The effect of the electrical stimulation was examined by confocal fluorescence microscopy using calcein-AM to stain live cells (Figure 8).

As can be seen from the images, the cells in the electrically stimulated bandages (for both A and B, lower sets) are more widely distributed. There appears to be a directionality reflected in the growth pattern of the cells, with a larger proportion of the cells towards the direction of the cathode (+), which is the expected behavior for galvanotaxis of human keratinocytes subjected to DC current (*39*). This suggests that the CNT-PVP films were capable of delivering biologically relevant electrical stimulation to human keratinocytes. The smaller more evenly distributed cell clusters would allow for better coverage of an affected wound site, and increase the mobility of the keratinocytes. Increasing the mobility of keratinocytes is a critical component of wound healing, as this has been shown to minimize the effects of scarring (*42*).

Wound Monitoring with CNT Bandage

Real-time monitoring of wounds *in situ* is an important technological goal that is gaining increasing attention from researchers. The wound healing process is a complex and dynamic series of events, making it difficult for a single treatment strategy to be applicable in every case. It would be preferred for medical professionals to have the ability to respond to changes in the wound environment as they occur, and not rely on invasive procedures such as bandage removal for periodic observation of wound sites. Reduction in bandage and dressing removal for wound assessment would be an additional benefit of such *in situ* wound monitoring, as it would it would reduce pain and limit the possibility of infection. Several optical strategies are currently being developed that rely

on *in situ* immunofluorescence techniques targeting specific growth factors and proteins. In addition to such advanced techniques are complementary methods to detect changes in wound site conditions such as pH and moisture. These *in situ* wound monitoring techniques offer promise of a future where bandages can keep medical professionals updated on the status of a wound and deliver therapeutic agents at their discretion.

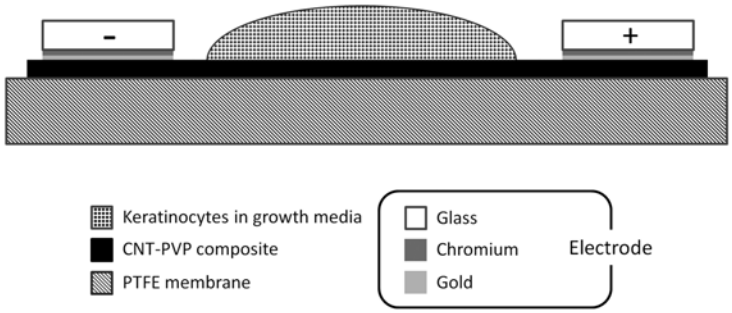

Figure 7. Schematic diagram of electrical stimulation experimental setup. Keratinocytes in a liquid growth media are deposited on a CNT-PVP composite film supported on a PTFE membrane. Two gold coated glass electrodes are placed on either side of the CNT-PVP film. There is a layer of chromium primer on the glass that allows for greater adherence of the gold.

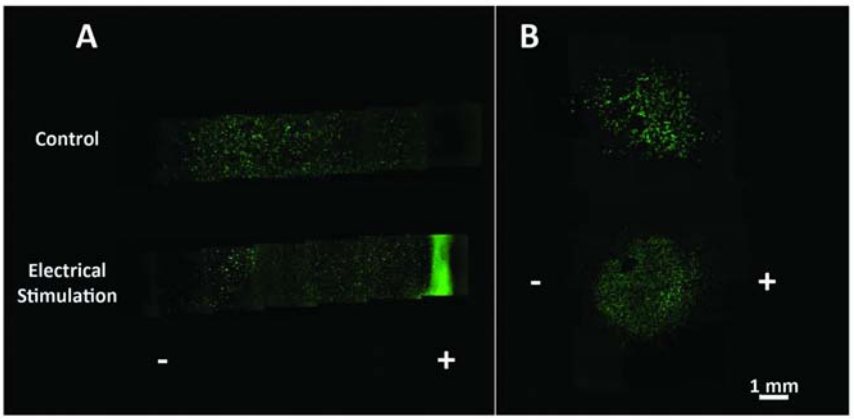

Figure 8. Confocal fluorescence imaging of live human keratinocytes at 4x magnification. The samples of both the 7 h (A) and 28 h (B) total incubation times show that the control set presents larger clusters of cells, while the electrically stimulated set shows a larger number of smaller clusters. The large green band seen on the electrically stimulated 7 h sample (A, lower) was identified by energy dispersive X-ray spectroscopy as NaCl. (see color insert)

