Portable Optoelectronic System for Monitoring Enzymatic Chemiluminescent Reaction

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Abstract This work presents a portable lab-on-chip system, based on thin film electronic devices and an all-glass microfluidic network, for the real-time monitoring of enzymatic chemiluminescent reactions. The microfluidic network is patterned, through wet etching, in a 1.1 mm-thick glass substrate that is subsequently bonded to a 0.5 mm-thick glass substrate. The electronic devices are amorphous silicon p-i-n photosensors, deposited on the outer side of the thinner glass substrate. The photosensors, the microfluidic network and the electronic boards reading out the photodiodes' current are enclosed in a small metallic box ($10 \times 8 \times 15\,cm^3$) in order to ensure shielding from electromagnetic interferences. Preliminary tests have been performed immobilizing horseradish peroxidase on the inner wall of the microchannel as model enzyme for detecting hydrogen peroxide. Limits of detection and quantification equal to 18 and 60 μM, respectively, have been found. These values are comparable to the best performances reported in literature for chemiluminescent-based optofluidic sensors.

Keywords Photosensors · Amorphous silicon · Microfluidics
Enzymatic reactions · Horseradish peroxidase · Anodic bonding

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B. Andò et al. (Eds.) *Sensors*, Lecture Notes in Electrical Engineering 539,
https://doi.org/10.1007/978-3-030-04324-7_25

1 Introduction

The combination of biosensing systems with microfluidic circuits improves the overall performance of the sensing analysis. The reduced dimensions and volumes in microfluidic channels allow first of all to work with much less sample than using standard equipment, making analysis on drops of blood or even the contents of single cells possible. More importantly, the short distances between analyte molecules and biorecognition elements reduce the diffusion times, which immediately yields a great gain in response time, significantly improving conditions for diffusion-limited processes [1]. Expanding from pure microfluidics to more fully developed lab-on-chip (LoC) solutions, entire sample preparation procedures, biorecognition elements and detection can be integrated in portable systems for health-care and diagnostics. Antibodies, aptamers and enzymes were immobilized by us into lab-on-chip devices for a variety of biosensing applications [2–4]. However, the integration of the sensing element has to be adapted to the technological steps necessary to combine the microfluidic network with the detection systems. For example, the integration of the detection system with the microfluidic network, can be an issue in terms of feasibility and sensitivity. The most used techniques are electrochemical and optical methods. In particular, optical methods rely on the use of fluorescence, absorbance and chemiluminescence. The last one is largely coupled with microfluidics since it does not require an excitation source and does not give background signals [5]. Lately, some groups started to work on the implementation of optical sensing elements directly on the microfluidic platform for chemiluminescence detection. Photosensors where integrated on silicon/PDMS chip for chemiluminescence detection [6, 7].

In this work, we present a novel lab-on-chip system based on a glass microfluidic channel with on-chip amorphous silicon photosensors (a-Si:H) [8] for the monitoring of chemiluminescent based enzymatic sensing assays. The photosensors are positioned underneath the microfluidic channel and allow monitoring the enzymatic assay. A layer of polymer brushes was grown on the inner wall of the microchannel and subsequently an enzyme was immobilized to it by peptide bond. As a proof of principle, horseradish peroxidase (HRP) was used as model enzyme to be anchored for detecting hydrogen peroxide (H_2O_2) in presence of luminol and 4-iodophenol.

2 System Structure and Operation

Figure 1 reports a cross section of the proposed lab-on-chip. It is constituted by two bonded glass substrates, which include both a microfluidic network and a-Si:H photosensors. Substrate 1 is a 1.1 mm-thick glass plate, that has been patterned by wet etching, in order to define the microfluidic network. Substrate 2 is a 0.5 mm-thick glass that has been anodically bonded to substrate 1. After the glass bonding, the a-Si:H photosensors have been deposited on the outer side of the thinner substrate and aligned with the microfluidic network. When a chemiluminescent reaction occurs inside the channels, as a result of a biomolecular recognition, the emitted radiation

