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Electrochemiluminescence for the identification of electrochemically active bacteria

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ABSTRACT

Electrochemically active bacteria (EAB) use extracellular electron transfer (EET) to exchange electron with extracellular acceptors. Previous studies regarding the measurement of EAB were based on either extracellular reduction or oxidation. In this work, we developed a simple electrochemiluminescence (ECL) assay for the identification and detection of EAB. The results of this proposed method revealed that EET of EAB influenced the content of dissolved oxygen and the formation of $Ru(bpy)_3^{2+\bullet}$ thus leading to qualitative changes of the ECL signal. EAB with the ability of extracellular reduction (such as *Shewanella oneidensis* MR-1) gave enhanced signal on ECL emission while those displaying the ability of extracellular oxidation (i.e., *Sulfobacillus acidophilus*) showed the opposite effect on ECL emission, but non-EAB (i.e., *Escherichia coli*) did not. These changes in ECL intensity were also proportional to the cell density that could be quantitatively detected in the concentration range of $(1.1 \pm 1) \times 10^5$–212 ± 2 CFU/mL (i.e. *Shewanella oneidensis* MR-1). Moreover, the measurement of the ability of EAB using this approach was in agreement with measurements using the dissimilatory Fe(III) reduction method. Compared to previous reports, this method displayed a continual and steady ECL signal that allowed accurate measurements of EAB. Most important, only a low cell density was needed in this $Ru(bpy)_3^{2+}$-based ECL method, which is beneficial for cell detection.

1. Introduction

Electrochemically active bacteria (EAB) are a kind of bacteria that are able to exchange electrons with extracellular acceptors (Myer and Nealson, 1988), i.e., metal minerals (Liang et al., 2016), humic substances (Lovley et al., 1996) and other substrates (Nielsen et al., 2010), as part of their cellular metabolism. Due to their unique extracellular electron transfer (EET) capability, EAB have been widely used in metal recovery (Lloyd, 2003; Liang et al., 2016), environmental bioremediation (Logan and Rabaey, 2012), electricity generation (Tender et al., 2000; Lovley, 2006) and the production of biofuels (Rabaey and Rozendal, 2010). Nevertheless, up to now, only a limited number of EAB have been identified (Koch and Harnisch, 2016), partly because it is difficult to measure their EET ability efficiently and as exact as possible. If this issue could be solved, more EAB species involved in the biogeochemical cycles will be detected.

Many methods, such as dissimilatory Fe(III) reduction (Lovley and Phillips, 1988), humic substance reduction (Coates et al., 1998), microbial fuel cells-based electricity generation (Biffinger et al., 2009) and the electrochromic methods (Yuan et al., 2014), have been applied to examine and characterize EAB. Detailed results regarding the electron-donating ability of EAB were obtained. Nevertheless, these methods have often been reagent-intensive and time-consuming when performing quantitative measurements. To resolve these issues, a simple colorimetric method for rapidly determining the ability of EAB was proposed (Zhou et al., 2015). It utilized the high peroxidase activity of membrane *c*-type cytochromes to oxidize tetramethylbenzidine leading to a measurable color change (Zhou et al., 2015). However, this method was limited to the identification of only those EAB with extracellular reduction ability. Since the ability of extracellular reduction and

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oxidation had to both be included when monitoring EAB, the deficiency in the available methods would bring about a false result for those EAB with extracellular oxidation ability. Although the extracellular oxidation ability could be determined by the conventional dissimilatory Fe (II) oxidation method, it was still limited to screen more types of unknown bacteria in the natural environment. Therefore, developing an effective and inclusive method to identify EAB is urgently required.

Electrochemiluminescence (ECL), a powerful analytical technique, has been extensively studied in the biochemistry due to its high sensitivity and selectivity (Li et al., 2017). Different from chemical or biological luminescence, ECL is a process driven by electron-transfer reactions that form the excited states species to emit light (Bard et al., 2000). This feature makes ECL much suitable for the identification of EAB because it can be sensitively influenced by microbial EET process. In addition, in comparison to cell-free solution, the electrochemical reaction will be slower in the presence of EAB (Lin et al., 2018), and the resulting ECL signal will be stable and last for longer time, which is beneficial for ECL measurement.

Aerobic EABs not only consume O_2 to meet their metabolism but also have the ability of transferring electrons to extracellular electron acceptors. Moreover, the ECL signal of $Ru(bpy)_3^{2+}$ is sensitively affected by dissolved O_2 at relatively low concentration of tripropylamine (TPrA) (Zheng and Zu, 2005). In point of this view, we developed a simple ECL system for identification of EAB in both qualitative and quantitative manner. This method provides an O_2-sensitive ECL mechanism for measuring both microbial extracellular reduction and oxidation ability, avoids the false diagnosis to EAB with one kind of ability, which also exploits a new field for the application of ECL.

