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# Single Electrochemical Impacts of *Shewanella oneidensis* MR-1 Bacteria for Living Cells Adsorption onto a Polarized Ultramicroelectrode Surface

Hassiba Smida,[a] François-Xavier Lefèvre,[a] Christine Thobie-Gautier,[a] Mohammed Boujtita,[a] Catarina M. Paquete,[b] and Estelle Lebègue*[a]

Single electrochemical impacts of *Shewanella oneidensis* MR-1 Gram-negative electroactive bacteria onto ultramicroelectrode surfaces are reported and in-depth analysed. Chronoamperometry measurements recorded onto 10 µm-diameter Pt and 7 µm-diameter carbon fibre disk ultramicroelectrodes in a 20 mM potassium ferrocyanide aqueous solution in the presence of living bacteria show an electrostatic attraction of *Shewanella* cells onto the ultramicroelectrode surface polarized at +0.8 V vs Ag/AgCl. Single current step events analysis and atomic force microscopy experiments confirm the adsorption of living bacteria following the collision onto the ultramicroelectrode surface. The bacteria washing step before the chronoamperometry measurements leads to lower current step events related to the smaller size of the living cells. The electrostatic attraction of the negatively charged bacteria onto the positively charged ultramicroelectrode surface polarized at the oxidation potential of ferrocyanide is clearly demonstrated.

## Introduction

These last years, different innovative analytical methods with high sensitivity and spatial-temporal resolution allowing qualitative and quantitative analysis at single-cell and subcellular levels have emerged.[1] The four major areas of analytical methods usually reported are electrochemical analysis, super-resolution microscopy, mass spectrometry imaging, and microfluidics.[1] The crucial and unique advantage of electrochemistry is to combine high sensitivity and easy handling with a light and portable instrumentation allowing to work with miniaturized devices for micro-biosensor applications.[2–4] In this way, the electrochemistry of discrete impacts onto ultramicroelectrode (UME) gives the opportunity of electrochemical analysis at single-cell level with high sensitivity.[1,5–7] Fast and easy handling instruments for detection of bacteria at the single cell scale (the highest possible sensitivity) have become reachable with the promising electrochemistry of single impacts onto a microelectrode surface.[7–12] Although this method is not efficient for selective bacterial detection yet, the observation of these single events can provide information on various individual nanoparticles in contrast to ensemble (bulk) measurements.[13–17] The main advantage of studying collisions of single entities is the low limit of detection (in principle, one single species) inherent to this electroanalytical method and the ability to study various single entities (e.g. cells, viruses, nanoparticles) in real time (dynamic measurement).[12,18–22]

Also, this approach is advantageous because it allows the direct characterization of the electrical interaction between a single bacterium and an UME under controlled conditions, in the absence of a biofilm (UME surface biofouling), and also minimizes the effect of secreted materials on the sensitivity of the electrochemical signal.[23] The recording of single collisions events in the chronoamperometry curve corresponding to bacteria impacts onto the UME surface is performed in an aqueous solution usually containing a redox active probe and about $10^{10}$ bacteria per milliliter.[10,12] The collision event signal observed is usually a current step when one bacterium impacts the UME, corresponding to a blocking effect because of the bacterium adsorption onto the UME surface.[8–10,12,24] In these conditions (presence of an aqueous redox probe and bacteria), the response signal expected from single bacterium collision event may also be a current spike corresponding to either the own electrochemical activity of the bacterium toward the redox probe and the UME applied potential or a bouncing effect of the bacterium which does not stick onto UME surface.[9,11,12,24,25]

To better analyse the bacteria adsorption process onto the UME surface during single electrochemical impact measurements, here we present optimized experiments of *Shewanella oneidensis* MR-1 (SOMR1) Gram-negative electroactive bacteria adsorbed onto carbon fibre (CF) and Pt UME surfaces polarized at the oxidation potential of ferrocyanide (+0.8 V vs Ag/AgCl)

