Rapid and Sensitive Detection of Total Coliforms and E. coli Using SWNT Membrane

Basic Information

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10. **Principal Investigators:**	Yu Lei

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1. Introduction

Escherichia coli, which belong to fecal coliform, is the most important indicator for fecal contamination in aqueous systems.[1] Therefore, the fast determination of *E. coli* is essential for monitoring the microbiological water quality and mitigating the risks associated with water contamination. *E. coli* have the ability to produce β-D-galactosidase and β-D-glucuronidase activity, both of which are inducible and can be employed in enzyme assays for the bacterial detection.[2]

In the past decades, a variety of technologies have been developed for determining the number of waterborne microorganisms in drinking water. Among all the techniques, amperometric method has demonstrated to be rapid, sensitive, cost-effective, and user-friendly.[3,4] Many efforts have been focused on the construction and modification of the working electrode with batch analysis mode or flow injection analysis mode.[5,6,7,8] A detection limit of 1×10^{6} cfu of *E. coli* mL^{-1} in 5 mL working solution was obtained using a tyrosinase composite biosensor without any pre-concentration or per-enrichment.[8] To the best of our knowledge, this is the best result for the electrochemical detection of *E. coli* reported to date. However, redundant operation, such as permeabilization step, was still included, therefore, inevitably increase the overall analysis time.

Carbon nanotube (CNT) has drawn considerable attentions due to its unique electrical and mechanical properties.[9] Recently, highly porous CNT membrane was prepared by chemical vapor deposition (CVD).[10] Enlightened by this study, we consider to fabricate free-standing CNT membrane electrode with a more straightforward method and apply it in the electrochemical detection of *E. coli*. Preferably, conductive free-standing membrane can make the bacterium of interest directly contact the electrode and keep them within a tiny space, therefore, greatly increase the efficiency of electron transfer and dramatically concentrate the bacteria without actually increase the number, respectively. At the end of this report, we also

demonstrate the electrodeposition of platinum (Pt) nanoparticles on the CNT membrane electrode, which makes it possible to further increase the sensitivity of this method due to the catalytic potential of Pt on the oxidation of the electroactive compounds applied in this work.

2. Experimental section

2.1 The fabrication of CNT membrane

0.4 g/L Single-walled carbon nanotubes (Unidym) and 10 g/L sodium dodecyl sulfate (SDS) were sonicated for 30 min by ultrasonic processor (Cole Parmer) in deionized (DI) water, and then diluted to different concentrations (5 mg/L or 10 mg/L) for further treatment. 5 mL CNT solution was filtrated through a Nuclepore polycarbonate (PC) membrane (Whatman, 47 mm diameter) with diverse pore sizes (0.4 μm or 0.05 μm). The CNT-PC was then thoroughly washed with DI water and dried at room temperature.

2.2 Bacteria cultivation and immobilization

Wild-type *E. coli* belong to the microorganism biosafety level Two (BSL-2) group, and all safety considerations concerning this group were accomplished through out the experiments.

Wild-type *E. coli* stock cultures, collected from Mirror Lake, Storrs, CT, were recovered overnight (10 hrs) in LB medium at 37 ˚C with shaking. 10 μL overnight culture solution was added in 10 mL fresh LB medium supplemented with 1 mM isopropyl β-D-galactropyranoside (IPTG) or methyl β-D-glucuronide sodium salt (MetGlu) for certain time (3 hrs or 5 hrs). Different numbers of *E. coli* were immobilized on pristine PC membrane (0.4 μm) and covered on the free-standing CNT-PC electrode to carry out the amperometric detection. In this way, the bacteria were confined between the two membranes. The immobilized *E. coli* was re-suspended in phosphate buffer solution (0.1 M, pH 7.4) for 10 min. 4-aminophenyl-β-D-galactopyranoside (4APGal, Sigma-Aldrich) or 8-hyroxyquinoline glucuronide (8-HQG, Sigma-Aldrich) was added in the system according to the purpose and the current response was recorded.

