In Vivo and In Vitro Feasibility Studies of Intraocular Use of Polyethylene Glycol–Based Synthetic Sealant to Close Retinal Breaks in Porcine and Rabbit Eyes

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PURPOSE. Absorbable polyethylene glycol–based synthetic sealant (PEG sealant) polymerizes under xenon illumination and forms a clear, flexible, and firmly adherent hydrogel. The intraocular biocompatibility of PEG sealant and efficacy for closing retinal breaks were evaluated.

METHODS. In an in vitro study, retinal detachment with a tear was created in porcine eyecups after vitreous gel removal. Polyethylene glycol–based synthetic sealant was applied to cover the tear and polymerized with a 40-second application of xenon light. Retinal adhesion strength was tested by forcefully squirting balanced salt solution (BSS) onto the retinal tear. Polyethylene glycol–based synthetic sealant was soaked in the BSS, incubated at 37°C, and the pH measured periodically over 72 hours. In an in vivo study, PEG sealant was injected into the vitreous cavity of the left eyes of rabbits. Ophthalmologic examinations were performed and bilateral ERGs were recorded simultaneously before and 28 days after injection. The eyes were enucleated for histological evaluation.

RESULTS. Adhesion of PEG sealant to the retina was good in BSS. A forceful squirt of BSS onto the retinal tear covered with PEG sealant did not detach the retina; the retinal tear without PEG sealant detached immediately. The pH of the BSS containing PEG sealant was between 7.2 and 8.2. No inflammatory reaction was observed in the eyes throughout 28 days of follow-up. The ERGs recorded before and after injection showed typical patterns. Histological examinations did not reveal any abnormality or inflammation.

CONCLUSIONS. Polyethylene glycol–based synthetic sealant appeared to effectively seal retinal breaks and was not toxic to the eye.

Keywords: retinal detachment, vitreoretinal surgery, FocalSeal, bioadhesive

Today, most rhegmatogenous retinal detachments are successfully treated with conventional surgical procedures, such as scleral buckling, vitrectomy with vitreous tamponade by air or gas, and cryotherapy or laser treatments with gas tamponade.1,2 However, these current surgical techniques can fail in some cases. Most failures are associated with proliferative vitreoretinopathy, and others are retinal detachments from congenital anomalies of the retina, such as those associated with peripapillary staphyloma with a retinal tear at the peripheral edge of the staphyloma or a posterior break in retinopathy of prematurity.3 Sealing retinal breaks appears to be a logical approach to prevent vitreous fluid from flowing through retinal breaks into the subretinal space and also to prevent the dispersion of RPE cells into the vitreous cavity, allowing RPE and other cells to proliferate and possibly leading to proliferative vitreoretinopathy.3

Some adhesives, such as cyanoacrylate, fibrin glue, sodium hyaluronate/carboxymethylcellulose absorbable film, mussel protein, TGF-β, and polysiloxanes, have been used to seal retinal breaks in the treatment of retinal detachment for many years4–15; however, each has its own drawbacks, such as potential ocular toxicity, difficulty in intraocular delivery, weak adhesive force, inflammatory response, and granulomatous tissue reaction. For these reasons and others, the use of glue has not yet become a standard procedure in the treatment of retinal detachment.

To make sealing retinal breaks easier and more effective, we studied the efficacy of polyethylene glycol–based synthetic hydrogel sealant (PEG sealant), which has been used to prevent air leaks from pulmonary resections in humans,16,17 for sealing and covering retinal breaks.

METHODS

Sealant

FocalSeal (Genzyme Corporation, Cambridge, MA, USA), which the US Food and Drug Administration approved as a sealant to limit air leakage after pulmonary resection, is an absorbable PEG sealant. With visible illumination from a xenon arc lamp...
Feasibility Studies of Intraocular PEG Sealant Use

In Vitro Study

Adhesion to the Retina. We first tested whether PEG sealant remains adherent to the retina in fluid. Fresh porcine eyes were obtained from a slaughterhouse less than 1 day after death. The anterior segment (cornea, iris, and lens) of the porcine eye was excised, and the vitreous was removed to make an eyecup. The retinal surface of the eyecup was kept wet throughout the experiment. A retinal detachment was created by making a retinal tear of approximately two disc diameters in the porcine eyecup. Polyethylene glycol–based synthetic sealant material degrades over a 6- to 9-month period by hydrolysis, releasing biocompatible components that are metabolized or cleared by the kidneys.16,17 The PEG sealant used in this study was provided by Genzyme Corporation.

