Int. J. Electrochem. Sci., 14 **(2019)** 2833 – 2845, doi: 10.20964/2019.03.45

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Bacteria-immobilized Prepartion as the Microbial Probe for Electrochemical Susceptibility Test

Xiao Han, Lizhi Chen, Di He, Jingting He, Ye Ma, Jingjing Wang, Chang Liu[*]

College of Pharmacy, Jinzhou Medical University, No. 40, Section 3, Songpo Road, Linghe District, Jinzhou City, Liaoning Province, 121001, China
[*]E-mail: liuchang@jzmu.edu.cn, hx_jzmu@163.com

Received: 24 August 2018 / *Accepted:* 18 December 2018 / *Published:* 7 February 2019

Electrochemical measurement of respiratory activity based on a multilayered composite of bacteria has been developed for susceptibility test. Here, we firstly report the successful fabrication of graphene oxide (GO)/polyvinyl alcohol (PVA)/*Escherichia coli* (*E. coli*)/GO/graphite rod (GR) composite, where the GO/GR composite was prepared by chemical oxidation and then the *E. coli* was confined at the surface of GO/GR composite by in site culture and crosslink of GO/PVA in sequence. Although it induced 29.07% decrease in the sensitivity of immobilized *E. coli* to toxic chemicals, the presence of GO/PVA could promote the storage stability for 20 days in term of relative bioactivity. The GO/PVA/*E. coli*/GO/GR composite was used as the bacterial probe in the electrochemical sensor for susceptibility test of antibiotics, and the IC50 values were determined to be 8.01 mg/L, 6.51 mg/L, 9.93 mg/L and 4.07 mg/L for gentamicin, amikacin, amoxicillin and cefixime, which were identical with those obtained with standard Kirby-Bauer disk susceptibility (KB) testing. The vancomycin, only affecting gram-positive bacteria, exhibited an inhibition ratio of 34.5% at the concentration of 16.0 mg/L in the electrochemical susceptibility test, agreeing with the result from KB testing.

Keywords: electrochemical biosensor, nanocomposite, antibiotic efficacy testing, immobilized microorganisms, electrochemical susceptibility test.

1. INTRODUCTION

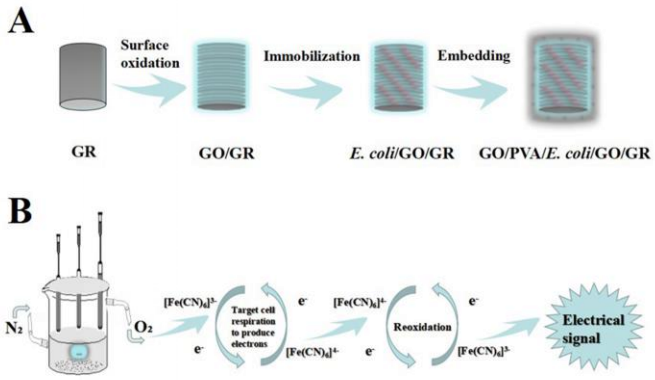
The investigations of clinically and daily appropriate antibiotics used reveal the main reason for the emergence of drug-resistant bacteria, as well as the importance of monitoring antibiotic effectiveness to control the drug-resistant bacteria [1-3]. Thus, a variety of methods have been developed to monitor and detect the bacterial susceptibility, both *in vitro* and *in vivo* [4,5]. To date, the electrochemical biosensor has been acknowledged as a rapid, sensitive, cost-effective and *in situ* diagnostic tool for susceptibility test [6-8].

Immobilized bacteria is actually favorable as sensing element in electrochemical biosensors for susceptibility test rather than free cells, as they can be used repeatedly and exhibit long-term storage stability [9-12]. Thanks to the appearance of macromolecule polymers, such as chitosan, agar, polyacrylamide and polyvinyl alcohol (PVA), which offers a possibility to ensure sufficient cells loading, bioactivity of immobilized cells and conformational stability in aqueous and special media [13-16]. Therefore, the electrochemical biosensors based on the biofilms composed of polymer and cells have attracted increasing attention and been applied widely in susceptibility test [17].

Carbon nanomaterials (CNs) like graphene oxide (GO) modified with abundant of hydroxyl and carboxyl groups by acid treatment have been proved to be an excellent auxiliary material for cell immobilization [18,19]. The opposite views pointed that the CNs could reduce the activity of cells [20], and moreover, the complicated preparation process and inherent characteristic of aggregation in the suspension have hindered the further application of CNs [21]. So new development of CNs synthesis technology and all-round studies on the biocompatibility of CNs are very necessary when the CNs are used in bacterial processes.

In recent, there is a growing interest to fabricate the composite of CNs and polymers, which can not only effectively maintain the stability of the CNs, but also simplify the operation steps by one-pot synthesis [22-27]. The entrapment of cells in PVA is known as an efficient method to construct a sensitive biological probe, and the optimal content of PVA was 10g/L according to our previous study [28]. Thus this study designed to prepare an excellent performance CNs/PVA composite material for the susceptibility test.

