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Evaluation of viscoelastic poly(ethylene glycol) sols as vitreous substitutes in an experimental vitrectomy model in rabbits

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Abstract

The aim of this study was to employ an experimental protocol for in vivo evaluation of sols of 5 wt.% poly(ethylene glycol) (PEG) in phosphate-buffered saline as artificial vitreous substitutes. A 20 gauge pars plana vitrectomy and posterior vitreous detachment were performed in the right eye of eight pigmented rabbits. Approximately 1 ml of the viscoelastic PEG sols was then injected into the vitreous space of six eyes. PEG with an average molecular weight of 300,000 and 400,000 g mol⁻¹ was used in two and four eyes, respectively. Two eyes received balanced salt solution and served as controls. Full-field electroretinography was carried out and intra-ocular pressure (IOP, palpation) measured pre- and post-operatively at regular intervals up to 41 days. The rabbits were killed and the eyes examined by retinal photography, gross macroscopic examination and histology. The viscoelastic sols were successfully injected and remained translucent throughout the post-operative period, with some inferior formation of precipitates. None of the eyes displayed IOP elevation post-operatively, but in three of the PEG sol injected eyes transient hypotony was noted. One eye sustained retinal detachment during surgery and another two in the post-operative period. ERG recordings confirmed preservation of retinal function in three out of four eyes injected with 400,000 g mol⁻¹ PEG. Histological examination revealed up-regulation of glial acidic fibrillary protein in Müller cells in PEG sol injected eyes, but normal overall morphology in eyes with attached retinas. The viscosity of the sol was not retained throughout the post-operative period, indicating the demand for polymer cross-linking to increase residence time. The results provide promising preliminary results on the use of PEG hydrogels as a vitreous substitute.

Keywords

Electrophysiology; Polyethylene oxide; Ophthalmology; Hydrogel; Vitreous

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 1–5, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.11.013.

1. Introduction

Synthetic vitreous replacement is an important part of vitreo-retinal surgery, for use as a tamponade in retinal detachment, diabetic retinopathy, trauma or possibly as a depot for long-term drug delivery. Currently gases, perfluorocarbon liquids or silicone oil are predominately applied clinically to separate water from the retinal rupture to prevent sub-retinal fluid accumulation leading to further retinal detachment. However, these agents have several disadvantages, including cataracts and elevation of the intra-ocular pressure (IOP), and they are unsuitable as long-term vitreous substitutes [1,2]. The vitreous is a hydrogel composed of collagen and hyaluronic acid, with reported values of 98–99% water, pH 7.0–7.4, with an elastic modulus of 4.2–4.7 Pa and a loss modulus of 1.9–3.7 Pa and a refractive index of 1.3345–1.3348 [3]. Synthetic hydrogels are a promising form of vitreous substitute that could tamponade water influx through a rupture and simultaneously provide a normal vitreous biochemistry. Material requirements include optical translucence and homogeneity, atoxicity and physiological isotonicity. The material must also permit transport of salts, nutrients and water and be stable to enzymatic or hydrolytic degradation, as well as being immunologically inert. In addition, it should be able to be administered through a 20 gauge needle without inducing structural damage to the gel. Therefore, an injectable hydrogel that cross-links in situ is theoretically an ideal solution. A wide variety of synthetic polymers, in solution or as cross-linked hydrogels, have been tested experimentally and shown to satisfy a wide range of necessary properties. However, to date no material has been successfully translated for clinical use that can meet all the desired properties for an ideal vitreous substitute [4–15]. Problems have included gel fragmentation, opacification, retinotoxicity and IOP elevation. In a prior study a polymer used in reconstructive surgery, poly(alkyl-imide) (Bio-Alcamid™), was tested as a potential substitute [16,17]. However, this material caused central retinal edema 1 day after surgery, accompanied by a pathological electroretinography (ERG) [18]. Poly(ethylene glycol) (PEG) is a synthetic water-soluble polymer that has been approved by the FDA for use in a wide range of biomedical applications, including injectable hydrogels [19]. It has also been tested in formulations for intravitreal drug delivery, repair of scleral incisions and sealing retinal detachments [20–22]. In this study we investigated the injection of viscoelastic sols of high molecular weight (>200 kDa) PEG in phosphate-buffered saline (PBS) into the vitreous bodies of rabbit eyes following vitrectomy. The molecular weights and concentration of PEG were chosen to approximate the mechanical properties of the natural vitreous. The aim of the study was to ascertain whether the PEG sols preserve normal IOP, retinal function and attachment as vitreous substitutes after experimental vitrectomy.

