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Effect of Microchannel Dimensions in Electrochemical Impedance Spectroscopy Using Gold Microelectrode

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Abstract

Microfluidic chip systems have been an area of interest for lab-on-a-chip and organ-on-a-chip studies in recent years. These chips have many advantages such as high efficiency, low sample consumption, fast analysis, durability and low cost. Today, electrochemical sensors are frequently applied in microfluidic chips because of their potential for label-free detection and low-cost production. A commonly employed electrochemical technique is electrochemical impedance spectroscopy (EIS), which captures changes in phase and amplitude as signal passes through the system under test. In the utilization of microelectrodes within microfluidic channels, noise becomes a problem in EIS measurements. In this study, EIS measurements were performed using microfluidic chips with various dimensions of width while the properties and dimensions of the microelectrodes were kept constant. It was found that the results of cyclic voltammetry (CV) cleaning and EIS experiments deteriorated when smaller than 1 mm wide-microchannels were integrated onto 100 µm wide microelectrodes. These finding sets the basics for on-chip electrochemistry experiments using microfluidic integrated microelectrodes and therefore is fundamentally important in future on-chip EIS measurements.

Keywords: Electrochemical sensing, microfluidic chip, lab-on-a-chip, organ-on-a-chip

1. INTRODUCTION

Electrochemical approaches using microelectrodes are useful for the development of rapid, label free and simple biological assays due to their enhanced electroanalytical capabilities, particularly for the sensitive measurement of the target analytes and the potential for the production of arrays for multi-analyte detection [1-5]. The combination of microfluidic approaches with electrochemical methods permits the development of a convenient and in-flow label-free lab-on-a-chip and organ-on-a-chip technologies for rapid detection methods [6, 7].

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One way to do so is to integrate microelectrodes with microfluidic channels.

Microelectrodes have one critical dimension on the scale of microns [8] and are produced in a number of geometries including discs, squares, bands and rings. They have the electroanalytical advantages of reduced iR drop, improved signal to noise, the rapid evolution of steady state diffusion profiles and a relative insensitivity to convection [9]. These properties make microelectrodes an excellent technology for healthcare applications where high sensitivity is required to measure low concentrations [10, 11]. The traditional method for producing microelectrodes is to encapsulate a metal wire in glass [12]. However, whilst effective for sensor production, analytical measurements are subject to significant error due to the variable electrode areas produced [13]. Microfabrication based on photolithographic processes has been demonstrated as a viable route to the production of consistent microelectrodes for analytical applications [12]. The ability to photo-lithographically pattern arrays of individually addressable microelectrodes allows the detection of multiple biomarkers on a single chip [14, 15].

In recent years, microfluidic systems have been developed for various sensor applications. This technology possesses many advantages such as system miniaturization, the ability to handle and process, reduced reagent volumes, vastly reduced instrumental footprint, facile parallelization through multiplexing, improved analytical performance, enhanced sensitivity, and the high analytical throughput [16]. The combination of microfluidic system with microelectrodes is not new however, to date, the performance of sensitive EIS measurements have not been outlined in detail.

In this study, we integrated a microelectrode sensor within a microfluidic chip in order to investigate effect of channels in EIS measurements. Four different microchannel with widths of 150, 300, 500 and 1000 μm were used in the design of the chips. The two-electrode system was applied to design of the microelectrodes. Reference/counter electrode and working electrode widths were 2 mm and 100 μm, respectively. The experiments were performed by using CV cleaning and EIS analysis to evaluate the electrochemical response for particular microchannel sizes. We believe that the results obtained in this study provides fundamental knowledge for the study of EIS measurements on-chip and this would then help construct easier integrated devices and speed up the efforts towards research on the development of in-flow label-free electrochemical on-chip sensors.

2. MATERIALS AND METHODS

2.1. Microfluidic Channel Design and Production

Four different widths of microchannel dimensions were designed (150, 300, 500, and 1000 μm). The length and depth of microchannels were kept constant and were 10 mm and 50 μm, respectively. Designs were drawn and converted into pdf format with AutoCAD and CorelDRAW software programs, and then sent to Çözüm Tanıtım (Turkey) for acetate mask production. Molds were prepared by using classical lithography methods in a clean room with acetate masks. In the mold preparation, firstly, the surface of a 4 inch silicon wafer (Prime CZ-Si, Nanografi, Turkey) was first washed with isopropanol, acetone and distilled water, then dried with N2 gas. Next the wafer was heated at 100 °C for 5 minutes to remove moisture on the surface. SU8 2050 (Nippon Kayaku, MicroChem, Japan) photoresist was spin-coated onto the cleaned silicon wafer and then was exposed to UV. The soft bake step was completed firstly for 1 minute at 65 °C, then 7 minutes at 95 °C. In the developing step, the developer removed uncross-linked SU-8 photoresist and the design appeared on the wafer. The hard bake process was carried out by placing silicon wafer on the heater for 5 minutes at 150 °C, producing the Si template. Following that, microchannels were fabricated by using soft lithography method by casting Polydimethylsiloxane (PDMS-Sylgard 184, Dow Corning, USA), which is silicone elastomer:crosslinker ratio of 9:1 [17]. Inlet and outlet of microchannels were opened with a 1 mm biopsy punch (Selles Medical, UK).