2.3 Electrodeposition of Pt

2 mL sulfuric acid (H_2SO_4, 0.1 M) with 1 mM hydrogen hexachloroplatinate (H_2PtCl_6) served as the electroplating solution and cyclic voltammetry (CV) was performed in the range of 0 to -1 V with a scan rate of 0.01 V/s for 10 cycles.

2.4 Equipments

The morphology of the pristine PC membrane, CNT-PC membrane, and Pt deposited CNT-PC membrane was examined by field emission scanning electron microscope (FESME, JEOL 6335F). All the electrochemical experiments were carried out using a home-made plate material evaluating cell (Figure 1) on CHI 601C electrochemical workstation (CH Instruments) at room temperature. A platinum electrode and a Ag/AgCl (0.21 V vs. SHE) electrode were used as the counter and reference electrode, respectively.

3. Results and discussions

3.1 Characterization of CNT-PC working electrode

Fig. 2 shows the SEM images of the as-prepared CNT-PC membrane with different CNT loadings (Fig. 2A and B) as well as the pristine 0.4 μm PC membrane (Fig. 2C) serving as the template. One can see that CNT networks were formed on the PC membrane with relative uniform distribution. The pores of PC membrane were totally covered by CNT. It is obvious that the higher CNT concentration gives more concentrated CNT networks, which is consistent with the fact that higher CNT concentration possesses a higher electrical conductivity. In addition, the as-prepared CNT-PC membrane also inherits the excellent mechanical strength of PC membrane, making it a promising free-standing electrode material in the sensor applications.

3.2 Electrochemical detection of 4-aminophenol and 8-hydroxyquinoline

4-aminophenol (4AP) and 8-hydroxyquinoline (8HQ) are the two electroactive compounds

which are the products of the enzymatic reaction in *E. coli*, and can be amperometrically determined by appropriate working electrode. The hydrodynamic voltammograms in Fig 3 shows that the optimal oxidation potential for 4AP and 8HQ are 0.3 V and 0.7 V, respectively. It is necessary to point out that the response with the CNT-PC membrane is also better than that with bare glassy carbon electrode (GCE) or modified GCE. Taking into account the free-standing capacity as well as the convenient operation, CNT-PC electrode is much superior to GCE.

Fig. 4 shows the effect of CNT concentration on the anodic current of 4AP and 8HQ. One can see that the CNT concentration does not affect too much on the amperometric response. In addition, both 4AP and 8HQ demonstrated good linear relationship with increasing the concentration of the analytes. Furthermore, the free-standing CNT-PC electrode is able to detect the analytes with the concentration as low as 500 nM or even lower. These traits ensure the good repeatability and high sensitivity of the constructed sensor in the application of *E. coli* detection.

3.3 Electrochemical detection of *E. coli*

β-D-galactosidase and β-D-glucuronidase can hydrolyze their corresponding substrates, 4APGal and 8-HQG, respectively, to produce the electroactive compounds (4AP or 8HQ), which can be oxidized on the working electrode and give current response.

Fig. 5 verifies that both β-D-galactosidase and β-D-glucuronidase activities in wild-type *E. coli* can be specifically induced by IPTG and MetGlu, respectively. The induced strain exhibited at least 100 times increase of the current response compared to the uninduced strain. This result is in accordance with the one in literature.[2] One can also see that IPTG can only induce the activity of β-D-galactosidase and vice versa for MetGlu. This provides more options with regard to the enzyme for *E. coli* detection. The decrease of each injection is consistent with Michaelis-Menten equation. However, due to the saturation of the active sites on the enzyme, the response from the first injection is more accurate to reflect to amount of *E. coli* than that from the later injections. Since the prices of the substrates applied in this system are normally high (0.1 g 4APGal for $ 52.4 and 0.05 g 8HQG for $ 52.8), low concentrations of the substrates are

desired in order to decrease the operational cost at utmost. Therefore, it is necessary to investigate the loading of the substrates. Fig. 6 examines the effect of concentration of 4APGal on the current response. In the investigated range, 10 μM 4APGal gives the best result. Even though further increasing the amount of substrate would produce bigger response, taking into account that the detection of low concentration of *E. coli* are always required, the concentration of 10 μM is high enough to reflect the total enzyme amount, which is equivalent to the total number of *E. coli* in the detection system.

