Fabrication of Interdigitated Microelectrodes in Microfluidic Channel

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***Abstract*—Biosensors have become widespread in various domains in biomedical and life science applications. The bioreceptor as the second main component along with transducer to meet the architecture of the biosensors. The bioreceptor's configuration should follow up the development of biosensors to catch up the research needs in simple, fast, and low cost techniques. Micro/Nanotechnology provides high transducers performance, where the development of bioreceptor has other tasks to be considered. The major tasks that should be taking into account to achieve the bioreceptor development are but not limited to selectivity, sensitivity, design and packaging. Interdigitated Microelectrode Array (IDMA) that lies on the surface of microfluidic channel (MFC) plays an important role in biosensors development. An easy, cheap, rapid process to fabricate IDMA using soft-photolithography technique will be unfolded. The beneficial of the new bioreceptor is applicable in many biomedical and biological applications such as biomarker detection, cancer clinical testing, detection of infectious microorganisms and viruses. Moreover, it is strongly contributing the development of biosensors and work as Aptasensor. The electrochemical impedance spectroscopy (EIS) is the approach that will imply to detect the variation on the surface of MFC as a sensing surface layer.**

Keywords-Biosensors, Bioreceptor, Aptasensor, MLoC, IDMA, DNA, EIS.

I. INTRODUCTION

The biosensors with traditional electrodes, which are made of three electrodes, are incapable to sense the tiny biomolecular in the areas of biomarker detection, cancer clinical testing, detection of infectious microorganisms and DNA. Therefore; the IDMA takes over this part of biosensors and imposed itself strongly on the areas for its capability in sensing the variation credit to microtechnology design that produces a new electrode configurations by adding one more electrode, which ultimately known as microelectrodes and ultraelectrodes arrays. The four electrodes configuration; which is implying reference electrode (RE), counter electrode (CE), the working electrode collector (WEC) and working electrode generator (WEG), made a new era of biosensors and consequently can be readily used as Aptasensors. DNA microarrays are a major Aptasensors application, which are known as a surface on which sequences from thousands of different genes are covalently attached to fixed probes. By using DNA microarrays technology, it allows a parallel analysis for the selective nature of DNA-DNA or DNA-RNA hybridization; simultaneously. DNA microarrays require optically active labels attached to the target DNA and fluorescence sensing. Some pioneering Aptasensors are one type of DNA microarrays technology, which employs electrochemical labels; redox-active molecules or enzymes, which produces an electrical current through conductive electrodes in case of complementary sequences. DNA microarrays allow highly parallel and low cost analysis. In fact, they take advantage of the capability to fabricate a large number of miniaturized detection sites on a substrate to extract information after exposition to the target DNA to be detected or recognized. The standard approach to DNA detection is based on optic or optic-like methods, which make use of a passive substrate for DNA immobilization and indirect detection of labels bound to the target DNA molecule under investigation. Each site is specifically bio-functionalized by means of DNA probe molecules of known sequence immobilized on its surface. Target molecules in the sample solution bind only to probes with complementary sequences; hybridization, thus, their presence at specific sites make known their composition [1]. A known single strand DNA (probe ssDNA) sequence is immobilized on a substrate; silicon, the unknown target (target ssDNA) sequence is labeled with a fluorescent or radio label and injected on the substrate. If the two sequences are complementary, they hybridize and form the double strand DNA (dsDNA) so the label is immobilized on the substrate as well. The passive substrate is then checked to verify the presence of the label rather than of the DNA molecule [1]-[4]. In terms of the transduction techniques used, the three main classes of biosensors are optical, electrochemical and piezoelectric. Out of the three; optical methods appear to be the most sensitive, with surface plasmon resonance and waveguide based devices being the technological spearhead. As for Electrochemical biosensors, they are cheaper than optical ones. They can be amperometric or impedimetric, depending on whether they monitor a current as a function of potential or the resulting sensor impedance as a function of frequency. The advantage of impedimetric methods is that, unlike amperometry, they do not need of enzymatic labels in order to detect. To overcome this problem, sample pre-treatment steps and signal amplification strategies are usually required [5].

