AN ESCHERICHIA COLI CONCENTRATOR USING MAGNETIC PARTICLES IN A MICROFLUIDIC CHANNEL FOR THE URINARY TRACT INFECTION (UTI) APPLICATION

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ABSTRACT

This paper reports a miniaturized lab-on-a-chip device to detect *Escherichia coli* (*E. coli*) from simulated urine using magnetic particles (MPs) in a microfluidic channel. The lab-on-a-chip device consists of two chambers connected in series, *E. coli* concentration and sensing chamber, integrated with a label free impedance sensor to detect the concentration of *E. coli* for urinary tract infection (UTI) application. The two chamber configuration is designed to reduce potential false-positive response caused by proteins in urine. The integrated impedance sensor in the concentration chamber shows little impedance change in different *E. coli* concentrations due to masking effect by proteins while the one in the sensing chamber shows a significant (30 k Ω) change between control (no *E. coli*) and a concentration of 6.4×10^4 CFU/mL, close to the threshold of UTI infection, which is above 10^5 CFU/mL. This demonstrates the effectiveness of the dual-chamber design to detect *E. coli* for the UTI application.

INTRODUCTION

Urinary Tract Infection (UTI) is one of the common infections in human, resulting in 8 million hospital visits in US annually [1]. The urine of UTI patients may contain a microbial strain, which can cause UTI, such as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella*, *Enterobacter*, *Enterococcus*, *Staphylococcus saprophyticus* and others [2]. Among them, *E. coli* is responsible for up to 80% of UTI [2]. Current standard methods of *E. coli* detection include multiple-tube fermentation, membrane filter and plate count [3]. Among them, the plate count method is the most widely used, which cultures *E. coli* on an agar plate and counts cultured colony forming units (CFU). If CFU exceeds 10^5 CFU/mL, the patient has a high chance of having symptomatic UTI [4]. Typically, people do not have symptoms below 10^5 CFU/mL [5]. The plate count method offers accurate diagnose, however it is not a feasible method when patients need an immediate medical treatment because it requires lengthy, from 24 to 48hrs, cultivation of urine samples and laborious procedure. In order to avoid such lengthy processes, alternative methods have been developed. Goodridge *et al.* used a fluorescent bacteriophage assay (FBA), Subramanian *et al.* developed immunosensor techniques to utilize antibody-derivatized surface plasmon resonance devices, and Wang *et al.* used piezoelectric crystals and enzyme-linked immunosorbent assays (ELISA) techniques [6-8]. Other researchers developed electrochemical impedance spectroscopy (EIS) to detect *E. coli* by monitoring the binding of antibody-antigen reaction [9-13]. All those methods offer a quick diagnose of *E. coli*; however capturing efficiency of *E. coli* is low.

In this paper, we take advantage of microfluidics in immunomagnetic separation (IMS) to overcome the limitation, low capturing efficiency. IMS uses magnetic particles (MPs) that are covered by anti-*E. coli* antibody to capture *E. coli*. The captured *E. coli* is transported via microfluidic channels and the impedance of *E. coli*-immobilized magnetic beads is measured to estimate the concentration of *E. coli*. The impedance results are verified by counting cultured colonies of *E. coli*, the current standard practice.

DESIGN

The schematic of the lab-on-a-chip device is illustrated in Figure 1. The *E. coli* concentrator/detector device consists of two mechanical valves [14] and two chambers, concentration and sensing, to reduce non-specific absorption by proteins potentially co-existing with *E. coli* in urine.

Figure 1: A schematic of the lab-on-a-chip *E. coli* concentrator/detector. Two separate (concentration and sensing) chambers capture and sense *E. coli*, respectively. Magnetic particles (MPs) inside the chambers are manipulated by external magnets.

Two mechanical valves control the fluidic stream to Outlet 1 and the sensing chamber. Two immunosensors to measure impedance are integrated in the concentration and sensing chambers and made of two gold (Au) electrodes. MPs were retained in the chamber by placing two permanent magnets above and beneath of the chambers. Figure 2 shows the length of the chambers as a function of *E. coli* capturing rate.

