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A Study of Vision Biomembrane Assembly using Photoreactive Protein Adsorbed Polypyrrole Film

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ABSTRACT

A protein based vision biomembrane was fabricated by adsorbing bacteriorhodopsin into electrochemically polymerized polypyrrole film substrate mainly through strong electrostatic interaction. The immobilized bacteriorhodopsin on the polypyrrole film was demonstrated by SEM and SRET. The light signal transducing function from the bacteriorhodopsin which was adsorbed into polypyrrole film was evaluated by electroretinogram(ERG). A wave form analysis of the electroretinogram indicated that the adsorbed bacteriorhodopsin retained its activity and light signal was obtained from the protein for at least one month.

Keywords: Vision biomembrane, Polypyrrole, Bacteriorhodopsin, Electroretinogram

1. INTRODUCTION

The successful development of a vision membrane would be a tremendous breakthrough for the treatment of the visually impaired people. Partial success has already been achieved by using semiconductors to create a non-biological artificial retina, however, this structure proved to be too complex to process functions inherent in a biological retina, accordingly, the resolution was too low[1].

Several research efforts are currently focusing on the development of a biological vision membranes employing photoreactive proteins such as bacteriorhodopsin[2-5]. Bacteriorhodopsin is a light harvesting protein possessing a number of intrinsic photoelectrical properties. It functions in the same manner as the human retinal protein rhodopsin, and can be extracted from the purple membrane of the *halobacterium halobium*. It is photochemically robust, environmentally stable, and possesses intrinsic photoelectrical properties that can produce an appropriate material for photovoltaic imaging devices[3]. A previous study with bacteriorhodopsin in a solution state retain their activity and exhibit differential responsivity, edge enhancement and motion detection[2].

The current study attempted to immobilize bacteriorhodopsin into solid-state conducting polymer polypyrrole and then evaluated whether the assembled membrane retained its visual function. Polypyrrole can transduce an electrical signal due to its electrical conducting characteristics[6]. The bacteriorhodopsin can be adsorbed into positively charged polypyrrole film mainly via strong electrostatic interaction[7].

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2. MATERIALS AND METHODS

2.1. Biomembrane assembly

Polypyrrole synthesis: The electrochemical polymerization of polypyrrole was performed in a two compartment cell with power supply(AFRDE5 Bi-potentiostat). The cell was equipped with a platinum rod cathode, platinum anode with a front side area of 35 cm^2 and saturated calomel electrode(SCE) as reference electrode. A reaction vessel contained 200 ml of a 0.2 M solution of distilled pyrrole(reagent grade, Aldrich) holding 0.2 M tetraethylammonium p-toluenesulfonate (TEA p-TS)(reagent grade, Aldrich) electrolyte in acetonitrile (HPLC grade, Sigma Chemical). Pyrrole was distilled at 130℃ to a colorless and purified form within 30 minutes prior to electropolymerization. The current density was held constant at 1.0 mA/cm^2. The reaction period was 4 hours. The film was first rinsed, soaked in pure acetonitrile, for one hour, then peeled off with a razor blade and tweezers. The free standing film was then soaked in 50 mL of pure acetonitrile for about 24 hours. Polymers were cut into 1 cm×1 cm with a razor blade. Before being used in the bacteriorhodopsin adsorbing, the samples were allowed to dry in air, placed on weighing paper and stored in polystyrene Petri dishes in the dark.

Biomembrane preparation by adsorbing bacteriorhodopsin into polypyrrole: In this experiment the bacteriorhodopsin-embedded purple membrane(PM) purchased from Sigma Chemical was used as a source of bacteriorhodopsin. One mg of the bacteriorhodopsin-embedded purple membrane fragments were suspended in 1 ml phosphate buffered solution(PBS:Dulbecco, pH:7.4) at a well of 24 well culture plate. A $1cm^2$ section of polypyrole film was then placed in the bacteriorhodopsin/PBS solution for 24 hours at 4 ℃ and the plate covered to prevent any evaporation. This time period also ensured the physical adsorption of the bacteriorodopsin into the polypyrrole film. Thereafter, the polymer film was gently rinsed twice with 1 ml PBS to remove any non-adsorbed protein, dried in air and finally stored in a Petri dish until further evaluation.

