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Miniaturized Electrochemical Biosensor based on Whole-Cell for Heavy Metal Ions Detection in Water

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Abstract

The heavy metals pollution represents one of the important issues in the environmental field since it is involved in many pathologies from cancer, neurodegenerative and metabolic diseases.

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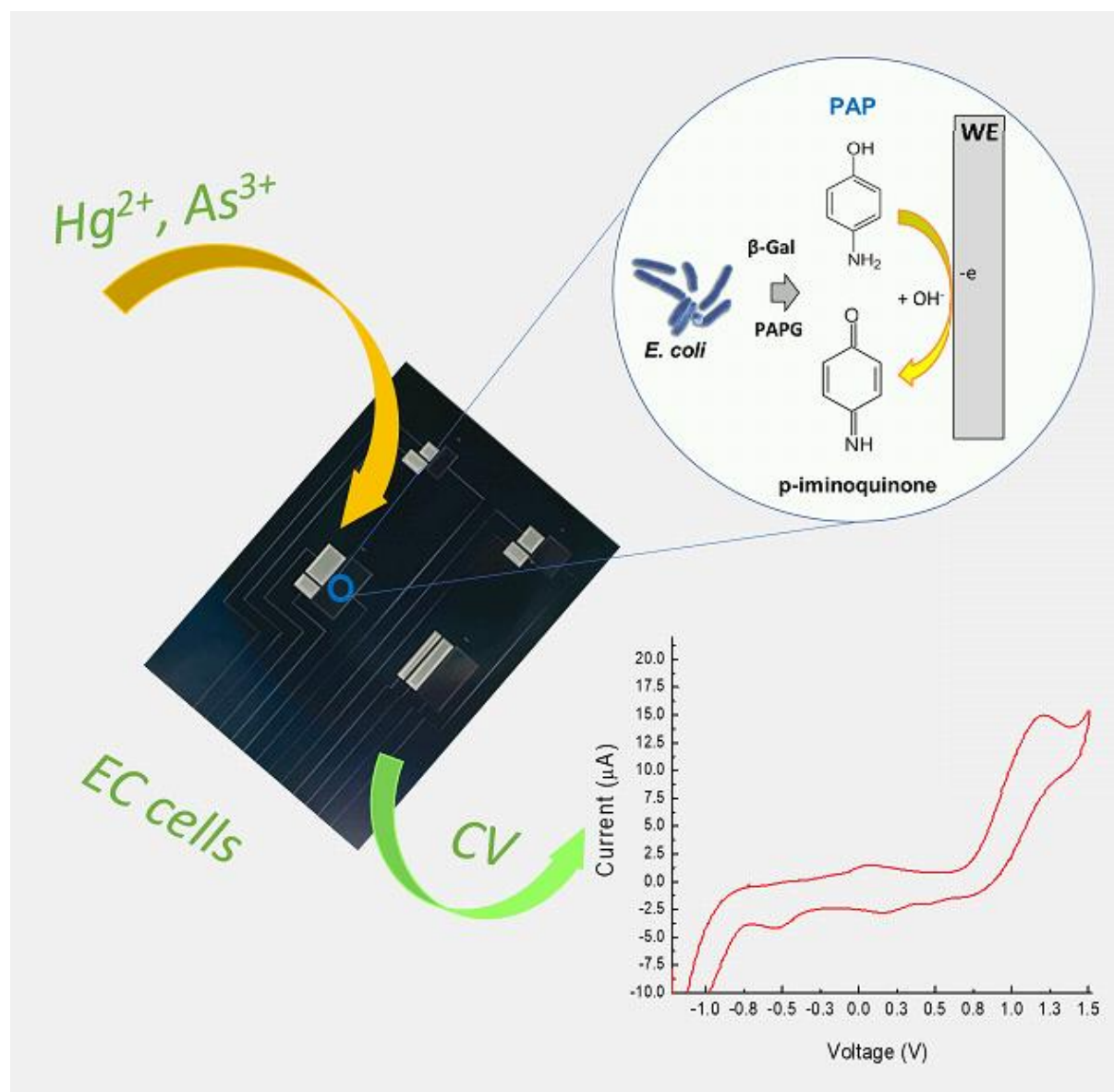
We propose an innovative portable biosensor for the determination of traces of trivalent Arsenic (As(III)) and bivalent mercury (Hg(II)) in water. The system implements a strategy combining two advanced sensing modules consisting in (a) a whole cell based on engineered *Escherichia coli* as selective sensing element towards the metals and (b) an electrochemical miniaturised silicon device with three microelectrodes and a portable reading system. The sensing mechanism relies on the selective recognition from the bacterium of given metals producing the 4-aminophenol (PAP) redox active mediator detected through a cyclic voltammetry analysis. The miniaturized biosensor is able to operate a portable, robust and high-sensitivity detection of As(III) with a sensitivity of 0.122 $\mu\text{A ppb}^{-1}$, LoD of 1.5 ppb and a LoQ of 5 ppb. The LoD value is one order of magnitude below of the value indicated to WHO to be dangerous (10 $\mu\text{g/L}$). The system was proved to be fully versatile being effective in the detection of Hg(II) as well. A first study on Hg(II) showed sensitivity value of 2.11 $\mu\text{A/ppb}$ a LOD value of 0.1 ppb and LoQ value of 0.34 ppb. Also in this case, the detected LOD was ten time lower than that indicated by WHO (1 ppb).

These results pave the way for advanced sensing strategies suitable for the environmental monitoring and the public safety.