2. Materials and methods

2.1. Chemicals

Luria–Bertani (LB) broth, agar, peptone and yeast extract were purchased from Guangdong Huankai Microbial Sci. and Tech. Co., Ltd, China. $Ru(bpy)_3^{2+}$ and TPrA were purchased from Sigma. The other chemical reagents, such as KH_2PO_4, $FeSO_4{\cdot}7H_2O$, are of analytical grade (Sinopharm Chemical Reagent Co., Ltd, China) and all the solutions were made of ultrapure water (Milli-Q Labo, Nihon Millipore Ltd, Japan).

2.2. Cell cultivation

Shewanella oneidensis (*S. oneidensis*) MR-1 (You et al., 2015), *Shewanella putrefaciens* (*S. putrefaciens*) SP200 (Zhou et al., 2015), *Azospirillum humicireducens* (*A. humicireducens*) SgZ-5T (Zhou et al., 2013), *Escherichia coli* (*E. coli*, ATCC 25922), *Staphylococcus aureus* (*S. aureus*, ATCC 6538), were grown aerobically in LB broth with shaking at 120 rpm at 30 °C for 25 h according to our previous reports (Zhou et al., 2013, 2015; You et al., 2015). *Sulfobacillus acidophilus* (*S. acidophilus*, DSM 10332) was cultivated in the main medium followed as (L^{-1}): 0.2 g yeast extract, 980 mL H_2O, 20 mL of a 50 × heterotrophic basal salts solution (7.5 g of $Na_2SO_4{\cdot}10H_2O$, 22.5 g of $(NH_4)_2SO_4$, 2.5 g of KCl, 2.5 g of $MgSO_4{\cdot}7\ H_2O$, 2.5 g of KH_2PO_4, 0.7 g of $Ca(NO_3)_2{\cdot}4H_2O$ dissolved in 1 L H_2O), pH 2.0. 20 mL of a ferrous sulfate solution (278 g L^{-1}, pH 1.8), serving as an electron donor, was subsequently added into the main sterilized medium through 0.22 μm water filtration membrane. *S. acidophilus* were then cultured aerobically at 45 °C for 90 h. Afterward, these bacteria were harvested by centrifugation (5000 rpm, 5 min), rinsed three times with ultrapure water and finally resuspended in fresh 0.1 M phosphate buffer solution (PBS, pH 7.4).

2.3. Cell counting

The streak plate method was used to determine the population density of bacterial colony. The collecting *S. oneidensis* MR-1 cells were gradually diluted to final optical densities at 600 nm (OD_{600nm}) of $x \times 10^{-5}$ with sterile 0.8% NaCl solution, respectively (OD_{600nm} of 0.03 was diluted to 2000-fold, 0.02 and 0.01 were diluted to 1000-fold, 10^{-3} was diluted to 100-fold, 10^{-4} was diluted to 10-fold). 100 μL of the diluted bacterial suspension was spread on the solid LB agar plate (2 g L^{-1} LB and 1.5 g L^{-1} agar), and the colonies formed after 36 h incubation at 30 °C were counted.

2.4. Electrochemical and ECL tests

All electrochemical measurements were carried out with three-electrode system at room temperature by a CHI 660E electrochemical workstation (Shanghai Chenhua Instruments Co., Ltd., China). An Ag/AgCl (sat. KCl) electrode and a Pt wire served as reference electrode and counter electrode, respectively. If not specially noted, all the potentials mentioned in this work were in reference to Ag/AgCl. A glassy carbon electrode (GCE) was taken as working electrode. It was in turn polished in 1.0, 0.3, 0.05 μm Al_2O_3 powder and rinsed with ultrapure water several times before the experiment. It was swept in 0.1 M PBS + 1 mM $K_3[Fe(CN)_6]$ solution (pH 7.0) to check its surface cleanliness. To investigate the effect of microbes on the diffusion process of the $Ru(bpy)_3^{2+}$ - TPrA system from bulk solution to electrode surface, chronoamperometry was used and the *i*-t curves were recorded at 1.2 V for 500 s. Oxygen in the solutions was removed by bubbling nitrogen gas.

ECL measurement was performed on a BPCL-1-TIC Ultra Weak Luminescence Analyzer (Institute of Biophysics, Chinese Academy of Sciences) (Yang et al., 2016). A commercial 5 mL cylindroid quartz cell was used as the ECL cell. It was placed in front of the photomultiplier tube that was set at 800 V during the detection. A bare GCE working electrode was then transferred into 1490 μL $Ru(bpy)_3^{2+}$ - TPrA - PBS solution (pH 7.0) that containing 10 μL of 1 mM $Ru(bpy)_3^{2+}$, 10 μL of 5.28 M TPrA, 1 mL of 0.1 M PBS and 470 μL H_2O in the detection cell. 10 μL of the bacterial suspension was uniformly added into the above solution in reference to $Ru(bpy)_3^{2+}$ - TPrA - PBS solution as the control experiment.