Real-Time Monitoring of Clot Drying as Proof-of-Concept

The electrical conductivity of carbon nanotubes was exploited to demonstrate a simple proof-of-concept for use of CNT-PVP films for *in situ* wound monitoring. A simulated clot was created by combining 1 mL of a 10 mg/mL solution of fibrinogen in Dulbecco's phosphate buffered saline (DPBS) solution with 48 μL of a 50 U/mL solution of thrombin in DPBS. These two solutions were combined on the center of bandage with the fibrinogen being added first. The initial electrical resistance of the film studied was approximately 600 Ω, and the addition of PBS to completely wet the film and the supporting PTFE membrane reduced this resistance to approximately 250 Ω. The CNT-PVP film wetted with PBS was given several minutes to equilibrate before testing began. The formation of the simulated clot on the surface of the bandage did not appreciably change the resistance. Once the clot formed, it was allowed to dry in ambient conditions (25 °C and 35% relative humidity). The studied was continued for 18 h and electrical measurements were digitally recorded by an automated LabVIEW program running on a desktop computer interfaced with a Keithley 2400 sourcemeter.

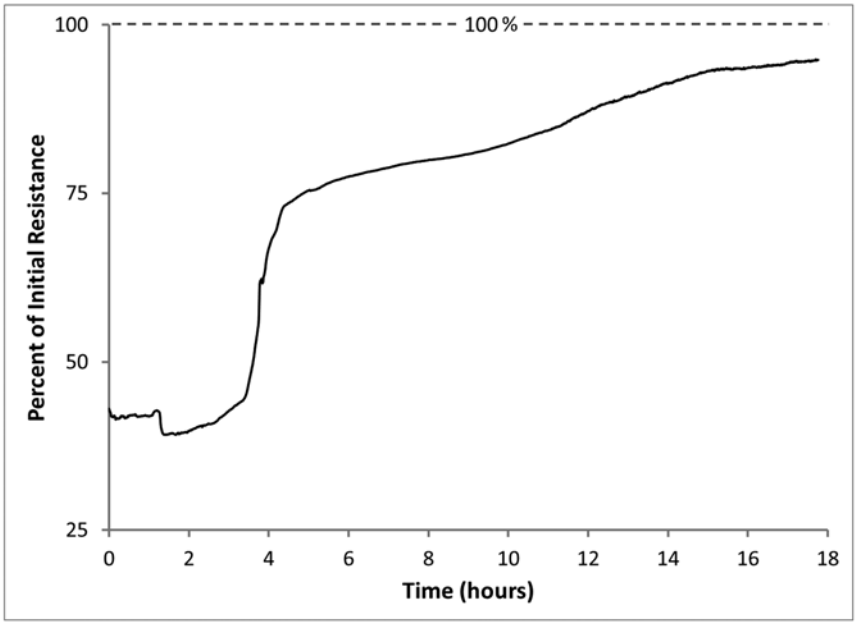

Figure 9. Electrical resistance monitoring of drying of a CNT-PVP bandage film with a simulated clot. Drying of the clot at 25 °C shows a sharp increase in resistance at 4 h, returning quickly to approximately 75% of the initial resistance (~600 Ω).

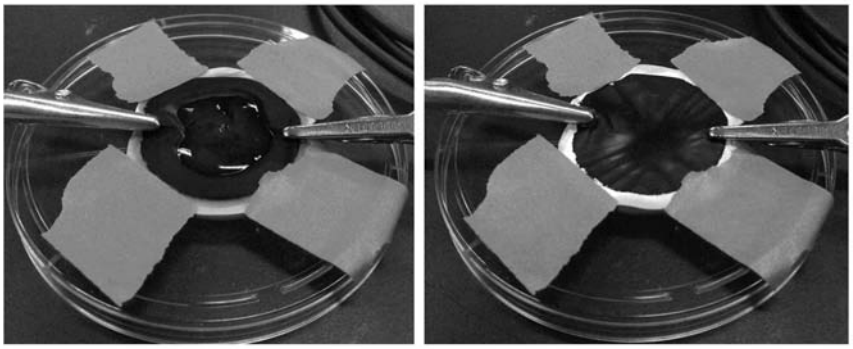

Figure 10. Simulated clot (fibrinogen and thrombin in PBS) on CNT-PVP film before (left) and after (right) drying for 18 h at 25 °C.