Fig. 7 demonstrates the limit of detection of wild-type *E. coli* with the constructed sensor. The bacteria were induced by IPTG for 3 hrs and diluted to 5×10^6 cfu/mL with LB medium. 1 mL culture solution was filtrated and 5×10^6 cfu of *E. coli* were immobilized on the pristine PC membrane. This result is comparable to the most impressive result applying electrochemical method published so far.[8] The total number of *E. coli* in that paper was also 5×10^6 cfu. As shown in Fig. 7, the current response of this number of *E. coli* is about 150 nA/cm^2. Theoretically, 30 nA/cm^2 is also distinguishable in the figure. Therefore, further decrease of the detection limit is still possible. Moreover, there is no permeabilization step and enzymatic reaction step in this method, which can save at least 1 hour.

3.4 Electrodepostion of Pt nanoparticles on CNT-PC membrane

CNT-PC electrodes with different pore sizes of PC membrane (0.4 μm or 0.05 μm) served as the substrate of Pt electrodepostion. Fig. 8 shows the SEM images of the as-prepared Pt-CNT-PC membranes. PC membrane with the smaller pore size (Fig. 8A) can generate the Pt nanoparticles with higher density of more uniform distribution. The average diameter of the particles is around 150 nm. The formation procedure and the effect of different electrodepostion substrates are still under investigation. However, we believe that the sensitivity of our method for the determination of *E. coli* can further be improved with the Pt-CNT-PC free-standing electrode due to the outstanding catalytic capacity of platinum along with the huge specific area of the nanoparticles.

4. Conclusions and future work

In this report, we successfully fabricated carbon nanotube networks on polycarbonate membrane. This CNT-PC membrane demonstrated good electrical conductivity and excellent mechanical flexibility. The as-prepared CNT-PC membrane was applied as free-standing working electrode in the oxidation of 4-aminophenol and 8-hydroxyquinoline, and further in the electrochemical detection of wild-type *E. coli*. 5×10^6 cfu of *E. coli* was determined without any pre-concentration and pre-culture. No permeabilization and enzymatic step were included in the operation. We further modify the CNT-PC with electrodepostion of Pt on it. The resulting Pt-CNT-PC membrane is expected to be more sensitive in the application of *E. coli* detection in our detection system.

In the future, the electrochemical property of the Pt-CNT-PC membrane will be investigated and its applicability in *E. coli* detection will be verified. We will also examine the optimal pH value of the working solution in the system. With the Pt-CNT-PC free-standing electrode in the optimized system, we will plot the curve of current vs. culturing time and demonstrate the detection of extremely low initial concentration of *E. coli* in water.

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Figure Captions

Figure 1. The plate evaluating cell applied through out the experiments.

Figure 2. The SEM images of the as-prepared CNT-PC membrane (400 nm pore size) with 2 mg/L CNT loading (A), 5 mg/L CNT loading (B), and without CNT loading (C).

Figure 3. Hydrodynamic voltammograms of 8HQ (A) and 4AP (B) at the concentration of 5 μM with the free-standing CNT (10 mg/L)-PC (400 nm pore size) electrode.

Figure 4. Current vs. time curve of 500 nM, 1 μM, and 5 μM of 4AP at 0.3 V and of 8HQ at 0.7 V with the free-standing CNT-PC (400 nm pore size) membrane. The red and blue line correspond to 5 mg/L and 2 mg/L CNT loading, respectively.