In this work, little graphite rod (GR) was modified with multilayered GO to yield an immobilizing matrix of GO/GR for *Escherichia coli* (*E. coli*) immobilization (*E. coli*/GO/GR). Then a GO/PVA composite was confined on the surface of *E. coli*/GO/GR (GO/PVA/*E. coli*/GO/GR) to fabricate some stable and biocompatible pellets with excellent layer-by-layer structure (shown in Scheme 1A). As shown in equation (Eqs. 1, 2 and 3), the synthetic co-substrate mediator can replace the nature co-substrate oxygen as an electron acceptor and then are re-oxidized at the surface of the electrode under a properly applied voltage. Ferricyanide was chosen to shuttle the electrons in this study because the NADH dehydrogenases as hexacyanoferrate (III) reductases are responsible for transporting intracellular electrons to terminal acceptor of hexacyanoferrate (III) [29,30]. Then biological activity of target cells could thus be determined by measuring the anodic current of ferrocyanide oxidation (shown in Scheme 1B). The optimum immobilization conditions were investigated in term of the relative biological activity of immobilized *E. coli* cells. Finally, the resultant GO/PVA/*E. coli*/GO/GR was used as a biocomposite in studying of susceptibility test for several common antibiotics, and the obtained results were compared with those obtained with Kirby-Bauer disk susceptibility (KB) testing [31,32].



Scheme 1. The preparation of GO/PVA/*E. coli*/GO/GR pellet by a layer-by-layer assembly (A) and the principle of electrochemical determination with ferricyanide ions as the mediator (B).

$$Normal\ TAC: CH_2O_{(aq)} + O_{2(g)} \xrightarrow[microorganism]{enzyme} H_2O_{(l)} + CO_{2(g)} \quad (1)$$

$$Ferricyanide\ substitution: CH_2O_{(aq)} + H_2O + 4[Fe(CN)_6]^{3-} \xrightarrow[microorganism]{\text{enzyme}} CO_2 + 4H^+ + 4[Fe(CN)_6]^{4-} \quad (2)$$

$$Electrode\ reaction: [Fe(CN)_6]^{4-} - e \xrightarrow[\text{oxidation}]{+450\ \text{mV}} [Fe(CN)_6]^{3-} \quad (3)$$

2. MATERIALS AND METHODS

2.1 Materials

Peptone, beef extract, soluble starch, acid hydrolyzed casein and PVA were obtained from Beijing AoBoXing Bio-tech co. Ltd (China). 3,5-dichlorophenol (DCP) was from Chengdu Gracia Chemical Technology Co. Ltd (China). Yeast extract, chitosan, glucose and glutamic acid were from Hants OXOID Ltd (England). $NaNO_3$, $KMnO_4$, H_2SO_4, HCl, NaCl, NaOH, Na_2HPO_4, KH_2PO_4, $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, boronic acid were purchased from Tianjin Chemical Reagent Factory (China). Graphite rods were purchased from Tianjin Changyuan Electronic co. Ltd (China). Gentamycin was from Henan Runhong Pharmaceutical co. Ltd (China). Amikacin was from Jiangsu Wuzhong Pharmaceutical Group Corporation (China). Cefepime was from Shandong Luoxin Biotechnology co. Ltd (China). Amoxicillin was from Sichuan Pharmaceutical.inc (China). Vancomycin was from Zhejiang Medicine co. Ltd (China). GO and PVA solutions with desired concentrations were prepared with sterilized phosphate buffer solution (PBS, 1.088% K_2HPO_4, 4.298% $NaH_2PO_4 \cdot 12H_2O$). *E. coli* ATCC 25922 was gifted from the First Affiliated Hospital of Jinzhou Medical University. It was cultured in the Luria-Bertani medium (LB, 1% peptone, 0.5% yeast extract, 1% NaCl, pH 7.4±0.2) and the Mueller-Hinton medium (MH, 0.2% beef extract, 0.15% soluble starch, 1.75% acid hydrolyzed casein, pH 7.4±0.2), respectively. Addition of 15% agar was used to prepare the MH solid medium. The standard

glucose-glutamic acid (GGA, 0.015% glucose, 0.015% glutamic acid) as nutrient substance was prepared.

Scanning electron microscopy (SEM, XL30ESEM FEG) was from Philips (Tokyo, Japan). Air clean bench (SW-CJ-IE) was from Shanghai Boxun Industrial co. Ltd (China). Air thermostatic shaking table was from Shanghai Fuma Laboratory Instrument co. Ltd (China). Pressure steam sterilizer (SYQ-PSX-280B) was made by Shenan, Shanghai. Electrochemical workstation (CHI832C) was obtained from Chenhua Instruments, inc (China).

2.2 Preparation of GO matrix to immobilize E. coli strains

In a typical synthesis procedure of GO/GRs, 150 g of GRs was added into 200 mL of H_2SO_4 and then the mixture was stirred for 20 min. Afterward, 5 g of $NaNO_3$ and 5 g of $KMnO_4$ were added into the mixture in an ice-water bath under vigorous stirring. When there were a large number of little bubbles, the mixture was transferred to a hot bath at 80 ºC and stirred for 1 min. The chemical reaction was terminated through the addition of deionized water. The GO/GRs were collected and washed with 10% HCl solution and deionized water until no sulfate residue. Then all rods were transferred into dialysis bag to neutralize (7.0±0.2) with deionized water. The resultant GRs with surface modification of multilayered GO structure were harvested and used as a biocompatible matrix for cell immobilization.

