

Resistance and potentiostatic based measurements of an antibody functionalized conductive polymer coated textile as a biosensor

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ABSTRACT

Effective food safety monitoring is challenged by the necessity to identify human infection capable microorganisms at sufficient infective doses and consumption levels. Regulatory agencies have developed methods for assessing food safety based on currently available technologies. Many of these technologies, however, require time-consuming amplification to produce a sufficient detection signal. While improvements have been made to these current technologies offering increased sensitivity and decreased sample time, the new field of nanotechnology offers the potential for dramatic improvements. The conformal thin-film deposition of conducting polymer via vapor deposition onto a fiber mat is an attractive platform to base an electrochemical biosensor utilizing resistance and potentiostatic measurements. We present our continuing work on the development of a field portable biosensor for the detection of pathogens (i.e. *E. coli* O157:H7) in food safety based on this platform.

Keywords: biosensor, conductive polymer, food safety

1 INTRODUCTION

There is a need for improved detection technologies, sampling methodologies and assays to meet current food safety needs. Researchers have been looking at ways to reduce the number of particulates in the homogenized sample and to reconcentrate the bacteria for detection. [1,2] In addition novel biosensor concepts are needed to reduce the weight of the current detection platform and the amounts of chemicals required to run them. Small foot print biosensors with multiplexing capabilities that are robust and very sensitive will meet these requirements. Current field “portable” detection technologies are heavy (50 lbs) and require generous quantities of chemicals to operate them. Small hand-held lateral flow devices are light but have sensitivity levels ranging from 10^5 to 10^7 cfu/g. Sensors platforms made from functionally derived melt-spun fibers have been prepared and conformally coated with conductive polymers to create electrochemical biosensor devices. The geometry of the membrane is such that it creates an extremely high surface area for increased

antibody attachment as compared to magnetic beads, conventional membranes, glass, and silicon wafers. [3,4] The antibody functionalized conductive polymer textile acts as a transduction element when a target is bound resulting in a measureable signal through resistive or potentiostatic measurements. Because the capture and detection platform are the same, the melt-spun membranes will not require flow devices or pumps allowing for the development of smaller biosensor foot prints. It is expected that the available surface area demonstrated by this technique may provide a one to two log increase in both sensitivity and capture efficiency with less than a two-hour response time needed in real-world sensing applications.

2 EXPERIMENTAL

2.1 Textile Preparation

A nonwoven polypropylene fiber textile was produced using a melt-spun process to fabricate the fibrous platform. Nanofiber production was performed with a Fiberio Forcespinning™ CycloneL-1000M/D with melt, solution and deposition capability which allowed us to create a polypropylene melt-spun membrane with high strength and flexibility characteristics. Oxidative chemical vapor phase deposition and polymerization is used to conformally coat the fibrous mat (textile) with a conducting polymer and functional group [5]. The substrate is simultaneously exposed to an oxidant and monomer vapors allowing for a uniform deposition. The copolymers of 3,4-ethylenedioxythiophene (EDOT) and 3-thiopheneethanol (3-TE) were placed in a monomer jar and delivered into the reactor. The reaction conditions are briefly as follows: 1) the monomer jar and feed lines were heated to 150°C to avoid condensation and pressure drop in the reaction chamber, 2) the substrate temperature will be maintained at 80° C by a temperature controlled stage, 3) the samples, melt-spun fiber mats embedded in paper framework were placed upside down in the reactor chamber, 4) iron chloride powder as oxidant was placed in a stainless steel crucible and resistively heated to ~350°C initiating sublimation to obtain iron chloride vapor, 5) the valve of the monomers vapor was opened and the reaction was allowed to continue until the desired polymer thickness is obtained.

The conductive polymer textile is further reacted utilizing the hydroxyl functional group imparted by the 3TE with the isocyanate group of the heterobifunctional crosslinker, p-Maleimidophenyl isocyanate (PMPI). An antibody to *E. coli* O157:H7 was then attached via sulfhydryl group.

2.2 Resistance Based Measurements

Resistance measurements were measured in 25 mM Tris Buffered Saline (TBS) (pH = 7.2) and dry using both a digital multimeter and four-point probe (R-Chek-RC2175, EDTM Corp).

2.3 Potentiostatic Measurements

Cyclic voltammetry (CV) was performed using an open source potentiostat (Cheapstat) [6]. The working electrode was either the conducting polymer textile with dimensions of 1x2 cm or a conducting polymer textile incorporated into a commercial screen printed electrode (SPE) (Figure 1). The reference electrode was Ag/AgCl and the auxiliary electrode was carbon on the SPE or a platinum wire. The starting potential was -990 mV and the ending voltage was 900 mV. The scan rate was 50 mV/s. A 50 mL electrochemical cell with TBS as the electrolyte was used to make measurements.

