

Immobilization of Photoelectric Dye on the Polyethylene Film Surface

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PE film was treated with fuming nitric acid at 80 °C for 20 min, resulting in introduction of COOH moieties on the film surface. The COOH's were reacted with ethylenediamine, whose amino groups were used for linking with (2-[4-(dibutylamino)phenyl]ethenyl)-3-carboxy-methylbenzo-thiazolium, photoelectric dye (NK-5962), which absorbs visible light and converts the photon energy to electric potentials. The dye molecules were immobilized on the PE film surface and they were able to stimulate chick retinal tissues on incidence of visible light. These facts hopefully lead to development of an artificial retinal prosthesis.

1. INTRODUCTION

Blindness diseases, such as hereditary retinitis pigmentosa or age-related macular degeneration, cause progressive degeneration of photoreceptor cells. But in these cases, the other retinal neurons and circuits are still alive and capable of fulfill their functions [1]. Based on these clinical findings, a number of research groups are

developing electrical implants that can be attached directly to the retina in an attempt to restore vision to patients suffering from retinal degeneration, using photodiodes as photoreceptor cells [2-4]. However there are several problems with the implants, i.e., their bulk around eye balls, poor biocompatibility of the constituent materials, low resolution in imaging and

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necessity of extra energy supply to the implant. One of the authors Matsuo has been measuring interaction between retinal tissues and various kinds of photoelectric dye molecules under visible light. Finally he has screened several dye molecules which have functions of the photoreceptor cells, and proposed that they may be better candidates for artificial photoreceptors in the future development of the retinal prostheses [5].

In this study, we have studied how to use the dye molecules effectively as a photoreceptor in the development of the retinal prostheses. For this purpose, the dye molecules have to be settled behind the retinal tissue in the eye ball. We chose polyethylene thin films as a substrate for the attachment because they are flexible and chemically stable, i.e., feasible to insert behind the retinal tissue and biocompatible. Among the screened dye molecules, we adopted (2-[4-(dibutylamino)phenyl]ethenyl)-3-carboxy-methylbenzo-thiazolium, (photoelectric dye, NK-5962, Fig.1), as a photoreceptor molecule. Although this chemical stability made the immobilization of the dye molecules difficult, the fuming nitric acid treatment enabled us to introduce COOH groups on PE film surface [6], which worked as anchors

for the dye molecules. Thus we made the dye-immobilized film and tested in vitro whether it was capable of stimulation of the retinal tissues on incidence of light.

2. EXPERIMENTAL

High density polyethylene (Sholex 6009) was melt at 180 °C and extruded from a circular orifice into air, while blowing air into a hole in the center of the orifice to maintain the film at the extrusion die diameter. Crystallization proceeded under elongational stress by drawing the extrudate to about 56 times. Thickness of resultant film (PE-EB film) was ca. 0.02 mm. Then the film was treated with fuming nitric acid at 80 °C for 10, 20, 25 minutes respectively, washed with water and dried in air [6]. Then the film was reacted with ethylenediamine in chlorobenzene in the presence of dicyclohexylcarbodiimide (DCC) at 35 °C for 48 hrs [7]. After the reaction, the film was washed with chlorobenzene and dried in air. The resultant film with NH₂ moiety on the surface was reacted with 2-[4-(dibutylamino)phenyl]ethenyl]-3-carboxymethyl-