For cell immobilization, *E. coli* cells were firstly inoculated into 20 mL of LB substrate and grown aerobically at 37 ºC for 6 h in a shaking incubation (120 r/min). Then 0.2 mL of LB suspension containing activated cells was added into 300 mL of freshly prepared LB substrate, and the purified GO/GRs were dispersed into this LB substrate at a ratio of 0.5% (w/w). The mixture was stirred at a speed of 120 r/min under 37 ºC for 6, 8, 10, 12, 16, 18, 20 and 24 h. Finally, the *E. coli*/GO/GR composites were separated from the reaction mixture and washed with deionized water thoroughly for further use.

2.3 Fabrication of GO/PVA/E. coli/GO/GR

PVA solution was prepared by dissolving different content of PVA in the PBS solution at 90 ºC, and the GO/PVA suspension was obtained by dispersing commercially available GO powder in the PVA solution *via* sonication for 30 min. For fabrication of GO/PVA/*E. coli*/GO/GR, the *E. coli*/GO/GR composites were firstly dispersed into the GO/PVA solution by stirring for 10 min, and then saturated boric acid was added as a crosslinking reagent of PVA to confine GO/PVA at the surface of *E. coli*/GO/GR composites. After treating the crosslinking process of PVA for 1, 3, 5, 10, 20, 40 and 60 min, the GO/PVA/*E. coli*/GO/GR with an excellent layer-by-layer structure was harvested by washing three times with fresh sterile PBS solution to remove the leakage cells and impurities. The surface morphology was examined by SEM.

2.4 Evaluation of cells bioactivity in batch process

In the bioactivity test, 10 mL of samples containing 45 mM potassium ferricyanide, standard GGA solution, the immobilized cells (*E. coli*/GO/GR or GO/PVA/*E. coli*/GO/GR) and the sterilized PBS as the supplement were incubated anaerobically using oxygen-free nitrogen for 60 min at 37 ºC. The DCP or antibiotics with different concentrations were present to study the sensitivity of immobilized cells. Chronoamperometry was employed to measure the anodic current of ferrocyanide formed during incubation, and the parameter was set at 450 mV with the pulse width of 20 s. The electrochemical experiments were performed with a CHI 832C electrochemical workstation (Chen Hua, Shanghai) at room temperature. While an ultramicroelectrode array made of 15 pieces of isolated Pt ultramicroelectrode with the diameter of 25 μm was used as a working electrode. An Ag/AgCl (3 mol/L KCl) electrode and a Pt stick were used as the reference and auxiliary electrode, respectively.

The relative bioactivities of the immobilized cells were measured with equation (Eq. 4):

$$A_{Ratio} = \frac{i_m}{i_0} \quad (4)$$

where i_m meant that the output of limiting current was from immobilized cells, and i_0 was a control value based on the empty immobilized matrix.

The susceptibility tests of immobilized cells to antibiotics in our research could be calculated by the following equation (Eq. 5):

$$Inhibition\% = \left(\frac{i_{nor} - i_{anti}}{i_{nor}}\right) \quad (5)$$

where i_{nor} was the anodic oxidation current of ferrocyanide ions produced by normal immobilized cells, and i_{anti} was that of the immobilized cells in the presence of the antibiotics.

2.5 KB testing

The media used in the KB testing was MH agar at about 4 mm deep, and the pH level of the agar was adjusted to 7.4. The wafers containing antibiotics with different concentrations were laid onto the agar plate where *E. coli* ATCC 25922 has been inoculated, and then the plate was incubated at 37 ºC for 16 h.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization of GO/GR

The GO/GR was characterized by SEM and showed multilayered GO structure on the surface of GR (Fig. 1A). As shown in Fig. 1B, this chemical modification resulted in a large number of cells loading and a great cells morphology because the formation of a more biocompatible environment and larger specific surface area for cell immobilization compared to bare surface of GR [33].

The culture time is a very important factor on the bioactivity of immobilized cells, and thus the

dependence of the bioactivity of immobilized *E. coli* cells on culture time was evaluated in form of relative bioactivity through Eq. (4) (Fig. 2). From Fig. 2, it was obvious that the relative bioactivity of the immobilized *E. coli* cells increased with the culture time firstly, and then decreased when the culture time was over 16 h, which suggesting that 16 h should be chosen as the optimal culture time for *E. coli* immobilization on the matrix of GO/GR. To further reveal the effect of culture time on the relative bioactivity of immobilized cells, SEM was used to study the changes at morphology of *E. coli*/GO/GR. Fig. 3 showed that the amount of immobilized cells on the matrix of GO/GR increased with the increase in culture time.