After remaining essentially constant for about 1 h, a sudden drop was noted, followed by a slow increase until about 3 h (Figure 9). The resistance significantly increased between 3h 15 m and 4 h 15 m, and then maintained a relatively steady increase for the remaining 14 h of the study. The final resistance at 18 h was approximately 95% the initial value, and the bandage material appeared to be completely dried with an obvious residue from the simulated clot remaining (Figure 10).

Conclusions

In summary, we have developed a novel nanocomposite material from the combination of SWNTs with PVP in aqueous media. The filtration of this aqueous suspension creates a high purity micro-porous film that can have antiseptic iodine available on the surface of a network of SWNT 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 an antiseptic bandage. This study showed that although PVPI is accepted as a low-toxicity antiseptic for mammals, the application of the CNT-PVPI material must be judicious, taking into account the inhibition of cell growth during the initial 48 hours. Electrical pulses sent through this SWNT 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. Moreover, future experiments will be required to integrate both an antiseptic strategy with an electrical stimulation strategy in the same material. To accomplish this, an antiseptic other than iodine or silver must be identified, and incorporated with the CNT-PVP material. Ultimately the goal of materials

development for modern wound care should be to develop "smart" bandages and dressings. These smart materials should both carry out *in situ* monitoring of wounds and deliver therapeutic agents at appropriate points in the wound healing process to accelerate recovery, reduce pain, and minimize scarring.

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References

1. Leibovich, S. J.; Ross, R. *Am. J. Pathol.* **1975**, *78*, 71–100.
2. Jenkins, L. S.; Duerstock, B. S.; Borgens, R. B. *Dev. Biol.* **1996**, *178*, 251–262.
3. Nanney, L. B.; King, L. E., Jr. In *The molecular and cellular biology of wound repair*, 2nd ed.; Clark, R. A. F., Ed.; Plenum Press: New York; 1996; pp 171−194.
4. Abraham, J. A.; Klagsbrun, M. In *The molecular and cellular biology of wound repair*, 2nd ed.; Clark, R. A. F., Ed.; Plenum Press: New York; 1996; pp 195−248.
5. Werner, S.; Smola, H.; Liao, X.; Longaker, M. T.; Krieg, T.; Hofschneider, P. H.; Williams, L. T. *Science* **1994**, *266*, 819–22.
6. Ghalioungui, P. *The Ebers papyrus. A New English Translation, Commentaries and Glossary*; Academy of Scientific Research and Technology: Cairo, Egypt, 1987; Vol. 1, pp 1−298
7. Moore, O. A.; Smith, L. A.; Campbell, F.; Seers, K.; McQuay, H. J.; Moore, R. A. *BMC Complementary Altern. Med.* **2001**, *1*, 2.
8. Meigs, C. D. *On the Nature, Signs, and Treatment of Childbed Fevers*; Blanchard and Lea: Philadelphia, PA, 1854; Vol. 1, p 104.

