Microelectronic Engineering 129 (2014) 70–76

Contents lists available at ScienceDirect

Microelectronic Engineering

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

Impedimetric detection of whole blood concentration for early detection of intraocular hemorrhage

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ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 9 July 2014
Accepted 24 July 2014
Available online 1 August 2014

Keywords:
Impedimetric measurement
Biochip
Intraocular hemorrhage
Whole blood

ABSTRACT

Early detection of retinopathy can substantially reduce the risk of visual impairment and blindness in diabetes mellitus (DM) patients. Screening tool such as Amsler grid may be useful for the early detection of maculopathy in DM patients, but the metamorphopsia by Amsler gird self-screening is subjectively, not objectively. Although some kinds of optical imaging instruments have been used for the early detection of retinopathy, self-testing regularly is still not possible. In this work, impedimetric detection of whole blood concentration is proposed to be a promising technique to detect intraocular hemorrhage. An intraocular implant chip is also discussed to integrate the impedimetric detection into a CMOS IC. Results revealed that the impedimetric measurement could indicate 5% (v/v) whole blood in intraocular irrigating solution. Such technique is a label-free, non-invasive, and real-time analytical method. The preliminary results showed that impedimetric measurement had high potential to be implemented to an intraocular implant chip.

1. Introduction

Proliferative diabetic retinopathy (PDR) is a retinal vascular disease, which is the abnormal proliferation of abnormal retinal tissue. PDR has the tendency of intraocular hemorrhage if fragile retinal neovascularized vessels rupture. The hemorrhage brings the retinopathy into a vicious cycle and retinal detachment develops eventually. It has been estimated that after 30 years, patients with PDR will increase up to 20% of persons with diabetes who have been treated with intensive glucose control. Nevertheless, well-controlled glucose is very difficult to achieve. The current survey from different countries portends a huge population of persons at high risk for diabetes-induced visual impairment.

Intraocular hemorrhage is the hallmark of PDR [1,2]. The blood clot blocks the light; therefore, vision decreases prominently. The clearance rate of intraocular hemorrhage was reported to become only 1% per day [3,4]. Consequently, vision recovery is slow if new vitreous hemorrhage happens. And the vision recovery is the slowest in PDR among all etiologies of intraocular bleeding [1,5]. Ziemianski et al. followed PDR patients for 3–10 years and reported 30% of intraocular bleeding PDR patients had final vision improved, 28% keeping stable, and 42% becoming worse. They reported 71% of vitreous hemorrhage patients had the final vision less than 5/200, and 50% of patients had the poorest vision because of unresolved blood clot [6]. However, the decomposing product of hemoglobin has toxicity for the retinal tissue. Saxena et al. reported only 0.6 mL or 45 mg of intraocular hemoglobin have the toxicity detectable for the neuroretina [7]. Doly et al. used Fe isotope injected into the rabbit eyes, and found the siderosis was in the inner nuclear layer [3,8]. Other reports found the morphologic degenerative change of Muller cells and retinal pigment epithelial cells in siderosis bulbi human eyes [8], including hyperpermeability of cell membrane [8,9], lysosomal fragility [9,10], and mitochondrial dysfunction [10]. Therefore, early detection of intraocular hemorrhage and timely treatment is the management of better visual prognosis in diabetic patients. If the patients can self-monitor the intraocular hemorrhage, they may have less

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http://dx.doi.org/10.1016/j.mee.2014.07.020
0167-9317/

chance of visual decrease and better chance of visual recovery. However, there is no commercial intraocular implant chip for the monitoring of intraocular hemorrhage.

In the past decades, because of the mature development of the microfabrication technology, microfluidic systems have been extensively reported to be implemented on various biomedical diagnostic applications [11,12]. They are envisioned as miniaturized and portable devices that can perform analyses based on different diagnostic protocols. Electrical impedance measurement is one of the analytical techniques and has been applied for the detection of cellular response [13–15], immunoassays [16,17], and DNA assays [18,19]. It is widely adopted because this technique is label-free, non-invasive, and real-time and the detection results are represented by electronic signals that can easily interface with miniaturized devices. A pioneer work of using impedance measurement to perform real-time monitoring of cellular responses was reported [20]. Interdigitated electrode was utilized and long-term cellular behavior was clearly shown by the impedance change across the electrode. The principle is when cells adhere and proliferate on the electrode surface, the effective surface area is reduced and total impedance across the electrode is hence increased. On the other hand, cell death leads to the release of cells from the electrode surface. That indicates the total impedance is decreased. Therefore, the impedance change can estimate real-time cellular behavior during culture. Based on this technique, cell proliferation and viability were respectively demonstrated to be monitored under different tested culture conditions [21–24]. In addition, electrical impedance measurement technique has been suggested to be the best method for the determination of blood coagulation among five methods including visible clotting under rocking, thromboelastograph, and mechanical measurements [25]. Real-time impedimetric monitoring of blood coagulation process was also demonstrated under temperature and hematocrit variations [26]. Analysis of the impedance change of the whole blood was reported to estimate the characteristics of blood coagulation process and the starting time of blood coagulation. To the best of our knowledge, there is no study to investigate the impedimetric detection of the whole blood concentration on a biochip.

