Direct electrochemistry and electrocatalytic mechanism of evolved *Escherichia coli* cells in microbial fuel cells†

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***E. coli* cells evolved under electrochemical tension in a microbial fuel cell possess direct electrochemical behavior due to the excretion of hydroquinone derivatives through a highly permeable outer membrane, and their catalyzed fuel cell demonstrates excellent performance.**

The microbial fuel cell (MFC) is a promising clean energy source for a sustainable power supply.[1–5] The mediatorless MFC is very attractive because of its advantages of high energy conversion efficiency and low manufacturing cost. Metal reducing microorganisms of *Geobacter*, *Rhodoferax* and *Shewanella* in the mediatorless MFC are reported to directly transfer electrons to the electrode in the electrocatalytic process.[1–3] Two models have been proposed to explain the electron transfer process in the mediatorless MFC. One is direct electron transfer *via* a physical contact between the bacterial outer membrane or membrane appendages and the electrode surface. Some *Geobacter* and *Shewanella* strains can evolve electronically conducting molecular pili to facilitate distant electron transfer.[2,3] Another model involves a secondary metabolites (endogenous redox mediators)-mediated electron transfer process, of which an example is that pyocyanine and phenazine-1-carboxamide produced by *Pseudomonas aeruginosa* mediate the electron transfer to the MFC anode.[6]

Recently a mediatorless MFC with *E. coli* as a biocatalyst has been reported by Zhang *et al.*[7,8] A natural selection process is proposed in their work to explain the direct electrochemistry of the evolved *E. coli* and possible mediator molecules excreted by *E. coli* cells are reported. A similar phenomenon is suggested by Wang *et al.*,[9] in which an *E. coli*-excreted redox compound works as a mediator for the electron transfer. However, the direct electrochemistry of *E. coli* cells has not been conducted systematically for explanation of the direct electron transfer process of the cells. In this work, we investigated the direct electrochemical behavior of MFC-evolved *E. coli* cells. The cells evolved through the electrochemical tension during fuel cell operation. We have discovered that a hydroquinone type endogenous compound produced by the evolved cells is responsible for the direct electrochemical redox behavior of *E. coli* cells and propose a membrane related mechanism for the quinone excretion. A mediatorless MFC is also fabricated to demonstrate its excellent electrocatalytic performance.

Original *E. coli* K-12 (ATCC 29181) was grown anaerobically at 37 °C for 12 h in a standard glucose medium, which was a mixture of 10 g glucose, 5 g yeast extract, 10 g $NaHCO_3$ and 8.5 g NaH_2PO_4 per litre. The bacterial culture was harvested by centrifugation at 6000 rpm at 4 °C and then suspended in 0.1 M phosphate buffer after washing three times. The concentration of *E. coli* cells was about 10^9 cells mL^{-1}. MFC-evolved *E. coli* K-12 cells were obtained from the long term discharged MFC in our previous study[4] and cultured in the same conditions as those used for the original cells. Electrochemical experiments were conducted in a three-electrode cell with Ag/AgCl (saturated KCl), carbon cloth (E-TEK, B1D, plain) and platinum film as the reference, working and counter electrode, respectively. Before each test, the whole cell was incubated anaerobically at 37 °C for 1 h to reach equilibrium. Fig. 1a shows a pair of well-defined redox waves on the cyclic voltammogram (CV) of evolved cells but no redox waves from the original cells. The ratio of anodic peak current to cathodic peak current produced from evolved cells is about 1 and peak potential is independent of the scan rate, indicating a reversible redox reaction behavior. The peak potential separation is 30 mV, which is equal to the theoretical value for a two-electron reversible reaction. The linear relationship between peak current and square root of scan rate (Fig. 1b) reveals that the electrochemical redox reaction is a diffusion controlled process. This indicates that the direct electrochemistry of evolved *E. coli* cells involves a diffusive, two-electron transfer redox species. CVs of evolved cells show a strong dependence on solution pH value (Fig. 1c). Both oxidation and reduction peaks shift negatively with the increase of pH, indicating a proton is involved in the redox reaction. The slope for the linear regression equation is −60.7 mV, suggesting that the number of electrons transferred in the redox reaction equals the number of protons involved. All the results discussed above reveal that the direct electrochemistry of the cells is enabled by a diffusive redox species, which should be excreted from the evolved cells. Relations of the growth of evolved *E. coli* cells and the corresponding anodic peak current *vs.* time are shown in Fig. 1d. The anodic current increases with the increment of the cell density in the first 24 h growing stage, and then the cell density remains almost constant (stationary phase) but the oxidation current still increases until 60 h to a plateau value, which may indicate that the excretion process to produce the