2. Materials and methods

2.1. Characterization of PEG sols

Sols of 5 wt.% PEG (Sigma Aldrich, St. Louis, MO) were prepared in sterile PBS, pH 7.4 (Invitrogen, Carlsbad, CA), with molecular weights of 300 and 400 kDa. The sols are referred to as S300 and S400, respectively, in subsequent sections. The pH was measured using a digital pH probe (Russell RL060P, Thermo Scientific, Waltham, MA). Dynamic rheology was performed using an AR G-2 with a 40 mm diameter 2° cone and plate set-up

(TA Instruments, New Castle, DE) at 10 rad s^{-1} oscillation and 5% strain. Refractive indices were measured using a hand held Brix refractometer (PAL-1, Atago, Tokyo, Japan). Sols were sterilized by ultraviolet irradiation for 12 h prior to surgery.

2.2. Animal selection and study protocol

The study was approved by The Regional Ethics Committee for Animal Experiments in Lund and also conformed to the ARVO Resolution on the Use of Animals in Vision and Ophthalmic Research.

Eight pigmented rabbits, aged 4 months, were used in the experiment. The right eye was operated upon and injected with 0.8–1.5 ml solutions following vitrectomy (two with S300, four with S400, two with balanced salt solution (BSS)) while the left eye served as a control. Examination including ophthalmoscopy and IOP measurement (palpation) was performed post-operative on days 6, 20 and 41. The rabbits were killed on day 41, at which time the eyes were grossly examined, photographed and prepared for histological examination.

2.3. Surgery

All procedures were performed by a clinically experienced vitreo-retinal surgeon. General anesthesia was provided with a combination of ketamine (35 mg kg^{-1}) and xylazine (5 mg kg^{-1}) intramuscularly. The right eye was instilled with cyclopentolate (1%) and phenylephrine (10%) for 30 min before surgery. Topical tetracaine (0.5%) was applied just before surgery. The conjunctiva was incised limbally at 270° from 9 to 6 o'clock with a vertical incision at 12 o'clock, creating two flaps. A 20G infusion cannula was sutured to the sclera in the 4 o'clock position 1 mm posterior to the limbus and BSS (Endosol, Allergan Medical Optics) infusion was commenced. Two 20G sclerotomies were made in the 10 and 2 o'clock positions. A BIOM 90-D lens (Oculus) was used to visualize the fundus and an Accurus surgical system machine (Alcon, Fort Worth, TX) was used for surgery. A standard endoilluminating light probe (Alcon) was introduced through the 10 o'clock sclerotomy (illumination level 80%) and a vitreous cutter (Innovit, Alcon) was inserted through the 2 o'clock sclerotomy. Posterior vitreous detachment (PVD) was created by positioning the vitrectomy probe at the margin of the disk and applying suction (100 mm Hg) while pulling on the probe. PVD was confirmed visually as the posterior vitreous cortex separated from the posterior pole. All vitreous in the central fundus (approximately 50% of the total volume) was removed, while the peripheral parts were left because of the risk of the instrument touching the comparatively large lens. In one case (No. 1, S300) the vitrector touched the retina, causing retinal rupture and anterior chamber bleeding. After vitreous removal, fluid–air exchange was performed, after which PEG sol was injected under visual control through a 19G needle. The amount of sol injected varied from 0.8 to 1.5 ml (Table 2). In one case some backflow during gel injection was noted (No. 6, S400). The sclerotomies and conjunctiva were sutured and 25 mg gentamicin and 2 mg betamethasone were injected subconjunctivally. No post-operative treatment was given. The eyes were examined externally daily and with an external ophthalmoscope on days 6 and 20. Images of the retinas were taken on days 20 and day 41 (RetCam, Clarity Medical Systems, Pleasanton, CA). Two eyes, used as controls, underwent the above surgery but were injected with BSS instead of PEG sol.