2.2. Characterization

Film thickness: The thickness of free standing polypyrrole film was measured by a micrometer (Mitutoyo, Japan) and was 60±1 μm.

Electrical conductivity: The electrical conductivity of polypyrrole was measured over one month using 4-point probe system(SR 100E, Changmin Tech).

Surface morphology: The surface morphologies of the polypyrrole and bacteriorhodopsin adsorbed polypyrrole were examined by scanning electron microscope (SEM S-2500 Hitachi; 15KV) after coating the samples with gold at 7mA for 4 minutes to 200Å film thickness .

Scanning Reference Electrode Technique (SRET): The Scanning Reference Electrode Technique(SP100 SRET) was used to evaluate the presence of bacteriorhodopsin in the polymer film. The SRET enables the imaging and quantification of real time localized electrochemical activity by measuring changes in micro-electrochemical activity in proximity of the specimen surface.

Electrophysiological test by electroretinogram: The electrophysiological studies of the biomembrane evaluated the wave deflection, amplitude and implicit time of the major components using an electroretinogram[8,9]. An electroretinogram in response to a short light stimulation consists of a series of negative and positive deflections. Studies made during the course of dark adaptation and variations in wave lengths or stimuli has provided a duplicity in the positive deflections(B waves). The use of higher flash intensities gave evidence of similar duplicity in the negative component(A waves)[10,11]. A silver

needle electrode(Grass, Model type E2) was attached to the anterior side of the polypyrrole membrane, a reference electrode to the posterior side, and a ground electrode to the plastic disc-plate where polypyrrole membrane was located. Light source(Xenon flash(Nihon Kohden, Model SLS-400)) was located 30 cm front of the polymer and flashed 8 times. The wave deflections in response to the light stimulation were amplified with an amplifier(Nihon Kohden, VC-10) and recorded on photographic film. After dark adaptation for 20 minutes, the biomembrane was exposed to different light intensities of 0.6 J, 2 J, 20 J with a stimulation interval of 2 seconds, duration time of 5 msec, and band filter 0.5~300 Hz. The sample biomembrane was measured for one month and the resulting waveforms were recorded to compare the amplitudes.

3. RESULTS AND DISCUSSION

3.1. Surface morphology of biomembrane

The surface morphologies of pure polypyrrole and bacteriorhodopsin adsorbed polypyrrole were observed with SEM. Their surface roughness reveals distinct difference as shown in Figure 1. The pure polypyrrole surface morphology of Figure 1(a) shows the typical nodular polypyrrole surface having abundant grooves, channels and craters. When the pyrrole was adsorbed with bacteriorhodopsin the characteristic texture became different being devoid of deep channels and smoother after covered with proteins as shown in Figure 1(b). The presence of proteins can be observed on the surface of the pyrrole film. The micrographs demonstrate the bacteriorhodopsin is physically well adsorbed on the surface of polypyrrole membrane.

3.2. Conductivity measurement of biomembrane

The conductivity of the polypyrrole measured by four point probe was ranged from 0.1 to 0.25 S/cm and remained stable for one month.

3.3. SRET evaluation of biomembrane

When observed using the SRET, the bacteriorhodopsin adsorbed polypyrrole exhibited a phase-separated surface, thereby indicating different electrical properties when the protein is adsorbed compared to when it is not (data not shown). This suggests that bacteriorhodopsin exists in a discontinuous pattern on the surface of polypyrrole film.

