LIGHT EMITTING DEVICES AND INTEGRATED ELECTROCHEMICAL SENSORS ON LAB-ON-CHIP FOR TOXICITY BIOASSAYS BASED ON ALGAL PHYSIOLOGY

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ABSTRACT

In the frame of water toxicity analysis, a portable, glass-based, lab-on-chip was developed, integrating three-electrode electrochemical microsensors and organic light-emitting diodes (OLED). The basic detection principle consists in monitoring electrochemically O_2-related, algal metabolism in presence of herbicides. Thus, aiming on Diuron herbicide detection, a concentration-dependent inhibition effect on photosynthetic oxygen production rate was evidenced in the [0 – 1 µM] range. Finally, OLED-based integrated system demonstrates higher detection characteristics than those using external white light source (sensitivity: 0.48 versus 0.26 nA/s/µM) and is highly promising for further integration of optical and electrochemical sensors enabling double complementary detection.

KEYWORDS

Lab-on-chip, microelectrode, OLED, O_2-related algal metabolism, herbicide detection, water toxicity analysis

INTRODUCTION

Assessment of water quality has become an increasingly important environmental issue in the past few years. Water pollution induced by agricultural and industrial activities is known to have a severe impact on all living organisms. This is associated to the excessive use of herbicides and pesticides to protect crops. These toxicants can easily penetrate the soil and are finally detected in ground and/or river waters. It is therefore a major challenge to monitor their different quantities. Although water toxicant detection is most commonly performed in laboratories, it is important to find alternative methods to existing analysis tools, more adapted to field detection. Biosensors and bioassays can eventually meet the implemented requirements and have attracted wide attention [1]. For instance, optical [2] and electrochemical [3] algal-based systems are proposed in literature for the detection of herbicides as their metabolism is highly influenced by such toxic species.

In this study, a portable, glass-based, lab-on-chip was developed to monitor algal metabolism and detect targeted herbicides. It involves three-electrode (Pt – Pt – Ag/AgCl) electrochemical microcells (ElecCell) to perform electrochemical analysis, as well as organic light-emitting diodes (OLED) to trigger algal photosynthetic activities.

EXPERIMENTAL

Electrochemical microcells (ElecCell) and organic light-emitting diodes OLED were assembled on a lab-on-chip. Six independent detection chambers were designed on each platform enabling simultaneous processing of different assays. Three fluidic chambers were dedicated to electrochemical detection and three to future fluorescence-based optical detection. The lab-on-chip structure was fabricated on glass substrate in order to be compatible with optical technology and associated measurement.

Fabrication procedure included electrochemical microcell integration on the glass substrate, fluidic microsystem implementation, and device packaging. A 3D model of lab-on-chip sensor platform, including 6 independent chambers is presented in Figure 1.

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Figure 1: 3D model of the lab-on-chip sensor platform.

Integration of electrochemical microcells

Concentric, three-electrode (Pt – Pt – Ag/AgCl) electrochemical microcells (ElecCell) were integrated on glass substrate through photolithography patterning and physical vapor deposition (PVD) according to a previously developed process [4,5]. Three PVD steps were performed in a row. First, a 200nm platinum layer was deposited on a 20nm titanium underlayer to form platinum-based working and counter microelectrodes. Then, a 400nm silver layer was deposited on top and was later oxidized in chloride-based solutions to form the integrated Ag/AgCl pseudo-reference microelectrode. Finally, a Si_3N_4 passivation layer, deposited by chemical vapor deposition (CVD), was patterned in order to insulate electrically the different microelectrodes and define precisely their active surface. To improve detection properties, specific attention was brought on working microelectrode design and functionalization. Thus, a microelectrode array involving 25 disks of 10µm diameter was realized and later functionalized thanks to a black platinum (Bl-Pt) electrodeposition process.

Implementation of the fluidic microsystem and device packaging

The fluidic microsystem was structured by patterning 250µm-thick SU8 photoresist. Dimensions were specified according to the ElecCell geometry as well as to requirements related to the optical system. Consequently, circular-shaped fluidic analysis microchambers were

designed with a 8mm diameter and therefore a 12.5µL volume. Finally, the fluidic platform was sealed by wafer bonding using a glass cover. Finally, device packaging and electrical connection were performed in order to obtain a plug-in, easy to use, lab-on-chip device for algal solution analysis (Fig. 2).

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Figure 2: Algal test solution inserted in one of the detection chambers of the lab-on-chip device.

