Capacitive Micromachined Ultrasonic Transducer (CMUT)-based Biosensor for Detection of Low Concentration Neuropeptide

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***Abstract*— Accurate detection of neuropeptides in cerebrospinal fluid (CSF) plays an important role in both in-depth studies and early diagnosis of neurological diseases. Here, we report a biosensor based on Capacitive Micromachined Ultrasonic Transducer (CMUT) which is capable of detecting low concentrations (pg ~ ng/ml) of a neuropeptide involved with the progression of Alzheimer's diseases, somatostatin (SST). A 10-MHz CMUT was fabricated and utilized as a physical resonant sensor which detects the change in the concentration of analyte through the mass-loading mechanism. The resonant plate was sequentially coated with protein G and antibodies to provide specificity to SST; Cysteine-tagged protein G layer enables controlled immobilization of antibodies in a well-oriented manner. The change in the resonant frequency of the CMUT sensor was measured after incubating the sensor in various concentrations of SST. The significant shifts in the resonant frequency were observed for SST concentrations in the range of 10 pg/ml ~ 1 ng/ml. Compared to the previously reported biosensors developed for SST detection, our sensor shows discernable responses for SST that are ~6 orders of magnitude lower in concentration. Thus, this work demonstrates the potential of the CMUT resonant sensor as a promising biosensor platform for detection of neuropeptides involved with neurodegenerative diseases that often exist in low concentrations in CSF.**

I. Introduction

Due to the ageing society, the number of patients associated with neurodegenerative diseases as well as the associated societal costs are continuously increasing. For example, today, approximately 47 million people are suffering from dementia, a disease which causes deterioration in memory and cognition. This number is projected to increase up to 131 million by 2050 [1]. Since the most common form of dementia is Alzheimer's disease (AD), a large amount of efforts has been dedicated to investigating the fundamental mechanism of AD progression and searching for biomarkers for early diagnostics over the past decades. Currently, the widely accepted concept of the pathogenesis of AD is the amyloid cascade hypothesis, which states that the pathological accumulation of the amyloid-β peptide (Aβ) is the main cause of neurodegeneration in AD [2]. The principal components of the hypothesis are Aβ and tau protein. However, pharmaceutical attempts that have targeted these two hallmarks have not been successful in clinical trials.

As an alternative, various neuropeptides and neurotransmitters have been proposed as biomarkers for early diagnosis of AD. Among these, somatostatin (SST) is a neuropeptide which modulates motor, sensory behavioral, cognitive, and autonomic functions. In the case of AD, the concentration of SST consistently decreases in the brain and cerebrospinal fluid (CSF) (AD: ~40 pg/ml; normal: ~120 pg/ml) [3, 5]. In addition, SST has been shown to be related to other neurological disorders such as mood disorder, epilepsy, Huntington's disease, and Parkinson's disease [3-6]. Therefore, an effective method to detect SST that exists in low

