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Bipolar (Bio)electroanalysis

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Abstract

This contribution reviews a selection of the most recent studies on the use of bipolar electrochemistry in the framework of analytical chemistry. Despite the fact that the concept is not new, with several important studies dating back to the middle of the last century, completely novel and very original approaches have emerged over the last decade. This current revival illustrates that scientists still (re)discover some exciting virtues of this approach, which are useful in many different areas, especially for tackling analytical challenges in an unconventional way. In several cases, this "wireless" electrochemistry strategy enables carrying out measurements that are simply not possible with classic electrochemical approaches. This review will hopefully stimulate new ideas and trigger scientists to integrate some aspects of bipolar electrochemistry in their work in order to drive the topic into yet unexplored and eventually completely unexpected directions.

1. INTRODUCTION

1.1. General

In classic electrochemical textbooks, most of the content usually refers to conventional three electrode setups, composed of working, counter, and reference electrodes (1). The electrode that is controlled in terms of potential is the working electrode, where either oxidation or reduction reactions take place. In bipolar electrochemistry (BPE), oxidation and reduction reactions occur simultaneously on the same electrode and, most importantly, the bipolar electrode (BE) is not directly connected to a power supply.

A major advantage of BPE is the straightforward and easy-to-implement experimental setup. The general strategy is to place a (semi)conducting object in a solution where an electric field is generated between two electrodes. The conducting object is by definition equipotential, but the potential gradient existing in the surrounding solution will lead to an inhomogeneous potential difference between the solution and the object, which varies along its main axis parallel to the electric field lines. The direct consequence of the resulting polarization is that one extremity of the object will preferentially be the site of oxidation processes, whereas the opposite extremity will be involved in reduction reactions. Consequently, BPE generates an asymmetric reactivity at the surface of conducting objects and therefore constitutes a method of choice for breaking the symmetry in electrochemical systems.

Application of BPE to the field of electroanalysis is rather recent despite the fact that the concept is absolutely not new, and several important studies date back to the middle of the last century. At that time, BPE was proposed more in the framework of industrial devices, for example, as a driving force in fluidized bed reactors (2–4). However, the current renaissance nicely illustrates that scientists have only recently discovered some new and exciting virtues of this approach, especially in the framework of micro- and nanotechnology. Over the last two decades, many groups worldwide were able to report completely different aspects of BPE, with unforeseen potential applications in many areas, ranging from materials science to catalysis or energy storage (5–10). A primary platform for scientists currently using this concept is to apply BPE to develop new strategies in analytical chemistry, ranging from classic (bio)analytical challenges (11) to more unconventional topics, such as tracking corrosion processes (12–14). Several very attractive features are responsible for this trend. One single pair of electrodes used to address several thousand or even millions of BEs is advantageous because it enables setting up high-throughput screening schemes of analytes. The low-cost design is another significant advantage. Finally, yet importantly, as for many other electrochemical techniques, the miniaturization of devices is rather easy and thus an essential advantage when thinking about key issues such as the integration into microfluidic systems. Consequently, numerous publications have appeared in the last decade based on studies using this concept to develop original and sometimes quite unconventional analytical approaches. Thus, it is the right moment to summarize in this review these latest achievements in the field of wireless electroanalytical chemistry. The goal is to present BPE as an appealing alternative to a broad audience of analytical scientists, hopefully convincing them that these types of experiments often allow measurements that are simply impossible with classic electrochemical approaches. We specifically discuss these latter aspects after an initial, short introduction to some theoretical aspects.

1.2. Theoretical Aspects of Bipolar Electrochemistry

In this first section, we consider the case of a conducting object immersed in a homogeneous electrolytic solution and exposed to an electric field (that is applied between two so-called feeder

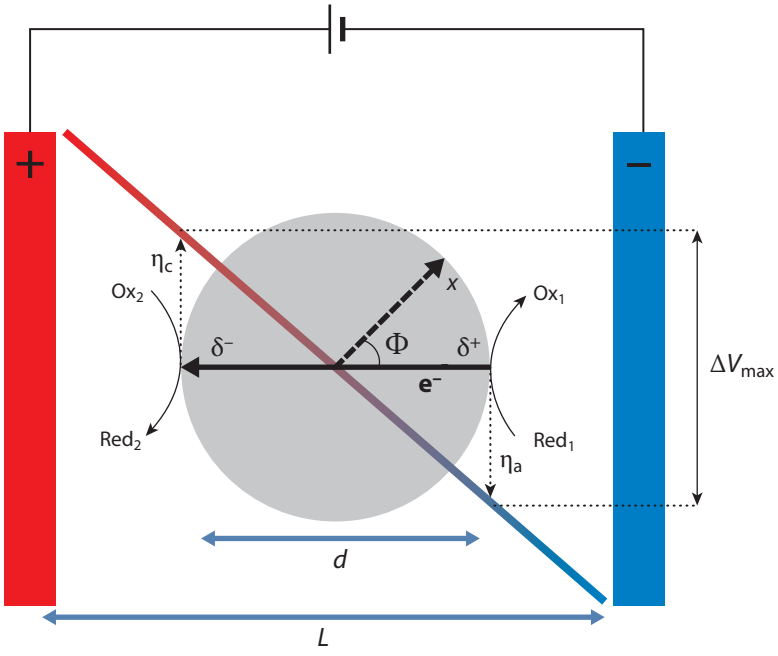

Figure 1

Scheme illustrating the polarization of a spherical conducting object with respect to the solution potential.

or driving electrodes). By definition, in general, the object is not directly in contact with one or both feeder electrodes (**Figure 1**). In the specific case of an open configuration, the object is not obstructing the electrochemical cell, thus allowing the electrolyte to still ensure ionic contact between the two feeder electrodes.

When these two electrodes are biased at potentials of V_a and V_c, respectively, an imposed potential difference V_{imp} is generated between them in the solution. This gives rise to an electric field E in the electrolyte expressed by

$$\mathrm{E} = \frac{V_a - V_c}{L} \qquad 1.$$

with L being the distance between the feeder electrodes. However, this is only a linear first-order approximation (see the bicolored diagonal line in **Figure 1**), neglecting additional potential drops that may occur at the electrode/electrolyte interfaces, as well as a distortion of the electric field by the presence of the bipolar object itself. As a result, anodic and cathodic polarization potentials η_a and η_c will be established at every position on the object. This polarization potential is a function of the localization x on the surface of the object that can be expressed as

$$\eta_x = \mathrm{E}\frac{d}{2}\cos\,\Phi \qquad 2.$$

for a spherical object, with d being the object diameter and Φ the angle between the electric field lines and a given position on the object. A maximum polarization potential difference is generated between the extremities of the object, and its value, $\eta_a - \eta_c = \Delta V_{max}$, is given by

$$\Delta V_{max} = \mathrm{E}\,d. \qquad 3.$$

If the amplitude of ΔV_{max} is sufficiently high and in the presence of appropriate redox-active species Red_1 and Ox_2, they can be electrochemically transformed by the following reactions:

$$Red_1 \rightarrow Ox_1 + n_1 e^- , \quad 4.$$

$$Ox_2 + n_2 e^- \rightarrow Red_2 , \quad 5.$$

with n_1 and n_2 being the numbers of electrons involved for each electrochemical reaction. If the two redox couples Red_1/Ox_1 and Red_2/Ox_2 have standard potentials of E_1° and E_2°, respectively, then ΔV_{max} has to be, in a first-order approximation, at least equal to the difference between the two standard potentials (the corresponding formal potentials under operating conditions might be used instead of the standard potentials):

$$\Delta V_{max} \geq E_1^\circ - E_2^\circ . \quad 6.$$

Consequently, the conducing object behaves as a BE, simultaneously promoting in a wireless way these two electrochemical reactions. Interestingly, it is possible to adjust the driving force by controlling the electric field in the solution.

