REAL-TIME IMPEDIMETRIC MUC1 APTASENSOR USING MICROFLUIDIC SYMMETRIC AU ELECTRODES

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Introduction

MUC1 is a surface glycoprotein which over-expresses in several types of cancer cells, making it an ideal marker for cancer detection. For MUC1 recognition, the S2.2 aptamer is a 25mer ssDNA selected *in vitro* and can affinitively and specifically bind to certain motifs within the MUC1 protein. Electrochemical impedance spectroscopy (EIS) has been proven as an effective method for ultrasensitive MUC1 aptasensing and cell detection [1]. Despite the high sensitivity and selectivity of EIS and the S2.2 aptamer, long reaction times and large sample volumes have hindered EIS biosensors for realistic bioanalysis.

The integration of microfluidics with EIS has a large potential for lowering the amount of usage during reactions and to meet real-time, portable, large-scale and high-throughput requirements. Though up to date, there hasn't yet been studies regarding real-time impedimetric aptasensing to the best of our knowledge. Thus, this research is dedicated to develop a real-time microfluidic impedance aptasensing platform for affinitive and selective detection of MUC1. The general scheme and setup is shown in Fig. 1.

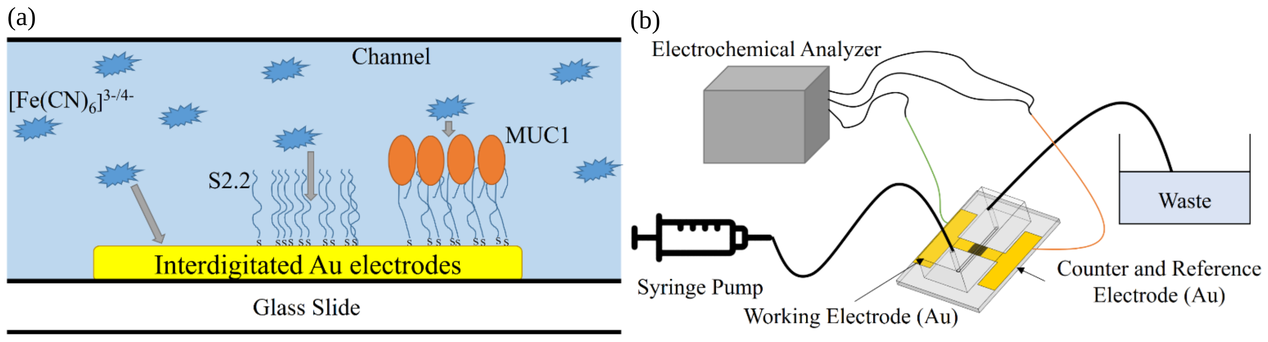

Figure 1: (a) Schematic of MUC1 impedimetric aptasensing using $Fe(CN)_6^{3-/4-}$ as the redox mediator in a microfluidic channel, (b) microfluidic system and electrochemical setup of this study.

Experimental

Interdigitated electrodes and the channel dimensions are designed (Fig. 2). S1813 photolithography followed by E-beam evaporation of a 20nm Ti adhesive layer and a consequent 80nm Au layer is applied. The soft lithography fabricated PDMS channel and the Au electrode glass slide are clipped together using a 3D printed fixture. The microfluidic system is set up using digital controlled syringe pump (Legato® 111, KD Scientific). A CHI614B electrochemical workstation is used for the following experiments. EIS and CV comparing different flow rates are conducted to evaluate the underlying microfluidic phenomena under flowing conditions and determine the optimal AC frequency for real-time impedimetric sensing. After the analysis of EIS, the aptasensor is fabricated by flowing through 10μM thiol-modified S2.2 aptamer (5'SH-$(CH_2)_6$-GCAGTTGATCCTTTGGATACCCTGG), followed by 10μM BSA to check the non-specific binding level. 200nM MUC1 (APDTRPAPG, the highly immunogenic epitope of the variable tandem repeat (VTR) region of MUC1 targeted by S2.2 aptamer) is lastly added to examine affinitive binding. Real-time data are measured at each stage.