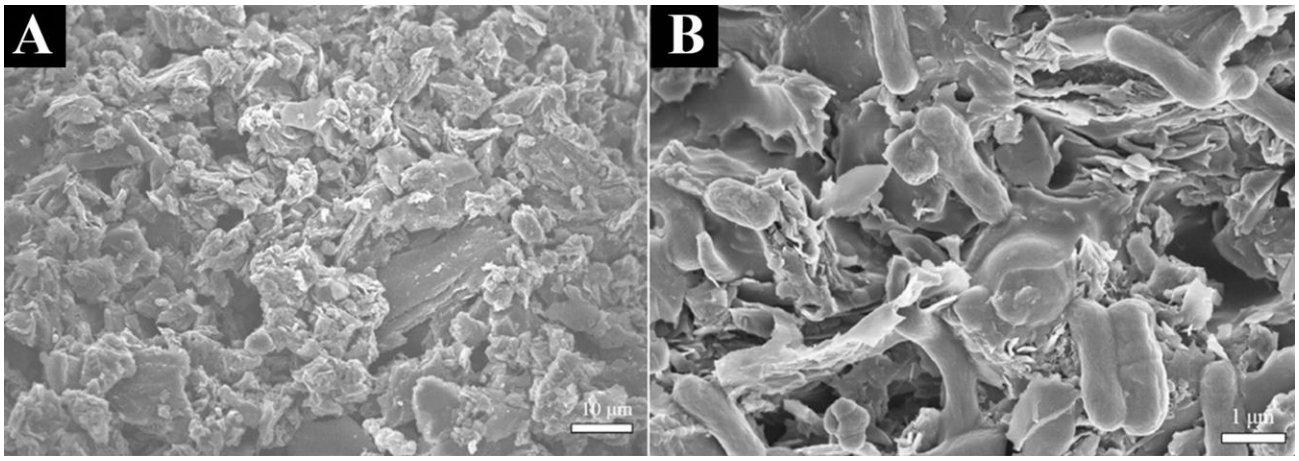


Figure 1. (A) showed the surface feature of GO/GR, (B) showed that the morphology of immobilized *E. coli* cells on the matrix of GO/GR at 12 h culture.

3.2 Immobilization of E. coli cells

The culture time is a very important factor on the bioactivity of immobilized cells, and thus the dependence of the bioactivity of immobilized *E. coli* cells on culture time was evaluated in the form of relative bioactivity through Eq. (4) (Fig. 2). From Fig. 2, it was obvious that the relative bioactivity of the immobilized *E. coli* cells increased with the culture time firstly, and then decreased when the culture time was over 16 h, which suggesting that 16 h should be chosen as the optimal culture time for *E. coli* immobilization on the matrix of GO/GR. To further reveal the effect of culture time on the relative bioactivity of immobilized cells, SEM was used to study the changes at the morphology of *E. coli*/GO/GR. Fig. 3 showed that the amount of immobilized cells on the matrix of GO/GR increased with the increase in culture time. However, the immobilized cells were shriveling and curving at the culture time of 24 h. These SEM images well explained the change in relative bioactivity of immobilized cells with the increase in culture time.

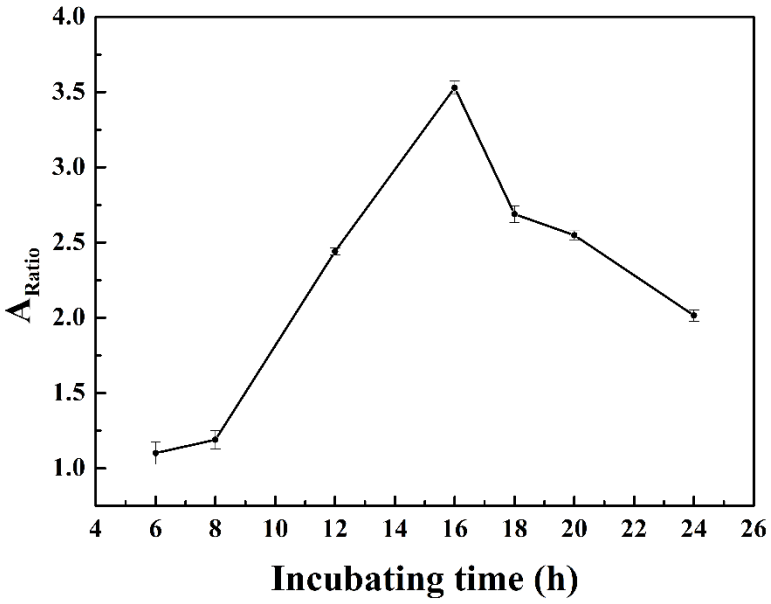


Figure 2. The effects of incubating time on the relative bioactivity of *E. coli* cells. Each result was the average of two or three samples.

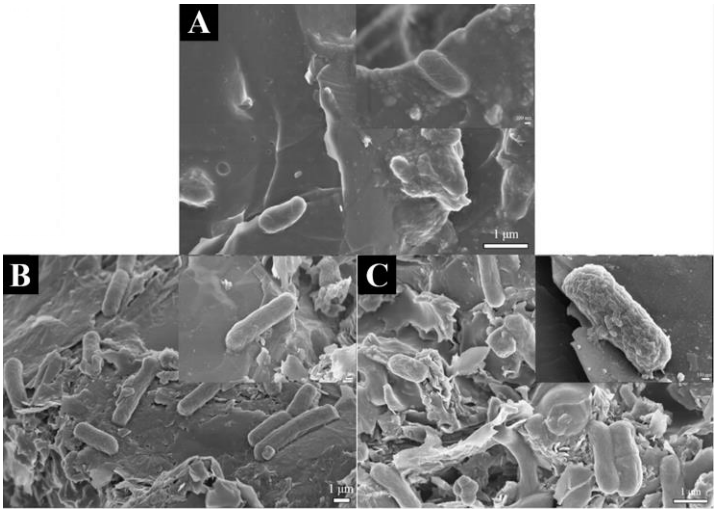


Figure 3. The change of surface characteristics of immobilized cells with culture time at 6 h (A), 16 h (B), 24 h (C).

