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Short communication

Highly sensitive detection of epidermal growth factor receptor expression levels using a capacitance sensor

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ABSTRACT

We developed a capacitance sensor with parallel plate geometry to measure epithermal growth factor receptor (EGFR) expression levels on cell membrane in real-time. We first proved correlations between capacitance changes and cell numbers settled down between electrodes, and then observed capacitance changes elicited by interactions between EGFR on membrane and EGF proteins in real time. Consequently, we confirmed that the EGFR expression levels of varied typed cells were successfully quantified. This approach can effectively distinguish differences of EGFR levels of cancer cells and normal cells in real-time. Also, up to 600% sensitivity enhancements and around 2.2 h on average sensing time saving were achieved by using the capacitance sensor over a conventional immunoassay technique. Such a capacitance biosensor can be extended to broad fields where the receptor–antibody reactions, the receptor–virus reactions or DNA hybridizations are involved.

1. Introduction

Cancer cells have specific own cancer biomarkers, such as epidermal growth factor receptors [1], vascular endothelial growth factors [2], carcinoembryonic antigens [3], prostate specific antigens [4] and cancer antigens [5]. Easy, rapid and accurate detection of those cancer biomarkers with high sensitivity plays an important role to understand biological mechanisms of cancer cells and develop clinical diagnosis systems at the incipient stage of cancer. Moreover, the development of detection systems can definitely involve researches to find a new medicine candidate for a cancer treatment.

Among biomarkers, an epidermal growth factor receptor (EGFR), which is a transmembrane glycoprotein on cell membrane, has considerable attentions as one of the effective cancer biomarkers in recent researches [6–8]. The high EGFR expression level have quite been discovered in tumor progression [9–13], cancer cell proliferation and metastatic phenotype [14–16]. In other words, observing over-expressed EGFR can indicate that the cells are likely to be identified as cancer cells [17,18]. In terms of clinical diagnosis, the EGFR has also been widely investigated to determine factors of tumorigenesis, such as angiogenesis and metastasis, since it has been reported that its over-expression is associated with colorectal, cervical, squamous and gastric tumorigenesis [19–22].

Thus, the development of measurement techniques has been desired to analyze the accurate EGFR expression levels of varied cells in real-time, and diverse techniques have been reported in recent decades. The conventional imaging methods including total internal reflection fluorescence microscopy [23,24] and fluorescence resonance energy transfer microscopy [25,26] can easily confirm the existence of the EGFR expression. However, most of the imaging techniques are not able to quantify the levels, and they require fluorescence labeling. The use of fluorescence labeling is accompanied by undesired side effects such as chemotoxicity on cells, photobleaching which disturbs a long-term measurement and label interferences on imaging. The other approaches which can measure the EGFR level are conventional sensing methods based on immunoassay or flow cytometry. Even though those techniques are simple and widely used, they still require minimal labeling and need long assay time. In addition,

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it is difficult to measure dynamic interactions on membrane in real-time.

A capacitance biosensor has been actively investigated as one of the representative label-free, non-invasive and real-time sensing techniques, which can overcome those limitations [27–30]. The capacitance are determined by dielectric properties and charge distributions between two electrodes, thereby the capacitance biosensor has wide availability for measuring various biological interactions with high sensitivity [31]. The capacitance biosensor can also provide morphological and physiological information of target cells with measuring polarizations induced by alternating current electrical fields [32].

In this study, we develop a capacitance sensor which is fabricated with parallel plate geometry to effectively observe the capacitance changes elicited by interactions between EGFR on membrane and EGF protein in real-time. To confirm stability and consistency of the sensor, we first prove correlation between capacitance changes and the number of cells settled down between electrodes, and then differences of EGFR expression levels based on capacitance changes are quantified with varied cancer cells. Those levels are compared to the levels of normal cells which have the same morphological properties to measured cancer cells, respectively. Through numbers of measurements, we confirm that the parallel plate-based capacitance sensor can enhance the sensitivity for EGF-level measurements and save total assay time over a conventional sensing techniques. Consequently, we suggest the proof-of-concept that the parallel plate based capacitance sensor can be an effective sensing system to measure the EGFR expression level with high sensitivity in real time.

