Separating and Detecting Escherichia Coli in a Microfluidic Channel for Urinary Tract Infection (UTI) Applications

by

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ABSTRACT

In this thesis, I present a lab-on-a-chip (LOC) that can separate and detect *Escherichia Coli* (*E. coli*) in simulated urine samples for Urinary Tract Infection (UTI) diagnosis. The LOC consists of two (concentration and sensing) chambers connected in series and an integrated impedance detector. The two-chamber approach is designed to reduce the non-specific absorption of proteins, e.g. albumin, that potentially co-exist with *E. coli* in urine. I directly separate *E. coli K-12* from a urine cocktail in a concentration chamber containing micro-sized magnetic beads (5 μm in diameter) conjugated with anti-*E. coli* antibodies. The immobilized *E. coli* are transferred to a sensing chamber for the impedance measurement. The measurement at the concentration chamber suffers from non-specific absorption of albumin on the gold electrode, which may lead to a false positive response. By contrast, the measured impedance at the sensing chamber shows ~60 kΩ impedance change between $6.4x10^4$ and $6.4x10^5$ CFU/mL, covering the threshold of UTI (10^5 CFU/mL). The sensitivity of the LOC for detecting *E. coli* is characterized to be at least $3.4x10^4$ CFU/mL. I also characterized the LOC for different age groups and white blood cell spiked samples. These preliminary data show promising potential for application in portable LOC devices for UTI detection.

To My Parents, Wife and Children

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My deepest and special thanks should go to my parents since they always have loved me, believed in me, and encouraged me in my study.

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1. INTRODUCTION

1.1…..Introduction

Human urine is commonly used in regular medical check-ups and tests to identify the cause of symptoms [1]. Urine is a rich source for evaluating overall health, especially kidney function, for instance, because urine contains hundreds of body wastes that kidneys filter from the blood. Urinary Tract Infections (UTI) are common kidney-related diseases in humans, especially women. They account for 8 million hospital visits annually in the US [2]. Moreover, UTI has a high recurrence rate: among individuals of a first infection, 20% has their second infection within six months, and 3% has a third infection within six months [3],[4]. *Escherichia Coli* (*E. coli*) is responsible for up to 80% of UTI [5],[6], and conventional detection methods, bacteria culture for a urine sample require 24 to 48 hours cultivation and labor-intensive procedures [7]. Being able to rapidly identify living cells without culturing is very important to medical diagnosis and treatment because microorganisms can cause severe diseases [8],[9], and some progress very quickly [10],[11]. Though more rapid test methods do exist, such as the dip-stick method for *E. coli* detection, and such method is based on nitrite and esterase and offers fast detection, but does not provide sufficient sensitivity [12].

1.2…..Background

Immunomagnetic separation (IMS) [13],[14] and impedance spectrometry (IS) techniques have been frequently used to capture and detect *E. coli* [33]. Some have been implemented in a miniaturized form of detector [33]. These IMS/IS techniques presented focus on detecting *E. coli* O157, which are a cause of foodborne illness [15]. Wright *et al.* used IMS to collect and detect *E. coli O157* from minced beef samples. They used magnetic beads coated with anti-*E. coli* antibodies to isolate *E. coli O157* from the beef samples and cultured them to count colony forming unit (CFU) [13]. Perez *et al.* presented IMS to capture *E. coli O157* and detected them electrochemically [14]. They also measured a calibration curve of CFU, mapped to the electrochemical response. Besides these work there are a number of prior work using IMS/IS techniques to collect and detect *E. coli* [16],[17],[18]. Varshney *et al.* used magnetic nanoparticles conjugated antibody for detection *E. coli O157* in food samples [33]. The nanoparticles immobilized biotin-labeled polyclonal goat anti-*E. coli* antibodies to separate and concentrate *E. coli* O157:H7 from ground beef samples. Then, the impedance of the cluster of the nanoparticles and *E. coli* were measured using interdigitated microelectrodes. The lowest detection limits of their biosensor in pure culture and ground beef samples were 7.4×10^4 and 8.0×10^5 CFU/mL. By miniaturizing electrodes and using nanoparticles, the sensitivity enhances and the flexibility of electrode fabrication improves. Miniaturized

electrodes also allows to perform IS in low conductivity solution since they require lower concentrations of electro-active ions to form double layer as compared to macrosized counterparts. An excellent review on miniaturized impedance biosensors for detecting bacteria is available in [32].

In this thesis, a lab-on-a-chip (LOC) is presented to separate *E. coli* and measure its concentration using magnetic beads (MBs) directly from a cocktail urine sample. The *E. coli* captured by MBs was evaluated by IS, and those IS measurements were further evaluated using the current standard practice of counting cultured CFUs. An *E. coli* concentration of 10^5 CFU/mL or higher in urine is typically considered a UTI, and several different *E. coli* serotypes can cause UTI [23],[24],[25]: O1 : K1 : H7, O6 : K15 : H31 ; Sm^R, O6 : K2 : H1, O18 : K1 : H7 ; Sm^R, and O4 : K6 ; Nal^R. Since UTI-related *E. coli* demands high safety requirements, *E. coli K-12* was used instead of serotypes such as O1 : K1 : H7 and O6 : K15 : H31 ; Sm^R. Normal urine does not contain any protein, or contains only trace amounts of it [26],[27]. For UTI patients, however, albumin may be excreted and contained in the urine. I attempted to make the closest possible simulation of UTI-infected urine sample by spiking albumin and *E. coli K-12* in cocktail urine. The LOC was also characterized using the simulated urine having different concentrations of *E. coli*, co-existing with while blood cells (WBCs).

1-3…..Motivation

Significant research efforts have focused on shortening analysis time, and on detecting *E. coli* with increasing sensitivity and high accuracy. One example is the work performed by Klodzinska *et al.,* who separated *E. coli* using Capillary Zone Electrophoresis (CZE) and the presence of negatively charged ions of carboxyl and phosphate groups on the bacterial cell wall [19]. The capillary surface was modified to separate *E. coli*, and spectrophotometry measured the concentration of bacterial cells. The high voltage (20kV) supply used in the work, however, could cause electromagnetic interference, posing a bottleneck in the development of a portable hand-held unit for use at a patient's bedside [20],[21]. In another example, Ruan *et al.* immobilized anti-*E. coli* antibodies on a planar electrode to detect *E. coli O157:H7* [22]. The surface of glass electrodes coated by indium tin oxide were modified and detected *E. coli*, but the capturing rate was low; this may lead false-negative results under the normal use. I believe that microfluidics in IMS may overcome such limitations. Despite of such success of many microsized platforms, few study have focused on detecting bacteria in urine for UTI applications. This motivates developing a micro-chip to integrated IMS/IS to detect *E. coli* for UTI applications.

