Antibody Immobilization on Conductive Polymer Coated Nonwoven Fibers for Biosensors

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ABSTRACT

Research is being performed to develop rapid and novel electrochemical biosensors for the detection of food pathogens at the US Army NSRDEC. The focus of this research is fiber based platforms to perform both capture and sensing functions in a single component. The biosensor is based on the use of nonwoven fiber membranes coated with a conductive polymer coating and functionalized with antibodies for biological capture. The study examines three methods: passive adsorption, gluteraldehyde cross-linking, and EDC/sulfo-NHS cross-linking, for the immobilization of antibodies onto the three dimensional conductive fiber surfaces in order to improve the specific capture of a target pathogen. The immobilization and capture capabilities for each method are analyzed through the use of fluorescent stains and labels. The fluorescence is measured and then imaged using a fluorescent microscope.

Keywords: biosensor, electrotextile, antibody, immobilization, fluorescence

1 INTRODUCTION

High-surface area electrospun membranes are versatile and can be applied to biosensor development.[1] One way is through the creation of electrotextiles. Electrotextiles are fabrics that can function as electronics and physically behave as textiles.[2] Electrotextile "smart membranes" can be designed for use with all forms of sensor signal transduction, however very little research has been done into the integration of electrotextile, biological, and electrical technologies to create novel biosensor systems for food defense. Previous work has been done to develop electrically active non-metallic textile coatings that are conductive using doped poly-pyrrole polymer.[3,4,5,6,7] By producing a conformal conductive polymer coating on non-woven microfibers an electrochemical biosensor electrode can be created that is less expensive than and has more surface area for attachment than its planar metal counterparts.[8] In addition these electrotextile electrodes are durable, disposable, and have the potential for minimal required attachment chemistry. With the attachment of biological recognition elements to the electrotextile electrode surface the electrodes would have the capacity to perform pathogen capture, concentration, and detection.

The creation of this type of electrode would simplify a food pathogen detection biosensor, allowing the overall system to be produced smaller and lighter than many current systems. Small foot print biosensors with multiplexing capabilities, environmental robustness and high sensitivity are needed for rapid presumptive testing. With membranes being designed to do the capture, the sample readers can be simplified and significantly reduced in size.

2 MATERIALS & METHODS

2.1 Materials & Apparatus

Polypropylene microfibers were coated with a doped conductive poly-pyrrole polymer and copolymerized with carboxylic acid functional 3-thiophene acetic acid (3TAA). This combination of co-polymers provides sites for covalent attachment of the bio-recognition (active) component of the biosensor to the fiber based platform. The fibers were cut into circular discs with a diameter of 6.35 mm. Durability of the fibers and coating as well as physical and electrical properties were previously characterized using techniques such as scanning electron microscopy and four point probe resistance measurements.

Two antibodies against *Escherichia coli* (*E. coli*) O157:H7 were used in this study. BacTrace antibody goat anti-*E. coli* O157:H7 unlabeled and BacTrace antibody goat anti-*E. coli* O157:H7 fluorescein isothiocyanate (FITC) labeled (KPL). Both antibodies were affinity purified. The antibodies were diluted in phosphate buffered saline (PBS) at a concentration of 4 μg/ml immediately before use.

Concentrated SYPRO tangerine protein gel stain Invitrogen) was diluted to 1x in PBS. It was shielded from light and stored at 4°C.

Escherichia coli (*E. coli*) O157:H7 Sakai strain was obtained from the Michigan State University Food Safety and Toxicology Center. Cell cultures were grown overnight in trypic soy borth (TSB) at 37°C. 1 mL of cells were removed and pelleted in a centrifuge for 1 min at 10,000 x g. The pelleted cells were washed with PBS and

pelleted again to remove the PBS. They were then resuspended in 50 µL of acridine orange (Invitrogen) stain and diluted in 950 µL of acridine orange buffer (0.1M phophate, 0.1M NaCl pH 3.8). The cells were mixed with the stain under gentle agitation for 15 minutes. After 15 minutes the cells were pelleted, washed with PBS, pelleted again, and then resuspended in 10 mL of PBS.

Fluorescence of the samples was measured using the Victor3 multilable counter (PerkinElmer).

