Continuous Adsorption of Poly(L-Lysine) Under an Applied Electric Potential Onto Single-Walled Carbon Nanotube-Coated Indium Tin Oxide Substrates

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Review of Literature

Today, infectious disease is the foremost cause of human fatality (WHO, *Top Ten Causes of Death*). Though modern medicine has appreciably diminished infectious diseases among humans, they still remain a major threat to human longevity. Ironically, many infectious diseases originate in the hospital. Such nosocomial infections reach seven digits annually, with nearly one million resulting from implantable devices (W. R. Jarvis et al., 2007). Moreover, the added cost of these infections, on average, varies from $15,275 (Roberts RR et al., 2003) to $38,656 (Zhan C et al., 2003) per infection. Because of the ironic nature of these infections and the controllability of the hospital environment, these infections are of special interest.

Various antimicrobial materials have been proposed to restrict pathogen growth on medical devices (A.J. Kugel et al., 2009), yet these efforts have been largely unsuccessful and/or lack efficiency. Recently, single walled carbon nanotubes (SWNTs) – in the form of membrane coatings and deposited aggregates – have been proven to be highly antimicrobial (S. Kang et al., 2007), and have been characterized in many ways as ideal antimicrobial materials. They have also been described as chemically stable and easy to functionalize. SWNTs are, therefore, effective in the inactivation of several pathogens (Upadhyayula et al., 2009).

Combining these nanomaterials with customized polymers could coalesce SWNTs' antimicrobial activity with the desirable structural, mechanical, and degradation properties of the polymers (Aslan et al., 2010). In order to do so, researchers have recently looked towards the construction of nanofilms. A method called layer-by-layer (LbL) assembly has lately gained prominence as a simplistic way to produce such thin (50nm – 1μm) multilayer polyelectrolyte nanofilms (J. B. Schlenoff et al., 2003). The idea behind this is layering oppositely charged polymers atop one another (and all on top of a substrate) (A. P. Ngankam and P. R. Van Tassel,

2005) resulting in a film created by adsorption. There are several advantages to such a method. Particularly attractive are the ease of the process, the resulting uniform films, and the possibility of coating objects of various shapes (Xingjie Zan et al., 2010).

However, the process behind creating LbL films to house carbon nanotubes is not perfect. In order to create this films, one must be very attentive - dipping and taking out the substrate from various polymer and buffer solutions every few minutes (over the course of several hours). This process thus proves to be extremely tedious, and has recently caused researchers to look elsewhere for an efficient way to fabricate polymer nanofilms of desired thickness. In order to do so, researchers have searched for and studied various methods that invoke continuous adsorption of polymer onto a substrate. Continuous adsorption allows for the creation of a polymer nanofilm using only one polymer (in LbL assembly of polymer nanofilms, at least two polymers are necessary to create a film). This is much more useful than the use of LbL assembly, as a smaller range of materials are necessary to create a film of desired thickness. Continuous adsorption involves the substrate (after it is prepped) being set into only one polymer solution, allowing the individual creating the films to be much less attentive than when he/she is creating a film via LbL assembly.

Continuous adsorption of polymer onto a substrate has been proven to be possible under the application of a small anodic potential to the substrate (A. Pascal Ngankam et al., 2007). Evidence of nearly linear continuous adsorption of poly(L-lysine) (PLL) - a polymer frequently used in the fabrication of nanofilms - over several hours is made obvious through the use of optical waveguide lightmode spectroscopy (OWLS) and an indium tin oxide (ITO) substrate, to which the electric potential was applied (Michelle A. Brusatori et al., 2003). After proving continuous adsorption under an applied potential to be successful, researchers turned towards

including various materials in these nanofilms produced via continuous adsorption. Because of the prevalence of infectious disease, many of these materials have been antimicrobial. With the recent introduction of carbon nanotubes (single-walled carbon nanotubes, or SWNTs, specifically) into the world of antimicrobial materials, heads have turned towards the possibility of using this allotrope of carbon in nanofilms created via continuous adsorption.

The purpose of this research is to help smooth the process of creating antimicrobial nanofilms (that can be used to coat medical devices and in various other biomedical applications). In this study, we will assess whether or not it is possible to achieve continuous adsorption of poly(L-lysine) onto a substrate that is coated in single-walled carbon nanotubes. It is hypothesized that continuous adsorption of PLL onto a substrate coated in SWNTs will be possible. It has already been proven that continuous adsorption of PLL onto a substrate without any coating is feasible (Ngankam et al., 2007) - the addition of the SWNTs to the substrate prior to the adsorption of polymer should not interfere with the ability of PLL to be continuously adsorbed under an applied electric potential. In fact, the extra exposed surface area and conductivity of the substrate that comes from the carbon nanotube coating should only facilitate the process of continuous adsorption, as this process is based on running an electric potential through a substrate. Thus, it is also hypothesized that continuous adsorption of PLL will occur at a higher rate when the substrate is coated in SWNTs.

