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Short communication

Monitoring change in refractive index of cytosol of animal cells on affinity surface under osmotic stimulus for label-free measurement of viability

Jina Park [a,b], Sung Il Jin [c], Hyung Min Kim [c], Junhyoung Ahn [d], Yeon-Gu Kim [e], Eun Gyo Lee [e], Min-Gon Kim [f], Yong-Beom Shin [a,b,*]

[a] Nanobiotechnology Major, Korea University of Science and Technology, 52 Eoeun-Dong, Yuseong-Gu, Daejeon 305-333, Republic of Korea
[b] Korea Research Institute of Bioscience and Biotechnology, 52 Eoeun-Dong, Yuseong-Gu, Daejeon 305-333, Republic of Korea
[c] Department of Bio & Nano Chemistry, Kookmin University, Seoul 136-702, Republic of Korea
[d] Department of Nano Manufacturing Technology, Nano Convergence Mechanical Systems Research Division, Korea Institute of Machinery and Materials, Daejeon 305-343, Republic of Korea
[e] Biotechnology Process Engineering Center, Korea Research Institute of Bioscience and Biotechnology, 52 Eoeun-Dong, Yuseong-Gu, Daejeon 305-333, Republic of Korea
[f] Department of Chemistry, Gwangju Institute of Science and Technology (GIST), Gwangju 500-712, Republic of Korea

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ABSTRACT

We demonstrated that a metal-clad waveguide (MCW)-based biosensor can be applied to label-free measurements of viability of adherent animal cells with osmotic stimulation in real time. After Chinese hamster ovary (CHO) and human embryonic kidney cell 293 (HEK293) cells were attached to a Concanavalin A (Con A)-modified sensor surface, the magnitudes of cell responses to non-isotonic stimulation were compared between live and dead cells. The live cells exhibited a change in the refractive index (RI) of the cytosol caused by a redistribution of water through the cell membrane, which was induced by the osmotic stimulus, but the dead cells did not. Moreover, the normalized change in the RI measured via the MCW sensor was linearly proportional to the viability of attached cells and the resolution in monitoring cell viability was about 0.079%. Therefore, the viability of attached animal cells can be measured without labels by observing the relative differences in the RI of cytosol in isotonic and non-isotonic buffers.

1. Introduction

Cell viability is a fundamental physiological characteristic of a cell, and has been of general interest in various kinds of cell-based biological research and applications. In general, cell viability has been determined by staining cells with vital dyes such as trypan blue (TB) and Methylene Blue. However, these dyes have some problems that the number of blue-stained cells increases by lapse of time (Jones and Senft, 1985) and determining viability may be inaccurate when viability is $<95\%$. Alternatively, fluorescent dyes such as Acridine Orange (AO), Alcian Blue (Fukudome et al., 2002), Congo Red (Kovács and Foote, 1992), ethidium bromide (EB) (Maria et al., 1997), and SYTOX Green (Breeuwer and Abee, 2000) have been used to assess cell viability. It is impossible to recover cells for further analysis and to continuously monitor over the entire culture procedure because all of these assays are cell invasive and require multiple steps and end-point detection (Hynes et al., 2003).

The release of specific molecules such as ^{51}Chromium (Vennström et al., 2008), iododeoxyuridine (Schneiderman et al., 1991; Gee et al., 1985), [^{3}H] praline (Vázquez et al., 2003), [^{75}Se] selenomethionine, and [^{3}H] uridine (Hu et al., 2000) from cells can also be utilized to measure cell viability, which may be toxic to the cells and limit sensitivity of the assay. Based on cellular metabolic activity, the bioluminescence-based ATP assay using luciferase (Kanges et al., 1984; Cree, 1998) and the MTT/XTT assay (Carmichael et al., 1987) also suffer from the limitation such as the requirement of cell lysis and lower sensitivity, respectively.

An ideal method of measuring cell viability would not use chemicals that are toxic and hinder measurements of cell viability, but remain sensitive enough to measure viability accurately in real time. A number of assays have been developed that allow cell viability to be assessed label-free and noninvasively such as the

* Corresponding author at: Korea Research Institute of Bioscience and Biotechnology, 52 Eoeun-Dong, Yuseong-Gu, Daejeon 305-333, Republic of Korea.
E-mail address: ybshin@kribb.re.kr (Y.-B. Shin).

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assay based on monitoring the metabolic activity of living cells via consumption of oxygen has been developed (O'Riordan et al., 2000). However, it has potential problems such as the self-quenching of reagent at high concentrations and phototoxic effects.

The ultimate objective of our research including this work is development of a method for label-free and real-time measurement of cell viability using a surface-refractometric sensor, overcoming the limitations of existing cell viability assays. In case of using a surface-refractometric sensor, however, target cells should be contacted with the sensor surface for measurement. Nevertheless, this method could be applied to most of cell systems since all cells would sink to contact with the sensor surface due to gravity. In this report, as a preliminary result, we assessed the feasibility of label-freely estimating the viability of animal cells adhering to solid surfaces using a metal-clad waveguide (MCW) sensor in real time by monitoring changes in the refractive index (RI) of cytosol due to osmotic stimuli. A recent study showed that surface plasmon resonance (SPR) can be used to monitor volume changes caused by osmotic stimuli in a confluent monolayer of cells (Robelek and Wegener, 2010). Compared to SPR, the MCW sensor with the extensive penetration depth (>1.5 μm) of the optical field is comparatively efficient for monitoring of changes in cell volume (Zourob et al., 2003). Intentional imbalances between osmolarity of the cytosol and ambient media cause the diffusion of water through the cell membrane and consequent alterations in cell volume and the RI of cytosol, which could be observed only in live cells. We investigated the correlation between the changes in RI of cytosol in cells adhering to a surface measured using the MCW sensor and their viability.

