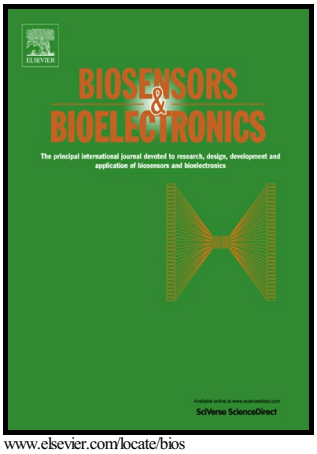
Rapid detection of *Escherichia coli* based on 16S rDNA nanogap network electrochemical biosensor

Jialin Zhang, Jiajia Wang, Xiaoqing Zhang, Fengjiao He



www.elsevier.com/locate/bios

PII: S0956-5663(18)30541-4
DOI: https://doi.org/10.1016/j.bios.2018.07.041
Reference: BIOS10630

To appear in: *Biosensors and Bioelectronic*

Received date: 5 May 2018
Revised date: 15 July 2018
Accepted date: 17 July 2018

Cite this article as: Jialin Zhang, Jiajia Wang, Xiaoqing Zhang and Fengjiao He, Rapid detection of *Escherichia coli* based on 16S rDNA nanogap network electrochemical biosensor, *Biosensors and Bioelectronic,* https://doi.org/10.1016/j.bios.2018.07.041

Rapid detection of *Escherichia coli* based on 16S rDNA nanogap network electrochemical biosensor

Jialin Zhang[a], Jiajia Wang[a], Xiaoqing Zhang[a,b], Fengjiao He[a*]

[a] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082,P.R. China*

[b] *School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, P.R. China*

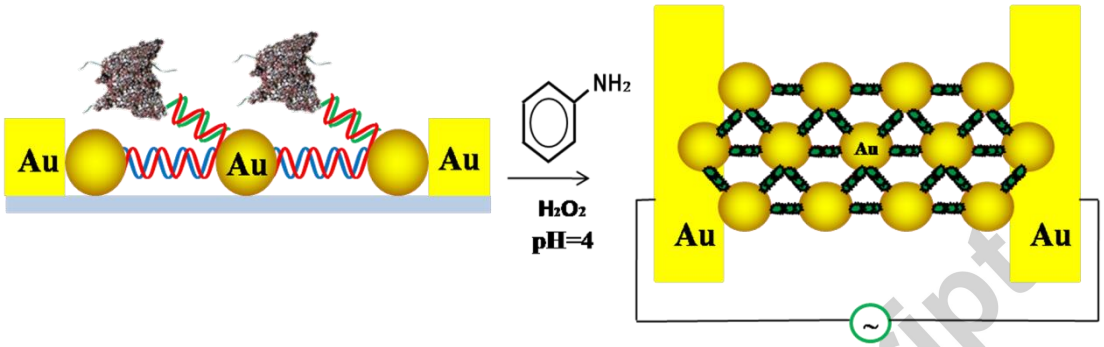
[*]Corresponding author. E-mail address: fengjiao87799232@hotmail.com (F. He)

Abstract

Simple, fast and effective detection methods of *Escherichia coli* (*E. coli*) are essential for human life and health. This article proposes a sensitive and rapid detection method of *E. coli* based on electrochemical biosensor using 16S rDNA as target biomarker, and using oligonucleotide probes and nanogap network electrodes as transducer elements. The nanogap network electrodes are prepared by interconnecting of gold nanoparticles through thiolated peptide nucleic acid (PNA) probes on the substrate of Au interdigital electrode. In the presence of specific 16S rDNA fragments, PNA capture probe hybridized to 5′ terminal of the fragments, and detection probe modified with horseradish peroxidase (HRP) hybridized to its 3′ terminal. HRP attached to the hybridized detection probe catalyzed the polymerization of aniline along target chain. Conductive connection was caused by polyaniline deposition between the interrupted gold nanoparticles and offered a conductimetric response of constructed sensor. Thus,

E. coli could be successfully detected with the detection limit of 100 CFU/ml. The detection time was less than 3 h. It would be widely used for rapid detection of *E. coli*.

Graphical Abstracts



The designed strategy for rapid detection of *Escherichia coli*

Keywords:

Electrochemical biosensor; Nanogap network electrodes; Escherichia coli; Polyaniline deposition.

1. Introduction

Food-borne illnesses caused by the bacterial pathogens have become a critical health issue worldwide (Scharff, 2012). Every year, almost 1.5 million people die of the disease caused by pathogens in drinking water and food sources (World Health Organization, 2008). As one of the most harmful bacterium related to food-borne diseases, *E. coli* live within digestive tracts of human and poultry, and cause the injury and pathological change in the gastrointestinal tract and urinary systems (Kaper et al.,

2004; Zowawi et al., 2015). For example, urinary tract infections are the most prevailing and difficult-to-treat infectious diseases all over the world, while the main cause of these diseases is infected by uropathogenic *E. coli* (UPEC) (Olanrewaju et al., 2017; Foxman, 2010; Nair et al., 2018; Moura et al., 2009). Therefore, clinical diagnosis as well as the water and food industry continued to demand rapid and sensitive detection of *E. coli*.

The most common methods to detect the presence of *E. coli* are based on bacterial culture. Although the method is accurate and reliable, it is time-consuming and takes up 48 h or even more to yield the result due to the long incubation periods (Gao et al., 2017; Hyun et al., 2013; Kaittanis et al., 2007). Automatic incubation detection systems like the VITEK-2 (bioMerieux SA) or BD Phoenix (Becton, Dickinson and Company) have been developed for detection of *E. coli* and can shorten detection time to 6-13 h, but these systems are not available for each laboratory and still face critical limitations include lengthy detection time and high cost (Leonard et al., 2017). In recent years, several new Culture-independent methods have been developed for detection of *E. coli*. Detection time, detection limits (LOD) and linear ranges of these techniques are summarized in Table 1. Though rapid and sensitive, methods such as surface plasmon resonance imaging (SPRi), fluorescence and surface enhanced Raman spectroscopy (SERS) are labor intensive and require expensive instrumentations, which limited their applications in resource-limited environments (Tlili et al., 2013). Chemiluminescence (CL) and colorimetry have poor selectivity for detection in complex samples (Zhang et al., 2015; Orenga et al., 2009). Electrochemical method can offer simple, sensitive and selective analysis using portable, miniaturized and low-cost devices, which has become an effective technique for rapid medical and environmental detection of *E. coli*. More

recently, electrochemical methods using ferrocene-peptide conjugate (Li et al.,2014), double functionalized Au@Pt/SiO_2 nanocomposites (Ye, et al.,2018), or gold nanoparticles modified graphene paper (Wang, et al.,2013), the LOD of *E. coli* detection reached 10^3 CFU/ml, 1.83 × 10^2 CFU/ml and 1.5×10^2 CFU/ml, respectively. However, there is a need to develop new electrochemical strategies to further enhance the sensitivity of *E. coli* detection.