Graphical Abstract

Sciuto and co-workers have described an innovative electrochemical miniaturized silicon device for the detection of heavy metal ions in water. The whole cell sensing element integrates engineered *Escherichia* on three planar microelectrodes structure. The selective sensing mechanism is based on the specific recognition of given metals from engineered *E.coli* producing the redox active mediator 4-aminophenol,

measurable by voltammetry analysis. The system was proved to be selective and effective for the detection of Hg(II) with a sensitivity of $2.11 \mu\text{A ppb}^{-1}$ and a LOD value of 0.1 ppb and for the detection of As(III) with a sensitivity of $0.122 \mu\text{A ppb}^{-1}$, LoD of 1.5 ppb



Keywords

Trivalent arsenic; Divalent Hg; water monitoring; whole-cell-based biosensing; electrochemical detection; silicon technology.

Introduction

The heavy metals pollution, as consequence of the industrial progress and the human activities, is one of the important issues in the environmental field. One of the most contaminated natural resource is water. Metals are often spilled in soils through the industrial and consumer wastes and accumulate in streams, lakes, rivers and water bringing a series of implications for the human safety (Masindi, Muedi, 2018; Verma, Dwivedi, 2013). Arsenic is one of the most toxic metals in the form of arsenite (AsIII) (ATSDR, 2007). It is frequently assumed by using drinking, food preparation and irrigation water that are contaminated. If concentrated at high levels, i.e. above the World Health Organization (WHO) provisional guideline value of 10 µg/L (or ppb), it becomes very dangerous for human safety (World Health Organization, 2020; Ravenscroft et al., 2009) bringing a series of cardiovascular diseases, diabetes, liver/prostate/bladder cancer, cognitive effects and youthful deaths (Hong, Song, Chung, 2014; Mandal, Suzuki, 2002).

Also, mercury represent a dangerous analyte. In its divalent inorganic form (Hg(II)), it is spilled in water as waste of industrial activities and accumulates over the WHO threshold value of 1 ppb becoming toxic and cause of severe neurological disturbances, skin rash and kidney failure.

Therefore, considering the arsenic and mercury toxicity the availability of effective sensing technologies able to detect and quantify these pollutants in water is extremely important. Unfortunately, many of the available detection systems have several limitations mainly due to the complexity of the analysis.

Even consolidated methods like Spectrometric (CV-AAS, AEF, CV-AFS), chromatographic and potentiometric technologies, with low detection limits (LoD) of

about 1 $\mu\text{g L}^{-1}$ or 1 part per billion (ppb), and a wide linear range for arsenic detection, present some drawbacks in terms of test time, expensive reagents, the need for sample pre-concentration, lab constraints related to bulky instrumentation, and the need for highly trained personnel (United States Environmental Protection Agency Office of Water, 1999; Yu, Wang, 2013; Bose, Rahman, Alamgir, 2011). Ultrasensitive method (LoD of 0.14 ppb) by colorimetric and surface-enhanced Raman (SERS) using glutathione functionalized Au-nanoparticles (Lia et al., 2020) and rapid colorimetric assay on Paper-based microfluidic device have been described (Morita, Kaneko, 2006; Martinez et al., 2010). However, the main drawback of these procedures are the complexity of the procedure (SERS-based method) and the specificity (colorimetric method) due to the cross-reactivity of other metallic species with the sensing module giving a false positive and the sensitivity (Lace et al., 2019; Yogarajah, Tsai, 2015).

Also in the case of quantification of mercury traces in water combined gas/liquid chromatographic-spectrometric methods (GC-AFS, HPLC-AAS, HPLC-ICP-OES, etc.) and SERS techniques (Kodamatani et al., 2011; Hashemi-Moghaddam, Saber-Tehrani, 2008; Guerrini et al., 2014) are used. These approaches, even if show a good reliability, high sensitivity (LoD of 0.14-0.17 ppb) and accuracy discriminating among multiple mercury species (as methylmercury, ethylmercury, phenylmercury and the mercury ions), however have the limitations above described of lab constraints, time and cost of analysis which make these methods unsuitable for on-site measurements.

In this sense, many attempts have been made towards the miniaturization of the entire detection system. The sensing platforms based on optical detection (both colorimetric

and fluorometric) introduced rapid and low-cost direct observations of colour and light changes induced by the reaction of metals with specific dyes in small sized devices, providing an improvement of time and miniaturization of analysis but keeping a limit of specificity, due to the cross-reactivity of other metallic species with the sensing module (Li et al., 2020; Morita, Kaneko, 2006; Martinez et al., 2010; Lace et al., 2019; Yogarajah, Tsai, 2015; Chen et al., 2015; Yang et al., 2018; Santangelo et al., 2014). However, the portability of these systems is still a challenge.

The electrochemical silicon-based technologies, in combination with the evolution of biotechnology, gave an opportunity to improve the sensing approach introducing new portable and easy-to-use devices able to solve the drawbacks described above. The effectiveness of this approach has been already demonstrated in several areas of medical field such as nucleic acids analysis (Petràlia, Cosentino, Sinatra et al., 2017; Petràlia, Sciuto and Conoci, 2017; Petràlia, Sciuto, Di Pietro, et al., 2017) glucose and biomolecules sensing (Petràlia et al., 2018) where the electrochemical systems provided a high miniaturization degree and rapid, highly-sensitive analysis (Petràlia, Sciuto, di Pietro et al., 2017). However, they present some drawbacks due to sample preparation introducing additional variability into the measurement (Toor, Sharma, Bansod, 2015), expensive fabrication costs due to functionalization of electrodes in case of enzyme-based sensors (Pujol et al., 2014; Fu et al., 2011) and weak specificity for electrochemical sensors based on Noble Metal nanoparticle-modified electrodes (Petràlia, et al, 2012; Majid et al., 2006; Kumar et al., 2017; Babar et al., 2019).

