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# Trends in single-impact electrochemistry for bacteria analysis

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## Abstract

Single-impact electrochemistry for bacteria analysis is a powerful technique for biosensing applications at the single-cell scale. The sensitivity of this electro-analytical method based on chronoamperometric measurements at an ultramicroelectrode polarized at the appropriate potential of redox species in solution, is widely demonstrated. Furthermore, the most recent studies display a continuous improvement of this sensitive electrochemical method in order to be able to identify different bacterial strains with better selectivity. To achieve this, several strategies such as the presence of a redox mediator are investigated for detecting and identifying the bacterial cell through its own electrochemical behavior. Both the blocking electrochemical impacts method and electrochemical collisions of single bacteria with a redox mediator are reported in this review and discussed through relevant examples. An original sensing strategy for virulence factors originating from pathogenic bacteria is also presented, based on a recent proof of concept dealing with redox liposomes single-impact electrochemistry. The limitations, applications, perspectives, and challenges of single-impact electrochemistry for bacteria analysis are briefly discussed based on the most significant published data.

## Keywords

Single-impact; Bacterial cell; Chronoamperometry; Ultramicroelectrode; Redox mediator; Blocking electrochemical collisions

## Introduction

Nowadays, the main objective in the detection and analysis of bio-entities such as bacteria is a low detection limit, a short assay time and a high selectivity in identification, ideally combined with easy-handling and cheap instrumentation [1]. In this way, the development of innovative analytical methods in terms of high sensitivity and spatial-temporal resolution allowing both qualitative and quantitative analysis at single-cell and subcellular levels is becoming a technological requirement [2]. Currently, the four major analytical methods usually reported are electrochemical analysis, super-resolution microscopy, mass spectrometry imaging, and microfluidics [2]. The main advantage of electrochemistry in biosensing applications is to combine high sensitivity and ease of use with the possibility of miniaturization at low cost [1,3–5]. Especially, new electrochemical techniques and various types of electrodes have been recently developed offering the feasibility of analysis at the single-cell level with high sensitivity and selectivity [2,6–9]. Nano-electrochemistry has been widely extended to various applications such as biosensing thanks to the continuous improvement of the instrumentation sensitivity, especially for individual entity electrochemical detection [9–12]. Single-impact electrochemistry provides a low limit of detection (in principle, one single species) inherent to this electro-analytical method and the ability to study single entities (cells, viruses, bacteria, nanoparticles...) in real-time through a dynamic measurement [12–20]. The electrochemical nano-impacts method or discrete collisions technique (stochastic events) consists in detecting single impacts of various micro- and nano-objects (entities) such as nanoparticles, cells, bacteria, vesicles, viruses, proteins in solution at a polarized ultramicroelectrode (UME) [18–25]. For each collision event, a specific signal is recorded in the chronoamperometry curve (current as a function of time) corresponding to an “impact” of the entity onto the UME surface. Crucial information can be reached from the analysis of the electrochemical impact event in the amperometry measurement, such as the concentration and the size of the colliding entity. In order to improve the quality of the results, interesting studies aim to investigate the role of the UME's nature, shape, and geometry on the electrical response [26,27]. For instance, the accuracy of particle size distribution is highly improved by carrying out single-impact electrochemistry with a hemispherical Hg UME than a disk Pt UME [26]. Also, circular shape microelectrodes with radii optimized according to the type of the analyzed cells improve the signal-to-noise ratio of the electric signal in single-particle detection [27].

Electrochemical nano-impacts (or collisions) of single bacteria are usually detected by recording a chronoamperometry measurement ( $i-t$  curve) at a UME biased at a redox potential of an electroactive species in an aqueous electrolyte containing between  $10^6$  and  $10^9$  bacteria per milliliter [16,28–31]. Since 2015, most of the reports in this area concern *Escherichia coli* (*E. coli*) as a model bacterium but this sensitive electro-analytical

technique has not proved to be efficient for selective detection yet, except for the discrimination between Gram-positive and Gram-negative bacteria [30,32]. In order to develop single-impact electrochemistry toward the identification of bacterial cells, several studies deal with the use of redox mediators for probing the electrochemical activity of the bacterium [32–34]. Also, an original and recent approach is to detect the virulence factors released by pathogenic bacteria rather than the cells themselves, based on redox liposomes single-impact electrochemistry [17]. All of these different strategies have a common crucial objective: finding an efficient way to make single-impact electrochemistry able to selectively identify bacteria at the single-cell scale. Reaching this objective will allow to offer the most attractive method for sensor applications because it will combine the previously-mentioned advantages: a low limit of detection, fast response and high selectivity in a cheap and easy-handling device. Only at this stage, the detection of single bacteria in real samples and complex matrices will be investigated.