Table 1. LODs, linear ranges, and assay time for detection of *E. coli* under different techniques

Technique	Time, h	linear range, CFU/ml	LOD,CFU/ml	Ref
SPRi	< 10	/	100	Bulard et al.,2015
Fluorescence	3	42-42×10^6	10	Pan et al.,2015
Colorimetry	<4	<10^3	50	Derd et al., 2013
CL	2	4.3×10^3-4.3×10^5	1.2×10^3	Zhang et al., 2014
SERS	/	<10^9	10	Zhu et al., 2018
Electrochemistry	/	10^3-10^7	10^3	Li et al.,2014
Electrochemistry	/	3.5×10^2-3.5×10^8	1.83 × 10^2	Ye, et al.,2018
Electrochemistry	/	1.5×10^2-1.5×10^7	1.5×10^2	Wang, et al.,2013

The 16S rDNA sequence contains high conservative regions across species (available for general bacterial detection) as well as variable regions (available for species typing) (Woo et al., 2008). It has advantages especially in the systematic classification of fastidious bacteria and slow growing strains (Schmid et al., 2001). Recently several studies based on detection of 16S rDNA using quantitative real-time PCR (qPCR) (Hünniger et al., 2015; Jiang et al., 2014; Jacob et al., 2013), sequencing (Wang et al., 2014), and surface plasmon resonance (SPR) (Shi et al., 2014) have been reported as effective tools for identification and detection of bacteria. However, qPCR-based

technique and sequencing are high-cost (Niemz et al., 2011; Thomas et al., 2014). SPR technology requires expensive equipment and is currently limited especially in direct detection of small molecules at ultralow concentrations (Yang et al., 2018; Chang et al., 2018; Su et al., 2012).

Electrical biosensor is particularly attractive for its low cost and easy popularization. The biosensor system enables to detect nucleic acids by converting hybridization events of nucleic acids between electrode pairs into measurable electrical signals (Chen et al., 2010). Combined with the compatibility with advanced semiconductor technology, integration, massive reproducible manufacturing as well as miniaturization, it is promising for the development of high-performance nucleic acids detection methods (Zhang et al., 2014). It has been reported that electrode pairs with a nanoscale gap remarkably increased detection sensitivity of nucleic acids (Porath et al., 2000), but the preparation of such a narrow gap electrode pair will significantly increase the cost because of the complexity of manufacturing process. In addition, enzyme labeling method can also improve the sensitivity of nucleic acid detection (Gerion et al., 2003).

Herein, a new 16S rDNA electrochemical biosensor was constructed for sensitive and rapid detection of *E. coli*. Nanogap network electrodes and enzyme labeling method were employed to increase the sensitivity of detection. The preparation method of nanogap network electrodes was simple and low-cost. Conductive connection was caused by deposition of polyaniline between the interrupted gold nanoparticles using enzymatic target-guided methods and offered a conductimetric response of constructed sensor. Proposed method showed high specificity and sensitivity for detection of *E. coli*. The detection limit of the constructed sensor was 100 CFU/mL and the detection time was less than 3 h. In addition, the performance of the constructed sensor in the bacteria

identification was evaluated by detecting *E. coli* in 45 simulated samples, the results showed no significantly difference to those detected by the bacteria culture method.

2. Material and methods

2.1. Reagents and Materials

2.1.1 Bacteria

Staphylococcus aureus (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*), *Enterococcus faecium* (*E. faecium*) and *Salmonella enteritidis* (*S. enteritidis*) were bought from the National Institute for the Control of Pharmaceutical and Biological Products. These bacterial strains were grown in a sterilized conical flask containing NB nutrient medium (meat extract 3 g, Bacto Peptone 10 g, NaCl 5 g, distilled water 1000 ml) for 18 h at 37 °C under shaking cultivation. The concentrations of the bacterial strains were determined by McFarland's turbidity method.

2.1.2 Reagents

Sodium Citrate, Chloroauric Acid ($HAuCl_4 \cdot 3H_2O$), Zonyl FSN-100 (FSN), Tris(2-carboxyethyl)phosphine (TCEP), 3-Aminopropyl triethoxysilane (APTES, 99%) and HRP Type II (200 units/mg) were bought from Sigma-Aldrich. Aniline (99.5%) and Hydrogen peroxide (H_2O_2) were obtained from Sinopharm Chemical Reagent Co, Ltd (SCRC). *N*-[Maleimidobutyryloxy]sulfosucci-nimide ester (Sulfo-GMBS) was obtained from Pierce Biotechnology, Inc. Alu I restriction endonuclease and Taqα I restriction endonuclease enzyme were obtained from New England Biolabs (NEB). The interdigital electrode was bought from Dalian Institute of Chemical Physics.

Hybridization and washing buffered solution (pH 8.5, 10 mM Tris-HCl, 1.0 mM EDTA and 0.10 M NaCl solution). Storage buffered solution (pH 7.4, PBS, 1% BSA and 1% Triton X-100).

2.1.3 Sequence

Thiol-terminated PNA was purchased from Panagene (Daejeon, Korea). All synthetic oligonucleotides were acquired from Sangon Biotech (Shanghai). The detailed sequences of detection probe, 16S rDNA fragment, 16S rDNA fragments with single-base, three-base, five-base mismatch and non-match were shown in the Table 2.

Table 2 The detailed sequences of oligonucleotide probes

Oligonucleotide Type	Sequences
PNA (capture probe)	5'–S-AGGAAGAAG-S–3'
DNA(capture probe)	5'–S-AGGAAGAAG-S–3'
16S rDNA fragment	5'–CTTCTTCCTGTTACCGTT–3'
Single-base mismatch	5'–CCTCTTCCTGTTACCGTT–3'
Three-base mismatch	5' –CATCATCATGTTACCGTT–3'
Five-base mismatch	5' –AAGCTCCGTGTTACCGTT–3'
Non-match	5' –AGATAAAGCAGGCGGAAC–3'
Detection probe	5' –AACGGTAAC–3'

2.2. Preparation of detection probe modified with HRP

Before modification, all nucleotide sequences were treated with pH 7.5 20 mM TCEP for 1 h to remove the disulfide bonds. 2 nmol of HRP was reacted with 10 nmol of Sulfo-GMBS and 20 nmol thiolated detection DNA in a mole proportion of 1:5:10, at 38 °C for 3 h. The unbound Sulfo-GMBS and DNA were removed using a Microcon centrifugal filter device unit (cut-off MW 30,000). Detection probes with a final

concentration of 2.5 μM were obtained by dilution with storage buffer and stored at -30 ^{o}C.