Samples were analyzed and photographed using a fluorescent microscope (Olympus BX41 with Q Color 3 camera and a QBC ParaLens Advance kit with blue excitation).

2.2 Methods

For this study, antibody-antigen binding assays were performed to determine the capture capability of the conductive membrane coatings. Attachment and capture efficiency were determined through the use of fluorescent labels that were measured using a fluorometer and visualized with confocal microscopy. Effects on the surface conductivity of the microfiber electrodes were measured with the use of a multimeter. Each immobilization and staining technique combination was performed in triplicate. Three samples received no immobilization chemistry and no fluorescent labeling to serve as a negative control. Each immobilization procedure was performed on twelve discs. Three discs from each method had FITC-labeled antibodies immobilized on them. Three discs from each method had non-labeled antibodies immobilized and were stained with SYPRO tangerine protein stain to determine the immobilization efficiency. Three discs from each sample were blocked with Tween-20 and then had acridine orange stained *E. coli* O157:H7 cells applied and three discs were exposed to stained cells, but without the Tween-20 blocking. Each of the stains were also applied to three discs without any antibodies immobilized to determine the background noise from the stains and non-specific binding.

2.2.1. Passive Adsorption

The conductive nonwoven fiber discs were washed with sterile distilled water and left to dry for 10 min. After 10 min 250 µL of antibody was applied to each and left to mix with gentle agitation. After 1 hr the discs were washed twice with PBS. Half of the discs then received a third wash with PBS while the other half was washed with PBS containing 0.1% Tween-20.

2.2.2. Gluteraldehyde Attachment

The conductive nonwoven fiber discs were washed with distilled water and left to dry for 10 min. After 10 min 25 µL of 2.5 mM gluteraldehyde was applied to each disc. The discs were incubated with the gluteraldehyde for 1 hour at 4°C. After 1 hour the discs were washed with distilled water and left to dry for 10 min. 250 µL of antibody was then applied to each disc. They were incubated for 15 min at 37°C. Following another wash with distilled water and 10 min dry time 50 µL of deactivating buffer was applied to each disc and left to react while they were incubated at 37°C for 15 min. The discs were washed twice with PBS. Half the discs then received a third wash with PBS while the other half was washed with PBS containing 0.1% Tween-20.

2.2.3. EDC / Sulfo-NHS Cross-Linking

The discs were washed with distilled water and left to dry for 10 min. After 10 min 200 µL of EDC and Sulfo-NHS in 2-(N-morpholino)ethanesulfonic acid (MES) buffer was added to each disc and left to react with gentle agitation for 15 min. The discs were then washed twice with MES buffer. A volume of 250 µL of antibody was added to each disc and they were left to react with gentle agitation for 4 hours. After 4 hours the discs were washed with MES buffer. Finally, the discs were washed twice with phosphate buffered saline (PBS). Half the discs then received a third wash with PBS while the other half was washed with PBS containing 0.1% Tween-20.

2.2.4. Protein Staining

A volume of 200 µL of the SYPRO staining solution was applied to three discs with antibodies that had been bound by each immobilization method. The stain was left to react for 1 hour and then each disc was washed three times with PBS.

2.2.5. *E. coli* O157:H7 Capture

A volume of 200 µL of 10^8 cfu/mL of *E. coli* O157:H7 cells stained with acridine orange were applied to twenty-four discs. Six had no antibodies applied to them. Six had antibodies attached using passive adsorption, six with gluteraldehyde, and six with EDC and sulfo-NHS. For each immobilization method three of the discs had been washed with PBS and three had been washed and blocked using PBST. The discs were incubated with the cells at 37°C for 15 min and then washed three times with PBS.

2.2.6. Fluorscence Measurement & Imaging

The negative control samples and samples stained with SYPRO tangerine were measured for fluorscence using a multilabel counter. They were excited at a wavelength of 490 nm and emission was measured at a wavelength of 595 nm.

The negative control samples and samples with FITC-labeled anti-*E. coli* O157:H7 were measured for

fluorescence at an excitation wavelength of 490 nm. Emission was measured at 535 nm.

The negative control samples and samples exposed to acridine orange stained *E. coli* O157:H7 were measured for fluorescence at an excitation wavelength of 490 nm. Emission was measured at 535 nm.

