EMBROIDERED BIOSENSORS ON GAUZE FOR RAPID ELECTROCHEMICAL MEASUREMENTS

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ABSTRACT

Electrochemical biosensors are useful diagnostic tools which possess the capacity for rapid detection of analytes in biofluids. Here, we report the first time embroidered electrochemical sensors on gauze for rapid measurements of wound biomarkers. Robust, flexible electrodes were successfully fabricated onto medical gauze and wound dressings via embroidery. Proof-of-concept was carried out by performing quantitative measurements of uric acid, a biomarker for wound healing, in simulated wound fluid. This gauze-based sensor exhibits excellent linearity from 0 μM to 800 μM. Additionally, we demonstrated that the gauze sensor works excellent and consistent when this is extra layer wrapping over it. Lastly, we show that this biosensor exhibits high specificity and good resilience against mechanical deformation, making it a promising platform for noninvasive wound monitoring.

INTRODUCTION

Electrochemical sensors are a promising technology for biochemical measurements due to their speed, small size, and high sensitivity [1]. Screen printing is broadly used for fabricating the electrochemical biosensors on flexible substrate, such as plastic [2] or tattoo papers [3]. However, the cost for such materials is relatively pricy for point-of-care testing in the developing world. Recently, wearable electronics have attracted more and more attention since they are flexible, robust, and can be easily integrated with wearable materials. Research in wearable sensing area has mostly focused on the integration of sensors onto wearable garments for monitoring physiological parameters such as temperature [4], heart rate [5, 6] and respiration rate [7]. Very little attention has been directed towards chemical sensor for measurement of analytes and biomarkers in bodily fluids, which could provide useful information to doctors or patients for further insight into the health or disease status. Recent work has focused on fabricating electrochemical sensors on flexible substrates such as plastic films and textiles for in vitro diagnostics and noninvasive health monitoring [8]. Several groups have also demonstrated smart bandages for wound monitoring using screen-printed electrochemical sensors [9, 10]. While these systems offer good analytical performance, screen-printed sensors are difficult to fabricate on textured materials such as woven gauze or wound dressings. To address this issue, we demonstrate a unique gauze-based embroidered electrochemical sensor, which is flexible, mechanically robust and compatible with woven materials.

Here we demonstrate a novel electrochemical sensor embroidered on gauze substrate. This sensor is mechanically robust, compatible and offers custom electrode geometries. We also show that this embroidered sensor can be used for quantitative measurement of uric acid, a biomarker for wound healing process. Additionally, this biosensor exhibits great linearity and excellent specificity and repeatability. Furthermore, we demonstrated that covering with one layer of gauze will not affect the linearity and accuracy of the senor. Lastly, durability testing shows that our gauze sensor can maintain its accuracy when subject to bending while taking the measurements, this making this gauze-based embroidered biosensor a promising platform for noninvasive wound monitoring.

MATERIAL AND METHODS

Materials and Reagents

Uric acid, glucose, L-lactate and albumin were purchased from Sigma-Aldrich (St. Louis, MO). Uricase and Ringer's solution (mammalian) were purchased from Fisher Scientific (Pittsburgh, PA). Silver/silver chloride (Ag/AgCl) and carbon inks were purchased from Conductive Compounds Inc. (Hudson, NH). Deionized (DI) water was generated using a Barnstead Smart2Pure water purification system. Simulated wound fluid was freshly made by adding 17g/L albumin to Ringer's solution. Samples were dissolved in the simulated wound fluid at room temperature. Samples were freshly prepared prior to experiments and remaining biochemical was used without further purification.

Thread Preparation

Each electrochemical sensor consists of three electrodes, a reference electrode (RE), working electrode (WE) and counter electrode (CE), which were fabricated using conductive thread. Briefly, carbon or Ag/AgCl ink was used to coat the polyester thread (Brothers International, Bridgewater, NJ). The coated thread was then cured at 120 ˚C for 30 min. Thread coated with carbon ink was used for the WE and CE, and thread coated with Ag/AgCl ink was used for the RE. For Ag/AgCl thread, soldering flux (Kester, Itasca, IL) was applied to the thread using a flux pen prior to the ink coating process to minimize oxidation of the ink.

Thread Characterization

SEM images were captured using a JEOL 6620V scanning electron microscope at 12 kV and 170× magnification.

Sensor Design

Electrochemical sensors were designed using AutoCAD software (Autodesk, Vernon Hills, IL) and converted into an embroidery file using SewArt software (S & S Computing). The digitized image was uploaded to a Brothers SE400 embroidery machine and ready for fabrication.