Figure 5. The effects of inducing agents on the current response of *E. coli* detection. 5×10^7 cfu of *E. coli* were immobilized and 25 μM of 4APGal or 8HQG was injected with the free-standing CNT (5 mg/L)-PC (400 nm pore size) electrode.

Figure 6. The effects of concentration of the substrate on the current response of *E. coli* detection. 1×10^7 cfu of *E. coli* were immobilized and different concentrations of 4APGal (10, 5, and 2 μM) was injected with the free-standing CNT (5 mg/L)-PC (400 nm pore size) electrode.

Figure 7. The current response of four successive injection of 4APGal (10 μM) for 5×10^7 cfu of *E. coli* with the free-standing CNT (5 mg/L)-PC (400 nm pore size) electrode. The bacteria were

induced by 1 mM IPTG for 3 hrs.

Figure 8. The SEM images of electrodeposition of Pt on CNT-PC membrane. The pore size of PC membrane was 50 nm (A) and 400 nm (B) with 5 mg/L of CNT loading.

Fig. 1

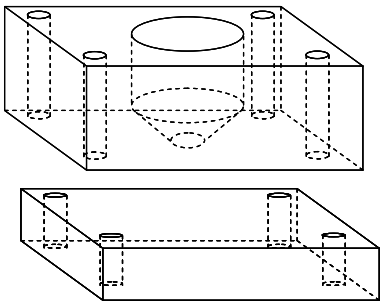
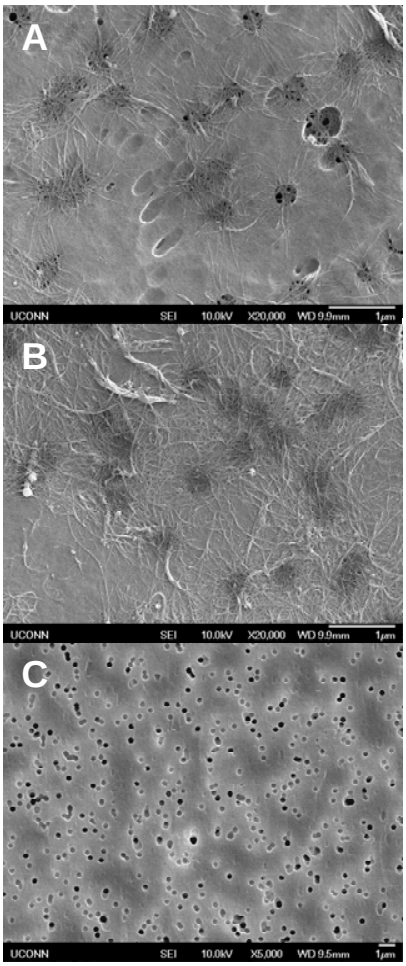


Fig. 2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C
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Fig. 3

