Detection of Mucin1 with a Microfluidic Impedimetric Aptasensor

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Mucin1 (MUC1) is a well-known glycoprotein which overexpresses on surfaces of tumor cells and is highly associated with breast, lung, colorectal and pancreatic cancers[1]. The rapid, sensitive, real-time and selective detection of such tumor markers are becoming more critical due to the arising demand of early diagnostics and point-of-care applications.

Aptamers are artificially synthesized DNA or RNA that are selected *in vitro* using Systematic Evolution of Ligands by Exponential Enrichment (SELEX)[2]. They can be specifically evolved to attain great affinity and selectivity against proteins such as MUC1. Due to the low immunogenicity and toxicity compared with antibodies[3], aptamer-based sensors (aptasensors) have attracted wide attention and are being more intensively studied during the past few years[4].

Among the variety of biosensing detection methods, electrochemical impedance spectroscopy (EIS) serves as a commonly used one regarding its ability for monitoring different stages during the fabrication process of aptasensors. EIS measures the current and its phase of an electrochemical system when giving oscillating voltages[5]. Upon binding of the analyte to the electrode surface, interfacial electron transfer kinetics gives rise to the change of the impedance measured at different oscillation frequencies. Moreover, the label-free, simple and highly sensitive features further strengthens its potential of integrating with other applications[6].

Microfluidics are recently recognized as an advantageous technique to perform bioanalysis. Not only can they lower the amount of usage during reactions, but also have the potential to meet real-time, portable, large-scale and high-throughput requirements[7].

In this study, a novel aptasensor for detecting MUC1 using microfluidic integrated gold electrode is fabricated and demonstrated. Scheme 1 illustrates the general procedure of this work. A 50nm gold layer was evaporated and physically deposited onto a 1.00×0.75 in glass slide, preceded by a 3nm chromium adhesive layer deposition. Two regions of gold make up the working and counter electrode of the chip, which represents a microfluidic two-electrode cell. The device is finally sealed by a previously fabricated PDMS flow channel and is ready to perform flow through experiments. To prevent the gold layer from being scraped off, another piece of copper tape was taped onto the exposed gold electrode surface. Figure 1 shows the sketch and photograph of the microfluidic chip device.

EIS comparing flowing and non-flowing aqueous solution inside the flow channel are performed prior to aptasensor fabrication of the chip, which is revealed in Figure 2. The solution is negative-pressured pumped through the channel using a peristaltic pump to avoid leakage due to high pressure. Results show that impedances measured at high frequencies tend to be more insensitive to flowing conditions while those at low frequencies do, proving that the flowing of the solution has a fundamental effect on electron transfer. Still, the configuration of the cell (e.g. Order of the working and counter electrodes) may also influence delays or leads of current. A previously discovered MUC1 aptamer S2.2[8] is thiolated at the 5’ end, prepared at 1μM and pumped through the flow channel for 1.5hr immobilization on the gold electrode surface. The outflow fluid is guided back to the original container, resulting in a recycling flow of aptamer probe immobilization. 11-Mercaptoundecanoic acid (MUA) is used as the blocking reagent, prepared at 1mM and is recycled pumped for 30mins. Finally, the chip is treated by 512nM MUC1 flow through. EIS methods are performed between each step for characterization of the gold electrode. Figure 3 plots the electrochemical impedance spectra of the previous procedures. Table 1 represents the fitted data according to a simplified Randles circuit. Self-Assembled-Monolayer (SAM) formed by covalent bonding of the gold electrode surface and the thiol group of S2.2-5’SH aptamer slightly increases the charge transfer resistance (R_{ct}). Moreover, blocking of MUA onto electrode greatly increases the series resistance (R_S). The binding of MUC1 onto the electrode further shifts right the whole plot, making R_S even bigger.

With the microfluidic impedimetric aptasensor, a novel and label-free method for detecting MUC1 has been proposed, further allowing portable, sensitive, and real-time detecting developments.

