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Functional Lipid Vesicles Based on Artificial Electric-Taster Sensor

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We have demonstrated the functional lipid vesicles based on artificial electric-taste sensor capable of identifying NaCl, HCl, sucrose and quinine. It used the membrane modified gold electrode with neutral, positive, negative, hydrophilic vesicles. The discriminative character of our taste-sensors has been confirmed by evaluating them against four basic taste solutions using electrochemical method. Each taste sensor was exhibited variations in electric current response when exposed to the four basic taste solutions. The principle component analysis (PCA) on our sensor was clearly recognized against four basic tastes.

Keywords: electrochemical detection; functional lipid vesicles; sensor; tastes

1. INTRODUCTION

The human tongue has the ability to distinguish between the four basic components of taste, sourness, saltiness, sweetness and bitterness. Artificial taste sensor systems, however, are important in various fields related to food and beverage industry; for instance, in maintaining consistent product quality [1–4]. In order to successfully mimic the

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human perception of taste, sensors must be able to discriminate the four basic tastes, exactly. To this point however, a satisfactory taste sensor has not yet been developed. Additionally, human sensory taste discrimination cannot distinguish each chemical material that comes in contact with the cell membrane, but they can respond to suppression effects [5].

The key point is that the proposed artificial taste sensor closely mimics the taste buds on the human tongue, but with much higher sensitivity, selectivity and stability. Recently, Peri *et al.* suggested that the mechanism of signal transduction may be induced by various mechanisms using multilamellar liposomes and taste cells [6]. It is believed that the detection and quantification of taste using artificial sensors may help to gain greater understanding of the complex system for classifying tastants in distinct groups.

Two major types of artificial tastes sensor have been reported which are classified by the sensor materials. In the first, the lipid monolayer was mixed with the various polymers and plasticizers [1]. The second type utilizes conducting polymers as transducers [3]. Although they are able to detect various tastes with a high degree of sensitivity, stability and rapidly, the fabrication process of such a variety of sensor membranes is costly and time consuming.

In our design, special attention has been given to these problems in designing a lipid vesicle sensor substrate that mimics the sensory taste cell membrane, and is useful for practical applications such as advanced artificial taste sensors. Lipid vesicles are relatively easy to prepare on a uniform size scale and modification upon the membranes with various functional ligands and taste receptors such as T1R1 and T1R3 G protein-coupled [7] on the membrane is readily achieved. Therefore, we have fabricated these artificial taste sensors based on amperometric detection with redox probe and altered the properties of the sensor membrane with various lipids. In the study, we have developed a novel electrochemical taste sensor, effective in the discrimination of tastes, utilizing various functional lipid vesicles (FLVs). With this sensor design, we attempt to discriminate the four basic tastes (bitterness, sweetness, saltiness and sourness) using principle component analysis (PCA).

2. EXPERIMENTAL MATERIALS AND METHODS

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dimiristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene-glycol)-2000] (PEG-2000) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA).

Stearic acid (SA), cholesterol (CH) chloroform, and 1-octadecanthiol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). N-(10,12-pentacosadiynoic) acetylferrocene (Fc-PDA) was synthesized via the previously reported method [8]. All other chemicals were of analytical reagent grade.

Four specifications of sensor membranes were prepared using differing lipid properties. The components and properties of the functional lipid vesicle surfaces (FLVs) are summarized in Table 1. The FLVs were fabricated by an extrusion-thaw method [9]. The FLV size was adjusted by extrusion through a polycarbonate filter with 50, 100, and 200 nm pores. The lipid vesicle size was confirmed, prior to immobilization, in all cases by Dynamic Light Scattering method (DLS-700 Ar; Otsuka Electronics Co., Ltd., Japan). A gold electrode array, 800 μm in diameter, was fabricated upon a glass surface utilizing the sputtering method and photolithography technique. Various FLVs were immobilized by incubating a 100 μl portion of the solution on the gold electrode for one hour. The electrodes were then carefully rinsed three times with a phosphate buffer saline (PBS) solution (pH 7.4). The sensor membranes were maintained at 4°C in humid conditions prior to measurement.

