Sensing and Bio-Sensing Research 6 (2015) 51–58

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Lab-on-chip system combining a microfluidic-ELISA with an array of amorphous silicon photosensors for the detection of celiac disease epitopes

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ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 6 November 2015
Accepted 9 November 2015

Keywords:
Lab-on-chip
Celiac disease
Microfluidics
On-chip detection
ELISA
Amorphous silicon photosensors

ABSTRACT

This work presents a lab-on-chip system, which combines a glass-polydimethilsiloxane microfluidic network and an array of amorphous silicon photosensors for the diagnosis and follow-up of Celiac disease. The microfluidic chip implements an on-chip enzyme-linked immunosorbent assay (ELISA), relying on a sandwich immunoassay between antibodies against gliadin peptides (GPs) and a secondary antibody marked with horseradish peroxidase (Ig-HRP). This enzyme catalyzes a chemiluminescent reaction, whose light intensity is detected by the amorphous silicon photosensors and transduced into an electrical signal that can be processed to recognize the presence of antibodies against GPs in the serum of people affected by Celiac syndrome.
The correct operation of the developed lab-on-chip has been demonstrated using rabbit serum in the microfluidic ELISA. In particular, optimizing the dilution factors of both sera and Ig-HRP samples in the flowing solutions, the specific and non-specific antibodies against GPs can be successfully distinguished, showing the suitability of the presented device to effectively screen celiac disease epitopes.

1. Introduction

Celiac disease (CD) is a chronic inflammatory disease of the small intestine that affects genetically susceptible individuals. This disease is unique in that the critical etiologic factor has been identified as the ingestion of gluten found in grains of wheat, barley, rye and triticale. Upon ingestions of those proteins, the symptoms showed by patients affected by CD can be severe, thus the diagnosis and treatment must be fast [1]. The diagnostic procedure is performed through serological tests and histological examination of at least one biopsy [23].

In the recent years, the area of serological testing for CD has developed greatly, since it is less invasive than biopsy [26]. Among all serological tests, recently, a lot of attention has been given to the analysis of antibodies against the deamidated gliadin peptides (GPs) due to their promising performance as compared to the most commonly used antigliadin antibodies (AGA), endomysial antibodies (EMA) and tissue transglutaminase antibodies (tTG). Recent studies have demonstrated that the detection of antibodies for GPs can be applied for the diagnosis of celiac disease and for monitoring the adherence of CD patients to gluten free diet (GFD). The determination of antibodies is generally based on enzyme linked immunosorbent assay (ELISA) based screening tests [28].

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The ELISA process includes a series of washing, mixing, and incubation steps, which are labor intensive and time consuming, which often takes several hours, sometimes even up to 2 days to perform one single assay. Most of the time required in a long immunoassay is mostly because of the long incubation time attributed to inefficient mass transport for the immunoagents to move from a solution to the surface, where the conjugation occurs because the immunoreaction itself is relatively rapid [17]. Moreover, the immunoagents used in immunoassays are relatively expensive. To improve the throughput of these processes, robotic systems can be used for fluid handling, but this solution is only available to wealthy laboratories and requires significant maintenance efforts and a large laboratory footprint. Therefore, there is a demand to develop an automated and miniaturized platform for immunoassay.

Miniaturized analytical equipment based on lab-on-chip device (LoC) [18], has been growing due the possible advantages which can be gained by the used of these devices compared to standard analytical equipment, [13] such as (i) consumption of low sample volume, (ii) rapidity of analysis, (iii) easy handling and (iv) multiple target analysis. These advantages can potentially improve the performance and reduce the operating cost of conventional immunoassays.

In particular, LoC have been extensively studied to develop point-of care (POC) devices in the field of health-care and diagnostic. In these devices, biological components are usually immobilized on a solid-

http://dx.doi.org/10.1016/j.sbsr.2015.11.003

state surface, which interacts with the analyte. The interactions are detected by using electrochemical or, more often, optical methods [14,21]. In this context, surface chemistry treatments of microchip play a critical role because they deeply influence the biomolecular recognition in terms of selectivity and sensitivity [13]. These chemical treatments may negatively affect the functionality of detection elements such as photodiodes, when these are integrated on the microsystem. As a consequence, microfluidic chips for POC are often based on off-chip detection (e.g. using CCD or CMOS sensors) with difficulty in producing low-cost and portable systems.

Recently, we reported the development of an ELISA-on-chip device for the detection of GPs [10]. Nevertheless, in this ELISA-on-chip device, the serum to be analyzed was handled manually, by spotting a few microliter of sample on the array. As a consequence, the analysis is time consuming and more exposed to inaccuracies of the personnel.

In order to improve this device, we combined a microfluidic network, chemically functionalized to anchor GPs (probe) for the biochemical recognition of GP antibodies, based on an ELISA, with an array of silicon amorphous photosensors (a-Si:H) for the detection of the biochemical reaction. This microfluidic ELISA relies on a sandwich immunoassay between antibodies against GPs (target) and a secondary antibody marked with horseradish peroxidase (HRP), which is used to obtain a chemiluminescence signal detected by the array of a-Si:H photosensors.

