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Electrical impedance spectroscopy of single cells in hydrodynamic traps

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ABSTRACT

This paper presents a new design of a microfluidic device combining hydrodynamic trapping and impedance spectroscopy measurements of single cells. Four microelectrodes integrated within cell traps enable impedance measurements in varying electrode pair configurations. To improve the impedance response of the microelectrodes, a modification using electrodeposition of a conductive polymer was applied. A considerable decrease of impedance magnitude was observed, and thus a significant enhancement of the useful frequency range was obtained. After electrode modification, the $12 \times 22\ \mu m^2$ electrodes were sensitive for frequencies ranging from 10 KHz to 2–5 MHz. Impedance measurements were carried out on single mouse oocytes with and without the surrounding glycoprotein matrix, called zona pellucida. Higher impedance values were obtained for zona pellucida-free than for zona pellucida-intact oocytes, reflecting the known high electrical conductivity of the zona pellucida.

1. Introduction

Enabling technologies of microfabrication have led to remarkable development of microfluidic systems for biosensing applications including sorting, manipulation, detection and characterization of cells [1–5]. Common detection methods in such micro devices include electrical and optical techniques [6,7]. Microfluidic platforms with optical detection methods usually require bulky and expensive off-chip instrumentation rendering them unsuitable for portable instruments [8].

With respect to miniaturization, integration and portability, increased focus has been devoted to electrochemical biosensors with particular interest to electrical impedance spectroscopy [9,10]. Two main categories of impedance-based microfluidic systems are reported in the literature: devices for characterization of the deposition of biomolecules on the sensor surface [11–13] and devices for cell analysis. This technique has been recently widely used as a non-invasive and label-free detection tool for single cells [14–21]. Impedance detection in microfluidic devices makes use of integrated microelectrodes to sense impedance variation caused by changes of the dielectric properties in a small detection volume [22].

Compared to conventional large area electrodes, microelectrodes offer many advantages, such as high sensitivity, spatial resolution, and high integration capability. However, microelectrodes exhibit large interface impedance caused by the well-known electrode polarization effect. This polarization effect is attributed to the formation of a double layer in response to an applied potential to the electrode. This mainly capacitive behavior is manifested in the low frequency range and can extend to frequencies up to 1 MHz depending on electrode size and measurement configuration [23,24]. The large interface impedance of the microelectrodes reduces the overall sensitivity and accuracy of the measurement [25]. The double layer acts as a capacitor masking the electric field in the detection region at low frequencies [26,27]. Theoretically, measurements can be performed at high frequencies to bypass the electrode polarization. But this is generally limited by the performance of the used measurement equipment and the parasitic effects of the device dominant in the high frequency range. Furthermore, measurements at low frequencies are of great interest in physiological analysis of single cells [19,28].

Many reported microfluidic devices used for characterization of single cells with electrical impedance spectroscopy are based on conductivity changes occurring in a detection region consisting

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of a trap site fitting the cell size [19]. Trapping mechanisms proposed involve hydrodynamic [17,29,30], dielectrophoretic [15] and negative pressure traps [31], respectively. Sensing microelectrodes scale with the trap size. The impedance measurement is usually carried out in a 2-electrode arrangement. This configuration suffers from the mentioned electrode polarization effect, influencing the signal spectrum in a broad frequency range reaching in some cases the available measurement bandwidth. Various approaches have been proposed to reduce the double layer effect, including analytically based correction or electrode modification techniques [28]. Using non-polarizable electrodes such as silver/silver chloride (Ag/AgCl) also overcome this effect, but Ag/AgCl electrode integration is complex and the electrodes suffer from fouling and degradation [2,28,32]. Since the double capacitance scales with the electrode-electrolyte interface area, a number of coating methods have been used to maximize the effective surface area of microelectrodes [26]. Commonly used materials to reduce the interface impedance include platinum black [19,33], iridium oxide [34], conducting polymers [28,35] and carbon-based materials [36,37].

Conducting polymers and carbon-based materials have been widely used for neural signal recording to maximize the interface surface area and to reduce the transfer resistance improving consequently the signal transport [26,38–40]. Because of their intrinsic conductivity, flexibility, and biocompatibility they are well suited for biosensing applications [35,41]. Poly(pyrrole-polystyrene) sulfonate (PPy:PSS) and poly(3,4-ethylenedioxythiophene) (PEDOT) as biocompatible conducting polymers are particularly suitable for covering biosensing electrodes, because these polymers can be electrochemically deposited and functionalized [35,42–44].