To confirm immobilization of FLVs on the gold electrodes, AFM measurements were performed promptly after immobilization. All tapping mode AFM images were obtained using a commercially available instrument (Dimension 3100; Veeco Instruments, USA) without environmental controls. A normal tapping mode silicon cantilever with an oscillation frequency of 350 kHz and a spring constant of 40 N/m (OTESP7; Olympus Optical Co. Ltd., Japan) was used for all AFM imaging. Electrochemical measurements were performed utilizing a BAS 100 B/W potentiostat (Bioanalytical Systems, Inc.)

TABLE 1 The Properties and Lipid Components Utilized in the Multichannel Electrode System

Sensor number	Property	Lipid component (abbreviation) and moral ratio
S1	Negative	POPC:DMPG:CH:FP:ODT = 7:3:1:1:0.1
S2	Positive	POPC:SA:CH:FP:ODT = 7:3:1:1:0.1
S3	Neutral	POPC:CH:FP:ODT = 10:1:1:0.1
S4	Hydrophilic	POPC:PEG:CH:FP:ODT = 7:3:1:1:0.1

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dimirystoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene-glycol)-2000] (PEG), Stearic acid (SA), cholesterol (CH), 1-octadecanthiol (ODT), N-(10,12-pentacosadiynoic) acetylferrocene (FP).

at room temperature. Electrodes consisted of a gold working electrode, a Ag/AgCl reference electrode, and a platinum-wire counter electrode (1 mm). Square-wave voltammetry (SWV) measurements were performed in a solution containing 100 mM PBS solution at a scan rate of 100 mV/s.

3. RESULTS AND DISCUSSION

The performance of each sensor was evaluated by observation and measurement of the current change before and after injection of the various basic taste solutions. The concentrations in some of the basic solutions tested were sub-threshold for human detection, for example, 1 mM of NaCl, sucrose, HCl. However, the solution concentration for bitterness, 0.1 mM quinine, is readily identifiable by humans. Initially, we assessed the immobilized density and cyclic voltammograms of FLVs both in the presence and absence of redox probe (Fc-PDA) utilizing 10 mM KCl as the initial solution. Figure 1 illustrates photographic and typical AFM images of the gold electrode for immobilized FLVs. FLVs were formed directly on the gold electrode, through interaction between the gold surface and thiol groups which allow a highly cohesive bond, and were immobilized as a uniform surface topology in the scan area. The FLV are similar in shape and size to an individual liposome (size: 50 nm).

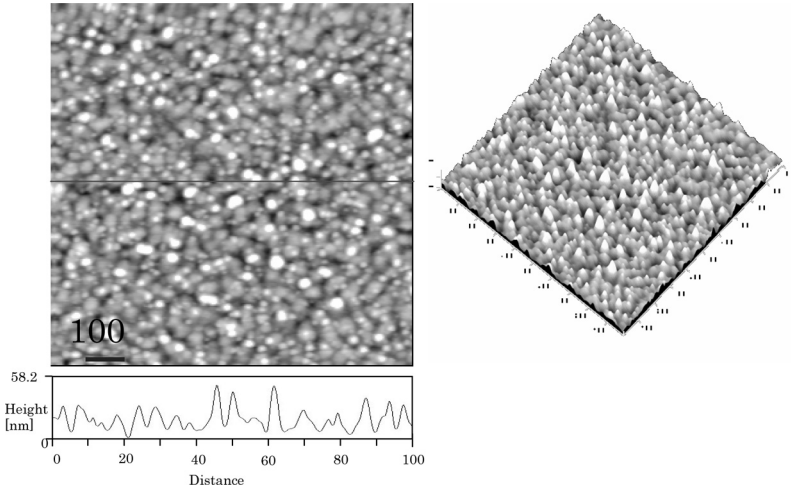

FIGURE 1 AFM topographic images of functional lipid vesicle modified gold electrode. The scan area is $1 \times 1\,\mu m^2$.