[a] Dr. H. Smida, F.-X. Lefèvre, Dr. C. Thobie-Gautier, Dr. M. Boujtita, Dr. E. Lebègue
Nantes Université, CNRS
CEISAM, UMR 6230
F-44000 Nantes, France
E-mail: estelle.lebegue@univ-nantes.fr
[b] Dr. C. M. Paquete
Instituto de Tecnologia Química e Biológica António Xavier
Universidade Nova de Lisboa
Av. da República, 2780-156 Oeiras, Portugal

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during electrochemical impact experiments (Figure 1). Based on our expertise in single impact electrochemistry, electroactive bacteria and on our previous preliminary work,[12,26–30] here we demonstrate that the frequency of living bacteria collisions, which is related to cells concentration (in conditions where the mass transport is controlled by diffusion), is in agreement with the electrostatic attraction of negatively-charged bacterial cells to the electric field of the UME surface polarized at +0.8 V vs Ag/AgCl. Atomic force microscopy (AFM) post-characterization of the UME surface confirms the bacteria adsorption (Figure 1), demonstrating that electrostatic attraction has a significant importance in the collision process of living bacterial cells to the positively charged UME surface (polarized at the oxidation potential of ferrocyanide).

## Results and Discussion

The applied potential for the chronoamperometry measurements performed in 20 mM $K_4Fe(CN)_6$ and 0.1 M PBS at pH 7.4 aqueous solution is +0.8 V vs Ag/AgCl (3 M KCl), corresponding to the steady-state current of the aqueous redox probe at CF and Pt disk UMEs (radial diffusion) as determined by cyclic voltammetry (Figure S1).[31] The theoretical steady-state current $i_{ss}$ (oxidation of $Fe(CN)_6^{4-}$), is given by Equation 1 below.[31]

$$i_{ss} = 4nFDr_eC_b \quad (1)$$

In Equation 1, $n$ is the electron stoichiometry ($n=1$), $F$ is Faraday's constant, $D$ is the diffusion coefficient of redox species ($6.7\times10^{-6}$ cm$^2$ s$^{-1}$ for $Fe(CN)_6^{4-}$),[32] $r_e$ is the UME disk radius ($r_e$(CF) = 3.5 µm and $r_e$(Pt) = 5 µm), and $C_b$ is the bulk concentration of redox species ($C_b$ = 20 mM). The steady-state current values observed in the cyclic voltammograms reported in Figure S1 (15 nA at CF UME and 23 nA at Pt UME) are in agreement with the theoretical limiting plateau current calculated from Equation 1 ($i_{ss}$(CF) = 18 nA and $i_{ss}$(Pt) = 26 nA).

In the absence of bacteria in solution, no current step is observed over a 600 s polarization time at +0.8 V vs Ag/AgCl in the blue *i-t* curve recorded at UME (Figure 2A and Figure S2A), as expected. In contrast, after addition of $10^9$ cells of SOMR1 bacteria sample in solution with $K_4Fe(CN)_6$ as redox probe (red *i-t* curve, Figure 2A), the stair step current response is detected and assigned to single bacteria impacts onto the UME surface, which locally block the flux of $Fe(CN)_6^{4-}$ to the electrode surface. The shape of the current step events is similar in the red *i-t* curves recorded at CF (Figure 2A) and Pt (Figure S2A) UMEs indicating that the type of electrode used is not a crucial parameter for single SOMR1 collisions in ferrocyanide. Note that only current steps with a current magnitude at least three times


**Figure 1.** Single electrochemical impacts of *Shewanella oneidensis* MR-1 onto an ultramicroelectrode (UME) polarized at the oxidation potential (+0.8 V vs Ag/AgCl) of the ferrocyanide redox probe in solution and post-characterization of the living bacteria adsorption onto the UME surface by atomic force microscopy.