In the present review, we will focus on the trends in single-impact electrochemistry for bacteria analysis including the direct detection of bacterial cells via the blocking method [16,28–31], the bacterial electrochemical activity via a redox mediator [32–34] and an original concept aiming to detect virulence factors from pathogenic bacteria via the nano-impacts of redox liposomes [17,20]. By presenting these three different methods of single-impact electrochemistry for bacteria analysis, we will discuss the current limitations and applications through different relevant examples. We will conclude this review with an outlook and our vision of the future challenges in this area.

### **Blocking electrochemical impacts of single bacteria onto ultramicroelectrode**

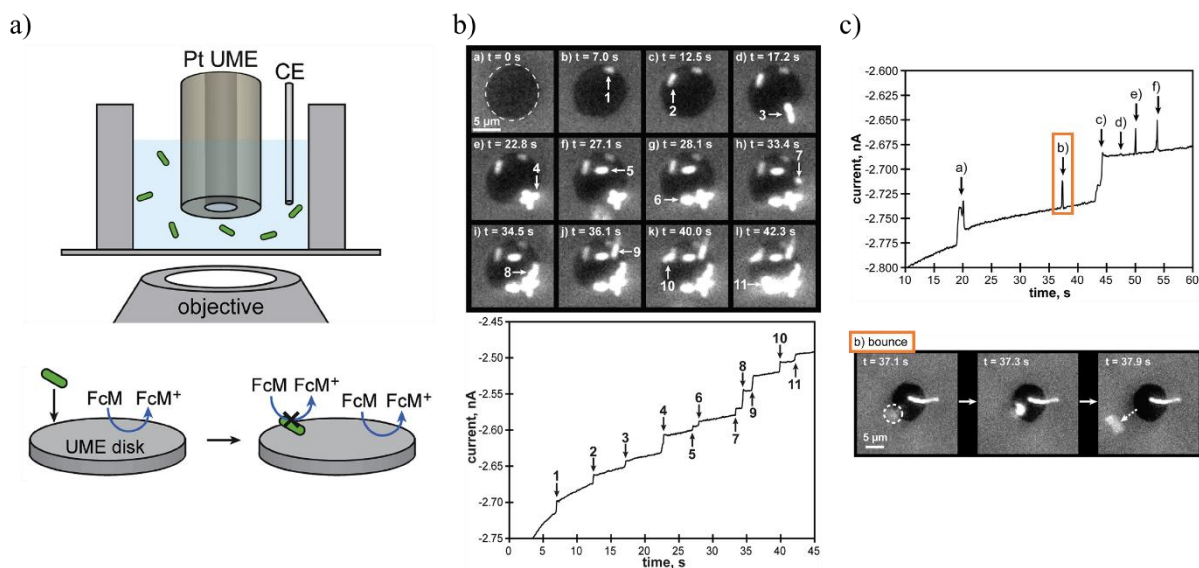
The blocking collisions method is initially reported by Quinn *et al.* for the detection of insulating polystyrene beads (1  $\mu\text{m}$  diameter) onto an Au microelectrode disk (5  $\mu\text{m}$  diameter) in a hydroxymethylferrocene (ferrocenemethanol) aqueous solution [21]. In the following years, this method has been widely extended to the detection of various micro- and nano-objects, with a specific interest in single collisions of bio-entities such as proteins, viruses, and bacteria [19,25,29,35]. Because of its versatility and efficiency for detecting various insulating particles in a short analysis time and with a high sensitivity, the blocking electrochemical impacts method has been quickly extended to cells and bacteria sensing [18,28]. The main advantage of the blocking collisions method is probably its easy-to-perform principle, consisting in detecting a “current step” in the chronoamperometry ( $i-t$ ) curve when a particle (entity) collides with the UME polarized at the redox potential of the aqueous electroactive probe [35]. Indeed, a microelectrode is biased at a potential where the electron transfer

reaction is at a diffusion-limited steady state in a solution containing the target particle and a redox species. When an insulating particle adsorbs onto the UME, it locally stops the diffusive flux of redox species to the electrode, leading to a drop of current (step-shaped transient) [30]. The magnitude of the current steps in blocking impact experiments depends on the size of the blocking object, the size of the UME, the type and the concentration of the redox probe, and the location of the blocking object adsorbed onto the UME [30,35]. Blocking electrochemical impacts have been extended to single bacteria detection for the first time in 2016 by Lee *et al.* with *E. coli* [29]. In this work, chronoamperometric measurements are performed with a carbon UME (10  $\mu\text{m}$  diameter) polarized at +0.6 V vs. Ag/AgCl in a solution containing 53 fM of *E.coli* living bacteria and at least 20 mM of potassium ferrocyanide. The authors have demonstrated a linear relationship between the collision frequency  $f_{\text{exp}}$  (number of step-shaped transients) and the *E. coli* concentration  $C_{\text{bac}}$ , as expected by Equation 1 below (in conditions where the mass transport is controlled by diffusion) [10,16,32].