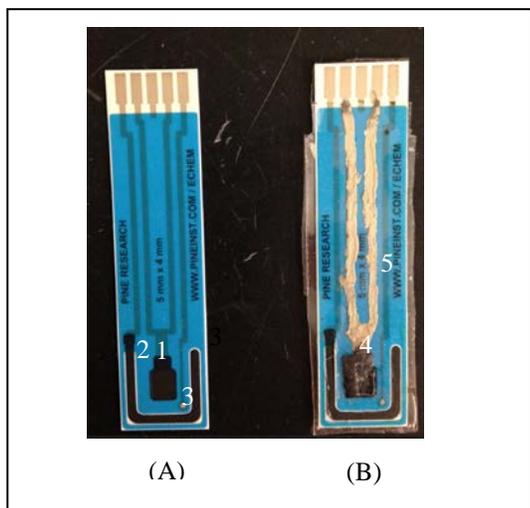


Figure 1: Comparison of SPE (A) and conductive polymer incorporated into SPE (B). 1) working electrode, 2) reference electrode, 3) auxiliary electrode, 4) CP membrane, 5) conductive paste

2.4 Bacterial Challenge

Conductive polymer membranes functionalized with anti-O157:H7 antibody at 4 μ g and 40 μ g were challenged with dilutions of *E. coli* O157:H7 to assess the capture and signal generation in resistance and CV in the biosensor platform.

3 RESULTS/DISCUSSION

Preliminary studies have shown that the textile is functionalized with antibody to cover the surface. Figure 2 details a confocal image of a membrane functionalized with antibody and stained with Syto9. The textile membrane offers excellent surface area for a binding platform incorporated into a biosensor.

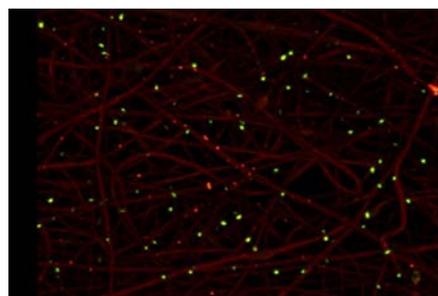


Figure 2: Preliminary studies with confocal microscope demonstrated reactivity of the OH functional group with the *E. coli* O157:H7 antibody.

Sheet resistance of different concentrations of monomers was measured before and after a 1 hour bacterial incubation. Table 1 details Resistance decreased with increasing concentration of EDOT as expected since EDOT has higher conductivity than 3TE. Additionally, the incubation with *E. coli* for 1 hour showed an increase in sheet resistance.

Concentration (EDOT:3TE)	Sheet Resistance (Ohm/Sq)		
	Start (dry)	Start (TBS)	Bacterial Incubation 1 hr
30:70	5000	4600	7000
50:50	4760	4500	8000
70:30	1630	4000	9800

Table 1: Sheet resistance of different concentrations on monomers deposited on textile.

SPEs with 4 μ g of antibody were exposed to dilutions of 10⁻⁵ and 10⁻⁴ *E. coli* corresponding to 700 and 7000 CFU/mL, respectively, with a control of culture broth (labeled as No *E. coli*). Cyclic voltammetry was used to probe the SPEs (Figure 3). A lower concentration of *E. coli*

displayed a lower current to voltage profile. The No *E. coli* profile is similar to the 10^{-5} dilution. This may be the limit of the sensor as fashioned in Figure 1. Additional concentrations of *E. coli* are needed to confirm this trend and understand the properties in more detail to assign a standard curve.

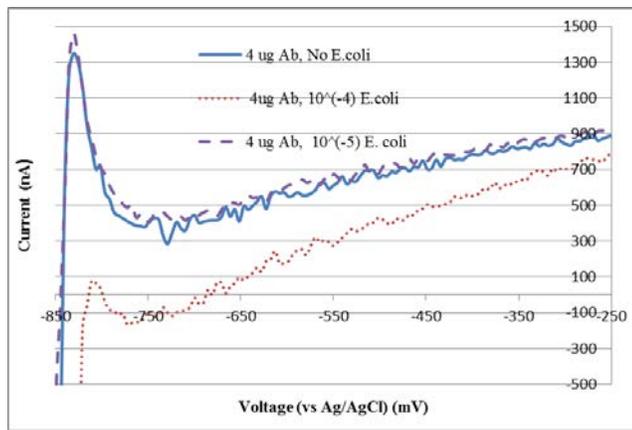


Figure 3: Cyclic voltammeter of antibody (4 ug) functionalized membrane exposed to 10^{-4} dilution (7000 CFU/mL) and 10^{-5} dilution (700 CFU/mL) of *E. coli*.

4 CONCLUSION

Resistance and potentiostatic measurements of antibody functionalized conductive polymer textiles offer the potential to rapidly detect *E. coli* O157:H7 in a field portable device. Continued studies will optimize the textile platform's resistivity/conductivity profile by assessing the concentration of monomers deposited on the fiber to offer increase signal detection. In addition, the fabrication of the electrode with incorporated conductive polymer membrane will be improved by investigating the feasibility of inkjet printing an electrode. The open-source potentiostat shows potential in being developed as an inexpensive field portable device for biosensing with the conductive polymer textile.

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