The samples that had been exposed to the fluorescent cells with the highest output fluorescence for each immobilization method were imaged using a fluorescent microscope to see the distribution of cells across the fibers.

A sample from each immobilization method that had not undergone staining was measured to determine the change in resistance across the membrane as compared to a fiber disc with no antibodies attached serving as a negative control. This was done three times and an average taken.

3 RESULTS & DISCUSSION

At an emission of 595 nm the average fluorescence of the non-treated fibers was 73.3 relative fluorescence units (RFU). The unstained immobilized fibers had an average fluorescence ranging between 80 and 82.7 RFU. The fiber discs not treated with antibody, but stained with SYPRO had an average fluorescence of 358.3 RFU. The discs that had antibodies immobilized via passive adsorption, gluteraldehyde, and EDC with sulfo-NHS and had been stained using SYPRO had average fluorescences of 382.7, 473.7, and 416.3 RFU, respectively. As can be seen in Figure 1, the samples with antibodies immobilized using gluteraldehyde showed the largest change in fluorescence, while passive adsorption showed the smallest change.

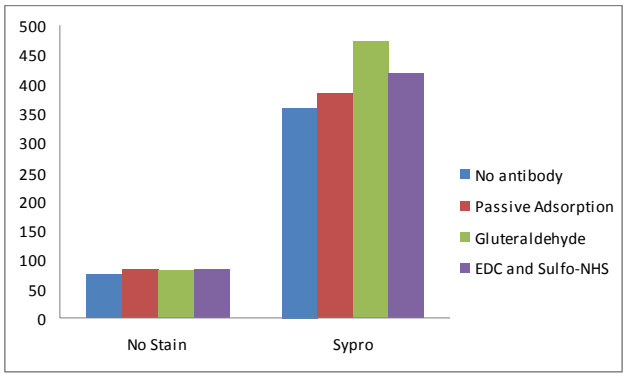

Figure 1: Fluorescence in RFUs using SYPRO tangerine protein stain with different antibody immobilization methods. (λ_{ex} 490=nm, λ_{em} 595=nm)

At an emission of 535 nm, the average fluorescence of the non-treated fibers was 25.7 RFU. The unstained immobilized fibers had an average fluorescence ranging between 36.7 and 42.0 RFU. The discs that had antibodies immobilized via passive adsorption, gluteraldehyde, and EDC with sulfo-NHS and had been labeled with FITC had average fluorescences of 15.3, 48.3, and 117.3 RFU, respectively. These results can be seen in Figure 2. The samples with antibodies immobilized using EDC and sulfo-NHS cross-linking showed the largest change in fluorescence, while passive adsorption showed a negative change.

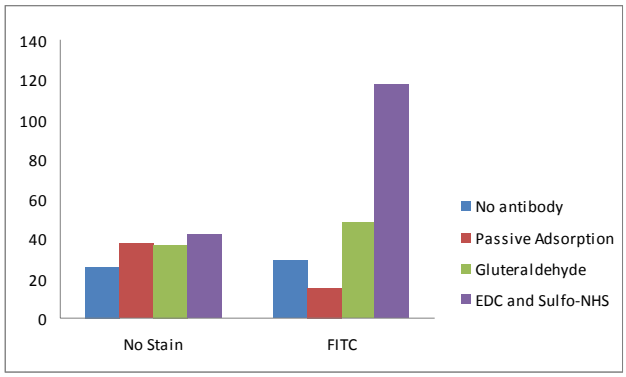

Figure 2: Fluorescence in RFUs using FITC-labeled antibody with different immobilization methods. (λ_{ex} 490=nm, λ_{em} 535=nm)

At an emission of 535 nm the fiber discs not treated with antibody, but exposed to acridine orange stained cells had an average fluorescence of 501.3 RFU. The average for the same samples blocked using Tween-20 was 159.3 RFU. The discs that had antibodies immobilized via passive adsorption, gluteraldehyde, and EDC with sulfo-NHS and had been exposed to the fluorescing *E. coli* cells without Tween-20 had average fluorescences of 445.0, 657.0, and 319.0 RFU, respectively. Those samples blocked using tween had average fluorescences of 230.3, 211.7, 194.3 RFU, respectively. The samples with antibodies immobilized using gluteraldehyde showed the largest change in fluorescence, while those immobilized using EDC and sulfo-NHS showed the smallest change. These results can be seen in Figures 3 and 4.