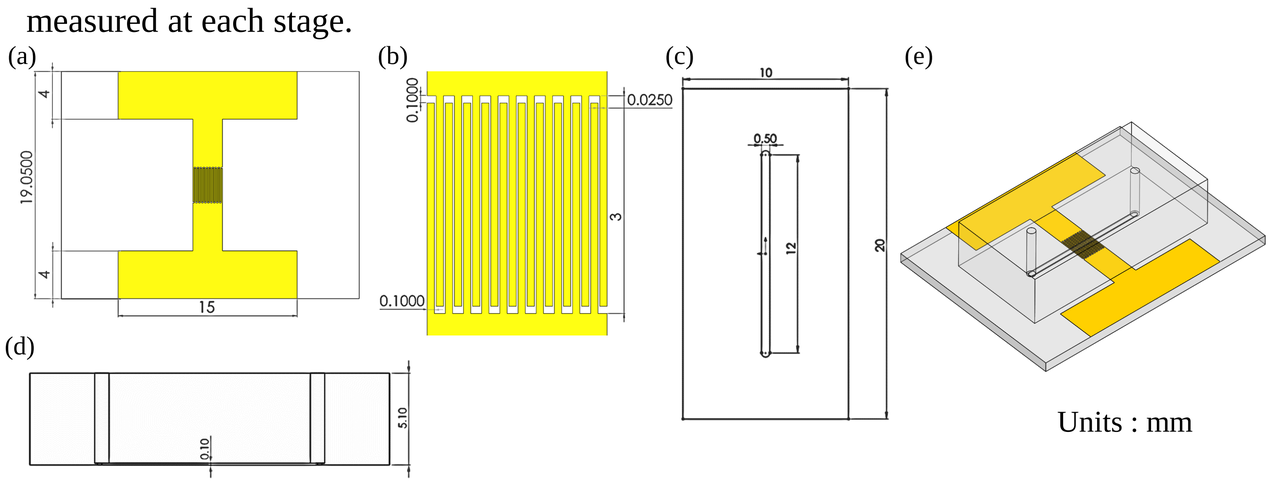

Figure 2: Schematic (a) top view, (b) center close-up view of the Au interdigitated electrodes, (c) top view and (d) side view of the microfluidic channel and (e) 3D view of the microfluidic channel conjugated interdigitated electrode chip.

Results and discussion

Interdigitated array electrodes of 100μm width each and 25μm apart are fabricated and the straight channel of 0.5mm width and ~100μm height is fabricated using SU-8 soft lithography and PDMS molding. A significant difference is present between 0μL/s and other flow rates in the cyclic voltammogram of the microfluidic Au interdigitated electrodes (Fig. 3). This is due to the active $Fe(CN)_6^{3-/4-}$ surface refresh in flowing conditions. Furthermore, at 0μL/s, the increase in scan rate enhances the current while scan rates at other speeds don't. The effect of impedance influenced by flow speed is secondly characterized by EIS, followed by affinitive aptasensing of MUC1. An increase in flow rate results in a decrease in total impedance and phase angle at low frequencies and the 45-degree line in the Nyquist plot due to diffusion effect at low frequencies is eliminated (Fig. 4). This is due to the corresponding faster surface refreshing rate of $Fe(CN)_6^{3-/4-}$.

The optimal real-time impedance measuring frequency is arbitrarily chosen as 100Hz, which corresponds to the intersection of the charge transfer and diffusion limiting region.