9. Benedek I. *Ignaz Philipp Semmelweis: 1818-1865*; Corvina Kiadó: Budapest, Hungary, 1983.
10. Lister J. *Antiseptic Principle in the Practice of Surgery*, read by Joeseph Lister before the Surgical Section of the Annual Meeting of the British Medical Association in Dublin, August 9, 1867. Reprinted in *Br. Med. J.* **1967**, *2* (5543), 9–12.
11. Luther, B.; Wirth, I. *Zentralblatt für Chirurgie* **1986**, *111* (22), 1389–97.
12. Worm, V. *Zentralbl. Chir.* **1976**, 9.
13. Winter, G. D. *Nature* **1962**, *193* (4812), 293.
14. Carterand, M. J.; Fife, C. E. In *Microbiology of Wounds*; Percival, S., Cutting, K., Eds.; CRC Press: 2010; pp 345–372.
15. Campistol, J. M.; Abad, C.; Nogue, S.; Bertran, A. *J. Cardiovasc. Surg. (Torino)* **1988**, *28*, 410–412.
16. Yang, W. R.; Moghaddam, M.; Taylor, S.; Bojarski, B.; Wieczorek, L.; Herrman, J.; McCall, M. *Chem. Phys. Lett.* **2007**, *443*, 169–172.
17. Suehiro, J.; Ikeda, N.; Ohtsubo, A.; Imasaka, K. *Microfluid. Nanofluid.* **2008**, *5* (6), 741–747.
18. Lin, Y. H.; Lu, F.; Tu, Y.; Ren, Z. F. *Nano Lett.* **2004**, *4* (2), 191–195.
19. Vohrer, U.; Kolaric, I.; Haque, M. H.; Roth, S.; Detlaff-Weglikowska, U. *Carbon* **2004**, *42* (5−6), 1159–1164.
20. Bianco, A.; Kostarelos, K.; Prato, M. *Curr. Opin. Chem. Biol.* **2005**, *9*, 674–9.
21. Mashal, A.; Sitharaman, B.; Li, X.; Avti, P. K.; Sahakian, A. V.; Booske, J. H.; Hagness, S. C. *IEEE Trans. Biomed. Eng.* **2010**, *57* (8), 1831–1834.
22. Lacerda, L.; Bianco, A.; Prato, M.; Kostarelos, K. *Adv. Drug Delivery Rev.* **2006**, *58*, 1460–70.
23. Shvedova, A. A.; Castranova, V. *J. Toxicol. Environ. Health, Part A* **2003**, *66* (20), 1909–1926.
24. Monteiro-Riviere, N. A.; Nemanich, R. J.; Inman, A. O.; Wang, Y. Y.; Riviere, J. E. *Toxicol. Lett.* **2005**, *155*, 377–384.
25. Jan, E.; Kotov, N. A. *Nano Lett.* **2007**, *7* (5), 1123–1128.
26. MacDonald, R. A.; Laurenzi, B. F.; Viswanathan, G.; Ajayan, P. M.; Stegemann, J. P. *J. Biomed. Mater. Res. A* **2005**, *74A* (3), 489–96.
27. Bravo-Sanchez, M.; Simmons, T. J.; Vidal, M. A. *Carbon* **2010**, *48* (12), 3531–3542.
28. Simmons, T. J.; Hashim, D. P.; Vajtai, R.; Ajayan, P. M. *J. Am. Chem. Soc.* **2007**, *129* (33), 10088–10089.
29. Betadine® Microbicides (which contain povidone-iodine) Product Information. Purdue Products L.P., 2005.
30. Houang, E. T.; Gilmore, O. J. A.; Reid, C.; Shaw, E. J. *J. Clin. Pathol.* **1976**, *29* (8), 752–755.
31. Simmons, T. J.; Lee, S-H.; Park, T-. J.; Hashim, D. P.; Ajayan, P. M.; Linhardt, R. J. *Carbon* **2009**, *47*, 1561–1564.
32. Müller, G.; Kramer, A. J. *Antimicrob. Chemother.* **2008**, *61* (6), 1281–1287.
33. Zhou, L. H.; Nahm, W. K.; Badiavas, E.; Yufit, T.; Falanga, V. *Br. J. Dermatol.* **2002**, *146* (3), 365–74.
34. Banwell, H. *Dermatology* **2006**, *212* (S1), 66–76.

35. Nahlieli, O.; Baruchin, A. M.; Levi, D.; Shapira, Y.; Yoffe, B. *Burns* **2001**, *27* (2), 185–188.
36. Balin, A. K.; Pratt, P. *Dermatol. Surg.* **2002**, *28*, 3.
37. Monteiro-Riviere, N. A.; Inman, A. O.; Wang, Y. Y.; Nemanich, R. J. *Nanomedicine* **2005**, *1*, 293–299.
38. Firme, C. P., III; Bandaru, P. R. *Nanotechnol., Biol., Med.* **2010**, *6*, 245–256.
39. Fang, K. S.; Farboud, B.; Nuccitelli, R.; Isseroff, R. R. *J. Invest. Dermatol.* **1998**, *111*, 5.
40. McCaig, C. D.; Rajnicek, A. M.; Song, B.; Zhao, M. *Physiol. Rev.* **2005**, *85*, 943–978.
41. Liopo, A. V.; Stewart, M. P.; Hudson, J.; Tour, J. M.; Pappas, T. C. *J. Nanosci. Nanotechnol.* **2006**, *6* (5), 1365–74.
42. Park, J-. C.; Park, B. J.; Suh, H.; Park, B. Y.; Rah, D. K. *Yonsei Med. J.* **2011**, *42* (6), 587–594.