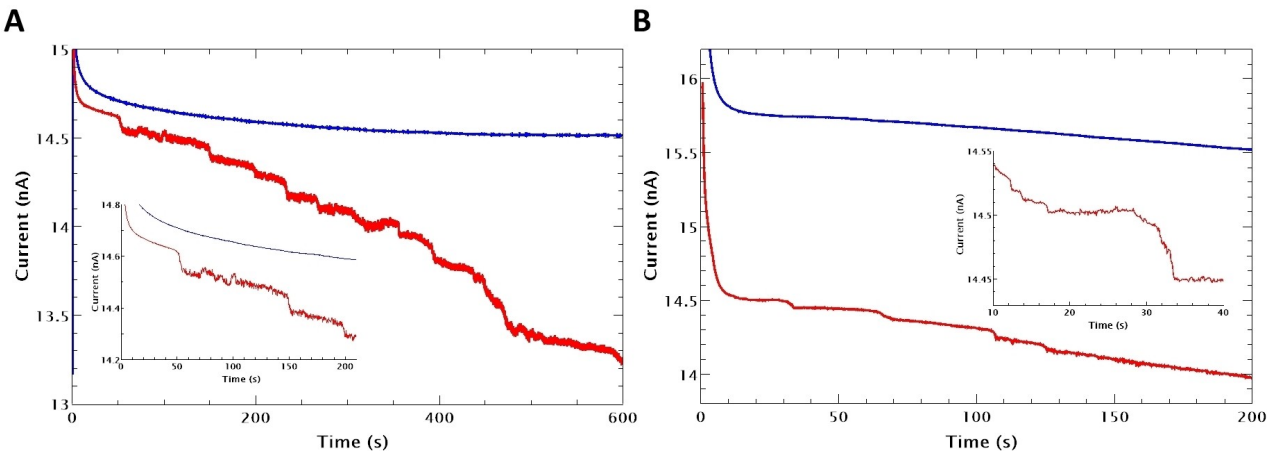


**Figure 2.** Chronoamperometry measurements recorded on 7 µm-diameter CF UME at +0.8 V vs Ag/AgCl in 2 mL of 20 mM $K_4Fe(CN)_6$ 0.1 M PBS at pH 7.4 as aqueous solution under inert atmosphere ($N_2$) in (blue) the absence and (red) presence of $10^9$ cells of (A) non-washed and (B) washed SOMR1 bacteria sample.


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higher than the noise were considered as collision events in our study (see Figure S3). Most of the time, a current decrease in the shape of stair steps is observed (Figure 2A and Figure S2A) and indicates that most of the bacteria which impact stick and remain adsorbed on the UME surfaces after collision. Also, the time scale of a single impact event is overall included between 0.2 and 1.0 s with a large majority inferior to 0.5 s.

These observations are in agreement with our previous work about blocking electrochemical impacts of single SOMR1 bacteria onto CF UME in a potassium ferrocyanide aqueous solution.[12] In order to keep the same UME surface available during single bacteria impact experiments and for evaluating the frequency of collisions in optimized experimental conditions, the chronoamperometry measurements were limited to 200 s (Figure 2B and S2B) in the following reported experiments. It is indeed visible that a higher frequency of bacteria collisions occurs in the first 200 s of the *i-t* curve, which is probably due to the partial covering of the UME surface by adsorbed bacteria. In this case, the effect of collisions is lower on the current and not visible after 200 s in the *i-t* curve and/or the electric field of UME is lower because of the high electrode surface coverage of bacteria. The difference of the background current in the *i-t* curves presented in Figure 2B is related to the UME polishing between each experiment.

Another interesting observation is that no current event signal is detected in the *i-t* curve when dead SOMR1 cells, killed with heat or sodium azide, are added in the electrochemical cell (Figure 3 and S4). While killing the cells with heat may lead to cell lysis, sodium azide prevents microbial growth by inhibiting respiratory chain,[33] and therefore does not affect the cell membrane structure of the bacteria. This suggests that the negatively charged living bacteria are attracted onto the positively-charged UME surface polarized at the oxidation potential of ferrocyanide, contrary to the dead cells. In addition, the increase of the current noise in the red *i-t* curve (Figure 3) from 50 s is assigned to the move of adsorbed living bacteria onto the UME surface. The heat treatment at 76 °C for 15 min was performed for killing SOMR1 cells without lysis, based on a published study.[34] In these conditions, the cells are dead (no growth on the LB agar plates) and no current step is observed in the corresponding blue *i-t* curves in Figure 3 and Figure S4. Hence this electrochemical method seems to be an interesting strategy to differentiate living and dead bacteria at the single cell scale.

