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Self-validating lab-on-a-chip for monitoring enzyme-catalyzed biological reactions

Bergoi Ibarlucea [a,1], Xavier Munoz-Berbel [a], Pedro Ortiz [a,2], Stephanus Büttgenbach [b], César Fernández-Sánchez [a,*], Andreu Llobera [a,b,**]

[a] Instituto de Microelectrónica de Barcelona, IMB-CNM (CSIC), Campus UAB, 08193, Bellaterra, Barcelona, Spain
[b] Institut für Mikrotechnik, Technische Universität Braunschweig, Alte Salzdahlumer Straße 201, 38124 Braunschweig, Germany

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ABSTRACT

This paper reports on a miniaturized and highly integrated lab-on-a-chip (LoC) combining both optical and electrochemical transduction modes for self-validating determination of biological analytes that undergo enzyme-catalyzed-reactions. This LoC monolithically integrates a biofunctionalized microfluidic mixer, also working as bioreactor, together with a measurement chamber, both fabricated in polydimethylsiloxane (PDMS) and applied for enzyme-mediated detection of glucose in continuous flow regime. The measurement chamber combines a multiple internal reflection (MIR) photonic cuvette and a tailor-made electrochemical cell, where gold electrode areas are defined by a silicon oxide passivation layer. Having separated mixing/reaction and measurement chambers greatly facilitates the functionalization process during the microsystem fabrication and the further calibration/rinsing steps carried out during the microsystem performance. A cascade bi-enzymatic reaction involving glucose oxidase (GOx) and horseradish peroxidase (HRP) together with azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) mediator is applied to carry out the glucose dual detection. The optical and electrochemical properties of the ABTS enable simultaneous absorbance and amperometric measurements, without cross-talk. The system requires low sample volumes below 15 μL and presents tunable analytical properties that can be adjusted by varying the flow rate. Linear ranges extending up to 1.6 mM and 2 mM glucose and limits of detection of 0.23 ± 0.02 mM and 0.064 ± 0.001 mM are achieved with the optical and electrochemical detection approaches, respectively, when operating simultaneously at 10 μL/min flow rate. The high degree of integration results in a minimization of dead volumes, reagent consumption and response time, providing with a high performance self-validating structure ideal for biomedical applications.

1. Introduction

The term lab-on-a-Chip (LoC) [1] refers to compact systems that integrate several steps of a (bio)chemical analytical process and are able to perform effective single or multiple detection in sample volumes in the μL-mL range. Reduction of the size does not only provide with a decrease of the fabrication costs, but, more importantly, enhances its performance in terms of higher diffusion mixing efficiency, improved heat transport, reduced reaction times and higher reaction yields as compared to their bulk laboratory counterparts.

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); LoC, lab-on-a-chip; GOx, glucose oxidase; HRP, horseradish peroxidase; LOD, limit of detection; MIR, multiple internal reflection; PBS(T), phosphate buffer saline (tween 20); PDMS, polydimethylsiloxane; PECVD, plasma-enhanced chemical vapor deposition; PGMEA, propylene glycol methyl ether acetate; PVA, polyvinyl alcohol; RT, room temperature; TEA, triethylamine; TESU, 11-triethoxysilyl undecanal; TIR, total internal reflection.

* Corresponding author.
** Corresponding author at: Instituto de Microelectrónica de Barcelona, IMB-CNM (CSIC), Campus UAB, 08193, Bellaterra, Barcelona, Spain.
E-mail addresses: cesar.fernandez@imb-cnm.csic.es, cesar.fernandez@csic.es (C. Fernández-Sánchez), andreu.llobera@imb-cnm.csic.es (A. Llobera).
[1] Present address: Institute for Materials Science, Max Bergmann Center of Biomaterials and Center for Advancing Electronics Dresden, TU Dresden, 01062, Dresden, Germany.
[2] Present address: Oxford Nanopore Technologies Ltd, Oxford, OX4 4GA, United Kingdom.

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To fully exploit the LoC concept, the means to carry out the selective detection and quantification of chemical species has to be included in the system. This is ubiquitously linked to both implementation of appropriate recognition elements such as biomolecules and incorporation of the appropriate transduction mechanism. Among the former, different approaches for the definition of microbioreactors in LoCs based on the immobilization of antibodies, enzymes or DNA strands [2–4] have already been presented to the scientific community. Similarly, different transduction mechanisms can be incorporated in LoC to transform the chemical signal being produced in the microbioreactors into a measurable signal. Optical or electrochemical approaches are preferred because they require components that are relatively easy to scale down and integrate [5]. In this context, miniaturized electrochemical systems based on different electrode configurations have already been demonstrated for analytical applications [6] and combined with microfluidic tools to facilitate analyte detection in different fields such as food analysis, applied to vitamins, toxins or antioxidants [7], or clinical analysis, as demonstrated by plenty of glucose biosensors [8]. They are compatible with microfabrication technologies and provide with high sensitivity and fast response [9] with low power requirements [10]. Among the different electrochemical transduction modes, those based on potentiometry [11], amperometry [12], impedance [13] or conductivity [14] have been implemented in LoC. On the other hand, different optical detection modes based on absorbance [15], fluorescence [16], chemiluminescence [17] or interferometry [18] have also been integrated in LoC systems using monolithic or heterogeneous integration approaches. Both optical and electrochemical transduction mechanisms can be coupled to biochemical reactions and, in some cases, they can provide with complementary analytical information. This is grounded on the fact that most of the optical transducers analyze the sample in bulk, whereas most electrochemical transducers measure an electrical property of the sample at the interface between the solution and the transducer. These attractive complementary features have been exploited in the development of dual optical/electrochemical LoC systems mainly showing three different strategies. Firstly, most of them incorporate different electrochemical and external optical detectors to carry out the simultaneous multiplexed analysis of several chemical species [19,20]. Secondly, some LoC architectures were developed where an electrochemically active analyte is oxidized on the electrochemical transducer surface resulting in a colored product that was optically measured off-chip [21,22]. Thirdly, integrated dual LoC systems have been developed with the ability to simultaneously measure the same target analyte, thus having a dual LoC with self-verifying analysis [5,23].