In this context, sensing strategies based on specific enzyme offer the advantage of selective detection. Direct enzymatic recognition of arsenic by arsenite oxidase (Male et al., 2007) or by indirect recognition through β -galactosidase have been reported

(Stocker et al., 2003) with good sensitivity and selectivity. However, both these technological approaches presents issues due to the production costs and stability of enzyme which degenerate after a long usage inhibition of the recognition activity.

More recently, whole-cell based biosensors received a widespread attention for their properties. Most of them are based on microorganisms, especially bacteria, that have been genetically modified in order to be sensitive towards the specific analyte of interest (Sciuto et al., 2019; Gu, Mitchell, Kim, 2004). These biosensors use engineered bacterium (generally an *Escherichia coli*) as sensing element that, each time it interacts and internalises the analyte to detect, produces a reporter protein that express a detection signal (optical, electrical and/or electrochemical). The genetic modification of the sensing element makes the whole-cell-based biosensor extremely customizable. Moreover, thanks to its intrinsic properties, the sensing bacterium is physiologically more robust than enzymes, in case of prolonged usage allowing a high degree of miniaturization and portability, due to the small size and the survival at different environmental conditions (Gilchrist et al., 2005). Finally, the genetic recognition of arsenic, based on the modified expression of the *ars* operon sequences, β -galactosidase or luciferase gene, guarantees a strong selectivity excluding the risk of cross-reactivity (Gui et al., 2017). However, the biotechnology has the main limitation of the sample management since the sensing bacteria are strictly dependant on the nutrients availability and the environmental parameters (pH, temperature, or ionic strength) and, usually, require a long time period to complete the genetic recognition of target, from the arsenic internalization to the reporter protein transcription and translation.

In this work we propose an innovative miniaturized electrochemical biosensing platform for the specific and high-sensitive quantification of metal ions in water sample. The sensor system exploits the synergy between two interfaced sensing modules: (a) a whole-cell-based module using an engineered *Escherichia coli* as whole-cell sensing element; (b) an electrochemical module based on a silicon chip, integrating electrochemical cells (EC-cell) composed by three planar microelectrodes, and a portable EC-reader, performing a cyclic voltammetry (CV) analysis. The whole-cell sensing element has been genetically modified to produce a redox active 4-aminophenol, as mediator, each time it interacts with the metal target in a highly specific manner. Thanks to the electrochemical detection of the mediator, the metal is indirectly detected and quantified. Sensitivity, robustness and selectivity of the sensing system have been fully studied for As(III) and preliminary investigated for Hg(II) proving the fully versatility of the system towards multiplex heavy metals detection.

Experimental section

Chemicals. ([Os(bpy)₂DPPZ]Cl₂) was prepared according the procedures reported in the work of Petralia, Sciuto, Di Pietro et al (2017). The engineered *E. coli* strains, specific for As(III) and Hg(II), the ampicillin antibiotic and the 4-aminophenyl β-D-galactopyranoside (PAPG) powder were provided by the EEM-Lab of the University of Lausanne and used as described in the section below. Filtered sterile 10X (1 mM) phosphate-buffered saline solution, or PBS, (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄ and 1.47 mM KH₂PO₄, pH=7, Fisher Scientific) was diluted 10-fold for usage. Sterile Luria Bertani (LB) broth and chloride potassium (KCl) were purchased from Sigma. Sodium arsenite (NaAsO₂), mercury chloride (HgCl₂) and cadmium

sulphate (CdSO_4) powders, from Sigma, were dissolved in filtered sterile 1 mM PBS and used for the analysis.

Whole-cell-based module. The whole-cell-based module is composed by a 20 μL polycarbonate squared chamber embedded on top of the microelectrodes of the detection module containing a sensing element for the As(III), which is obtained by the molecular cloning experiment described in the work of Cortés-Salazar et al. (2013). This consists in an *E. coli* strain that has been genetically modified by the integration of a recombinant plasmid (pMV132-arsR-ABS) containing an *ars* operon promoter sequence (P_{ars}) fused downstream to the *arsR* and *lacZ* gene. Once prepared for the analysis, the sensing element is mixed to a water sample, added with a specific amount of metallic target, and spotted into the sensing chamber for the sensing test.

Electrochemical module. The electrochemical (EC) module of the biosensing system consists of (a) a miniaturized device and (b) a portable electronic system to perform the analysis. The EC device reported in Fig. 1, was manufactured using the VLSI technology on a 6" silicon wafer substrate, according to the procedure described in Petralia, Sciuto and Conoci et al., 2017. It is a silicon chip contains 4 EC-cells for multiplexing analysis, each contains three planar microelectrodes so structured: a working electrode (WE) made in platinum, counter and reference electrode (CE, RE) made in gold through sputtering process. Table 1 contains the size of each EC cell. The electrode-to-electrode distances are 100 μm . The silicon chip is integrated with a polycarbonate ring creating 4 microchambers (20 μL total volume) and electrically isolated from the substrate by a thermally grown silicon oxide layer (Fig. 1c). The whole device is fixed on a plastic holder for easy handling. The EC device is connected with a customised board developed by STMicroelectronics acting as

miniaturised potentiostat and used to perform the electrical driving of CV measurements on samples (Fig 1b-c).