2.5. Spectroscopic measurement

Fluorescence spectra (Hitachi F-4600, Japan; slit: 10 nm; PMT voltage: 440 V) of $Ru(bpy)_3^{2+}$ were measured in the PBS solution with or without different concentrations of *S. oneidensis* MR-1 cells.

3. Results and discussion

3.1. Electrochemistry and ECL of $Ru(bpy)_3^{2+}$ in different EAB solutions

$Ru(bpy)_3^{2+}$, one of the most popular ECL reagents, emits strong ECL signal in the presence of coreactant of TPrA (Bard et al., 2000; Kanoufi et al., 2001). As shown in Fig. 1a, the oxidation peak at 0.73 V in the cyclic voltammograms (CVs) for the $Ru(bpy)_3^{2+}$ - PBS solution only appeared in the presence of TPrA. The corresponding ECL intensity of $Ru(bpy)_3^{2+}$ significantly increased with the addition of TPrA when the potential scan ranged from −0.4 to 1.2 V (Fig. 1b). These results indicated that TPrA assisted the electro-oxidation process of $Ru(bpy)_3^{2+}$ on the surface of GCE electrode, which agreed well with former studies (Bard et al., 2000; Kanoufi et al., 2001).

Although complicated ECL mechanisms are proposed for the $Ru(bpy)_3^{2+}$ - TPrA system (Miao et al., 2002), the electron exchange between $Ru(bpy)_3^{3+}$ and TPrA makes the possible occurrence of energy transfer from EAB to oxidizing/reducing luminescent species (Schemes S1 and S2). Once *S. oneidensis* MR-1 cells ($OD_{600nm} = 6.7 \times 10^{-3}$) were added into the $Ru(bpy)_3^{2+}$ - TPrA - PBS solution (Fig. 1b), the ECL intensity increased about 2.4-fold and the onset potential of the ECL emission was ca. 0.05 V lower in *S. oneidensis* MR-1 than in the control experiment (see the inset of Fig. 1b). It was noted that no ECL signal

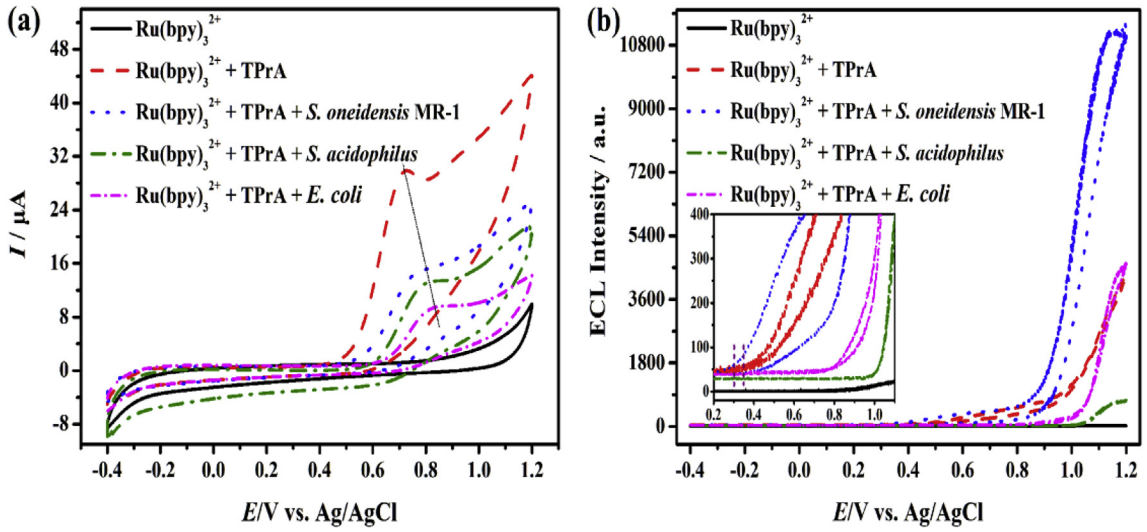


Fig. 1. CVs (a) and ECL (b) of a bare GCE electrode in the $Ru(bpy)_3^{2+}$ (solid line), $Ru(bpy)_3^{2+}$ - TPrA (dash line), $Ru(bpy)_3^{2+}$ - TPrA - *S. oneidensis* MR-1 (dot line), $Ru(bpy)_3^{2+}$ - TPrA - *S. acidophilus* (dash dot line) and $Ru(bpy)_3^{2+}$ - TPrA - *E. coli* (short dash dot line) PBS solutions; The insert is the image of ECL curves in limited potentials ranging from 0.2 to 1.1 V; scan rate = 50 mV s^{-1}; the concentration of cells: $OD_{600nm} = 6.7 \times 10^{-3}$.