The application of self-verifying analytical systems is fundamental when analyzing molecules of clinical interest in order to ensure measurement reliability. This is of particular relevance in patients suffering from chronic pathologies requiring a continuous and strict control. In most of these cases, low-cost, simple and miniaturized systems for in-house analysis have been developed and are mostly based on paper technology. Unfortunately, up to now paper-based systems suffer from poor precision due to the low homogeneity of the substrate and the difficult control over the liquid flow. Such limitations are particularly important in the case of point-of-care diagnostics.

In an attempt to tackle this issue, a miniaturized LoC integrating absorbance and amperometric transducers in a single measurement chamber as well as a biofunctionalized mixer/bioreactor, both of them fabricated in PDMS in a one-step fabrication process, for the self-verifying determination of analytes of clinical interest (i.e. glucose) is here presented. Unlike previously reported self-verifying systems, the resulting LoC present unique and improved characteristics ideal for analysis of biological samples. First of all, it incorporates for the first time a mixer element also working as enzymatic bioreactor that enables carrying out sample conditioning automatically with very low sample and reagent volumes. Second, separating the mixing/reaction and measurement areas facilitates the enzyme functionalization process in the reaction chamber during the system fabrication and allows for the implementation of cleaning/calibration steps of the optical and electrochemical transducers. The latter is achieved by pumping the corresponding solutions through an auxiliary channel directly into the measurement chamber without going through the mixer/reactor component and thus making sure that the activity of the immobilized enzymes was not compromised. This is possible by also integrating different fluidic inlets and outlets and two passive unidirectional valves for managing the corresponding solutions. The high degree of integration of the different fluidic elements significantly reduces sample and reagent volumes as well as dead volumes within the system. Hence, assay times are drastically shortened and the cost-per-assay reduced. The resulting system has been applied to the dual selective detection of glucose in continuous flow regime. Nevertheless, this LoC approach has the potential to be used for the precise analysis of other optically/electrochemically active analytes of clinical interest with a low probability of incorrect readings provided by the dual detection.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA), ethanol 99%, triethylamine (TEA), sodium cyanoborohydride ($NaBH_3CN$), tween 20, potassium hexacyanoferrate (II) ($[Fe(CN)_6]^{4-}$, ferrocyanide), potassium nitrate (KNO_3), glucose oxidase (GOx), horseradish peroxidase type VI (HRP) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt were from Sigma-Aldrich (Munich, Germany). Potassium hexacyanoferrate (III) ($[Fe(CN)_6]^{3-}$, ferricyanide) was from Panreac (Castellar del Vallés, Spain). 11-triethoxysilyl undecanal (TESU) was from ABCR GmbH & Co. (Karlsruhe, Germany). EPON SU-8 25 photoresist and propylene glycol methyl ether acetate (PGMEA) were from MicroChem Corporation (Newton, MA, US). The polydimethylsiloxane (PDMS) Sylgard 184 elastomer kit was from Dow Corning (Midland, MI, USA).

2.2. Design of the dual detection LoC

The LoC presented in this work, shown in Fig. 1, combines a multiple internal reflection (MIR) photonic cuvette [24] bonded over an electrochemical cell consisting of a glass substrate where gold electrodes are patterned. Upstream, an enzymatically functionalized Tesla mixer (thus also playing the role of reactor) is incorporated, resulting in a miniaturized and highly integrated structure, allowing for fast (seconds) and quantitative dual detection of analytes of clinical interest with small sample volumes (total volume = 12 μL). For simplification, each part is separately discussed below.

2.2.1. MIR system

The MIR is an optofluidic structure composed of an 11 mm long zigzagging fluidic channel (total volume = 2.7 μL). Optical interrogation of the sample is performed by using micro-optical elements incorporated in the MIR structure as detailed below.

Light inlet/outlet structures contain self-alignment elements and PDMS biconvex lenses for suitable coupling/collection of the light. Self-alignment elements are undulating PDMS microchannels for the accurate and stable positioning and clamping of the fiber optics at the MIR structure. Undulating configuration also minimizes fiber optics breakage risk. When positioned in this element, fiber optics are perfectly aligned to the optical axis of the PDMS