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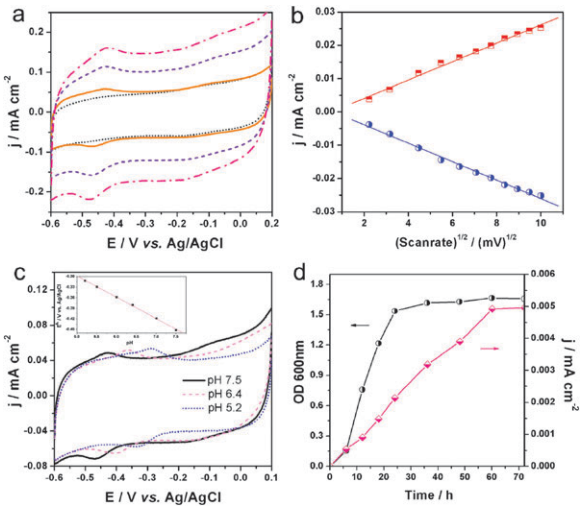

Fig. 1 Electrochemical behavior of *E. coli* cells. a: Cyclic voltammograms of original *E. coli* cells at 30 mV s^{-1} (dot) and evolved *E. coli* cells at 5 mV s^{-1} (solid), 20 mV s^{-1} (dash), 80 mV s^{-1} (dash–dot). b: Function of peak current *vs.* (scan rate)$^{1/2}$. c: Cyclic voltammograms of evolved *E. coli* cells in phosphate buffers with different pH values. The inset is the relationship between $E^{0\prime}$ and pH. d: Variation of cell density and oxidation current of evolved *E. coli* cells during anaerobic growth.

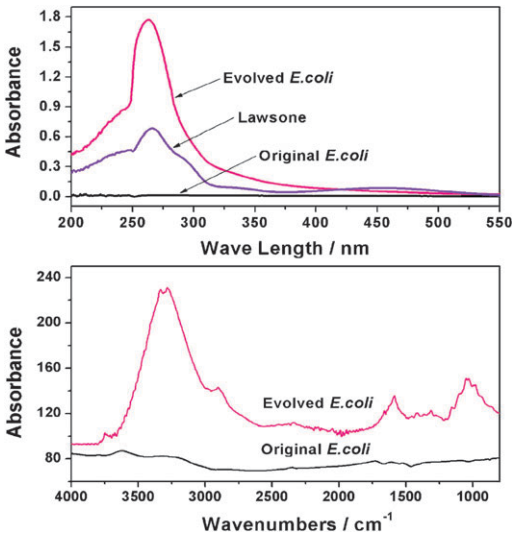

Fig. 2 UV–Vis absorption and FTIR spectra of different *E. coli* cells.

diffusive redox species continues even after the cells stop growing. When evolved *E. coli* cells were removed from the electrolyte, the supernatant still exhibited similar redox behavior (data not shown here). This may be direct and solid evidence that the direct electrochemistry is caused by certain kinds of endogenous mediators produced from evolved *E. coli* cells rather than the cell membrane or appendages. The endogenous redox mediator can serve as a reversible terminal electron acceptor to transfer electrons from the bacterial cell to the MFC anode, especially in batch cultures, effectively facilitating the electron transfer.[10]