Experimental procedure for analysis. To prepare the samples for the analysis, the *E. coli* sensing element was plated 24 h in LB-agar added with ampicillin 100 µg/ml. The day after, few colonies of the plate were suspended in 20 mL of fresh LB broth and incubated overnight at 21°C – 150 rpm. The last 3 h cells were incubated at 37°C – 150 rpm until the optical density (OD_{600nm}) was around 0.6. Subsequently, the culture was diluted 2-fold in tap water (final volume of 10 mL) and used to prepare the following mixes: 800 µL of diluted *E. coli* culture + 100 µL of 2.5 – 5 – 10 – 50 – 100 ppb NaAsO₂ (water as zero) + 100 µL of 10 mM PAPG. Once induced by incubation at 30°C – 350 rpm – 2 h, 20 µL of the induced mixes have been immediately spotted into the microchambers of the EC device and analyzed by CV. For each concentration, samples have been prepared and measured in triplicate. The experimental procedure was repeated by substituting the NaAsO₂ with HgCl₂ (Hg(II)) and CdSO₄ (Cd(II)) to test the cross-reactivity.

Electrochemical measurements. CV measurements were carried out by using the portable electronic board linked to a laptop via USB connection (Fig. 1C). RealTerm and MATLAB software were used to manage, respectively, the electronic settings for the CV experiments and the collection and post-processing of data coming from the board. The experiment was set using a scan rate of 10 mV/s and a voltage range of –1.5 V / +1.5 V, and focusing on the anodic peak of PAP at around +1.2 V. Before each test, the platinum surface of the WE was activated by 50 sweeps of CV in KCl 0.1 M. The LoD was obtained by measuring 3 replicas of a blank sample and calculating the mean result and the standard deviation (SD) according to the following

equation $LoD = 3 \times SD_{Regression} / Slope$, while the Limit of Quantification was calculated by the follows equation $LoQ = (LoD/3) \times 10$. The delta current signal (I_{delta}) was calculated as the difference between the current recorded in whole-cell biosensor in presence of the metal analyte and the current signal with 0 ppm of analyte (blank) in the voltage region +1.0 V/+1.4 V.

Results and Discussions

The electrochemical miniaturised silicon chip used in the present study is reported in Fig.1. It integrates four types of layout of EC-cells configured with three planar microelectrodes (WE, CE and CE), featured by different sizes and shapes (Fig 1a). Table 1 reports both the physical dimensions and the areas of each electrode/cell-type. The silicon chip has been integrated with plastic PC ring to create a reaction sampling chamber of 25 μ L for each EC cell and mounted on a customised electronic board for driving the electrical signals. The final whole portable EC system was connected to PC to automatically manage the reading process (Fig. 1b-c).

The first part of our study was focused on the evaluation of the EC-cells performances of the 4 layouts present in the EC chip. With this purpose, we carried out electrochemical measurements using Osmium based redox probe ($[Os(bpy)_2DPPZ]Cl_2$) at concentration ranging from 0.1 μ M to 100 μ M in KCl 20mM. Figure 2 reports the square wave (SW) intensity signals recorded at the oxidation peak of Os complex of 0,76V. The data show that four cells exhibit different performances with current intensities of $EC1 > EC2 > EC4 > EC3$ in the whole explored Osmium concentration range (Fig. 2(a)). A linear behavior was observed in the concentration range of 0 - 50 μ M (Fig. 2(b)) leading to values of sensitivity (slope of the linear curves illustrated in Fig. 2 (b)) of 0.12 $\mu A \mu M^{-1}$ (EC1), 0.09 $\mu A \mu M^{-1}$ (EC2), 0.05 $\mu A \mu M^{-1}$ (EC4) and 0.01 $\mu A \mu M^{-1}$ (EC3), respectively. These sensitivities values can be

correlated with both the area of WE (Fig 2 (c)) and the WE-CE distance cell parameters (Fig 2 (c)). Data show that EC1 cell present both the highest WE area and smallest WE-CE distance and it has been found to be the best performer in terms of both current intensities (Fig 2(a)) and sensitivity value (Fig. 2(c-d)). In case of EC2, even if it has the same WE area of EC1, it is nonetheless featured by the higher WE-CE distance and exhibits both current intensities (Fig 2(a)) and sensitivity values (Fig. 2(c-d)) lower than EC1. For EC4 and EC3 the worst performances (current intensities and sensitivity values) can be correlated to both the lower WE area and the shorter WE-CE distance (both the lowest for EC3). The results were also confirmed using other redox probe, such as $\text{Fe}^{\text{II}}(\text{CN})_6$ (data not showed).

Based on the above reported results we selected layout 1 of the EC chip for the heavy metal detection through the whole-cell sensing element using the engineered *Escherichia coli*. The principle of the sensing method is schemed in Fig. 3. More in details, thanks to the genetic modification carried out on *E. coli*, when As(III) metal goes into the bacterium cell, the ArsR protein is released from its DNA binding site (located to the downstream of *ars* operon promoter sequence P_{ars}) and bind the As(III) metal. This process has been designed taking inspiration to the intrinsic arsenate resistance mechanism of bacteria (Cervantes et al., 1994). When ArsR-As(III)'s binding event occurs, P_{ars} is free to increase the expression level of *lacZ* gene, resulting in the synthesis of β -galactosidase (Stocker et al., 2003; Cortés-Salazar et al., 2013). The enzyme, then, cleaves the 4-aminophenyl β -D-galactopyranoside (PAPG) and produces the 4-aminophenol (PAP). Once released by the *E. coli* cells, PAP interacts with the WE inside the EC device and triggers a redox exchange producing a current signal that is then detected and quantified by CV analysis to give the final output. Depending on the amount of As(III) present in the sample, *E. coli*

produces a correspondent concentration of PAP that is electrochemically detected. Fig. 4(a) reports CV results obtained by using concentration of As(III) from 0 to 100ppb. As shown, PAP oxidation peaks are centred from +1.0 V to +1.2 V. By going from the lowest concentration of 2.5 ppb (red line) up to the highest 100 ppb (orange line), the peak intensities increased over the background (0 ppb sample) (blue dashed arrow). It can be observed a voltage shifts of the PAP oxidation peak as illustrated in the inset of Fig. 4(a) (green dashed arrow). These shifts correspond to + 50mV for 2.5ppb, +150 mV for 10ppb, + 300 mV for 50ppb and +350mV for 100ppb. They can be explained by the gradual adsorption of the redox product of PAP over the platinum surface of WE, affecting the electrolytic process (Chandrashekar et al., 2011; Wudarska et al., 2015; Salavagione et al., 2004; Amanullah et al., 2010).