2.4. Full-field ERG

A standardized full-field ERG was recorded 7 days before surgery and 41 days post-operatively on the right eye using a Nicolet Viking analysis system (Nicolet Biomedical Instruments, Madison, WI) as previously described [23]. During examination the rabbits were sedated with Hypnorm (0.2 mg ml⁻¹ fentanyl and 10 mg ml⁻¹ fluanisone), 0.1 ml kg⁻¹ intra-muscularly, and the pupils were dilated with Cyclogyl (1% cyclopentolate hydrochloride) to a pupil diameter of 8–9 mm. After 30 min of dark adaptation a Burian–Allen bipolar ERG contact lens electrode was applied to the topically anesthetized cornea, together with a subcutaneous ground electrode on the neck. The lens was lubricated with methylcellulose (2%). Responses were obtained with a wideband filter (–3 dB at 1 Hz and 500 Hz), stimulating with single full-field flashes (30 μs) with dim blue light (Wratten filters Nos. 47, 47A and 47B) and of white light (0.8 cd s m⁻²). Cone responses were obtained with 30 Hz flickering white light (0.8 cd sm⁻²) averaged from 20 sweeps. The luminances of the three different light stimuli refer to the light reflected from the Ganzfeld sphere.

2.5. Tissue preparation

On day 41 the rabbits were killed and the eyes were dissected, grossly examined and fixed for 1 h in 4% formalin, pH 7.3, in 0.1 M Sørensen's phosphate buffer (PB). After fixation the specimens were washed with 0.1 M Sørensen's PB, and then washed again using the same solution containing sucrose of increasing concentrations (5–25%). The specimens were sectioned at 12 μm on a cryostat, and every tenth slide was stained with hematoxylin and eosin (H&E) according to standard procedures. To explore Müller cell activation, glial fibrillary acidic protein (GFAP) immunolabeling was performed by washing sections from each eye in 0.1 M sodium phosphate-buffered saline, pH 7.2, with 0.25% Triton X-100 (PBS/Triton) and incubating them with the primary antibody (anti-GFAP, clone G-A-5, Boehringer Mannheim Scandinavia, Bromma, Sweden) diluted 1:4 with PBS/Triton with 1% bovine serum albumin overnight at +4 °C. After incubation the slides were rinsed in PBS/Triton, incubated with fluorescein isothiocyanate (FITC)-conjugated antibodies for 45 min, rinsed, and mounted in custom-made anti-fading mounting medium. Unoperated eyes served as controls. For negative controls the same labeling procedure without the primary antibody was performed on both the normal left and the operated right eyes of the animals.

3. Results

3.1. Sol physical properties

Table 1 shows the physical properties measured for the sols. The addition of PEG to PBS, pH 7.4, resulted in a decrease in pH, which remained above 7 in all cases. Both the elastic and loss moduli increased with increasing molecular weight at a constant PEG concentration of 5 wt.% in PBS. Refractive indices decreased slightly with increasing molecular weight, with an approximate n_D of 1.339 at 25 °C converted from Brix% in all sols. While the sols permitted transmission of visual information, the sols were not as clear as pure water (Fig. 1A). Complete transparency of a vitreous substitute is required for clinical use. Transparency appeared unchanged when heating the sols from 4 to 37 °C. S300 and S400 could be drawn into a syringe via a 19 gauge cannula and this appeared unchanged at 37 °C.

