The Study of an Impedimetric Microfluidic Chip Design for Mucin1 Aptasensing

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Introduction

Mucin 1(MUC1) is a glycoprotein which plays an important role within the human body such as cell signaling and defense of pathogens. Overexpression of MUC1 on cancer cell surfaces promotes cell survival and tumor angiogenesis. Therefore, the detection of MUC1 has become a new trend in early detection of cancer and therapy. Aptamers are synthesized DNA or RNA which serves as suitable sensing elements for targets such as MUC1 or other proteins and can achieve excellent affinity and selectivity. Electrochemical impedance spectroscopy (EIS) is a powerful electrochemical detection technique regarding its ability for monitoring different stages during the fabrication process of aptasensors. Moreover, the label-free, simple and highly sensitive features further strengthen its potential of integrating with other applications. Microfluidics are recently recognized as a technique advantageous for performing 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.

In this study, a novel aptasensor for detecting MUC1 using microfluidic integrated gold electrode is fabricated and demonstrated. Parameter fitting of the equivalent circuit is performed after each step during the fabrication and protein detection. An LOD of 0.42nM is achieved and the fitting result suggests several varying element parameters influenced by the concentration of MUC1, which are all shown below. The simple, label-free and low reagent usage detection of MUC1 using this microfluidic impedimetric aptasensor is achieved.

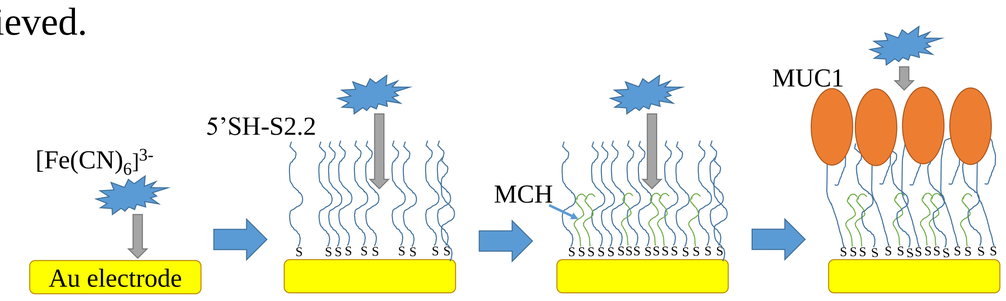

Figure 1. General scheme for the fabrication and detection process of the microfluidic impedimetric aptasensor

Experimental

Micro Gold Electrode and Microfluidic Chip Fabrication

The micro gold electrode is fabricated on a 25.4x25.4mm diamond knife cut glass slide. Symmetric electrodes which are each 0.3x0.3mm in dimension and 0.4mm apart are designed. S1813 photolithography followed by thermal evaporation of a 20nm Cr adhesive layer and a 80nm Au layer is applied. PDMS microfluidic channels are molded using a previously constructed aluminum master. The PDMS channel and gold electrode glass slide are finally clipped together using a 3D printed fixture. Elasticity of the PDMS clamped on the slide resists solution leakage from inside the channel. This assembly-style making of the microfluidic chip permits flexibility for further modification and characterization.

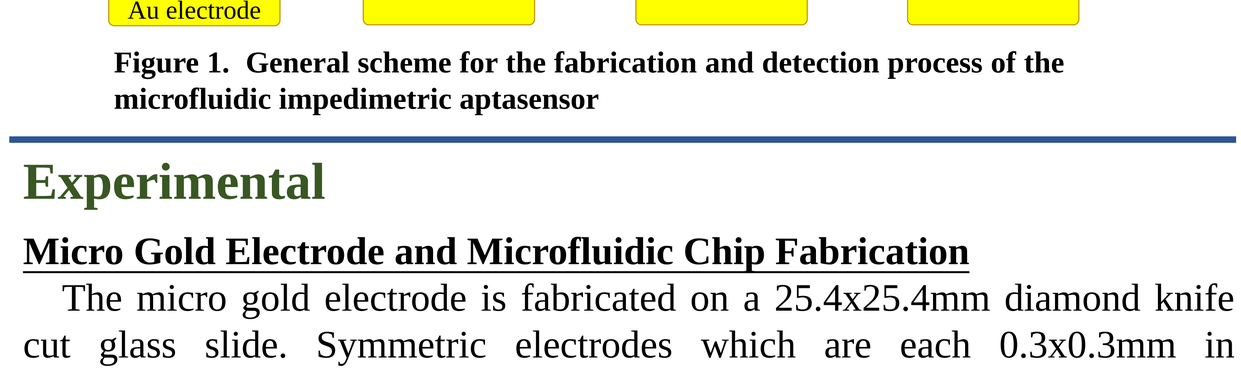

Figure 2. (a) Fabrication process, (b) 3D view and (c) photograph of the impedimetric microfluidic chip. (d) Top view and (e) zoom in of micro gold electrode

Equivalent Circuit Fitting of Symmetric Micro Gold Electrode

Two parallel connected Randles circuit, each containing a constant phase element(CPE), charge transfer resistance(R_{ct}), Warburg element(Z_W) and a shared solution resistance(R_S) are founded, representing the two symmetric micro gold electrodes within the chip. Impedance values of the same elements are set the same due to the alike conditions given to the symmetric pair. A previously written model-fitting program is applied using a self-defined error(ε, as in Equation 1) minimization parameter fitting algorithm.