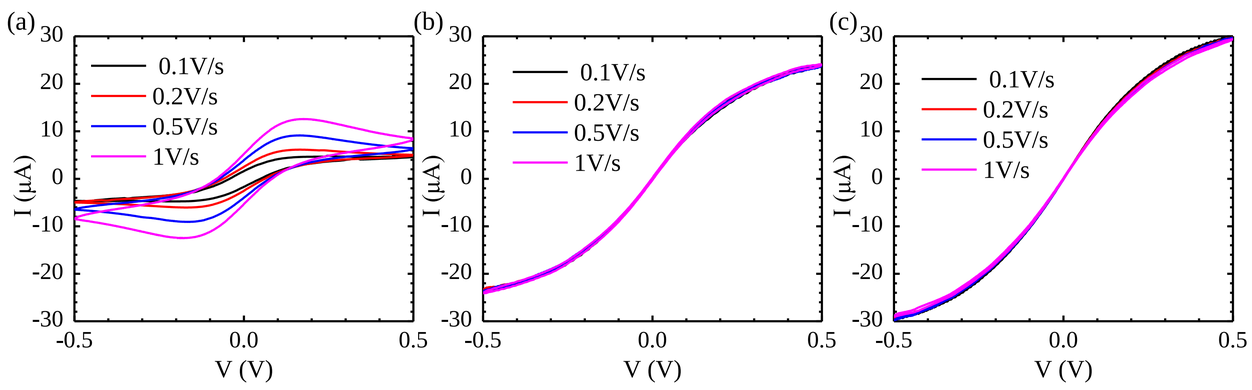

Figure 3: Cyclic voltammogram on microfluidic bare Au electrode comparing different flow rates and scan rates. (a) 0μL/s, (b) 0.2μL/s and (c) 0.4μL/s. The solution is 5mM $Fe(CN)_6^{3-/4-}$ in 10mM Tris-HCl buffer (5mM $MgCl_2$, 50mM KCl, pH = 7.4).