1-4…..Thesis Outline

This thesis is organized as follows. The design analysis of device, fabrication method and experimental methodology are presented in chapter 2. Chapter 3 describes the procedure of preparation for experiment, and experimental result. Finally, conclusion is expressed in chapter 4.

2. PROPOSED METHODOLOGY

In this chapter, device design, fabrication and experiment methodology are described in detail. The design analysis is presented in section 2-1. Section 2-2 presents a method to fabricate the device. The experimental methodology is presented in section 2-3.

2-1…..Design

The design of the *E. coli* separator is illustrated in Fig. 2-1. Two chambers, one for concentration and the other for sensing, are connected in series with two mechanical valves [28] to control the fluidic stream. The dimension of the chambers and inlets is 1.8 × 3.0 × 0.05 mm^3 (W×L×H), and that of the microfluidic channels is 0.5 × 5.0 × 0.05 mm^3 (W×L×H). The two gold electrodes for IS measurement are 1.0 × 1.5 mm^2 (W×L), and these are placed in the concentration and sensing chambers. MBs are retained and transported inside and between the chambers using two permanent magnets (K&J Magnetic, Inc.) placed above and beneath the chambers.

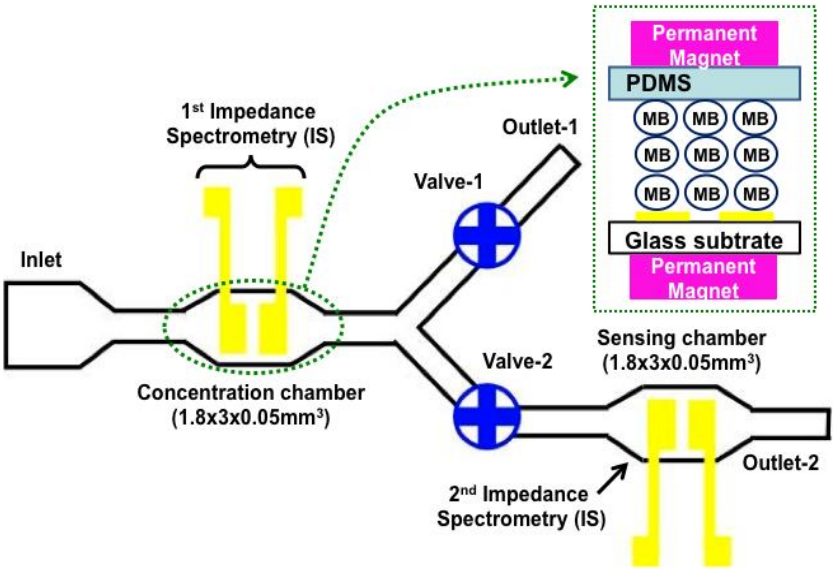


Figure 2-1: Schematic of the *E. coli* separator lab-on-a-chip (LOC) integrated with on-chip impedance spectrometry (IS) for UTI applications.

2-2…..Fabrication

I used the Replica-Molding (REM) technique, which transfers surface patterns on a mold to an elastomeric material. A silicon substrate and PDMS were used for the mold and elastomeric material, respectively. The fabrication of the silicon mold is shown in Fig. 2-2 requiring two-mask process using AZ 4330 photoresist and one Deep-RIE (Reactive Ion Etching), 50 μm deep, step.

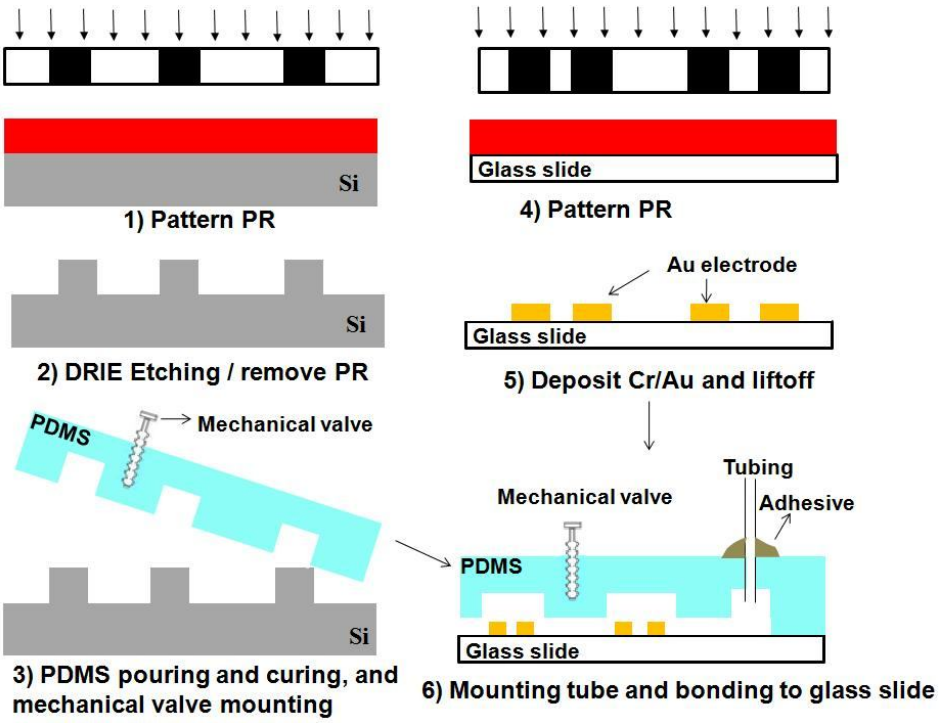


Figure 2-2: Fabrication process flow of the LOC *E. coli* separator integrated with on-chip IS.

Once the silicon mold was fabricated, PDMS was prepared by mixing silicone elastomer and curing agent in a 10:1 ratio and pouring it on the mold. After curing, the PDMS replicate was peeled off and assembled; two screws were mounted and used for mechanical valves. The gold electrodes, chrome/gold (10 nm/200 nm), were lithographically patterned on the glass slide. The glass slide and PDMS replicate were treated by oxygen plasma (Harrick Plasma) for 1 min at 100 W, and bonded, then cured for 20 min at 80 °C in the oven. The fabricated LOC is shown in Fig. 2-3.