3.3 Synthesis and characterization of GO/PVA/E. coli/GO/GR

The entrapment of cells in PVA has been proved to be an efficient method to construct a sensitive biological probe, and the optimal content of PVA was 10 g/L according to our previous study [28]. GO is well known for its excellent biocompatibility so it was used as an auxiliary material for the synthesis of GO/PVA nanocomposite. As shown in Fig. 4, the doped GO effectively improved the porosity of the polymer matrix, which could facilitate the mass transfer between the external environment and entrapped cells. Fig. 5 showed that the relative bioactivity of immobilized cells increased firstly and then decreased

rapidly with the increase in the GO concentration.

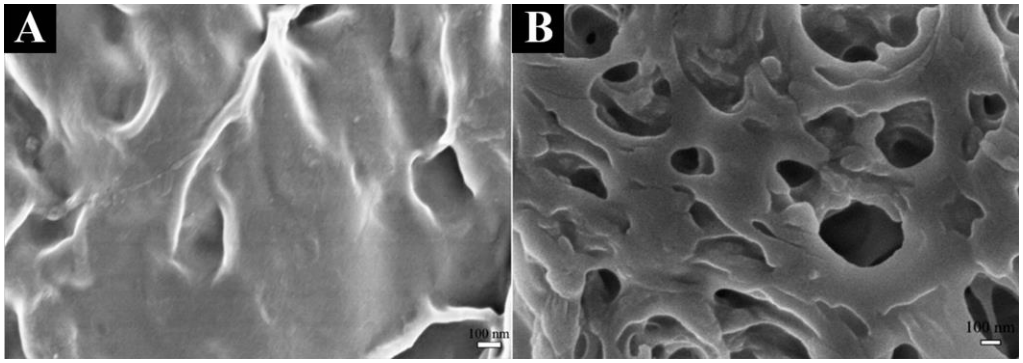


Figure 4. The surface characteristics of PVA (A) and GO/PVA nanocomposite with GO content in 5 mg/L (B).

The highest relative bioactivity of GO/PVA/*E. coli*/GO/GR in this work was obtained at 2.8 when the concentrations of PVA and GO powder were 10 g/L and 0.5 g/L, respectively. We believed that the suitability of hydroxyl and carboxyl structures chemically modified on the GO surface served a friendly environment for cells entrapment. But the concentration of GO was more than 0.5 g/L, there might be detrimental effect on the entrapped cells regarding membrane destruction and the oxidative damage induced by the radicals [34]. Comparing with the results of relative bioactivity shown in Fig. 2 and Fig. 5, the GO/PVA composite seemed to show some negative effects on the relative biological activity of immobilized cells because the PVA film could hinder the mass transfer, even though the doped GO already improved the porosity of GO/PVA composite.

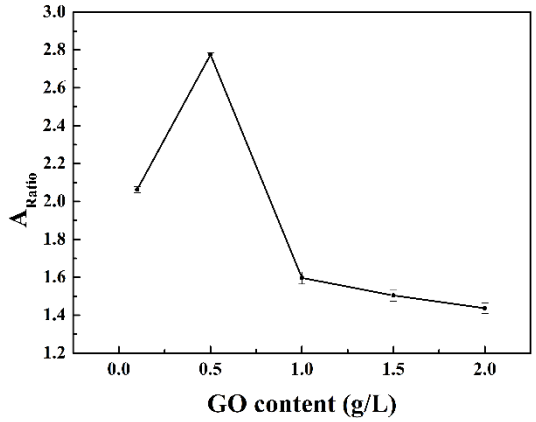


Figure 5. The effects of GO content on the relative bioactivity of GO/PVA/*E. coli*/GO/GR. Each result was the average of two or three samples.

3.4 The effects of PVA crosslinking time

Although it has been widely used as a crosslinking reagent, the effect of saturated boric acid on the bioactivity of immobilized cells should be investigated because of its well-known toxicity. As shown in Fig. 6, the relative bioactivity of immobilized cells decreased significantly while the crosslinking time

increased from 1 to 60 min, suggesting that the applied crosslinking time should be controlled strictly to avoid a serious toxic effect of saturated boric acid on the immobilized cells.

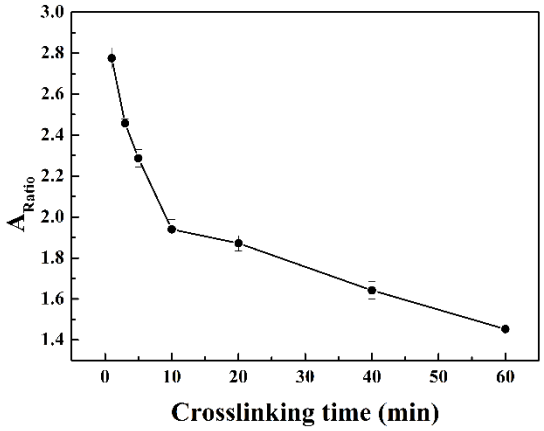


Figure 6. The effect of saturated boric acid on the relative activities of immobilized cells at crosslinking time in the range of 1-60 min. Each result was the average of two or three samples.