Bacterial cells can synthesize redox compounds such as cytochromes and quinones, the mobile electron carriers in electron transport chains.[11] It is difficult to identify through CV experiments which of them is responsible for the electron transfer of *E. coli* cells. Cytochrome c3, which has low redox potential ($E^{0\prime} = -233$ mV), has been isolated from anaerobically grown *Shewanella putrefaciens*.[12] Some quinones are also widely used in MFCs as low-redox-potential mediators. The pH dependent electrochemical behavior of the evolved cells is similar to that of quinone–hydroquinone redox couples. To determine what kind of molecule plays the key role in the direct electron transferring behavior, UV–Vis and FTIR spectra of the compounds in the supernatant were examined. Fig. 2 shows UV–Vis spectra and FTIR spectra of the supernatant isolated from the electrochemical cells. The supernatant of the original *E. coli* cells has no obvious signal over the background in both spectral measurements. The supernatant of the evolved cells displays strong absorption at 263 nm in the UV–Vis spectrum, indicating the aromatic structure of the compound. In comparison to the spectrum of 2-hydroxy-1,4-naphthoquinone (HNQ, lawsone, 50 nM), a widely used exogenous mediator, the supernatant of the evolved cells exhibits almost the same peak as that of HNQ near 263 nm. There is no absorption in the visible area, suggesting the supernatant may not contain cytochromes. The strong band at 1590 cm^{-1} in the FTIR spectrum indicates the vibration of the C=C stretch in the aromatic ring. The broad strong peak at 3284 cm^{-1} should be assigned to –OH stretching. The characteristic bands (–C=O–) of quinone located over the frequency range of 160–1800 cm^{-1} are not shown in the spectrum. This suggests that the redox compounds in the supernatant might be hydroquinones rather than quinones. Combining the electrochemical data and results of UV–Vis and FTIR spectra, we can conclude that the compounds in the supernatant should be hydroquinone derivatives. According to the the absorption value at 263 nm, it can be estimated that the concentration of the hydroquinone derivatives is around 150 nM. Further investigations are needed to confirm the exact chemical structures of the redox compounds.

For bacterial cells, quinones and hydroquinones are always in the quinone pool located at the cytoplasm membrane. The redox state of the quinone pool depends on the aeration and in the absence of oxygen, the quinone pool is reduced. Thus the hydroquinones are dominating molecules in the quinone pool. This is in accordance with the results described above. Although hydroquinones can diffuse freely in the membrane and periplasmic space, it is difficult for them to move across the cell wall. Evolved *E. coli* cells were observed to excrete endogenous hydroquinone derivatives while the original cells could not. There must be a pathway for the quinones to move through the cell wall. To investigate the mechanism, we examined the surface of *E. coli* cells with an atomic force microscope (AFM, SPM 3100, Veeco Instruments Inc., USA). 100 μL bacteria suspension were dropped onto the mica surface. After 10 min incubation at room temperature, the substrate was rinsed with distilled water three times, followed by drying with a stream of N_2. All AFM measurements were carried out in the tapping mode at ambient temperature in air. The micrographs of the cell surfaces show that the evolved cell has a much rougher surface morphology than the original cell (Fig. 3). Some large clusters appear on the surface of evolved cells with pores deeper and larger than those of the original cell. The SEM micrographs of both cells display the same phenomena (see ESI†). From section curves, it can be observed that pores on the surface of evolved *E. coli* cells are

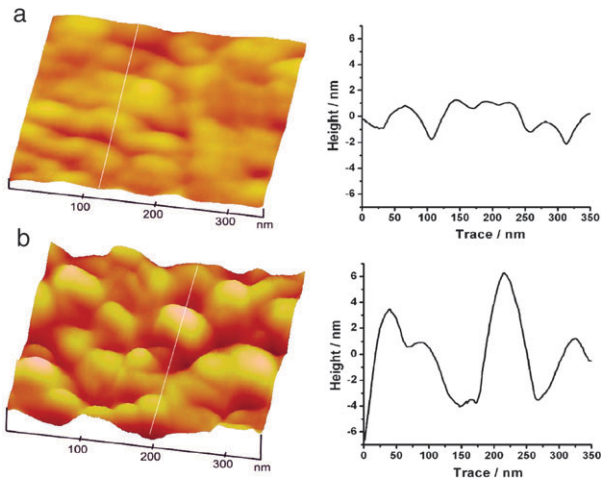

Fig. 3 AFM topograph and section analysis of original (a) and evolved (b) *E. coli* cell.

