BIOFUNCTIONALIZED LAB-ON-A-CHIP WITH DUAL READOUT

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ABSTRACT

A dual (electrochemical and optical) Lab-on-a-Chip is presented, which monolithically combines a Multiple Internal Reflection and a biofunctionalized mixer that also plays the role of reactor. Here, there is no need to include an enzyme reservoir, resulting in a reduction of dead volumes. This approach was validated by functionalizing the mixer/reactor with horseradish peroxidase and glucose oxidase, being possible to detect glucose optically and electrochemically under continuous flow conditions, with a limit of detection of 6.3 ± 0.4 mM at 100 µL/min (20 s response time) and 0.113 ± 3 x 10^{-3} mM at 10 µL/min (1 min response time).

KEYWORDS: biofunctionalization, Lab-on-a-Chip, Photonic lab-on-a-chip, microfluidics, reactor.

INTRODUCTION

Most of the reported Lab-on-Chips (LOCs) are based either in optical or electrochemical transduction methods. Since both approaches are relatively easy to scale down and integrate [1], the selection of one of them ultimately depends on the nature of the analyte to be detected. In many cases, however, the integration of the two transduction methods in a single LOC is justified, since simultaneous electrochemical and optical measurements can provide with either complementary information or enhanced performance. The integration of such transducers with microreactors also offers several advantages such as: high efficiency and repeatability, large surface areas for fast mass transfer, and control of reaction time and conditions through changes in the microfluidics [2]. However, many of the reactor approaches found in the literature need to follow several fabrication steps such as the fabrication of additional materials like gold patterns [3], the mechanization of several layers [4], or make use of very low flow rates [5] that increase the response time.

In this work, a dual (electrochemical and optical) LOC (DLOC) is presented, which monolithically combines a Multiple Internal Reflection (MIR) and a biofunctionalized mixer (also playing the role of reactor and therefore being called mixer/reactor). This approach avoids the necessity to add an enzyme reservoir in the system, resulting in a reduction of the dead volumes, keeping the advantage of the low reagent consumption that is associated to the LOC concept. Validation of this concept was done by functionalizing the mixer/reactor with horseradish peroxidase (HRP) and glucose oxidase (GOx), being possible to detect glucose both optically and electrochemically and in continuous flow.

DESIGN

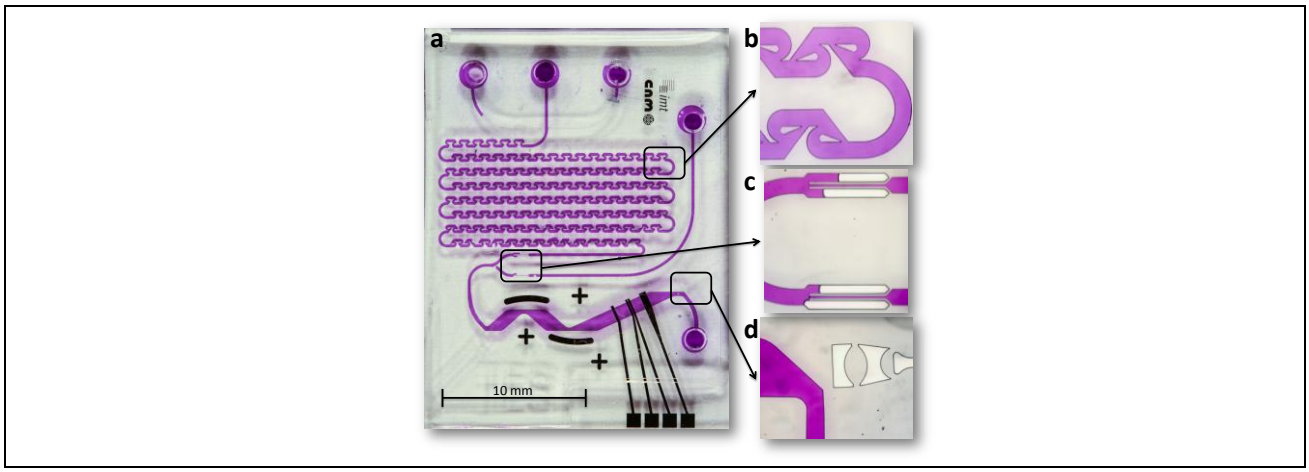

Figure 1: (a) Optical and electrochemical Lab-On-a-Chip composed of mixer and MIR. Close view of the (b) mixer structure, (c) passive valves and (d) biconvex lens.