2.2. Microelectrode design and production

Gold (Au) electrodes were fabricated on glass substrate via thermal evaporation. The microelectrode design consists of two electrodes, namely a 2 mm-wide counter (CE) / reference electrode (RE) and a 100 µm working electrode (WE), as shown in Figure 1. The length of the electrodes was 12 mm. Electrical connections were made via the contact pads attached to the electrodes and the dimensions of the electrodes were 10*10 mm. Alignment markers (with microchannel shape) were also designed around the microelectrode to ease the integration. Before deposition, electrode patterns were obtained using classical optical lithography method on glass by using the positive photoresist Az-9260, according to a protocol described elsewhere [18]. Firstly, the substrates were then rinsed with isopropyl alcohol, DI- water, and blow-dried with nitrogen gas. After cleaning, the substrates were coated with 2.5 µl positive photoresist AZ- 9620 using a single substrate spin processor. Before exposure, the photoresist coating was soft baked for 2 min 45 s at 110°C. The photoresist-coated substrates were placed under the pattern mask with scotch tape and exposed to an in-house developed UV light source for 70 s. After exposure, the photoresist on the substrates was developed in AZ 400K Developer for 2 min 30 s and rinsed with DI water.

To improve Au adhesion to glass, a 100 nm thickness chromium (Cr) layer was initially deposited on substrate; next, a 300 nm thick Au layer was coated to the Cr layer. Lift-off was performed using acetone to remove photoresists from substrate and hereby microelectrodes were produced.

2.3. Assembly of the Chip

Figure 1 demonstrates a schematic view of the microchannels and microelectrodes to be integrated. For the assembly, the electrodes and channels were first washed with acetone, then dried with nitrogen and finally surfaces were treated using a custom-made plasma device. Straight after the plasma treatment, the exposed surfaces were brought together for bonding and left on a heater for 2 h at 80 °C. Precise bonding process of two platforms was performed with the help of the alignment marker. Electrically conducting adhesive cold soldering silver epoxy (M.G. Chemicals, Ontario, Canada) was applied on the electrode terminals in order to create electrical connections between clips and electrodes. The electrodes were then kept on a hotplate set to 60 ° C for 24 h to cure the epoxy.

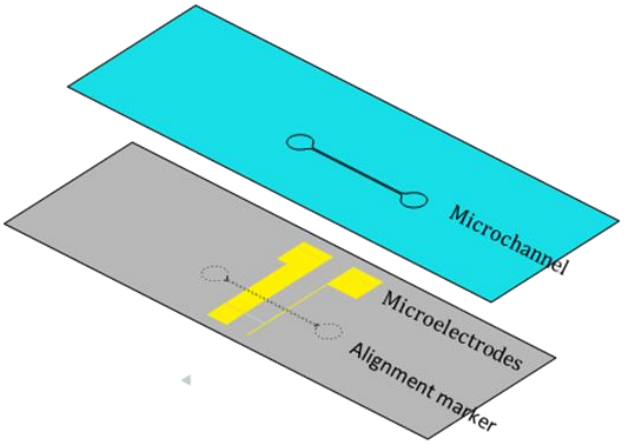

Figure 1 A schematic view of the microchannel and microelectrodes

2.4. Electrode Pretreatment and Electrochemical Measurement

0.1 M H2SO4 was injected into the microchannels for cleaning the surface of the microelectrodes by a micropipette. The inlet and outlet of chips were closed by using stopper after filling microchannel and applied CV (100 cycle) by a potentiostat device (PalmSens PS4, Houten, the Netherland). CV was implemented by sweeping the potential from −0.4 to 0.8 V at 100 mV / s scan rates. After CV cleaning, H2SO4 was removed by deionized water (DI) from the electrode surface, then soluble redox mediator (1 mM ferri/ferrocyanide (Fe(CN)6]3-/4-) was injected by micropipette into the microchannels to measure EIS between 100kHz and 0.1Hz frequencies. Randles equivalent circuit carried out in order to determine the charge transfer resistance (Rct) fitting.