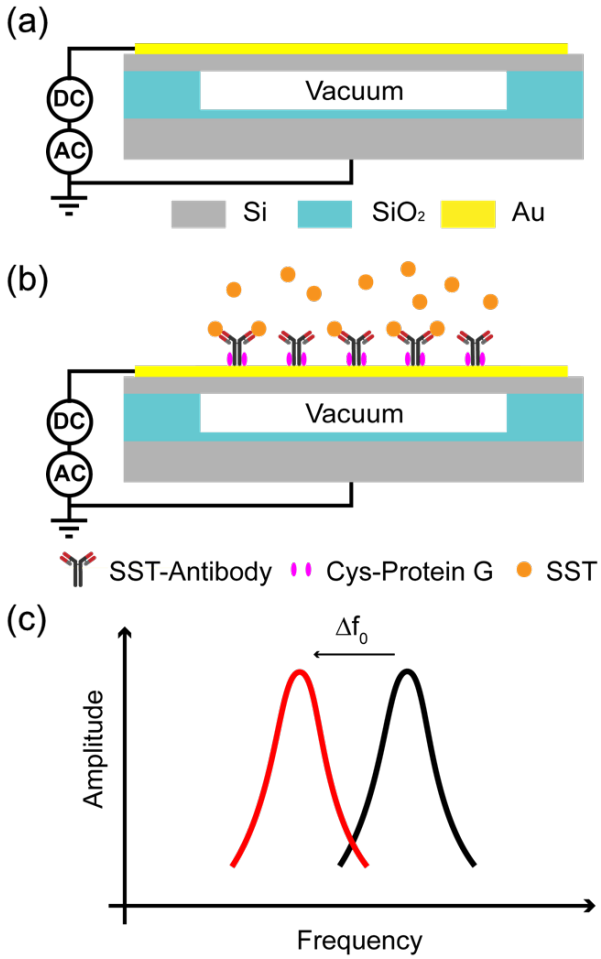

Figure 1. Conceptual illustration of schematics and principle of operation of the CMUT-based biosensor for SST detection: (a) cross-section of a single cell of a CMUT device, (b) functionalization layers on the CMUT-based biosensor, and (c) shift in the resonant frequency of the CMUT cell due to the mass-loading mechanism.

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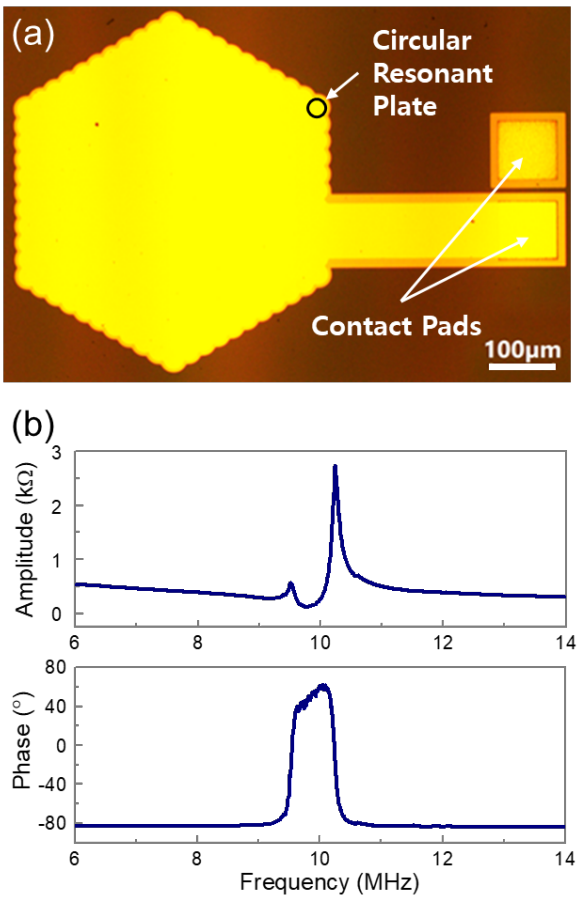

Figure 2. (a) Optical picture of the fabricated CMUT (b) Measured impedance characteristics of the fabricated CMUT.

concentrations in blood and CSF would enable in-depth studies of the AD progression and potentially serve as an early diagnostic tool for AD. However, previously reported sensor platforms developed for SST detection show limited detection range (~ μg/ml), which is ~6 orders of magnitude higher than the typical SST concentrations in CSF [6].

Acoustic resonant sensors based on the mass-loading effect such as quartz crystal microbalance (QCM) [7, 8], surface acoustic wave device (SAW) [9], and film bulk acoustic resonator (FBAR) [10] offer competitive advantages as biosensors such as label-free detection and relatively high mass sensitivity. Among these acoustic resonant sensors, a capacitive micromachined ultrasonic transducer (CMUT) is an attractive platform. The top resonating plate is backed by a vacuum cavity which not only reduces air damping and thus increases quality factor but also allows for straightforward functionalization of the top plate [11, 12].