These considerations reveal that the two most important key parameters for bipolar electrochemical reactions to occur are (*a*) the external electric field E and (*b*) the size of the object d, as indicated by Equation 3 (see **Figure 1**). The latter can become problematic when trying to achieve bipolar electrochemical reactions on nanosized objects. Another issue that is intrinsically related to the open configuration is that the total current flowing through the cell is divided into two contributions. One fraction flows through the BE as a real faradaic current and triggers the bipolar electrochemical reactions. The second fraction, which can be considered as a bypass current, flows through the solution, without driving electrochemical reactions at the BE, and is strictly related to ionic flux. In order to minimize the latter one, it is preferable to work with solutions of high resistance. Another option is to use a so-called closed configuration of the bipolar electrochemical cell. This setup corresponds to a situation where the BE is the only existing current path, meaning that everything around the BE is made out of an insulator, and de facto the cell is divided into two independent compartments (15, 16). This has several important advantages, especially for objects with dimensions in the micro- and nanometer ranges, and also for analytical applications (16–19). The fact that no bypass current exists is especially interesting for cases where high current efficiencies are required. In the extreme case, almost the entire externally applied potential drop occurs between the extremities of the BE. Last but not least, with the cell separated into two independent compartments, the closed configuration allows a physical separation of the reducing agents from the oxidizing ones. This might be an advantage if it is mandatory to isolate reaction products from reactants for chemical compatibility reasons, or if one wants to employ different solvents in the two compartments for reasons of solubility. Each of these two configurations of BPE has its advantages and drawbacks, and therefore using one or the other will essentially depend on the specific requirements of a given analytical challenge.

1.3. Experimental Considerations

Remarkably different setups of BPE have been used over the last decades, ranging from ordinary beakers with two conducting wires acting as feeder electrodes, to microfluidic devices with specifically designed electrode patterns. The electrolytic solution can be a simple aqueous electrolyte or more exotic electrolytes such as ionic liquids. In the following sections, some general aspects

are presented that should give the reader a brief overview and allow them to choose the most appropriate cell for a given analytical task.

BPE has quite a long history, as mentioned above. Since the early 1970s, the basic concept of BPE has been used for developing different types of bipolar electrochemical cells. In this context, classic cells have been based on electrodes that are stacked between feeder electrodes in either an open or closed configuration. For the closed configuration, the absence of bypass currents is a major advantage (vide supra). Many BEs can be found in industrial stacks (20), and their shape can vary from plates (21, 22) to porous conductive membranes (23), but their use in the framework of analytical chemistry is rather limited. Bipolar cells packed with beads seem to be more interesting in this context. In this case, the BEs are conducting objects usually in the size range of millimeters to submicrometers, packed in an ordered or random way (24, 25). The bipolar particles should be sufficiently far apart to allow their efficient polarization in bulk and avoid mutual screening effects of the electric field, as such cross talk would be deleterious for the efficiency of the device.

Equation 3 indicates that performing BPE becomes increasingly difficult when decreasing the size of the object, as it is necessary to apply higher electric fields to achieve the same polarization potential difference between the extremities of the BE. This is especially true for addressing micro- and nano-objects. Theoretically, electric fields on the order of hundreds of kV/m are then necessary, causing various practical problems such as gas bubble formation, excessive ohmic heating, and massive convection in the cell. One way around this intrinsic problem is to perform BPE in a capillary electrophoresis (CE) setup, initially proposed for analytical applications (26). The analog setup, adapted for capillary-assisted bipolar electrodeposition (CABED) (27), was the first successful attempt to use CE equipment for bipolar electrodeposition and also constitutes the first example of bipolar electrodeposition in the bulk of an electrolyte. CE is classically employed for separating chemicals in analytical chemistry. Usually, the capillary is inserted into a chamber where its two extremities are dipped into the electrode compartments containing the solvent. The electrodes are located outside the capillary in such a way that bubbles generated by the electrolyte decomposition cannot enter it. After being filled with a solution of interest, voltages of up to 30 kV are applied, resulting in electric fields of up to 150 kV m^{-1} for a 20-cm-long capillary. An ultraviolet-visible (UV-vis) detector is located close to the outlet of the capillary, monitoring the composition of the solution leaving the capillary due to the electroosmotic flow. Performing BPE with CE equipment has several advantages (28). The first is due to the high electric fields that can be applied, which is an extremely important parameter for the polarization of small objects. A second attractive feature is the intrinsic presence of liquid flow owing to the electroosmotic flow within the capillary. This flow continuously drives the bipolar objects from the inlet to the outlet. Finally, the capillary as a bipolar electrochemical reactor provides a high surface area for heat dissipation and a restrictive current path that allows decreasing bypass currents.

Although the CABED setup allows addressing thousands and even millions of nanoparticles simultaneously in the bulk of a solution, it also has drawbacks. First, it uses a CE device that is rather expensive. Maybe the strongest restriction of the CABED approach is that the modification takes place in a narrow capillary with an inner diameter in the range of 100 μm, making the process poorly adapted for scaling-up the production of modified particles for industrial applications. In order to partially overcome these limitations, capillaries with much larger diameters have been used, not only in the context of bipolar electrodeposition (29), but also to address bipolar nanoobjects in the framework of analytical challenges (30, 31).

However, even in this case, the capillary contains at most a few milliliters of solution. Therefore, an alternative setup consists of using a cell composed of one reaction compartment, in which the bipolar objects and the reagents are located, separated from the two electrode compartments by two membranes (32). The feeder electrodes, which are immersed in the solvent containing only

supporting electrolyte, are connected to a high-voltage power supply. Rather inert materials such as gold, platinum, or carbon are typically employed as feeder electrodes. Polymer or sintered glass membranes with satisfactory mechanical stability and permeability are used as separators to limit the interference of bubble formation and solvent evaporation as well as convection, occurring in the electrode compartments when applying high electric fields, with the reaction compartment. Another obvious advantage of this setup is that the electroactive precursors will not undergo parasitic reactions at the feeder electrodes, as they are only present in the reaction compartment, delimited by the membranes. However, one has to be aware that such separators induce an additional potential drop and thus lower the effective electric field in the reaction compartment.

In addition to the setups described above, several more special configurations in terms of cell design, electrodes, and experimental conditions have been developed in recent years and are described in more detail in the following sections.