2.3. Preparation of AuNPs-PNA nanogap network electrode

AuNPs with average diameters 10 nm were prepared by the reduction of $HAuCl_4$ with Sodium citrate (Zu and Gao, 2009). 0.05 wt % FSN was added into the colloid solutions for the formation of FSN blocking layers. The purified PNA probes were treated with pH 7.5 20 mM TCEP for 10 min to reduce disulfide bond between the thiolated PNA chains. Subsequently 2 μM thiol-modified PNA capture probes were added to a 0.14 nM solution of the FSN capped gold NPs at a mole proportion of 200:1 and mixed for 1 hour at room temperature. 10 mM phosphate buffer (pH 7.5) and 1.0 M NaCl were added and incubated for another hour. The FSN blocking layers keep gold NPs steady efficiently in the high salt media and hedge the unspecific adsorption of the PNA on the surface of gold nanoparticles (Zu and Gao, 2009). The mixed solution was centrifuged at 13.2 K rpm for 15 min and the supernatant was removed, the conjugates were washed with 10 mM phosphate buffer (pH 7.5) solution for five times until PNA in washing solution could not be detected by UV-Vis spectrophotometer. Finally, AuNPs-PNA conjugates suspension was ready by adding 0.1 M PBS in tube (Fig. 1A). The surface of electrode substrate was modified with APTES (Ma et al., 2004). Then AuNPs-PNA conjugates suspension was dropped onto the top of modified interdigital electrode at 38 °C for 60 min. Subsequently the electrode was cleaned with pure water and dried under nitrogen flow. Thus the AuNPs-PNA network electrode with the PNA linked nanogap was formed.

2.4. Detection of E. coli

To obtain the *E. coli* 16S rDNA, 1 mL of bacteria solution (10^8 CFU/ml) in centrifuge tube was centrifuged at 8 K rpm for 5 min. After the supernatant was abandoned, 20 μL of 5 % Chelex-100 solution (5 g Chelex-100 in 100 ml 0.5 % SDS, 1 % Tween 20 and 1 % NP40 buffer) was added. The mixed solution was placed in 100 ° C water bath for 10 min and then in ice bath for 1 min. Subsequently the solution was centrifuged at 12 K rpm for 10 min and the supernatant solution was added in Cutsmart Buffer. 1 μL of Alu I restriction enzyme and 1 μL of Taqα I restriction enzyme was added and incubated at 37 ° C for 10 min and then at 65 ° C for 10 min. After denatured at 95 °C for 15 min, the fragments solution was transferred into the detection cell which containing AuNPs-PNA network electrode and detection probes which modified with HRP enzyme. Hybridization was carried out for about 1 h at 38 °C. Subsequently the electrode was thoroughly cleaned with pH 9.5 washing buffer to remove unbound probes. A mixture of aniline /H_2O_2 (30 mM /2.0 mM) in the pH 4.0 0.1 M HAc-NaAc buffer was then added to detection cell and stayed for 40 min. The electrode was cleaned with washing buffer solution and ultra pure water thoroughly to remove unreacted monomers and enzymes, followed by drying under nitrogen flow. HCl gas was doped with polyaniline for about 15 s. The conductance was measured thereafter under ambient conditions with a TH2816 LCR meter. The datas were obtained from average value of five times measurements.

2.5. Detection in the simulated aqueous samples

A total of 45 simulated aqueous samples were performed to evaluate the constructed sensor. 1 mL of *E. coli* (10^3 CFU/mL) was added to 2 mL of drinking water as the

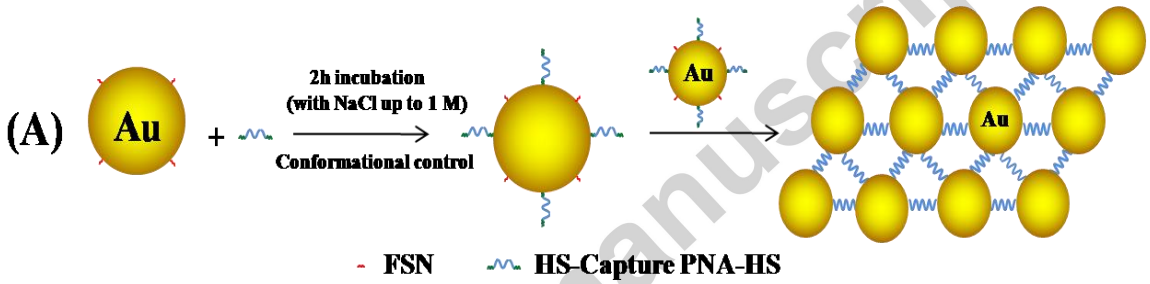
positive control, and 3 mL of sample without *E. coli* was regarded as the negative control. Each sample was divided into two equal segments. The two parts were identified by traditional bacteria culture method and constructed sensor, respectively.

3. Results and Discussion

3.1. The constructed strategy of proposed biosensor

The designed strategy of biosensor is illustrated in Fig. 1B. The AuNPs-PNA nanogap network electrode was mounted in the detection cell. Here PNA was used as capture probe. PNA is unnatural nucleic acid analogue. Its neutral pseudopeptide backbone is consisted of *N*-(2-aminoethyl)glycine units that are ligated through a methylene carbonyl to the four nucleobases (Egholm et al., 1993; Ratilainen et al., 2000). In the presence of specific 16S rDNA fragments, the PNA capture probe hybridized to a nine-nucleotides long recognition sequence at the 5′ terminal of fragments, and the detection probe modified with HRP hybridized to a nine-nucleotides long recognition sequence at its 3′ terminal (Fig. 1C). Thus, HRP was immobilized on the surface of the electrode. As the pseudopeptide backbone of PNA probes is uncharged, when aniline/H_2O_2 buffer was added to the detection cell, the protonated aniline monomers were distributed along the surface of the target biomarker through electrostatic interactions. HRP catalyzed arrangement of aniline monomers in a head-to-tail orientation along the target DNA strand in the nanogap of the electrodes. After a doping treatment with HCl vapors, conductive connection was caused by polyaniline deposition between the interrupted gold nanoparticles and the presence of *E. coli* was detected by electrochemical biosensor.