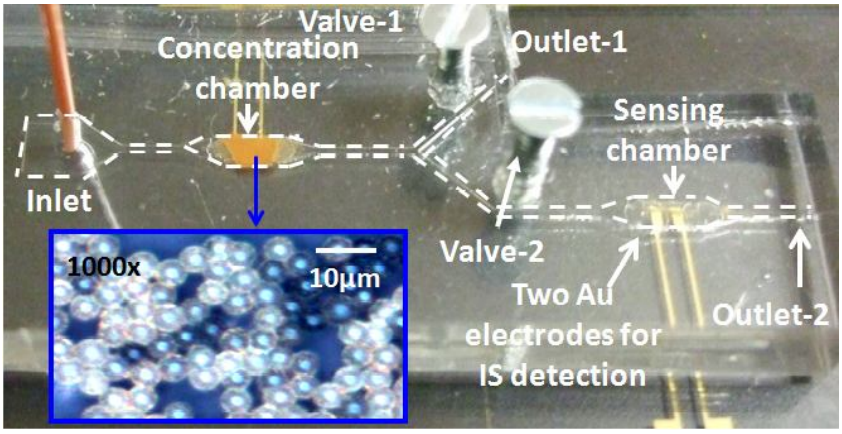


Figure 2-3: Fabricated LOC *E. coli* separator integrated with on-chip IS

2-3….. Methodology

MBs were conjugated with anti-*E. coli* antibodies. First, 400 mM of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 100 mM of N-hydroxylsulfosuccinimide (NHS) were freshly prepared in DI (de-ionized) water and mixed with MBs to activate COOH-terminated self-assembled monolayers (SAMs). After 15 min of incubation, the mixture of MBs with NHS and EDC was washed with PBS solution. The anti-*E. coli* antibody was prepared with 10 mM NaAc (sodium acetate), pH 5.3 solution, and the anti-*E. coli* antibody and NaAc mixture was mixed with MBs to conjugate with activated COOH-terminated SAM on the surface of the MBs. After completing the antibody bonding to the COOH-terminated SAM, the unbound, activated COOH-terminated SAM was blocked by ethanolamine [29],[30].

There are several example of prior art that modeled the equivalent circuit of *E. coli* on thin film electrodes and *E. coli* conjugated with

magnetic particles [33]. Radke *et al.* modeled *E. coli* O157:H7 on interdigitated electrodes [31]. The equivalent model of *E. coli* consists of the resistance of the cytoplasm, the resistance of the cell membrane, and the capacitance of the cell membrane. Additionally, the parasitic capacitance includes the double-layer capacitance at the electrode surface and stray capacitance associated with the oxide layer sandwiched between the gold electrodes and silicon. Varshney *et al.* presented an equivalent circuit model of magnetic nanoparticles attached to *E. coli* on a thin film electrode [33]. The model consists of double layer capacitance, stray capacitance (associated with electrodes, shielding, wires, cables, and adhesives), and resistance of medium or bulk that represents the cluster of magnetic nanoparticles and *E. coli*. The equivalent model was characterized by a series of measurements, which fit very well to the model. It is very difficult to model the undefined number of magnetic particles and *E. coli* clusters between sensing electrodes. One cannot assume a distributed model of a cluster of a magnetic particle and *E. coli* since non-uniform electric field density exists inside the chamber.

A non-Faradaic equivalent circuit is used to model IS measurements of the LOC as shown in Fig. 2-4. The equivalent circuit consists of two double layer capacitances (C_{dl}), connected in series with a bulk medium resistor (R_s), and a stray capacitor (C_s) connected in parallel with C_{dl} and R_s [32],[33]. C_{dl} comes from the effect of ionic species near the electrode surface. R_s reflects the conductivity change in bulk medium

and charge transport across the bulk solution. C_s represents stray capacitance associated with shielding, electrodes, and the adhesion layer between the gold electrode and glass. Contributions to the total impedance value can be expressed by the equations below:

$$|Z_1| = \sqrt{R_s^2 + \frac{1}{(\pi f C_{dl})^2}},\ |Z_2| = \sqrt{\frac{1}{(2\pi f C_s)^2}},\ \frac{1}{|Z_{total}|} = \frac{1}{|Z_1|} + \frac{1}{|Z_2|} \quad (1)$$

where f is frequency, Z_1 is the impedance of R_s and C_{dl}, Z_2 is the impedance of C_s, and Z_{total} is the total impedance of Z_1 and Z_2. The current cannot pass through C_s at low frequency; this results in an open circuit, and only the C_{dl} and R_s path is active and contributes to total impedance. At high frequency, however, current passes through C_s, and R_s and C_{dl} can be ignored.

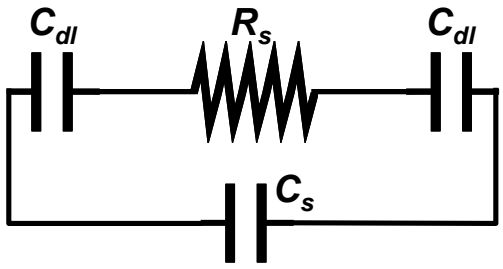


Figure 2-4: Equivalent circuit model for the IS.

3. EXPERIMENTAL RESULTS

In this chapter, the experiment procedure and result are presented. Section 3-1 reports what reagent and analyte are used for experiments. Then test procedure and test set-up are described in section 3-2. In section 3-3, capture efficiency is presented and the experiment results are reported in section 3-4.

3-1…..Reagent and analyte

Affinity-purified rabbit anti-*E. coli* antibody (1.0 mL, 4.6 mg/mL) was purchased from ViroStat (Portland, ME) and diluted with PBS (0.01 M, pH 7.4) from Fisher Scientific. MBs from Spherotech, Inc. were 4.5 μm in diameter and coated by COOH-terminated SAMs on the surface. The activating reagents that couple anti-*E. coli* antibody to the COOH-terminated SAMs on the MB are EDC and NHS, as purchased from Pierce. NaAc was obtained from Mediatech, Inc. Ethanolamin from Sigma was used as a blocking buffer. One tenth molar solution of mannitol (Sigma-Aldrich) in DI water from Millipore (Milli-Q, 18.2MΩcm) was used for washing and IS measurement. Simulated urine cocktail was prepared by mixing ingredient A and B (Table 3-1) in 1 L of sterilized DI water. *E. coli K-12* (ATCC 10798) was obtained from American Type Culture Collection (Rockville, MD). The pure culture of *E. coli K-12* was prepared in tryptic soy broth (BD, Franklin Lakes, NJ) at 37°C for 24 h. Pure cultures were serially diluted in PBS and surface plated on nutrient agar

plate (PML Microbiologicals), incubated at 37°C for 20 to 24 h, to check CFUs at different concentrations.

Table 3-1.

Simulated urine cocktail composition.