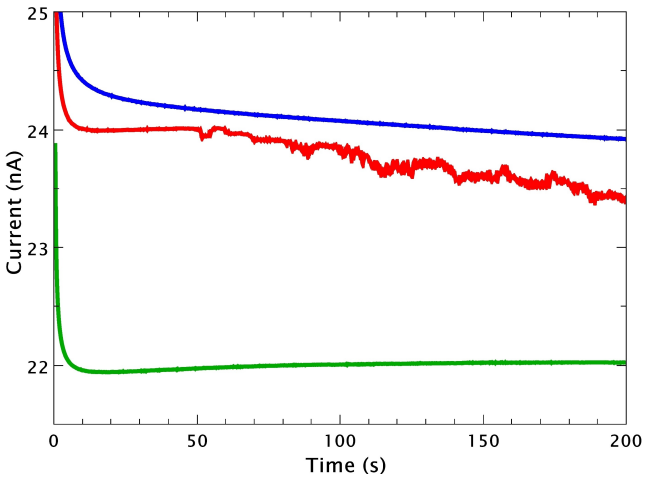


**Figure 3.** Chronoamperometry measurements recorded on 10 μm-diameter Pt UME at +0.8 V vs Ag/AgCl in 2 mL of 20 mM $K_4Fe(CN)_6$ 0.1 M PBS at pH 7.4 as aqueous solution under inert atmosphere ($N_2$) in the presence of $10^9$ cells of (red) living and dead SOMR1 bacteria sample killed with sodium azide (green) and by heat treatment (blue).

The current step magnitude in electrochemical blocking experiments is related to several parameters such as the particle position onto the UME surface (edge effect), as well as provides an overall idea of the size of the adsorbed particles (Table S1).[35–37] A simple estimate of the radius of the disk surface occupied by a single particle adsorbed onto the UME surface ($r_{ads}$) can be calculated from the following equation.[35]

$$r_{ads} = r_e\sqrt{\frac{\Delta i_{ss}}{i_{ss}}} \quad (2)$$

In Equation 2, $r_e$ is the UME disk radius, $\Delta i_{ss}$ is the current step magnitude, and $i_{ss}$ is the steady-state current previous the analysed current step (see Figure 1). Although Equation 2 does not take into account the edge effect inherent in electrochemical blocking experiments on disk UMEs and the shape of the particle, which is not spherical in the case of SOMR1 bacteria,[38] it allows to have an estimated value of the size of a single adsorbed cell.[35]

Comparing the *i-t* curves recorded in the same experimental conditions between non-washed (Figure 2A) and washed (Figure 2B) SOMR1 bacterial cells, it is easy to observe that the current steps magnitude is higher for non-washed cells ($\Delta i_{ss} = 80 \pm 30$ pA on CF UME and $\Delta i_{ss} = 70 \pm 30$ pA on Pt UME) than for washed cells ($\Delta i_{ss} = 60 \pm 30$ pA on CF UME and $\Delta i_{ss} = 60 \pm 40$ pA on Pt UME) over a batch of experiments with the same culture at different concentrations (more than 70 counted events). This can be due to the fact that the unwashed cells contain proteins and unbound reagents at the cell-surface that contribute to the collision process to the UME surface. Indeed, the values of the average radius ($r_{ads}$) of the disk surface occupied by a single SOMR1 cell adsorbed onto the UME surface (Table S2), confirm the lower size of washed bacteria (loss of approximatively 8.5 % and 14.5 % on the $r_{ads}$ values after washing SOMR1 cells onto CF and Pt UMEs, respectively). This result suggests that the bacteria washing in 0.1 M PBS at pH 7.4 is efficient for removing outer-cell components that are attached to the cells like proteins or unbound reagents. Furthermore, this comparison between non-washed and washed bacterial cells highlights the sensitivity of the electrochemical blocking impact method which is able to show a slight difference in the discrete event signals at the single-cell scale. Considering that the size of the rod-shaped SOMR1 cells is about 1.5 μm in length and 0.5 μm in width,[38] the average radius value $r_{ads}$, estimated at ca. $0.25 \pm 0.09$ μm (Table S2) is within an order of magnitude of the actual cell dimensions.