Bioassays and electrochemical analysis of algal photosynthetic activities

Chlamydomonas reinhardtii green algae were cultivated at 25°C in high salt medium (HSM) thanks to time-regulated light/dark (16h/8h) cycles. Algal cell concentration was measured by a multisizer Coulter counter in order to ensure a constant value for the different experiments. After leaving algae in dark for a sufficiently long time period, herbicide detection tests were finally conducted by mixing algal solutions with different Diuron concentrations (range: 0 – 1 µM, in agreement with maximum acceptable value implied in Canada: 0.64 µm). Using the (Pt/Bl-Pt – Pt – Ag/AgCl) ElecCell, O_2 monitoring was finally performed by chronoamperometry with an applied potential of -0.7 V versus integrated Ag/AgCl. All the experiments were performed at ambient temperature (~ 22°C).

Thanks to its realization on a glass substrate, the glass-based lab-on-chip was fully compatible with optical technology and associated measurement (Fig. 2). As a result, it allowed herbicide detection while using a non-integrated, halogen, white light source. However, in order to eventually obtain portable and autonomous analysis microsystem, the light source used for the stimulation of algal photosynthetic activities should be integrated on the final lab-on-chip device. Consequently, blue OLED devices were fabricated on a glass substrate through multilayer evaporation of PEDOT:PSS/NPB/PCAN/Alq_3/Al and placed on top of the detection chamber, in direct contact with the lab-on-chip glass cover (Fig. 3). It was specifically designed in order to have a good overlap between the OLED electroluminescence spectrum and the *Chlamydomonas reinhardtii* algae absorption band (Fig. 4).

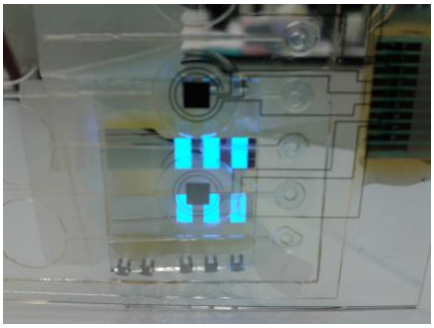

Figure 3: Integration of blue OLED devices on the lab-on-chip glass cover.

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Figure 4: Comparison of emission spectra of OLED and halogen white light source with algal absorption spectrum.

RESULTS AND DISCUSSION

O_2 measurement in control algal solutions

Using the halogen white light source, dark and light periods were alternated in control algal solutions and O_2 concentration was monitored by chronoamperometry (Fig. 5). When light is on, photosynthesis is responsible for oxygen production and therefore for the O_2-related cathodic reduction current increase. On the contrary, when light is off, algae are consuming oxygen due to respiration and the cathodic reduction current decreases. Prior to further analysis, it was checked, using non-algal control solutions that current variations were not related to illumination interferences (result not shown).

Thanks to these first experiments, it appeared that current follows roughly linear variations with time and therefore that algal metabolisms can be studied according to to the slope of the current versus time.

Furthermore, a compromise was found concerning algal concentration in order to yield well-defined differences between oxygen production and consumption and high signal-to-noise ratio. This leads to an optimal concentration around 13×10^6 cells/mL. This value was chosen for the following experimental campaigns.

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Figure 5: Current measurements of algal respiration and photosynthesis using a (Pt/Bl-Pt – Pt – Ag/AgCl) ElecCell device integrated on a lab-on-chip.

Diuron detection using halogen white light source

First experiments were performed using a halogen white source illumination. Light-induced oxygen production was monitored in real time and O_2 production rate decrease with increasing Diuron concentration points out the toxicant effect (Fig. 6). Nevertheless, respiration rates before turning on the light were found to be not identical. Since Diuron herbicide targets only photosynthetic activity, the causes of this variability are difficult to determine. They can be related to algae culture intrinsic properties, sampling protocol and/or biofouling phenomena.

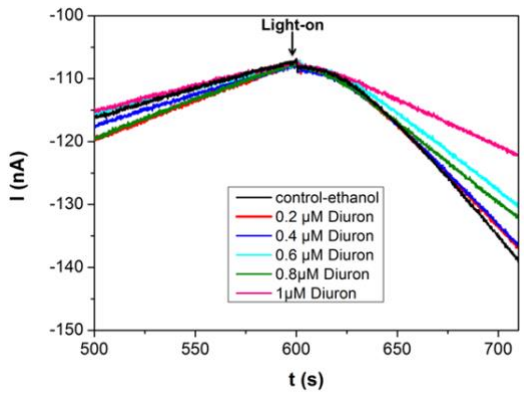

Figure 6: Algal O_2-amperometric responses for various Diuron concentrations using a (Pt/Pt-Bl – Pt – Ag/AgCl) ElecCell-based lab-on-chip device.