The pH of PEG sealant in BSS Plus (Alcon Ltd.) was measured periodically. The pH of BSS Plus was approximately 7.4, and that of BSS was approximately 7.0. BSS Plus was used for pH measurement because the pH of BSS Plus was closer to that of the human vitreous. Three samples were prepared in glass tubes: 0.2 mL photopolymerized sealant soaked in 5 mL BSS Plus, 0.2 mL unphotopolymerized sealant dissolved in 5 mL BSS Plus, and 0.1 mL of the solution was injected into the left vitreous cavity of each study animal (sealant group). The needle was advanced under direct visualization through a contact lens under an operating microscope toward the region of the optic disc as closely as possible to the retina. In the control animals (BSS group), 0.1 mL BSS was injected into the vitreous cavity of the left eye without PEG sealant. Polyethylene glycol–based synthetic sealant solution and BSS were injected into the vitreous cavity, but not applied to the retinal surface. The surgeon and examiner were not blinded to the solution injected (BSS or PEG sealant solution) or to the subsequent examinations.

In Vivo Study

Dutch pigmented normal rabbits (Kitayama Labes Ltd., Nagano, Japan), each weighing 2.0 to 3.0 kg, were used. The study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Intravitreous Injection. All procedures were carried out with sterile techniques under a surgical microscope. Twelve rabbits were divided into two groups (six rabbits in the sealant group and six rabbits in the BSS group). The left pupil of each rabbit was dilated with 0.5% phenylephrine and 0.5% tropicamide eye drops. Rabbits were anesthetized by an intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine (5 mg/kg). Topical anesthesia (0.4% oxybuprocaine hydrochloride drops) was applied to the eyes. A 10-mL volume of BSS Plus, 0.2 mL unphotopolymerized sealant dissolved in 5 mL BSS, and 0.1 mL of the solution was injected into the left vitreous cavity of the left eye without PEG sealant. Polyethylene glycol–based synthetic sealant solution and BSS were injected into the vitreous cavity, but not applied to the retinal surface. The surgeon and examiner were not blinded to the solution injected (BSS or PEG sealant solution) or to the subsequent examinations.

Electroretinogram. Under general and topical anesthesia and with the pupils dilated, an ERG was recorded before and 28 days after the intravitreal injection of PEG sealant solution. Contact lens electrodes (LW-102; Mayo, Aichi, Japan) with built-in white light-emitting diodes were placed on the corneas of both eyes, and a ground electrode was attached to the ear. The ERG was recorded from both eyes simultaneously with an ERG recording system (LE-5000; Tomey, Aichi, Japan). This apparatus combines a stimulus instrument, amplifier, and recorder. The frequency band ranges from 0.5 to 300 Hz. The luminance values of the stimuli were 0.36, 3, and 20 cd·s/m².

The recording started with the weakest stimulus after 30 minutes of dark adaptation. No background light was used. The light stimulus was delivered every 40 seconds and four responses were averaged at each light intensity. Amplitude and implicit time ratios were calculated between the right and left eyes. A two-tailed paired t-test was used in the statistical analysis to examine the relation before and after intravitreal injection. An unpaired t-test was used in the statistical analysis to examine relations between the sealant and BSS groups.

Histology

Rabbits were killed with an overdose of pentobarbital 28 days after injection, and the eyes were enucleated. After enucleation, all eyes were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde solution, dehydrated in a series of increasing alcohol concentrations, and embedded in paraffin. Sections cut at a 4-μm thickness were stained with hematoxylin and eosin and examined under a light microscope (Nikon Eclipse 80i; Nikon, Tokyo, Japan).

RESULTS

Adhesion of PEG sealant to the retina was good in BSS; the PEG sealant remained adhered 24 hours after application (Fig. 1). The forceful squirt of BSS onto the retinal tear covered with PEG sealant did not detach the retina, whereas the retinal tear without PEG sealant detached immediately after the BSS squirt (Fig. 2).