The DLOC is shown in Figure 1. It includes a mixer and a Multiple internal reflection system (MIR). The mixer makes use of the Coanda effect to split and recombine counter propagating liquid flows [6]. Up to three fluidic inlets can be simultaneously used with the mixer and an additional auxiliary inlet has been implemented to directly pump to the MIR the samples that do not need to be mixed or that could negatively affect the immobilized enzymes on the mixer/reactor. Passive unidirectional valves at both downstream the mixer and the auxiliary inlet assure no liquid counter propagation. The MIR [7] consists of a zig-zag microchannel and two air mirrors that make the light path match with the microchannel geometry, enlarging the optical path to enhance the sensitivity without increasing the system's size. Light in/out coupling is done with fiber optics clamped into self-alignment elements ended with biconvex lens for correcting the beam divergence.

GOx and HRP were immobilized on the walls of the mixer/reactor . As the sample downstream propagates, a reaction between the glucose and the redox mediator 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) present in it and the immobilized enzymes occurs. GOx catalyzes the oxidation of D-glucose by oxygen to produce D-gluconolactone and H_2O_2. H_2O_2 is the substrate for HRP, which catalyzes the reduction to H_2O with the concomitant oxidation of ABTS redox mediator to $ABTS^{+\bullet}$, that presents absorbance peaks at 420, 650, 740 and 835 nm (Figure 2a). ABTS presents an oxidation limiting current at 0.3 V vs. Au pseudo-reference electrode (Figure 2b), being possible in this way to detect glucose optically and electrochemically as it arrives already reacted to the MIR zone.

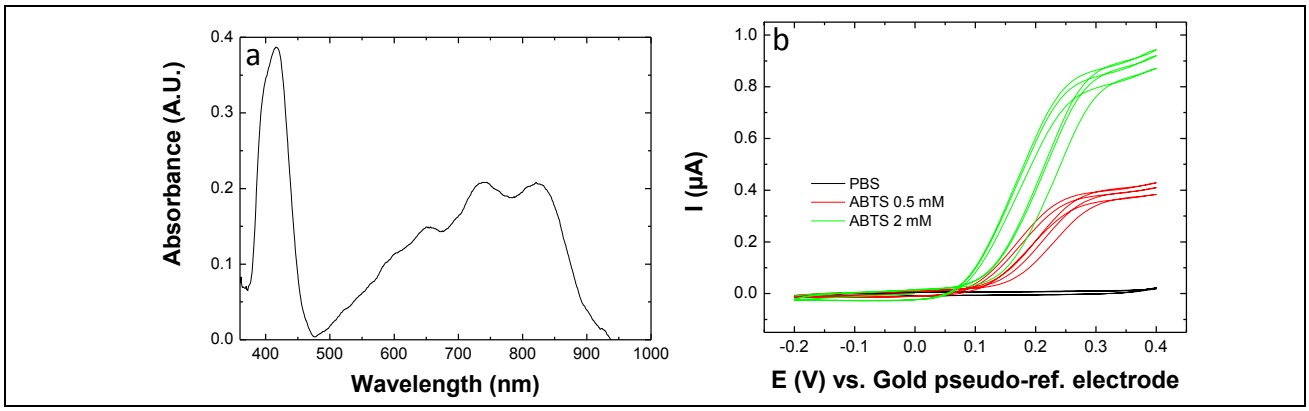

Figure 2: (a) Absorbance spectrum of ABTS•+. (b) Cyclic voltammetry of ABTS in PBS buffer.