II. LITERATURE SURVEY AND LIMITATIONS OF CURRENT METHODS

Label-free techniques are thoroughly investigated, in view of the fact that they avoid expensive reagents and pre-treatment steps. Recently, several approaches have

been proposed, based on piezoelectric materials [6], microfabricated cantilevers [7][8]. Other techniques detect the variations of electrical properties of electrode–solution interfaces induced by DNA recognition [9]-[11]. Within this category, a merit can be made between devices where sites make use of passive components or semiconductor sensors.

Aptamers are small single stranded synthetic oligonucleotides, which provides a unique 3D conformational structure, therefore; it can be acquainted and bound with high specificity and selectively to almost any kind of target, which makes them a powerful molecular recognition element that can be used widely in different applications, such as biosensors; aptasensors [12][13][14].

III. IDMA Fabrication process

So far, the simplicity and thus the low-priced in fabricating any sensing surface are always a figure of merit in MEMS fields, therefore, IDMA has been produced using soft-photolithography technique, where high cleanroom classification is not required as CMOS technology.

With the intention of decreasing the costs of the bioreceptor with a new microelectrode configurations a low-priced photolithographic masks are sought. A low-cost mask fabrication process involves the use of transparency masks. Whereas these masks suffer from less accurate features, this hitch can straightforwardly come over it using the Step-and-repeat cameras, which is known as steppers. The reduction feature can be accomplished using projection lithography tools, which lies into two major classes; scanning and step-and-repeat cameras systems. In scanning technique, reflective optics is employed and using mirrors rather than lenses to expose pack of light through mask aperture onto the wafer while the mask and wafer are moved concurrently. In scanning process, the scanned speed of the wafer, the aperture width, and the intensity of the light are the parameters that responsible on defining the dosage of the exposure light, which leads to a good pattern on the wafer similar to the mask. In steppers technique, where the refractive optics is employed; the pattern on the wafer performs by transforming each image field, which is one rectangular, one-step at a time that causes significantly feature reduction with high-resolution imaging. Stripper are good techniques to attain minimum feature sizes reached up to 250-nm levels but it did not work below this level as the technology keep growing up and Nanotechnology features and Nanopores have become indispensable and inevitable demand. Consequently, other photolithography techniques are proposed mainly the hybrid step-and-scan approach, which was introduced by SVG Lithography. This technology can attain a fraction of what stepper reached [15].

The IDMA as a sensing surface layer through the binding process in any biosensor architecture passes through fabrication process that implies specific procedures using soft-photolithography techniques, such process is capable to bring IDMA to light in easy and inexpensive technique, which is readily as follows:

i. The sensing surface layer (SSL) is started by designing the mask using CleWin 4.0 that is a layout editor designed Windows-based, spontaneous, comprehensible editor for photolithographic mask layouts with 1 nm resolution.

ii. The substrate of SSL is made of glass with a dimension 1.21 mm × 1.0 mm.

iii. The material for WEC and WEG is gold and platinum for CE and silver and HCl for RE, and aluminum for external contact.

A. Photolithography protocol and process

After finishing the mask designing and having four masks as shown below in the figure, and then after the photolithography process starts in the yellow room and the SSL passed through cleaning step to remove any contamination and residual attached to it, Figure 1(a). The process involves one protocol repeated for each mask. Figure down below illustrates all of the steps.

i. Starting with spinner and coating the photoresist, soft-baking, aligning, developing the UV-exposed photoresist, sputtering gold for WEC and WEG, remove the entire photoresist, Figure 1(b).

ii. Starting over for the next layer for reference electrode (RE) where the silver; Ag, is sputtering and then dunk it in HCl to have AgCl for the RE, and then clean up the SSL for next step, Figure 1(c).

iii. Making the counter electrode (CE) and the only difference is the sputtering step where platinum (Pt) is used instead, Figure 1(d).

iv. The last step, which is making Ohmic contact by sputtering Aluminum (Al), Figure 1(e).