The pH of the BSS containing PEG sealant was between 7.2 and 8.2 at all time points.

No inflammatory reaction was observed in the eyes in which PEG sealant solution had been injected into the vitreous throughout 28 days of follow-up. Slit-lamp and indirect ophthalmoscopy examinations showed normal conjunctiva, corneas, aqueous, crystalline lens, vitreous, and retinas at all time points. No cell or flare in the anterior chamber or the vitreous was observed.

The ERGs recorded before and after injection showed the typical components of an ERG: an a-wave followed by a rapidly
rising b-wave. Oscillatory potentials were recorded on the ascending slope of the b-wave with stronger stimulus intensities. Figure 3 shows an ERG from the right eye (noninjected eye) and left eye (PEG sealant–injected eye) simultaneously recorded 28 days after the injection of PEG sealant solution.

There was no significant difference in the sealant group or BSS group in the amplitude ratios or implicit time ratios of the a-waves, b-waves, and oscillatory potentials of the ERGs at any stimulus intensity level before and after injection (Fig. 4). There was also no significant difference in the amplitude ratios or implicit time ratios of the a-waves, b-waves, and oscillatory potentials of the ERG at any stimulus intensity level between the sealant group and BSS group. We did not isolate oscillatory potentials from the recordings because of the device we used, but were able to recognize oscillatory potentials on the ascending slope of the b-wave with stronger stimulus intensities. Figure 3 shows an ERG from the right eye (noninjected eye) and left eye (PEG sealant–injected eye) simultaneously recorded 28 days after the injection of PEG sealant solution.
potentials from flash ERGs recorded under the strongest stimulus condition (20 cd·s/m²). We measured the amplitudes and implicit times of the oscillatory potentials and calculated the amplitude and implicit time ratios. The Table shows the average amplitude and implicit time of each wave before and after injection at the strongest stimulus intensity in both the sealant and BSS groups.

Histological examination with a light microscope did not reveal any abnormality or inflammation, such as the existence of inflammatory cells, epiretinal membrane, retinal edema, or disorganization or atrophic changes of the retinal layers in either group 28 days after injection (Fig. 5).

DISCUSSION

The PEG sealant is a water-soluble polyethylene glycol-based synthetic hydrogel sealant. This liquid is polymerized under visible xenon illumination, and becomes a clear, solid, flexible, and firmly adherent hydrogel. In our in vitro study, PEG sealant was easily delivered to a retinal tear with a 27-gauge needle and photopolymerized into a flexible mass. With PEG sealant on the retinal tear, the retina remained attached despite a forceful squirt of BSS.

Cyanoacrylate, a glue that has been evaluated by a number of investigators, is already used to treat retinal disorders in human eyes, such as giant tears caused by perforating injury, proliferative vitreoretinopathy associated with inferior retinal breaks or retinotomy, retinal detachment associated with retinal breaks within a choroidal coloboma, recurrent macular holes, and breaks after dissection of the preretinal membrane during open-sky vitrectomy in retinopa-

![Figure 3](https://example.com/figure3)

**Figure 3.** An ERG recorded simultaneously from the right eye (blue line) and left eye (red line) 28 days after the injection of PEG sealant solution into the left eye. The numbers to the left of the ERG are the stimulus intensities. Scale bar: 100 μV and 25 ms.

![Figure 4](https://example.com/figure4)

**Figure 4.** Amplitude ratios and implicit time ratios between the right and left eyes of the a-wave, b-wave, and oscillatory potentials (OPs) at all stimulus intensity levels before and after injection in the sealant and BSS groups.
thy of prematurity. However, its rapid polymerization makes application and intraocular delivery extremely difficult, even when mixed with oil, which delays polymerization. Furthermore, cyanoacrylate forms a hard mass rather than a thin sheet or membrane, and is inadequate to cover a large retinal tear. Polyethylene glycol–based synthetic sealant appears more suitable than cyanoacrylate to seal retinal breaks, including large retinal breaks, not only because it is easy to deliver, but also because it forms a flexible, thin sheet rather than a hard mass.