Chronoamperometry measurements (200 s) were recorded at +0.8 V vs Ag/AgCl at a Pt UME in 2 mL of 20 mM $K_4Fe(CN)_6$ and 0.1 M PBS at pH 7.4 with different volumes of washed


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SOMR1 cells added in the redox probe solution (Figure 4A). The number of current step events observed (frequency of single bacteria impacts) over 200 s in the *i-t* curves (see Figure S3 to count current step events in the chronoamperometry curves) can be plotted as a function to the concentration of washed SOMR1 bacteria sample in the electrochemical cell (Figure 4B). As observed, the collision frequency increases with the concentration of bacteria in solution in the four *i-t* curves reported in Figure 4A. The difference of the current profile in the *i-t* curves presented in Figure 4A is related to the UME polishing between each experiment. The frequency of impact events is linearly proportional to the concentration of living bacteria in this experimental range (from 1.4 to 10 $10^8$ cells $mL^{-1}$ of washed SOMR1 bacteria sample in the electrochemical cell) as presented in Figure 4B. This assumption must be considered carefully because it is not easily reproducible from an experiment to another, and it is not observed at the CF UME. Especially below a minimum concentration ($1.4\times10^8$ cells $mL^{-1}$) and above a maximum concentration ($1.1\times10^9$ cells $mL^{-1}$) of bacteria, no current step (impact event) is observed in the *i-t* curves because of a too much low and high concentrations, respectively.

The experimental frequency $f_{exp}$ of single current step in the associated *i-t* curve (red curve in Figure 4A) is calculated at $0.04\pm0.01$ Hz. This frequency value corresponds to a bacteria concentration $C_{bac}$ of about $4\times10^9$ cells $mL^{-1}$ (7 pM) according to Equation 3 below (in conditions where the mass transport is controlled by diffusion).[11,12,17]

$$C_{bac} = \frac{f_{exp}}{4Dr_eN_A} \quad (3)$$

In Equation 3, $D$ is the diffusion coefficient of bacteria ($4.5\times10^{-9}$ $cm^2\,s^{-1}$), $N_A$ is Avogadro's number, and $r_e$ is the UME disk radius ($r_e$(Pt) = 5 μm). The diffusion coefficient $D$ is estimated for rod-shaped particles such as SOMR1 bacteria with a length ($a$ = 1.5 μm) and a width ($b$ = 0.5 μm)[38] based on the two equations below for circular cylinders.[39,40]

$$D = \frac{k_BT}{3\pi\eta a}\left[\ln\left(\frac{a}{b}\right) + w\right] \quad (4)$$

$$w = 0.312 + 0.565\frac{b}{a} - 0.1\frac{b^2}{a^2} \quad (5)$$

In Equation 4, $k_B$ is Boltzmann's constant, $T$ is temperature, and $\eta$ is the solution viscosity.

The bacteria concentration $C_{bac}$ determined from the experimental frequency $f_{exp}$ of current steps (red curve in Figure 4A) and Equation 3 is in agreement with the concentration determined from $OD_{600}$ ($1\times10^9$ cells $mL^{-1}$). The electrostatic attraction between the negatively charged surface of living SOMR1 and the positively-charged UME surface is confirmed by the experimental collision frequency. In this way, the adsorption of single living bacteria onto the UME surface following-up the impact is highly favourable and supported by the stair step shape of collision events. In contrast, a different behaviour is observed (repulsive effect) when these experiments are performed in a 20 mM ferricyanide solution with a cathodic polarization (−0.4 V vs Ag/AgCl).[12] In this case, no current step event is observed in the chronoamperometry measurement, as well for living and dead (killed with sodium azide) SOMR1 bacteria. This opposite behaviour is assigned to the electrostatic repulsion of the negatively charged UME surface polarized at the reduction potential of ferricyanide on the negatively charged cells. The cells cannot be adsorbed onto the UME surface in these conditions.