was found coming from the TPrA - PBS solution with *S. oneidensis* MR-1 cells, indicating that the ECL response was generated from Ru $(bpy)_3^{2+\bullet}$ (Fig. S1). In contrast, only 14% of the initial ECL intensity was obtained for *S. acidophilus* in the $Ru(bpy)_3^{2+}$ - TPrA - PBS solution (dash dot line in Fig. 1b). No significantly increased ECL signal was detected in the presence of *E. coli* (short dash dot line in Fig. 1b) as the reaction went on. These distinct results might be due to their different EET abilities and this hypothesis could be confirmed by following indisputable facts. As shown in Fig. S2, the redox peaks of *c*-type cytochromes occurred in *S. oneidensis* MR-1 and *S. acidophilus* (Clark and Norris, 1996; You et al., 2015; Liang et al., 2016), but did not occur in *E. coli*. Moreover, according to previous reports (Clark and Norris, 1996; Liang et al., 2016), *S. oneidensis* MR-1 could reduce Fe(III) to Fe (II) and *S. acidophilus* was able to oxide Fe(II) to Fe(III), but *E. coli* could not. In addition, the oxidation peak current sharply dropped in the order of *S. oneidensis* MR-1, *S. acidophilus* and then *E. coli* (Fig. 1a), which was also owing to their EET abilities. As indicated in Fig. S3, *S. oneidensis* MR-1 having higher redox current and lower impedance in $[Fe(CN)_6]^{3-}$ solution presented a weaker obstacle to electron transfer than *S. acidophilus* and *E. coli* did. Meanwhile, the oxidation peak potentials positively shifted in the presence of cells (Fig. 1a), illustrating the existence of an interaction between the electro-oxidation process of $Ru(bpy)_3^{2+}$ - TPrA and microbes.

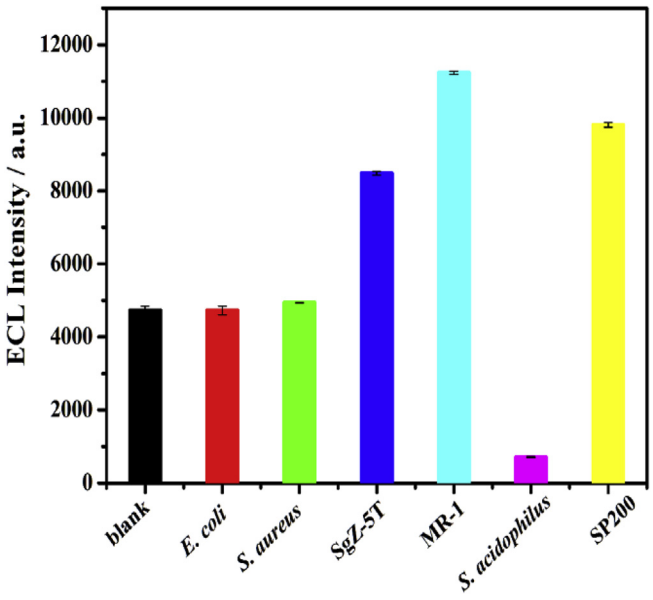


Fig. 2. Effect of different types of bacteria ($OD_{600nm} = 6.7 \times 10^{-3}$) on ECL intensity; $Ru(bpy)_3^{2+}$, 6.7 μM; TPrA, 30 mM; PBS, 67 mM; pH, 7.0.

3.2. Identification of EAB

After showing these above-mentioned bacteria had distinct ECL signals, we then examined whether more types of microbes could be identified by ECL. Not much increase in ECL intensity was expectedly found in the presence of *S. aureus* cells in comparison to the cell-free Ru $(bpy)_3^{2+}$ system (Fig. 2). *S. oneidensis* MR-1 displayed a higher ECL intensity than SP200 and SgZ-5T, which could mainly be attributed to their different EET abilities. These results were consistent with their ability of Fe(III) (ferrihydrite) reduction: *S. oneidensis* MR-1, SP200 and SgZ-5T produced 2.92 mM (Zhou et al., 2015), 2.52 mM (Zhou et al., 2015) and 0.63 mM Fe(II) (provided in Fig. S4) after 12 days of incubation with lactate as electron donor, respectively. In contrast, *S. acidophilus*, displayed the opposite effect on ECL intensity (Fig. 2). These findings demonstrated that ECL could be used to identify EAB. However, this observation then raised the question of how EAB interacted with $Ru(bpy)_3^{2+}$ - TPrA and further effected the ECL emissions.

3.3. ECL mechanism for EAB

To answer these questions, we chose *S. oneidensis* MR-1, to study their electron-transferring effect on the ECL emission of $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$ - TPrA. As shown in Fig. 3a, $Ru(bpy)_3^{2+}$ exhibited a low ECL intensity while the potentials shifted from −0.4 to 1.2 V. No significant change occurred regarding the ECL intensity with increased CVs scans. Upon addition of *S. oneidensis* MR-1 cells (Fig. 3b), an approximately 24% higher ratio of ECL intensity was produced by Ru $(bpy)_3^{2+}$ - *S. oneidensis* MR-1 compared to $Ru(bpy)_3^{2+}$ only, which might be result from a decrease in O_2. Similar results were also found in their fluorescence spectra as the intensity of the band centered at 622 nm, ascribed to $Ru(bpy)_3^{2+}$, increased as a function of the concentration of *S. oneidensis* MR-1 (Fig. 3c). It could be observed that *S. oneidensis* MR-1 did not show any fluorescence emission in the range from 555 to 780 nm. This enhancement might be also due to the decrease of dissolved O_2.