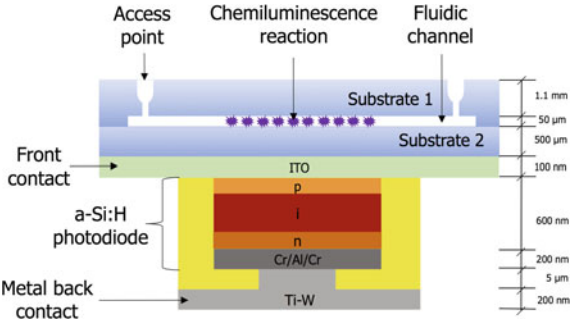

Fig. 1 Cross section of the all-glass lab-on-chip (not in scale): the bottom glass substrate hosts the the a-Si:H sensors on the bottom side, while the top substrate hosts the microfluidic network

is absorbed by the a-Si:H photodiodes, which produce photocurrents proportional to the emitted light. The a-Si:H are p-type doped/intrinsic/n-type doped stacked layers, whose thickness and energy gap has been optimized to detect the spectrum of the chemiluninescent spectrum.

The proposed lab-on-chip structure features therefore on-chip chemiluminescence detection which offers several advantages with respect to off-chip methods and in particular:

- reduction of the distance between the radiation source and the photosensors and as a consequence limited light diffusion and optical losses;
- absence of focusing optics which implies a higher degree of compactness of the system.

3 System Fabrication

The fabrication steps of the lab-on-chip have been optimized in order to keep the compatibility between microfluidics, microelectronics and biochemical requirements. In particular, the deposition and patterning of the a-Si:H photosensors should not affect the chemical surface composition of the glass channels, while the chemical procedures needed to implement the biomolecular recognition and to produce the chemiluminescent signals should not degrade the optoelectronic performance of the a-Si:H photodiodes.

Taking into account these specifications the fabrication of the lab-on-chip has been performed through the following technological steps (see Fig. 2):

- realization of fluidic networks on the bondside of a 1.1 mm-thick Borofloat 33 substrate by wet etching (25% hydrofluoric acid (HF)) yielding a 50 µm-deep and 110 µm-wide channel;
- definition of trenches, through the powderblast technique, in the 1.1 mm-thick glass (at the non-bond side). These trenches define the position of the inlets and outlets but at this phase of fabrication they are not in contact with the microfuidic channels (see Fig. 2b);

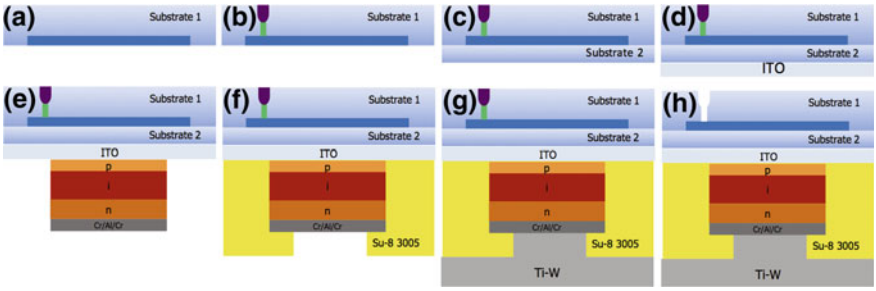

Fig. 2 Fabrication steps of the lab-on-chip: **a** Definition by wet etching of the microfluidic network in substrate 1. **b** Definition of the trenches for the microfluidic access. The purple region is the part removed by powderblasting. The green region is still glass. **c** Anodic bonding of the two glasses. **d** Deposition of the Indium Tin Oxide bottom contact of the photosensors. **e** Deposition by PECVD and patterning by reactive ion etching of the a-Si:H p-i-n junction. **f** Deposition and patterning of the SU-8 3005 insulation layer. **g** Deposition and patterning of the TiW top contact of the sensors. **h** Complete opening of the inlets and outlets of the microfluidic network by laser drilling

- anodic bonding of this substrates to a 0.5 mm-thick BF33 substrates [9];
- realization of the a-Si:H photodiodes by standard microelectronic techniques, including Plasma Enhanced Chemical Vapor Deposition for the deposition of the amorphous silicon layers [8] on the not-bonded side of the 0.5 mm-thick substrate;
- complete opening of the inlets and outlets by laser drilling.