In order to confirm the adsorption of single living bacterial cells onto UME surfaces following electrochemical impact experiments performed in ferrocyanide at the polarization potential of the aqueous redox probe (+0.8 V vs Ag/AgCl), a post-characterization by atomic force microscopy (AFM) was achieved after a chronoamperometry measurement for cells

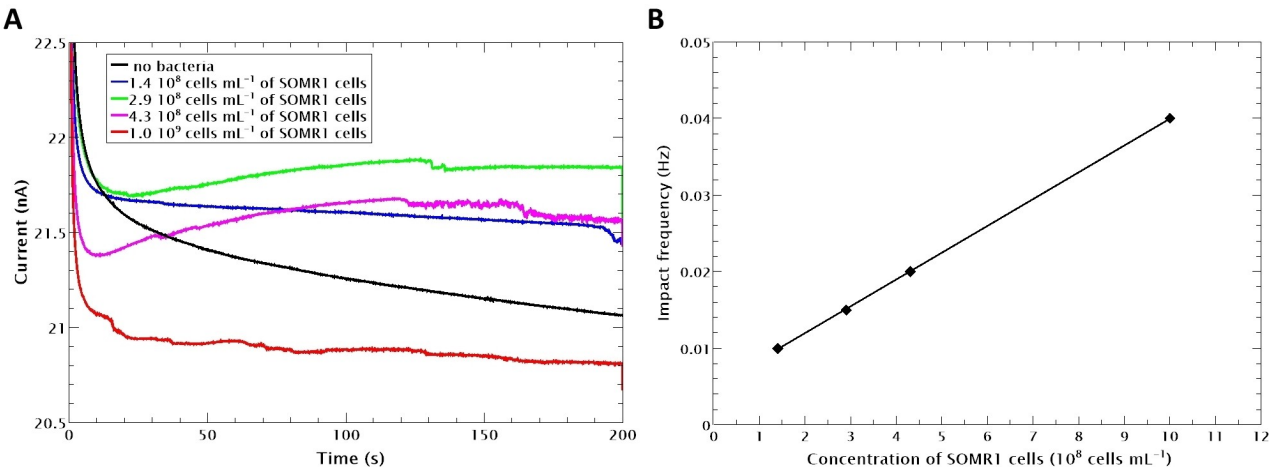


**Figure 4.** (A) Chronoamperometry measurements (200 s) recorded on 10 μm-diameter Pt UME at +0.8 V vs Ag/AgCl in 2 mL of 20 mM $K_4Fe(CN)_6$ 0.1 M PBS at pH 7.4 as aqueous solution under inert atmosphere ($N_2$) in the presence of different concentrations of washed SOMR1 bacteria. (B) Frequency of current steps (single impacts) in function to the concentration of washed living bacteria sample in the electrochemical cell extracted from the corresponding *i-t* curves (A).


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adsorption (Figure 5). It is noticeable to precise that without an appropriate polarization such as the oxidation potential of ferrocyanide applied to UME, no bacteria are adsorbed onto the electrode surface. The observation of bacteria by AFM after the chronoamperometry measurement is a real challenge to keep the SOMR1 cells onto the UME surface without any polarization. In these conditions, the number of cells adsorbed onto the UME surface cannot be correlated to the number of impact events because of the cells lost during the transfer and the addition of water for keeping the UME surface wet between electrochemical and AFM experiments. For this reason, the post-characterization by AFM was reproduced at least three times and at different potentials (anodic/cathodic) for controlling the attractive/repulsive effect of the polarization on single SOMR1 impacts and bacteria adsorption onto the UME surface. As a control, Figure 5 shows the AFM images of the bare CF (Figure 5A) and Pt (Figure 5B) UME surfaces. The presence of adsorbed bacteria is observed both onto the CF (Figure 5C) and Pt (Figure 5D) UME surfaces after a chronoamperometry experiment at +0.8 V vs Ag/AgCl in 2 mL of 20 mM $K_4Fe(CN)_6$ 0.1 M PBS at pH 7.4 as aqueous solution with $10^9$ cells of SOMR1 bacteria sample. The shape and the size of cells observed by AFM are in good agreement with the morphology of SOMR1 bacteria.[38] This result confirms the electrochemical data where the living bacteria which impact stick and remain adsorbed on the UME surface after collision because of the electrostatic attraction of negatively-charged SOMR1 cells to the positively-charged UME surface (polarized at the oxidation potential of ferrocyanide).

