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Electrochemical As(III) Whole-Cell Based Biochip Sensor

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1. INTRODUCTION

The development of an electrochemical sensor for living cell toxicity detection by coupling biological engineering and electrochemical techniques is presented. In presence of a toxic agent many different bacteria develop a natural resistance mechanism based on the selective detection of the toxic species and its pumping out from the cell (*e.g. E.coli* has a natural resistance to As(III)).[1,2,3] Therefore, toxic effects in bacteria can be accurately detected when coupling a genetically modified signaling cascade to the toxicity mechanism. Such 'reporter bacteria' can produce a measurable signal, which can be read out by various means such as electrochemistry.[1,4] Here we describe the use of a genetically engineered *E. coli* for detecting low arsenic concentrations in tap and ground water from Switzerland with a microchip containing 16 individually addressed electrochemical cells for high-throughput environmental monitoring of arsenic in potable water. The main advantages of the present approach over previously reported arsenic bacterial bioreporters are the faster response time and the higher sensitivity achieved (LOD = 0.8 ppb).[1]

2. PRINCIPLE OF As(III) BIOSENSING AND ELECTROCHEMICAL DETECTION

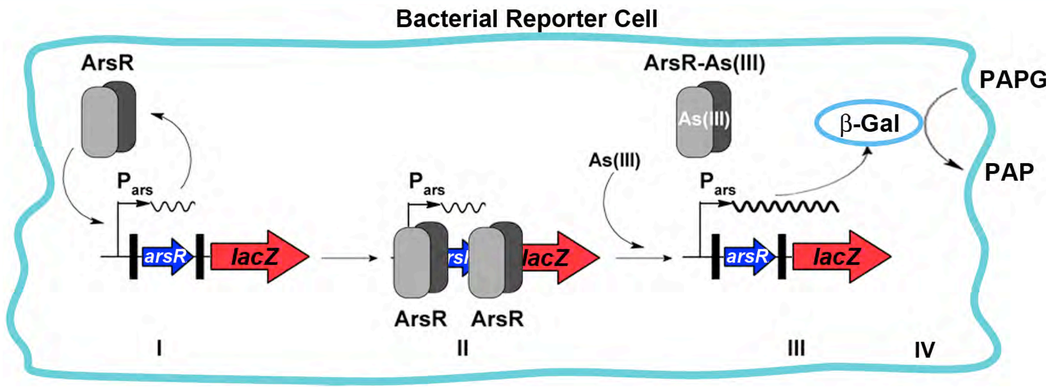

Figure 1. Principle of the As(III) bioreporter. I) The ArsR repressor protein (sensing protein) is synthesized from the arsR promoter (Pars). II) ArsR binds to two binding sites on the DNA and prevents expression of the reporter gene (*LacZ*) and the subsequent formation of beta-galactosidase (β-Gal). III) When As(III) is present, ArsR loses its affinity for the binding sites on the DNA and the transcription of the *arsR* and *LacZ* genes takes place and as a consequence β-Gal is synthesized. IV) **The activity of the expressed β-Gal can be detected by following the product of its reaction (p-aminophenol, PAP) with p-aminophenyl galactopyranoside (PAPG).[1,2]**