The preparation of nanogap network electrode is simple and low-cost. PNA probe with thiol at both ends connected the adjacent gold NPs on the substrate of 20 μm interdigitated gap of Au interdigital electrode and functioned as both the bridge molecule (less than 4 nm) and probe molecules. In this case, the gold NPs were equivalent to the nano-electrodes, thereby forming the nanogap network electrode. The nanogap network electrodes possessed larger surface area and showed higher capture ability to target. It significantly lowered the minimum detection limit. Meanwhile, as its gap of electrodes was less than 4 nm, the constructed sensor showed a high sensitivity to conductimetric response (Porath et al., 2000).



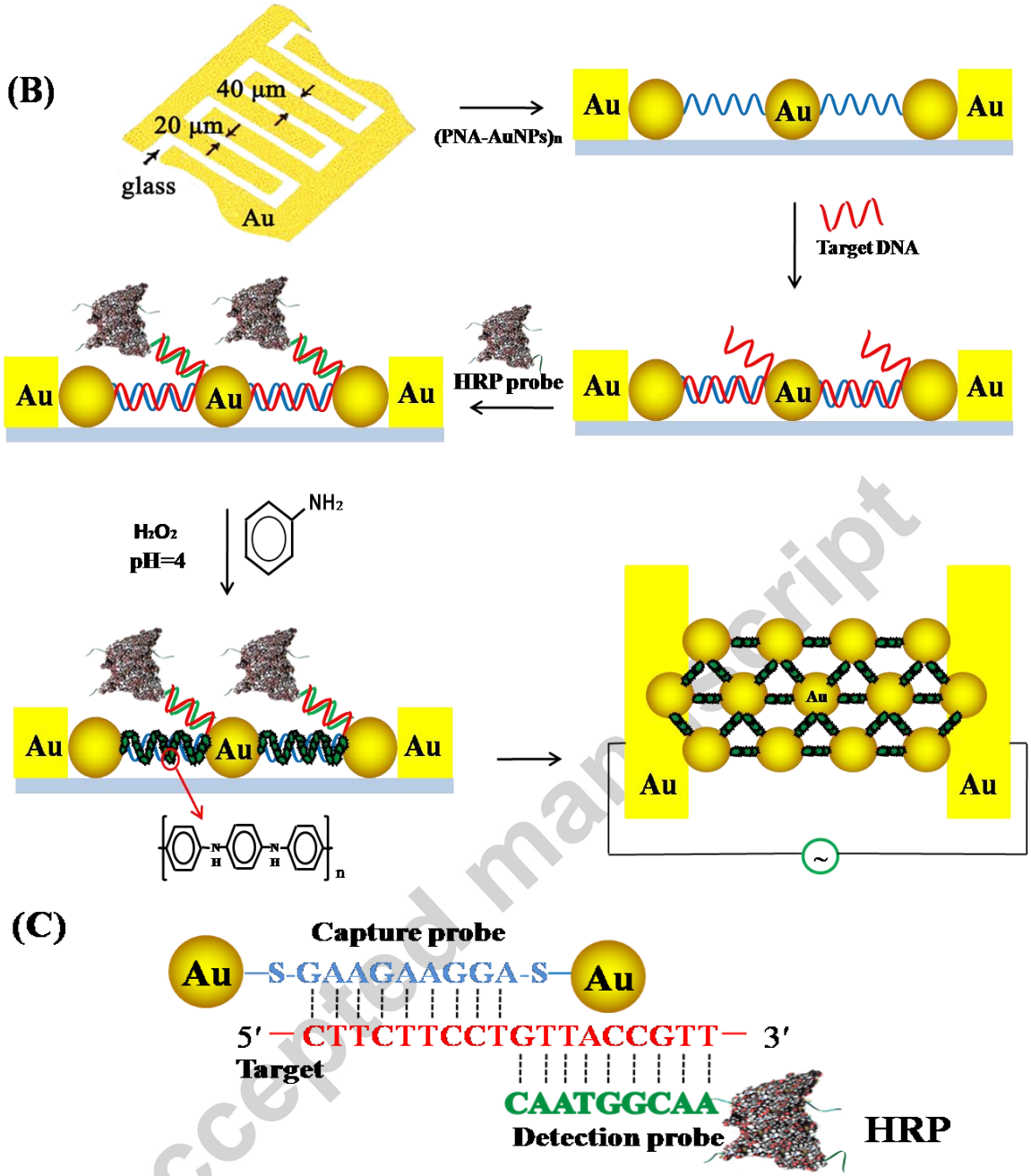


Fig. 1. The designed strategy of 16S rDNA electrochemical biosensor. (A) Preparation strategy of AuNPs-PNA conjugates. (B) Biosensor assembly procedure. (C) Visualization of the hybridization probes.

3.2. Selection of the biomarker

The conservative region and variable region of 16S rDNA of *E. coli* species were confirmed by alignments of the 16S region with Vector NTI 9.0 software package.

Array Designer 4.0 and Primer Premier 5.0 software were used to design the specific biomarker by considering the thermodynamic properties such as a melting temperature (Tm = 55 ± 0.3 °C) and steady secondary structures against the variable sequence of *E. coli*. The DNA fragment (5′-CTTCTTCCTGTTACCGTT-3′) was chosen as the biomarker. It showed the highest degree of similarity to 16S rDNA sequences of *E. coli* culture collection reference strains by using BLASTN analysis in NCBI database.

3.3. Characterization

Transmission electron microscopy (TEM) of naked AuNPs (inset) and AuNPs -PNA conjugates was shown in Fig. 2A, compared with TEM image of naked AuNPs (inset), due to the PNA connection, the distance between adjacent AuNPs was much smaller than that of naked AuNPs. The existing of AuNPs-PNA layers can be further confirmed by the UV−visible spectroscopy measurement and the results were shown in Fig. 2C. A 3 nm red shift of absorption peak from 521 nm to 524 nm indicated the binding of AuNPs with PNA. TEM image of polyaniline deposited AuNPs-PNA layer was shown in Fig. 2B. Compared with TEM image in Fig. 2A, it can be clearly seen that the surface of AuNPs was not smooth, and the connection was formed between adjacent AuNPs, which indicated that polyaniline was formed on the surface of AuNPs and in the nanogap between adjacent AuNPs and caused conductive connection between the interrupted gold nanoparticles. The existing of polyaniline deposition can be further confirmed by Raman spectrum shown in the Fig. 2D. The stretching vibration appeared at 1568 cm^{-1} was belong to C = C in the quinone ring; 1474 cm^{-1} was to C = C in the benzene ring; 1370 cm^{-1} was to Ar-N in the aromatic amine; The bending vibrations

appeared at 1161 cm^{-1} and 813 cm^{-1} were corresponding to C-H of benzene ring in and out of plane, indicating the deposition of polyaniline.