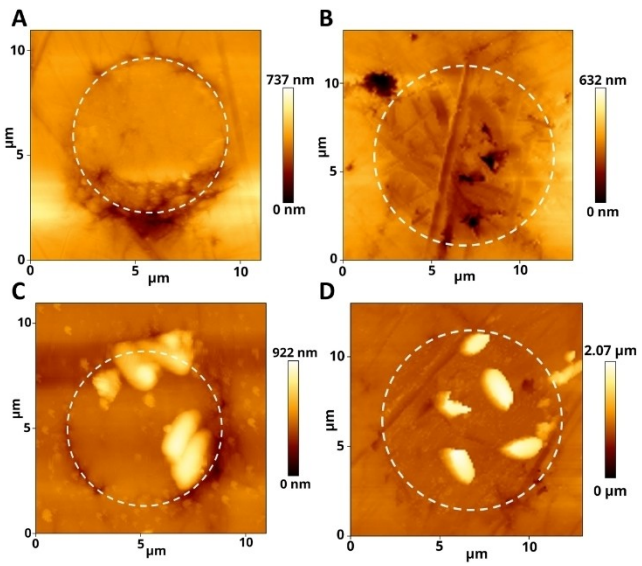


**Figure 5.** AFM images recorded in water of (A and C) CF and (B and D) Pt UME surfaces (A and B) before and (C and D) after a chronoamperometry measurement at +0.8 V vs Ag/AgCl in 2 mL of 20 mM $K_4Fe(CN)_6$ 0.1 M PBS at pH 7.4 as aqueous solution under inert atmosphere ($N_2$) in the presence of $10^9$ cells of living SOMR1 bacteria sample. The circle in white dashed line is added for showing the UME disk on the AFM images.

## Conclusion

In this work, single electrochemical impacts of SOMR1 Gram negative electroactive bacteria onto a carbon fibre and Pt ultramicroelectrodes polarized at the oxidation potential (+0.8 V vs Ag/AgCl) of the ferrocyanide redox probe in solution are optimized and analysed. The electrochemical blocking method data confirm that living bacteria stick and remain adsorbed onto the ultramicroelectrode surfaces according to the shape of current stair steps observed in the chronoamperometry measurements. The washing of living bacteria sample with phosphate buffer previous the electrochemical experiments is crucial to efficiently remove outer-cell components attached to the cells that may alter the results. The average radius value of the disk surface occupied by a single living bacterium adsorbed onto the UME surface is in agreement with the size of the rod-shaped SOMR1 cells. The analysis of the *i-t* curves indicates that the electrostatic attraction of the negatively charged *Shewanella* cells to the positively charged UME surface seems to be a specific behaviour of living bacteria, highlighting a possible method to differentiate living from dead cells, which requires to be deepened. AFM images recorded in water after a chronoamperometry measurement in the presence of living bacteria confirm the adsorption of single cells onto the CF and Pt UME surfaces.

These experiments should be repeated by coupling the electrochemical experiments to optical microscopy measurements in order to confirm our observations in real time with a high temporal and spatial resolution. Also, this work should be extended to non-electroactive bacteria in order to study the influence of the extracellular electron transfer on the electrochemical single impact events.

## Experimental Section

**Reagents**. All chemicals were reagent grade and used as purchased without further purification. Water used in each experiment was deionized water. Potassium ferrocyanide trihydrate (98.5%) was purchased from Acros Organics. Phosphate buffer solution at 1.0 M and pH 7.4 (25 °C) was purchased from Sigma Aldrich and stored at 3 °C. Potassium and sodium chloride were purchased from Sigma Aldrich. Phosphate buffered saline (PBS) used for all experiments was composed to 0.1 M commercial phosphate buffer solution, 50 mM KCl and 50 mM NaCl (pH 7.4 at 25 °C) and was stored at 3 °C. Luria–Bertani (LB) medium, LB agar plates, sodium azide (99.5%) and glycerol were obtained from Sigma Aldrich.