Ingredient A	
CaCl2.H2O	1.77g
Na2SO4	4.86g
MgSO4.7H2O	1.46g
NH4Cl	4.6g
KCl	12.13g
Urea	25g
Creatinine	1.1g
tryptic soy (0.25-0.5%)	5 or 2.5g
Ingredient B	
NaH2PO4.2H2O	2.66g
Na2HPO4	0.87g
NaCitrate	1.17g
NaCl	13.545g
Urea	25g
Creatinine	1.1g
tryptic soy (0.25-0.5%)	5 or 2.5g

3-2…..Test procedure and test setup

Fig. 3-1 illustrates the test setup of the fabricated LOC *E. coli* separator/sensor. Three different syringe pumps contain MBs with anti-*E. coli* antibody, mannitol solution, and *E. coli K-12* in cocktail urine, and deliver them to a six-way valve. An LCR meter (Agilent E4908A) measured from 10 Hz to 1 MHz at the concentration and sensing chambers.

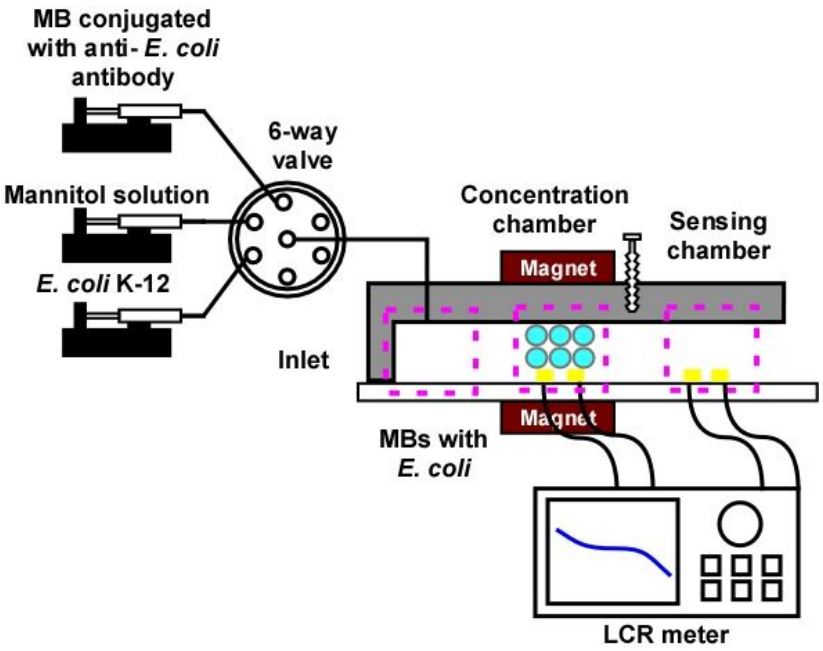


Figure 3-1: Schematic of characterizing the fabricated LOC *E. coli* separator integrated with on-chip IS.

Fig. 3-2 depicts the test procedure. First, when valve-2 closed, mannitol solution flew into the separator at 10 μL/min; MBs with anti-*E. coli* antibody were inserted as shown in Fig. 5(a). The mannitol solution was used to allow *E. coli* to live and enable impedance measurement as it has high impedance. Two permanent magnets were placed on the top and bottom of the concentration chamber and retained the MBs, which were then washed by mannitol solution for 30 min at 10 μL/min. 5 μL of cocktail urine containing different concentrations of *E. coli K-12* and albumin were injected through the chip. Once MBs captured *E. coli K-12* in the cocktail urine, mannitol solution again flew to wash the concentration chamber for 30 min at 10 μL/min, and then IS was performed using a LCR meter (E4980A Precision LCR Meter from Agilent Inc.). When valve-1 closed

and valve-2 opened, the two permanent magnets above and beneath the concentration chamber were relocated at the sensing chamber as shown in Fig. 5(b). Mannitol solution then transported MBs with *E. coli K-12* from the concentration to sensing chamber at 10 µL/min. Then, the impedance was measured. Both electrodes pairs in the concentration and sensing chambers were used as they were without any surface modification. At the end of each measurement, the *E. coli* separator was washed with 0.1 M sodium hydroxide for 30 min, DI water for 30 min, 0.1 M hydrochloric acid for 30 min, and DI water for 30 min.

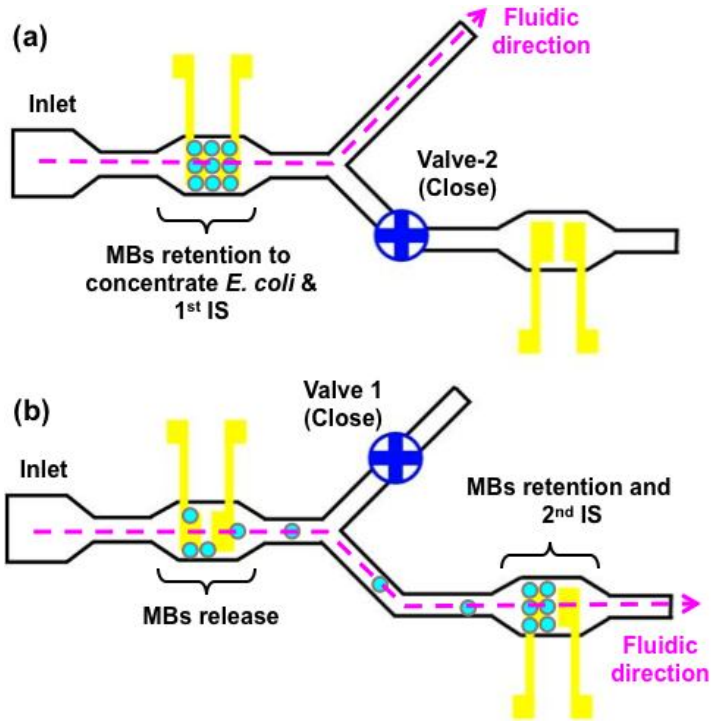


Figure 3-2: Test procedure: (a) MBs retention, direct *E. coli* separation from cocktail urine, and IS measurement in the concentration chamber and (b) MBs' transfer and retention and IS measurement in the sensing chamber.

Table 3-2 shows the step-by-step procedure of the microchip operation and associated time to complete each step. The total analysis time is roughly 100 mins. Please note that this does not include fabricating microchips and immobilizing MBs with anti-*E. coli* antibodies. I assume these steps are performed before the analysis. I used mannitol solution to rinse microfluidic channels and chambers. The rinsing step is to ensure I remove all unnecessary entities to enhance sensitivity of the microchip.