The effect of crosslinking time on the long-term storage ability of GO/PVA/*E. coli*/GO/GR was also investigated. When the GO/PVA/*E. coli*/GO/GR was stored in the PBS solution at 4 °C, the gradual exfoliation of PVA/GO film could be observed within 5 and 10 days at the crosslinking times of 1 and 20 min, respectively. In contrast, when the GO/PVA/*E. coli*/GO/GR was stored within 20 days in a dry vessel at 4 °C, no obvious exfoliation of PVA/GO could be observed during storage in the case of crosslinking time of 10 min. But there were no obvious differences in the residual bioactivity between PBS storage and dry storage (shown in Fig. 7A and Fig. 7B). Apparently, the exfoliation of PVA was not the main influencing factor for bioactivity loss comparing with the toxicity of saturated boric acid during storage time. Considering the preservation period, the toxicity of boric acid, bioactivity and morphology changes, all PVA/GO/*E. coli*/GO/GR were treated in the saturated boric acid at 10 min and then stored in the dry vessel at 4 °C. Based on optimized immobilization and preservation conditions, the immobilized cells of GO/PVA/*E. coli*/GO/GR showed higher stable performance than that of the immobilized cells by other immobilization methods in previous studies [35-39].

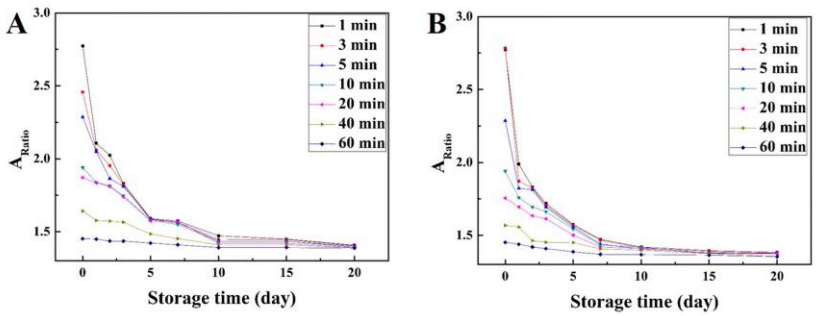


Figure 7. The changes of relative activities of immobilized cells depending on the crosslinking time and storage time in the PBS solution (A) and dry condition (B) at 4 °C storage. Each result was the average of two or three samples.

3.5 The sensitivity of immobilized cells to toxin

The immobilized cells have been widely used as biological probes, and the electrochemical method is often introduced to detect the physiological state of target cells when encountering toxin. Fig. 8 showed the results of limiting current before and after 1 h incubation of GO/PVA/*E. coli*/GO/GR suspension in ferricyanide and GGA standard solution containing amikacin (amikacin was substituted by PBS in the positive control solution). Apparently, the respiration of *E. coli* cells was inhibited and the oxidation current of the ferrocyanide decreased comparing with the result of positive control after amikacin was added. Moreover, DCP was chosen as a model toxin, and to which the toxicity on the immobilized *E. coli cells* in the form of *E. coli*/GO/GR, PVA/*E. coli*/GO/GR and GO/PVA/*E. coli*/GO/GR were determined, and the IC50 values were measured to be 11.3, 29.8 and 18.7 mg/L，respectively (Fig. 9A). According to the results, *E. coli*/GO/GR exhibited the highest sensitivity to DCP. With the increase in the storage time, the sensitivity of immobilized *E. coli* cells to DCP decreased due to the adverse effects of storage time on the immobilized cells, but these effects were obviously weakened while the immobilized cells in the form of GO/PVA/*E. coli*/GO/GR due to the protection of external GO/PVA film (Fig. 9B).

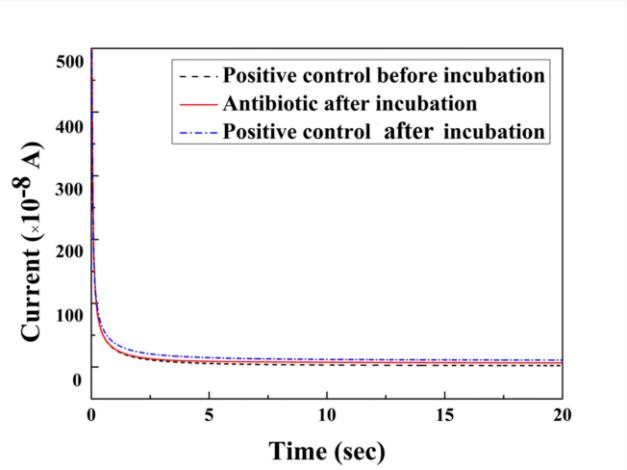


Figure 8. The curves recorded electrochemical signals of a Pt ultramicroelectrode (diameter 25 μm, 15 pieces) before and after 1 h incubation of positive control, and the effect of 9 mg/L amikacin on immobilized *E. coli*.

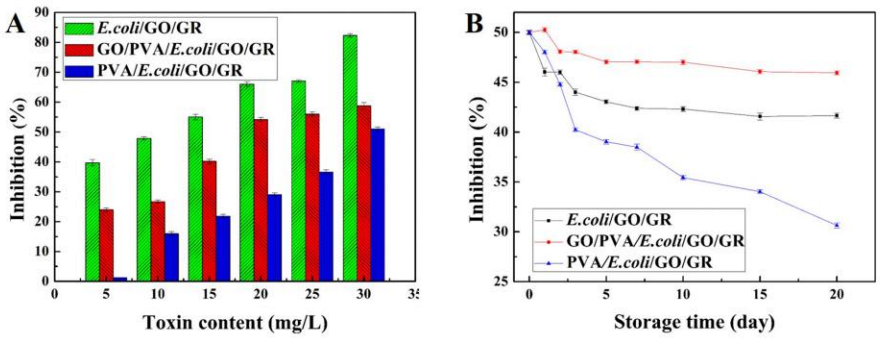


Figure 9. The inhibition of DCP on the immobilized cells in form of *E. coli*/GO/GR, GO/PVA/ *E. coli*/GO/GR and PVA/ *E. coli*/GO/GR (A). The effects of storage time on the sensitivities of immobilized cells to DCP (B). Each result was the average of two or three samples.