Methods

Poly(L-lysine) and Poly(L-glutamic acid) were both used in this study. In order to make the polymer solutions to be used in the nanofilms created, 0.01 grams of each polymer concentrate was dissolved into HEPES buffer to create 100 mL of solution (concentration =

0.1g/L). Several substrates to which continuous adsorption of PLL would be tested were set aside. These substrates were (bio)sensor chips of different compositions - one of silicon titanium oxide (STO) and one of indium tin oxide (ITO). The latter chips were used when an electric potential was applied in order to test for continuous adsorption of polymer. For each nanofilm created, Optical Waveguide Lightmode Spectroscopy (OWLS) was used to determine the amount of polymer adsorption onto ITO and STO sensor chips that were placed into the OWLS 210 Biosensor machine. The machine fed back the dynamic refractive index of the chips being coated. The increasing amount of polymer adsorbed onto the substrates was noted by the increasing refractive index of the substrate and polymer complex. Prior to the addition of polymer onto each substrate, some ITO and standard sensor chips were dip-coated in a SWNT solution (0.01 g/L) made with chloroform and sonicated frequently to prevent aggregation of SWNTs. These coated chips were placed in the OWLS 210 machine and PLL was run through.

The dynamic refractive index was observed using a 2010 iMac with Biosensing software. After a saturated level of PLL was obtained on the chip (determined by a constant refractive index over several minutes), an electric potential of 1.5V was hooked up to the OWLS 210 machine and the resulting dynamic refractive index was observed. It was noted that a constantly increasing refractive index over an extensive period of time would suggest continuous adsorption. These tests were ran with and without voltage, as well as with and without a SWNT coating on each type of chip (as controls). In order to clean any substrate that were previously coated in SWNTs, the substrate was placed in a beaker of pure chloroform. The beaker was then placed in a bath sonicator for one hour, and the substrate was returned to a basin of DI water in order to rinse off any remnant chloroform. The chips could then be used as substrates for the fabrication of other polymer films.

Several OWLS tests were run employing non-continuous adsorption of PLL and PGA (poly(L-glutamic acid)), simulating the typical layer-by-layer method of creating polymer films of desired thickness (Figures 1 and 2).