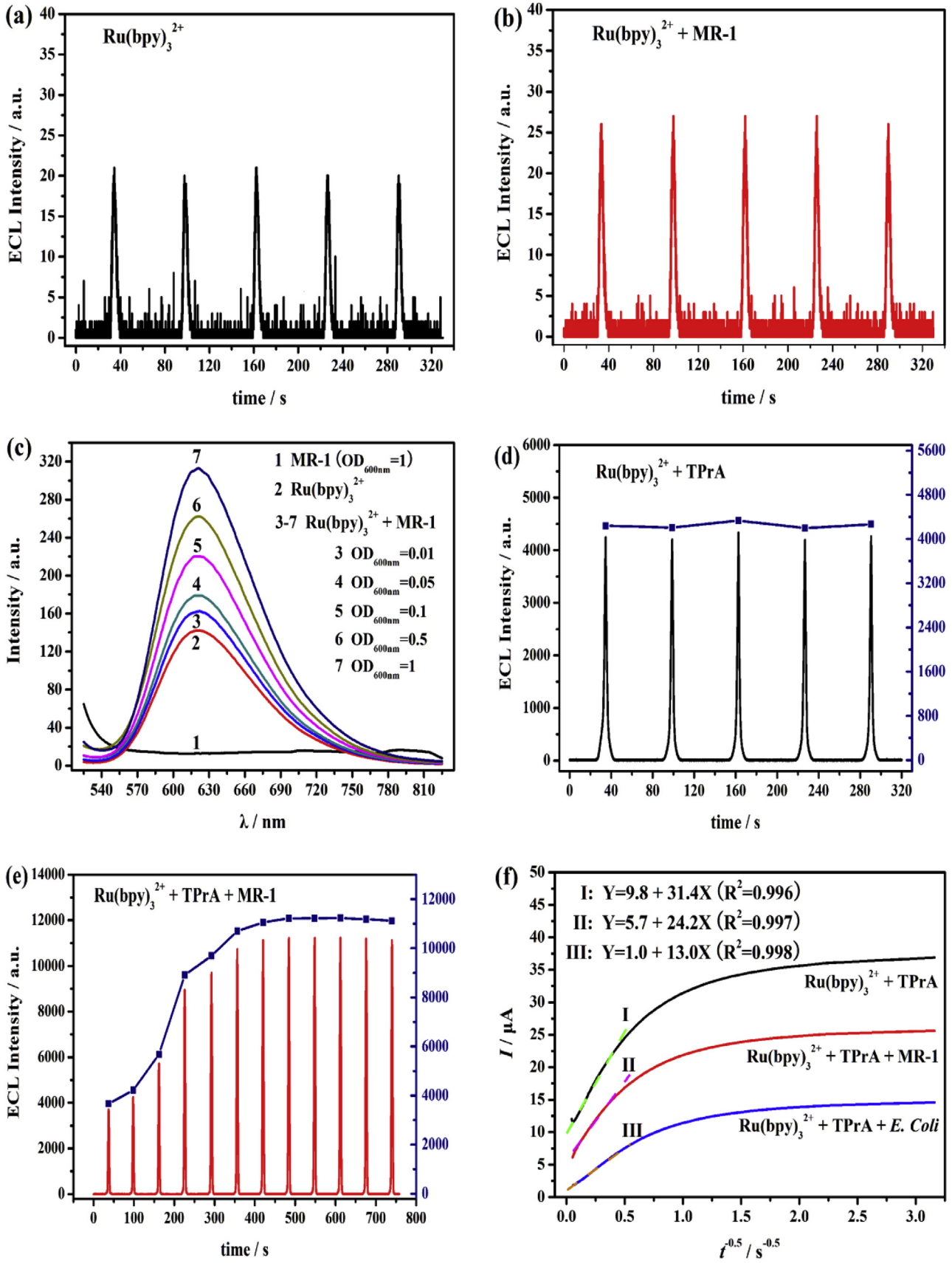


Fig. 3. ECL of a bare GCE electrode in the $Ru(bpy)_3^{2+}$ - PBS solution without (a) or with (b) *S. oneidensis* MR-1 cells; (c) Fluorescence spectra and intensity of Ru $(bpy)_3^{2+}$ before and after adding *S. oneidensis* MR-1 cells as functions of concentration (OD_{600nm}: 1–0.01); excited wavenumber: 465 nm; (d,e) ECL of a bare GCE electrode in the $Ru(bpy)_3^{2+}$ - TPrA - PBS solution without (d) or with (e) *S. oneidensis* MR-1 cells; (f) *i*-t curves of bare GCEs in the $Ru(bpy)_3^{2+}$ - TPrA - PBS solution with or without microbes; initial potentials: 1.2 V; the value of OD_{600nm} was adjusted to 6.7×10^{-3}; three repetitions.