2. ANALYTICAL APPLICATIONS

2.1. Analytical Readout Schemes

In the last decade, different strategies have been developed to use BPE as an analytical technique. In all the configurations, the BE has a sensing site to detect the analyte and a reporting site to quantify the analyte (33) (**Figure 2**). The simple experimental setup is composed of a generator connected to the anode and the cathode to apply the electric field and a conducting object located between the two electrodes, which will be the BE. In certain cases, an additional instrument to measure the signal (e.g., light) emitted from the reporting site is required. As mentioned above, the open configuration offers various possibilities to carry out experiments for analysis and sensing (30). For example, an open configuration based on the use of nanopipettes has been reported. In this configuration, the inner wall of a nanopipette tip is decorated by a metallic layer (34, 35), and wireless electroanalysis has been performed at the tip of the nanopipette (36). In the case of the closed configuration, several approaches have been reported, depending on the type of

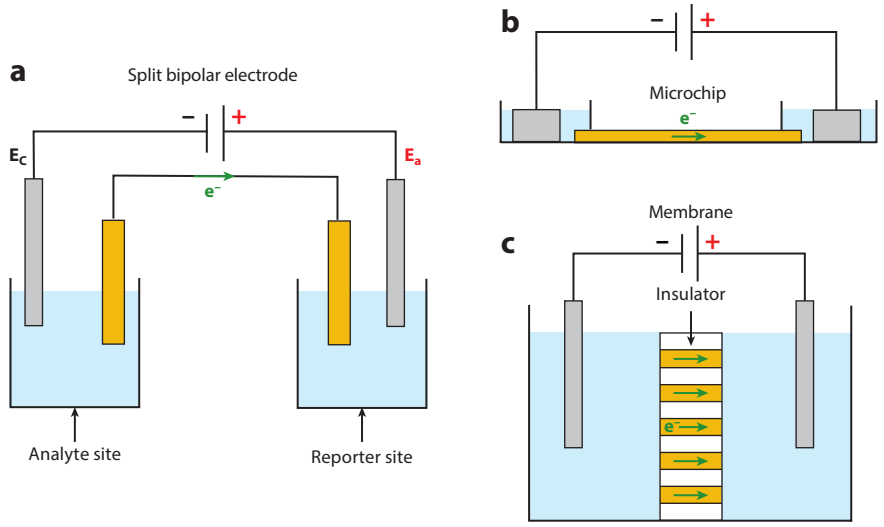

Figure 2

Schemes illustrating different possible setups for a closed bipolar electrochemical cell.

BE connecting the two compartments. Microchips with electrode arrays and membranes filled with conducting materials as well as split BEs, connecting the two independent compartments via an external wire, have been used in this context. One major advantage of BPE is the ability to simultaneously address a large number of objects to carry out multiple detections. Therefore, chips containing several BEs have been prepared by (photo)lithography to generate micrometer-sized electrodes that allow parallel analysis (37–44).

An interesting alternative to (photo)lithography, which is expensive, is the use of paper-based microsystems (45–50). This approach, based on screen-printing technology, represents an affordable strategy to develop even quite sophisticated devices. Chips can also be made by simply using an indium-doped tin oxide (ITO) slide with polydimethylsiloxane (PDMS) slices (51, 52). When using a membrane to separate the sensing and reporting compartments, the membrane channels can be filled with conducting material to create a massive array of nanoelectrodes, which will ensure the faradaic charge transfer between the two compartments (53–55). While in most cases the BE is a single object, it is also possible to connect two individual electrodes, located in the respective compartments, with a wire to make them behave as one single BE. Such an electrode is called a split-BE. The split-BE offers the possibility to spatially separate the sensing and reporting compartments at large distances. This method has been used to connect a carbon electrode with another carbon electrode (56, 57), a semiconductor (58), or a screen-printed electrode (59). Two microelectrodes can also be connected together (60–62). Furthermore, a suitable configuration is also to couple a bipolar electrochemical reaction with measurements with a potentiostat (56, 63–65).

Recently, an original setup without any feeder electrodes was proposed. The anodic and cathodic poles of the generator were directly connected to an ITO plate. The internal resistance of the ITO electrode induces a potential gradient along the electrode, and thus a variable polarization with respect to the solution that can trigger anodic and cathodic reactions at opposite locations on the surface of the plate (66).

Most of the reporting sites use an optical readout based on electrochemiluminescence (ECL) (38, 39, 67, 68). In order to trigger an ECL reaction, an electroactive luminophore is dissolved in the solution. The bipolar electrochemical process initiates a series of chemical reactions that will trigger light emission. The two most employed ECL-active dyes are tris(2,2′-bipyridine)ruthenium ($[Ru(bpy)_3]^{2+}$) and 3-aminophthalhydrazide (luminol). Usually, a sacrificial coreactant has to be present in the solution to promote the excited state of the luminophore. Tri-*n*-propylamine (TPA) and hydrogen peroxide (H_2O_2) are employed with $[Ru(bpy)_3]^{2+}$ and luminol, respectively. Fluorescence emission is another type of optical readout, for example, by using a fluorogenic reaction that will convert an electrochemical signal into a fluorescent signal (69). The redox couple resazurin/resorufin is interesting, because resazurin is a weakly emissive molecule, while resorufin is a much brighter luminophore (70). Instead of light emission, an electrochemically induced change of color (i.e., electrochromism) can also be employed as an optical readout (71). In this context, methyl viologen (MV^{2+}) is often used because the di-cation and the radical cation MV^{+} are colorless and purple, respectively (72, 73). The potential-induced color change of Prussian blue is also a good alternative, as has been demonstrated, for example, in the frame of potentiometric-sensing arrays composed of closed BEs (74). To detect the light emitted by the reporting site, a simple digital camera (75) or smartphone (42, 76) enables quantitative measurements. However, for more precise monitoring, the emitted light is mainly recorded with a microscope equipped with a charge-coupled device (CCD) camera (62, 77, 78) or a photomultiplier tube (52, 79). The light from the reporting site can also be guided to the camera by an optical fiber (59, 80, 81). Non-optical readout concepts are equally available, such as potential or current measurements (82–84), electromechanical actuation (85–87), or the dissolution of metals (88).

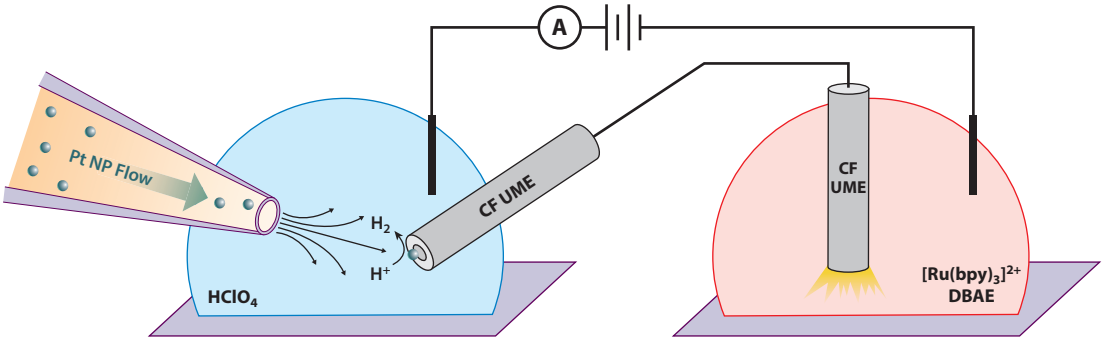

Figure 3

Scheme of the experiment coupling Pt nanoparticle (NP) collisions to anodic electrochemiluminescence (ECL) across a split-BE (bipolar electrode) composed of two carbon fiber ultramicroelectrodes (CF UMEs). The microjet collision and the reporting ECL were characterized on two separated inverted microscopes. ECL was achieved by using $[Ru(bpy)_3]^{2+}$ luminophore and 2-dibutylaminoethanol (DBAE) co-reactant. Other abbreviation: $[Ru(bpy)_3]^{2+}$, tris(2,2′-bipyridine)ruthenium. Adapted with permission from Reference 61; copyright 2020 John Wiley & Sons.