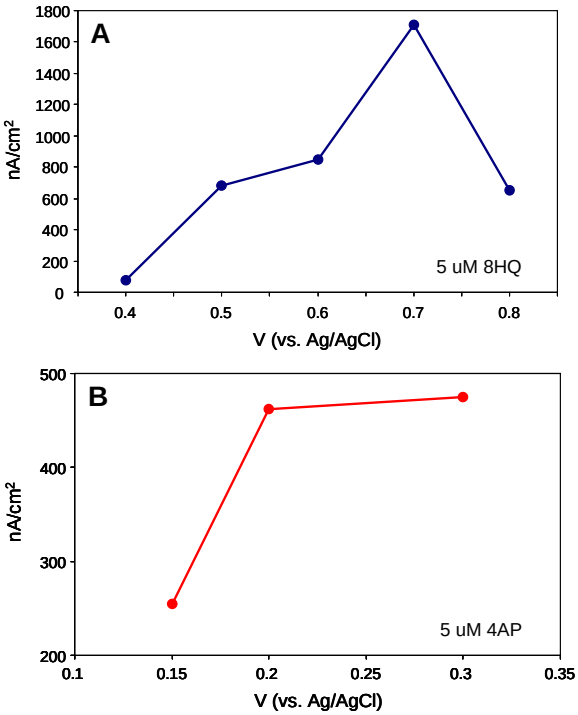
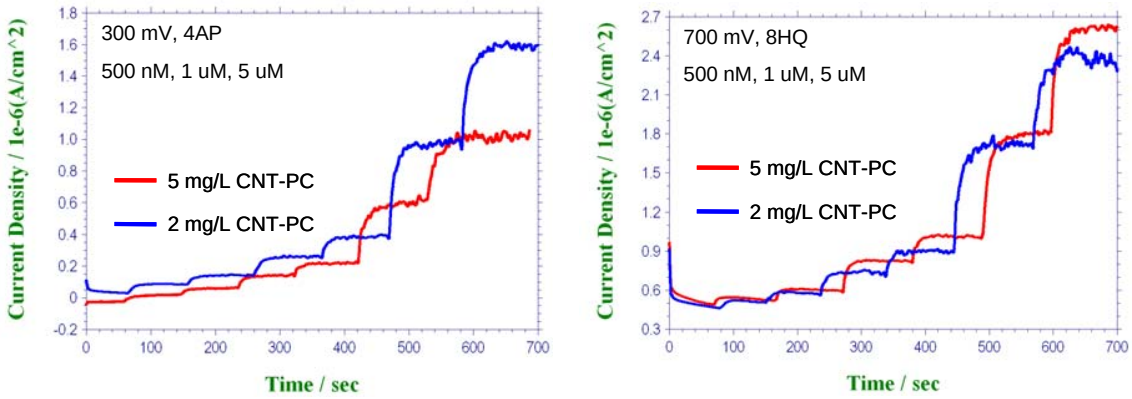

A
1800
1600
1400
1200
1000
800
600
400
200
0
nA/cm²
5 uM 8HQ
0.4
0.5
0.6
0.7
0.8
V (vs. Ag/AgCl)
B
500
400
300
200
nA/cm²
5 uM 4AP
0.1
0.15
0.2
0.25
0.3
0.35
V (vs. Ag/AgCl)

Fig. 4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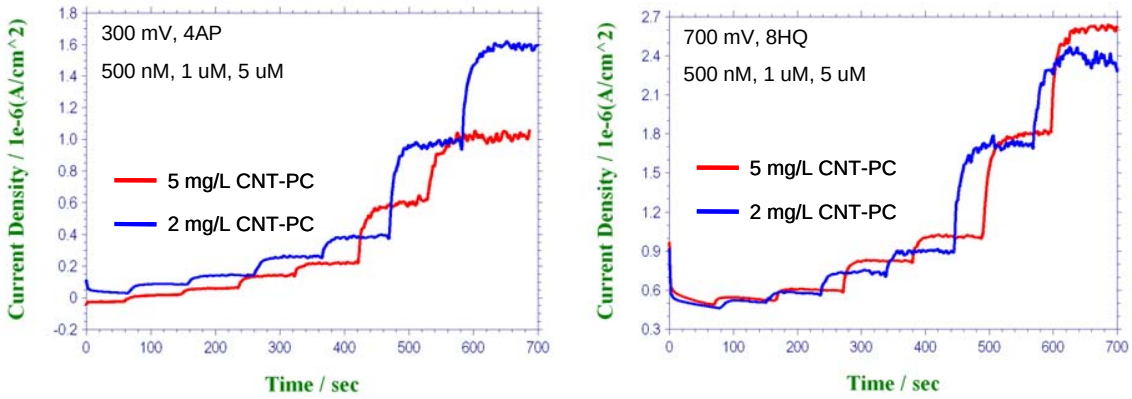
5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec
700 mV, 8HQ
500 nM, 1 uM, 5 uM
5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec