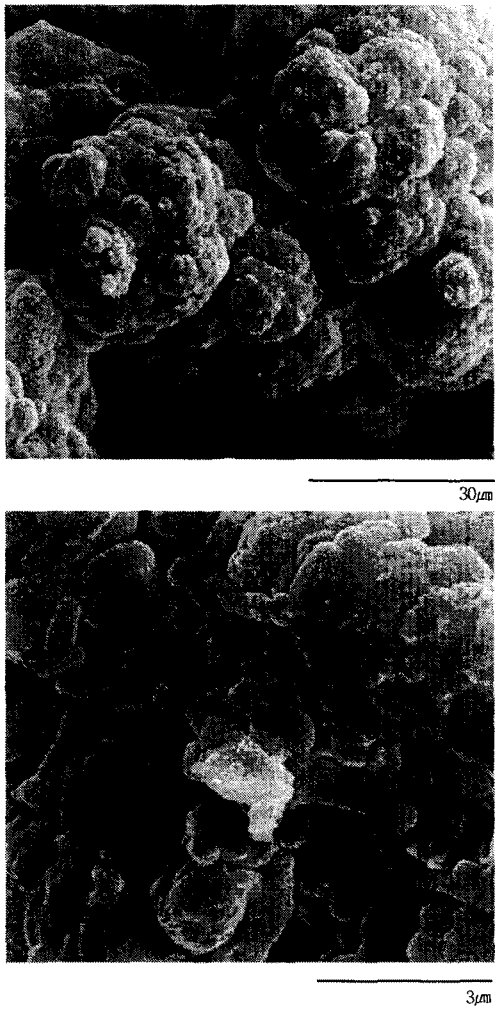


Figure 1. Scanning electron micrographs of surface morphology (a) polypyrrole (b) bacteriorhodopsin adsorbed polypyrrole

Figure 1(a) shows the pure polypyrrole surface morphology. The grooves and channels on the polypyrrole surface became filled up and smoother after being adsorbed with proteins as shown in Figure 1(b).

3.4. Electroretinogram test of biomembrane

In the initial electrophysiological test, a negative impulse was observed after a light stimulus, and the larger the light impulse the larger the resultant amplitude. In the ensuing test, similar impulses were observed yet no significant variance in the amplitude as shown in Table 1 and Figure 2. The result demonstrated that the proteins adsorbed on the polypyrrole film were stable for a period of one month.

Table 1. Data of wave amplitude(μV) which is generated from bacteriorhodopsin adsorbed polypyrrole obtained by electroretinogram

date	intensity of stimulus light		
	0.6J	2J	20J
1 day	17	68	600
30 days	12	27	450

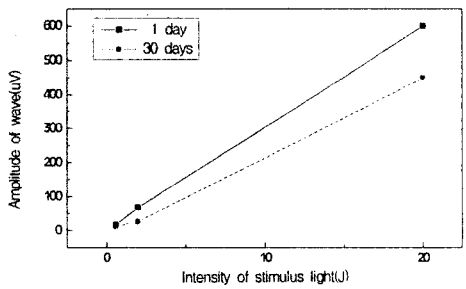
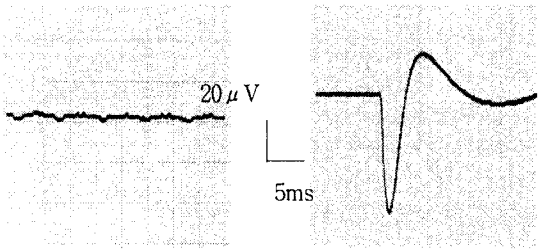


Figure 2. Amplitude(μV) of wave which is generated from bacteriorhodopsin adsorbed polypyrrole obtained by electroretinogram

The wave forms resulting from the power stimulation of 0.6 J, 2 J, 20 J were similar to those obtained from human rhodopsin(Figure 3). The amplitude was proportional to the stimulation power. In the electrophysiological test, a characteristic wave form was obtained from bacteriorhodopsin adsorbed polypyrrole film, indicating that the adsorbed protein retained its photoresponsiveness, and this function remained stable over one month.



(a) (b)

Figure 3. Wave forms generated from films obtained by electroretinogram (a) polypyrrole film (b) bacteriorhodopsin adsorbed polypyrrole film

4. CONCLUSION

In an attempt to recreate a retinal membrane, a vision biomembrane was fabricated by adsorbing bacteriorhodopsin, which functions in the same manner as the human retinal protein rhodopsin, into a polypyrrole substrate. The light signal transducing function from the adsorbed bacteriorhodopsin into the conducting polymer membrane was evaluated by an electroretinogram. The wave analysis by electroretinogram indicated that the adsorbed bacteriorhodopsin retained its activity and an effective light signal was obtained from the protein, as shown in human retina. To achieve a more stable biomembrane, it is necessary to increase both the binding force and specific binding between the bacteriorhodopsin and conducting polymer substrate. Therefore, the biotin-avidin binding system can be utilized in the biotinylation of the protein and polymer[12]. These findings demonstrated that the functionality of photoreactive proteins adsorbed into thin

conducting polymer membranes can be maintained over an extended period of time. Accordingly, protein-immobilized biomembrane systems may be suitable for further application in a vision functioning membrane.

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