A sodium hyaluronate/carboxymethylcellulose absorbable film (Seprafilm; Genzyme Corporation) is a nontoxic, inert, nonimmunogenic, and biocompatible membrane that adheres strongly to moist tissues. Sueda et al. previously demonstrated that the film adhered well to the retina using bovine eyecups. However, the film needed to be delivered in a dry condition and only a small piece of the film could be placed into the vitreous cavities. This means that film application is difficult when patching peripheral and large breaks, and it is suitable only for small, posteriorly located retinal breaks. On the other hand, with PEG sealant, a single liquid can be easily delivered into the vitreous cavity with a 27-gauge needle. No bulky, heavy instrument, such as a special syringe pump for hydrophobic silicone oil injection, is necessary for intraocular delivery.

### TABLE

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<tr>
<th></th>
<th>Before Injection</th>
<th>After Injection</th>
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<tr>
<td></td>
<td>Right (Noninjected Eye)</td>
<td>Left (Injected Eye)</td>
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<td></td>
<td>BSS</td>
<td>Sealant</td>
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<tr>
<td><strong>a-wave</strong></td>
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<td>Amplitude</td>
<td>136.6 ± 20.2</td>
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<td>128.3 ± 25.5</td>
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<td>Sealant</td>
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<td>Amplitude</td>
<td>125.0 ± 0.7</td>
<td>122.2 ± 1.5</td>
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<tr>
<td>Implicit time</td>
<td>12.2 ± 1.7</td>
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<tr>
<td><strong>b-wave</strong></td>
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<td>Amplitude</td>
<td>557.6 ± 55.5</td>
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<td>522.6 ± 41.0</td>
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<td>30.1 ± 0.9</td>
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<td>30.1 ± 2.3</td>
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<td>Amplitude</td>
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<td>51.4 ± 0.8</td>
</tr>
<tr>
<td>Implicit time</td>
<td>49.1 ± 3.5</td>
<td>49.8 ± 3.3</td>
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Values are means ± SD. OP, oscillatory potentials.

FIGURE 5. Histological examination with a light microscope does not reveal any abnormality or inflammation in either the (A) BSS group or (B) sealant group. Scale bar: 100 μm.
Fibrin glue, which is usually refined from bovine blood or from human autologous serum, is less likely to cause a foreign body reaction, but doubts exist as to the adhesive qualities of fibrin in the posterior segment.\textsuperscript{11,25} Coleman et al.\textsuperscript{12} treated giant retinal tears by applying fibrin glue to the edges of the breaks after vitrectomy; however, none of the giant tears remained closed after surgery because the fibrin glues were effective for only 4 to 6 days. Although TGF-\(\beta\) also sealed retinal breaks, cryotherapy and internal tamponade were still necessary. The TGF-\(\beta\) seems to have the same disadvantage as fibrin glue, namely, temporary adhesion.\textsuperscript{13} Polyethylene glycol–based synthetic sealant, developed as a firmly adherent hydrogel that can seal air and fluid leaks in pulmonary resection, seemed to have better adhesive qualities than fibrin glue or TGF-\(\beta\).

The pH of PEG sealant in BSS was within an acceptable range as an intravitreal solution. The pH of a sodium hyaluronate/carboxymethylcellulose absorbable film in BSS was measured previously with the same modality as in our experiment, and was demonstrated to be within an acceptable range.\textsuperscript{24}

The ophthalmologic examinations and histological evaluations in our in vivo study revealed that intravitreal PEG sealant injection had no unfavorable inflammatory effect on the eye. The intravitreal injection of PEG sealant was found to have no effect on retinal function, as there was no significant difference in ERG results between the study and control groups. A similar in vivo study with a sodium hyaluronate/carboxymethylcellulose absorbable film was performed by Sueda et al.\textsuperscript{23} and demonstrated no retinal toxicity. Cyanoacrylate, on the other hand, was reported to have significant cytotoxicity and reactivity to the retina.\textsuperscript{8–10}

Other adhesives\textsuperscript{3,13} evaluated for retinopexy were mussel protein and polysiloxanes. Mussel protein adhesive (Cell-Tak; Becton Dickinson, Bedford, MA, USA) caused an inflammatory response.\textsuperscript{13} Adhesive polysiloxanes are advantageous in that they can be delivered in an aqueous environment, but cause a localized granulomatous tissue reaction.\textsuperscript{6}

