PAPER BASED IMPEDIMETRIC IMMUNOASSAY FOR LABEL FREE DETECTION OF URINARY ALBUMIN

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ABSTRACT
This study presents a label free sensing mechanism for analyzing immunoreactions on paper. This is particularly important in diagnosis of conditions like chronic disease (CKD) which occurs in stages and thus quantitative detection of target protein is vital. We have fabricated a simple paper based immunosensor that analyzes impedance variations to detect human serum albumin (HSA). Screen printed interdigitated microelectrodes were used for sensing with a lower limit of detection of 0.375mg/ml. This disposable paper sensor and the novel surface modification used for microprobe synthesis can be further extended to the detection of a wide variety of immunoreactions.

KEYWORDS: Label free, impedance, microprobes, immunosensor.

INTRODUCTION
Paper-based biosensors have emerged as an alternative technology for fabricating simple, low-cost, portable and disposable analytical devices for several applications in areas including clinical diagnosis, food quality control and environmental monitoring. Paper is an attractive sensing platform as it allows passive liquid transport through capillary action without the need for external pumping and is biocompatible allowing immobilization of a wide variety of biomolecules. Furthermore, paper has tunable properties such as hydrophilicity, flexibility, permeability and reactivity and is thus an ideal material for disposable one time tests. Several techniques have been used to tune the properties of paper which include inkjet¹, wax printing² or screen printing technology³, making paper based sensors amenable to in-situ fabrication with interest to be delivered or even produced in areas with limited resources. While paper as a substrate has been widely used for label assisted calorimetric detection, there still remains several opportunities for paper based devices to replicate other modes of detection, in particular electrochemical detection, while still being simple and low cost. Furthermore, calorimetric assays might not be very useful when testing colored fluids like blood. Electrical impedance analyses is a useful label free alternative that is insensitive to color contamination and quantitative measurements can be made. Impedance based biosensors have been widely researched for detection of immunoreactions as they possess attractive characteristics which include electrode mass production ability, low cost instrumentation and easy integration with microprocessor based electroanalytical platforms. Furthermore, interdigitated microelectrodes as used in this research show promising advantages in impedance sensing due to fast attainment of steady-state, low ohmic drop, an increased signal-to-noise ratio and rapid kinetics of reaction.

EXPERIMENTAL
The fabrication of the paper based immunosensor consists of interdigitated microelectrode printing followed by paper patterning and bio-probe immobilization as illustrated in Fig. 1a. Screen printing allows transfer of the nano particulate silver ink onto the filter paper substrate except in areas that are made impermeable by use of a blocking stencil. The printed electrodes are thermally cured in an oven at
150°C for 30 min. Next, the paper is patterned by spraying UV-curable resin with the aid of a PMMA mask that is made using laser cutting. The mask is then removed and the paper is exposed to a 400W high pressure mercury lamp (dominant wavelengths of 253 and 365nm) for curing the resin to complete the hydrophobic/hydrophilic patterning and an image of the paper sensor can be seen in Fig. 1b.

The microprobes used in this study were synthesized using alumina micro particles that were coated with conductive polyaniline using a surface modification technique followed by conjugation with antibody. To obtain the coating, 3g of aniline monomer, 8.4115 g of DBSA, 7.35 g of ammonium persulfate (APS) and 2 g of Al₂O₃ micro particles were used. The alumina micro particles were added to aniline monomer solution and mixed using ultrasonication for 1hr. Next, DBSA was added which reduces the solution pH for more adhered PANI binding and also acts as a dispersant to prevent agglomeration of the alumina micro particles. APS solution was then added and the solution was allowed to react for 2hr which resulted in standard oxidative polymerization of the monomer. The PANI coated micro particles were obtained after washing and filtration steps in acetone and DI water followed by drying are referred to as PANDB. Before conjugation, the antibody (anti-HSA) was oxidized in a solution of sodium metaperiodate and sodium acetate. This step oxidizes the hydroxyl groups (-OH) on the carbohydrate moieties of the antibody to aldehyde groups (-COH) which can then be attached to the amino group (NH₂) present on the PANDB micro particle surface. This allows the fragment crystallizable region of the antibody to be attached to the PANDB surface while the fragment antigen binding region is available for binding.

The sensing mechanism is illustrated in Fig. 1c where the microprobes conjugated with antibody serve as the test while without antibody serve as the control. The microprobes are dropped on paper surface by pipetting and immobilized using a suction pump. After drying, the initial impedance before immunoassaying ($Z_0$) is measured. Different concentration of protein solution is then dropped followed by a washing step with DI water. After drying for 10 minutes using suction, the impedance after immunoassaying ($Z_1$) is measured. The normalized impedance variations are obtained using the equation shown in Fig. 2 (inset). All impedance measurements were made using an LCR meter (Wayne Kerr Electronics, WK 6420).
RESULTS AND DISCUSSION
Results for impedance variations of test and control before and after immunoassay show a value which is 2.08 times higher for the specific interaction of HSA with anti-HSA (Fig. 2a). Furthermore the impedance change is higher for increasing HSA concentrations, thus showing the viability of obtaining quantitative detection on paper.

CONCLUSION
In summary, this paper based impedimetric sensor allows rapid and label free detection in immunoassay. Future works include clinical testing of HSA in urine and use of a portable impedance sensing device for point of care diagnosis.

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