A small amount of PEG remained insoluble, observed by addition of a blue hydrophilic dye to the sols. This fraction was larger in S400 compared with S300 (Fig. 1B).

3.2. Post-operative evaluation

The results are summarized in Table 2. During surgery one eye had a retinal rupture and bleeding in the anterior chamber due to contact with a surgical instrument (No. 1, S300). In all other eyes the PEG sols were injected without complications. 6 days post-operatively two eyes injected with the PEG solutions displayed hypotony, with an IOP of approximately 0 mm Hg (No. 1, S300, No. 8, S400), while the remaining four had an IOP of approximately 10–15 mm Hg. Hypotonic eyes had a slight cataract superiorly, but the majority of the lens was clear. No signs of inflammation were observed. The eye with a per-operative retinal rupture displayed a retinal detachment of approximately 40% (No. 1, S300), while the remaining eyes displayed a normal retina. The vitreous space was clear in the central and superior part. However, all eyes containing PEG displayed small white aggregates in the inferior vitreous body (Fig. 2). 20 days post-operatively this precipitate was still present, but appeared to be reduced in quantity. At this time one eye had total retinal detachment (No. 1, S300) (Fig. 3A). Two eyes had peripheral retinal detachments (No. 2, S400, No. 6, S400). No inflammation was observed externally or internally (Fig. 3C and E). A slight cataract was noted in two of the PEG injected eyes (No. 2, S400 and No. 5, S300) and one BSS eye. IOP was not elevated in any eye, but two eyes with detachment were hypotonic, between 0 and 5 mm Hg (No. 1, S300, No. 6, S400). At final examination and dissection, 41 days post-operatively, the vitreous was clear in all animals (Fig. 3B, D, and F), and the IOP was normal in all eyes. Minimal cataract was noted in three eyes. Precipitates were found in the inferior vitreous in two eyes (No. 6, S400, No. 8, S400), but was reduced compared with earlier examination time points. The retina was completely detached in two eyes (No. 1, S300, No. 6, S400) and some folding was also noted (No. 4, BSS, No. 5, S300, No. 6, S400) (Fig. 3F). The sols in all eyes were completely resorbed.

3.3. Histology

In H&E stained sections three of the PEG sol injected eyes and the two BSS injected eyes displayed a normal retinal morphology (Fig. 4A and B and Table 2). The eye with early clinically diagnosed retinal detachment (No. 1, S300) displayed destruction of the retinal layers, choroidal inflammation and dissolved retinal pigment epithelium (RPE) (Fig. 4C). In another eye with detachment (No. 6, S400) the outer and inner photoreceptor segments were missing and some cell loss in all nuclear layers was evident. In one eye (No. 5, S300) a minimal central detachment was found, together with inflammatory cells in the outer nuclear layer (ONL) and sub-retinal space (Fig. 4D). The macroscopically observed precipitates seen in No. 2, S400 were observed in histological sections as brown clumps on the retinal surface, and also in an area within the inner retinal layers, with the presence of macrophages (Fig. 4E and F).

3.3.1. Immunofluorescence labeling—In BSS injected eyes GFAP labeling was comparable with normal unoperated controls with discrete labeling of Müller cells seen only in the periphery (Fig. 5A). All eyes injected with PEG sols displayed up-regulation of glial fibrillary acidic protein (GFAP), as a sign of Müller cell activation (Fig. 5B). The eye with

surgically induced total retinal detachment displayed disorganized Müller cells (No. 1, S300), whereas in all other eyes Müller cells displayed the normal vertical arrangement.