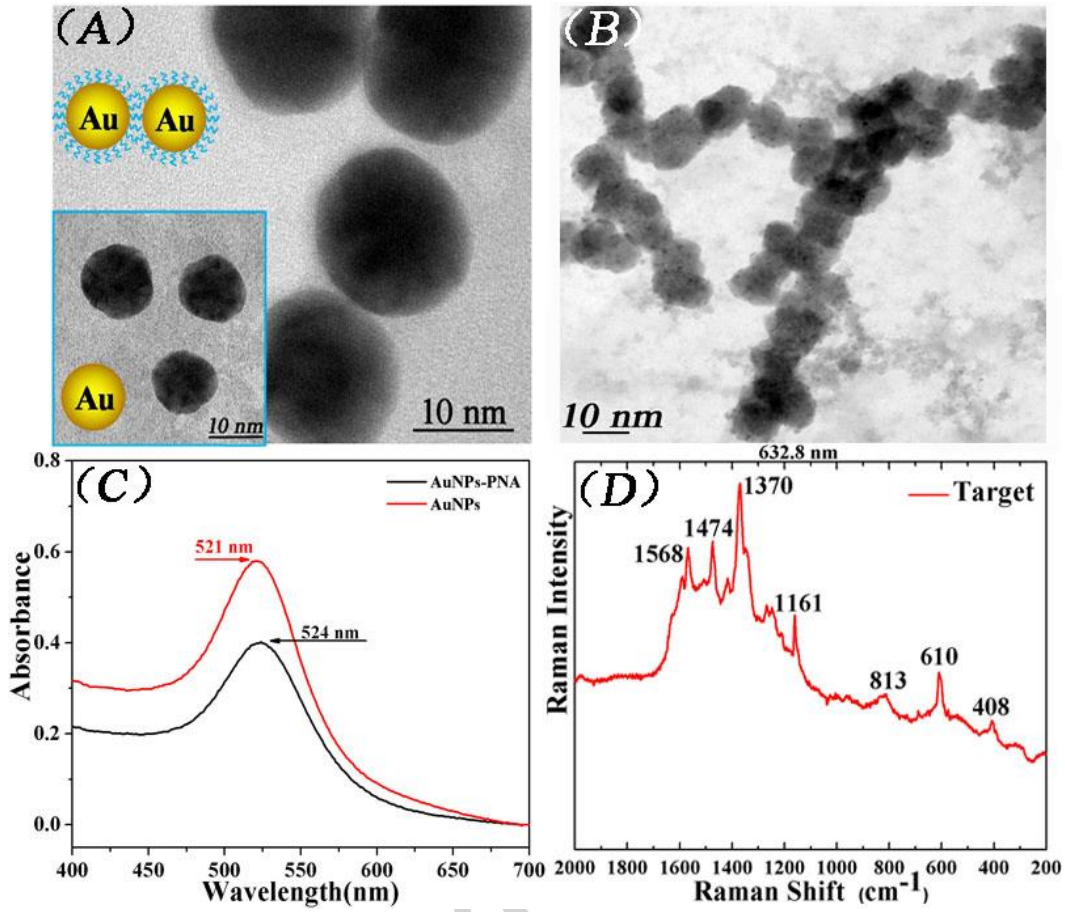


Fig. 2. Characterization. (A) TEM images of AuNPs-PNA conjugates and naked AuNPs (inset). (B) TEM image of AuNPs-PNA layer after target hybridization and polyaniline deposition. (C) The UV−visible spectra of naked AuNPs and AuNPs-PNA conjugates. (D) Confocal Raman Characterization of AuNPs-PNA layer after polyaniline deposition.

3.4. Response of constructed sensor to 16S rDNA fragments

16S rDNA fragments in range of 1 nM to 10 fM were used as analytes and the conductance was detected. In the absence of 16S rDNA fragments, there was no significant detectable electrical signal even after aniline deposition. The conductance increased with the increasing of concentration of 16S rDNA fragments. And a linear

relationship between the conductance and the concentration of 16S rDNA fragments in the range of 10 fM to 1 nM was obtained. $G = 2.8 + 1.18 \times 10^{-4}C_i$. Where C_i was the concentration of 16S rDNA fragments (fM). The regression coefficient R^2 reached 0.98. The detection limit was 5 fM. The value was estimated by using 3 × (SD / k), where SD is the standard deviation of the electrical signal for the blank sample without 16S rDNA fragments, and k is the slope of response curve in the linear region.The sensitivity of biosensor device was 2 orders of magnitude higher than silver enhancement approaches (Park et al., 2002).

3.5. Effect of polyaniline deposition time on detection

The polyaniline deposition time on detection was investigated and the results were shown in Fig. 3. It can be seen that conductance increased with the increasing in deposition time before it was less than 40 min, and conductance value reached a platue after 40 min deposition. Therefore, 40 min was chosen for detection.

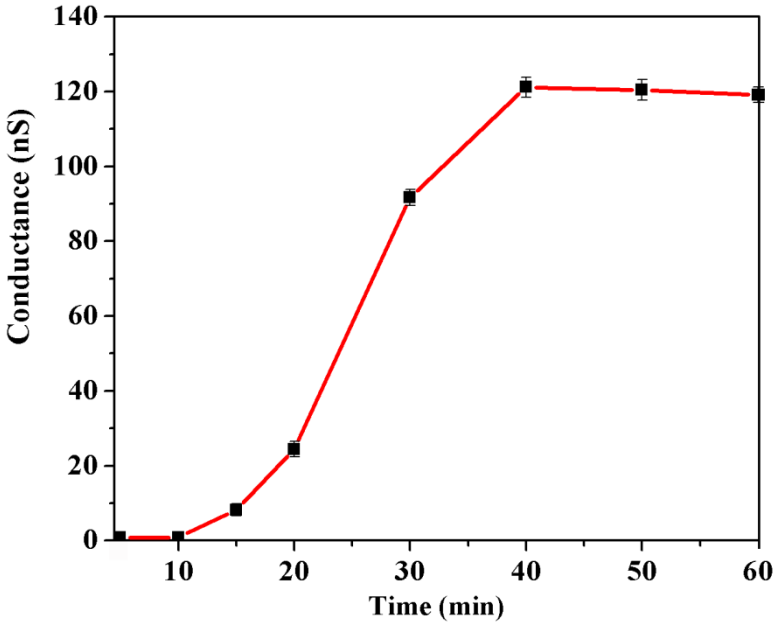


Fig. 3. Effect of polyaniline deposition time on conductance. Note: the concentrations of 16S rDNA fragments were at 1 nM. Error bars indicate standard deviation (n = 5)**.**