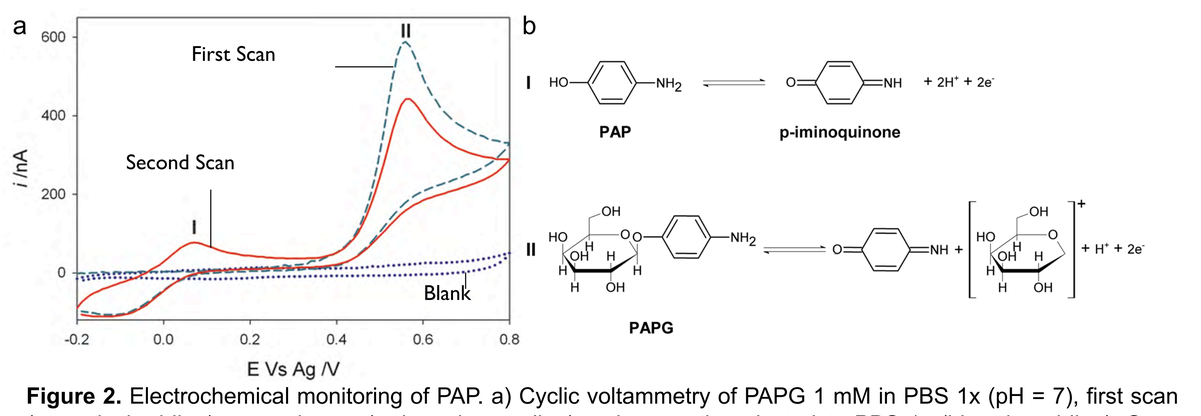

Figure 2. Electrochemical monitoring of PAP. a) Cyclic voltammetry of PAPG 1 mM in PBS 1x (pH = 7), first scan (cyan dashed line), second scan (red continuous line) and supporting electrolyte PBS 1x (blue dotted line). Scan rate = 20 mV/s. b) Electrochemical reactions taking place at I (*i.e.* PAP oxidation) and II (*i.e.* PAPG oxidation). **By working at a constant potential of 0.05 V Vs Ag, the production of PAP can be monitored electrochemically.**

3. EXPERIMENTAL SETUP AND PROTOCOL

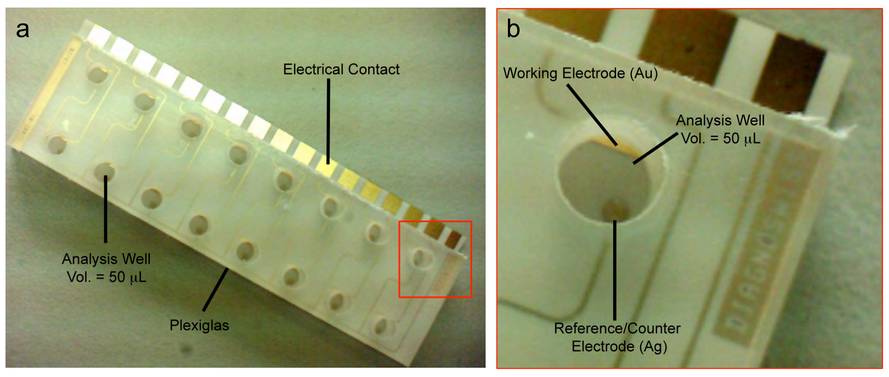

Figure 3. a) Optical photography of the microchip employed for the stop flow measurements of As(III). The microchip contains **16 independent electrochemical sensors** each one consisting of a two-electrode setup (working electrode = Au, counter/reference electrode = Ag) and a well made in Plexiglas (see Figure 3b).

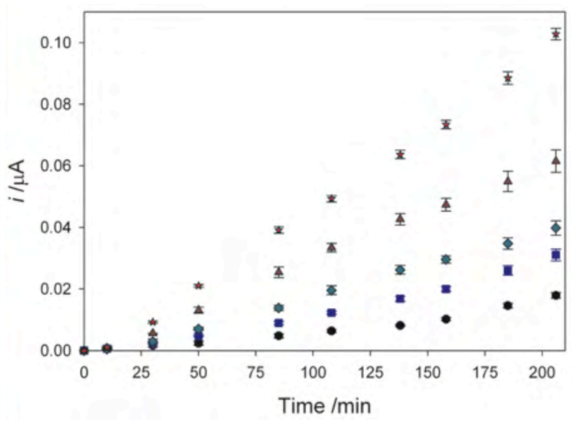

Figure 4. Amperometry detection of PAP as a function of time for different As(III) concentrations: 0 µM (0 ppb, Filled Black Circles), 0.05 µM (3.75 ppb, Filled Blue Squares), 0.1 µM (7.5 ppb, Filled Cyan Diamonds), 0.2 µM (15 ppb, Filled Brown Triangles), 0.4 µM (30 ppb, Filled Red Starts). The error bars represent the calculated standard deviation from triplicates. **The recorded response is proportional to the As(III) concentration.**

Protocol:

1) A given volume of the sample containing arsenic is mixed with a defined volume of the *E. coli* bioreporter suspension.

2) Immediately, 25 µL of the As(III)-*E. coli* solution are mixed inside the analysis well with 25 µL of 10 mM PAPG in 1xPBS (pH = 7). Final *E. coli* cells concentration ≈ 1 x 10^8 cells mL^{-1}.

3) Amperometric detection of PAP is started (see Figure 4).