Among the optical detection technique, chemiluminescence, in which light is produced as a result of a chemical reaction, is particularly suited for its application in miniaturized assays, offering high specificity and detectability [25] combined with rapidity and simplicity, avoiding the need for additional optical systems as radiation sources and optical filters. On the other hand, the advances in microfabrication have enabled high density, chip-scale integration of optical components, such as photodetectors, and integrated waveguides [29] in lab-on-chip system. In particular, the integration of hydrogenated amorphous silicon (a-Si:H) [16] photodetectors within a lab-on-chip can lead to a very compact system. Indeed, the a-Si:H technology, thanks to its low-temperature processing (below 250 °C), enables the fabrication of high performance photosensors on glass and polymers, ideal supports for lab-on-chip device. The possibility to use transparent substrates reduces the distance between the photosensors and the site where the luminescence takes place [2,6,27] while the very low dark current in reverse bias condition coupled with a quite high photosensitivity in the ultraviolet [4], visible [19,24] and near infrared [3] determine an effective competitiveness of a-Si:H photosensors with respect to cooled CCD detectors [20] and prove their wide applicability in a variety of analytical formats.

The device presented in this paper overcomes the integration issues deriving from combining the on-chip sensor fabrication and the surface functionalization based on polymer brushes [7–9]. In addition, the proposed device permits the simultaneous detection of multiple GPs antibodies. The development of a LoC based on a microfluidic ELISA, with integrated optical sensing for the detection of antibodies against GPs was never reported before. This LoC system is engineered to reuse the optical sensing part of the chip for multiple patients, while the chemically functionalized microfluidic network can be easily removed after the analysis of one serum sample. This LoC is valuable to (i) verify adherence of CD patients to the GFD, (ii) permit the screening of multiple antibodies in one analysis, (iii) perform the analysis using a little amount of serum, thus resulting less invasive for the patient, (iv) and more importantly represents a novel instrument for the diagnosis of CD.

2. Experimental

2.1. Materials

All reagents were purchased by Aldrich Chemicals. 2-Hydroxyethyl methacrylate (HEMA) was distilled prior to use, whereas the other chemicals were used without further purification. 2-Bromo-2-methyl-propionic acid 3-trichlorosylanyl-propyl ester was synthesized following a reported procedure [15]. Methanol (analytical reagent grade) was used without further purification, while toluene was distilled over sodium.

Synthetic gliadine peptides PQPQLPYPQ (VEA) or FPGQQQPFPPQQP (31–43), anti-VEA and anti-DEC rabbit antisera and secondary antibody anti-rabbit marked with HRP were provided by Primm (Milan, Italy).

Chemiluminescent substrate SuperSignal® ELISA Pico was purchased by Thermo Scientific.

All the gases utilized for the deposition of the amorphous silicon layers (silane, diborane, methane and phosphine) and the dry etching processes were provided by Rivoira.

2.2. Equipments

Water was purified with a Milli-Q pulse (MILLIPORE, R = 18.2 MΩ·cm) ultra-pure water system.

The a-Si:H layers were deposited by a Glasstech Solar Inc. (GSI) Plasma Enhanced Chemical Vapor Deposition system working at 13.56 MHz constituted by three ultra high-vacuum (UHV) chambers sharing a load-lock chamber for the sample transportation. Each UHV chamber uses a turbomolecular pump coupled with a mechanical pump for the achievement of an ultra-high pre-deposition vacuum and a roots pump coupled with a backing pump during the deposition process.

All the microelectronic processes for the fabrication of the a-Si:H photosensor array have been performed in a clean room, which includes a vacuum evaporation system (Balzers 510) and a sputtering system (Materials Research Corporation, Orangeburg, New York, USA) for the deposition of the metal layers and the Indium Tin Oxide (ITO) film acting as contact electrodes. The photolithographic processes were performed using TAMARACK 152R mask-aligner for mask reproduction, a Reactive Ion Etching system (from IONVAC PROCESS, Italy) for dry etching of the amorphous silicon and ITO films and a chemical bench for wet etching of the metal films.

The current–voltage (I–V) curves of the photosensors were acquired by means of a Keithley 236 Source Measure Unit, utilized in the Voltage-Source/Current-Measure mode.

The sensor quantum efficiency was measured by using a dual arm optical set-up, which includes a tungsten lamp acting as light source, a monochromator (model Spex 340E from Jobin-Yvon) able to transmit a narrow band of wavelengths chosen from the wide range of wavelengths available at its input, a beam-splitter located at the monochromator output to minimize the effect of a lamp instability, a UV-enhanced crystalline silicon diode (model DR 2550-2BNC from Hamamatsu) used as reference, lenses (from Melles-Griot) acting as focusing optics and two Keithley 236 Source Measure Units, which acquire the currents of the reference diode and the a-Si:H sensor.

A custom low-noise readout electronic board [22] measures the photosensor currents that are induced by the chemiluminescent reactions occurring inside the microfluidic channels. The circuit operation relies on a charge sensitive amplifier (the DDC118 Current-Input Analog-to-Digital Converter from Texas Instruments), which integrates the currents flowing through the a-Si:H diodes and converts it to a 20-bit digital signal. The chip has eight identical input channels to simultaneously monitor eight sensors of the photosensor array. The DDC118 output signal is acquired by a PIC18F4550 microcontroller from Microchip that also provides the communication interfaces, both USB and UART, to a personal computer for the subsequent signal elaboration.

2.3. Methods

2.3.1. Synthesis of the PHEMA brush layer and its functionalization

Glass and oxidized silicon substrates were immersed in a Piranha solution (H_2SO_4: H_2O_2 3:1) for 20 min, copiously rinsed with Milli-Q water and then dried with a stream of nitrogen. Subsequently, the substrates were incubated in a solution of 0.2% of 2-bromo-2-methyl-