Apparently, the sensitivity of immobilized cells to toxin was affected by the immobilization method and storage time. The relevant studies revealed that the GO/PVA/*E. coli*/GO/GR displayed a balance between the sensitivity and the storage time, which was thus to be employed in our further work.

3.6 The susceptibility of GO/PVA/E. coli/GO/GR to antibiotics

Fig. 10 showed the susceptibility of immobilized *E. coli* cells to five commonly used antibiotics, such as gentamicin, amikacin, amoxicillin, cefixime and vancomycin. All antibiotics tested except vancomycin showed profound inhibition effects on *E. coli* ATCC 25922 after 60 min incubation, and the IC50 values were 8.01, 6.51, 9.93 and 4.07 mg/L, respectively. Because it only affects gram-positive bacteria, the inhibition ratio of vancomycin to *E. coli* ATCC 25922 with the concentration of 16.0 mg/L was 34.5%.

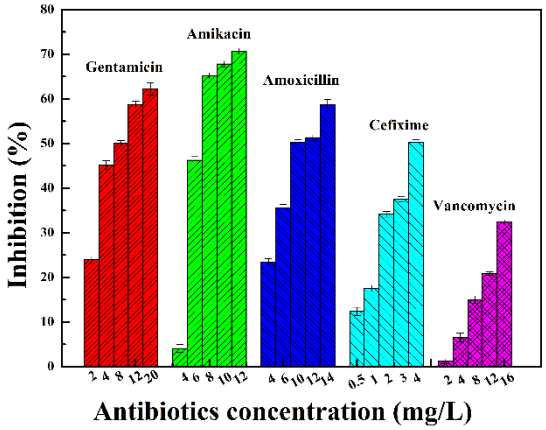


Figure 10. Inhibition of antibiotics on immobilized *E. coli* at different concentrations. Each result was the average of three samples.

Standard KB testing was further employed as a comparison to the advanced electrochemical method based on immobilized cells. As listed in Table 1, the medium susceptibility of all antibiotic except vancomycin could be obtained by KB testing at the concentrations identical with their IC50 values obtained by the electrochemical method. The test concentration of vancomycin was 16.0 mg/L in the KB test, and a low susceptibility was obtained, agreeing with the results obtained with the electrochemical method. Apparently, the results based on our method were in good agreement with the results of standard KB testing. But the electrochemical method based on immobilized cells of GO/PVA/*E. coli*/GO/GR was a more convenient and time-saving approach than the traditional studies [40-41].

Table 1. The results of standard KB testing for five different antibiotics.

	Gentamicin	Amitacin	Amoxicillin	Cefixime	Vancomycin	Blank
Zone of inhibition(mm)	10.2	11.4	12.1	12.8	8.8	0
Sensitivity	medium	medium	medium	medium	low	null

4. CONCLUSION

In this study, the GO/PVA/*E. coli*/GO/GR had been successfully prepared with chemically oxidized GRs as matrices and GO/PVA as an embedding material to immobilize *E. coli*. The preparation method was simple and cost-effective, and the bioactivity of the resultant GO/PVA/*E. coli*/GO/GR could be maintained for more than 20 days in a dry vessel at 4 ºC. By coupling with the electrochemical method, the GO/PVA/*E. coli*/GO/GR had been used as the bacterial probe for toxicity test and susceptibility test of antibiotics. Noticeable, the present results of susceptibility test of five commonly used antibiotics were identical with those obtained with standard KB test, demonstrating the feasibility of the electrochemical method based on GO/PVA/*E. coli*/GO/GR in the rapid susceptibility test of antibiotics.

ACKNOWLEDGEMENTS
This work was supported by the Training Foundation of Liaoning Province for University Students' Innovation and Entrepreneurship, China (201510160000007). We acknowledge the technique and source support of The First Affiliated Hospital of Jinzhou Medical University.

References

1. J. Inchai, J. Pothirat, C. Bumroongkit, L. Limsukon, W. Khositsakulchai and C. Liwsrisakun, *J.Intensive Care*, 3 (2015) 9.
2. D.Y. Cho, K. Hoffman, D. Skinner, C. Mackey and D.J. Lim, *Int. Forum Allergy Rhinol.*, 4 (2017) 352.
3. C.T. Chang, K.M. Doerr and C.M. Whipps, *J. Fis. Di.*, 40 (2017) 1473.
4. R. Delgado, *Enferm. Infecc. Microbiol. Clin.*, 26 (2008) 28.
5. B. Ibarlucea, T. Rim, C.K. Beak, JABM. de Visser, L. Baraban and G. Cuniberti, *Lab. on a chip.*, 17 (2017) 4283.
6. H.Y. Li, X.L. Wang and Z.Y. Yu, *J. Solid State Electr.*, 18 (2014) 105.
7. J. Li, Y.L. Liu, X.Q. Zhu, G. Chang, H.P. He, X.H. Zhang and S.F. Wang, *ACS Appl. Mater. & Interfaces*, 9 (2017) 44231.
8. G. Yammouri, J. Mandli, H. Mohammadi and A. Amine, *J. Electroanal. Chem.*, 806 (2017) 75.
9. G.M. Wen, S.M. Shuang, C. Dong and M.M.F. Choi, *Chin. Chem. Lett.*, 23 (2012) 481.
10. J.C. Ni, Q.X. Wang, W.Q. Yang, M.M. Zhao, Y. Zhang, L. Guo, B. Qiu, Z. Lin and H. Yang, *Biosens. Bioelectron.*, 86 (2016) 496.
11. Z. Zhao, Z. Xie, Z. Wang, Y.C. Tao, X.D. Niu, X. Huang, L. Liu and Z. Li, *J. Biosci. Bioeng.*, 121