Sensor Fabrication

With the uploaded image, the embroidery machine starts to fabricate on medical-grade gauze stacked with a stabilizer film (World Weidner, Ponca City, OK). After the sensors were embroidered, the stabilizer was removed. Uricase solution (10 mg/mL) was drop casting on the WE and waited at least 1 hour before using. Individual sensors were cut and stored at ambient conditions prior to experiments.

Electrochemical Measurements

Amperometric measurements were performed using a multichannel electrochemical workstation (GeneFluidics, Inc. Irwindale, CA). For single analyte measurements, 15 µL of smaple was dispensed from bottom of the sensing region using a pipette, followed by the application of a 350 mV bias potential after 1 min. All measuremtns were performed at room temperature under ambient conditions using new sensors.

Statistical Analysis

Data were presented as mean ± SD. For comparisons between bent biosensors and unbent biosensors, one-tailed Student's t-test was used. P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Thread Characterization

Conductive threads were characterized using SEM to evaluate the effectiveness of the thread coating process. SEM was used to further visualize the thread surface morphology after the coating process. For the carbon-coated thread, we can observe that the entire length of thread is uniformly coated with ink with negligible blotching or defects (Fig. 1b & c). Ag/AgCl-coated thread also exhibited similar surface coverage (Fig. 1d &e). These results also suggest that the enzyme immobilization process has negligible impact on the surface morphology and the coating thickness.

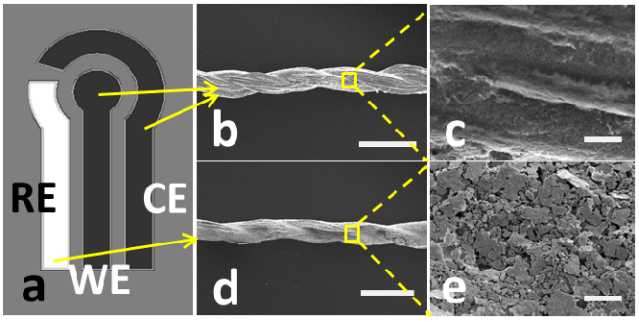

Figure 1: (a) Schematic of the electrochemical sensor. SEM images of Ag/AgCl-coated thread (b) and carbon-coated thread (d). Scale bar, 500 µm. Close-up SEM images showing the surface of the Ag/AgCl thread (c) and carbon thread (e). Scale bar, 10 µm.

Sensor Embroidery

Embroidery is an important process where graphical patterns are designed by AutoCAD and subsequently sewn onto fabrics using threads, thus it provides the biosensor with great design flexibility and mass customization at the desired location s and substrate. A few embroidery parameters can affect the how the pattern is embroidered (stitch density, stitch length, stitch angle and so forth), thus affecting the sensor performance. For instance, decreasing the stitch separation distance resulted in a higher stitch density which improved electrode uniformity. However, using a higher stitch density required a larger amount of thread which increased the electrical resistance of the electrodes. We determined that a stitch separation distance of 0.2 mm and stitch length of 0.5 mm produced consistent uniformity while minimizing the electrode resistance.

With the optimized embroidery parameters, we successfully embroidered biosensors on fabricated embroidered electrochemical sensors with tightly-stitched, discrete electrodes allowing for excellent signal consistency and signal-to-noise ratio (SNR). As shown in Fig. 2, our embroidered sensors can be fabricated onto different types of gauze and wound dressing materials, making it versatile for various clinical applications. Additionally, due to the hydrophilic nature of the thread, embroidered sensors can readily absorb liquid samples for chemical reactions, thereby enhancing the detection performance.

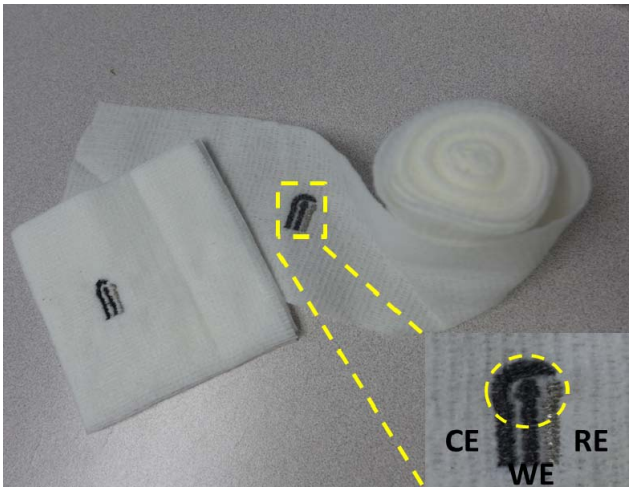

Figure 2: Embroidered electrochemical sensors on medical gauze and wound dressing. Inset shows a close-up view of the sensor.