It can be also observed that the *E. coli* strain exhibit a proper broad peak at about 1V (Fig. 4(a) curve 0 ppb). This is probably due to the biological matrix of the cellular line. The reduction peak for dissolved oxygen was observed in the range -0.5 V -1.1 V.

The analysis of CV signal intensities is reported in Fig. 4(b-c). Data showed a linear current increase of PAP peaks when As(III) concentration is from 2.5 ppb to 50 ppb and a slope change from 50 ppb to 100 ppb (Fig. 4(b)). It is noteworthy that the sensor shows a detectable signal at 2.5ppb of As(III) metal below the WHO threshold (10 ppb).

The linear trend was deeper investigated using As(III) concentrations of 2.5 ppb, 5ppb, 10ppb and 50 ppb. The current intensities of CV curves as function of the As(III) concentration are plotted and linearly interpolated in Fig. 4(c). The analysis of the curve show sensitivity of $0.122 \mu\text{A ppb}^{-1}$ LoD of 1.5 ppb and a LoQ of 5 ppb. These values, lower than the acceptable limit of arsenite in water as defined by the WHO (LoD 10 ppb), indicate the proposed approach as a potential candidate for the

quantification of the pollutants in water. It is noteworthy that, compared to our previous system using the whole-cell detection for As(III) (Cortés-Salazar et al., 2013), the proposed platform has a sensitivity about 40 times higher (0.12 $\mu\text{A/ppb}$ versus 0.0028 $\mu\text{A/ppb}$). This finding could be reasonably explained by the best performance of our silicon-based EC-cells, of which planar electrodes assure a higher current intensity signal, as confirmed by the comparison of the intensity current values recorded by two systems. In particular water samples containing As(III) in concentration 10 ppb, interrogated with the proposed platform gives a current intensity value of 1.5 μA , while the previous system gives a very low current value of 55 nA. It is noteworthy that this highly sensitive integrated detection was obtained without any additional reagent consumption, since the enzyme is directly produced by the sensing cells, giving an extreme precise quantification of As(III) in a small sample of water (just 20 μL), with a linear trend revealed at low concentration range, well below the 10 ppb guideline value.

The selectivity of the miniaturized biosensor was studied by testing the whole As(III)-*E. coli* cell portable sensor towards Hg(II) and Cd(II) metals. Results are summarised in Fig. 5. When Hg(II) and Cd(II) metals are present in the sampling water solution, no PAP signals were detected at in the range +1.0 V/+1.2 V, while the reduction peaks for dissolved oxygen were still present the range -0.5 V -1.1 V (Fig 5(a-b)). Figure 5c reports the plots of the delta currents of both Hg(II) (red line) and Cd(II) (blue line) compared with those showed by As(III) (green line; data extracted from Fig 4b). The results confirm the specific genetic recognition operating inside the *E. coli* cells with the activity of Pars promoter enhanced only by As(III) species inducing the *arsR*-mediated expression of β -galactosidase and the production and release of the PAP redox mediator. The selectivity is guaranteed without the need of

any chemical reagent and enzymatic or nanostructural functionalizations, that can degenerate after long usages and affect the quality and cost of screening.

In order to assess the multiplex capability of our portable platform, we also included an additional test using a genetically modified *E. Coli* strain specific for the Hg(II) species. This strain was modified in order to trigger the β -galactosidase expression, thus the PAP production, once interacted with Hg(II) species revealed in water. We preliminary investigated this assay using the following amount of Hg(II): 0, 0.25ppb, 1ppb, 2.5ppb and 10 ppb. The recorded I delta currents values are plotted in fig. 6(a). A linear behaviour was observed in the range 0-2.5 ppb leading to a sensitivity value of 2.1 μ A/ppb, LOD value of 0.1 ppb and LoQ value of 0.34 ppb (inset of Fig 5a). These starting data are optimal, considering the 1ppb WHO tolerance value for total Hg(II) in water. Good specificity was also observed since no signal was recorded in presence of unspecific As(III) and Cd(II) species (Fig 6(A)).

Optimization tests are in progress to improve the sensing performances of the system towards Hg(II) species.

Conclusions

In this work we propose an innovative, miniaturized electrochemical biosensing platform for the specific and high-sensitive quantification of metal ions in water sample. The platform synergically interfaces a biosensing module based on whole-cell using engineered *Escherichia coli* and a miniaturised electrochemical silicon chip integrating 4 type of EC cells configured with three planar microelectrodes and a portable EC-reader. The electrochemical characterization of the silicon chip using Os complex redox probe shows that that EC1 cell obtains the best EC performances in terms of current intensity respect to the other ECs configuration (EC2-EC4). This was

attributed to the highest WE area and shortest WE-CE distance of EC1 that was chosen for the sensing test towards the heavy metals. The sensing mechanism relies on the selective recognition from a specific engineered *E. coli* towards a given metals producing the 4-aminophenol (PAP) redox active mediator detected through a cyclic voltammetry analysis. The miniaturized biosensor is able to operate a portable, robust and high-sensitivity detection showing for As(III) a sensitivity of $0.122 \mu\text{A ppb}^{-1}$, LoD of 1.5 ppb and a LoQ of 5 ppb. The system was proved to be fully versatile, being effective in the detection of Hg(II) as well. A preliminary study on Hg(II) showed sensitivity value of $2.11 \mu\text{A/ppb}$ a LOD value of 0.1 ppb and LoQ value of 0.34 ppb. It is noteworthy that the LoD values were below of the values indicated to WHO to be dangerous ($10 \mu\text{g/L}$ for As and 1 ppb for Hg).