Keywords: Impedimetric aptasensor, Mucin1, Microfluidics

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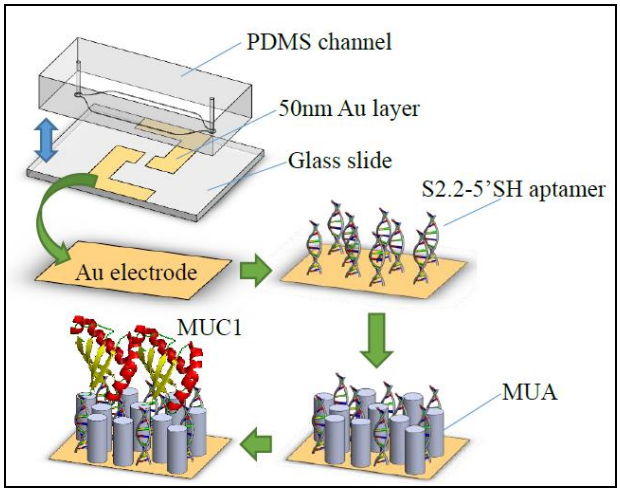
Figures and tables



Scheme 1. Fabrication of the microfluidic impedimetric aptasensor. A two-electrode microfluidic gold electrode chip is fabricated, followed by immobilization of thiolated S2.2-5'SH MUC1 aptamer, MUA blocking and MUC1 protein detection.

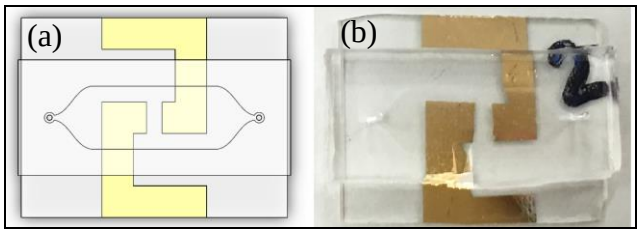


Figure 1. (a) Sketch and (b) photograph of the two-electrode microfluidic gold electrode chip with PDMS flow channel.

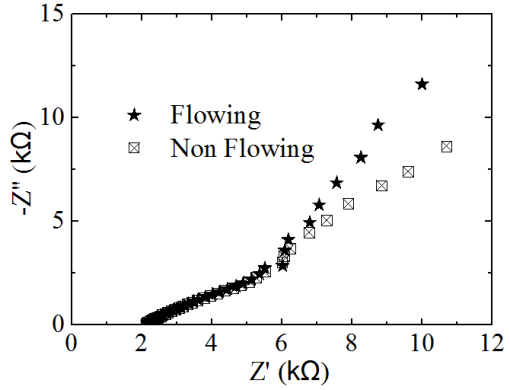


Figure 2. Electrochemical impedance spectra of flowing and non-flowing aqueous solution (5mM $Fe(CN)_6^{3-/4-}$ and 0.1M KCl as redox probe in PBS buffer) inside microfluidic channel.

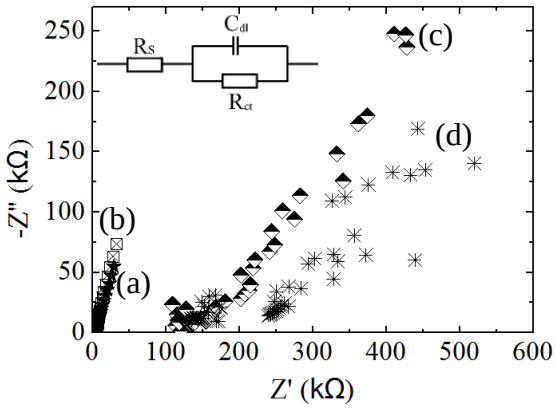


Figure 3. Electrochemical impedance spectra of (a) the bare Au electrode, (b) the Au/Apt electrode (10μM S2.2-5'SH aptamer 1.5hr immobilization), (c) the Au/Apt/MUA electrode (1mM MUA 30min immobilization) and (d) the 512nM MUC1 treated Au/Apt/MUA electrode (512nM MUC1 30min immobilization).

Table 1. Series resistance (R_S) and Charge transfer resistance (R_{ct}) for microfluidic gold electrode.

	R_S (kΩ)	R_{ct} (kΩ)
Bare Au Electrode	2.604	62.31
Au/Apt Electrode	2.731	104.7
Au/Apt/MUA electrode	113.0	241.7
512nM MUC1 treated Au/Apt/MUA electrode	136.2	156.6