The IDMA system lies in 1.21 mm × 1.0 mm, and the length of each finger is 162.6 μm, the width is 5 μm and the space 20 μm, as shown in Figure 2. The entire SSL with the entire feature is shown in Figure 3.

IV. Electrochemical oligonucleotide Method

Electrochemical oligonucleotide; Aptamers (DNA or RNA), detection presents several advantages over other methods, which is suitable to be integrated on the multi-labs-on-a-single-chip (MLoC) system [16]. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) are the efficient methods of probing the interface properties of the modified surface of interdigitated microelectrodes [17]. Therefore, the fabrication of the aptasensor; biosensor, was characterized by EIS and CV . The detection is based on the change of the current response before and after the aptamer-target recognition reaction.

V. Aptasenosr components and functions

Electrochemical oligonucleotide is an aptasensor that can be treated as electrochemical impedance spectroscopy (EIS). While EIS technique measures the impedance of a system over a range of frequencies, and therefore, the frequency response of the system, including the energy

storage and dissipation properties, it is given away and then represented using bode or Nyquist plot. As it is well known in a complex system, the impedance is the antagonism to the flow of alternating current (AC). Consequently, this technique has employed for aptasensor; i.e. aptamers DNA or RNA.

The reaction mechanism of EIS that dominates at certain frequencies along with its response identify the rate-limiting step. The size and cost of potentiostat are counted to be the major limiting factors in mass production and field use of electroanalytical instruments, therefore; CMOS potentiostat was designed and proposed not for its performance alone, which is comparable to bench-top instruments, but also for its tiny size, power consumption and low cost.

A. Signal Processing Unit

The signal-processing system, as shown in Figure 4, is a key component of the IC CMOS potentiostat. The current flows through working electrode is converted to frequency using a signal processing unit, which is Analog Frequency convertor unit (AFC), the frequency of which is proportional to the concentration of the Aptamers; i.e. DNA or RNA.

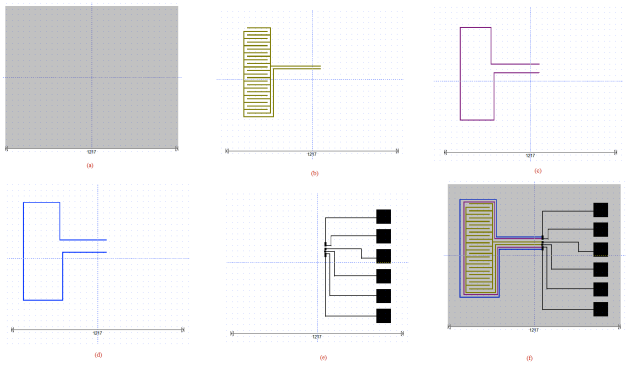

Figure 1. The soft-photolithography steps: (a) Substrate (b) WEC & WEG (c) Reference electrode (RE) (d) Counter Electrode (CE) (e) Ohmic Contact, IDMA system.

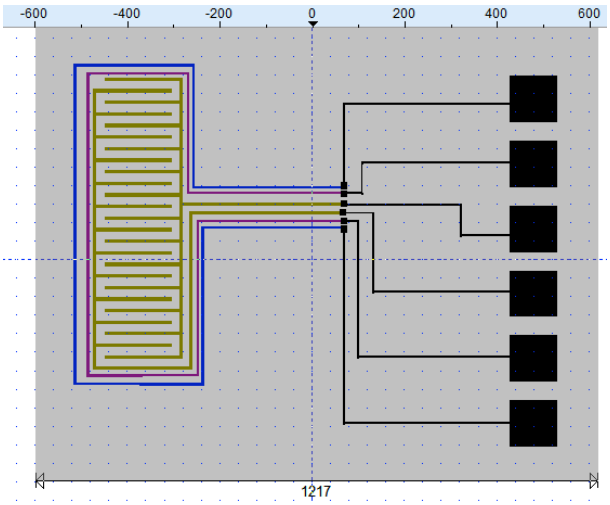

Figure 2. The entire system of SSL.