Table 3-2

Step-by-step procedure and required time

Step #	Description	Time [min]
1	Preparation of *E. coli* sample	5
2	Capture the MBs using two permanent magnets in the concentration chamber	10
3	Flow manitol solution to rinse the microfluidic channels/chambers	30
4	Flow *E. coli* sample	5
5	Flow manitol solution to rinse the microfluidic channels/chambers	30
6	Perform impedance spectroscopy measurements at the concentration chamber	5
7	Move and immobilize *E.coli*-conjugated MBs inside the sensing chamber	10
8	Perform impedance spectroscopy measurements at the sensing chamber	5
	Total time required to complete the procedure	***100 mins***

3-3…..Capture efficiency

One of biggest advantage of using IMS is in capture efficiency (CE). Prior art using IMS reported up to 100% CE [33],[34]. I characterized CE in a single chamber configuration using a standard fluorescent intensity

measurement. MBs having anti-*E. coli* antibodies were placed inside the chamber and held by permanent magnets on the top and bottom of the chamber. After packing the chamber I flew 3 μL of 10^7 *E. coli*/mL that were conjugated with red fluorescent dye and chemically dialyzed (E-2863, Invitrogen Inc.). Then, the permanent magnets were removed, and MBs were collected with *E. coli* at the outlet. The fluorescent intensity was measured using an inverted microscope. The measured fluorescent intensities were compared to that of *E. coli* before being captured by MBs. It was repeated the experiments for a different length of the column from 1 to 6 mm as shown in Fig. 6. As the column length increased, CE improved; however the applied fluidic pressure also increased. At the length of 3.2 mm, the fluidic pressure reaches 100 psi, structural failure point, based upon the geometry. This determined the channel length as 3 mm.

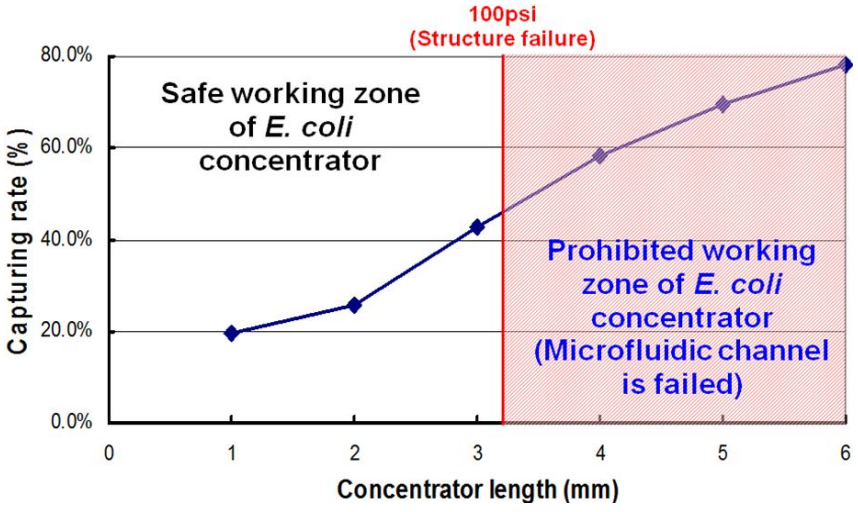


Figure 3-3: Capture efficiency (CE) of the *E. coli* separator as a function of the concentrator length.

3-4…..Results

Fig. 3-4 shows the bode plot and phase angle of the IS measurement and fitted impedance spectra for 6.4 x 10^8 CFU/mL of *E. coli K-12* attached to the antibody of the MBs retained in the sensing chamber. The fitting was generated by ZSimp software [35],[36]; mean errors of impedance and phase angle shown in Table 3-3 were 6% and 0.8°, respectively. Based on the phase angle, there are two distinct regions in the impedance spectra, which correspond to two elements in the equivalent circuit. The double layer capacitance can be measured below 10 Hz; however I was not able to measure it because the LCR meter cannot measure below 10 Hz. When the frequency is between 10 Hz and 10 kHz, current cannot pass through C_s, resulting in an open circuit in the equivalent circuit; only the C_{dl} and R_s become active, but R_s is more dominant because captured *E. coli* membrane becomes an insulator that increases the impedance [32],[37]. By contrast, C_{dl} and R_s become inactive at higher frequencies (above 10 kHz); the *E. coli* membrane becomes electrically invisible and the value of C_{dl} is larger than *Cs,* contributing all the impedance change at a frequency above 10 kHz.

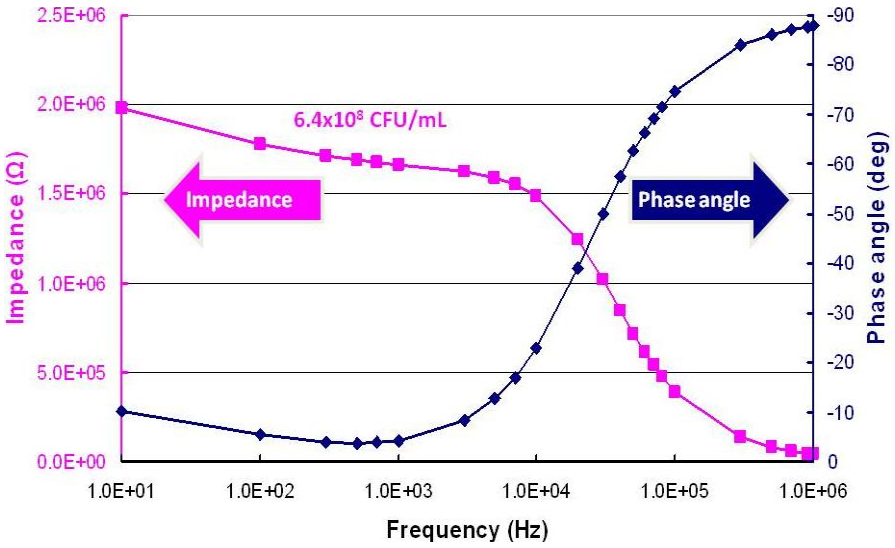


Figure 3-4: IS measurement of 6.4×10^8 CFU/mL *E. coli*.

Table 3-3.

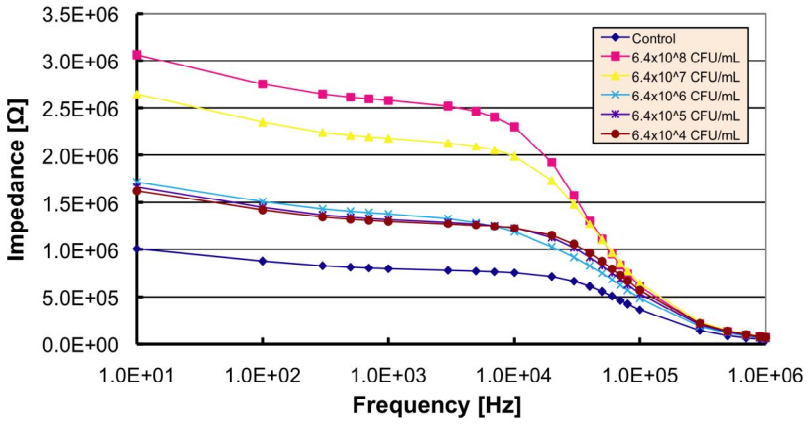
Measured and fitted values of each component in IS equivalent circuit.