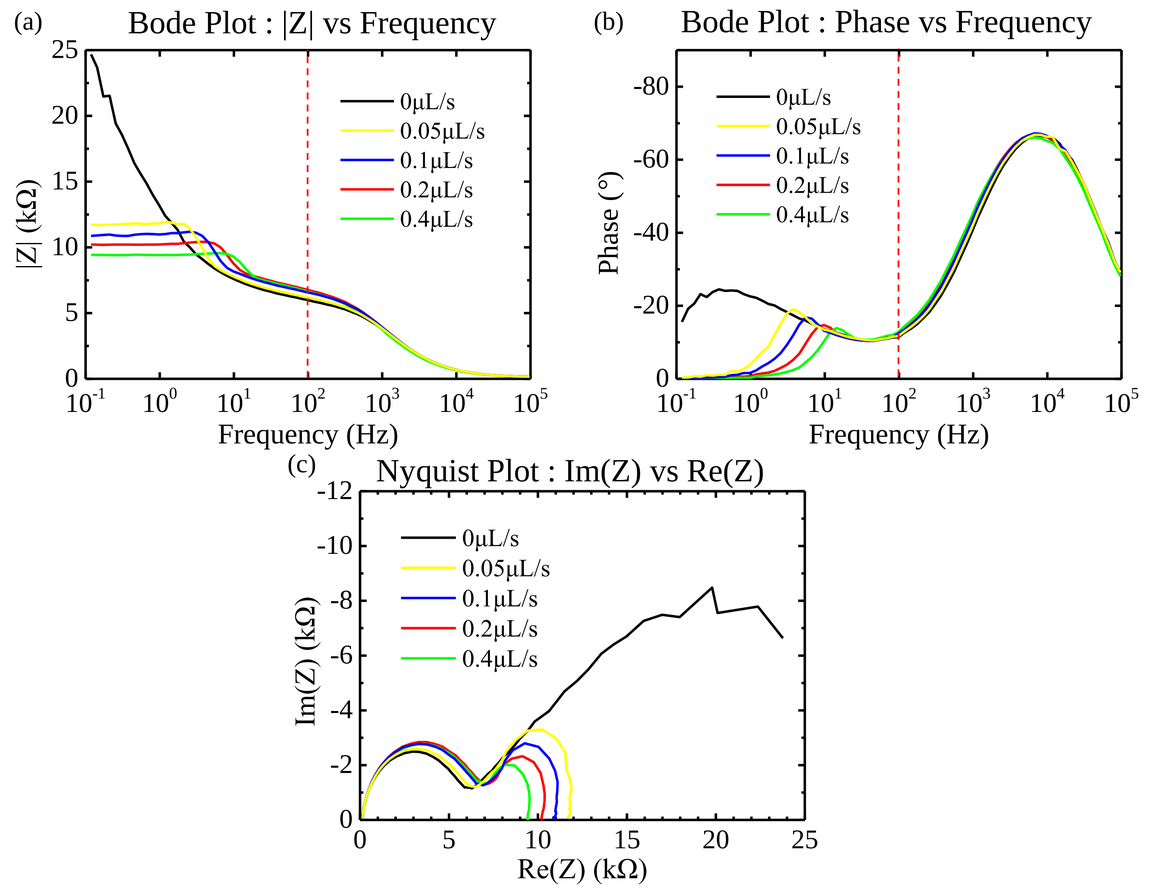

Figure 4: EIS spectra on microfluidic bare Au electrode comparing different flow rates. (a) |Z| vs frequency, (b) Phase vs frequency bode plot and (c) Nyquist plot. E_{init} = 0V, V_{amp} = 5mV, 5mM $Fe(CN)_6^{3-/4-}$ in 10mM Tris-HCl buffer (5mM $MgCl_2$, 50mM KCl, pH = 7.4).

The total impedance and phase angle increased after aptamer immobilization (Fig. 5), although the further increase in impedance after BSA flow-through indicates a strong non-specific binding to the not fully covered Au electrode. MUC1 binding results an increase in phase angle and the imaginary part of impedance and a decrease in the real part of impedance.

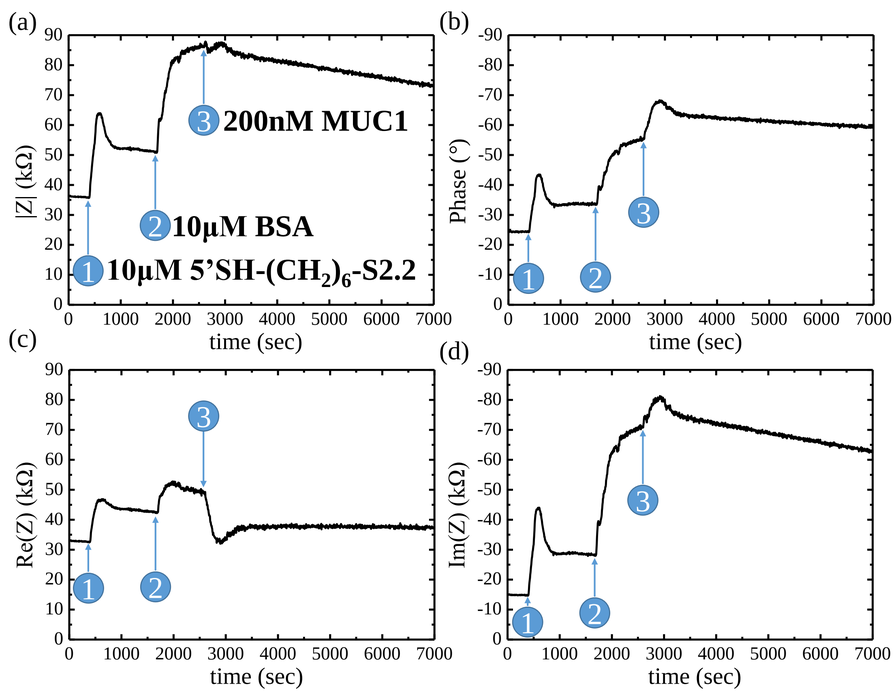

Figure 5: Plots of Real-time impedimetric aptasensing of MUC1. (a) |Z| vs time, (b) Phase vs time, (c) Re(Z) vs time and (d) Im(Z) vs time. Frequency = 100Hz, V_{amp} = 5mV, flow rate = 0.2μL/s and all samples were dissolved in 10mM Tris-HCl buffer.

Conclusion

In summary, a real-time impedimetric aptasensor for detection of MUC1 is developed and studied using microfluidic symmetric Au electrodes. Though further improvement on sensitivity and selectivity are needed to be realized, the label free, real-time and simple characteristics of this system widens its potential for portable and multiplex developments.

[1] X. Liu, Y. Qin, C. Deng, J. Xiang and Y. Li, "A simple and sensitive impedimetric aptasensor for the detection of tumor markers based on gold nanoparticles signal amplification", *Talanta*, 2015, p. 150-154.