**Bacterial strain and growth conditions**. All the manipulations were performed under sterile conditions to avoid contamination with other microorganisms. Pure culture of *Shewanella oneidensis* MR-1 (SOMR1) was grown aerobically in LB medium. First a small amount of cells from the SOMR1 bacterial strain sample was spread with a sterile tip on a plate with LB agar which then was placed in an incubator (STUART, SI600) overnight at 30 °C for cells growth. A single colony was collected using a sterile tip and incubated in an Erlenmeyer containing 20 mL LB liquid medium which was placed in an orbital shaker (STUART, SI600) overnight at 30 °C and 150 rpm. The growth of the bacterial culture was checked by measuring the optical absorbance at 600 nm (UV-Visible spectrometer, Analytikjena SPECORD® 210 PLUS). The optical density at 600 nm ($OD_{600}$) of


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the undiluted cells was approximately 4.0, consistent with the exponential growth phase.[41] The dead SOMR1 bacteria sample was prepared by adding 20 mM of sodium azide in the culture at room temperature overnight. The death of bacterial cells was check on a LB agar plate. The heat treatment for killing bacteria was carried out at 76 °C for several time durations between 2 and 60 min. The SOMR1 bacteria solution used in the electrochemical experiments was prepared by centrifuging 1 mL of overnight grown culture or dead bacteria sample for 15 min at 2500 rpm to remove the LB growth medium, and resuspended in the same volume of 0.1 M PBS aqueous solution at pH 7.4. For the washing SOMR1 cells, an additional washing step with 0.1 M PBS aqueous solution (pH 7.4) was performed. The bacteria solution in PBS was prepared just before the single electrochemical impact experiments and used immediately within in maximum 2 hours.

**Electrochemical measurements**. The electrochemical experiments were carried out at room temperature (20 ± 3 °C) with a three-electrode cell placed in a Faraday cage (BioLogic FC-45) and a SP-300 potentiostat (BioLogic) with an ultra-low current module using the EC-Lab software. For all recorded chronoamperometry *i-t* curves, the sample interval (in sampling time) was 100 ms. A Pt wire and a Ag/AgCl (3 M KCl) were used as a counter electrode and a reference electrode, respectively for all electrochemical measurements. As a working electrode a 10 μm-diameter Pt from CH Instruments and a 7 μm-diameter carbon fibre (CF) from BAS disk ultramicroelectrodes (UMEs) were used. Before each electrochemical experiment, the Pt and CF UMEs were mechanically polished using wetted fine grid silicon carbide paper from Struers (4000-grid SiC) and washed in water. All solutions of the electrochemical cell were degassed under $N_2$ for 5 min prior to each measurement. The UME was then immersed in the solution of the electrochemical cell, connected as a working electrode and the electrochemical measurement was launched straight away in a matter of a few seconds (max. 5 s of elapsed time). All data and results presented in the manuscript and Supporting Information are reproducible and were repeated at least three times in similar experimental conditions. The concentration of washed SOMR1 bacteria in the electrochemical cell for a volume of 70 μL (optimal volume for our electrochemical experiments) added in 2 mL of redox probe is estimated at ca. $10^9$ cells $mL^{-1}$ (2 pM) after dilution of the stock solution with $OD_{600} = 4.0$.[42]

**Atomic force microscopy measurements**. Atomic force microscopy (AFM) experiments were carried out with a BRUKER JPK Nanowizard4 using Qi mode. All AFM experiments were performed in water (droplet deposited on the UME surface after single bacteria impact experiment) with an applied force of 0.12 nN. Sensibility and spring constant of cantilever were determined by thermal noise method. qp-SCONT probe from Nanosensor was used with a curvature radius of 10 nm approximately, cantilever nominal spring constant of 0.1 N/m and resonant frequency of 14 kHz. All the AFM experiments were performed immediately within in maximum 1 hour after the chronoamperometry measurement and the UME surface was gently washed with distilled water to remove the electrochemical solution.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Keywords:** Single electrochemical impacts · Ultramicroelectrode · Electroactive bacteria · Chronoamperometry · Atomic force microscopy

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