$$C_{\text{bac}} = \frac{f_{\text{exp}}}{4D\tau_e N_A} \quad (1)$$

In Equation 1,  $D$  is the diffusion coefficient of the bacteria,  $N_A$  is Avogadro's number, and  $r_e$  is the UME disk radius.

After this first proof of concept by Lee *et al.* on the single *E. coli* bacteria detection by blocking electrochemical impacts [29], the principle has been extended to other bacteria in several recent studies, using *E. coli* as a model bacterium [16,28,30]. Ronspees *et al.* have investigated blocking electrochemical collisions of single *E. coli* (Gram-negative bacteria) and *Bacillus subtilis* (*B. subtilis*, a Gram-positive bacteria) using simultaneous fluorescence microscopy (Fig. 1) [30].



**Fig. 1** a) Schematic of the electrochemical cell used for bacteria impact experiments coupled with simultaneous fluorescence microscopy (top) and blocking collisions of single bacteria (bottom). b) Simultaneous electrochemistry and fluorescence microscopy for a blocking collision experiment using *E. coli*. Each bacterial adsorption event at a 10  $\mu\text{m}$  Pt UME polarized at +0.385 V vs. Ag/AgCl observed by microscopy (top) is related to a corresponding step transient in the  $i-t$  curve (bottom). c) Chronoamperometry curve recorded at a 10  $\mu\text{m}$  Pt UME polarized at +0.385 V vs. Ag/AgCl during a blocking collision experiment involving *B. subtilis* (top) and coupled to fluorescence microscopy imaging (bottom). Adapted with permission from [30]. Copyright © 2018 Elsevier.

As illustrated in Fig. 1a, when an *E. coli* cell adsorbs onto a 10  $\mu\text{m}$  Pt UME biased at the oxidation potential of ferrocenemethanol (FcM), a current step is observed in the  $i-t$  curve. This corresponds to the blocking effect on the diffusive flux of FcM to the UME surface, as the redox probe is no longer available for undergoing oxidation to ferroceniummethanol (FcM<sup>+</sup>) [30]. Fig. 1b shows the correlation between electrochemical measurements and simultaneous fluorescence microscopy imaging for each bacterial adsorption event detected by step transients in the  $i-t$  curve [30]. This observation with *E. coli* is in agreement with the previous study of Lee *et al.* [29], confirming the adsorption of the cell onto the UME surface following the collision [30]. The novelty in the work of Ronspees *et al.* is the extension of the blocking experiments to *B. subtilis* (Fig. 1c), which displays the opposite behavior of *E. coli*'s [30]. Indeed, in the same experimental conditions (FcM as a redox species and 10  $\mu\text{m}$  Pt UME as a working electrode), the signal of collision events in the  $i-t$  curve with *B. subtilis* is a spike (blip shape), which returns to the baseline current after 0.2–0.8 s (Fig. 1c) [30]. Based on the simultaneous fluorescence microscopy imaging (Fig. 1c), these blip-shaped collision transients are due to short-lived bounce events of bacteria at the UME, in contrast with the step-shaped transients observed in Fig. 1b due to the adsorption of *E. coli* at the UME [30]. This work provides the first example of different species of bacteria leading to different transients (current signals) in a blocking collision experiment, and thus opens the way to the selective detection of bacteria [30].

Following a similar strategy, Lebègue *et al.* have investigated the electrochemical single impacts of electroactive *Shewanella oneidensis* (*S. oneidensis*) Gram-negative bacteria in two different aqueous redox probes (either potassium ferrocyanide or potassium ferricyanide) by applying the appropriate redox potential at a 7  $\mu\text{m}$  carbon UME [16]. In this study, a typical stair-step current response is detected for single *S. oneidensis* impacts at the potential of steady-state current of potassium ferrocyanide ( $E \geq +0.3$  V vs. Ag/AgCl), corresponding to the adsorption of cells onto the UME [16]. In contrast, current spikes (blips) are observed for *S. oneidensis* collisions in the chronoamperometry measurement recorded at the potential of steady-state current of potassium ferricyanide ( $E \leq +0.1$  V vs. Ag/AgCl), assigned to short bounce events [16]. These observations point to the attraction/repulsion of negatively charged *S. oneidensis* bacteria by the polarized carbon UME surface as a function of the applied potential [16]. These results have been further confirmed in a recent report dealing with single impacts of *S. oneidensis* onto the UME surface, combined with atomic force microscopy measurements [31].