3. RESULTS AND DISCUSSIONS

EIS is a label-free method which is frequently applied in molecular level measurements [6, 19].

Microelectrodes play a major role in the development of highly sensitive sensor platforms in EIS technology. Microelectrodes are considered to increase sensitivity in low concentrations and volumes when combined with micro systems. However, improvements in the analysis are still required due to the problem of noise when miniaturized. The presence of noise prevents collection of accurate CV and EIS measurements. Therefore, it will not be possible to take more complex measurements at molecular level. In this study, we evaluated the effect of microchannel size while keeping the size of the electrode constant. Figure 2 depicts CV cleaning graphics for 150, 300, and 1000 µm wide microchannels. In electrodes with micron-size, currents are in the pico- to nano-ampere range, therefore two electrode systems are often used [20]. In our study, a two electrode system containing WE and CE/RE were designed.

The lifetime has been set to 100 CV cycles. The noise became visible in the narrowest microchannel, 150 µm -wide channel (Figure 2a), while the widest microchannel 1000 µm (Figure 2c) did not demonstrate any noise in the CV graph and also displayed better oxidation and reduction peaks. In addition, the current amplitude was also seen affected. Figure 2a and Figure 2b show that current is less than 1 nA in noise containing chips. In the other words, 150 µm microchannel has the highest noise and shows the lowest current in the analysis. Therefore, it is clear that the cleaning process was better for wider channel dimensions of more than 300 µm width where the signal was not affected by noise.

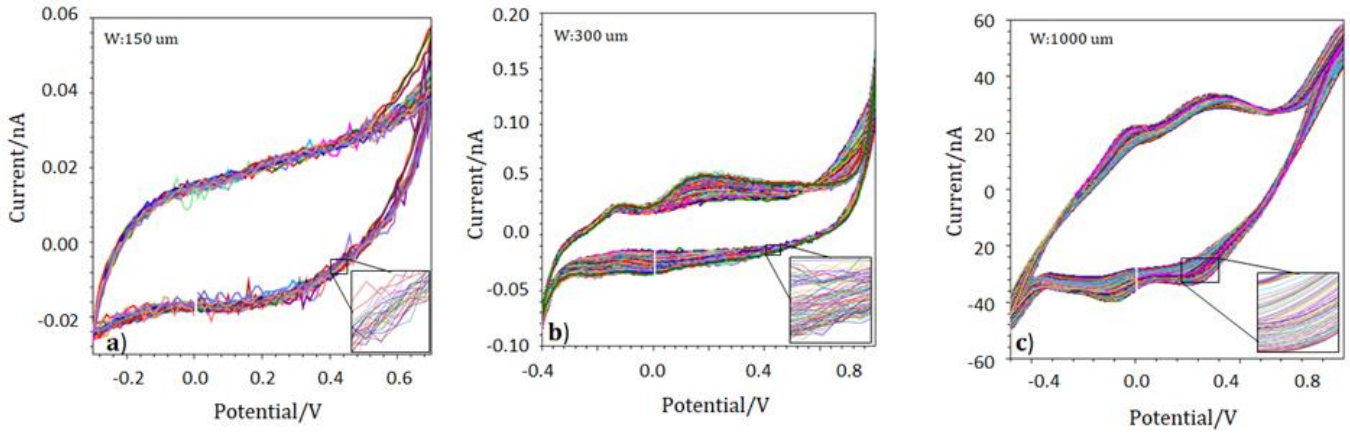

Figure 2 CV cleaning graphics for microchannels widths of a)150 µm ,b)300 µm, and c) 1000 µm.

Figure 3 demonstrates the EIS graphs after CV cleaning for 4 different types of microchannel integrated microelectrodes in the presence of 1 mM FF. The EIS response shows a semicircle transitioning into a 45° line, which is typical of a charge transfer reaction followed by diffusional behavior from the redox couple in solution [21]. Of particular importance to these experiments was the semicircular portion of the plot which was used to extract the Rct, which is indicative of electron transfer rates at the sensor surface [4]. Figure 3a shows that a highly complex graphic appears on the EIS plot for the narrowest microchannel 150 µm experiment. In the second microchannel with 300 µm dimension, the noise decreases on the EIS plot as seen in Figure 3b. The 500 µm microchannel shows slightly better EIS plot (Figure 3d). It is clear that the EIS plots do not display a normal semicircle transitioning in narrow microchannels, as did not demonstrate normal CV graphs in the cleaning process. However the experiments with 1000 µm dimension depicts a semicircle transitioning approximately without noise in the EIS plot. Thus, we concluded that the wider channels of about 1 mm dimension in width produces more scientifically meaningful EIS respond.