The platform miniaturization, together with the limited fluidic movement, make of the proposed assay a potential portable and easy-to-use system for unspecialized personnel for outdoor application. These results pave the way for advanced sensing strategies suitable for the environmental monitoring and the public safety.

Author Contribution

E.L.Sciuto contributed to experimental execution of the tests and data analysis; S. Petralia contributed to chip preparations, testing and data analysis; Jan Roelof van der Meer contributed to data verification and methodology definition; S. Conoci contributed to work conceptualization of the work and writing;

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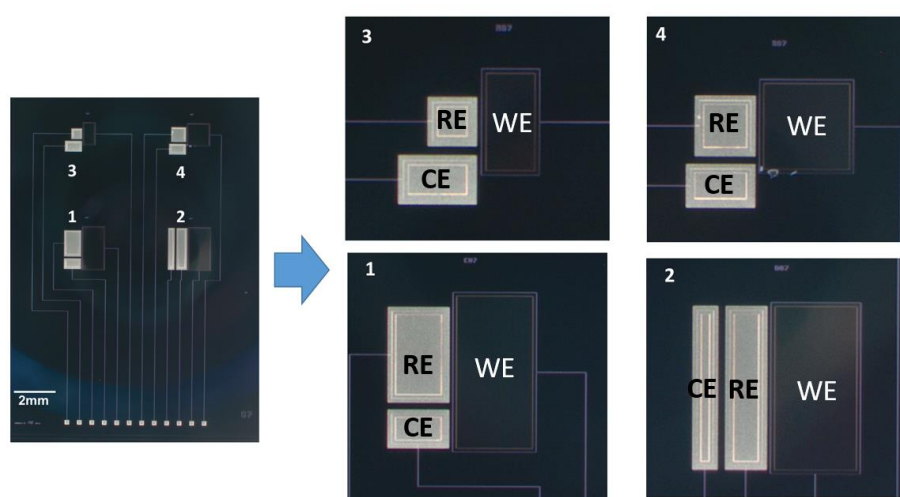
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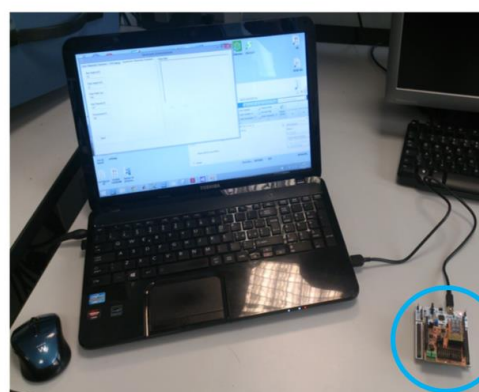
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Figures

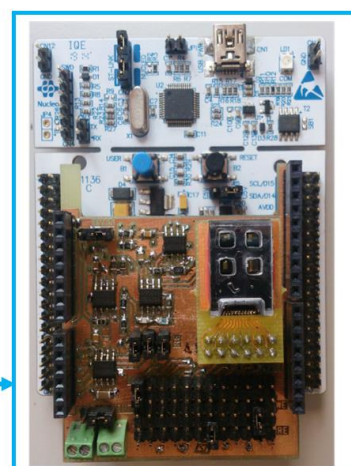
Figure 1. (a) Miniaturised electrochemical silicon chip integrating 4 layout of EC cells with three planar microelectrodes made in Pt (WE) and Au (CE and RE). Table 2 reports the electrodes dimensions; (b) whole portable system EC-reader connected to the PC; (c) details of the portable system EC-reader: electrochemical silicon chip connected to the reading board



(a)



(b)



(c)

Figure 2. (a) Electrochemical response (SW current intensities) of EC-Cells at various Os redox probe amount; (b) linear interpolation of current intensities versus Os amount; (c) Correlation of Sensitivity/WE area; (d) Correlation of Sensitivity/WE-CE distance. The error bars represent the standard deviation from a triplicate.

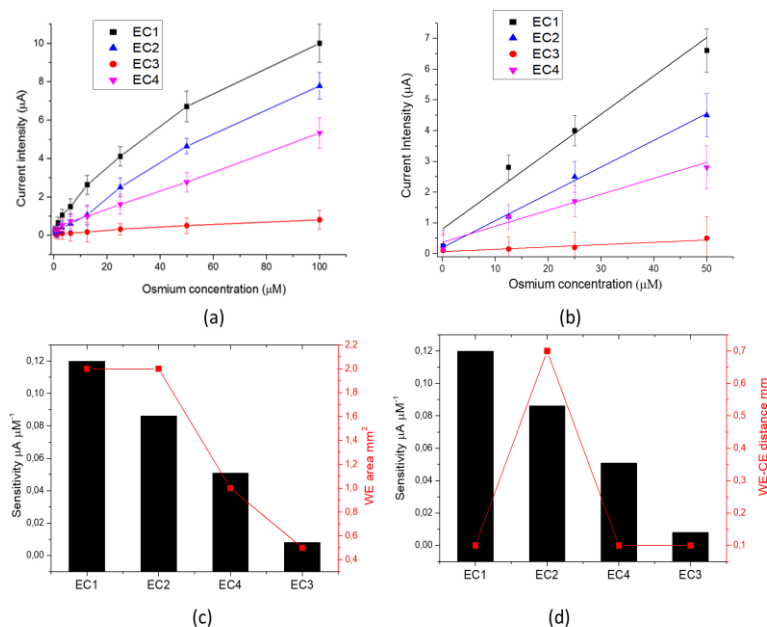


Figure 3. (a) Details of the EC cell used for the sensing; (b) Principle of the biochemical method based on whole-cell sensing using the engineered *E. coli* as sensing element; (c) Example of CV signal output (obtained using 50 ppb of As(III)).