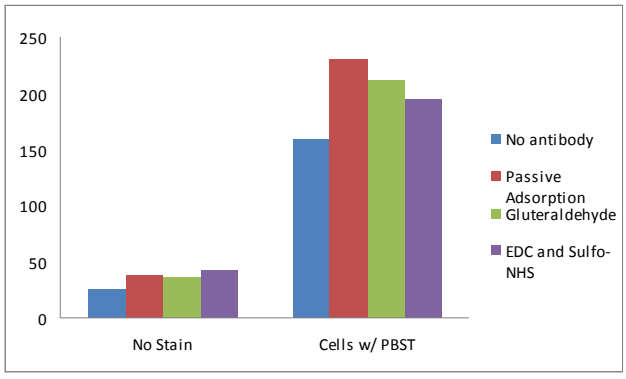

Figure 3: Fluorescence in RFUs using acridine orange stained *E. coli* O157:H7 cells with different antibody immobilization methods and blocked using PBST. (λ_{ex} 490=nm, λ_{em} 535=nm)

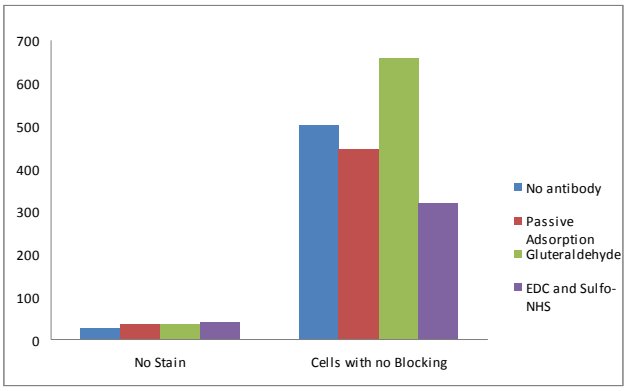

Figure 4: Fluorescence in RFUs using acridine orange stained *E. coli* O157:H7 cells with different antibody immobilization methods and no blocking agent. (λ_{ex} 490=nm, λ_{em} 535=nm)

The results show that covalent binding of antibodies to the fiber surfaces, either using gluteraldehyde or EDC and sulfo-NHS, leads to better antibody immobilization than passive adsorption. The results from the antibody staining as well as the FITC labeling of the antibodies both showed higher fluorescence measurements than the samples that used passive adsorption. They also had higher output flourescences that the negative controls.

The results from the samples exposed to the fluorescently stained cells showed EDC and sulfo-NHS cross-linking to have a lower fluorescence than passive adsorption and gluteraldehyde. While some variability is expected due to non-specific binding, it is probable that while the EDC and sulfo-NHS is binding the antibodies to the surface, it is binding them in an orientation that prevents them from capturing the target bacterial cells. Taking this into consideration, the method for antibody immobilization on the conductive polymer coated nonwoven fibers that shows the most promise is covalent binding using gluteraldehyde.

The addition of the Tween-20 as a blocking agent to the discs prior to the addition of bacterial cells significantly reduced the average fluorescence from the attachment of cells to the fiber discs. These results show that non-specific attachment and entrapment are significant issues to consider when using conductive fibers for the capture of bacterial cells. However, the addtion of a blocking agent can greatly reduce the effects of non-specific binding.

4 CONCLUSIONS & FUTURE WORK

The long-term goal of this research is to develop a rapid and novel electrochemical biosensor for the detection of pathogens. Ultimately, the nonwoven membrane platform will be capable of being dipped into a test sample to act as a pathogen collector and then can be inserted into the electrochemical cell to complete the biosensor circuit by also functioning as the working electrode.

The initial results from this study show that biological recognition elements can be attached to conductive polymer coated non-woven fibers. This technology will be extremely useful in the formation of electrotextiles for use in biosensor systems.

Future research will include exploring different washing solutions, blocking agents, and attachment chemistries. PBST will be used for all washing steps in order to remove unbound proteins. The use of bovine serum albumin (BSA) as a blocking agent before the application of bacterial cells will also be explored. Attachment and capture studies will be conducted using avidin-biotin based immobilization chemistry. Finally, the effect each immobilization method has on the fibers' conductivity will be studied.

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