2.2. Electroanalysis

BPE can be used to perform various types of electroanalytical measurements, either to characterize materials or to detect analytes. For example, Eßmann et al. (82) investigated the bifunctional water-splitting catalyst Co_2B for hydrogen evolution reaction and oxygen evolution reaction. Using this approach, all of the key parameters for both reactions (overvoltage, half-cell potential, and catalyst stability) were obtained in a single experiment by using a split-BE. Defnet & Zhang (61) used two carbon microelectrodes as a split-BE to study transient collision events of single Pt nanoparticles. The split-BE converted the amperometric signal generated for each collision at the sensing site into an optical signal on the reporting site (**Figure 3**). On single Pt nanoparticles (70 nm), the fast transient hydrogen evolution reaction (~400 ms) at the cathodic side was measured via the ECL signal at the anodic side. This study opens up the potential to image transient redox processes when large arrays of closed BEs are used.

Han et al. (89) demonstrated that a nanopipette (~60 nm in diameter) can be used to observe transient BPE on a single silver nanoparticle (Ag NP, 50 nm). When the particle approaches the nanopore aperture, it is possible to oxidize one side of the particle and to produce H_2 on the other side due to the focusing of the electric field. These experiments allow the study of gas generation on nanocatalyst materials.

An original method that combines the advantages of BPE and electrochemical microscopy has been developed under the name of scanning bipolar electrochemical microscopy by Santos et al. (62). This recent technique employed two microelectrodes in the split configuration to observe small changes in local O_2 concentrations at a photocatalyst (Mo-$BiVO_4$) revealed by the ECL signal on the reporting site.

It is also possible to detect heavy metals (Cu^{2+}, Cd^{2+}) in water by using potential-resolved multicolor ECL with the split configuration based on the combination of a screen-printed electrode and a simple glassy carbon electrode (59). BPE has also been used to develop new types of H_2O_2 sensors, either by changing the morphology of the electrode (75) or by playing with the composition of the solution at the reporting site (79). Using nanoporous ITO at the sensing site, Seo et al. (75) reported a linear detection range of 0.02–5 mM and a limit of detection (LOD) of ~20 μM for H_2O_2. The LOD was divided by a factor of ten compared to bare ITO. Hao et al. (79) also employed all-inorganic perovskite $CsPbBr_3$ quantum dots at the reporting site to detect H_2O_2.

The quantum dots emit an ECL signal at the anodic pole simultaneously with the reduction of H_2O_2 at the cathodic pole, leading to a linear detection range of 1–200 mM and a LOD of 50 μM.

Ibañez et al. (81) developed bipolar spectroelectrochemistry. They used UV-vis spectroscopy with optical fibers in the reflection mode to observe oxidation and reduction reactions on the same BE. The setup allows separation of the electrochemical signal into two different optical signals from the two compartments. The behavior of redox molecules such as $[Ru(bpy)_3]^{2+}$, $[Fe(CN_6)]^{4-}$, and $[IrCl_6]^{3-}$, but also conducting polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT), have been studied with this method. The coupling of these two techniques promises to offer interesting analytical possibilities in the future. In addition to the readout schemes used above, a completely new approach appeared recently, based on the mechanical deformation of a conducting polymer triggered by BPE. In this case, the analyte is oxidized at one extremity of a bipolar polymer strip, whereas a reduction of the conducting polymer itself occurs at the opposite side. The latter is accompanied by an exchange of ions, which leads to shrinking or swelling of the polymer and finally to a well-controlled macroscopic deformation of the bipolar object (90–92). The rate and amplitude of deformation are directly correlated with the concentration of analyte; this approach could thus be successfully used to detect various analytes, ranging from H_2O_2 to glucose (85), with good linear range and detection limits. An additional aspect, which has been explored based on this detection approach, is the enantioselective recognition of chiral analyte molecules. When the positively polarized side of the polymer strip is postmodified with an enantioselective material, either a chiral metal (86) or intrinsically chiral polymers (87), the oxidative conversion becomes stereoselective. Therefore, deflection of the polymer at the δ^- extremity will be more pronounced in the presence of one of the enantiomers or even allows absolute chiral recognition, providing an on/off answer when one or the other molecular antipode is present in solution (**Figure 4**).

2.3. Bioelectroanalysis

Different biological entities have been used in combination with BPE to design highly selective and sensitive analytical devices. For reasons of clarity, the literature survey in this section is structured based on the nature of the biochemical ingredient.

2.3.1. Enzymes. Enzymatic systems have often been combined with BPE, mainly to develop original bioanalytical platforms exploiting the versatility of BPE. For the analytical applications, as already discussed, a major challenge associated with BPE is the readout of the signal due to its wireless feature. Because the electrochemical activity of a BPE system can be converted quantitatively into an optical signal, as reported initially by Manz's group (93), the different optical readout methods discussed previously, such as ECL, fluorescence, colorimetry, and electrochromism (6, 33, 94), have also been used for bioelectroanalysis. For BPE, redox enzymes are exploited to convert target analytes into redox-active or optically active species. Bohn and coworkers (95) described a multiplexed closed bipolar sensor for the simultaneous detection of lactate, glucose, and uric acid. The activity of these specific oxidases was coupled with an electrochromic reaction occurring in a separated reporter cell in such a way that the magnitude of the color change was correlated with the concentration of the enzymatic substrates in the readout compartment. Moreover, the device was fabricated as a paper-based carbon electrode, and the color change was simply recorded by a smartphone. Such a straightforward readout makes the approach quite attractive, and this is reinforced by the disposable character of the device. Based on the electrochromism of Prussian blue films, a resettable and reprogrammable enzymatic keypad lock system was presented for the direct detection of ascorbic acid and glucose by the naked eye (96). Fluorescence-based reporting