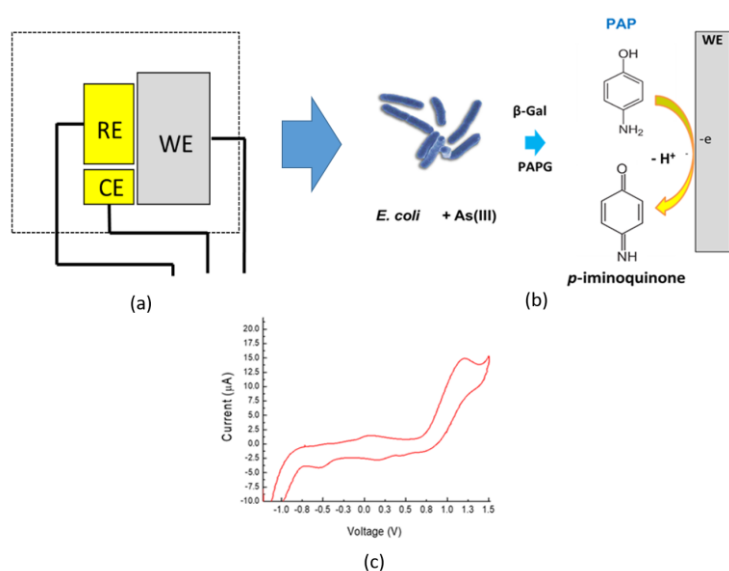


Figure 4. (a) CV curves of arsenic sensitive *E. coli* strain in presence of As(III) at 0 (black line), 2.5ppb (red line), 10ppb (blue line), 50 ppb (green line) and 100 ppb (orange line) and relative current increase (blue dashed arrow). Inset: detail of PAP peak voltage shift (green dashed arrow); (b) CV trend for *E. coli* samples induced by As(III) in water; (c) calibration curve calculated at 2.5 ppb to 50 ppb of As(III). The error bars represent the standard deviation from a triplicate.

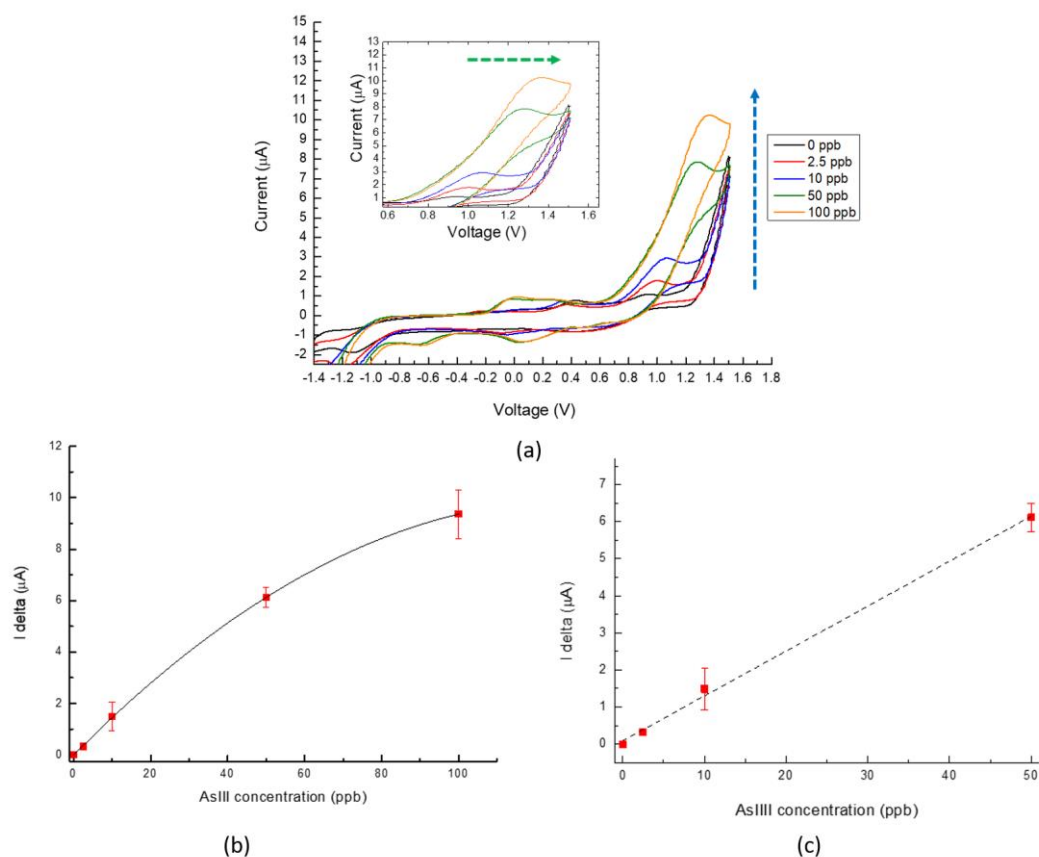


Figure 5. (a) CV curves of *E. coli* cells in presence of Hg(II) at 0 (black line), 2.5ppb (red line), 5 ppb (yellow line), 10ppb (blue line) and 50 ppb (green line); (b) CV curves of *E. coli* cells in presence of Cd(II) at 0 (black line), 2.5ppb (red line), 5 ppb (yellow line), 10ppb (blue line) and 50 ppb (green line); (c) comparison of PAP current values measured on *E. coli* samples (specific for As(III)) treated with different concentration of As(III) (green line), Hg(II) (red line) and Cd(II) (blue line). The error bars represent the standard deviation from a triplicate.