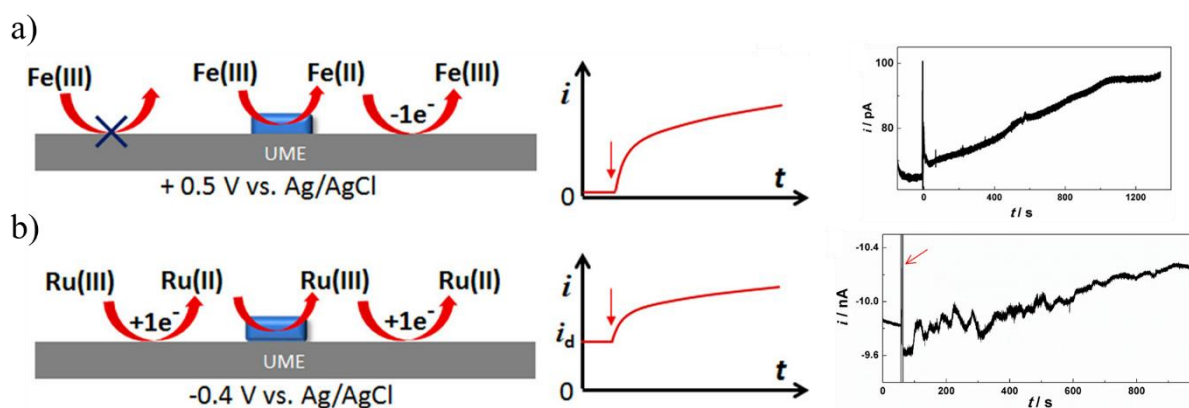
Furthermore, a study by Thorgaard *et al.* dealing with collision events of single *Lactococcus lactis* (*L. lactis*) Gram-positive bacteria at Pt disk UMEs has elucidated the influence of electroosmotic flow on the transport of cells near the electrode [36]. The *L. lactis* velocity due to convection caused by the electroosmotic flow exceeds that of electrophoresis at the edge location of the UME disk, leading to the transport of the cell away from the electrode [36]. These results suggest that electroosmotic flow is significant for particle transport in stochastic collision experiments in solutions of low ionic strength [36].

In order to conclude this section dealing with blocking electrochemical impacts of single bacteria onto UME, we have reviewed the most significant studies in this area, which mainly concern *E. coli* as a model Gram-negative bacterium [28–30]. Also, we have highlighted a possible strategy (electrostatic attraction/repulsion) to improve the selectivity and the identification of single cells with the electrochemistry of single impacts. In this perspective, the work of Gao *et al.* offers great opportunities in the detection and identification of single bacteria by electrochemical collision technique through the specific redox activity of the cell toward a redox species [28].

### **Electrochemical collisions of single bacteria with a redox mediator**

In a study reported by Gao *et al.* about the electrochemical collisions of two kinds of Gram-negative bacteria, *E. coli* and *Stenotrophomonas maltophilia* (*S. maltophilia*), the signal produced by the electrochemical activity of bacterial cells reducing (or oxidizing) redox species has been presented as a selective strategy to identify different types of bacteria (Fig. 2) [28]. Indeed, the redox activity of *E. coli* cells has been detected via the ability

of these bacteria to reduce ferricyanide species in a 100 mM  $\text{K}_3\text{Fe}(\text{CN})_6$  solution with a Pt UME polarized at the oxidation potential of ferrocyanide (Fig. 2a) [28]. In this case, Fe(III) is reduced by the *E. coli* bacteria colliding with the UME surface and the produced Fe(II) species get oxidized back at the UME (Fig. 2a) [28]. The resulting current increases with the number of adsorbed bacteria (Fig. 2a) but the flux of Fe(II) produced by a single bacterium is much too low to be detected in the  $i-t$  curve [28]. Alternatively, *S. maltophilia* bacteria have been detected in an aqueous solution containing 10 mM  $\text{Ru}(\text{NH}_3)_6^{3+}$ , which is reduced to Ru(II) at the Pt UME, using the cells' ability to regenerate the redox mediator (by oxidation) during single collisions (current amplification, Fig. 2b) [28]. As indicated by the authors, this experimental setup is more complicated and the signal is harder to interpret because of two opposite processes occurring simultaneously: the blockage of the  $\text{Ru}(\text{NH}_3)_6^{3+}$  diffusion to the UME and the regeneration of this species by the adsorbed cells (Fig. 2b) [28]. Nevertheless, this work allows to broaden single bacteria collisions to the specific redox activity of the cells in order to identify them from a unique electrochemical signature [28].