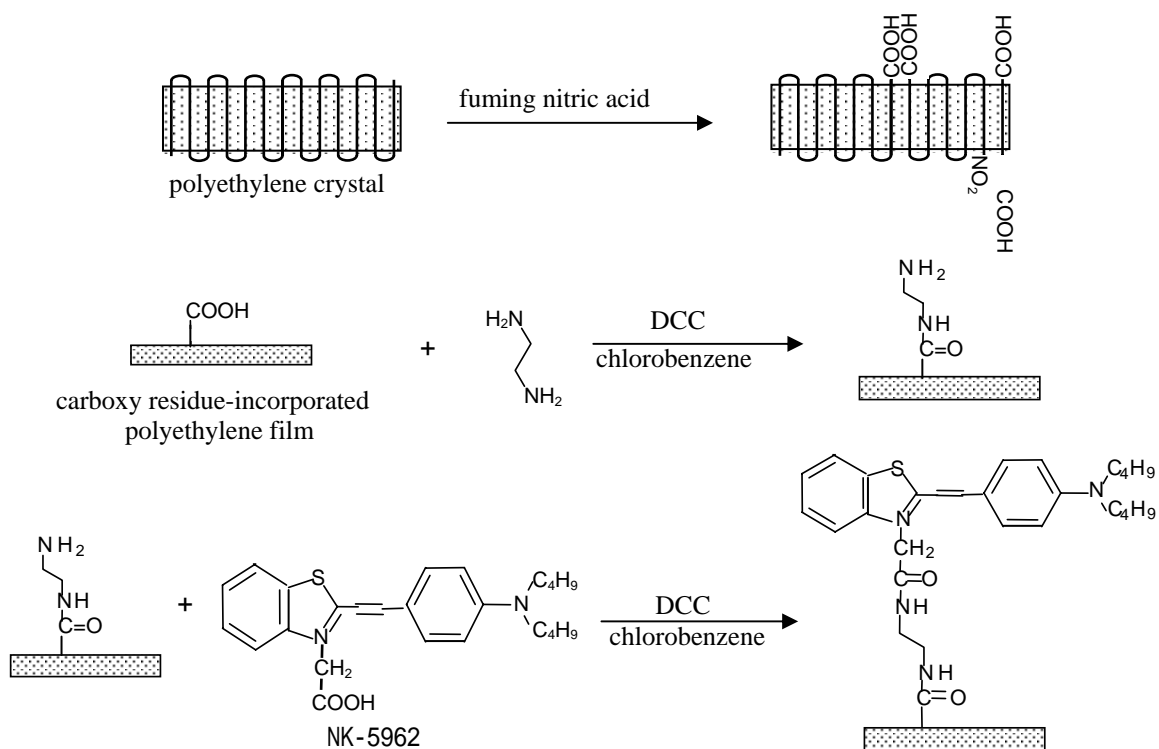


Fig. 1 Immobilization processes of photoelectric dye on PE film surface.

benzothiazolium (NK-5962) in chlorobenzene in the presence of DCC at 35 °C for 24 hrs then washed and soaked in chlorobenzene. Measurements of infrared and visible light absorption spectra were carried out with Paragon 1000 FT-IR and Lambda 40 (PERKINELMER), respectively.

3. RESULTS AND DISCUSSION

3.1 Morphology of PE-EB film

According to the scanning electron microscopy, the PE-EB film is fundamentally composed of stacked lamellae with their normal along the extrusion direction (Fig.2). The lamellar thickness was estimated to be ca.18 nm by the x-ray small angle scattering. Wide angle x-ray diffraction showed that the polymer chains were accommodated orienting perpendicular to the lamellar with folding on the lamellar surface. Closer analysis by the pole figure method made the b-axis orientated in the plane of the film and also normal to the extrusion direction [8]. From the data we roughly estimated the density of the chain foldings on the film surface to be ca.75000/micrometer².

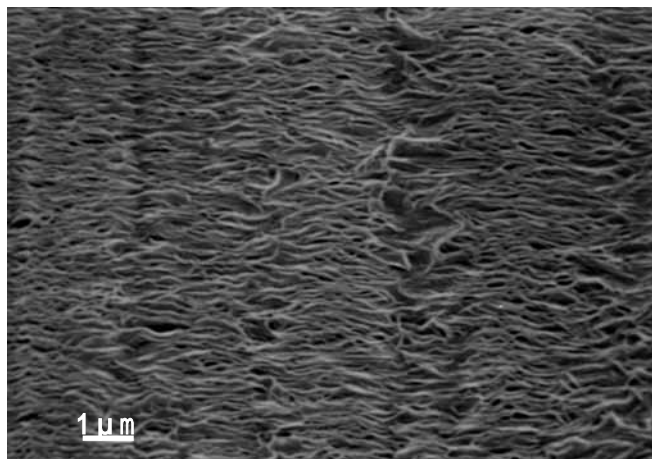


Fig. 2 Scanning electron micrograph of PE-EB film.

