QCM DETECTION OF MEMBRANE PROTEIN-LIGAND INTERACTIONS USING CELL-DERIVED LIPOSOMES

Makoto Yamanaka, Takashi Yasuda
Kyushu Institute of Technology, Japan

ABSTRACT

We present a novel QCM (quartz crystal microbalance) sensor for detecting interactions between membrane proteins and their ligands using liposomes derived from human cells. The liposome production is induced by stimulating human lymphocyte cells with NaB (sodium butyrate). Liposomes produced in this method already retain specific membrane proteins that were expressed in the host cells. The membrane proteins on the liposomes maintain their original structures and functions. In this research, we applied the cell-derived liposomes to the detection of membrane protein-ligand interactions as follows. First, liposomes having BCR (B-cell receptor) were derived from human lymphocyte cells, Ramos. Next, the liposomes were separated from the cells by filtration and micronized by sonication. Then, the liposomes were immobilized on the surface of a QCM sensor chip using cell-anchoring molecules. Several experiments demonstrated that ligand detection is achieved by frequency reduction with application of the antibody against BCR.

KEYWORDS

Liposome, Human lymphocyte, Membrane protein, Quartz crystal microbalance, Ligand detection

INTRODUCTION

Most membrane proteins such as receptors and transporters on the surface of a cell play important roles in signal transduction between the inside and outside of a cell and are closely involved in many diseases. In development of novel drugs such as receptor agonists and antagonists, detailed analyses of interactions between membrane proteins and drugs require the membrane proteins to be isolated from cells and transferred onto liposomes and other phospholipid membranes in order to avoid complex cellular responses. However, isolation and purification of membrane proteins require many steps and procedures due to their inherent hydrophobic property and complex structures [1]. Therefore, the conventional method for inserting membrane proteins into liposomes during or after the liposome preparation invites a heavy risk of degeneration or disruption of membrane proteins, which makes it difficult to maintain their original structures and functions. Alternatively, several researchers have succeeded in preparing membrane protein-containing liposomes (proteoliposomes) without isolation and purification of membrane proteins. Akiyoshi's group reported a novel approach for cell-free synthesis of membrane proteins inside giant liposomes [2]. Also, Yoshimura's group proposed a baculovirus-liposome membrane fusion method for reconstituting membrane proteins on liposomes [3]. However, those methods cannot handle human-derived membrane proteins which are required for drug development, and cannot modify liposomes with various lipids such as cholesterol and glycolipid which are also present on cell membranes and significantly affect structure and function of membrane proteins [4, 5].

On the other hand, we previously reported that cell-sized liposomes can be derived directly from cell membranes by stimulating human lymphocytes with NaB in a manner shown in Fig. 1 [6, 7]. Liposomes derived in this method already retain specific membrane proteins that were expressed in the host cells (Fig. 2). The proteins maintain their original structures and functions that are necessary for drug discovery, and the liposomes have the same membrane composition as the host cells. Moreover, if human lymphocytes are transfected with specific genes in advance of liposome derivation, our method can produce liposomes having human membrane proteins with complex structures such as transporters and GPCRs (G protein-coupled receptors). In this research, we applied the cell-derived liposomes to a novel biosensor based on a QCM which detects the binding of biological molecules with the membrane proteins on the liposomes.

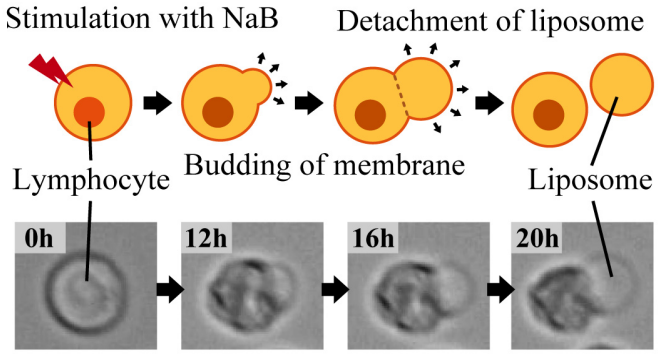

Figure 1. Schematic and photographs of liposome derivation from a human lymphocyte.