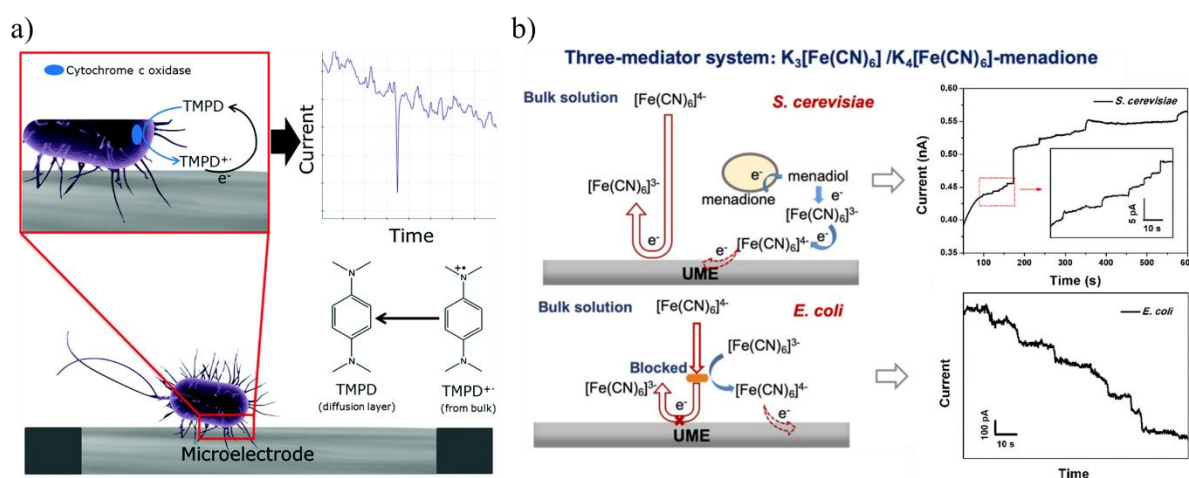


**Fig. 2** a)  $\text{Fe}(\text{CN})_6^{3-}$  is reduced by *E. coli* bacteria and the resulting  $\text{Fe}(\text{CN})_6^{4-}$  is oxidized at the UME (left). Chronoamperometry measurements recorded at +0.5 V vs Ag/AgCl in 100 mM  $\text{Fe}(\text{CN})_6^{3-}$  show collisions of *E. coli* cells onto Pt UME (right). The arrows indicate when the bacteria are added to the solution. b)  $\text{Ru}(\text{NH}_3)_6^{3+}$  is reduced at the UME and then regenerated by the *S. maltophilia* bacteria (left). Chronoamperometry measurements recorded at -0.4 V vs Ag/AgCl in 10 mM  $\text{Ru}(\text{NH}_3)_6^{3+}$  show collisions of *S. maltophilia* cells onto Pt UME (right). Adapted with permission from [28]. Copyright © 2018 American Chemical Society.

The first reported strategy to detect single bacteria impacts is to decorate the *E. coli* cells with Ag nanoparticles, which can be oxidized during stochastic collisions at a UME biased at the appropriate potential [23,37]. Nevertheless, this technique requires a pretreatment of the cells and is not easily applicable to selective bacteria detection and identification. Hence, the most promising strategy to analyze single label-free living bacteria through a specific electrochemical response seems to be electrochemical collisions with a redox mediator (Fig. 3).



For the detection of *E. coli* by impact electrochemistry, the work of Couto *et al.* deals with the use of *N,N,N',N'*-tetramethyl-*para*-phenylene-diamine (TMPD) as a redox mediator, which enables the electrochemical recognition of bacterial cytochrome c oxidases without the need of cell lysis (Fig. 3a) [32]. Here, the strategy consists in applying a reduction potential (-0.15 V vs. SCE) to the microelectrode, where the radical cation  $\text{TMPD}^{+\cdot}$  species present in solution are reduced to TMPD within the diffusion layer [32]. Because *E. coli* cells include cytochrome c oxidases in their membranes, they are able to oxidize extracellular TMPD to  $\text{TMPD}^{+\cdot}$ . When the bacteria enter the diffusion layer, a feedback redox cycle is created and a reductive current spike (current increase) is detected in the chronoamperometry measurement, corresponding to the regeneration of  $\text{TMPD}^{+\cdot}$  in the periphery of the bacterium (Fig. 3a) [32]. This technique is an efficient method to discriminate living bacteria from dead ones (no cytochrome c oxidase activity) and it also shows the expected linear relationship between the collision frequency and the *E. coli* concentration in solution (see Equation 1) [32]. The main advantage of this strategy is the easy setup of experiments, which does not need a bacteria pretreatment nor cells adsorption onto the UME surface for observing a reproducible electrochemical signal [32]. However, this method is not selective to *E. coli* bacteria because numerous cells have cytochrome c oxidases in their outer membrane [38].