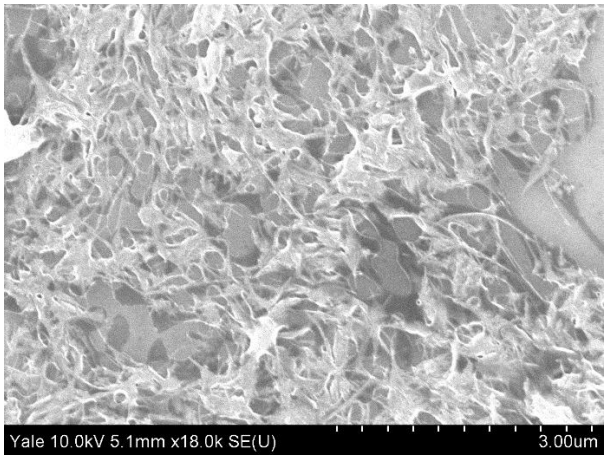

Figure 1
Scanning electron microscopy (SEM) image of one polymer (PLL) layer

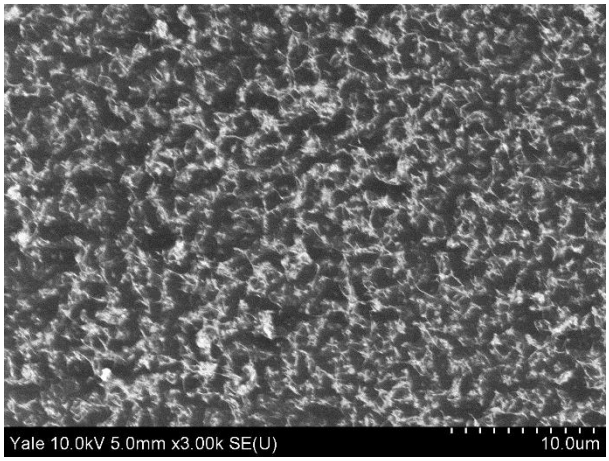

Figure 2
SEM image of two polymer (PLL + PGA) layers

The resulting films on the ITO and standard sensor chips were compared to continuously adsorbed films (of only PLL) using the dynamic refractive index graph output from the previously mentioned biosensing software. In order to assess the accuracy of these tests, the "peaks" graphs for each nanofilm fabricated were studied. "Peaks" graphs measure the photocurrent running through each substrate as it is in the OWLS 210 machine, rotating between an angle of -5° and +5°. The higher and more consistent the peaks in the graph (illustrating high photocurrent), the more reliable the dynamic refractive index graph of the substrate as measured concurrently by the OWLS 210 machine. The peak graphs for each substrate and polymer complex were looked at in order to see how the substrates and polymers used affected the accuracy of the OWLS 210 machine in assessing the dynamic refractive index of each complex.

To analyze the data/results, an output graph (from the above mentioned biosensing software) of the dynamic refractive index of the standard or ITO sensor chip was studied. The

amount that the refractive index of the chip changes over time was used in order to determine the extent of adsorption of polymer onto the chips, and helped determine whether or not continuous adsorption of polymer was occurring. It was apparent that if a continuously growing refractive index persisted over a long period of time (with no change in input to the OWLS 210 machine), then the supposition of continuous adsorption would be supported.

Results

Before running any OWLS tests, the peaks graphs (as per the description above) for several substrates were examined in order to make sure that the OWLS 210 machine would output an accurate reading for dynamic refractive index. The peaks graphs for ITO and STO chips with and without SWNT coatings (and after cleaning those with SWNTs with chloroform) were assessed. In general, they demonstrated that a sufficient amount of light from the HeNe laser was penetrating the substrate, allowing for an accurate reading of the varying thickness of the substrate as polymer layers were adsorbed. The most important peaks graphs for this study are in Figure 3.

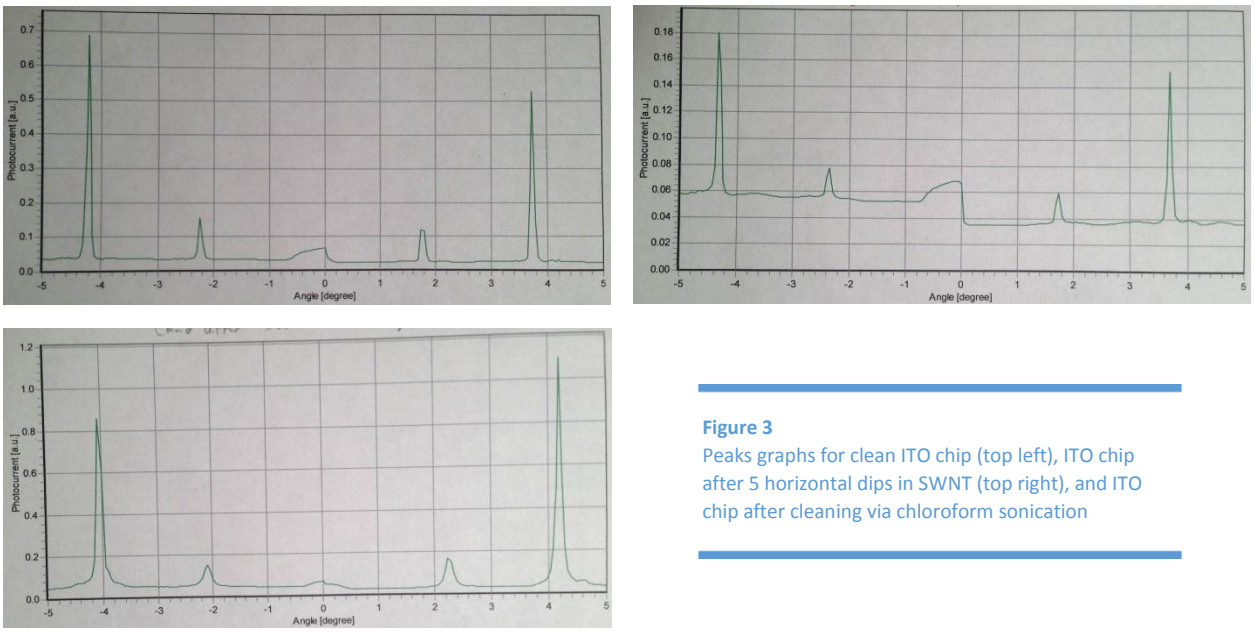

Figure 3
Peaks graphs for clean ITO chip (top left), ITO chip after 5 horizontal dips in SWNT (top right), and ITO chip after cleaning via chloroform sonication