2. Experimental

2.1. Fabrication of capacitance biosensor platform

The fabrication process of the proposed capacitance biosensor is schematically illustrated in Fig. S1. The capacitance sensors were simply fabricated by a standard photolithography and subsequent lift-off processes. For this, a 1.4-μm-thick photoresist (PR, AZ5214) mold was first defined on a glass substrate. A 100-nm-thick gold (Au) film was then deposited on the mold substrate after depositing a 5-nm-thick chromium (Cr) adhesion layer using a thermal evaporation technique. After removing the unnecessary portions of the metal film in a sonication bath containing acetone, the 6-channel parallel plate capacitors were obtained A 50-nm-thick insulating silicon dioxide (SiO_2) layer was deposited on the capacitive electrodes using a chemical vapor deposition (CVD) process and subsequently patterned using a buffered oxide etchant (BOE) with photolithographically defined PR etching mask. The distance and overlapped length of the electrode pair are 50 μm and 200 μm, respectively. Finally, an acrylic well, which was purchased from Thermo Fisher Scientific (Lab-tek chamber slide w/cover LOT# 1060336) was attached to the sensor substrate with a polydimethylsiloxane (PDMS, Sylgard 184 kit, Dow Corning) bonding layer for convenient measurements in a liquid environment. The fabricated capacitance biosensor platform was pictured as shown in Fig. S2.

2.2. Sample preparation process for the capacitance measurement

Cells from activated cultures were carefully collected with cell scrapper and centrifuged at 260 × *g* for 5 min and then washed twice with PBS. Cells were redistributed in DMEM solution and incubated in medium for 1 h on a rocker platform to enable regeneration of EGFRs. For the capacitance measurements, the cultured

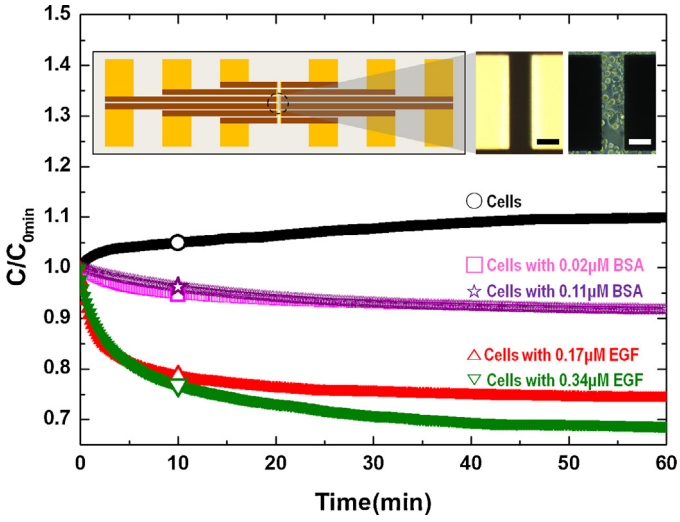

Fig. 1. Time dependence of the capacitance (C/C_0), where C_0 is the initial capacitance, for L-929 cells treated with the different concentrations (0, 0.17 and 0.34 μM) of EGF. For the comparison, the capacitances of cells with the 0.02 and 0.11 μM of BSA were measured. Schematic inset describes 6-channel parallel plate capacitor and the optical image where cells are placed.

cells were kept in PBS buffer during experiments. Prior to measurements, the capacitance sensor was carefully sterilized in ethanol under UV radiation. Then, 250 μl of cell suspension was added to each well and EGF or BSA was added in each well. The time dependent capacitance was measured by using α-analyzer (Novocontrol Co.) at a frequency of 5 kHz with a 0.1 V_{rms}. The electrical connection between capacitance sensor and α-analyzer was performed via silver paste. The capacitance was recorded every 5 min for the single capacitance sensor and maintained for 1–3 h. The fabricated sensor was mounted on an optical microscope (Olympus PME).

3. Results and discussion

Prior to the measurment of capacitance, we checked biocompatibility of the capacitance sensor from electrical stimulations, the cytotoxicity of alternating current (AC) signal were measured with L-929 fibroblast cells using a CCK-8 (modified MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay as shown in Fig. S3. The cells which were exposed to AC signals with 1.5 V for 5 min kept over 90% viability and no dependency on frequency of electrical fields from 0.1 mHz to 10 MHz. In addition, when the cells are placed inside AC electric fields, the cellular dielectric responses are characterized by two major dispersions in the ranges of mHz to GHz, which it is termed α-dispersion (low frequency) and β-dispersion (radio frequency) [33]. To find the characteristic frequency in our sensing system, we measured the capacitance of untreated and treated HDF cells with 0.17 μM EGF at frequencies ranging from 5 kHz to 1 MHz (Fig. S4). We could not find any significant change in frequency-dependent capacitance of untreated HDF cells, whereas the capacitance of treated HDF cells with 0.17 μM EGF was changed in the frequency region around few kilo Hertz. It indicated that the dynamics from the adsorption of EGF were observed in the frequency region around few kilo Hertz. For that reason, we considered the cellular response at the frequency of 5 kHz in following experiments.

Fig. 1 shows the time dependent capacitance of L-929 cell suspension at 5 kHz in absence and presence of EGFs which are intended to attach to EGFRs on cell membrane. The L-929 cells with population from 10 to 50 were settled down between the two gold electrodes of our sensor as shown in the inset of Fig. 1. The sensor measured integrated electrical data from the cells, and