	Measured	Fitted	Error
Rs	1.68 MΩ	1.68MΩ	0.3%
Cs	3.8 pF	3.9 pF	0.1%

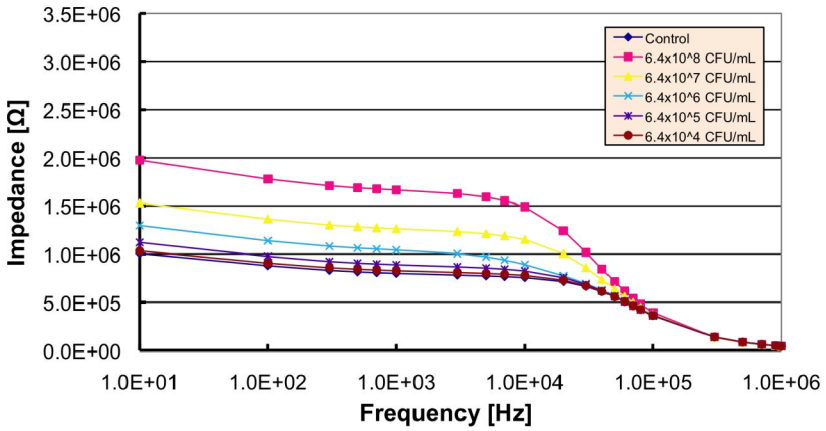
I spiked *E. coli K-12* and albumin in urine cocktail and repeated the separation/detection procedure in the concentration chamber (Fig. 3-5(a)). Large impedance change was observed between high *E. coli* concentrations (6.4×10^8 CFU/mL and 6.4×10^7 CFU/mL), and the control (MBs with anti-*E. coli* antibody). On the other hand, low *E. coli* concentrations (6.4×10^5 CFU/mL and 6.4×10^4 CFU/mL) showed almost no impedance change. I believe this is due to the masking effect of albumin; albumin absorbed on the electrodes, thus low concentration *E.*

coli cannot be well discriminated. Moreover, impedance changes between the low concentration samples and the control are very large. The changes may cause a false-positive response. The albumin in the urine cocktail was absorbed on the gold electrode by hydrophobic interaction in the concentration chamber [38],[39]. In the higher *E. coli* concentration, *E. coli* was the dominant factor to increase R_s, medium resistance, but in the lower concentration, albumin absorbed on the gold surface resulted in constant impedance change.

Fig. 3-5(b) shows the IS measurement in the sensing chamber where MBs conjugated with *E. coli* were transported from the concentration chamber. Compared to the IS measurement in the concentration chamber, lower *E. coli* concentrations became clearly distinguishable in the sensing chamber. The impedance difference between 6.4 x 10^5 CFU/mL and 6.4 x 10^4 CFU/mL was approximately 60 kΩ at 1 kHz.



(a)



(b)

Figure 3-5: IS measurement for different *E. coli* K-12 concentrations ranging from $6.4x10^4$ to $6.4x10^8$ CFU/mL in the concentration chamber (a) and the sensing chamber (b).

Fig. 3-6 summaries the impedance change at 1 kHz in the sensing chamber. 60 kΩ resistance change was observed between the 6.4×10^4 CFU/mL and 6.4×10^5 CFU/mL; the threshold of UTI is 10^5 CFU/mL [23],[24]. Therefore, the IS measurement suggests that the LOC separator/detector is capable of evaluating urine samples for UTI.

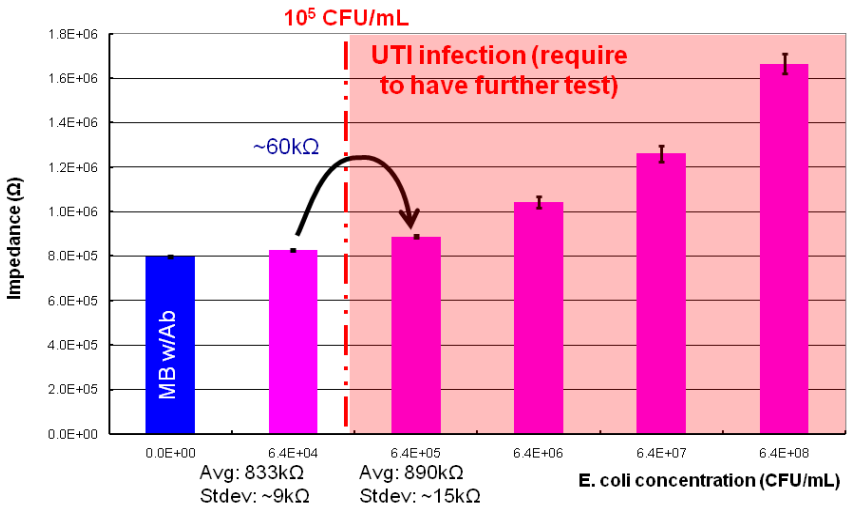


Figure 3-6: Impedance spectrum of different *E. coli* concentrations at 1kHz in the sensing chamber.

Fig. 3-7 shows the sensitivity characterization of the LOC. I used the same simulated urine samples and spiked *E. coli* from 3.4 x 10^7 CFU/mL down to 3.4 x 10^3 CFU/mL. Impedance spectrums at 1 kHz in both concentration and sensing chambers were measured and compared. The sensitivity, slope of the bars, in the concentration chamber is lower than that in the sensing chamber. Above 3.4 x 10^6 CFU/mL, the impedance increases significantly in both concentration and sensing chambers. The minimum detectable concentration of *E. coli* follows the similar trend. The data between 3.4 x 10^3 and 3.4 x 10^4 CFU/mL in the concentration chamber are indistinguishable while the data in the sensing chamber are clearly distinguishable. This suggests the minimum detectable concentration of *E. coli* of the LOC is at least 3.4 x 10^4 CFU/mL. However, below 3.4 x 10^4 CFU/mL, it is too close to the control (control has 112 kΩ and the concentration of 3.4 x 10^4 CFU/mL has 113 kΩ). The 3.4 x 10^4 CFU/mL minimum detectable concentration is lower than the threshold of UTI (10^5 CFU/mL).