**Fig. 3** a) Schematic representation of *E. coli* detection by impact electrochemistry. Bacteria are diffusing toward the microelectrode, oxidizing TMPD to  $\text{TMPD}^{+\cdot}$ , which is regenerated at the microelectrode (44  $\mu\text{m}$  diameter carbon working electrode) polarized at  $-0.15$  V vs. SCE, resulting in a reductive current spike. Adapted with permission from [32]. Copyright © 2018 Royal Chemical Society. b) Schematic representation of the electron transfer process of different microorganisms in the three-mediator system (left) and the corresponding  $i-t$  curves of *S. cerevisiae* (top) and *E. coli* (bottom) collisions (right). Carbon fiber electrode (diameter: 7  $\mu\text{m}$ ),  $E = +0.7$  V vs. Ag/AgCl. Adapted with permission from [34]. Copyright © 2022 American Chemical Society.

In order to quantify the redox activity of two bacterial species: the Gram-negative *E. coli* and the Gram-positive *B. subtilis*, thionine ( $\text{TH}^+$ ) has been used as a redox mediator electrostatically adsorbed on the bacterial surface, forming the bacterium-TH complexes [33]. Thionine can be reduced by the bacterial cell and when a single

bacterium-TH complex collides onto the UME (5  $\mu\text{m}$  diameter carbon fiber), the reduced thionine is re-oxidized at an appropriate potential (+1.0 V vs. Ag/AgCl) and generate a current spike in the  $i-t$  curve [33]. By quantifying the charge flowing during the current spikes, these experiments have shown a higher reactivity of *E. coli* cells ( $6.8 \times 10^{-18}$  mol of redox mediator reduced per bacterium) towards the TH mediator compared to *B. subtilis* ( $3.5 \times 10^{-18}$  mol of redox mediator reduced per bacterium) [33]. This work demonstrates an interesting method based on the electrochemical collision technique for quantifying the bacterial redox activity with a mediator at the single-cell level, which is of great significance to better understand electron transfer processes in microorganisms and thus, improve the design of microbial electrochemical technologies [33].

