Synthesis and Light-Induced Surface Potential Observation of Retinal Prosthesis Using Polyethylene Thin Films Immobilized with Photoelectric Dyes

Tetsuya Uchida*, Makoto Nitta, and Syoko Kanashima

Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-naka, Kita-ku Okayama 700-8530, Japan
tuchida@cc.okayama-u.ac.jp

Photoelectric dyes that absorb light and convert photon energy to electric potential have previously been shown to stimulate retinal neurons in cultures. As such, we have designed a new type of retinal prosthesis using polyethylene films coupled with photoelectric dye molecules. In this study, the electric potentials on the film were observed using a scanning Kelvin probe microscope under light and dark conditions. As a result, electric potentials were observed on the film, which were dependent on light intensity and wavelength. Furthermore, the electric potentials showed a rapid on–off response to light.

Keywords: retinal prosthesis, photoelectric dye, surface potential

1. Introduction

Photoreceptor cells in the retina initiate vision by altering electric potentials through absorption of light. We designed the present study to investigate the feasibility of utilizing dye-immobilized thin films to develop artificial thin films (retinal prosthesis) that are biologically compatible with the functions of photoreceptor cells. To prepare a dye-immobilized thin film, we attempted to chemically immobilize a photoelectric dye [1] on a polyethylene (PE) thin film and cause an electric potential difference in the molecules through light absorption. However, it was difficult to chemically modify the surface of a thin film as PE is chemically stable. Therefore, we developed a new method for selectively introducing carboxyl groups to the molecular chain by folding portions of PE and immobilizing a photoelectric dye (Figure 1) [2–4].

We previously demonstrated that blind mice with a disease related to photoreceptor cells regained vision after insertion of this dye-immobilized thin film [4]; thus, in this study, we investigated the feasibility of human clinical trials.

![Figure 1. Synthesis of dye-coupled polyethylene film](image)

The synthesis of the dye-immobilized thin film was optimized for this study. Furthermore, we clarified the requirements for using thin films as retinal prostheses to quantitatively evaluate the properties of prepared samples.
2. Experimental

2.1. Preparation of a dye-immobilized thin film [4]

2.1.1. Preparation of a PE thin film

A Teflon film was placed on an aluminum plate and high density polyethylene powder (mol. wt. $1.5 \times 10^5$) 30mg was placed in the center. The plate was then sandwiched between another aluminum plate and Teflon film. Vacuum pressing was implemented at 160°C using a vacuum heating press (IMC-11FD, Imoto machinery Co., Ltd.). After pressing, the sample on the aluminum board was rapidly cooled in ice water to create a crystallized PE thin film (thickness: $30 \pm 5 \mu m$).

2.1.2. Fuming nitric acid treatment

The crystallized PE thin film and fuming nitric acid (100 mL) were placed in a four-neck flask with a Dimroth condenser. The flask was placed in an oil bath at 80°C. The treatment time was varied between 4 and 16 min with 2-min intervals. The treated thin film was washed with distilled water and dried for 24 h.

2.1.3. Diamine modification

The thin film treated with fuming nitric acid was immersed in a stoppered flask containing chlorobenzene (75 mL), ethylenediamine (2.6 μL), and N,N'-dicyclohexylcarbodiimide (DCC, 8.25 mg). The contents were stirred and reacted at 50 rpm for 48 h using a shaking water bath (NTS-4000AM, EYELA) at 35°C. After reaction, the thin film was washed with distilled water and dissolved in vacuo.

2.1.4. Dye immobilization

After chlorobenzene (75 mL) was placed in a stoppered flask, a photoelectric dye (2-[2-[4-(dibutylamino)phenyl]ethenyl]-3-carboxymethylbenzothiazolium bromide, NK-5962, 20 mg) and DCC (8.25 mg) were added, which was followed by dispersion through ultrasonic irradiation for 10 min using an ultrasonic bath (2510J-MT, BRANSON). The diamine-modified thin film was then immersed in the flask. The contents were stirred and reacted at 50 rpm for 48 h using a shaking water bath at 35°C. After the reaction, the thin film was washed with water and chlorobenzene. Then, the thin film was dried in vacuo.

2.2. Physical property tests

2.2.1. Tensile tests

The tensile tests on the thin film treated with fuming nitric acid as well as the dye-immobilized thin film was conducted using a tension and compression testing machine (SV-201NA, IMADA-SS Co.).

2.2.2. Measurement of ultraviolet-visible (UV-Vis) absorbance

The absorbance of the dye-immobilized thin film was measured using an ultraviolet-visible spectrophotometer (U-1900, HITACHI). Furthermore, the absorbance of the dye solution (methanol) adjusted to an arbitrary concentration was determined to create standard curves. The density of dye on the thin film surface was calculated according to the absorbance of the dye-immobilized thin film and its standard curves.