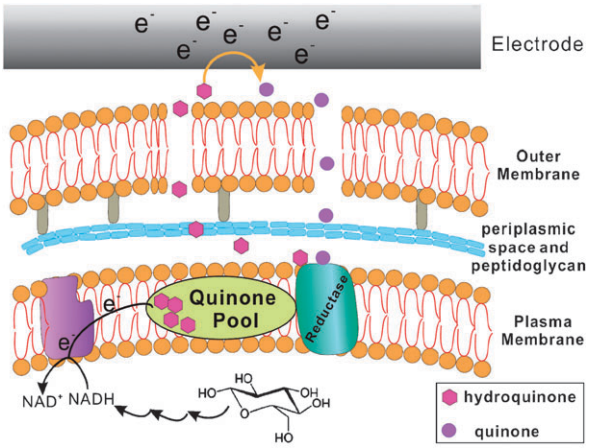

Fig. 4 A hypothetical mechanism for extracellular electron transport of evolved *E. coli* cells.

about 5–7 nm in depth. The cell wall of an *E. coli* cell is generally 9–10 nm thick, and consists of a 2–3 nm peptidoglycan inner wall and a 7 nm outer membrane. It is possible that the outer membrane of evolved cells has changed under long term electrochemical tension in the environment and the pores have enhanced permeability to hydroquinones. Thus excreted hydroquinones can reach the electrode surface to transfer electrons through the membrane.

In the electron transport chain of bacteria quinones accept electrons from donors such as NADH and pass electrons to acceptors such as O_2 (aerobic) or fumarate, NO_2, NO_3 (anaerobic). In an anaerobic electrochemical cell only containing phosphate buffer solution, there is no electron acceptor for *E. coli* cells. The accumulated hydroquinones can only deliver electrons to the electrode surface. This may be the reason for the electrochemical redox behavior of *E. coli* cells in phosphate buffer even without glucose. When glucose was added to the suspension, it increased the oxidative current and enhanced the electron transfer rate (see ESI†). Apparently, this can be attributed to the increment of electron donors – NADH, the product of glucose oxidation under catalysis in *E. coli* cells, which enhances the mediated electron transfer rate through hydroquinone redox reactions. Reduction of quinones in the plasma membrane is accompanied by dehydrogenation of NADH,[13] and thus the concentration of NADH is the limiting factor for the quinone redox reaction. This indicates that the electron flow in this system might be: NADH → quinone → hydroquinone → electrode. The electro-oxidized quinones can move into cells and be reduced by quinone reductase[14] located at the plasma membrane to become hydroquinones again. The possible quinone redox cycle and electron transport path in this membrane-electrode system is illustrated in Fig. 4. Hydrophilicity of the derivative is important for the cross-membrane-diffusion and related reductases in the redox reaction need to be identified in further investigations.

To evaluate the performance of these evolved *E. coli* cells in MFC, a mediatorless MFC with the same configuration as in our previous study[4] was constructed and the nanostructured polyaniline–titanium dioxide composite was used as anode materials. This mediatorless MFC delivered a maximum power density of 1300 mW m^{-2} (see ESI†), corresponding to a current density of 3390 mA m^{-2} at a cell potential of 340 mV, which is much higher than previously reported for an *E. coli* mediatorless MFC[15] and comparable with the original *E. coli*/HNQ MFC.[4] Considering the concentration of HNQ might be much higher than that of the endogenous mediators produced by evolved *E. coli* cells as previously described, these endogenous mediators can efficiently enhance the electron transfer rate between the electrode and *E. coli* cells.

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