Thus, in this study, we developed a CMUT-based biosensor for the detection of SST in relatively low concentrations (pg ~ ng/ml) (Fig. 1). Since the orientation of immobilized antibodies on the substrate directly affects the performance of sensor [13], we used Cysteine-tagged protein G as the linker layer for antibodies to achieve a well-oriented immobilization. Protein G, an immunoglobulin (IgG)-binding protein, binds to the heavy chain in Fc region of various antibodies, especially IgG isotypes [14]. Because Cysteine consists of a thiol group, by Cysteine tagging, the protein G can be self-assembled on the Au surface with a well-oriented manner [15]. After immobilization of antibodies on the sensor, we measured the frequency shifts of the functionalized CMUT-based biosensor to different concentrations of SST ranging from 10 pg/ml to 1 ng/ml, which is a common range of concentrations of SST in CSF.

II. Principle of Operation of CMUT

The primary component of CMUT as a biosensor is a capacitive cell (Fig 1(a)). Each cell consists of a circular plate supported by silicon dioxide posts, which resembles a standard parallel-plate capacitor (Fig. 2). The silicon plate deposited with a thin gold (Au) film serves as the top electrode. The bottom electrode (highly doped silicon substrate) is separated by a vacuum-sealed cavity from the top electrode. When the DC bias voltage is applied across these two electrodes, the resonant plate is deflected by the electrostatic force. This small deflection results in an effective electromechanical coupling. Under the DC bias, the circular plate is then actuated by superimposing an AC voltage.

The resonant frequency of CMUT is determined by the material properties and geometries of the plate and the applied bias voltage (Fig. 2(b)). For a circular plate, the resonant frequency of CMUT is inversely proportional to the square root of the mass of the plate. Hence, an additional mass on the membrane of CMUT induces a decrease in the resonant frequency. By tracking this frequency shift, the loaded mass is estimated. The relation between the frequency shift and the loaded mass is

$$\Delta f = -1/2\ (\Delta m/m)\, f. \qquad (1)$$

III. Experimental Methods

A. Immobilization of SST Antibodies

Prior to functionalization, the Au surface of CMUT plate was cleaned for 2 min with piranha solution (3:1, 98% H_2SO_4 / 30% H_2O_2) and the CMUT array was immersed in 100 ng/ml of Cys-protein G solution for 12 h to form a self-assembled linker layer. After functionalization of protein G, the Au surface was cleaned with phosphate-buffered saline, 0.05% Tween 20, pH 7.4 (PBST) and distilled water to remove any non-specific bindings on the Au surface. After drying with nitrogen, the device was immersed in a diluted antibody solution for 4 h to immobilize SST antibodies (IgG) on the protein G self-assembled layer.

B. Characterization of Functionalization and SST Binding

The frequency shifts of CMUT elements were measured using an impedance analyzer to characterize each step of functionalization: initial (bare Au), self-assembly of protein G, immobilization of SST antibodies, and SST binding. Before measuring the frequency shift at each step, the CMUT surface was dried to avoid any undesired effects. After drying, to reduce systematic errors in frequency measurements, the same DC bias voltage was applied to the device for at least 5 min to achieve stabilization. For each experiment, a total of 12 elements (*i.e.* individual sensors) was characterized to obtain statically significant results. Moreover, for visualization of protein G layer on the Au surface, the fluorescent-labeled