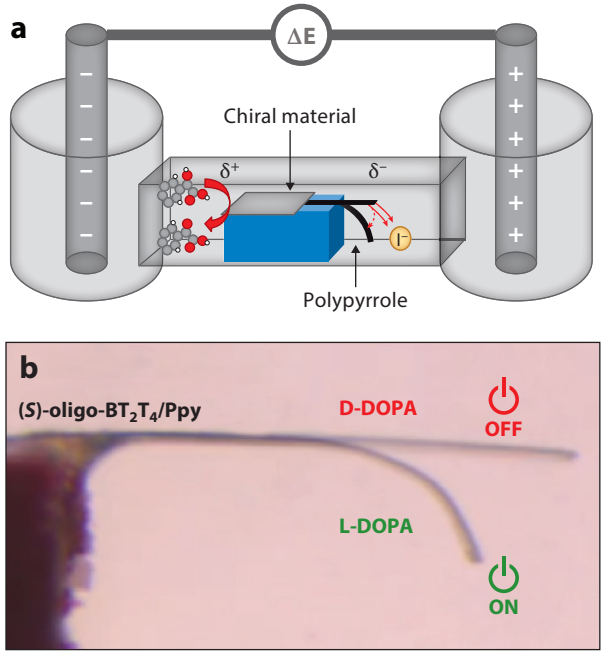

Figure 4

(*a*) Scheme of the experimental setup allowing a bipolar electromechanical readout of analytical information. Panel adapted with permission from Reference 86; copyright 2019 Royal Society of Chemistry. (*b*) Superposed optical microscopy pictures of the wireless bending induced on a polypyrrole (Ppy) strip in the presence of the wrong enantiomer (off state) or the good enantiomer (on state) of 3,4-dihydroxyphenylalanine (DOPA) when an intrinsically chiral polymer (oligo-BT_2T_4) is used as a selector on the positively polarized extremity of the polymer strip. Panel adapted with permission from Reference 87; copyright 2020 American Chemical Society.

reactions were also combined with BPE and enzymes. The oxidation of dihydroresorufin into resorufin, a highly fluorescent dye, was successfully applied for the detection of glucose and H_2O_2 using enzyme-modified electrodes at the reporting site (57). ECL, which is a very sensitive analytical method (97), was also coupled with BPE in closed and open configurations (46, 98, 99). Indeed, the by-products of the enzymatic activity of dehydrogenases and oxidases, such as NADH and H_2O_2 may react with $[Ru(bpy)_3]^{2+}$ or luminol and generate orange-red or blue ECL emission, respectively. The multiplexed detection of glucose, lactate, and choline was reported using the corresponding oxidases on a BE array together with luminol ECL (39).

A paper-based fluidic device operating in a closed bipolar mode was fabricated by a wax and carbon ink-based screen-printing process (100). The quantitative ECL-based analysis of H_2O_2 and glucose was demonstrated in four complex samples (human serum, urine, wine, and glucose injection). The band-shaped, paper-based fluidic device represents an interesting configuration with easy and low-cost fabrication procedures. Multicolor ECL emission has also been reported in an open bipolar configuration for single conducting objects as well as for a dispersion of micro- and nano-objects (31, 68).

Because BPE enables one to electrochemically address a multitude of conductive objects (25), bulk ECL imaging was combined with enzymatic coreactant production for the sensitive remote detection of two different analytes, namely, choline and glucose (30). It is noteworthy that bipolar ECL imaging was performed simultaneously with dehydrogenase-type and oxidase-type enzymes,

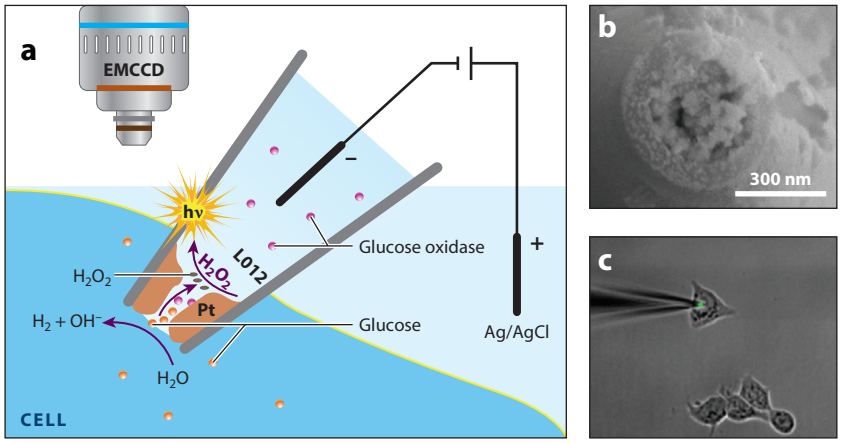

Figure 5

(*a*) Scheme of the bipolar electrochemiluminescence (ECL) detection at a nanopipette tip with the inner walls decorated by a Pt deposit. The tip is inserted into the cytosol for intracellular wireless analysis. (*b*) Scanning electron microscope (SEM) image of the Pt deposit at the nanopipette tip (front view). (*c*) Overlaid bright-field and ECL images of the nanopipette showing the detection of intracellular glucose in single living nonstimulated HeLa cells. The green spot originates from the ECL emission confined at the nanopipette tip (36). Other abbreviation: EMCCD, electron multiplying charge-coupled device. All panels adapted with permission from Reference 36; copyright 2020 John Wiley & Sons.

leading to concomitant orange-red and blue ECL emissions, respectively. In addition, this original approach provides a global chemical view through one single ECL image of inhomogeneous samples such as a biochemical concentration gradient formed in a capillary (30).

The selectivity of the enzymes has been recently combined with the confinement of the voltage drop in spatial restrictions by Jiang and coworkers to perform intracellular wireless analysis of single cells by bipolar ECL (36). As described previously in Section 2.1 and reported by several groups, the voltage drop is confined within the nanopipette tip (35, 89, 101–103), which generates ECL emission at very low voltage. The inner walls of a nanopipette tip were decorated by a Pt deposit that is used as an open bipolar ECL device (**Figure 5**). The electric field provokes the sorting of various molecules from the intracellular space into the nanopipette, where they react with L012 (8-amino-5-chloro-7-phenylpyrido[3,4-d]pyridazine-1,4(2H,3H)-dione), a luminol analog with high ECL efficiency, and produce a light signal. The synergetic action of the nanopipette and the bipolar enzyme–based ECL approach allows the in vivo measurement of the intracellular concentrations of hydrogen peroxide, glucose, and the intracellular sphingomyelinase activity (i.e., hydrolase) with the corresponding enzymes. This original strategy, which requires a remarkably low voltage, minimizes an eventual bias of the cellular activity and provides a novel concept in the field of single-cell electrochemical analysis.

As described above, most enzyme-based developments using BPE are focused on bioanalytical applications. However, BPE has also been coupled with enzymatic systems to investigate enzyme activity and construct enzymatic cells. The versatility of BPE was applied to prepare both a biocathode (bilirubin oxidase immobilized in a thiophene-based film) and a bioanode (glucose dehydrogenase immobilized on Au nanostructures) for mediatorless/membraneless biofuel cells (104). The assembled glucose/O_2 cell offers a power output of 146 $\mu W\ cm^{-2}$, with an open circuit voltage of 0.54 V. It illustrates an interesting application of BPE in the field of bioenergy. Conzuelo and coworkers (105) reported the electrical coupling of different photosystems and the resulting

photobioelectrochemical cell. Photosystems 1 and 2 were embedded and wired to the underlying electrode using an osmium-complex-modified redox polymer in a closed setup. The response upon illumination was studied, and the effects of different parameters such as pH, temperature, electron acceptor, or inhibitor concentration were investigated. Such a photobioelectrochemical study of the enzymatic performances provides interesting insights into the working principle of closed bipolar systems. Another enzymatic system, cytochrome c, was also studied in a four-electrode, closed bipolar cell, and direct electron transfer has been demonstrated between cytochrome c and the aqueous pole of a BE in aqueous-organic electrolyte solutions (106). This interesting approach facilitates the investigation of electron transfer between cytochrome c and redox mediators at the interface between two immiscible electrolyte solutions, which mimic soft biomembranes. The wireless feature of BPE, combined with the selectivity provided by the enzymes, offers a wide range of bioanalytical sensing strategies with disposable miniaturized devices.