Figure 2: Design criteria for concentration and sensing chamber: the length of the concentrator chamber is determined by the structural failure limit, 100 psi.

As the length of the chamber increases the capturing rate enhances. Practical limitation exists; as the length increases the fluidic pressure increases as well. The current structure of the lab-on-a-chip device allows up to 100 psi where the structural failure starts. Thus, we chose the length of the chambers as 3 mm.

Fabrication

Similar to our previous approaches, we used a replica molding technique using a silicon mold [15]. This two-masks process is shown in Figure 3.

Figure 3: Fabrication flow: forming the top using the replica molding technique (1-3) and the bottom substrate that has electrodes for impedance measurements (4-6).

The silicon mold fabrication starts from photoresist (PR) coating and patterning for the concentrator, followed by Deep RIE etching, $\sim 10 \mu\text{m}$. PDMS (Polydimethylsiloxane) was mixed (10:1) with base and curing agents and then poured on the silicon mold. After 20 min curing in an oven at 80°C , the PDMS replicas were peeled off, and two mechanical valves were assembled on the PDMS replica [14]. Then, PR was coated on the glass slide substrate and lithographically patterned. Cr/Au films for electrodes were evaporated (Edward II). The surfaces of the glass slide and PDMS replica were treated by oxygen plasma (Harrick Plasma) for 1 min at 100 W. Finally the PDMS top was bonded to the glass slide, and they were cured in the oven at 80°C for 40 min. Figure 4 shows the fabricated lab-on-a-chip device.

Figure 4: Fabricated lab-on-a-chip *E. coli* concentrator/sensor (permanent magnets are not shown).

EXPERIMENTAL

Reagent

PDMS (Sylgard 184 elastomer kit) was obtained from Dow Corning. MPs from Spherotech, Inc were $4.5 \mu\text{m}$ in diameter and coated with COOH-terminated self-assembled monolayers (SAM) on the surface. Sodium acetate (NaAc), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Pierce and GE health. Phosphate buffered saline solution (PBS) from Fisher scientific was used as a washing solution. Simulated urine was a cocktail of $\text{CaCl}_2\cdot\text{H}_2\text{O}$, Na_2SO_4 , $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, NH_4Cl , KCl, Urea, Creatinine and tryptic soy. *E. coli* K-12 (ATCC 10798) was obtained from American Type Culture Collection. The pure culture of *E. coli* K-12 was prepared in a tryptic soy broth at 37°C .

Test setup

MPs were immobilized with anti-*E. coli* antibody. MPs coated with COOH-SAM were mixed with EDC and NHS in order to activate the functional group of SAM, followed by PBS wash. The anti-*E. coli* antibody was blended with 10 mM NaAc, and the mixture of them was mixed with MPs to conjugate the anti-*E. coli* antibody with activated COOH-terminated SAM on the surface of MPs. In order to avoid non-specific absorption, Bovine serum albumin (BSA) was added with MPs. We prepared different concentrations of *E. coli* solution (100%, 10%, 1%, 0.1%, and 0.01%) with BSA in simulated urine.

Figure 5 shows the characterization setup. A syringe pump delivered MPs, mannitol solution and a mixture of *E. coli*, albumin and simulated urine to the concentration chamber. Permanent magnets were used for manipulating MPs. MPs, mannitol solution, and the sample were flowed by $5 \mu\text{L}/\text{min}$, $2 \mu\text{L}/\text{min}$ and $2 \mu\text{L}/\text{min}$, relatively. A LCR meter measured impedance at concentration and sensing chambers and the data were recorded via LabVIEW.

Figure 5: Characterization setup of *E. coli* lab-on-a-chip device.