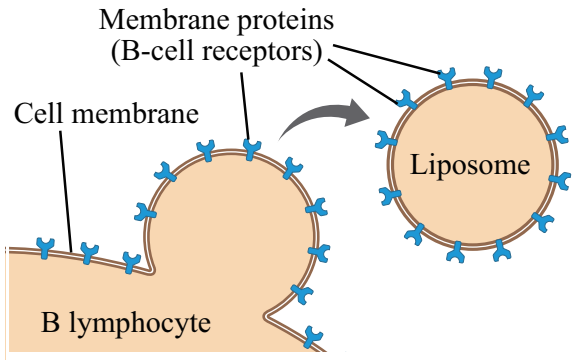

Figure 2. Schematic of transfer of membrane proteins from a cell to a liposome.

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METHOD

The sensor was prepared in the following procedure. BCR (B-cell receptor), which is one of the membrane proteins expressed in human B lymphocytes, Ramos, was used as a sensor probe. First, the liposomes having BCRs on their surfaces were derived from cell membranes of Ramos by chemical stimulation with 10 mM NaB (Fig. 3(a)). Secondly, the liposomes in cell culture medium were separated from the cells with a filter having many pores of 5 μm in diameter (Fig. 3(b)). Then, they were micronized into tiny vesicles smaller than 1 μm in diameter by means of sonication treatment (Fig. 3(c)).

Next, a SAM (self-assembled monolayer) with cysteamine and cell-anchoring molecules (NOF, OE-040CS) was formed on the surface of an Au electrode of a QCM sensor chip (Nihon Dempa Kogyo, NAPiCOS PSA10A). Finally, when the micronized liposomes were seeded on the electrode, they were immobilized on the electrode because the hydrophobic oleyl groups of cell-anchoring molecules bound with the liposome membranes (Fig. 4). Note that if the liposomes were not preliminarily micronized, they would make large mechanical disturbance on the electrode and cause unstable measurement signals. Also, the liposome micronization has the effect of uniformizing distribution in the membrane proteins on the sensor. Thus, the QCM sensor was prepared in order to detect a ligand of BCR.

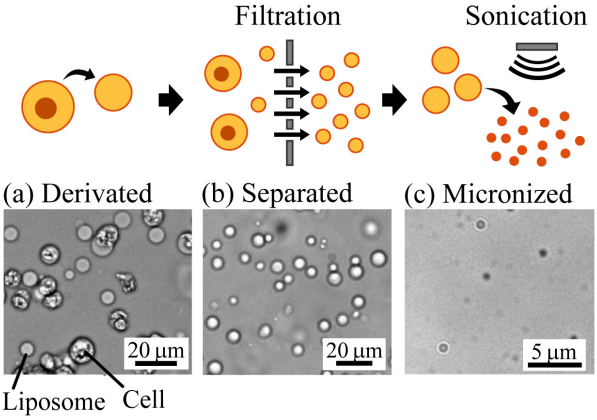

Figure 3. Schematic and photographs of the prepared liposomes.

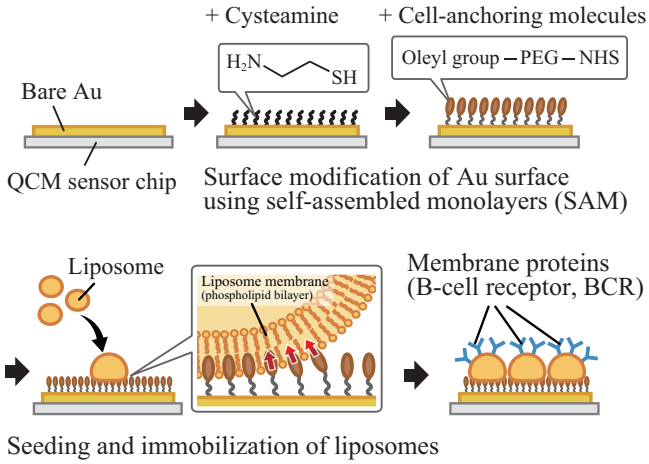

Figure 4. Liposome immobilization on the Au electrode of a QCM sensor.

EXPERIMENT

The detectability of the fabricated sensor was evaluated as follows. The sensor chip was equipped with a microchannel and a sample injector that is used for HPLC (high-performance liquid chromatography) (Fig. 5). BSA (bovine serum albumin) was used as a blocking agent for non-specific adsorption. Also, the anti-BCR antibody (IgG) was used as a ligand of BCR. The solutions including BSA and anti-BCR were injected sequentially into the microchannel for 10 minutes at a flow rate of 5 μL/min and then were reacted to the liposome-immobilized electrode.