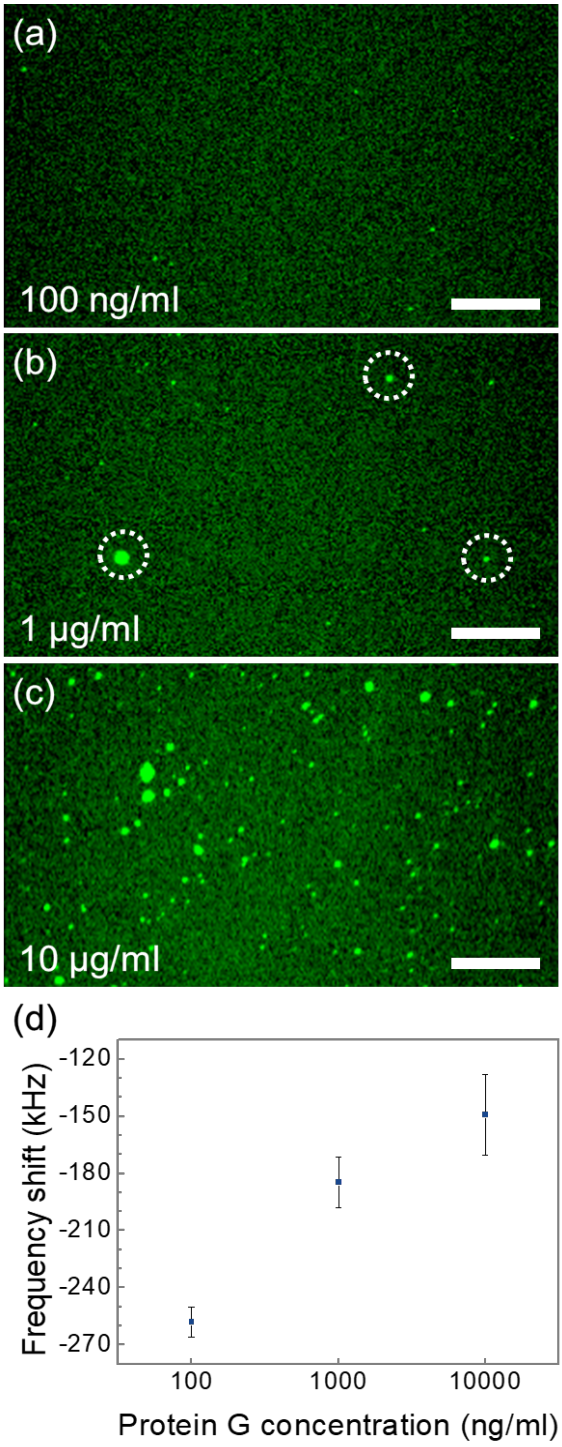

Figure 3. (a) Fluorescent images of the immobilized fluorescent-labeled antibodies on various concentrations of protein G layer coated on a gold film. (b) Frequency shifts after SST antibody immobilization on protein G layer with different concentrations. The scale bar is 10 μm.

antibodies (IgG) were applied to the protein-G-treated Au surface.

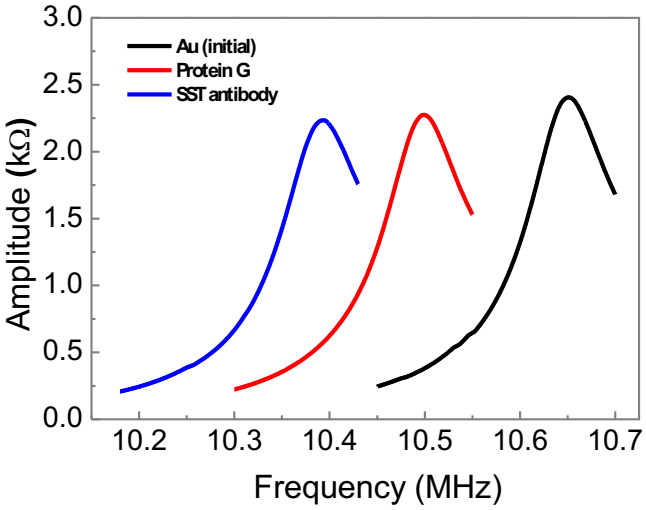

Figure 4. Measured resonant frequency of a CMUT element at each stage of functionalization.

IV. Experimental Results

A. Optimization of Protein G Concentration

Finding an optimal condition for the antibody and capturing biomolecules is important to maximize the sensitivity of a biosensor and to achieve reliable results. Therefore, to optimize the concentration of protein G, we first optically observed the immobilization of protein G on the gold substrate by using fluorescent-labeled antibodies (IgG). As expected, the fluorescent intensity of the protein G coated substrate was higher for a higher concentration of protein G (Fig. 3(a)). However, for the concentration higher than 100 ng/ml, spots which appeared to be an aggregation of protein G were observed.