3.6. Specificity study

A specificity study was performed to detect 16S rDNA fragment, 16S rDNA fragment with single-base, three-base, five-base mismatch and non-match using PNA as capture probes. For comparison, the other experiments were performed using DNA as capture probes. The experiment results were shown in Fig. 4. For detection of 16S rDNA fragment, both PNA and DNA capture probe showed similar conductance response. But for single, three, five-base mismatch 16S rDNA fragment and non-match 16S rDNA fragment, the difference in the response using PNA as capture probe was more remarkable. These may caused by two reasons. One was because uncharged pseudopeptide backbone of PNA probes alleviated the electrostatic interaction between PNA and HRP, thus minimizing non-hybridization-related uptake of HRP and reducing the background response signal. The other one was the less stability of PNA-DNA mismatch hybridization complex than that of DNA-DNA mismatch hybridization complex, so higher mismatch sensitivity for PNA capture probe than DNA capture probe (Egholm et al., 1993; Sosniak et al., 2009).

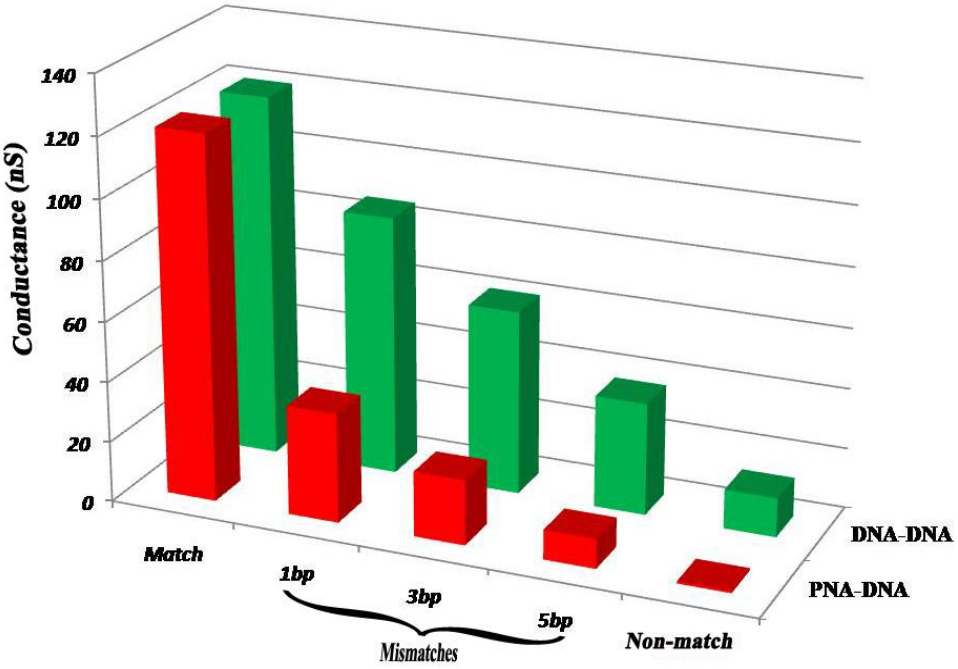


Fig. 4. Conductometric response for single-base, three-base, five-base mismatch and non-match oligonucleotide sequences compared to match sequence. The concentration of analytes was 1 nM.

The Gram-positive bacteria such as *S. aureus* and *E. faecium*, Gram-negative bacteria such as *P. aeruginosa*, *K. pneumonia* and *S. enteritidis* are common in the actual samples for detection of *E. coli* and may interfere with detection of *E. coli*. These bacteria at the concentration of 10^8 CFU/mL were detected by constructed sensor in order to evaluate the selectivity. In addition, blank experiments were performed using the sample without bacteria (blank sample). The detection results were shown in Fig. 5. There is no significant detectable signal changing toward the detection of *P. aeruginosa, S. aureus, K. Pneumonia, E. faecium, S. enteritidis* and the blank sample. However, the average value of five determinations of conductance for *E. coli* was significant. These results indicated that the constructed sensor showed high specificity and sensitivity for detection of *E. coli*. Many types of experimental bacteria [including *P. aeruginosa, S. aureus, K. Pneumonia, E. faecium* and *S. enteritidis*] did not interfere with the current *E. coli* sensor.

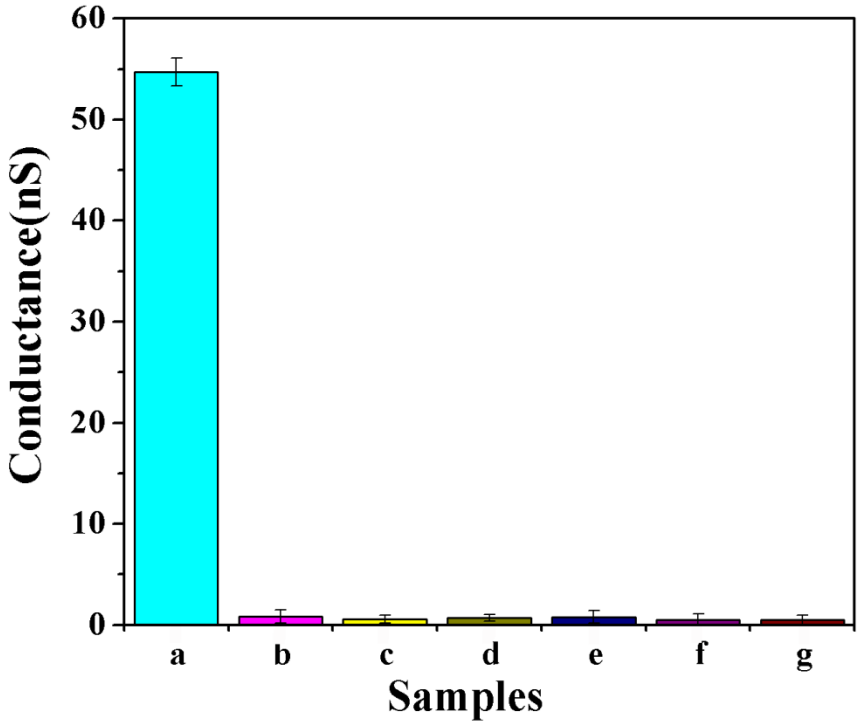


Fig. 5. The conductance values of constructed sensor to different strains. (a) *E. coli*, (b) *P. aeruginosa*, (c) *S. aureus*, (d) *K. Pneumonia*, (e) *E. faecium*, (f) *S. enteritidis*, (g) the blank sample. The concentrations of samples were at 10^8 CFU/ml. Error bars indicate standard deviation (n = 5)**.**