3.4. Electroretinography

Full-field ERG measurements were obtained pre- and post-operatively in all eight eyes (Table 3). All but one case followed the histological results. No residual response could be measured in the two eyes with total retinal detachment (No. 1, S300, No. 6, S400). Another eye with a minimal central detachment also displayed no response (No. 5, S300). Two eyes displayed supernormal responses to blue light (No. 4, BSS) and blue and white light (No. 7, S400). The other eyes did not display marked changes relative to pre-operative baseline recordings (No. 2, S400, No. 8, S400, No. 3, BSS).

4. Discussion

The vitreous is a hydrogel composed of collagen and hyaluronic acid, with reported values of 98–99% water, pH 7.0–7.4, with an elastic modulus of 4.2–4.7 Pa and a loss modulus of 1.9–3.7 Pa and a refractive index of 1.3345–1.3348 [3]. 5 wt.% PEG sols with average molecular weights between 200,000 and 400,000 g mol⁻¹ had viscoelastic properties of the same order of magnitude as the natural vitreous. However, none of the individual materials completely matched both the elastic and loss moduli of the natural vitreous. Using PBS, the pH values of the resulting sols were slightly above 7, and both sols had similar refractive indices (1.339 at 25 °C) to the natural vitreous. PEG sols presented an attractive vitreous substitute to test in an experimental vitrectomy model, especially given promising results indicating ocular biocompatibility of PEG hydrogels [20–22].

The sols formed some small white aggregates in the inferior vitreous body in vivo, resulting in a lack of total transparency, which is not ideal for clinical use. Both eyes injected with S300 had retinal detachment and loss of electrical function (No. 1, No. 5). In one eye the retinal detachment was caused during surgery (No. 1). In the other, a minimal central detachment and sub-retinal ONL inflammation developed in the post-operative period as observed upon histological examination (No. 5). It is uncertain why this eye had no post-operative ERG responses after 41 days, but inflammation may be partly responsible. The tolerability of S300 was inconclusive, limited by the small sample size. In three out of four eyes treated with S400 the PEG sol had a minimal effect on retinal morphology and electrical function (No. 2, No. 7, No. 8). One eye with S400 developed a post-operative retinal detachment (No. 6). However, all eyes injected with PEG sols exhibited elevated GFAP expression, indicative of retinal stress and Müller cell activation, which was not observed in BSS treated eyes. S400 affected the retina to a lesser degree than a previously tested material, poly(acrylimide), which caused retinal edema [18].

Following removal of the natural vitreous, metabolic and physiological changes occur within the eye, involving oxygen concentration, osmotic balance and molecular transport that may affect both the lens and the retina [24,25]. The normal morphology and non-pathological ERG responses observed in three out of four eyes injected with S400 may indicate that the nutritional demands of the retina are met by the PEG sol. The supernormal rod ERG (blue light) responses observed in one BSS eye (No. 4) and one S400 eye (No. 7)

may potentially be related to protein kinase C (PKC) α down-regulation and GFAP up-regulation, which warrants further investigation [26]. IOP was measured by digital palpation, which is not the most accurate and reliable method for IOP measurement. However, we were primarily interested in detecting any elevation in IOP, for which palpation was adequate. The PEG sols did not result in IOP elevation, an important clinical consideration. One eye was hypotonic post-operatively (No. 8 S400) and returned to normal within 20 days. Hypotonia in the first week following vitreo-retinal surgery is not uncommon, and is usually related to mild inflammation of the ciliary body. The rabbits were not treated with anti-inflammatory eye drops, as is common practice clinically, which may explain this phenomenon. Our protocol for vitrectomy in the rabbit eye is well established, and from experience IOP returns to normal within 1–2 weeks. The PEG sols were not retained in the vitreous body throughout the post-operative period. In retinal detachment surgery 3–7 days are required for laserpeky to form a permanent adhesion between the retina and choroid/RPE, and complex cases of proliferative vitreo-retinopathy (PVR) may require a tamponade for several months [27]. Despite a small sample size, the data provides evidence that in their current form PEG sols are not ideal due to their interaction with the retina and limited retention time. Steps should be taken to minimize interaction with the retina. Cross-linking of the polymer may improve biocompatibility and retention time, as well as the mechanical properties [28]. Future work will focus on cross-linking PEG following injection, so that the resulting viscoelastic insoluble gel is retained for a longer period in the vitreous body. This could be achieved by, for example, the use of PEG with thiol and acrylate functional groups to form cross-links in situ [29,30].