When TPrA was added into the $Ru(bpy)_3^{2+}$ - PBS solution, the ECL intensity changed little (Fig. 3d). When *S. oneidensis* MR-1 was present in the $Ru(bpy)_3^{2+}$ - TPrA - PBS solution, the ECL intensity first increased rapidly and then achieved a stable platform after 6 repeated CVs scans (Fig. 3e). It was immediately reduced to zero after the applied potential was removed (Fig. S5). More interestingly, the resulting stable ECL intensity lasted for several potential scans. Similar results were also observed in the $Ru(bpy)_3^{2+}$ - TPrA - PBS solution with *E. coli* cells (Fig. S6). These phenomena occurred only in the presence of TPrA, suggesting that the oxidation process of TPrA was largely hindered by microbes, leading to a strongly diffusion-limited process. By calculating the slopes from $i\text{-}t^{-0.5}$ curves according to Cottrell equation (1) and (2) (von Stackelberg et al., 1953), control experiments exhibited a higher value than those with *S. oneidensis* MR-1 and *E. coli* cells (Fig. 3f), revealing that *S. oneidensis* MR-1 was a weaker obstacle to diffusion than *E. coli*. This result was in accordance with the observation in CVs depicted in Fig. 1a.

$$i(t) = \frac{nFAD^{1/2}C^*}{\pi^{1/2}t^{1/2}} \quad (1)$$

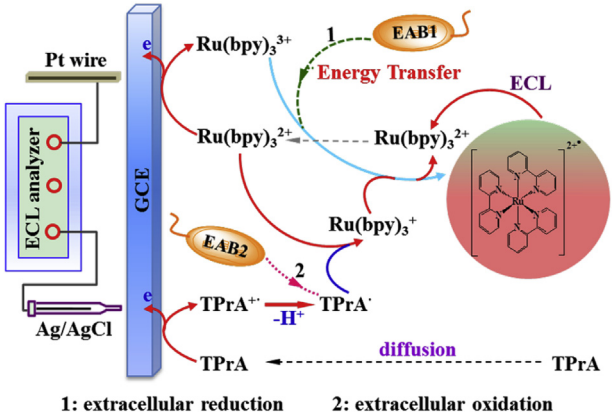
And

$$D = \frac{i(t)^2\pi}{(t^{-0.5})^2(nFAC^*)^2} = \frac{(slope)^2\pi}{(nFAC^*)^2} \quad (2)$$

Where *F*, *A* and *C** were Faraday's constant, electrode area and the bulk concentration of the redox species, respectively. n and D were the stoichiometric number of transferring electrons and the diffusion coefficient of the redox species.

According to the results mentioned above, we proposed that EAB might affect the $Ru(bpy)_3^{2+}$ - based ECL through an O_2 effect, energy transfer and diffusion limitation (Scheme 1). At the beginning of the reaction, EAB hindered the diffusion of TPrA from bulk solution to electron surface as far as its oxidation process, which thereby reduced the formation of $Ru(bpy)_3^{+}$. However, this diffusion-limited restriction was remarkably not found on the oxidation of $Ru(bpy)_3^{2+}$. EAB with an ability of extracellular reduction (such as MR-1), might react with the electrogenerated species $Ru(bpy)_3^{3+}$ to generate $Ru(bpy)_3^{2+}$ using a relatively slow reaction rate. Meanwhile, O_2 was consumed by EAB at a relatively slow rate. As a result, less amount of $[Ru(bpy)_3^{2+}]^{\bullet}$ would be generated by the interaction of $Ru(bpy)_3^{+}$ with insufficient $Ru(bpy)_3^{3+}$ species. Thus, the recorded ECL intensity was lower than that of cell-free system.

As the potential scans went on, increased amounts of intermediate reactive radicals were produced, and the dissolved O_2 within the ECL reaction layer was gradually reduced by MR-1. It was not negligible



Scheme 1. Mechanism of the $Ru(bpy)_3^{2+}$ - based ECL for the identification of EAB; EAB with the ability of extracellular reduction gave an enhanced signal on ECL emission while those displaying the ability of extracellular oxidation showed decreased ECL intensity, and non-EAB did not.

that MR-1 could transfer electrons to oxidizing species to change the ECL signal. While the amount of electrogenerated $[Ru(bpy)_3^{2+}]^{\bullet}$ was equal to its own consumption under the synergistic effect of O_2 and microbial EET ability, the highest ECL intensity was achieved and lasted for a longer time. On the contrary, EAB with the ability of extracellular oxidation (i.e., *S. acidophilus*) were assumed to react with strongly reducing species $[Ru(bpy)_3^{2+}]^{\bullet}$ or $TPrA^{\bullet}$, thus leading to a decrease in the ECL intensity.