3.7. The response performance of different concentrations of E. coli

The conductometric responses of different concentrations of *E. coli* detected by constructed sensor were shown in Fig. 6. When the constructed sensor detected the sample without bacteria (blank sample), there was no significant detectable signal even after 40 min of aniline deposition. The detectable signal increased with the increasing of *E. coli* concentration. The conductance increased linearly with the log of *E. coli* concentrations in the range $1 \times 10^3 - 1 \times 10^8$ CFU/mL. The associated regression equation was $G = 6.60 + 6.09 \times \log C$, $R^2 = 0.985$. Different concentrations of *E. coli* were detected five times. The relative standard deviation (RSD) values were between 1.8% and 3.5%. The limit of detection was 100 cfu/mL. The value was estimated by

using 3 × (SD / k), where SD is the standard deviation of the electrical signal for the blank sample, and k is the slope of response curve in the linear region. The detection time was less than 3 h.

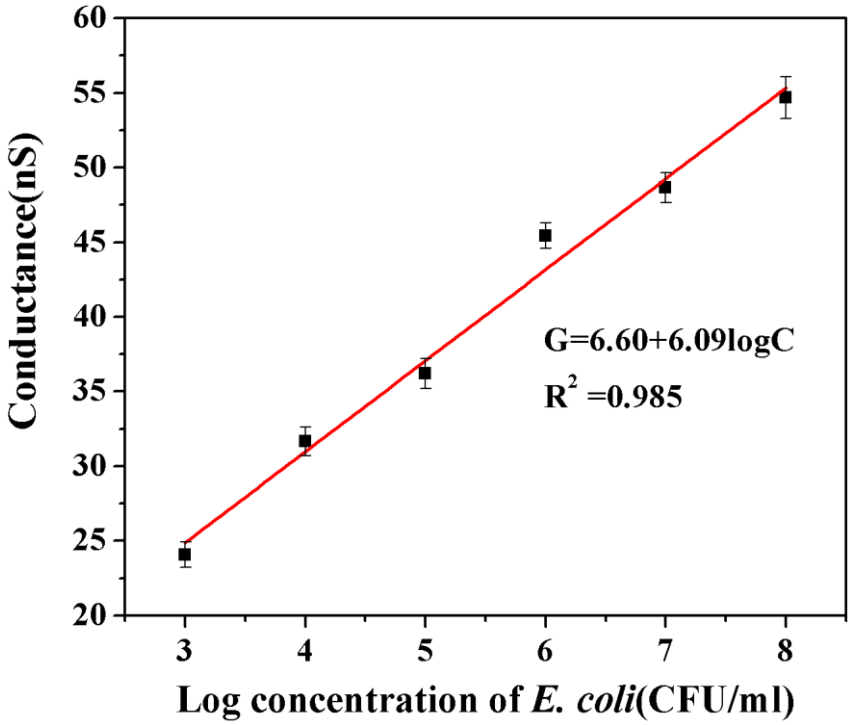


Fig. 6. The response curves of constructed sensor for conductance change and concentration of *E. coli* (CFU/ml). Error bars indicate standard deviation (n = 5).

To investigate the repeatability of the constructed sensor, ten sensors have been prepared for parallel experiment and the relative standard deviation (RSD) for the detection of *E. coli* at the concentration of 10^3 CFU/mL was 3.8%. This result showed that the constructed sensor has good repeatability. When the sensors were stored at 4 °C over 1 weeks, the difference of the detectable signals was insignificant (RSD = 4.3%), which showed that the constructed sensor has good stability.

3.8. Detection of simulated aqueous samples

The constructed sensor was evaluated by a total of 45 simulated aqueous samples including 13 positive samples and 32 negative samples for identification of *E. coli* by traditional bacteria culture method. The results were shown in Table 3. Among 13 positive samples, only a single sample was not detected by constructed sensor, one explanation could be that the failure of bacterial DNA extraction or contamination by bacteria during germiculture. Among 32 negative samples, 3 samples tests by the sensor were positive. The reason for that may be relevant to the low concentrations (less than 10^3 CFU/mL) of the bacteria in the samples or slow growth of bacterial even antibiotic effect. According to the value of the McNemar test, there was no significant change between the two methods ($P = 0.625 > 0.05$). The sensitivity and specificity of the constructed sensor were evaluated by using the results from the traditional bacteria culture method. The sensitivity was 92.3% (12/13) and the specificity was 90.6% (29/32). In addition, for identification of the bacteria, bacteria culture method typically takes 48 h or even more, whereas by constructed sensor the detection time was less than 3 h. Therefore the constructed sensor is simple, rapid and accurate.

Table 3 The results of constructed sensor for 45 simulated samples evaluated against that of the culture method.

constructed sensor	Bacteria culture method		Total
	Positive	Negative	
Positive	**12**	**3**	**15**
Negative	**1**	**29**	**30**
Total	**13**	**32**	**45**

4. Conclusions

A new electrochemical biosensor based on nanogap network electrodes and enzymatic target-guided methods is proposed for detection of *E. coli*. The constructed sensor was successfully used for detection of *E. coli* with the low minimum detection limit of 100 CFU/ml, and the detection time was less than 3 h. It provides a new path for rapid detection of *E. coli* with selectivity and specificity.

Acknowledgements

This research work was supported by the National Natural Science Foundation of China (No. 21275042) and the Hunan Province Science and Technology Plan Project (No. 2015JC3072).

References

Bulard, E., Chaud, P., Roget, A., Calemczuk, R., Fort, S., Livache, T., 2015. Anal. Chem. 87, 1804-1811.

Chang, Y.F., Wang, W.H., Hong, Y.W., 2018. Anal. Chem. 90, 1861−1869.