(2016) 645.
12. A.R. Mohammad, C. Umma and K. Ashok, *Int. J. Biol. Macromol.*, 87 (2016) 488.
13. X. Wang, S.S. Kim, D. Khang, H.H. Kim and C.J. Kim, *Biochem. Eng. J.*, 112 (2016) 20.
14. W.H. Chen, P.J. Liu, L.Z. Min, Y.M. Zhou, Y. Liu, Q. Wang and WF. Duan, *Nano-micro Lett.*, 10 (2018) 47.
15. V. Grumezescu, G. Socol, A.M. Grumezescu, A.M. Holban, A. Ficai, R. Trusca, C. Bleotu, P.C. Balaure, R. Cristescu and M.C. Chifiriuc, *Appl. Surf. Sci.*, 302 (2014) 262.
16. RGF. Costa, C. Ribeiro and LKC. Mattoso, *J. Appl. Polym. Sci.*, 127 (2013) 446.
17. G. Pavanello, M. Faimali, M. Pittore and A. Mollica, *Water Res.*, 45 (2010) 1651.
18. N. Wang, H.Y. Zhao, X.P. Ji, X.R. Li and B.B. Wang, *Chin. Chem. Lett.,* 25 (2014) 720.
19. J. Li, Y. Yu and J. Qian, *Analyst*, 139 (2014) 806.
20. R. Kaur, S. Verma, P. Joshi, SP. Singh and M. Singh, *J. Nanosci. Nanotechnol.*, 18 (2018) 6765.
21. Y.G. Shi, G.J. Zeng, D.Z. Xu, M.Y. Liu, K. Wang, Z. Li, L.H. Fu, Q.S. Zhang, X.Y. Zhang and Y. Wei, *Mater. Sci. Eng. C*, 80 (2017) 404.
22. Y.N. Ni, F.N. Zhang and S. Kokot, *Anal. Chim. Acta.*, 769 (2013) 40.
23. P. Singh, S.K. Pandey, J. Singh, S. Strivastava, S. Sachan and S.K. Singh, *Nano-Micro. Lett.*, 8 (2016) 193.
24. D. Soma and S. Mitali, *J. Pharm. Anal.*, 4 (2014) 351.
25. H. Gao, J.Y. Lu and Y.R. Cui, *J. Electroanal. Chem.*, 592 (2006) 88.
26. S.A. Bhat, M.A. Rather, S.A. Pandit, P.P. Ingole and M.A. Bhat, *J. Electroanal. Chem.*, 783 (2016) 280.
27. Y.J. He, L.Y. Luo, S.Q. Liang, M.Q. Long and H. Xu, *J. Colloid Interface Sci.*, 525 (2018) 126.
28. C. Liu, Y. Xu, X. Han and X. Chang, *Environ. Toxicol. Chem.*, 327 (2017) 329.
29. C. Liu, D.M. Yong, D.B. Yu and S.J. Dong, *Talanta*, 84 (2011) 766.
30. A. Tizzard, J. Webber, R. Gooneratne, R. John, J. Hay and N. Pasco, *Anal. Chim. Acta.*, 522 (2004) 197.
31. I. Ali, M. Shabbir and N. Ul Iman, *J. Pak. Med. Assoc.*, 67 (2017) 1161.
32. G. Ferro, A. Fiorentino, M.C. Alferez, MI. Polo-Lopez, L. Rizzo and P. Fernandez-ibanez, *Appl. Catal. B, Environmental*, 178 (2015) 65.
33. Y. Zhang, H.L. Hao and L.L. Wang, *Appl. Surf. Sci.*, 390 (2016) 385.
34. K. Charoenkitamorn, S. Chaiyo, O. Chailapakul and W. Siangproh, *Anal. Chim. Acta.*, 1004 (2018) 22.
35. M. Priti, H. Carlos and L. Yu, *Biosens. Bioelectron*., 21(2015)523.
36. A. Erol and D. Erhan, *Biosens. Bioelectron*., 20(2005)1263.
37. Y. Lei, P. Mulchandani and W. Chen, *Electroanalysis,* 15(2003)1160.
38. H. LI, X. Hu and H. Zhu, *Int. J. Electrochem. Sci*, 12(2017)7.
39. L. Liu, L. Sheng, S. Guo, D. Li, C. Liu, L.Qi and S. Dong, *Biosens. Bioelectron*, 25(2009)523.
40. W. Yang, O. Chan and T. Wu, *J. Microbiol Immunol Infect*., 49(2014)41.
41. T.G. Ribeiro, A. Novais and L. Peixe, *J. Antimicrob Chemother*, 71(2016)1169.