These graphs display how a high level of photocurrent was able to run through the substrate in the OWLS 210 machine when the substrate is new and not coated with anything. Further, when the substrate had been dipped five times in a SWNT-chloroform solution, a smaller quantity of photocurrent flowed through the substrate. After the substrate had been cleaned via placing it in chloroform and allowing it to bath sonicate for an hour, the optimal level of photocurrent flowed through the substrate in the OWLS 210 machine.

After determining that an appropriate amount of light from the HeNe laser was penetrating the substrate in the OWLS 210 machine at various angles, we were able to move on to adding polymer to each substrate (and assessing the increasing thickness of the growing film by observing the dynamic refractive index) while in the OWLS 210 machine. The uncoated STO chip displayed a rather predictable dynamic refractive index graph. Each layer of polymer added to the refractive index of the substrate and polymer complex a value of about 0.0001. The addition of the polymers PLL and PGA to the substrate followed a course that was to be expected: after each polymer added to the refractive index by about 0.0001, the refractive index stabilized at this new value; it could be assumed that the substrate had reached the current level of saturation for that polymer. This pattern continued as alternating layers of PLL and PGA were added, confirming that layer-by-layer assembly of polymer nanofilms does not involve any continuous adsorption. When the STO chip was coated in a layer of SWNT, the dynamic refractive index graph for layer-by-layer assembly of PLL and PGA mirrored the layer-by-layer graph of the clean STO chip.

Again, these results were mirrored when the substrate was changed to an ITO chip (Figure 4). However, the ITO chip allowed us to apply an electric potential in order to possibly induce continuous adsorption. When using an uncoated ITO chip and applying an electric potential of 1.5V after one layer of PLL was adsorbed and reached its level of saturation, past research (Ngankam et al., 2007) suggesting that one can induce continuous adsorption of PLL onto an ITO chip (under an applied potential) was supported: the refractive index of the substrate and film complex continued to grow linearly, without any apparent level of saturation of PLL.

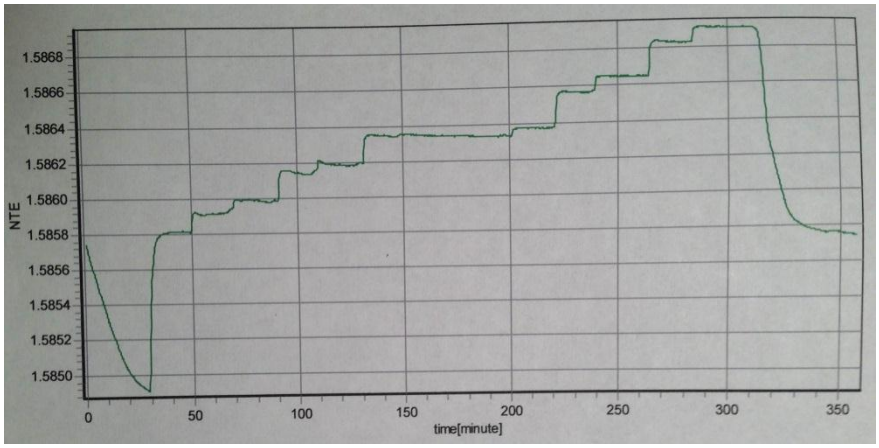

Figure 4
Layer-by-layer adsorption of PLL and PGA onto a SWNT-coated ITO substrate

The ITO chip coated with SWNT also demonstrated significant evidence of continuous adsorption: after applying 1.5V to the substrate and polymer complex after the substrate had saturated with PLL, the refractive index of the complex continued to grow over the next hour without any clear sign of imminent saturation of PLL (Figure 5). The growth of the film through

