ENZYME-DOPED POLYESTER THREAD COATED WITH PVC MEMBRANE FOR BLOOD UREA NITROGEN AND GLUCOSE DETECTION IN HUMAN WHOLE BLOOD

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ABSTRACT
This research presents a novel technique for detection in human whole blood utilizing a novel enzyme-doped thread with PVC membrane coating for on-site urea and glucose detection on a thread-based microfluidic device. The enzyme can be directly applied on the thread without delicate pretreatment or surface modification process. Whole blood sample is firstly mixed with RBC lysis buffer to prevent from blood coagulation. The lysed RBC and other solid pieces are simultaneously filtered away while electrokinetically flowing in the thread-based microfluidic system. A thin layer of PVC membrane is coated on the enzyme-doped thread to further fix the applied enzyme and to prevent from the rapid evaporation of the running buffer due to the Joule heating effect.

KEYWORDS: Human whole Blood, enzyme, PVC membrane

INTRODUCTION
Solid-state sensors incorporated with enzyme-modified sensing electrodes or microbeads is the most common scheme for on-site analyze of urea, glucose and creatinine in the human serums.[1] However, it is critical for integrating solid-state sensor with microfluidic channels for sample separation and transportation in a single chip. Alternatively, thread-based microfluidic system provides a simple and promising way for separating and on-site electrochemical detecting whole blood samples.[2] This study further extended the capability of the developed thread-based microfluidic system. The polyester thread is used as the filter to exclude the undesired solid pieces flow into the detection sites.

THEORY
This research presents a novel technique for detection in human whole blood which utilizes a novel enzyme-doped thread with a PVC (polyvinylchloride) membrane coating for on-site urea and glucose detection on a thread-based microfluidic device. The enzyme can be directly applied to the thread without delicate pretreatment or a surface modification process. The passing biomolecules are digested by the enzymes and then electrochemically detected downstream. With this approach, CE-EC detection with on-site bio-reaction can be simply achieved. The whole blood sample is first mixed with a RBC lysis buffer to prevent blood coagulation. The lysed RBC and other solid pieces are simultaneously filtered away while electrokinetically flowing through the thread-based microfluidic system. A thin layer of PVC membrane is coated on the enzyme-doped thread to further fix the applied enzyme and to prevent rapid evaporation of the running buffer due to the Joule heating effect. In addition, the PVC coated thread can be operated at a higher separation electric field of 500 V/cm due to the reduction of buffer evaporation.

Figure 1 shows the concept of this work. Various enzymes were applied onto the thread-based microfluidic system and the thread was then coated with a thin PVC layer. The enzyme immobilized was then sealed with PVC layer for high performance bioanalytical detection.

EXPERIMENTAL
Figure 2 shows the simplified fabrication process for producing the proposed thread-based microfluidic system. The details for the fabrication process can be found in the previous report. A concave master mold was first fabricated on a 2-mm thick aluminum plate using a micro CNC machine. The convex electrode structures were then produced on a PMMA substrate by hot embossing (Fig. 3-4 (A)). The working and counter electrodes for electrochemical detection were then produced by sputtered Cr/Au layers and the reference electrode was with Cr/Pt layers (Fig. 3-4 (B)). Polyester threads of around 200-µm in di-
ameter were fixed on the PMMA substrate as the liquid routes for CE operation (Fig.3-4 (C)). Specific enzymes including urease, glucose oxidase (GOD), horseradish peroxidase (HRP) and catechol (mediator) were then directly applied on different sites of the thread with a 2-µL pipette (Fig. 3-4 (D)). A thin PVC solution was sprayed on the desired region with the assistance of a plastic mask (Fig. 3-4 (E)). The PVC solution would form a thin membrane covering the thread after evaporating the solvent. Enzyme-doped polyester thread coated with PVC membrane was finally produced for simultaneously EC detection of urea and glucose. Figure 3 shows the experimental setup for the CEEC system.

RESULTS AND DISCUSSION

Figure 4 shows the SEM image for the thread with and without PVC coating. It is clear that a PVC layer of around 50 µm was successfully coated on the polyester thread (Fig. 4a) and constrained the thread bundle (Fig.4b). Figure 5 shows the photo images of the polyester threads with 2-µL whole blood without and with RBC lysis buffer. It is clear that the blood coagulation happened if no lysis buffer was applied. Figure 6 presents the measured EOF mobility of the threads with and without coating and conventional glass channel. Show that the EOF mobility of the thread-based microfluidic system is much higher than that of conventional glass-based microfluidic system. The calibration curves for detecting urea and glucose are presented in Fig. 7. Thus, results Figure 8 presents the electropherogram for detecting human whole blood with the developed thread-based microfluidic device. The calculated BUN and GLU-AC in concentration for 3.87 mM and 4.94 mM. The developed microchip device provides a low-cost and high performance way for detecting blood sensor. The whole blood sample is firstly mixed with an RBC lysis buffer to prevent blood coagulation. The lysed RBC and other solid pieces were simultaneously filtered away during electrokinetic flow in the thread-based microfluidic system. A thin layer of PVC membrane is coated on the enzyme-doped thread to further protect enzyme and to prevent the rapid evaporation of the running buffer due to the Joule heating effect. Enzyme-modified polyester thread coated with a thin layer of PVC was used as the liquid route and the reactor for converting urea and glucose into ammonium ions and hydrogen peroxide.

CONCLUSION

This study developed a novel technique to form a PVC coated thread doped with various enzymes of urease, glucose oxidase and horseradish peroxidase for on-site bio-sample separation, bio-catalytic reaction and electrochemical detection. Utilizing the technique of doping on the thread can provide several advantages, those of avoid dissociation of H₂O₂ before reaction with GLU, preventing interference of mixed samples, and providing sufficient transport to react urea. The products were then separated and detected using the CE-EC detection scheme. With this approach, a sealed microfluidic channel embedded with various enzymes could be easily produced. Results showed that the thread with PVC coating exhibited higher electro-osmotic mobility in comparison with that of the conventional glass microfluidic channel. In addition, the surface of PVC coated thread could have porous structures which sustain good cooling effect that can provide a higher operation voltage than the uncoated one, resulting in a better detection performance. The novel device developed in the present study provides a simple yet high performance method for rapid detection of bio-samples.

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REFERENCES


Figure 1: Schematic of the enzyme-doped thread coated with PVC membrane for high-performance CE-EC detection.

Figure 2: Schematic for the simplified fabrication process for the thread-based microfluidic device.

Figure 3: Experimental setup for the developed thread-based CE-EC detection system.

Figure 4: SEM images showing the polyester threads with (A)(B) and without PVC coating (C)(D). Note that (A)(C) are eagle view and (B)(D) are cross section view.

Figure 5: Photo images of the polyester threads with 2-μL whole blood without RBC lysis buffer (A) and with RBC lysis buffer on (B).

Figure 6: Measured EOF mobility of the threads with and without PVC coating and conventional glass channel.

Figure 7: Measured current responses for detecting various concentrations of urea and glucose solutions.

Figure 8: Electropherogram for detecting human whole blood with the developed thread-based microfluidic device. The calculated BUN and GLU-AC in concentration for 3.87 mM and 4.94 mM.