2.3.2. DNA. DNA is a classic bioanalytical target in the field of biosensors. In that context, one can discriminate two classes of electrochemical sensors that use DNA as a key ingredient. The first strategy, which was historically developed, relies on the immobilization of single-stranded DNA (ssDNA) at the surface of an electrode in order to use such a modified surface to detect the complementary sequence through DNA hybridization. In the second approach, DNA is not necessarily the analytical target, even if nucleic acid recognition processes are involved. For DNA sensing, BPE offers many advantages in comparison with conventional electrochemistry: (*a*) Because two electrochemical reactions are coupled across the BE, it is an ideal way to separate the sensing process and the reporting reaction; (*b*) a large ensemble of BEs can be employed in parallel, thus allowing high-throughput screening or multiplexing; and (*c*) the special case of the closed configuration offers the possibility to use two different electrolyte solutions in the two half-cells that are physically separated (aqueous/organic, different chemical composition, pH, etc.). The first example of wireless DNA sensing with ECL readout was originally proposed by Crooks and colleagues (107). From that early report, the same team and others were able to imagine additional schemes for DNA detection that were already reviewed several times (6, 33, 108, 109).

Focusing on the most recent contributions, an enhanced ECL detection strategy on a closed BE was proposed for the detection of prostate-specific antigen (PSA) (110). In this report, the target was recognized on the cathode part of the BE, whereas the anode served as a reporting pole. For the latter, a bottom up approach was adopted with the immobilization of the ssDNA probe prior to hybridization, with the complementary strand labeled with a gold nanoparticle (Au NP). This NP catalyzes anodic ECL involving luminol and H_2O_2, offering an excellent readout signal. Another strategy that involves the same Au NP–modified DNA was reported for the detection of cancer cells (111). As before, these Au NPs catalyze ECL, but they also act as seeds for the chemical reduction of Ag^+ cations released from the anodic dissolution of Ag. In that case, the formation of the Ag layer surrounding the Au NPs completely quenches the ECL emission of luminol. An original approach based on a direct ohmic loss principle was also proposed for the DNA-mediated detection of thrombin (56). The authors studied in detail the influence of key experimental parameters on the iR drop of a closed BPE device. They rationalized this effect by the peak separation of a potassium ferro-/ferricyanide redox probe, enabling a new class of biosensors by connecting sensing units with tunable resistance. The principle was exemplified with a thrombin aptamer in combination with an Au-modified complementary DNA. Again, these Au NPs were used to mediate the growth of Ag, which directly affects the resistance in the gap.

An ECL strategy for the detection of the HIV genome was also designed on a closed split-BE (112). Anthraquinone disulfonic acid was used as an electroactive reporter that intercalates with hybridized DNA on the sensing site and is reduced at the cathodic pole. Luminol is oxidized

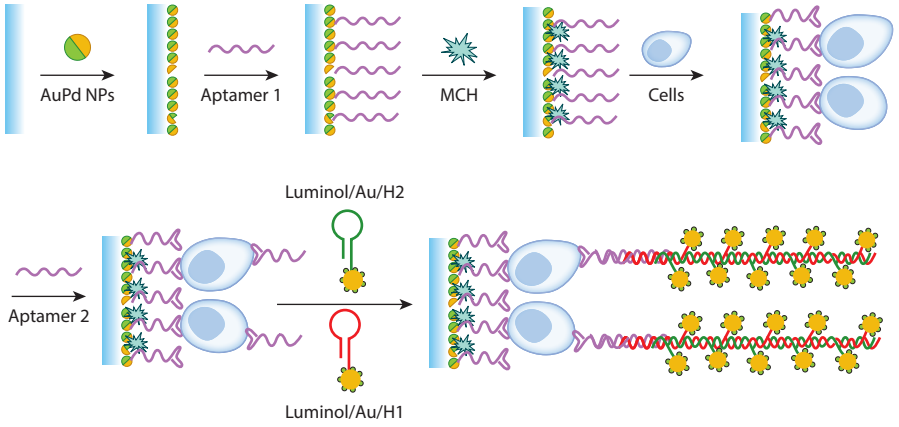

Figure 6

Illustration of a bottom-up sandwich-type assembly process of a highly sensitive cytosensor (limit of detection of 40 cells/mL) developed in Reference 47. Abbreviations: MCH, 6-mercapto-1-hexanol; NP, nanoparticle. Adapted with permission from Reference 47; copyright 2018 Elsevier.

on the anode, leading to an ECL signal that is directly correlated with the amount of short specific HIV oligonucleotide targets (dynamic range from 0.1 to 300 nM). A paper-based coupling between BPE and ECL was used for the rapid detection of Hg^{2+} (113). In this original strategy, the divalent cation mediates two thymine-enriched ssDNA probes to form a duplex where the ECL-active $[Ru(phen)_2dppz]^{2+}$ dye can readily intercalate. This organometallic complex features a dipyridophenazine (dppz) ligand that is responsible for the quenching of luminescence in aqueous media. However, the ECL signal is restored upon intercalation thanks to the local environment surrounding the DNA. It is noteworthy that the same intercalator was also used directly for the detection of pathogenic bacteria (48). Hydrophilic channels were wax-screen-printed on filter paper, whereas the BE and the two driving electrodes were screen-printed into the channels by using a carbon ink. Polymerase chain reaction (PCR) amplification of the bacterial DNA was therefore detected by the ECL signal originating from the complex when intercalated into DNA.

Other strategies that take advantage of in situ hybridization chain reactions (HCRs) were also proposed (47, 51). In a first example, luminol-modified Au NPs were immobilized at the surface of capture cancer cells via HCR with two hairpin DNA molecules (47) (**Figure 6**). The ECL coreactant was released from the target cells, leading to an ECL signal from luminol that was directly related to the number of cancer cells in the sample with an LOD of 40 cells/mL. An HCR-induced ECL amplification strategy was also reported for the detection of DNA and H_2O_2 (51). The ECL signal that occurred at the anode of the BE was coupled with the electrochemical signal at the cathode, which was triggered by target DNA and amplified through the HCR process. The calibration curve showed a wide linear relationship between the strength of the ECL signal and the DNA concentration (from 0.1 nM to 0.5 μM).

Recently, a smart area controllable interface was designed for the detection of tetracycline. It was based on a Janus glassy carbon bead, half covered by Au through BPE and functionalized with methylene blue (MB) and a DNA walker (114). In the presence of tetracycline, an aptamer that was partially hybridized with the DNA walker was released from the surface to enable the formation of a new duplex with the walker and MB. The latter was cleaved from the surface with an endonuclease, which released MB and triggered ECL detection in a closed configuration.