Uric Acid Detection

Uric acid is highly correlated with wound severity, which makes it a highly specific indicator of wound status and infection. To demonstrate the usefulness of our gauze-based embroidered sensor for biomolecular detection, we used it for electrochemical measurements of uric acid in simulated wound fluid. Briefly, 20 µL of sample was dispensed onto the sensing area (Inset of Fig. 2) and quickly soaked up by the biosensors due to the high wettability of the thread and gauze fabric. Amperometric measurements at different concentrations of uric acid were performed after 1 min, which was sufficient time for the sample to be fully absorbed and generate a stable electrochemical reaction. As shown in

Fig. 3, this sensor exhibits a lower detection limit of 100 µM and excellent linearity between 0 – 800 µM with a correlation coefficient (R^2) of 0.995, which spans the clinical range in open wounds [11].

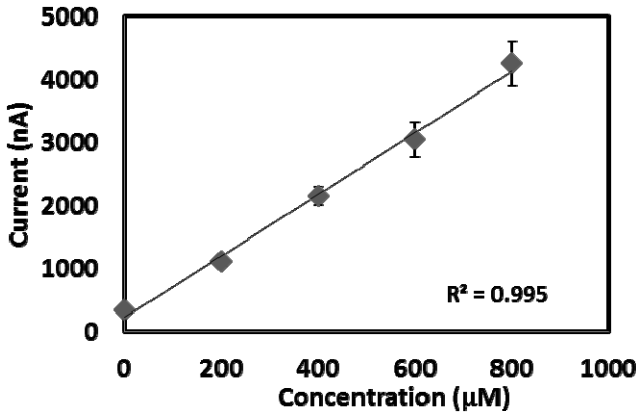

Figure 3: Electrochemical measurements of uric acid in simulated wound fluid. Values are average over the final 10 sec of the amperometric signal. Each data point represents the mean ± standard deviation (SD) of three separate measurements.

Assay Specificity

The specificity of the electrochemical sensor was briefly studied by performing independent measurements of glucose (100 mg/dL), lactate (100 mg/dL), uric acid (100 mg/dL) and simulated wound fluid using gauze sensors. The data is plotted as the signal to background ratio (SBR). From this plot (Fig. 6), we observe that a significant detection signal is generated only for the uric acid sample (SBR ~7), while the irrelevant analytes generate SBRs similar to that of the simulated blank control. These results indicate that the sensor exhibits good specificity with minimal interference due to analyte cross-reactivity.

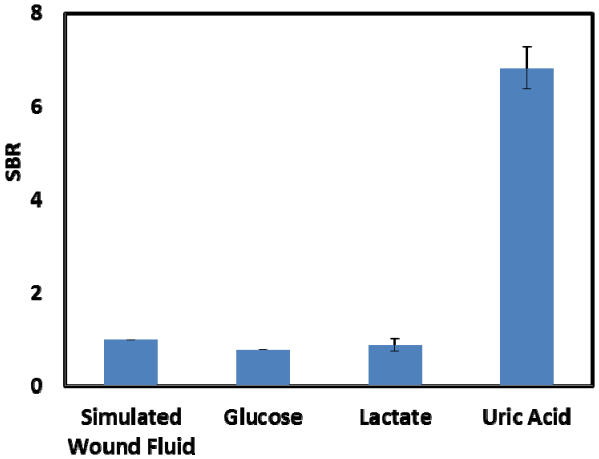

Figure 4: Specificity of the uric acid assay. Measurements were performed using glucose, lactate and uric acid in simulated wound fluid at a concentration of 2 mM, 10 mM and 400 µM respectively. Each bar represents the mean ± SD of three separate measurements.

Sensor Coverage Test

Gauze needs to be wrapped several layers over the wound area in real circumstance. However, wrapping over the sensor might affect the sensor performance. To mimic and study such effect, we carried out experiment measuring uric acid in simulated wound fluid with the uncovered sensor (Fig. 4a) and sensor covered with one layer of gauze (Fig. 4b). As shown in Fig. 4c, there was no significant difference ($p>0.137$) in the detection signal when the sensor was covered.