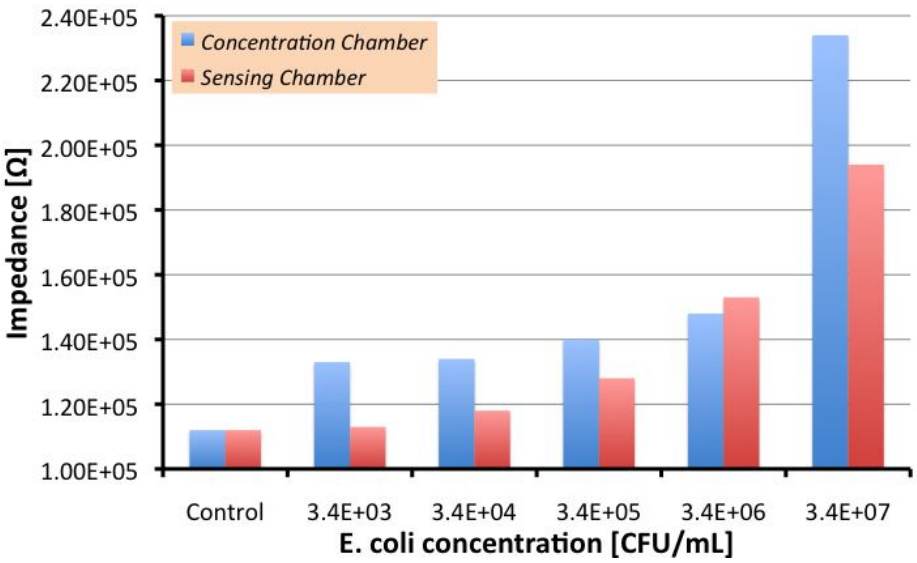
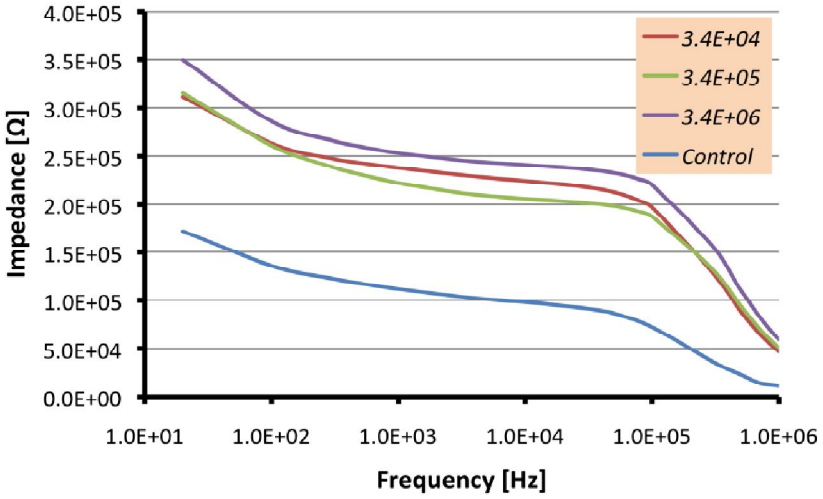


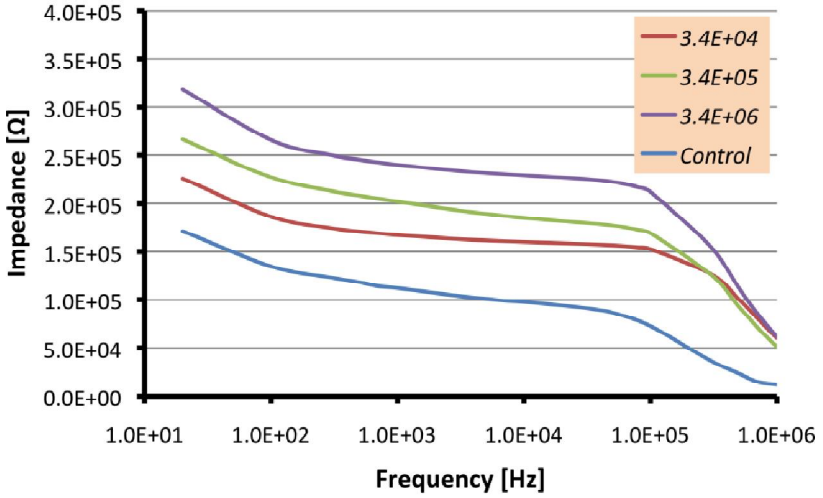
Figure 3-7: Sensitivity of the microfluidic device to detect *E. coli* (at 1 kHz).

WBC may present in urine. Having more than 10 WBCs/μL in urine is considered to be a high chance of UTI [40]. I spiked 35 cells/micro-L WBC (from Innovative Research, Inc.) in the simulated urine cocktail having 3.4 x 10^4, 3.4 x 10^5, and 3.4 x 10^6 CFU/mL *E. Coli* to see how WBC impacts the impedance measurements at concentration and sensing chambers, respectively as shown in Fig. 3-8. One can see a substantial gap of impedance measured between the control and the rest in the concentration chamber (Fig. 3-8(a)). Also the impedance measurements of three concentrations (3.4 x 10^4, 3.4 x 10^5, and 3.4 x 10^6 CFU/mL) overlap each other, which I believe it is due to the protein screening effect. On the other hand, the data in the sensing chamber (Fig. 3-8(b)) shows a different pattern; there is no abrupt change of the measured impedance as the concentration increases. The impedance is roughly proportional to the

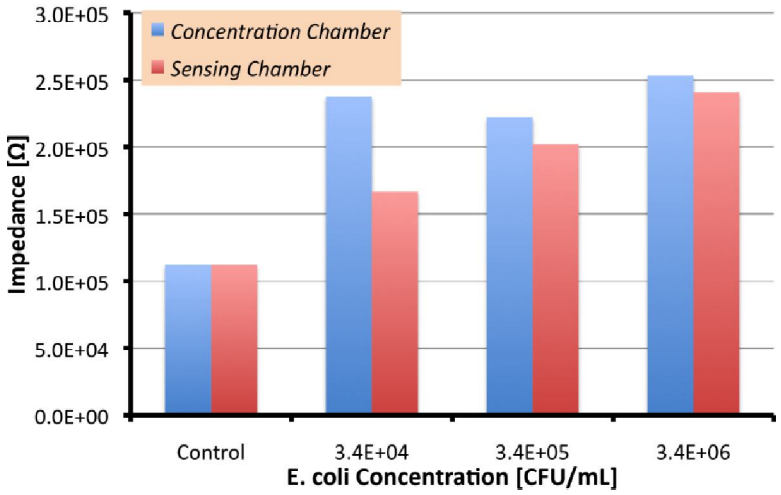
E. coli concentration as shown in Fig. 3-8(c). It is clear to observe that the measurements in the sensing chamber offered more consistent results than the ones in the concentration chamber when the samples were spiked by WBC.