In this paper, AFC has been built in a standard CMOSP35 process with no post-processing requirements.

B. Potentiostat

The main features of the potentiostat that should be considered are:

i. The size of the device
ii. The cost of potentiostat,
iii. The power consumption.

Figure illustrates the schematic of entire Aptasensor which is made of integrated CMOS potentiostat, electrochemical cell and the signal-processing system, where the potentiostat is setting the voltage between the working electrode and stable reference electrode. The other components, such as the control amplifier, exist to perform each its own task and let the aptasensor work properly, as it has been unfolded somewhere in a previous work [18].

The integrated potentiostat was composed of a potential controller and a current–frequency converter. The potential controller consisted of two operational amplifiers (TIA) and two load transistors.

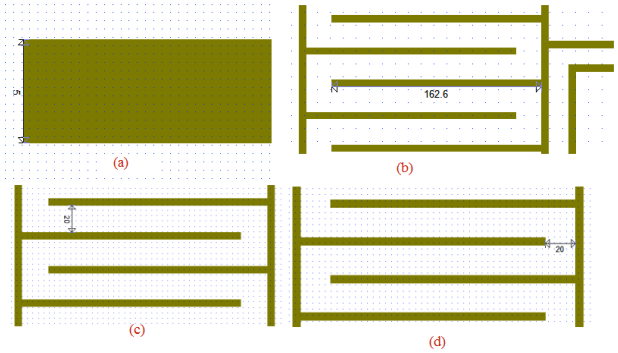

Figure 3. The dimensions of the finger and spaces in between them (a) The finger width (b) length (c) space between each finger (d) the space between the tip of the finger and the other electrode.

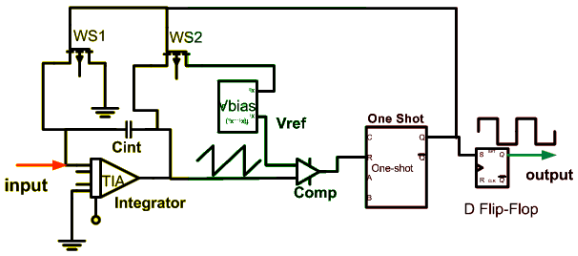

Figure 4. Signal processing unit.

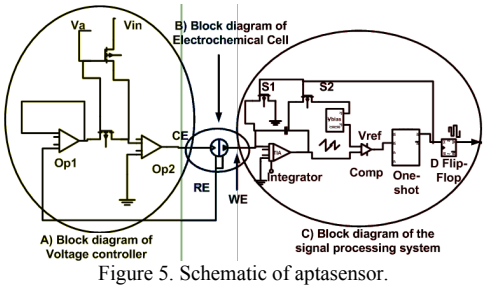

Figure 5. Schematic of aptasensor.

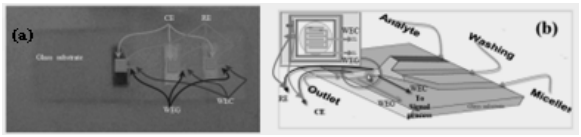

Figure 6. Electrochemical Cell with four electrodes.

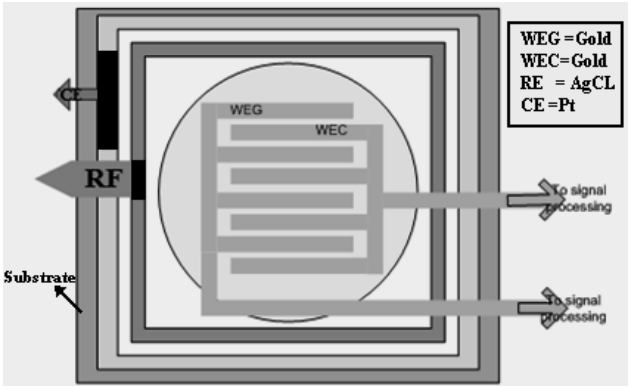

Figure 7. The schematic of MFC with four electrodes.