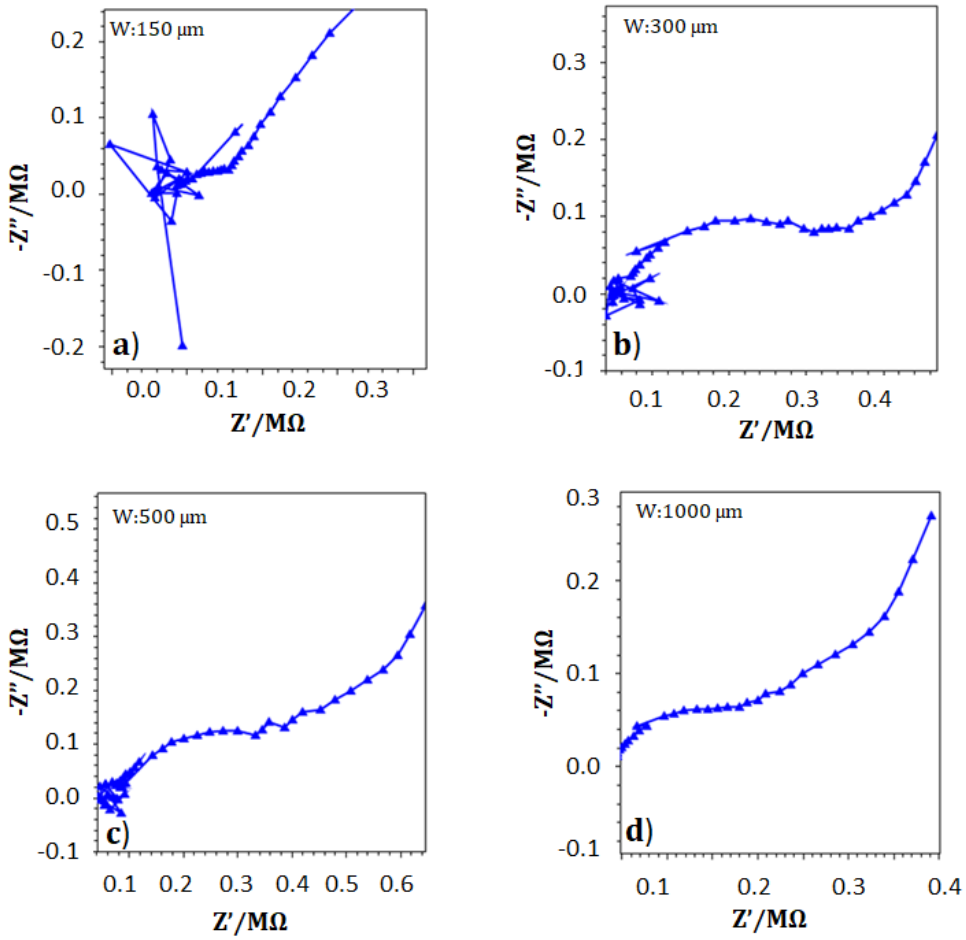

Figure 3 Graphs showing EIS results a) 150 µm, b)300 µm, c) 5000 µm, d 1000 µm

Horny et al. [22] applied a microchannel with 300 µm width and obtained EIS results without any noise. They used a working electrode with 30 µm wide working electrodes, while we applied a working electrode of 100 µm width. In this regard, it is clear that microelectrode sizes are inversely proportional to microchannels in obtained EIS results. Another important factor in EIS measurements is design and structure of microelectrodes. Ayliffe et al. [23] reported EIS results by using the microelectrodes and microchannels with width of the 0.5 and 10 µm, respectively. They designed a different design in study, where the working and other electrodes were placed against each other. It seems several parameters inter-play role in the production of an optimum signal.

In our study, we found that the size of the microchannels directly affects the EIS results. It is reasonable to think that the length and depth of the microchannel also affect the electrochemical output. This research suggests that miniaturization is not always the best option and a cross-over point also exists in the use of microfluidic integrated microelectrodes. We therefore presume that the combination of different design parameters for a particular study should be conducted for certain applications depending on the need.

4. CONCLUSIONS

In this study, we reported that fidelity of EIS measurements in microfluidic chips is dependent on microchannel dimensions. The best results were obtained when microchannels with 1000 µm widths were used. In narrower channels, noise was manifested in the EIS plot and CV cleaning.

As a result, when the working electrode width is 100 μm, microchannels width of 1000μm and above will be most suitable for performing EIS experiments with low noise. Further studies can be conducted for different electrochemical measurements and applications.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The authors contributed equally to the study.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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