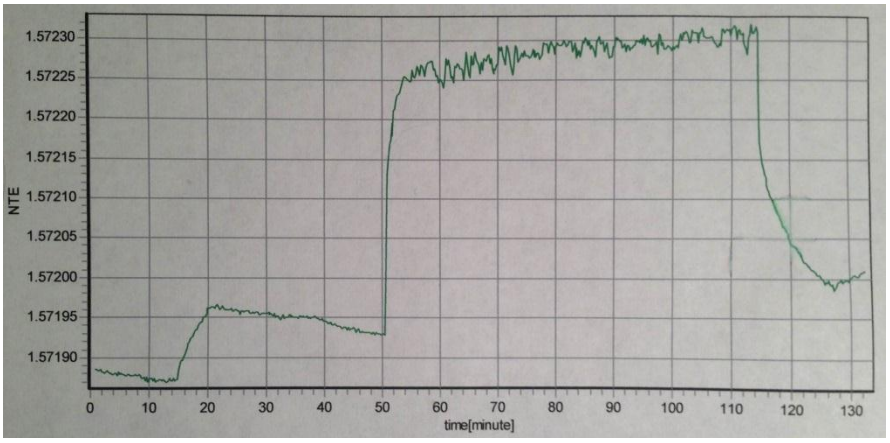

Figure 5
Continuous adsorption of PLL onto a SWNT-coated ITO substrate under an applied potential of 1.5V (at 50 min)

the continuous adsorption of PLL is evidenced by this increasing refractive index (about 0.0007/hr). Though this rate of increasing refractive index is not any greater than that of the continuous adsorption of PLL onto the uncoated ITO chip test, it suggests that continuous adsorption of PLL onto a SWNT-coated substrate is indeed feasible. Multiple tests run through the OWLS 210 machine proved this increasing rate of refractive index to be reproducible.

Discussion

Researchers have been searching for ways to combat harmful pathogens. In the past, several methods for controlling infectious diseases have been presented, including the use of silver (Davies et al., 1997), the boiling of liquids, and the use of reverse osmosis for water purification (Joyce et al., 2001). The use of carbon nanotubes has recently been suggested as a method of inactivating bacteria. Past studies have supported the antimicrobial activity of carbon nanotubes (single-walled carbon nanotubes, or SWNTs, especially), and have brought them into the world of antimicrobial materials (S. Kang et al., 2007).

In order to incorporate SWNTs into any practical applications, they should be worked into a polymer nanofilm to combine the antimicrobial activity of the SWNTs with the desirable structural and mechanical properties of specific polymers (Aslan et al., 2010). Layer-by-layer nanofilm assembly is currently one of the most prolific methods of creating such films to which antimicrobial materials can be applied (A. P. Ngankam et al., 2005). However, this method is extremely tedious and requires the use of two different polymers in order to create a nanofilm of desired length. In order to combat such tedium, the formation of nanofilms by continuous adsorption of polymer under an applied potential has been introduced (A. P. Ngankam et al., 2007).

Though the ability of PLL to be continuously adsorbed (under an applied potential) onto an ITO substrate has already been supported, research regarding the possibility of PLL being continuously adsorbed onto a SWNT-coated ITO substrate has not been conducted prior to this study. This research has supported the hypothesis that continuous adsorption of PLL under an applied potential onto SWNT-coated ITO substrate is possible. However, the rate of continuous adsorption onto the SWNT-coated ITO substrate does not appear to exceed the rate of continuous adsorption onto an uncoated ITO substrate, as was originally hypothesized. The inherent conductivity of SWNTs did not seem to play any role in aiding continuous adsorption, even though this technique of adsorbing polymer is based on running an electric potential through a substrate.

This research brings us one step closer to SWNT-polymer nanofilms that are antimicrobial and easy to make. Unlike films created via layer-by-layer assembly, films made via continuous adsorption of polymer (in this study, PLL) allow for the use of fewer materials. By utilizing fewer materials in the fabrication of these nanofilms, we are able to create antimicrobial films that are simple to fabricate and pose a smaller danger in potential biomedical applications (e.g. smaller chances of adverse responses from individuals who come into contact with the films). The antimicrobial films produced in this study display significant promise in various applications (creating antibacterial coatings for medical devices in order to reduce nosocomial infections, etc.).

Conclusion & Future Research

This study has supported the objective that continuous adsorption of PLL onto a SWNT-coated substrate is possible under an applied electric potential. It opens the door to the

fabrication of uniform, easy-to-make, antimicrobial nanofilms. Such nanofilms will be able to be used to coat medical devices in order to prevent and reduce infectious disease and potentially save millions of lives. As we progress with this research, we will incorporate these nanofilms created via continuous adsorption (under an applied potential) of a single polymer into hospital settings, and determine how effective they actually are in inactivating bacteria and preventing infectious disease. Further research must also involve extensive testing of the potential toxicity of carbon nanotubes to humans, as researchers have yet to determine fully whether or not carbon nanotubes introduce long-term adverse effects.

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