(a)



(b)



(c)

Figure 3-8: Impedance spectrum of simulated urine samples having 35 WBCs/micro-L in three different *E. coli* concentrations in the (a) concentration and (b) sensing chambers. (c) Impedance comparison of concentration/sensing chambers at 1 kHz

Human urine can be very diverse such as volume per day and *pH* of different age/sex groups [41]. I attempted to compare impedance responses of three different urine cocktails as shown in Fig. 3-9: 20 yr-old, 40 yr-old, and 60 yr-old female, respectively. I chose urine cocktails [41] for female only since up to 40% of women will develop UTI at least once during their lives [42]. The impedance measurements of the controls for three samples were largely different: 232 kΩ for 20 yr-old control, 135 kΩ for 40 yr-old control, and 799 kΩ for 60 yr-old control. This is partially because I used three different micro-chips. However it is notable that the impedance changes as a function of *E. coli* concentration showed different patterns from one from the other urine cocktails. The impedance of 40 yr-old samples showed larger increase over that 20 yr- and 60 yr-old

samples. It is hard to draw a conclusive evidence of any fundamental mechanism that describes the discrepancy of the three groups of samples; yet the measurements strongly suggest that the presented micro-chip must be calibrated for diverse nature of urine samples.

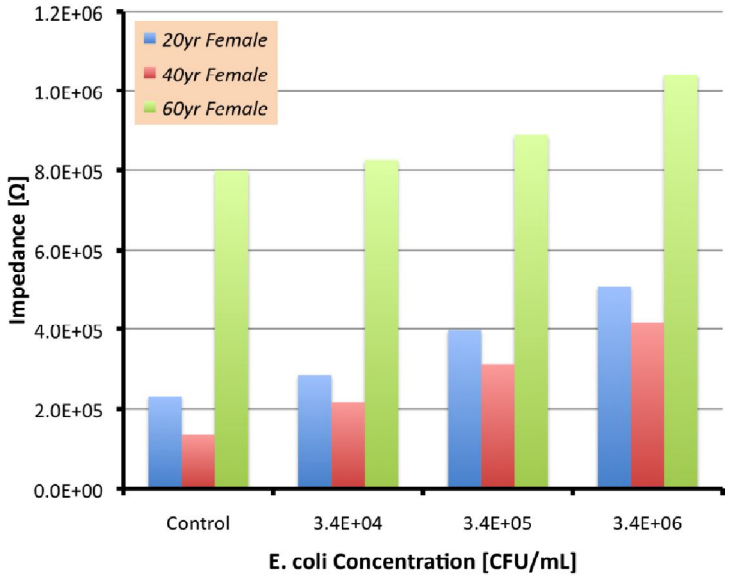


Figure 3-9: The effect of diversity on human urine compositions; comparison of measured impedance of 20 yr-old (blue), 40 yr-old (red), and 60 yr-old (green) female urine cocktails as a function of spiked *E. coli* concentration.

4. CONCLUSION

4-1…..Summary

In this thesis I present a micro-chip to study UTI using simulated urine samples. The target micro-organism to detect is *E. coli* which are responsible for 80 % of UTI. I designed a LOC which is equipped with dual chamber configuration that separates and measures the concentration of *E. coli* for UTI applications. MBs were conjugated with anti-*E. coli* antibody to capture *E. coli* and were transported from concentration to sensing chambers. The integrated electrodes allowed IS to measure the concentration of the captured *E. coli*. The IS measurements between concentration and sensing chambers showed that non-specific binding from albumin could cause a false-positive response. I believe that this result comes from a hydrophobic interaction between the gold electrodes in the concentration chamber and albumin in UTI-infected urine. The IS measurement in the sensing chamber showed a significant impedance change to evaluate UTI: ~60 kΩ, between 6.4 x 10^4 CFU/mL and 6.4 x 10^5 CFU/mL. Sensitivity of the LOC was characterized to be at least 3.4 x 10^4 CFU/mL, which is less than the threshold of UTI (10^5 CFU/mL). I also characterized the LOC for the interference of WBCs to impedance measurements and for different urine cocktail, and no significant interference and discrepancy were observed.

4-2…..Future works

This preliminary results using *E. coli K-12* may suggest that the LOC *E. coli* separator/sensor can be used to detect UTI *E. coli* in human urine. Before testing a target bacteria, UTI *E. coli*, however, I will need to optimize the capture rate of the *E. coli* separator and increase the sensitivity of IS measurements.

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APPENDIX A

DATA COLLECTED AUGUST–DECEMBER 2010

TABLE A-1

Test procedure

Step	Description
1	MBs are mixed with EDC, NHS, acetate and antibody for immobilization of antibody on MBs
2	Preparation of *E.coli* sample using cultured *E. coli* sample
3	Capturing the MBs using permanent magnet on the concentration chamber
4	Flow manitol solution using syringe pump by 10μL/min
5	Flow *E. coli* sample using syringe pump by 1μL/min
6	Flow manitol solution using syringe pump by 10μL/min
7	Measure impedance change using LCR meter
8	Moving and capturing MBs to sensing chamber by permanent magnet
9	Measure impedance change using LCR meter

TABLE A-2

Fabrication procedure

Step	Process
1	Patterning Si wafer by AZ 4330 photoresist
2	Hard bake photoresist for Deep RIE
3	Deep RIE for 50um etching
4	Removing photoresist by Acetone
5	Mixing silicone elastomer with curing agent by 10:1 ratio for PDMS
6	Molding PDMS on Si wafer
7	Curing for 40 min in 120℃ oven
8	Peeling off on Si wafer
9	Assembling two mechanical valves and one nanotube
10	Cleaning glass slide by Acetone, Isopropyl-alcohol and DI water
11	Pattering AZ4330 photoresist
12	Depositing Cr/Au by 10nm/200nm by sputter
13	Lift off Cr/Au by Acetone
14	Cleaning surface of glass slide and PDMS by oxygen plasma for 1min.
15	Bonding PDMS and glass slide
16	Curing device for 20min in 80℃ oven.

TABLE A-3

The age dependency of the composition of urine in the healthy women

(mmol/24h) [41]

Age group	20's	40's	60's
Parameter			
Volume (ml)	1277	1261	886
pH	6.27	6.14	5.89
Titratable acid	19.66	25.52	21.64
Ammonium	32.36	37.15	30.74
Sodium	148.3	153	78.6
Potassium	43.26	54.36	37.07
Calcium	3.54	4.41	1.8
Magnesium	5.41	4.08	2.98
Chloride	123.9	133.1	70.4
Phosphorus	22.64	21.2	14.19
Sulfate	18.79	20.01	10.59
Uric acid	3.04	3.07	1.94
Oxalic acid	0.353	0.376	0.299
Citric acid	2.06	1.94	1.33
Creatinine	10.56	11.5	6.94
Ionic strength (mol/l)	0.208	0.218	0.186
Ionic calcium	1.58	2.2	1.17
Relative super-saturation	5.67	7.18	5.9