Chen, X., Guo, Z., Yang, G.M., Li, J., Li, M.Q., Liu, J.H., Huang, X.J., 2010. Mater. Today. 13, 28-41.

Derda, R., Lockett, M. R., Tang, S.K.Y., Fuller, R.C., Maxwell, E.J., Breiten, B., Cuddemi, C.A., Ozdogan, A., Whitesides, G.M., 2013. Anal. Chem. 85, 7213-7220.

Egholm, M., Buchardt, O., Christensen, L., Behrens, C., Freier, S.M., Driver, D.A., Berg, R.H., Kim, S.K., Norden, B., Nielsen, P.E., 1993.Nature. 365, 566-568.

Foxman, B., 2010. Nat. Rev. Urol. 7, 653-660.

Gao,W., Li, B., Yao, R., 2017. Anal. Chem. 89, 9836-9842.

Gerion, D., Chen, F.Q., Kannan, B., 2003. Anal. Chem. 75, 4766-4772.

Hyun, J.C., Cesar, M.C., Hakho, L., 2013. Nature Nanotechnology. 8, 369-375.

Hünniger, T., Felbinger, C., Wessels, H., 2015. J. Agric. Food Chem. 63, 4291-4296.

Jacob, E., Elissa, F., Nicholas, V., 2013. Environ. Sci. Technol. 47, 13668-13676.

Jiang, X.R., Shao, N., Jing, W.W., Sui, G.D., 2014. Talanta. 122, 246-250.

Kaper, J.B., Nataro, J.P., Mobley, H.L., 2004. Nat. Rev. Microbiol. 2, 123-140.

Kaittanis, C., Naser, S.A., Perez,J.M., 2007. Nano Lett. 7, 380-383.

Leonard, H., Halachmi, S., Segal, E., 2017. ACS. Nano. 11, 6167-6177.

Li, Y.X., Afrasiabi,R., Fathi, F., Wang, N., Xiang, C.L., Love, R., She, Z., Kraatz, H.B., 2014. Biosens. Bioelectron. 58, 193-199.

Ma, Y., Zhang, J., Zhang, G., 2004. J. Am. Chem. Soc. 126, 7097-7101.

Moura, A., Nicolau, A., Hooton, T., 2009. J. Appl. Microbiol. 106, 1779-1791.

Nair,S., Ascanio, G., Escobedo, C., 2018. Biosens. Bioelectron. 106, 105-110.

Niemz, A., Ferguson, T.M., Boyle, D.S., 2011.Trends.Biotechnol. 29, 240-250.

Olanrewaju, A.O., Ng, A., Robillard, A., 2017. Anal. Chem. 89 , 6846-6853.

Orenga, S., James, A.L., Manafi, M., Perry, J.D., Pincus, D.H., 2009. J. Microbiol. Meth. 79, 139-155.

Porath, D., Bezryadin, A., De, V.S., Dekker, C., 2000. Nature. 403, 635-638.

Park, S.J., Taton, T.A., Mirkin, C.A., 2002. Science. 295,1503-1506.

Pan, W.X., Zhao, J.W., Chen, Q.S., 2015. J. Agric. Food Chem. 63, 8068-8074.

Ratilainen, T., Holmen, A., Tuite, E., 2000. Biochemistry. 39, 7781-7791.

Schmid, M., Schmitz Esser, S., Jetten, M., Wagner, M., 2001. Environmental Microbiology. 3, 450-459.

Scharff, R.L., 2012. J. Food Prot. 75, 123-131.

Shi, D.C., Huang, J.F., Chuai, Z.R., Chen, D., Zhu, X.Y., Wang, H., Peng, J., Wu, H.Y., Huang, Q., Fu, W.L., 2014. Biosens. Bioelectron. 62, 280-287.

Su, L.C., Chang, C.M., Tseng, Y.L., Chang, Y.F., Li, Y.C., Chang, Y.S., Chou, C.E., 2012. Anal. Chem. 84, 3914-3920.

Sosniak, A.M., Gasser, G., Metzler, N., 2009. Org. Biomol. Chem. **7**, 4992-5000.

Thomas, S., Rajan, A.C., Rezapour, M.R., Kim, K.S., 2014. J. Phys. Chem. C. 118, 10855-10858.

Tlili, C., Sokullu, E., Safavieh, M., Tolba, M., Zourob, M., 2013. Anal. Chem. 85, 4893-4901.

Wang, H.Y., Du, P.C., Li, J., Zhang, Y.Y., Zhang, W., Han, N., Woo, P., Chen, C., 2014. J. Med. Microbiol. 63, 433-440.

Wang, Y.X., Ping, J.F., Ye, Z.Z., Wu, J., Ying, Y.B., 2013, Biosens. Bioelectron. 49, 492-498.

World Health Organization, 2008 Safer water, better health Report. Available at:http://www.who.int/quantifying_ehimpacts/publications/saferwater/en/.

Woo, P.C., Lau, S.K., Teng, J.L., Tse, H., Yuen, K.Y., 2008. Clin. Microbiol. Infect. 14, 908-934.

Yang, M., Xiong, X., He, R., 2018. ACS Appl. Mater. Interfaces. 10, 5933-5940.

Ye, L.X., Zhao, G.Y., Dou, W.C., 2018. Talanta. 182, 354-362.

Zu, Y.B., Gao, Z.Q., 2009. Anal. Chem. 81,8523-8528.

Zowawi, H.M., Harris, P., Roberts, M.J., 2015. Nat. Rev. Urol.12, 570-584.

Zhang, J., Nie, H.G., Wu, Z., Yang, Z., Zhang, L.J., Xu, X.J., Huang, S.M., 2014. Anal. Chem. 86, 1178−1185.

Zhu, T.F., Hu, Y.J., Yang, K., Dong, N., Yu, M., Jiang, N.J., 2018. Microchimica. Acta. 185, 1-9.

Zhang, Y., Tan, C., Fei, R., Liu, X., Zhou, Y., Chen, J., Chen, H., Zhou, R., Hu, Y., 2014. Anal. Chem. 86, 1115-1122.

Zhang, R.K., Li, G.K., Hu, Y.F., 2015. Anal. Chem. 87, 5649-5655.

Highlights

1. A 16S rDNA electrochemical biosensor has been developed for sensitive, rapid detection of *Escherichia coli* based on nanogap network electrodes and enzymatic target-guided methods.

2. The proposed biosensor showed high specificity and sensitivity to detection of *Escherichia coli*.

3. The detection time to *Escherichia coli* was less than 3 h and the detection limit was 100 CFU/mL.