We investigated the relational effects of FLV vesicular size upon the experimental data; due to the inverse relationship between sensitivity of the sensor and larger liposome diameter. This is due to the fact that the larger liposome diameter has an inhibitory effect upon the electron transfer from membrane to substrate. Figure 2(a)

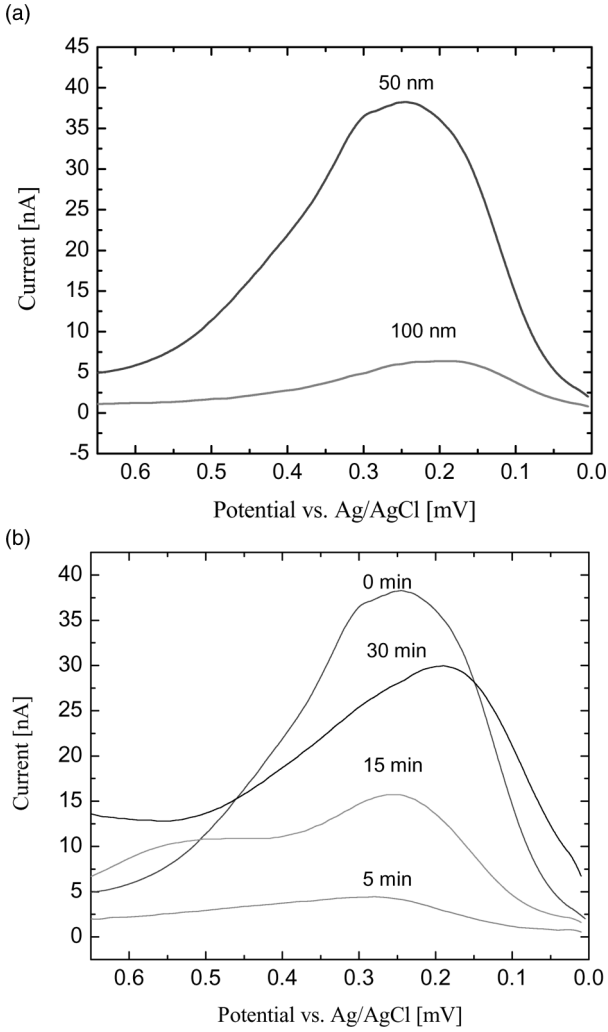

FIGURE 2 (a) The size of lipid vesicles which are prepared with controls of 50, 100, 200 nm diameter and the effect on the electric current responses and (b) the refresh time utilizing the PBS buffer solution with 50 nm lipid vesicles.

displays SWV results obtained depending upon the size of S1 membrane residing on the electrode. The 50 and 100 nm size FLVs presented measurable electric current responses for the initial solution. However, in the case of 200 nm sized FLVs, the electric current responses become negligible (approaching 0) due to the difficulty of electron transfer to the gold substrate. The current has the ability flow through the surface of the membrane to the gold substrate similar to the sol-gel film entrapped liposome electrodes proposed by Stevens *et al.* [14]. That study proposed that electron transport may occur via the lateral diffusion mechanism, on the liposome bilayer membrane and sol-gel film, to a metal electrode via the micro scale cracks within the thin gel film. For this reason, the electron transport of small size FLVs occurred more readily than the larger sizes.

Additionally, we examined the reusability of the testing membrane by using buffer solution rinsing. The S3 sensor membrane may be reused as the electrical signal returned to its initial value when the sensing unit was immersed in PBS. This is dependant upon immersion (refresh) time. Figure 2(b) illustrates the current response changes according to refresh time. We observed that an S1 sensor membrane immersed in PBS for thirty minutes returned to, approximately, its initial value. Therefore, sensor membrane contamination may be diminished, even in cases where the sensors were used to detect an ionic solution (NaCl). This also implies that any electrostatic interaction between the lipid vesicle membrane and taste solutions is a reversible process.

Figure 3 illustrates the type of response obtained for the set of sensors, where S1 carries a negative charged property, S2 carries a positive charged property, S3 is neutral charge, and S4 has a hydrophilic property. The measured current responses were reproducible within ca. 3%, which permitted distinction of and between the various taste solutions. However, it is proposed that the four basic tastes are immediately localized (or bound) to the sensor membranes within one minute because electrochemical measurements were performed for that elapsed time period. These extremely rapid rates of taste accumulation in FLVs may have inhibited the redox reaction. Of special note; the amphipathic taste quinine (bitterness) significantly increases the electrical current response of the sensor membrane.