2.3.3. Antibodies. Historically, ECL has been commercialized on the diagnostic market for the detection of specific antigens. Today, the number of ECL-based immunoassays performed per year reaches almost two billion. While most of these tests are performed by using a conventional electrochemistry setup, BPE can offer an interesting alternative with many different experimental configurations.

Li, Wang, and colleagues (53) proposed a multichannel closed BPE array based on a polyethylene terephthalate (PET) membrane. The multichannel PET membrane was etched in basic conditions before modification of the inner walls of the channels with Au nanofibers. This approach led to an array of BEs with a controllable ion track density from $10^4/cm^2$ up to $10^8/cm^2$. It was combined with the $[Ru(bpy)_3]^{2+}$/TPA system to generate the ECL output and applied to the detection of multiple targets, including dopamine, α-fetoprotein, and carcino-embryonic antigen (CEA). The same antigen target was also detected based on an electrochromic readout by the same authors (72). The CEA aptamer was preanchored on the bipolar anode for capturing CEA through affinity binding, resulting in steric hindrance and lower current. Prussian blue was used as an electrochromic indicator to report CEA concentration (typically in the ng/mL range) and distinguish samples of cancer patients from unaffected ones.

The ability of Ag@Au nanoparticles to quench luminol ECL, as discussed in Section 2.3.2, was also recently exploited in the context of antibody-based bipolar detection. This enables the measurement of PSA in a wide dynamic range from 0.1 μg/mL down to 0.1 pg/mL, which fulfills the clinical requirements for PSA determination (110). The possibility to apply this ECL test to real human serum samples was confirmed at a ng/mL PSA concentration and validated by comparing the results with a standard method. Another sensing platform for PSA monitoring in human blood serum was based on a multicolor ECL configuration in a closed bipolar setup (67). This approach combines the immobilization of an Au/Ag core-shell structure (**Figure 7**) with potential-resolved ECL, using a mixture of red and green luminophores ($[Ru(bpy)_3]^{2+}$ and $Ir(ppy)_3$, respectively) with TPA as a coreactant. The variation of interfacial potential at the poles of the BE results in an ECL color change and was used for the quantification of clinical biomarkers in a sandwiched antigen assay. Based on the same philosophy, a bidirectional ECL color switch was used for detecting multimarkers of prostate cancer (37). This time, the authors selected an original cyclometalated complex of iridium, featuring difluoro-phenylpyridine (df-ppy) and picolinate (pic) ligands. The bidirectional color change from blue to red could be observed with either an increase or a decrease of applied voltage, due to the dual excitation properties of $Ir(df\text{-}ppy)_2(pic)$. This ECL switch was applied to the detection of three biomarkers of prostate cancer such as PSA, μRNA, and sarcosine. Finally, spatially resolved ECL ratiometry was adapted to detect PSA by using another ECL dye, namely graphite-like carbon nitride ($g\text{-}C_3N_4$) (115). The cathode of the closed BE is modified with $Au@g\text{-}C_3N_4$ (blue emitter), which is coupled with the anodic ECL emission of $[Ru(bpy)_3]^{2+}$. The corresponding PSA biosensor has a calibration curve with a linear range from 1 to 200 ng/mL, and the measurements are in good agreement with the reference method used for clinical diagnostics.

In an effort toward parallel screening of samples, a very simple but efficient closed BE array was developed. The authors used an array of polymer-made wells that can be addressed individually. The proof-of-principle was achieved with an array composed of seven sensing compartments connected with a single reporting one (52). The ECL reporting was achieved by using $[Ru(bpy)_3]^{2+}$ dye together with ammonium oxalate coreactant. This sensing platform was applied to the detection of CEA biomarker with bimetallic Pd/Pt nanocrystals and a classic primary antibody/antigen/secondary antibody sandwich immunoassay. An impressive LOD down to 0.001 ng/mL was readily achieved.

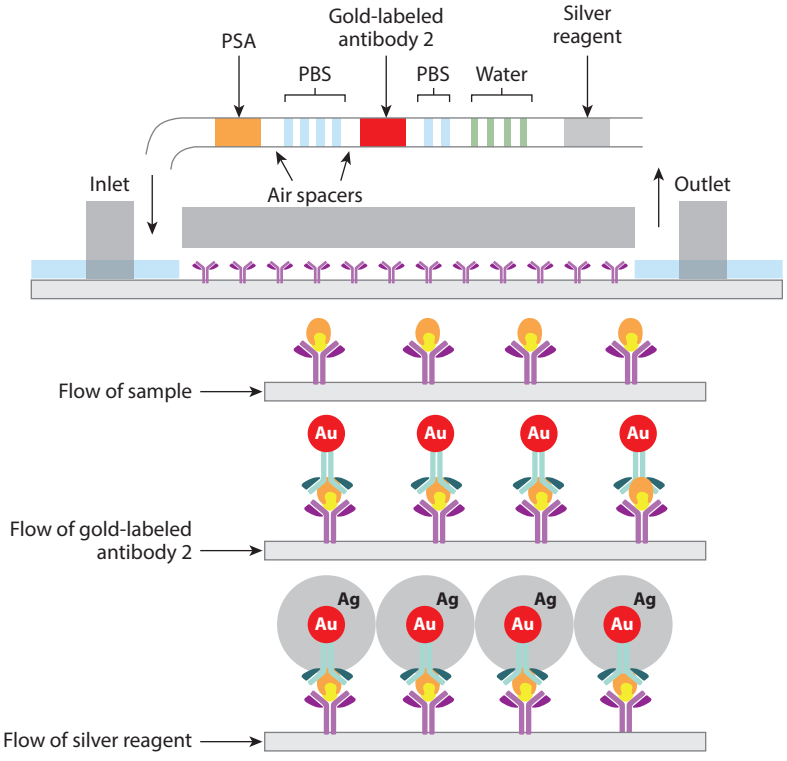

Figure 7

Stepwise injection of multiple (bio)chemical reagents into the bipolar electrochemistry cell for the immunoassay reported in Reference 67. The secondary antibody is labeled with a gold nanoparticle that acts as a seed for Ag deposition. Abbreviations: PBS, phosphate-buffered saline; PSA, prostate-specific antigen. Adapted with permission from Reference 67; copyright 2017 American Chemical Society.