Moreover, we measured the frequency shifts of CMUT elements after the immobilization of SST antibodies on the protein G layer. For a higher protein G concentration, the mass of protein G layer on the sensor was higher, but we observed smaller frequency shifts (Fig. 3(b)). This is probably due to the aggregation of protein G at higher concentrations which hinders the binding of SST antibodies. Furthermore, a larger variation in the frequency shifts was observed across 12 elements at higher concentrations (Fig. 3(b) error bar) due to random aggregation of protein G affecting the uniformity of the layer. Thus, for this work, to achieve a uniform and reliable protein G layer, we used protein G concentration of 100 ng/ml.

B. Immobilization of SST Antibodies

Prior to the SST binding, we confirmed that CMUT elements were successfully functionalized at each stage by measuring the frequency shifts (Fig. 4). Specifically, we observed distinctive shifts after functionalization of protein G and immobilization of SST antibodies. The immobilization in a protein G (100 ng/ml) solution for 12 h resulted in a decrease in the resonant frequency by approximately 150 kHz. SST antibodies were then immobilized by immersing the CMUT elements in the SST antibody solution for 4 h. The binding of SST antibodies on the top Au electrode surface resulted in a negative frequency shift of approximately 100 kHz.

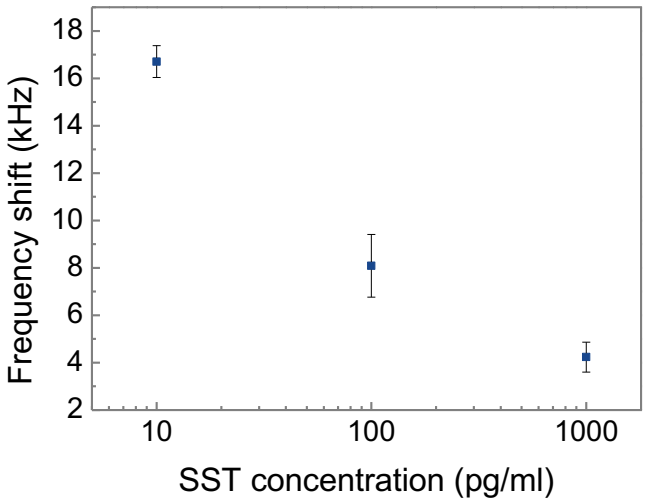

Figure 5. Averaged frequency shifts of 3 CMUT biosensors measured after binding to different concentrations of SST.

C. SST Detection using the CMUT Sensor

Since we aimed to develop a biosensor for the early diagnostics of AD progression, we tested the sensitivity of our sensor in the concentration range that is clinically relevant. We observed distinctive resonant frequency shifts across various concentrations of SST from pg ~ ng/ml (Fig. 5). However, if the sensing mechanism was due to the mass loading, negative frequency shifts should have been observed (1). However, we observed positive frequency shifts when our sensor was exposed to both the control (PBS) and the SST solution. There are two possible explanations for this phenomenon: stress [16] and removal of non-specific binding of functionalization layer (either SST antibodies or protein G). For the latter case, if the removal of non-specific binding is larger than the mass change due to the SST binding, a positive frequency shift could be observed. Thus, as future work, we plan to conduct further optimization on the functionalization layer to minimize the non-specific binding and investigate the mechanism behind positive frequency shifts. Nevertheless, the tendency of frequency shifts of CMUT to different concentrations of SST is clear and the sensitivity is relatively high. Thus, the CMUT-based biosensor has the potential as the platform for detection of low concentration neuropeptide such as SST.

V. Conclusion

In this work, we reported a successful detection of low concentration of SST in the order of pg ~ ng/ml in a buffer solution using a newly developed CMUT-based biosensor. Cys-protein G was immobilized on the sensing surface of CMUT through the SAM followed by immobilization of SST antibodies. Discernable frequency shifts were observed in response to the different concentrations of SST. Although positive frequency shifts were observed for both the control and the SST binding, the direction of the frequency shift was consistent across the pg ~ ng/ml range. Future works include investigating several hypotheses to explain the positive frequency shifts such as stress and removal of non-specific binding of antibody and protein G, further optimization of functionalization, and conduction detection at other range of SST concentrations. Thus, this work on label-free detection of low concentration of SST shows that the CMUT is a promising biosensor platform for detection of low concentration of neuropeptides.

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