Fig. 5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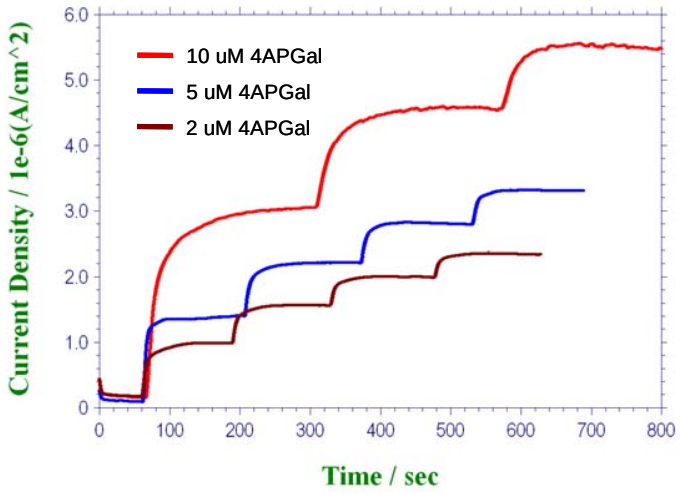
500 nM, 1 uM, 5 uM
5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec
700 mV, 8HQ
500 nM, 1 uM, 5 uM
5 mg/L CNT-PC
2 mg/L CNT-PC
Current Density / 1e-6(A/cm^2)
Time / sec

Fig. 6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5 uM 4APGal
2 uM 4APGal
Current Density / 1e-6(A/cm^2)
Time / sec
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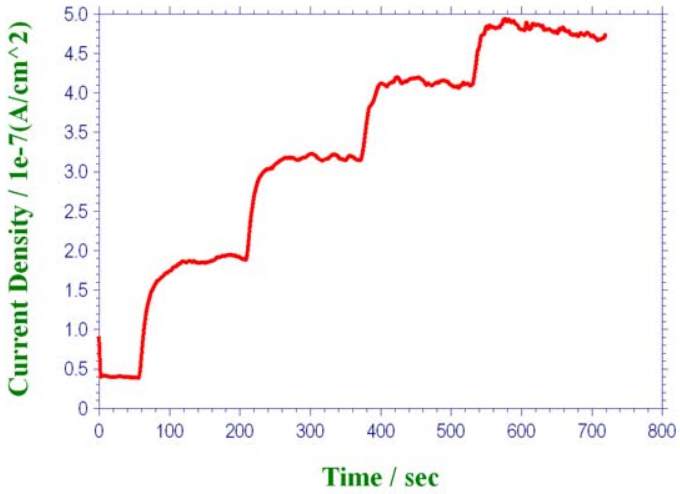
4.0
5.0
6.0
0
100
200
300
400
500
600
700
800

Fig. 7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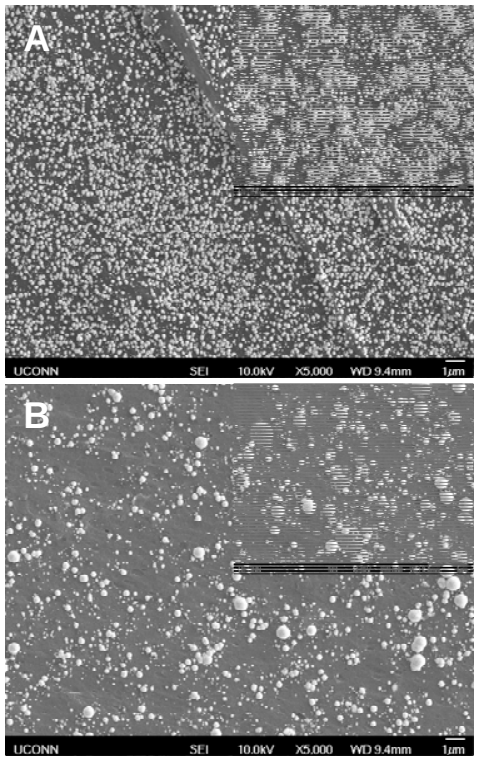
0
0
100
200
300
400
500
600
700
800
Time / sec

Fig. 8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B
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