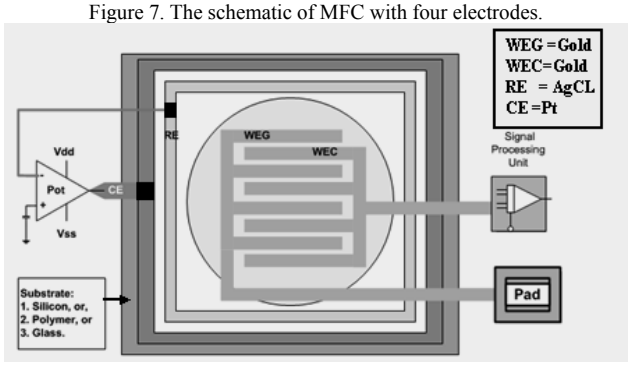

Figure 8. Aptasensor with IDMA.

The main function of CMOS potentiostat is to control the potential difference at the interface between the solution droplet and working electrodes; i.e. WEC, WEG. When an electrochemical reaction occurs, the input voltage (V_{in}) is read and the voltage at the counter electrode (CE) is adjusted, so that the potential difference between the voltages at the reference electrode (RE) and working electrodes is equal to V_{in}. On the other hand, the current–frequency converter is composed of two operational amplifiers, one-shot, D flip-flop and a load transistor. The purpose of this unit is to convert the electric current through the working electrodes into a digital signal. The open-loop gain and cutoff frequency of the operational amplifiers were more than 86 dB and roughly 500 kHz, respectively, by setting the control voltage (Vctrl); as shown in Figure 5.

Principally; the electrochemical cell is made of three electrodes; counter, reference, and working electrodes, fabricated using soft-photolithography technology as shown in

Figure 6 shows a cell called Microfluidic Channel (MFC) and it is developed to have four electrodes instead of three, to handle aptamers DNA or RNA detecting.

Figure 7 illustrates the MFC that is made of interdigitated microelectrodes (IDMA) using Au as a good material for immobilization, and the working electrode collector (WEC) and working electrode generator (WEG). Also, where the reference will make of Ag/AgCl and Pt for counter electrode is fabricated either on a glass or a polymer material substrate.

This paper will develop and boost the biosensors' performance by using fully IC potentiostat working off the chip with interdigitated microelectrodes as microfluidic cell (MFC). Aptasensor incorporated with IDMA is implemented for detecting DNA sequence, using four electrodes, reference, counter, WEC collector, and WEG generating electrodes, along with a readout circuitry that amplifies the output signal and applies it for current-to-frequency convertor, which includes integrator, comparator, one-shot and D-flip-flop.

VI. RESULTS AND DISCUSSIONS

Figure 8 illustrates the setup for detecting DNA sequence. This integrated CMOS electrochemical aptasensor is capable of performing impedance spectroscopy, potentiometry, voltammetry, and ion-sensitive detection. In this work, we explore the limits of form-factor achievement with electrochemical detection by constructing an integrated sensor directly on an electronically active CMOS substrate. The active CMOS biosensor described here includes off-chip WEs and full potentiostat electronics, including two Op-amplifier for Electrochemical Cell (ECC), current-input analog-to-Frequency converters (AFCs), and data-processing circuitry; a lot of work in this area have been reported [19][20]. CMOS integrated potentiostat has been fabricated and conduction-based DNA sensor arrays have been proposed.

Electrochemical sensing of biomolecules eliminates the need for the bulky and expensive optical instrumentation required in traditional fluorescence-based sensing assays.

VII. CONCLUSION

Aptasensor based on impedimetric techniques has been designed and implemented for detecting DNA sequence away from labeling and optical expenses. This device will achieve the target, which is the speed of detecting and cost. Aptasensor is one out of four biosensors on a chip. The varieties techniques on MLoC system allows the research to switch to any other technique rapidly and easily.

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