These results indicate that the poor electron transport has occurred due to quinine strongly binding to the FLVs membrane; because the quinine simultaneously possesses both amphipathic and hydrophobic properties. Conversely, the electric current response to nonelectrolytic substances, such as sucrose, is based upon a different mechanism than

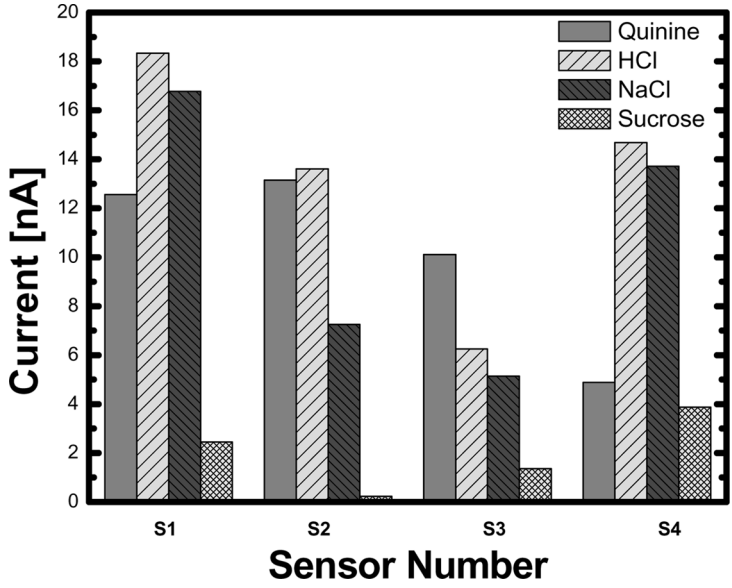

FIGURE 3 Current responses for four basic tastes against various sensor membranes. (a) 1 mM NaCl,; (b) 1 mM HCl; (c) 1 mM sucrose; (d) 0.1 mM quinine.

electrolytic taste substances. There is no significant change in the current response attributable to the translocation of sucrose through liposomal membrane [6]. However, differing responses from various sensors can be obtained when applied against the four basic tastes. We can discriminate the four basic tastes to graphical description utilizing principle component analysis (PCA). PCA is a statistical technique for the reduction of input data dimensions, and is largely used for feature extraction. It captures the relevant information in a set of input data providing a lower (but informative) dimension representation of the original data. The PCA results in Figure 4 clearly demonstrate the capability of the artificial taste sensor in distinguishing four basic tastes. However, at this time, we are unable to provide a cogent hypothesis by which to explain exactly what electrochemical mechanism occurs between the vesicular membrane and taste solution at the molecular level. However, this FLVs sensor design allows us to take advantage, in practical use, of its superior performance in detecting given materials pertaining to specific types of taste.

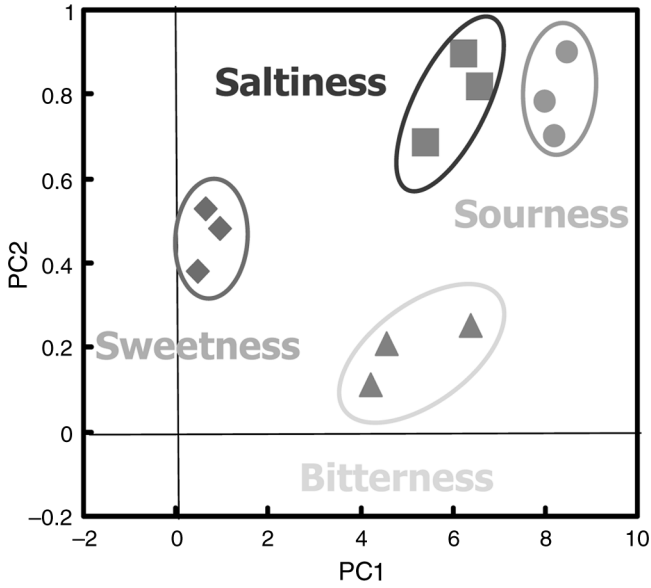

FIGURE 4 Principle component analysis plot of various sensor membranes composed of neutral, positive, negative and hydrophilic for the four basic tastes.

4. CONCLUSIONS

Higher sensitivity to the four basic tastes was successfully achieved by utilizing a novel design for a FLVs based on taste sensor. The sensor design, proposed herein, possesses the ability to perform measurable discrimination between tastes simply, rapidly and with high reproducibility. Additionally, the proposed FLVs design displayed responses superior to human sensory threshold evaluations. The FLVs is an artificial model which mimics the function of human tongue sensory cells. The design model itself is easily modified utilizing various materials such as specific receptor proteins on the human tongue.

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