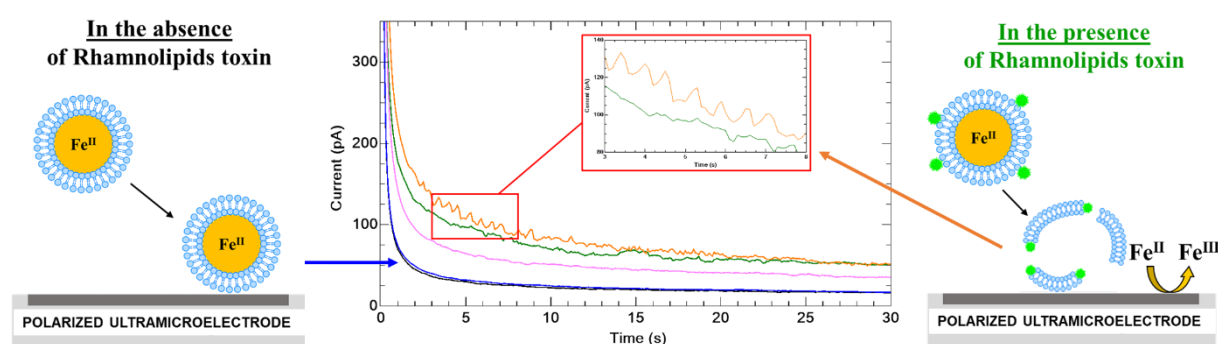
In a recent study, Chen *et al.* have reported a three-mediator system ( $\text{K}_3\text{Fe}(\text{CN})_6$ ,  $\text{K}_4\text{Fe}(\text{CN})_6$ , and menadione) for achieving redox activity analysis and selective identification of single *Saccharomyces cerevisiae* (*S. cerevisiae*, a yeast) without the use of antibodies (Fig. 3b, top) [34]. In the three-mediator system, there is an initial current due to the continuous oxidation of  $\text{K}_4\text{Fe}(\text{CN})_6$  to  $\text{K}_3\text{Fe}(\text{CN})_6$  at the carbon fiber UME (diameter: 7  $\mu\text{m}$ ) polarized at +0.7 V vs. Ag/AgCl (corresponding to the diffusion-limited steady state) [34]. Thus, single collisions of microorganisms onto the UME surface involve a blocking effect on the potassium ferrocyanide diffusion near the UME surface (as discussed in the first section of this review) [34]. However, the redox reactions between the *S. cerevisiae* cells and mediators also reduce  $\text{K}_3\text{Fe}(\text{CN})_6$  to  $\text{K}_4\text{Fe}(\text{CN})_6$  and increase the local concentration of  $\text{K}_4\text{Fe}(\text{CN})_6$  at the same time. The hydrophobic mediator (menadione) can selectively penetrate through the *S. cerevisiae* membrane and get access to its intracellular redox center, then can further react with  $\text{K}_3\text{Fe}(\text{CN})_6$  in the bulk solution (Fig. 3b, top left) [34]. Hence, the corresponding  $i-t$  curves for this microorganism in the three-mediator system show positive current step transients, combined with a steady state current increase (Fig. 3b, top right), corresponding to the impacts and adsorption of *S. cerevisiae* cells onto the UME surface [34]. In contrast, different electrochemical collision signals have been observed when the three-mediator system experiments are extended to *E. coli* (Fig. 3b, bottom) and *Staphylococcus aureus* (*S. aureus*, Gram-positive) bacteria (not shown here) [34]. Indeed, both *E. coli* and *S. aureus* only generate downward current steps because the blocking effect of mediator diffusion suppresses their redox activities (illustrated in Fig. 3b, bottom left for *E. coli*), thus only blocking collisions are observed in the  $i-t$  curves (shown in Fig. 3b, bottom right for *E. coli*) for these two types of bacteria in the three-mediator system [34]. Due to the opposition between the current signal generated by *S. cerevisiae* and the ones from *E. coli* and *S. aureus* in the three-mediator system, *S. cerevisiae* can be selectively detected and distinguished in the mixed microbial suspension [34]. Hence, this works provides a proof of concept for the selective detection of single *S. cerevisiae* cells from a mixed microbial consortium, based on the

electrochemical collision technique [34]. However, the electron transfer processes for fungi and bacteria differ significantly, thus the three-mediator system may not be suitable for selectively detecting similar bacterial strains in a mixed microbial suspension.

In conclusion of this section, we have reviewed the most significant studies dealing with electrochemical collisions of single bacteria with a redox mediator, by presenting some examples aiming to reach a better selectivity in the detection and identification of microorganisms. We have highlighted that the hardest challenge in this area is to design an efficient and versatile system for the analysis of several types of bacteria, with minimum preparation, in order to selectively detect and identify single living cells in real time based on the electrochemistry of impacts onto UME. Following an original and innovative strategy, a recent proof of concept presented in the next section has demonstrated the highly-sensitive detection of a bacterial virulence factor thanks to the redox liposomes single-impact electrochemistry [17].

#### Virulence factors detection by redox liposomes single-impact electrochemistry

In this last section, the detection of a sub-micromolar concentration of Rhamnolipid (RL) virulence factor by electrochemical single impacts of redox liposomes is reported (Fig. 4) [17]. RL is a glycolipidic biosurfactant secreted by *Pseudomonas aeruginosa* (*P. aeruginosa*), pathogenic Gram-negative bacteria involved in nosocomial infections [17]. The virulence factors such as RL are known to interact with the outer lipid bilayer membrane of eukaryotic cells and this property can be extended to liposomes and vesicles for pathogenic bacteria sensing [39].



**Fig. 4** Bacterial virulence factor detection by redox liposomes single-impact electrochemistry: chronoamperometry recorded at +0.7 V vs. Ag/AgCl on 10  $\mu\text{m}$  Pt UME in the presence of 500 nM RL and 3 pM redox DMPC liposomes (with encapsulated 0.5 M  $\text{K}_4\text{Fe}(\text{CN})_6$ ) for different incubation times. Adapted with permission from [17]. Copyright © 2022 Wiley Online Library.