EXPERIMENTAL

The glass substrate contains microband gold electrodes underneath the MIR, including a reference electrode, two working electrodes and a counter electrode, with a passivating silicon oxide layer on their edges to enhance robustness. All the micro-optical and microfluidic elements were monolithically fabricated in polydimethylsiloxane (PDMS) by replica molding. Bonding between the substrate and the PDMS was done by exposing both to an oxygen plasma and using deionized water (DI H_2O) as lubricant for their alignment.

For the biofunctionalization, a previously published protocol was followed [8]. PVA was adsorbed onto the microchannels by incubating a 1 mg/mL PVA solution for 1h at room temperature (RT). Then they were rinsed with DI H_2O and dried. This step was followed by a silanization carried out incubating at RT a 99.6% ethanol solution containing 2% 11-triethoxysilyl undecanal (TESU) and 2% TEA for 1h. After rinsing the microchannels with 99.6% ethanol, the DLOCs were kept for 2h at 80ºC. TESU presents an aldehyde group that works as anchoring point for proteins through their amine groups of its lysine residues, by forming a Schiff base that is stabilized by reducing it to a secondary amine by using sodium cyanoborohydride ($NaBH_3CN$). The silanized DLOCs were filled with a carbonate buffer solution pH 8.0 containing GOx, HRP and 5 mM $NaBH_3CN$. After 1h incubation at RT, a 0.1 M phosphate buffer saline solution pH 7.0 containing 0.02% (v/v) Tween 20 (PBST) was pumped to remove the nonspecifically adsorbed proteins. The DLOCs were kept in PBS at 4 ºC until their use.

RESULTS AND DISCUSSION

A calibration plot in a range from 10 to 100 mM glucose was measured (Figure 3a) optically and electrochemically. A linear range from 10 to 90 mM for optical detection and from 10 mM to 60 mM for electrochemical detection were considered, obtaining limits of detection (LOD) of 8.9 ± 0.5 mM and 6.3 ± 0.4 mM respectively and sensitivities of (0.037 ± 0.002) x 10^{-2} A.U./mM and -6.6 x 10^{-4} ± 3 x 10^{-5} μA/mM respectively. Simultaneous measurements were performed showing absence of crosstalk between detection modes and identical fast response of 20 s (Figure 3b).

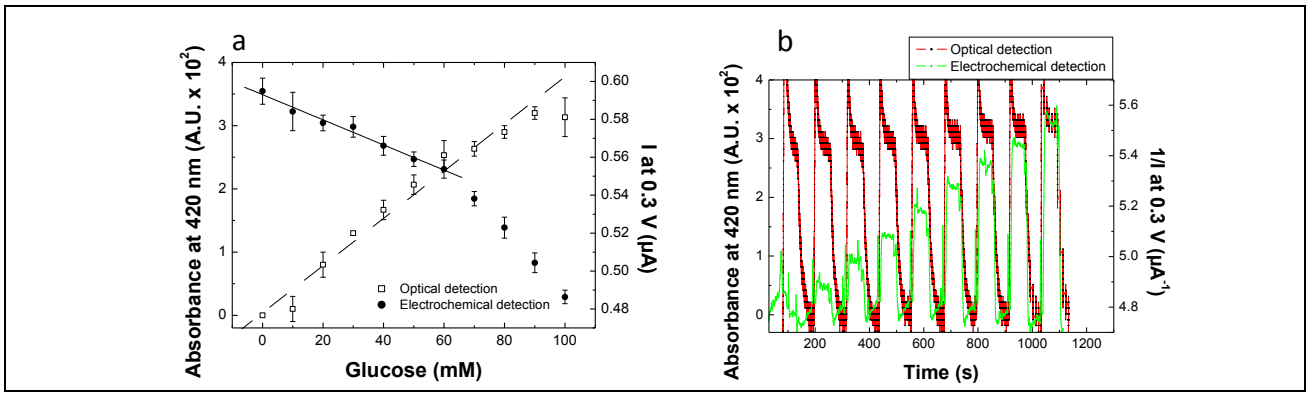

Figure 3: (a) Electrochemical and optical measurements of glucose at 100 μL/min. (b) Simultaneous measurements through time for glucose concentrations from 10 μM to 90 μM.