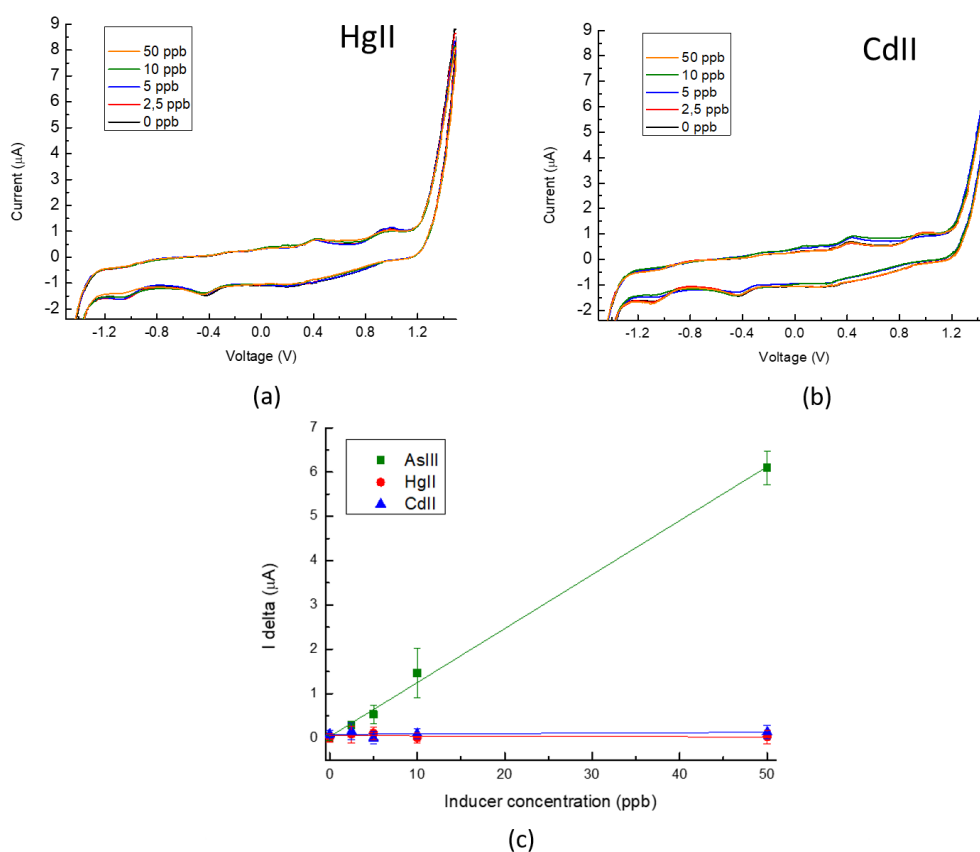


Figure 6. (a) CV trend of mercury sensitive *E. coli* strains induced by 0.25 ppb; 1 ppb; 2.5 ppb; 10 ppb Hg(II) in water: the insert shows a calibration curve calculated for the low range concentration (0.25 ppb to 2.5 ppb) of Hg(II) in water. (b) comparison of PAP current values measured on *E. coli* samples (specific for Hg(II)) treated with different concentration of As(III) (green line), Hg(II) (red line) and Cd(II) (blue line). The error bars represent the standard deviation from a triplicate.

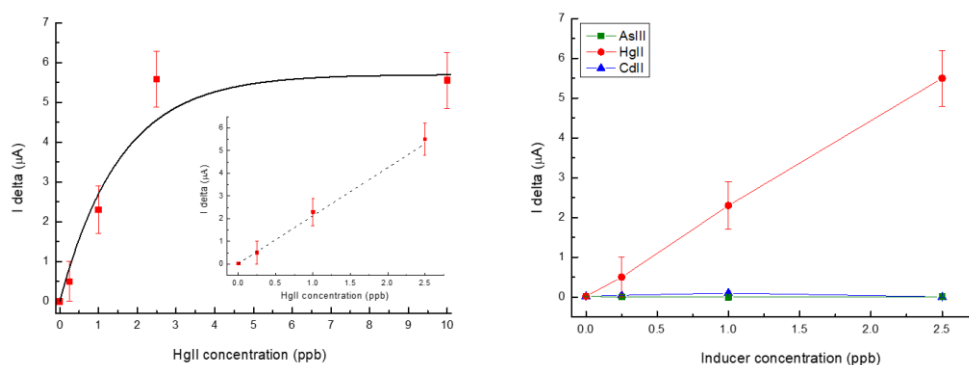


Table 1- EC-cells dimensional features

EC-cell type	WE (mm)	WE area (mm ²)	CE (mm)	CE area (mm ²)	RE (mm)	RE area (mm ²)
1	1 × 2	2.0	0.8 x 0.5	0.4	0.8 x 1.25	1.0
2	1 × 2	2.0	0.3 x 2	0.6	0.5 x 2	1.0
3	0.5 × 1	0.5	0.8 x 0.5	0.4	0.4 x 0.4	0.16
4	1 × 1	1.0	0.8 x 0.5	0.4	0.7 x 0.5	0.35