2.2.3. Measurement of infrared absorption (IR) spectra

The IR spectra of the thin film treated with fuming nitric acid and the dye-immobilized thin film were measured using an infrared spectrophotometer (IRAffinity-1, SHIMADZU). The resultant IR spectra were used to observe chemical modification of the thin films and evaluate the amount of introduced functional groups.

2.2.4. Contact angle measurement

Water contact angle of the dye-immobilized thin film was measured with a contact angle gauge (CA-D type, KYOWA INTERFACE SCIENCE).

2.2.5. Measurement of light-induced surface potentials

2.2.5.1. Measurement using surface potential microscopy (SPoM)

The surface potentials of the dye-immobilized thin film were measured using a scanning probe microscope (Nanoscope IIIa Interleave Mode, Digital Instruments) and probe for SPoM (Point Probe®, Nano World). To observe the height information and electric potentials, atomic force microscopy (AFM) and SPoM of Nanoscope IIIa
were respectively used.

2.2.5.2. Measurement using a scanning Kelvin probe (SKP)

The surface potentials of the dye-immobilized thin film were measured using a scanning Kelvin probe (SKP5050, KPTechnology). The strength and wavelength of the light irradiation were controlled by a surface photovoltage spectroscopy (SPS) module (SPS040, KPTechnology).

3. Results and Discussion

3.1. Consideration of fuming nitric acid treatment time

Figure 2 shows IR spectra of the crystallized thin film treated with fuming nitric acid at 4, 14 and 16 min.

The carboxyl group peak at 1710 cm$^{-1}$ ($\text{ACOOH}$) increased with increasing fuming nitric acid treatment time. We evaluated the amount of introduced carboxyl groups according to this peak and the PE methyl group peak at 1460 cm$^{-1}$ ($\text{AMe}$). Table 1 lists the absorbance and ratios of carboxyl and methyl groups of the thin film treated with fuming nitric acid. The results are indicated by treatment time.

As shown in Table 1, the absorbance and amount of introduced carboxyl groups increased in proportion to reaction time.

We also examined the mechanical characteristics of the thin film treated with fuming nitric acid. Figures 3–6 show the relationship between treatment time and mechanical characteristics, i.e., elastic modulus, yield strength, rupture strength, and rupture elongation from the tensile tests.

| Treatment time (min) | Absorbance $\text{ACOOH}$ | Absorbance $\text{AMe}$ | Absorbance ratio ($\text{ACOOH}/\text{AMe}$) |
|---------------------|---------------------------|-------------------------|------------------------------------------|
| 4                   | 1.00                      | 30.52                   | 0.033                                    |
| 6                   | 1.04                      | 32.16                   | 0.032                                    |
| 8                   | 1.10                      | 29.99                   | 0.037                                    |
| 10                  | 1.12                      | 25.25                   | 0.044                                    |
| 12                  | 1.15                      | 25.14                   | 0.046                                    |
| 14                  | 1.24                      | 21.82                   | 0.057                                    |
| 16                  | 1.46                      | 19.94                   | 0.073                                    |

Figure 2. IR spectra of the PE thin film treated with fuming nitric acid

Figure 3. Relationship between elastic modulus and fuming nitric acid treatment time of the PE thin film

Figure 4. Relationship between yield strength and fuming nitric acid treatment time of the PE thin film
Rupture elongation was found to be the mechanical characteristic most significantly related to treatment time. The relationship among the rupture elongation, absorbance ratios, and fuming nitric acid treatment time are shown in Figure 7.

When fuming nitric acid treatment time was increased, the amount of introduced carboxyl groups increased, while the rupture elongation decreased. Consequently, the thin film became brittle. It is considered preferable to contain many carboxyl groups, as they can act as a means for introducing dyes. Therefore, we examined the mechanical characteristics of the thin film in more detail by creating stress–strain curves of 14 and 16 min treatments (Figure 8).

The thin film treated for 14 min showed a necking phenomenon induced by deformation of polymer chains, whereas the thin film treated for 16 min was not extended at all. In other words, the thin film treated for 16 min was very brittle. Accordingly, we considered 14 min to be the best
treatment time, as the film had the most amount of carboxyl groups, thus maintaining the mechanical characteristics of the polymer material.

3.2. Examination of amount of immobilized dye

IR spectra of the thin film were measured after fuming nitric acid treatment, diamine modification, and dye immobilization to examine the reaction on the thin film surface. The IR spectra are shown in Figure 9.

The thin film treated with fuming nitric acid showed a carboxyl group peak at 1710 cm\(^{-1}\). After the diamine-modification, the carboxyl group peak was decreased. This may be because some carboxyl groups were converted to amino groups upon diamine modification. Regarding the dye-immobilized thin film, absorption caused by C–N stretching in the photoelectric dye (NK-5962) was observed at 1520 cm\(^{-1}\). In other words, the photoelectric dye was selectively coupled to carboxyl groups via ethylenediamine.