As a result, a normalization step was conducted in order to eliminate this variability. It consists in setting an average "reference" value $\bar{x}$. for the different respiration slopes (x_i) and applying a correction coefficient to all the photosynthesis slopes (y_i).

$$y_i^* = y_i \times \frac{\bar{x}}{x_i} \quad (1)$$

Through this approach, sensor calibration curves were defined by studying the photosynthetic O_2 production rate for different Diuron concentrations (Fig. 7). For the two light intensities tested, a linear decrease was obtained between both parameters, allowing to define the Diuron detection sensitivity. Values of 0.26 and 0.1 nA/s/µM were calculated for light intensities of 600 and 1800 µE/m²/s respectively.

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Figure 7: Calibration curves (normalized oxygen production rates versus Diuron concentration) for two different light conditions using halogen white light source.

Light intensity is known to play an important role in algal metabolism. At low intensity levels, photosynthesis is enhanced when light intensity is increased as more photons are captures by pigments and processed through the chloroplast reaction centers [6]. However, at high intensity levels, reaction centers of chloroplast becomes saturated or even damaged. An inhibition of photosynthetic activity is thus observed [7]. Our result demonstrates that, when illumination conditions are too high, they are responsible for such inhibition phenomenon.

Finally, it should be noted that herbicide detection was also performed using natural lake water to cope with real samples analysis. Thus, Diuron detection sensitivity around 0.26 nA/s/µM was still obtained for a 600 µE/m²/s light intensity (Fig. 8).

Diuron detection using blue OLED as light source

Given the fact that blue OLED devices were integrated on the ElecCell-based lab-on-chip at the system level, a non-algal control measurement of current monitoring through light/dark periods was conducted. In contrast with previous control measurement carried out with the non-integrated halogen white light source, an increase in the cathodic reduction current was observed when the light was turned on (result not shown). This current increase was attributed to the temperature increase induced by OLED heating. Indeed, the temperature on the lab-on-chip backside was measured around 35°C after a two minutes blue OLED illumination. In order to compensate this phenomenon, the illumination rate recorded for the non-algal control solution was subtracted from the photosynthetic O_2 production rate determined for different Diuron concentrations. Experiments were performed in natural lake water samples in the [0 – 0.6 µM] Diuron concentration range (Fig. 8). Detection sensitivity obtained when using the integrated blue OLED light source was estimated at around 0.48 nA/s/µM that corresponds to almost double the value obtained while using the non-integrated halogen white light source. This sensitivity improvement was related to the better overlap between the blue OLED emission spectrum with the major *Chlamydomonas reinhardtii* algae absorption band (Fig. 4).

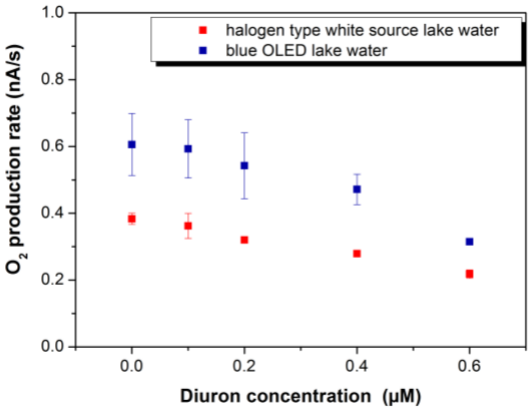

Figure 8: Calibration curves obtained in lake water samples using as light sources, either an integrated blue OLED (blue points) or a halogen white light source (red points).

CONCLUSION

A portable device for in-situ herbicide detection, based on algal physiology, was developed. The fabricated lab-on-chip platform consists in three fluidic chambers integrating (Pt/Bl-Pt – Pt – Ag/AgCl) electrochemical microcells (ElecCell) and three chambers dedicated to further optical fluorescence-based detection. The effect of Diuron herbicide was validated using the lab-on-chip devices with culture medium solutions. Illumination was first supplied through a

non-integrated, halogen white light source. Thus, a Diuron concentration-dependent decrease in the photosynthetic oxygen production rate was demonstrated for two different light intensities: 600 and 1800 $\mu E/m^2/s$. Diuron detection sensitivity of approximately 0.26 and 0.1 nA/s/µM were respectively obtained, evidencing photosynthesis inhibition phenomena due to too high intensity levels. Then, in order to obtain an autonomous system, the same experiments were successfully carried out in natural lake water samples, with an integrated blue OLED used as light source for the excitation of photosynthetic mechanism. Taking into account undesired heating phenomena, Diuron detection was found to gain a factor two (value around 0.48 nA/s/µM in the [0 – 0.6 µM] concentration range) evidencing that photosynthetic apparatus was more effective when the blue OLED light source is used compared to the halogen white light source.

These first results demonstrate that the proposed lab-on-chip biosensor can effectively follow the change in photosynthetic activity induced by Diuron herbicide and reflected through a modification in oxygen production rate. It can therefore be an efficient indicator of water pollution. Nevertheless, further studies should be conducted to still improve detection properties while optimizing the OLED photoluminescence spectrum, illumination power and integration process.

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