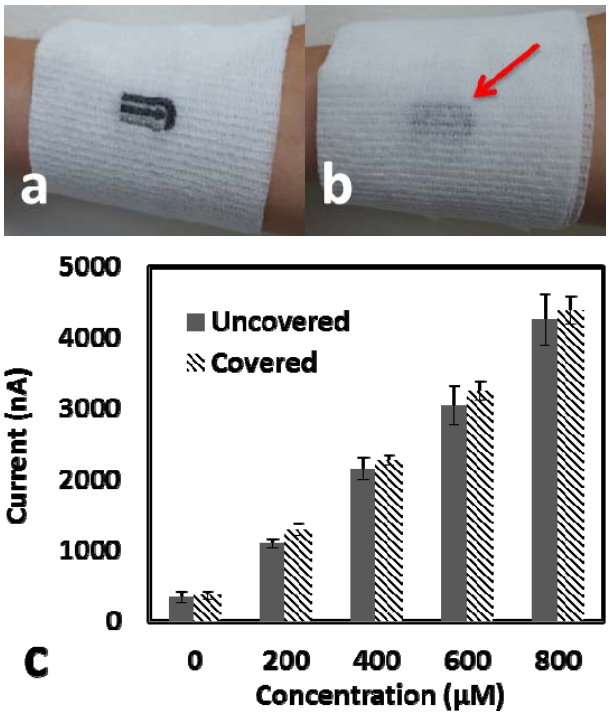

Figure 5: Photographs of an uncovered sensor (a) and sensor covered with one layer of gauze (b). Red arrow indicates the location of the sensor. c) Electrochemical measurements of uric acid in simulated wound fluid using uncovered sensors (solid) and covered sensors (striped). Each bar represents the mean ± SD of three separate measurements.

Sensor Durability Test

Since wound dressings experience natural mechanical stress from bending due to the curvature, movement, and flexing of the human body at the application site. We examined the biosensor performance in response to deformation occurring simultaneously while the measurement was being carried out. Comparative signals to each uric acid concentration from biosensors positioned flat in the reader and mechanically bent sensorsshow no significant difference ($p > 0.181$) on the biosensor performance under different mechanical conditions (Fig. 5). Furthermore, the signals from bent sensors still maintained a highly linear response ($R^2 = 0.994$) similar to that of the flat sensor throughout the tested concentration range with low SDs among multiple measurements. These data reveals that mechanical deformation during measurement has a minimal impact on the performance of our embroidered biosensors as the response from the bent sensors is nearly identical to those from the flat sensors. These results also suggest that our

embroidered biosensor will be able to maintain its accuracy and reproducibility under instances of repeated deformation for in vitro diagnostic testing or on-body wearable sensing.

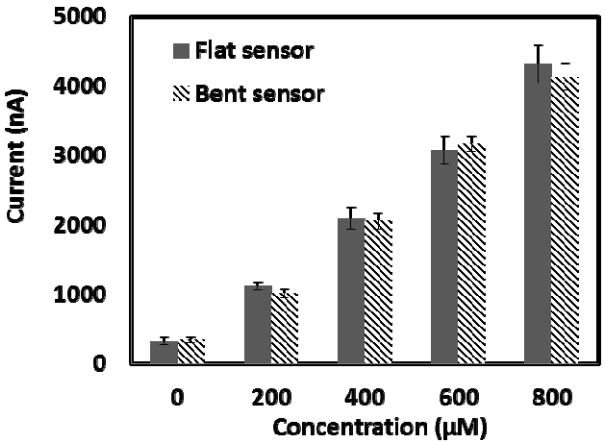

Figure 6: Electrochemical measurements of uric acid in simulated wound fluid using flat sensors (solid) and sensors that have undergone bending (striped). Each bar represents the mean ± SD of three separate measurements.

CONCLUSIONS

We developed a unique gauze based embroidered electrochemical sensor for quantitative analyte detection. The sensor was fabricated using special made conductive thread onto medical graded gauze. SEM images were used to study the surface morphology after coating process. For proof of concept, this platform was used for amperometric measurements of uric acid, a biomarker for wound healing process, in simulated wound fluid. Results indicate that our embroidered gauze sensor exhibits high linearity, repeatability and assay specificity. Considering for practical case, we further demonstrated that covering the sensor with one layer of gauze will not affect the sensor performance. Experiments to evaluate the performance of our biosensor in response to mechanical deformation showed its capability to produce consistent and accurate measurement in response to repeated bending prior to and during testing. Future work will focus on wound healing process monitoring and detection using raw clinical specimens (e.g. wound fluid).

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