The detection principle in this proof of concept is based on the weakening of the liposomes' lipid membrane caused by the interaction with RL, which leads to the breakdown of the liposomes upon impact at a UME, and

the release/electrolysis of its encapsulated redox probe (Fig. 4, right) [17]. Briefly, the redox liposomes single-impact electrochemistry consists in detecting the oxidation of ferrocyanide encapsulated in liposomes during single collisions onto the polarized UME surface, corresponding to current spikes in the  $i-t$  curve [20]. According to previous data, no current spike is observed in the  $i-t$  curves in the presence of redox 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) liposomes (with encapsulated potassium ferrocyanide) because they do not spontaneously break during impact onto the UME surface (Fig. 4, left) [13,15]. The addition of an optimal concentration of surfactant is required for weakening the liposome's lipid bilayer in order to facilitate its breakdown and the release/electrolysis of its encapsulated redox probe during the impact onto the UME [13,15]. This characteristic specific to the redox DMPC liposomes have been used for detecting the lowest concentration of RL reported in the literature for electrochemical sensing, by using the ability of this virulence factor to act as a biosurfactant on the liposome lipid membrane [17]. Hence, with RL species in solution, current spikes corresponding to the electrolysis of the encapsulated redox probe released from weakened liposomes during the collision onto the UME can be detected in the chronoamperometry measurement (Fig. 4, middle) [17]. These preliminary results based on redox DMPC liposomes have allowed the detection of a low concentration of RL (500 nM) in an aqueous phosphate buffer solution in less than 30 minutes thanks to the electrochemistry of single impacts at a Pt UME [17]. Nevertheless, the challenge of this work is to be successfully extended to other toxins and other types of liposomes in order to reach selective detection, which is a crucial parameter for biosensor applications [17]. Also, this electrochemical biosensor concept must be optimized for the detection of virulence factors from the supernatant of pathogenic bacteria, which is a significantly more complex mixture [17].

This short section highlights an original and elegant strategy to detect pathogenic bacteria with high sensitivity via their production of virulence factors and the redox liposomes single-impact electrochemistry. Nevertheless, the selectivity of this method for the detection and identification of several types of bacteria through their virulence factors also needs to be improved.

## **Outlook**

The trends in single-impact electrochemistry for bacteria analysis have been reported and discussed in this review. The two main current techniques in this area are the blocking electrochemical impacts of single bacteria and the electrochemical collisions with a redox mediator. The blocking collisions method is a very efficient and easy-handling strategy for bacteria detection at the single-cell scale onto the UME surface. Nevertheless, the

collision signal (current step transient) does not allow to identify the adsorbed bacterium, thus this technique is only suitable for discriminating single cells by electrostatic attraction/repulsion according to their own charge and the UME polarization. In contrast, the use of redox mediator(s) is a promising strategy for identifying single microorganisms according to their specific electrochemical activity with the mediator. The principle is based on the electron transfer of the single bacterium via the redox mediator, which is detected at the polarized UME by an increase of the current signal (current spike or step). The challenge of this technique is to be able to selectively detect the activity of the target's proteins or enzymes via the redox mediator in order to improve the specific single-bacteria sensing, and hence the identification. Indeed, the sensing of proteins and enzymes in the bacterial cell is a way to detect a specific electrochemical signature related to the bacteria strain thanks to a redox mediator. Also, for improving the instrumentation of this electrochemical technique, which is usually performed in a three-electrode cell with analytical microelectrodes as working electrodes, a microfluidic device with an ultramicroelectrode array will be probably the most appropriate system for an electrochemical biosensor application. A recent and original way has also been presented, dealing with the bacterial virulence factors sensing based on the redox liposomes single-impact electrochemistry, which is an indirect method to detect pathogenic bacteria via their secreted species. Although this proof of concept needs to be deeply investigated, this promising technique is also very efficient for the highly sensitive detection of virulence factors in a short time of analysis.

From an overall perspective, single-impact electrochemistry is an easy-handling and versatile technique for highly sensitive bacteria analysis at the single-cell scale. In our opinion, the next big challenge in this research area is to make this method selective for the identification of a given bacterial strain mixed within several different microorganisms (complex mixture). This is currently the most sensitive method to detect bacteria at the single-cell scale in a short analysis time, however the selectivity still needs to be improved for a possible extension to sensor applications and fundamental purposes. In the future, the coupling of electrochemistry and specific imaging techniques, such as fluorescence microscopy, will be essential to study the single bacterium behavior in real time with a high spatial and temporal resolution [20]. In addition to chronoamperometry measurements, new individual particle detection techniques, such as electrochemical impedance spectroscopy, could be interesting for single bacteria analysis, especially for evaluating the UME surface polarization effect on the adsorption of the cells [40].

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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**Graphical abstract**