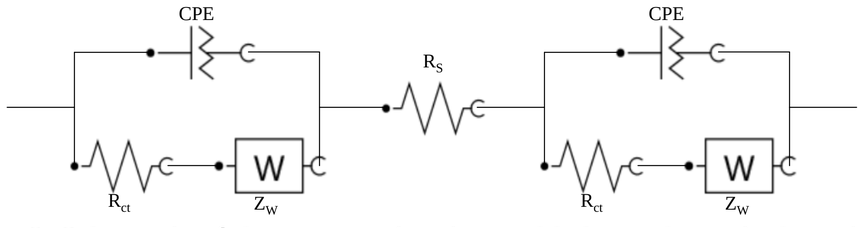

Figure 3. Schematic of the symmetric micro gold electrode equivalent circuit.

$$\varepsilon = w_{|Z|/\emptyset}\sum_{i=1}^{n_f}\left[\log\left(\frac{|Z|_{cal,i}}{|Z|_{in,i}}\right)\right]^2 + \sum_{i=1}^{n_f}\left(\emptyset_{cal,i} - \emptyset_{in,i}\right)^2 \quad \textbf{(Equation 1)}$$

Results and discussion

Calibration curve from phase angle bode plot

Thiol group modified MUC1 aptamer(5'SH-S2.2) is flown through the channel at 1μM for 13hr, followed by 1mM 6-mercaptohexanol(MCH) blocking for 1hr. MUC1 is serial diluted from 1μM to 0.98nM, flown through from low to high concentration for 45min and directly characterized by EIS. All steps are realized at a flow rate of 1μL/s with negative pressure and the outflow fluid is guided back to the original container, resulting in a recycling flow of immobilization. The phase angle between 100Hz and 10kHz decreased gradually within each fabrication and detection step, especially after blocking of MCH. We can observe a linear relationship between the phase angle and logarithm MUC1 concentration. Taking the phase angle of MCH blocking as a baseline and performing regression, 8105Hz serves as the most ideal frequency for it maximizes the baseline phase angle difference and the linear regression correlation coefficient at the same time(Fig.4(c)). The LOD determined from the phase angle at 8105Hz is 0.42nM(3 times the concentration at intersection of baseline) and the sensitivity is 0.92°/log(nM).

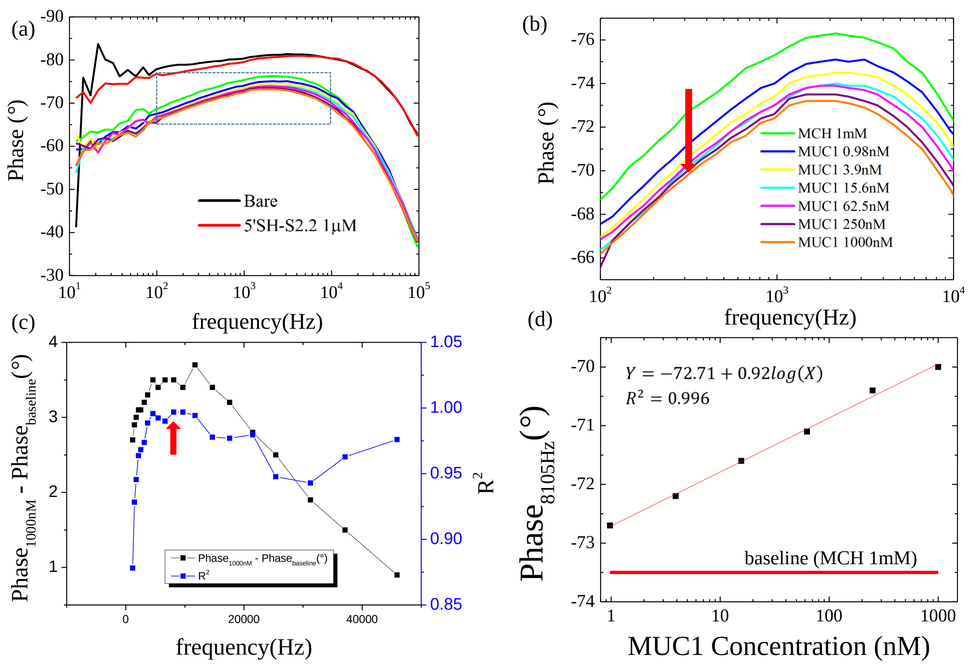

Figure 4. (a) Phase angle (Φ) vs frequency plot and its (b) zoom in. (c) Phase angle difference and the square of the linear regression correlation coefficient between MUC1 concentration and phase angle at different frequencies. (d) Phase angle vs MUC1 concentration at 8105Hz.

Equivalent circuit fitting and analysis

Further analysis is performed using equivalent circuit fitting. Solution resistance show low correlation with protein concentration, agreeing with presumed conditions. CPE capacitance(Q_{dl}) slightly increase with protein concentration and exhibited a mere selectivity against thrombin. The CPE also gradually approach a resistance due to the decline of n as the protein concentration increase.

Figure 5. Value of (a) solution resistance, (b) charge transfer resistance, (c) CPE capacitance, (d) CPE n factor, plotted with MUC1 and thrombin concentration and its linear regression line.

Conclusion

A microfluidic impedimetric aptasensor for detection of MUC1 is developed and studied. The label free, low reagent usage and flexibility of this system widens its potential for portable and real-time developments. Still, equivalent circuit fitting of the symmetric micro gold electrode further strengthen its ability to uncover the underlying phenomenon on this miniaturized system.