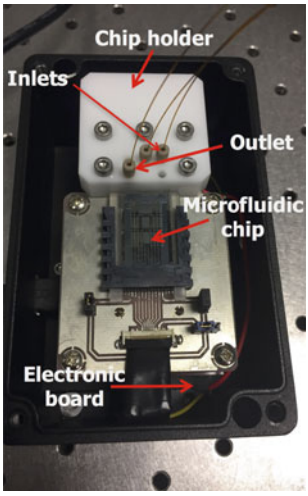

Fig. 3 Picture of the portable optoelectronic system: the metallic box includes the lab-on-chip, the chip holder for the inlet and outlet connections and the electronic boards for the photosensor read-out. A cover lid (not shown in the figure) with two small holes (for microfluidic access) shields the a-Si:H photodiodes from the external radiation

The lab-on-chip and the read-out electronics have been connected together through a custom made connector and enclosed in a metallic box ($10 \times 8 \times 15\,cm^3$) which ensures shielding from external radiation and electromagnetic interferences. The free-side of the lab-on-chip is also inserted in a Teflon-holder to ensure a sealed connection to the external tubes providing the reagents for the chemical treatments of the inner channels. A picture of the whole system is reported in Fig. 3.

4 Test of the System

The functionality of the entire lab-on-chip has been tested by using horseradish peroxidase (HRP) as model enzyme for detecting H_2O_2. Recognition of this molecule is of great interest because it plays an important role in atmospheric and biochemical processes [10]. The test has been performed by functionalizing, at first, the inner walls of the microfluidic channels. The functionalization procedure envisages the following steps:

- growth of PHEMA on the channel wall by atom transfer radical polymerization;
- flowing of succinic anhydride (SA) solution in order to obtain carboxylic functional moieties PHEMA-SA;
- flowing of a solution of n-hydroxysuccinimide (NHS) to achieve NHS esters functional groups (PHEMA-NHS);
- insertion of a solution of horseradish peroxide (HRP) and incubation over-night at 4 °C to form the PHEMA-HRP brush layer;
- rinsing with blocking buffer for 30 min at 1 μ L/min.

Once the functionalization was accomplished, the enzymatic reaction was conducted by mixing into the microfluidic chip, through two separate inlets, a solution of luminol 1 mM and 4-iodophenol 0.1 mM and a solution of H_2O_2 at different concentrations. The HRP, immobilized on the microfluidic channels, acts as a biosensors for the H_2O_2, catalyzing its reaction with luminol and yielding a chemiluminescent signal proportional to the concentration of hydrogen peroxide. The reaction was monitored by reading-out the sensor photocurrents connected to the low noise electronics. Results demonstrate a limit of detection (LoD) down to 18 μM and an excellent linearity of photosensor response up to 250 μM. These values are within the range of practical interest for this molecule and are comparable to the best performances reported in the literature for chemiluminescent-based optofluidic sensors [11]. We have also verified that, after washing with piranha (solution of sulfuric acid and hydrogen perodixe) and rinsing with deionized water, the same microfluidic chip can be re-used for further analysis.

5 Conclusions

This work has presented a miniaturized lab-on-chip system for monitoring chemiluminescent enzymatic reaction. It is the combination of an all-glass microfluidic chip and a-Si:H photosensors. The microfluidic chip is achieved by anodic bonding of two glass substrates. The a-Si:H diodes are deposited directly on one side of microfluidic chip for the on-chip detection of the chemiluminescent signals generated inside the channels. The whole fabrication process has been designed in order to satisfy the compatibility between microfluidics, electronics devices and surface chemical treatments.

The developed system has been tested in the detection of H_2O_2, achieving a LoD of 18 μM. This result demonstrates the successful integration of the different technologies for detection and quantification of molecule whose biochemical recognition exploits chemiluminescent signals.

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