Figure 10 shows the UV-Vis spectrum of the dye-immobilized PE thin film.

The peak showing dye absorption was detected at 539 nm. The absorbance of the dye in the methanol solution adjusted at predetermined concentrations was measured. The standard curve created is shown in Figure 11.

The density of dye on the dye-immobilized thin film calculated from the standard curve was \(4.7 \times 10^{-8}\) mol/cm\(^2\), i.e., there were more than 100 trillion dye molecules per square millimeter.

3.3. Examination of biological compatibility according to water contact angle

Water contact angle of the dye-immobilized thin film was 80.4° ± 1.4°. Since the best angle for cell growth is 75°–80° [5], the prepared dye-immobilized thin film was considered biologically compatible.

3.4. Performance evaluation according to light-induced surface potentials

3.4.1. Light-induced surface potentials measured by SPoM

Figure 12 shows an image of the dye-immobilized thin film observed in the phase mode of SPoM.

![Figure 10. UV-Vis spectrum of dye-immobilized PE thin film](image1)

![Figure 11. Standard curve of the dye (NK-5962)](image2)

![Figure 12. SPoM image of the dye-immobilized thin film. (Phase mode under light)](image3)

![Figure 13. SPoM image of the dye-immobilized thin film. (Phase mode under light irradiation and interception)](image4)
As shown in the picture, electric potentials were generated over the entire surface of the dye-immobilized thin film. We also examined surface potentials varied by light. Figure 13 shows the result under light irradiation and interception.

Phase differences became large under light irradiation and small under light interception. This variation was observed immediately after light switching. This suggests that dye-immobilized thin films are very responsive to light.

Furthermore, surface potentials were quantitatively measured in the potential mode. Figure 14 shows an image of the dye-immobilized thin film observed in the potential mode of SPoM.

The electric potentials varied by several hundred mV after light switching. The photoreceptor cells of the human eye vary by only several ten mV of electric potential after light absorption [6]. Therefore, we consider that dye-immobilized thin films meet the criteria of retinal prosthesis in terms of electric potential.

3.4.2. Light-induced surface potentials measured by SKP

3.4.2.1. Light intensity dependence of light-induced surface potentials

Light-induced surface potentials were measured by changing the light intensity. The results are shown in Figure 16.

Figure 16. Light intensity dependence of the light-induced surface potentials of the dye-immobilized PE thin film

As shown, the surface photovoltage was dependent on light intensity. The surface photovoltage increased when the light intensity increased. The electric potentials reached 100 mV around 300lx (light intensity = 2500). The measurement was conducted with a light intensity...
of 300lx.

3.4.2.2. Light responsivity of light-induced surface potentials

We examined the light responsivity of the dye-immobilized thin film by switching light irradiation and interception. The result is shown in Figure 17.

![Figure 17](image1.png)

Figure 17. Light responsivity of light-induced surface potentials

Surface potentials were generated immediately upon switching from dark to light and reduced to zero in the reverse case. This proves that dye-immobilized PE thin films are highly responsive to light.

3.4.2.3. Dependence of light-induced surface potentials on amount of immobilized dye

We measured light-induced surface potentials of the dye-immobilized thin film with different dye concentrations (Figure 18).

![Figure 18](image2.png)

Figure 18. Dependence of light-induced surface potentials on amount of immobilized dye

Surface potentials increased when the amount of dye immobilized to the PE thin film surface increased. This demonstrated that light-induced surface potentials increase in proportion to amount of immobilized dye.

3.4.2.4. Wavelength dependence of light-induced surface potentials

Light-induced surface potentials were measured by changing the wavelength of light irradiation. The result is shown in Figure 19.

The peak of the wavelength was observed in the same position as the wavelength of the absorbance of the dye-immobilized thin film. This indicated that electric potentials are not generated outside visible light regions.

4. Conclusion

The present study demonstrated successful chemical immobilization of a photoelectric dye on a PE thin film, examination of the IR spectra of these films, optimization of fuming nitric acid treatment time based on the results of the IR spectra and tensile tests, and calculation of the density of dye on the dye-immobilized thin films by UV-Vis spectrophotometry.

We also measured water contact angle of the dye-immobilized thin films to examine their biological compatibility.

SPoM was used for observation of the potentials, from which we found that electric potentials were generated over the entire surface of the dye-immobilized thin film. Furthermore, we proved their high light responsivity by switching light irradiation and interception, measured surface potentials of the prepared dye-immobilized thin film using SKP, and dependence on light intensity, amount of immobilized dye, and wavelength.

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