2.3.4. Cells. The progressive improvement and miniaturization of BPE's concepts and techniques now also allow us to tackle more challenging biological entities such as living cells. Consequently, BPE has been applied not only to the detection and imaging of cancer cells (38, 40, 78, 115) but also to the on-chip monitoring of oxygen consumption by cell aggregates (41) and to cell capture and lysis (43, 116). Xu and coworkers (38) first developed a dual-BE array chip generating $[Ru(bpy)_3]^{2+}$ ECL and/or luminol ECL in two separate reservoirs. In the presence of DNAzyme and H_2O_2, orange ECL emission decreased due to the quenching effect of H_2O_2, whereas blue ECL emission appears. The resulting ratiometric sensing principle provides better sensitivity and enables distinguishing cancer cells from normal ones because the normal cells produce less H_2O_2 and G-4 DNAzyme than do cancer cells. The sensitivity was further improved by combining anodic dissolution and ECL detection on a similar bipolar sensing platform (111). This analytical approach shows a very low detection limit down to 5 cells/cm^2. Cell metabolism was also monitored by designing a closed BPE device containing both analytical and reporter chambers (41). Dioxygen consumption by cell aggregates at the cathodic poles of BPE arrays was converted to ECL signals at the anodic poles. The authors exploited the advantages of a closed configuration to detect many samples without cell damage. The reported analytical approach may be used for high-throughput assays of cell aggregates for drug screening and cell differentiation.

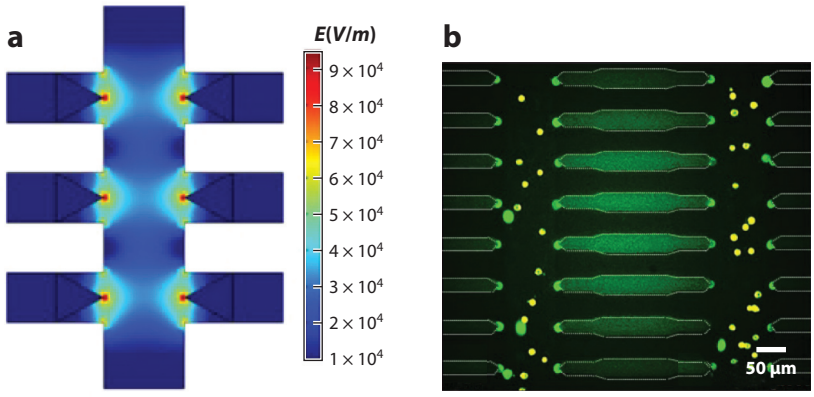

Figure 8

(*a*) Two-dimensional simulation of the electric field distribution across a single microchannel flanked by 6 bipolar electrode (BE) tips. (*b*) Fluorescent image showing the capture of single MDA-MB-231 cells (*green*) in the presence of Jurkat cells (*yellow*) by applying an AC field using a BE array. Figure adapted with permission from Reference 116; copyright 2017 American Chemical Society.

In addition to purely analytical applications, selective high-throughput, single-cell capture based on dielectrophoresis has been reported using BPE (116). Among cell manipulation methods, dielectrophoresis provides specific advantages, such as improved selectivity, high cell viability, and antibody-independent separation. The isolation of cells is based on their dielectric properties, which interact with an electric field gradient to create a movement. Li & Anand (116) designed an original dielectrophoresis device by integrating BEs. They demonstrated the wireless control of the distribution of an AC electric field at a BE array to specifically capture single cells. The developed BE arrays allow imposing an AC field across insulating barriers, such as channel walls, which provokes the simultaneous capture of cells across parallel microchannels. The wireless feature enables remarkably flexible device architectures with branched microchannels, increasing the throughput by offering 1,408 potential capture sites. The reported high-throughput, parallel-channel device was made of 32 parallel microchannels, and each channel had 22 pockets extruded at each side. The corresponding 1,408 micropockets provided discrete capture sites with defined volume, thus enabling single cell capture (**Figure 8**). Only two feeder electrodes were used to create the dielectrophoresis effect in an array format. This approach was further improved when combined with parallel electrical cell lysis in a single microfluidic platform (43). The specific capture of individual cells at such an array of wireless electrodes is a powerful technique and opens up promising possibilities to perform electrochemical analysis on the captured cells.

3. CONCLUSION AND OUTLOOK

In this review, we presented a selection of the most recent progress concerning the use of BPE in analytical chemistry. Completely new and extremely original approaches have emerged over the last ten years. The considerable increase in the number of studies using BPE for an analytical purpose can be explained by several important advantages of BPE compared to classic electrochemistry.

1. BPE can be performed without physical contact between the bipolar object(s) and the power supply. The wireless nature of the experiments allows straightforward miniaturization and

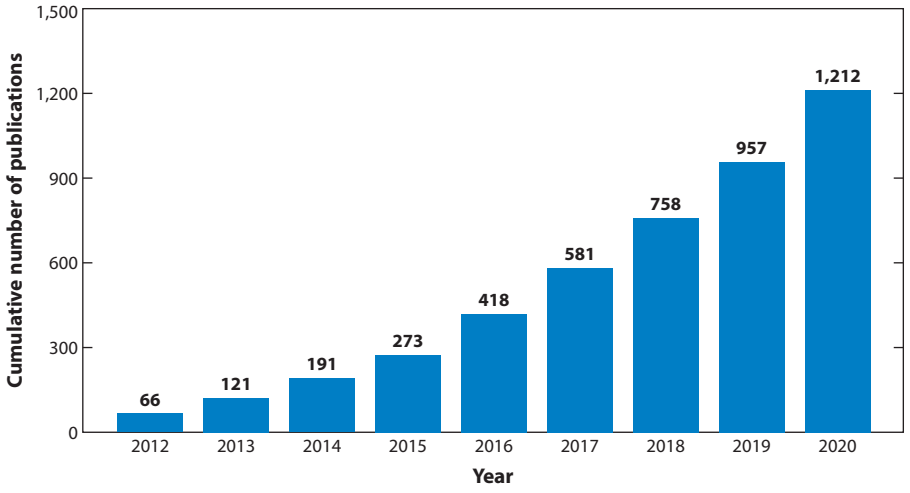

Figure 9

Histogram of the evolution of the cumulative number of publications containing the keyword "bipolar electrochemistry" in the title or abstract. Data from Google Scholar, accessed August 2020.

an easy integration of BPE in microfluidic structures, which is especially interesting in the context of designing efficient analytical systems, such as sophisticated lab-on-chip devices. Furthermore, this feature gives access to experiments that are difficult or completely impossible to conduct with a more classic electrochemical setup.

2. As a direct consequence of the previous point, the technique enables researchers to address in parallel thousands or millions of objects with just a single pair of feeder electrodes. Naturally, this immediately opens up a broad avenue for many new types of experiments, especially when thinking, for example, about high-throughput screening of analytes or (electro)catalytic features of materials and particles.
3. BPE can be considered as a true low-cost technique, which in most cases only needs very simple instrumentation that can be easily implemented in every laboratory and handled even by inexperienced students (**https://www.youtube.com/watch?v=bifEBSB1_9Y**).

The direct consequence of all these advantages is that the technique attracts a growing number of users, as can be seen in the histogram in **Figure 9**, tracing the cumulative number of publications containing the keyword "bipolar electrochemistry" in the title or abstract.

Obviously, these publications concern not only analytical chemistry, but also many other areas for which BPE is an attractive concept, ranging from materials science to catalysis and from energy storage/transformation to biology. However, it appears that studies in which analytical chemistry is the main focus represent an increasing portion in the last ten years. We expect that this trend will continue and hope this review might contribute to stimulating new ideas and motivate other scientists to integrate some aspects of BPE in their own work in order to drive the topic into yet unexplored and sometimes completely unexpected directions.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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