Because of the importance of the detection of intraocular hemorrhage, impedimetric detection of whole blood concentration is proposed to be a possible analytical solution for the minimalized devices. In this study, a biochip has been developed for the preliminary study of the intraocular implant chip. The biochip is composed of a glass substrate with measuring electrodes and a polydimethylsiloxane (PDMS) layer for providing a reservoir containing samples. The electrodes are gold material and are fabricated by microfabrication technology. Whole blood in a certain dilution is then loaded to the reservoir and contacted with the electrodes. The presence of blood cells on the electrode surface causes the impedance change across the electrodes. Hence, the presence of blood can be estimated by the electrical signal in this fast and easy technique. Based on this method, a proposal of an implantable miniaturized chip is also discussed and suggested to have potential to be developed to a commercial product. An illustration shown in Fig. 1 is provided to show the concept of the implant miniaturized chip for self-testing of intraocular hemorrhage by the patients. The proposed implant is $1.2 \times 0.635\ mm^2$ and consists of four building blocks including electrode array, CMOS electrical impedance sensing (EIS) integrated circuit (IC), battery, and radio-frequency (RF) antenna. They are heterogeneously integrated by using a substrate-to-substrate connector such as the through-silicon via used in the three dimensional stacked integrated circuits (3DIC). The anatomical location has been set at the bottom of the eye such that the ability of the detection of intraocular hemorrhage can be maximized. The proposed system is coated by a biocompatible moisture and dielectric barrier

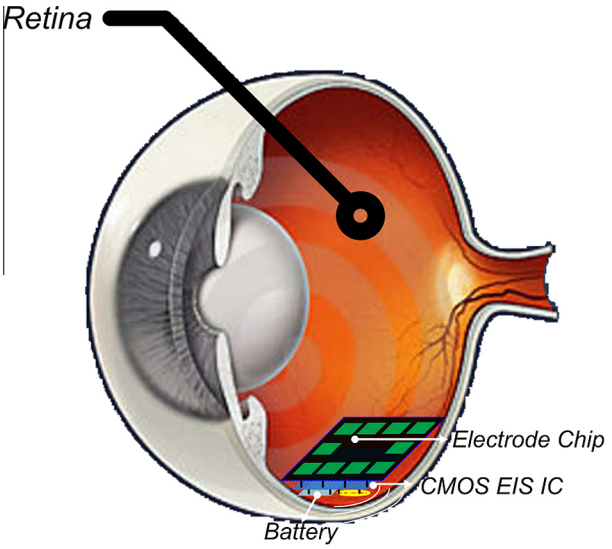

Fig. 1. Conceptual drawing of the proposal intraocular hemorrhage detection system.

such as Parylene C after being fabricated and experimentally characterized. The antenna can be used as both data and power links such as the inductive coupling technique. With the help of the antenna, data can be transmitted outside the eye and the battery can be charged wirelessly, which removes the transcutaneous external supply of sample.

2. Preliminary study of the intraocular implant chip using the biochip

2.1. Design and fabrication of the biochip

Impedimetric detection technique is a label-free, non-invasive, and real-time analytical method. Typically, a pair of electrodes as an electrical transducer is utilized to measure the impedance change caused by the existence of the biological substances. In this study, a biochip was fabricated for the study of impedimetric detection of whole blood concentration. The design of the biochip is shown in Fig. 2. It consists of a glass substrate with 8 pairs of interdigitated electrodes and a PDMS layer providing a sample reservoir. There were 11 fingers with 30 μm gap between in each pair of electrodes. The fabrication process of the electrodes is briefly described. After thoroughly cleaning of the glass substrate, a layer of Cr/Au (300 Å/2000 Å) was deposited by thermal evaporator. Hence, photolithography and wet etching were respectively performed to fabricate the interdigitated electrodes on the glass substrate. On the other hand, the PDMS layer was prepared by mixing PDMS pre-polymer and curing agent (Sylgard® 184, Dow Corning, USA) at 10:1 ratio. After solidification, a hole of 7 mm in diameter was punched on the PDMS layer. By oxygen plasma treatment, it was bonded to the glass substrate with proper alignment to fabricate the biochip.

Blood is a bodily fluid in animals and is composed of blood cells suspended in blood plasma. Plasma is mostly water (92% by volume) and contains small biomolecules, such as proteins, glucose, mineral ions, hormones, and carbon dioxide, which are difficult to be detected by label-free methods. The blood cells are mainly red blood cells (6–8 μm), white blood cells (10–12 μm), and platelets. When the whole blood is loaded to the sample reservoir of the biochip, blood cells are deposited on the electrode surface. Because cell membranes are very poor conductors at low frequency (below