3.4. Quantitative measurement of EAB

Quantitative detection was also important for the identification of EAB because the concentration of $Ru(bpy)_3^{2+}$ and the cell density could have an effect on the ECL intensity. Since most of the studied microbes were grown under neutral conditions, the pH value in ECL measurement was selected at 7.0. The ECL intensity increased with the concentration of $Ru(bpy)_3^{2+}$ when raised from 6.7 to 33.5 μM (Fig. 4a). The corresponding linear regression equation was determined as $I = -226.0 + 47.5$ C with the regression coefficient (R^2) of 0.997. In order to avoid an ECL intensity beyond the detection range, 6.7 μM Ru $(bpy)_3^{2+}$ was chosen, which could satisfy the sensitivity of ECL detection. It could be shown that the ECL intensity of $Ru(bpy)_3^{2+}$ - TPrA system increased as a function of the change in *S. oneidensis* MR-1 concentration in the range of 6.7×10^{-4} – 6.7×10^{-3} (Fig. 4b). Different from common chemical reactions, ECL responses of $Ru(bpy)_3^{2+}$ to *S. oneidensis* MR-1 displayed a key point where the ECL intensity was equal to the initial ECL intensity. It was noted that this point was different for microbes, i.e. OD_{600nm} of 2.7×10^{-3} for *S. oneidensis* MR-1, 1.3×10^{-3} for SgZ-5T and 6.7×10^{-3} for *E. coli* (Figs. S7 and S8). However, this point was not found for *S. acidophilus* (Fig. S9). To further evaluate the bacterial effect on the ECL intensity, we took the intensity difference $\Delta I = I - I_0$ as an index according to previous reports (Zhang et al., 2018), where I and I_0 were the ECL intensity with and without *S. oneidensis* MR-1 cells, respectively. As indicated in Fig. 4c, ΔI was significantly correlated linearly with *S. oneidensis* MR-1 concentration in the OD range of 6.7×10^{-4} – 6.7×10^{-3}. The linear regression equation was $\Delta I = -4297.9 + 1650.9$ C* (where C* indicated the 1000-fold value of OD_{600nm}), with a value of $R^2 = 0.998$ (Fig. 4c). The concentration between 6.7×10^{-4} and 1.3×10^{-4} was also measured and an equation of $\Delta I = -4858.6 + 2955.7$ C* ($R^2 = 0.999$) was obtained (Fig. S10). Interestingly, the value of ΔI was higher than zero only when the cell density was over 2.7×10^{-3}, otherwise it was lower than zero. The occurrence of these phenomena was due to the synergistic effect of O_2 and microbial EET ability.

To sensitively measure low concentrations of *S. oneidensis* MR-1 cells, we increased the concentration of $Ru(bpy)_3^{2+}$ to 16.75 μM. A similar dependence of ECL intensity on *S. oneidensis* MR-1 concentration increasing from 6.7×10^{-7} to 2×10^{-4} was also observed (Fig. 4d). The linear regression equation here was $\Delta I = -8865.2 + 20758.8$ C* and the value of R^2 was achieved at 0.998 (Fig. 4e). The detection limit in this approach was 212 ± 2 CFU/mL ($OD_{600nm} = 6.7 \times 10^{-7}$) which could be calculated from the linear regression equation shown in Fig. 4f. Considering that only the living cells were detected in the streak plate method, the cell detection limit was lower. In addition, this method was also suitable for the EABs transferring electrons only via mediators, i.e., *Pseudomonas aeruginosa* PA01 (Fig. S11). Compared with earlier reports using colorimetry (Wen et al., 2014; Zhou et al., 2015), this method offers an excellent detection performance between $(1.1 \pm 1) \times 10^5$ and 212 ± 2 CFU/mL toward *S. oneidensis* MR-1. Only a low cell density was needed to be used in this proposed method, which is of benefit for the rapid cell detection especially for those slow-growing EAB.