As a result, significant reductions of resonant frequency of the sensor were obtained in real time. Figure 6 shows frequency reductions obtained in response to a series of anti-BCR antibody dilutions (10, 20, 30, 40, 60, 80 and 100 μg/mL). In order to characterize the binding ability of anti-BCR (Fig. 7), the mass of the anti-BCR which bound to the membrane proteins was calculated using the Sauerbrey equation which is defined as the following equation:

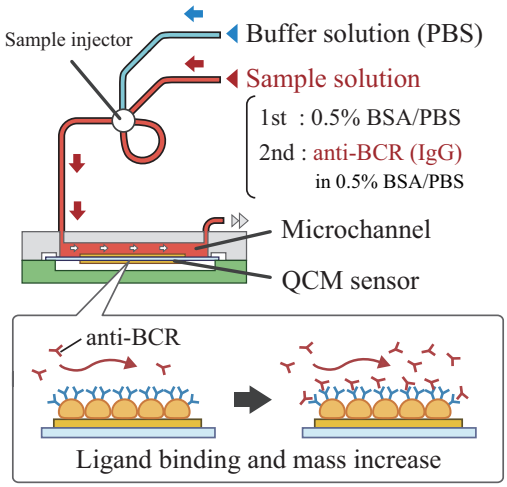

Figure 5. Schematic of the total sensing system.

$$\Delta f = -\frac{2 f_0^2}{A\sqrt{\rho_q \mu_q}} \Delta m = -2.86 \times 10^{10} \Delta m \quad \cdots\cdots\cdots\cdots (1)$$

where Δf is frequency change; Δm is mass change; f_0 is the resonant frequency; A is the piezoelectrically active crystal area; ρ_q is the density of quartz; and μ_q is the shear modulus of quartz for AT-cut crystal.

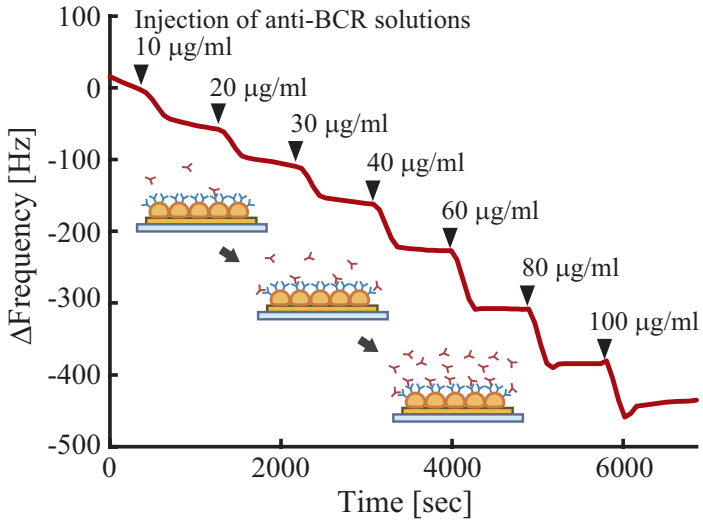

Figure 6. Frequency reduction in response to different concentrations of anti-BCR solutions.

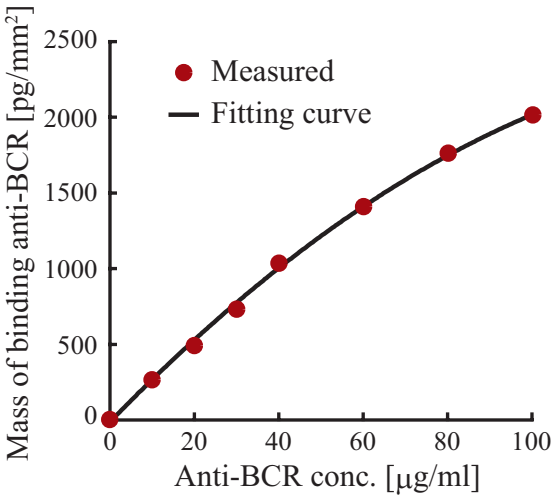

Figure7. Characterization of the binding ability of anti-BCR.

CONCLUSIONS

The present method to produce liposomes from cells permitted membrane proteins to be obtained without their degeneration and disruption. Thus, the QCM sensor having the cell-derived liposomes immobilized on the electrode provided measurement data that were highly relevant with the true binding abilities between membrane proteins and their ligands. In the near future, we will obtain more experimental data using other various membrane proteins which can be expressed on lymphocyte cells by gene transfection. Our sensors will be powerful tools for drug discovery, especially for development of peptide drugs or antibody drugs which affect disease-related receptors.

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CONTACT

Makoto Yamanaka yamanaka@life.kyutech.ac.jp