2. Experiments

2.1. Materials

The materials used in this study are as follows: phosphate-buffered saline (PBS), sucrose, poly-L-lysine (PLL), glutaraldehyde, Concanavalin A (Con A), polyvinylpyrrolidone, monobasic sodium phosphate, dibasic sodium phosphate, sodium hydroxide (NaOH), trypan blue (TB), HEDS (hydroxyethyl disulfide) assay kit, the detail information of which is described in Section S-1 of the Supplementary information.

2.2. Cell preparation

The CHO and HEK293 cells were grown in shake flasks (polycarbonate Erlenmeyer flask, Corning, USA) using HyClone SFM4CHO Cell Culture Media and HyClone SFM4HEK293 Cell Culture Media (Thermo Scientific Rockford, USA) with L-glutamine and 2.2 g/L sodium bicarbonate, respectively. The cultures were performed in an ordinary humidified cell incubator at 37 °C and 5% CO_2 with shaking.

2.3. Preparation of sensor chip and cell attachment

The MCW sensor chips were fabricated with the procedure described in the previous studies (Im et al., 2012; Kim et al., 2014), which is depicted in Section S-2 of the Supplementary information in detail. The surface of the fabricated MCW sensor was modified as illustrated in Fig. 1. The sensor chips were immersed in 5 N NaOH for 10 min with sonication and washed with DW. The sensor chips were soaked in 0.01% PLL solution overnight to produce an amine surface on the chip, followed by washing with DW. To create an aldehyde functionalized surface on the sensor chip, the chips were treated with 2.5% glutaraldehyde for 2 h and then washed with DW. The sensor chips were treated with Con A solution (1 mg/mL) for 2 h at room temperature and washed with DW to adhere cells on the sensor surface.

The CHO and HEK293 cell suspensions were diluted to a concentration of 1×10^6 cells/mL with culture medium, and 1 mL of the diluted cell suspensions was dropped on the Con A-modified sensor surfaces and reacted for 10 min. To remove unattached cells, the chip was washed via mild shaking with sterilized PBS three times and kept in the same buffer until measurement. To obtain attached cells with various viabilities, the attached cells were left alone in isotonic buffer at room temperature to starve.

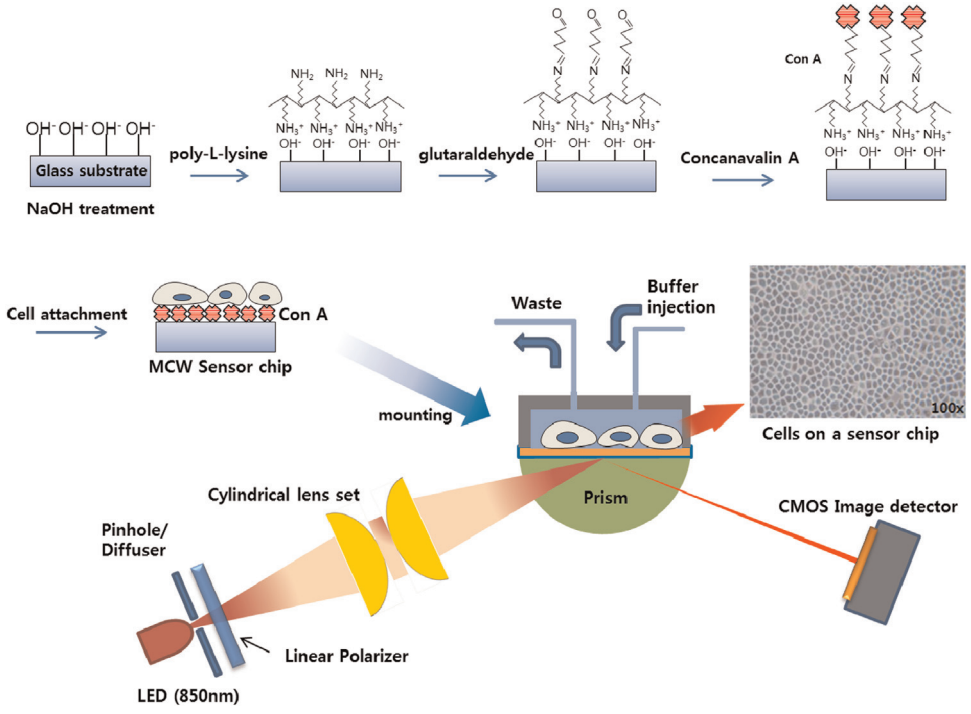

Fig. 1. Procedure of the modification of the sensor surface for attachment of cells and the schematic of the metal-clad waveguide (MCW) sensor system used to measure the change in the refractive index of the cytosol of CHO cells.