3.2 Introduction of COOH moieties on the PE film surface

Infrared spectra of PE-EB film treated with fuming nitric acid at 80 °C is shown in Fig.3. Absorbance around 1710 cm⁻¹, characteristic of carboxyl groups, was

increased with the treatment time. Duration of the treatment was critical for preparing suitable substrate for artificial retinal prostheses, i.e., short duration introduced too small number of reactive groups, while too long duration resulted in weakening or degradation of the polyethylene film. After several experiments under various conditions, we decided the duration should be 20 minutes. In order to evaluate how many chain foldings exist on the film surface, the film was treated with fuming nitric acid at 80 °C for 12 hrs. Gel permeation chromatography proved that all chain foldings in the film were cut completely under this reaction condition. With this sample, ratio of the IR absorption coefficient of COOH to that of CH₂ was 0.50. For the film treated for 20 min, the corresponding ratio was 0.11, suggesting that content of COOH groups was ca. 22% of that of the EB-PE film with which was subject to complete scission of the chain foldings. If we assume that one scission introduce two COOH groups, we can calculate COOH density on the film surface. As mentioned above, the original EB-PE film has ca.75000 foldings per the surface area of 1 micrometer². So the density of COOH's would be approximated to be twice of the 22% of 75000, i.e., 33000 per 1 micrometer², though this might be rough estimation.

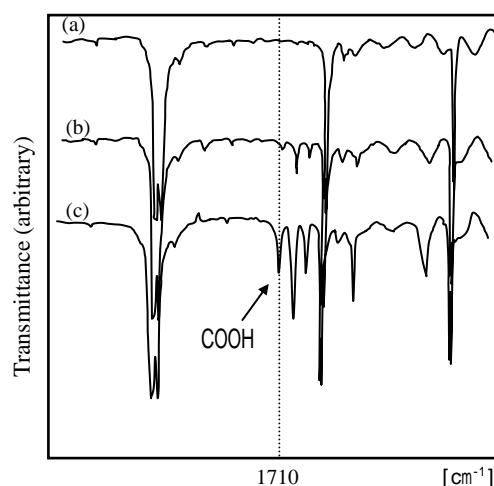


Fig. 3 Infrared spectra of PE-EB film treated with fuming nitric acid at 80 °C. (a) original film, (b) treated for 10 minutes and (c) treated for 20 minutes

Table 1 $A_{\text{COOH},1710\text{cm}^{-1}}/A_{\text{CH}_2,1470\text{cm}^{-1}}$ and COOH content of the fuming nitric acid treated PE films.

Fuming nitric acid treatment	$A_{\text{COOH},1710\text{cm}^{-1}}/A_{\text{CH}_2,1470\text{cm}^{-1}}$	COOH [%]*
10 min	0.02	4
20 min	0.11	22
12 hrs	0.50	100

*COOH [%] = $(A_{\text{COOH}}/A_{\text{CH}_2})$ of the treated film (10 min, 20min,) / $(A_{\text{COOH}}/A_{\text{CH}_2})$ of the treated film (12hrs)

3.3 Immobilization of the dye molecules on the polyethylene film surface

The COOH's were capped with ethylenediamine. IR spectrum of Fig.4b showed COOH's more than 80 % disappeared forming amide links leaving amino groups at the ends. These amino groups reacted with COOH's of dye molecules, showing an absorption band at 1520 cm^{-1} characteristic of aromatic $-\text{C}=\text{N}-$ (Fig.4 c). This absorption looks very weak but it might be the dye molecules were so bulky it could not penetrate into the film for the reaction with internal $-\text{NH}_2$'s.