4. QUANTIFICATION IN TAP AND GROUND WATER

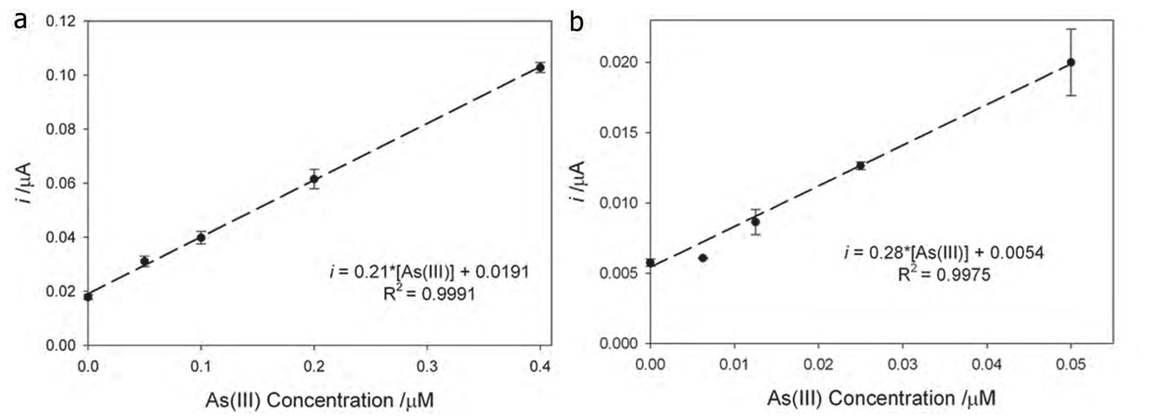

Figure 5. a) Calibration curve for As(III) at high concentration range (*i.e.* 0.05 µM to 0.4 µM or 3.75 ppb to 30 ppb) determined with the data obtained from Figure 4. b) Calibration curve for As(III) at low concentration range (*i.e.* 0.0125 µM to 0.05 µM or 0.94 ppb to 3.75 ppb) determined in a separated experiment (data not shown). **LOD = 0.0105 µM or 0.8 ppb.** The error bars represent the calculated standard deviation from triplicates.

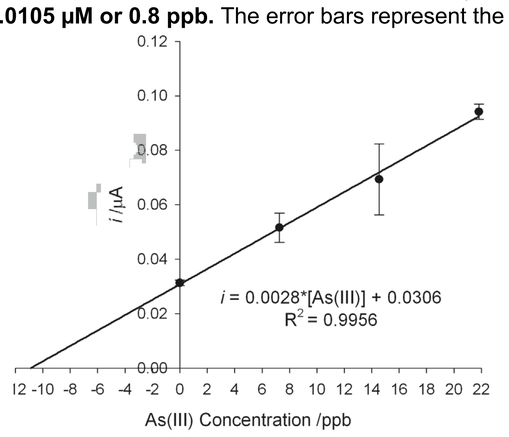

Table 1. Arsenic (III) quantification in ground water samples from Switzerland and Romania.

	As(III) Concentration /ppb	
Sample No.	Bioreporter	Reported (AAS)*
1	4.8 ± 0.4	3.3 ± 1.0
2	7.7 ± 1.6	6.5 ± 0.8
3	18.7 ± 1.2	17.8 ± 1.1
4	58.4 ± 1.7	66.0 ± 6.6

* AAS = Atomic Absorption Spectroscopy

Figure 6. Quantification of As(III) in four different samples of ground water from Switzerland and Romania employing the bacterial bioreporter by standard addition method. Only one quantification curve is shown here, but the results for all the samples are summarized in Table 1. The samples were neutralized previous to analysis with Pyrophosphate. The error bars represent the calculated standard deviation from triplicates. **The response of the bacterial bioreporter is reliable for the on-site quantification of arsenic in ground water.**

5. CONCLUSIONS AND PERSPECTIVES

Our results demonstrate the feasibility of coupling electrochemical and biological engineering methods for quantitative living cell toxicity detection in a high throughput microchip format. Therefore, monitoring of the water pollutant As(III) can be performed with a high selectivity and sensitivity. Indeed, the developed biochip has a LOD of As(III) in potable water equal to 0.8 ppb, with a linear response in the range of concentrations tested (*i.e.* 0.9 ppb to 30 ppb). According to the world health organization (WHO) an As (III) concentration in potable water higher than 10 ppb represent a risk for humans, and therefore the proposed approach is a very good alternative for the portable quantification of As(III) in water samples as demonstrated by the analysis of three natural water samples from Switzerland and Romania showing a very good agreement with the reported results by AAS. Furthermore, this strategy can be extended to the analysis of other toxic agents in the environment.

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