Test Procedure

MPs conjugated with anti-*E. coli* antibody were injected inside the concentration chamber. Two permanent magnets were placed above and beneath of the chambers to control MPs release and retention. When valve-1/2 were open/close, we retained MPs in the concentration chamber and flew $10 \mu\text{L}$ of K-12 *E. coli* and albumin ($1 \text{mg}/\text{mL}$) in simulated urine as shown in Figure 6(a). After *E. coli* was captured by anti-*E. coli* antibody on the MPs, we flew $90 \mu\text{L}$ of 0.1 M mannitol solution and the impedance sensor in the concentration chamber measured the impedance. When valve-1/2 were close/open and the two permanent magnets guided the MPs conjugated with *E. coli* to the sensing chamber, the impedance of the MPs was measured again as shown in Figure 6(b). The mannitol solution was used to allow *E. coli* alive at high solution impedance.

Figure 6: *E. coli* concentration and impedance measurements procedure: a) MPs retention, *E. coli* insertion, and measurement in the concentration chamber, b) MPs release, retention, and measurement in the sensing chamber, and c) *E. coli* conjugated by MPs (after gram stain).

RESULT

Figure 7 shows the immunosensor measurement in the concentration chamber. In the range of 6.4×10^4 to 6.4×10^6 CFU/mL, covering the threshold of UTI (10^5 CFU/mL), the measured impedance changes were almost negligible. This suggests that albumin in the simulated urine was absorbed on the surface of Au electrodes by the hydrophobic interaction to mask the impedance changes [16-21]. Due to the masking, the impedance measurements did not reflect the change of the *E. coli* concentrations.

Figure 7: Measured impedance data in the concentration chamber.

Figure 8: Measured impedance data in the sensing chamber.

Figure 8 shows the impedance measurement in the sensing chamber. As *E. coli* concentration increases, the impedance increases because the membrane of *E. coli* behaves as an insulator [3]. Comparing with the results in the concentration chamber, the impedance changes were bigger in the range from 6.4×10^4 to 6.4×10^6 CFU/mL. This is because albumin in the sample was removed effectively in the concentration chamber by hydrophobic interaction of albumin with Au electrodes. This result demonstrates the need of the two chamber configuration to detect *E. coli*. Figure 9 compares the impedance changes and traditional cultured colony forming unit method, the current standard practice, as a function of the concentration of *E. coli*. The impedance change at 1 kHz was 30 kΩ between control (no *E. coli*) and 6.4×10^4 CFU/mL, which is large enough to distinguish using a commercially-available portable impedance meter [22]. As the concentration of *E. coli* increases the impedance consistently increases. The threshold of UTI, 10^5 CFU/mL, was shown as a reference in the figure. The impedance data was further verified by counting cultured colonies of *E. coli* in Figure 9(b). We believe that the dual-chamber configuration allows reducing false positive UTI detection in the urine.

Figure 9: a) Impedance data at 1 kHz, b) *E. coli* CFU on a culturing agar plate at the different *E. coli* concentrations.

CONCLUSION

We demonstrated a dual-chamber lab-on-a-chip device to detect *E. coli* in simulated urine for UTI applications. The dual chamber configuration allows detecting the concentration of *E. coli* at the presence of protein in the sample. Impedance data at the concentration chamber results show that impedance changes were very little between 6.4×10^4 and 6.4×10^6 CFU/mL due to hydrophobic interaction of albumin on Au electrodes. Albumin masks the electrodes surfaces so that the measured impedance cannot be responsive to the concentration of *E. coli* in the sample. On the other hand, the impedance data in the sensing chamber did not show such masking effect because albumin was removed effectively in the concentration chamber. The impedance change between the control (no *E. coli*) and 6.4×10^4 CFU/mL was approximately 30 k Ω . Our successful detection result suggests that the dual chamber lab-on-a-chip device may be useful for diagnosis of symptomatic UTI.

Future work includes a reproducibility testing of the *E. coli* lab-on-a-chip device and improving sensitivity of impedance measurement by changing the design of the electrodes for distinguishing asymptomatic and symptomatic UTI.

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