Among other previously described adhesives with limitations in adhesion, delivery, or safety, polyethylene glycol hydrogel, including PEG sealant, seems to be a better adhesive as a retinal glue. Margalit et al.\textsuperscript{25} tested the suitability of some biologic adhesives for ophthalmic use in a study of three polyethylene glycol hydrogels, commercial fibrin sealant, autologous fibrin sealant, mussel adhesive, and three photocurable adhesives, and proved that hydrogels were superior for intraocular use in terms of consistency, adhesiveness, stability, impermeability, and safety. There were two disadvantages of hydrogels used as retinal glue: one was the need to mix the two components of the glue (one gel and the other liquid) for polymerization,\textsuperscript{24} and the other was the long duration required for polymerization, which was 2 to 5 minutes in the previous study.\textsuperscript{25} Because of these disadvantages, the application area is limited to the posterior pole of the eye. When applied to peripheral breaks, the liquid descends as the result of gravity from the area of application. Moreover, when liquid glue is applied to retinal breaks, the glue tends to slip under the retina. Sueda et al.\textsuperscript{13} partially solved these problems by using a double syringe system and a modification to the DuraSeal dural sealant (Confluent Surgical, Waltham, MA, USA), of which the curing time was 8 to 10 seconds. However, it was still difficult to handle the liquid glue, as they observed a case with glue entering the subretinal space in their experimental retinal detachment surgery.

Polyethylene glycol–based synthetic sealant fundamentally appears to solve the disadvantage of hydrogel, as it is a single liquid and photocurable. Polyethylene glycol–based synthetic sealant is considered to be suitable as a retinal glue as it photopolymerizes with xenon intraocular illumination, which we already use in daily surgery. Because liquid glue is unnecessary for curing the gel, it is less likely for the glue to slip under the retina, and the glue can be applied to the retinal periphery.

At present, the intraocular tamponade materials widely used in clinical practice are gases, perfluorocarbon liquids, and silicone oils. Although they are not sealants, they are hydrophobic materials that have high surface tension with the aqueous humor, and form an interface with the aqueous environment of the eye that can be effective in closing retinal breaks and holding the retina in place against the retinal pigment epithelium. On the other hand, PEG sealant is a hydrophilic material and closes retinal breaks by adhering to the retina. Although these tamponade materials and the PEG sealant are totally different in terms of hydrophobicity and hydrophilicity, they share the characteristic that they interrupt open communication between the subretinal space/RPE cells and the preretinal space. Silicone oils and carbon liquids need to be removed, as they are not soluble materials, but PEG sealant does not need to be removed, as it is water soluble.

This study has several limitations. First, in our in vitro study, we tested the adhesion quality of PEG sealant using cadaveric eyecups, in which the mechanisms of retinal adhesion are significantly impaired and changed overall. Second, we did not detect extensive ERG impairment in our in vivo study, although there was a lack of measurements under photopic conditions and 30-Hz flicker in our ERG examinations. Third, possible retinal toxicity needs to be discussed. In our in vivo study, we tested the unpolymerized form of the PEG sealant rather than the polymerized form. Furthermore, toxicity assessments of retinal contact with PEG sealant will be needed. Long-term follow-up and immunohistochemical analyses, such as glial fibrillary acidic protein, CD68, and Iba1 immunolabeling also will be needed to determine possible toxicity. Further studies are warranted to evaluate the efficacy and safety of PEG sealant as a retinal break sealing material by performing vitreous surgery for experimental retinal detachment using animals. The experiment should contain the following procedure: (1) making an experimental retinal detachment with a break during vitrectomy; (2) after performing fluid-air exchange, apply PEG sealant to cover the retinal breaks; and (3) perform air-fluid exchange and finish the operation without intraocular tamponade. It also needs to be confirmed that no sealant-related mechanical complication occurs, as PEG sealant swells after being polymerized from water absorption. Polyethylene glycol–based synthetic sealant appeared to effectively seal retinal breaks and was not toxic to the eye. Polyethylene glycol–based synthetic sealant has the potential to be used as a material for sealing retinal breaks.

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References