It was also confirmed by the visible light absorption spectra. In Fig.5 the spectra of NK-5962 in chlorobenzene were shown. In solution, there was a strong absorption peak with maximum at 570 nm, while after their immobilization on the EB-PE film the peak became weak and blue shifted.

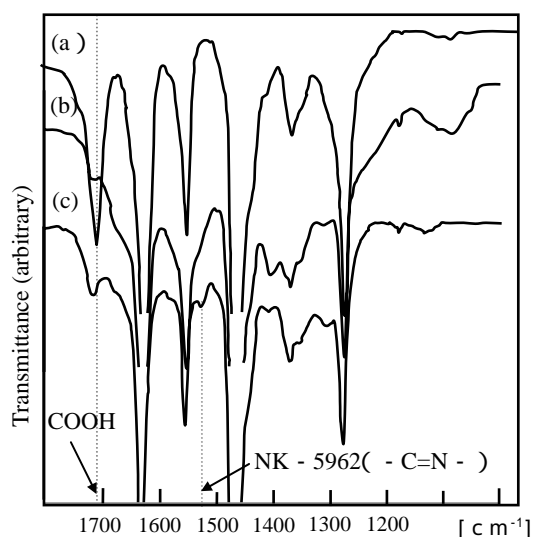


Fig.4 Infrared absorption spectra.

- (a) after fuming nitric acid treatment
- (b) after reaction with ethylenediamine
- (c) photoelectric dye immobilized PE film

extraction of the physically adsorbed molecules with chlorobenzene, this absorption became very weak but still detectable. Thus it was concluded that the photoelectric dye, NK-5962, was successfully immobilized on polyethylene film surface by this method. It is plausible that more than half of the COOH's on the film surface have reacted with NK-5962, so more than 17000 dye molecules exist on area of 1 micrometer^2 of the film surface. In general, the retina cell is larger than 100 micrometer^2 in area, so more than 1.7×10^6 dye molecules would be ready to stimulate one cell.

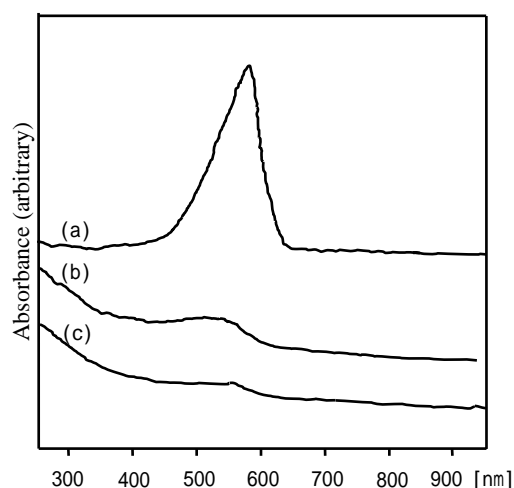


Fig.5 visible light absorption spectra.

- (a) NK-5962 in chlorobenzene
- (b) after rinsed the dye immobilized film
- (c) after soaked the dye immobilized film for 48 hours

Fluorescent emission from chick embryonic retinal tissues, in which retinal photoreceptor cell have not developed yet, has recently studied [9]. The retinal tissues placed on the dye-immobilized PE film emitted fluorescence on incidence of visible light. On the contrary, when the retinal tissues were removed apart from the dye-immobilized film, the fluorescence in the retinal tissues returned to a low level. These results show that the photoelectric dye, immobilized on the PE film surface, could stimulate neurons in the retinal tissues.

4. CONCLUSION

1. By using the fuming nitric acid treatment, COOH's were introduced on PE film surface.
2. A photoelectric dye (NK-5962) was immobilized through covalent bonds using the COOH's on the polyethylene film surface.
3. The dye immobilized on PE film can stimulate chick retinal tissues on incidence of visible light.

5. REFERENCES

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