When measurements at 10 µL/min were done, the measurable range decreased to 200 µM – 2 mM (linear range up to 1.6 mM for optical detection and up to 2 mM for electrochemical detection) and the response time increased to 1 min (Figure 4). The absorbance peak at 750 nm could also be plotted. LOD and sensitivity values for optical measurements at 420 nm and 750 nm, and for electrochemical detection, were 0.21 ± 0.15 mM and (4.2 ± 0.3) x 10^{-2} A.U. /mM, 0.225 ± 0.017 mM and (2.15 ± 0.16) x 10^{-2} A.U. /mM, 0.113 ± 3 x 10^{-3} mM and -0.0101 ± 3 x 10^{-4} µA/mM, respectively.

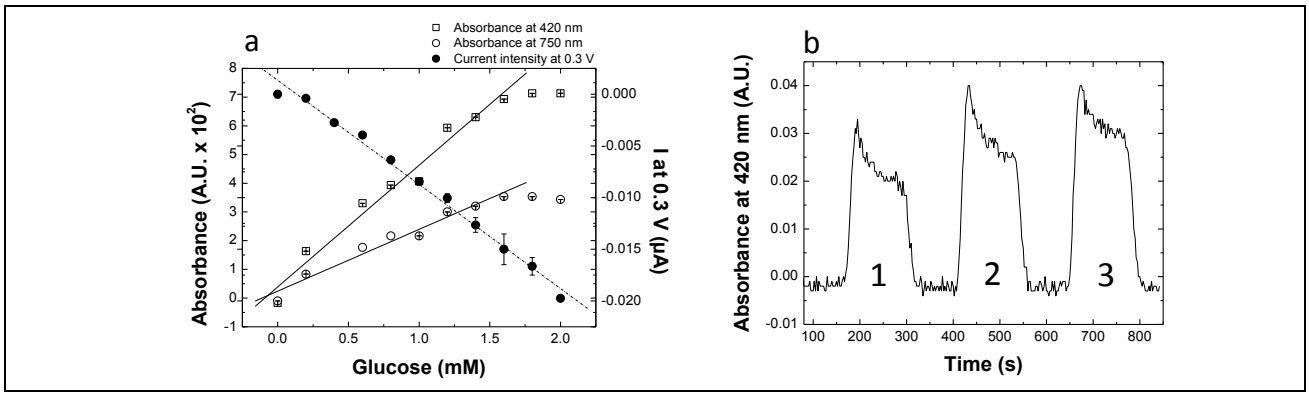

Figure 4: (a) Optical and electrochemical measurements of glucose at 10 µL/min. (b) Optical detection glucose through time at 10 µL/min. Peaks 1, 2 and 3 represent injections of 600, 1200 and 1800 µM glucose respectively.

The DLOC provides with a LOD in or under the physiological level depending on the flow rate used, and offering additional advantages as compared to a previously presented DLOC [1]: reduced response time and sample volume, inclusion of the mixer and MIR in a single substrate, reduction of the reagent consumption by immobilizing the enzymes.

CONCLUSION

A biofunctionalized DLOC that combines optical and electrochemical readout integrated with a mixer that plays the role of reactor and capable of performing continuous flow measurements has been presented. It has been tested for the analysis of glucose by immobilizing GOx and HRP at mixer walls. The results show that glucose can be measured at the physiological level using a relatively high flow rate (100 µL/min) and with fast system response (20 s). Simultaneous measurements show absence of crosstalk and identical fast response, enhancing the reliability of the DLOC by making it self-verifying. By manipulating the flow rate, the LOD can be improved while keeping the response time short (60 s).

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