We developed a microfluidic device for impedance spectroscopy characterization of single cells. A hydrodynamic trapping mechanism was used in combination with four integrated electrodes in the capture chamber, providing a versatile measurement scheme. Impedance measurements of the bare gold electrodes in the device revealed a capacitive electrode behavior over almost the entire frequency bandwidth of the measurement (100 Hz–25 MHz), whereas PPy:PSS modified gold microelectrodes introduced a remarkable resistive plateau. Thus, improved electrode interface impedance and device sensitivity were achieved maintaining the size of the microelectrodes. Using the improved microelectrodes, electrical impedance spectroscopy measurements of mouse oocytes were performed. Oocytes in vitro fertilization (IVF) is an established procedure in human and animal assisted reproduction. IVF is routinely employed when natural mating fails, or when livestock and genetic strains of mice must be cloned and propagated. IVF critically depends on the quality of oocytes, especially on the state of the zona pellucida, a gelatinous outer layer of extracellular matrix that spontaneously hardens and becomes impenetrable to sperm during oocyte isolation and culture [45]. Conventionally, oocytes are cultured in non-microfluidic environments. Oocyte quality is usually judged microscopically by an experienced observer [46–48], but IVF success is nevertheless highly variable since it involves subjective visual inspection. In addition, these methods are time consuming and require expensive labor equipment. Therefore, observer-independent characterization methods would be very useful. In the last years, many microfluidic platforms for oocytes manipulation have been reported [49–54]. Still, for quality and viability assessment, optical techniques are established also in these approaches [52,55–60]. While in the case of optical methods, staining and fluorescence techniques are used, and contrast effects are evaluated, dielectric properties are interrogated when using electrical measurement techniques. Recently, a selection method using dielectrophoresis has been reported [61]. Quality control of mammalian oocytes based on electrical impedance spectroscopy has however, not been published yet to the best of our knowledge. Here, impedance spectra clearly distinguished zona pellucida-intact from zona pellucida-free oocytes suggesting impedance as a novel, non-destructive criterion to judge the quality of oocytes meant for in vitro fertilization.

2. Materials and methods

2.1. Chip design and fabrication

Fig. 1A shows the design of the microfluidic chip, which mainly consists of a microfluidic channel with four embedded trapping sites defining the detection regions of the device. The trap structures are arranged along the main inlet microchannel and featuring a narrow cross connection region to the outlet channel. A similar hydrodynamic trapping mechanism has been demonstrated by Di Carlo and colleagues [62]. In our novel approach, four electroplated gold electrodes are integrated in each capture chamber for electrical impedance spectroscopy. One pair of planar electrodes is placed on the bottom and two parallel facing vertical electrodes are located on the sidewalls of the trap, respectively. The microfluidic chip has a dimension of 10.6 mm × 14.3 mm with a microchannel width of 100 μm. Each trapping site is 86 μm wide.

The microfluidic chip was fabricated on a glass wafer using standard photolithography techniques. Fig. 1B illustrates the microfabrication process. Metal films, Cr/Au (30 nm/100 nm), were deposited using electron-beam evaporation. This metal film stack was used as the seed layer for the first electroplating process to form the bottom electrodes. AZ9260 photoresist (AZ Electronic Materials, Somerville, USA) was spin-coated on the substrate and patterned to define the electrical connection pads and leads for the bottom electrodes. An electroplating process yielded a gold layer with a thickness of 3 μm. After stripping-off the photoresist layer and wet-etching the unpatterned metal regions, a 4 μm thin SU-8 negative photoresist layer (SU-8 5, MicroChem Corp., Newton, USA) was deposited and lithographically patterned. This SU-8 layer formed the first passivation layer with the exposed bottom electrodes, each having an area of 12 μm × 22 μm. For the subsequent electroplating process a second metal film stack, Cr/Au (30 nm/100 nm), was deposited using electron-beam evaporation again. A thin AZ9260 photoresist was used to pattern the contact pads and leads for the sidewall electrodes. Subsequently, electroplating of a 3 μm thick gold layer was performed. The photoresist was then removed and a second thick AZ9260 layer was deposited and patterned with patterns for rectangular electroplating holes for the vertical electrodes. After electroplating, gold electrode posts with a height between 12 μm and 14 μm were obtained. The metal seed layer was etched and a second passivation layer of SU-8 photoresist (SU-8 5) was deposited and patterned leaving only the detection region and the contact pads exposed. This passivation layer is necessary to make electrical insulation for the interconnection lines of the sidewall electrodes passing underneath the main microchannel. To define the microchannel with the embedded trap structures, a thick SU-8 photoresist (SU-8 100) was spin-coated and patterned. The width of the trap structure was designed to be wider than the distance between the two sidewall electrodes to make sure that the electrodes are suspended in the capture chamber. The thickness of the SU-8 layer was controlled to be 95 μm. This value ensures the function of the device according to the hydrodynamic trapping mechanism. Finally, the wafer was diced to obtain individual chips.

To seal the SU-8 microchannel and to provide inlet and outlet for the fluidic interconnections, Poly(dimethylsiloxane) (PDMS) covers were fabricated using a standard soft lithography process. For the irreversible bonding of the PDMS lid on the SU-8 microchannel, a chemical gluing method was used employing a surface functionalization process [63,64]. Briefly, the surface of the PDMS