4. Conclusions

In summary, we developed a simple ECL assay for the identification

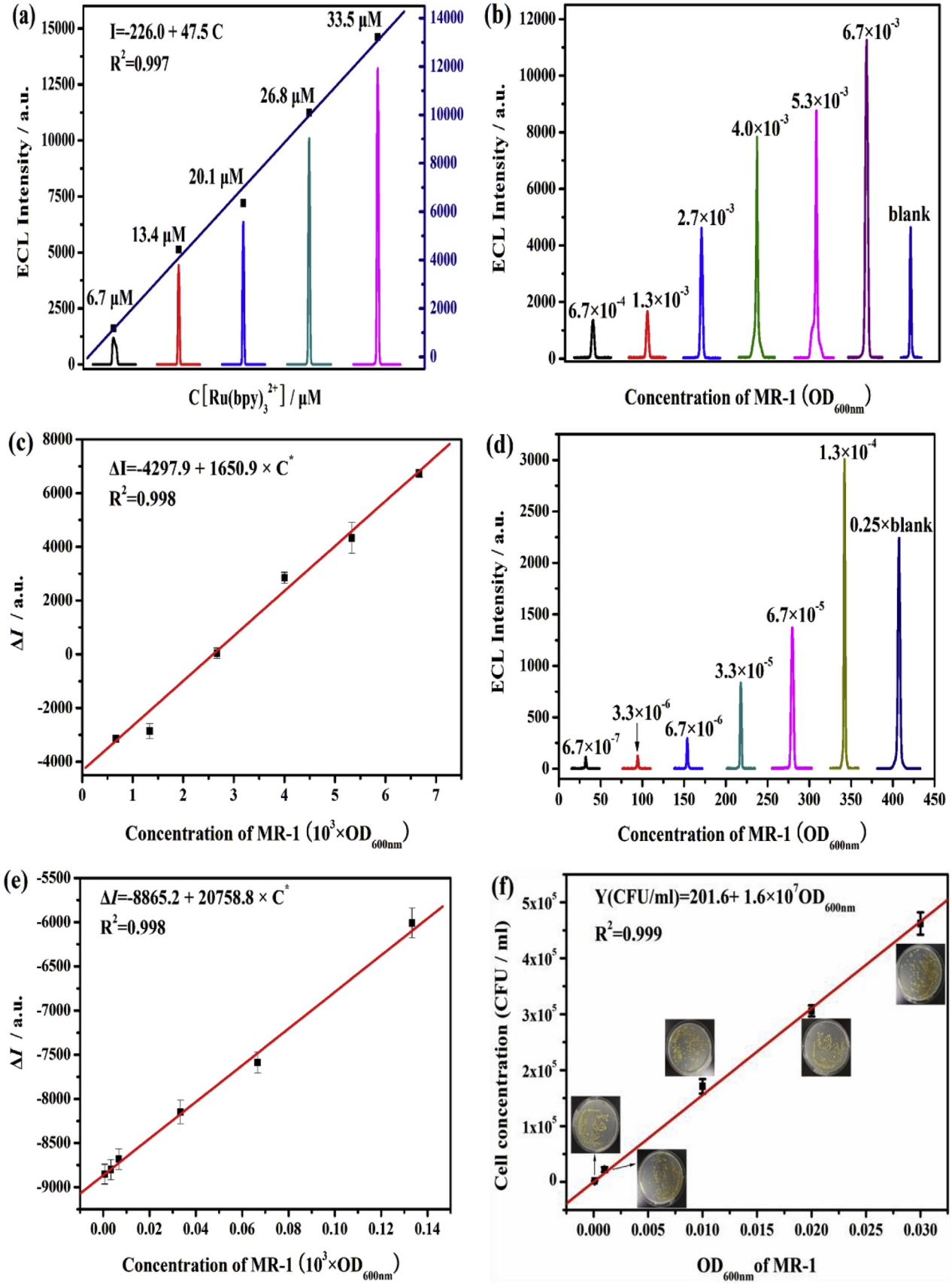


Fig. 4. (a) Effect of the concentration of $Ru(bpy)_3^{2+}$ on ECL intensity in the presence of diffusion-limited (OD_{600nm} = 6.7 × 10^{-4}); $Ru(bpy)_3^{2+}$, 6.7 μM. (b,d) ECL intensities of $Ru(bpy)_3^{2+}$ system as functions of diffusion-limited concentration and (c,e) calibration curves between ECL intensity and diffusion-limited concentration; (b,c) OD_{600nm}: 6.7 × 10^{-4} – 6.7 × 10^{-3}, $Ru(bpy)_3^{2+}$: 6.7 μM; (d,e) OD_{600nm}: 6.7 × 10^{-7} – 1.3 × 10^{-4}, $Ru(bpy)_3^{2+}$, 16.75 μM. TPrA, 30 mM; PBS, 67 mM; pH, 7.0; (f) the relationship between OD_{600nm} and population density of cells.

and detection of EAB. Based on the synergistic effect of O_2 and microbial EET ability, EABs with different EET ability could be qualitatively identified. Good linear relationships between ECL intensity and cell concentration were established in this assay. Compared to previous work on only Fe(III)-reducing bacteria, this study displayed advantages in the identification of extracellular reduction and oxidation ability from EAB. However, limitations also existed in the detection mode in this ECL assay (i.e., one sample for each time) and the false results may be caused by the cytochromes released from the lysed cells. In the future, high-throughput ECL techniques for cells measurement will be developed to identify EAB.

CRediT authorship contribution statement

Le-Xing You: Project administration, Writing - original draft. **Nian-Jia Chen:** Data curation. **Lu Wang:** Project administration, Investigation. **Jie Chen:** Formal analysis, Validation. **Su-Fang Qin:** Formal analysis, Validation. **Christoper Rensing:** Writing - review & editing. **Zhen-Yu Lin:** Methodology. **Shun-Gui Zhou:** Methodology.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.04.062.

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