5. Conclusion

In the present study aqueous sols of PEG and PBS were tested as potential vitreous substitutes in a rabbit model. Sols of 5 wt.% PEG with a molecular weight of 400,000 g mol⁻¹ in PBS were shown to have mechanical and optical properties similar to the natural vitreous and were well tolerated by the retina, with minimal histological or electrophysiological changes, with the exception GFAP up-regulation over a period of 41 days. However, the sols were not retained in the posterior body throughout the post-operative period. These results indicate the use of a cross-linked PEG hydrogel as a potential artificial vitreous substitute.

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Fig. 1. Poly(ethylene glycol) (PEG) sols. (A) Transparency of PEG sol (left) compared with distilled water (right). (B) Addition of hydrophilic blue dye to PEG sols showed a hydrophobic precipitate. The hydrophobic region was larger the higher the molecular weight of PEG. (Left) S400; (right) S300.

Fig. 2.
Ophthalmoscopic examination 6 days post-operatively. PEG injected eyes displayed a white precipitate in the inferior vitreous body. DPO, days post-operatively.

Fig. 3.

Images of retinas 20 days post-operatively (A, C, and E) and eyes after dissection 41 days post-operatively (B, D, and F). (A and B) No. 1, S300. Total retinal detachment caused during surgery (A) with no sol remaining (B). (C and D) No. 7, S400. Eye injected with PEG sol was normal with retina attached (C) and no sol remaining (D). (E and F) No. 4, BSS. Eye injected with balanced saline solution displayed attached retina (E) and minimal retinal folds (F). DPO, days post-operatively.

Fig. 4.

H&E stained sections. (A and B) No. 4 BSS (A) and No. 7 S400 (B) displayed normal retinal morphology. (C) No. 1, S300. Total destruction of the retina with choroidal inflammation and dissolved retinal pigment epithelium (RPE). (D) No. 5, S300. The retina had some elongation of Müller cell nuclei, large cells in the ONL (black arrow) and sub-retinal inflammatory cells (white arrows). (E and F) No. 2, S400. Brown precipitate and macrophage invasion on retinal surface (E) and inner retinal layers (F) (white arrows). Scale bar 400 μ m (C), 100 μ m (E), 50 μ m (A, B, D, and F). DPO, days postoperatively; VITR,

vitreous up; SCL, sclera down; NFL, neurofilament layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, photoreceptor inner segments; OS, photoreceptor outer segments; RPE, retinal pigment epithelium.

Fig. 5. Glial fibrillary acidic protein (GFAP) immunofluorescence stained sections of the peripheral retina. (A) No. 3, BSS. Eye injected with balanced saline solution shows labeling of the normal arrangement of vertically arranged Müller cells. (B) No. 2, S400. Eye injected with PEG sol S400 displayed up-regulation of GFAP in Müller cells. Scale bar 50 μ m. DPO, days post-operatively.

Table 1

Sol physical properties.

Name	PEG molecular weight (kDa)	pH	Elastic modulus G' (Pa)	Loss modulus G'' (Pa)	Refractive index at 25 °C (Brix %)
S300	300	7.13	0.74	4.68	5.1
S400	400	7.06	7.59	20.56	5.0

Table 2

Pre-, per-, and post-operative data.

Case No. vitreous substitute	Injection volume Surgical notes	Day 6	Day 20	Day 41	Dissection	H&E	GFAP
No. 1, S300	0.8 ml Surgery Retinal rupture and anterior chamber bleeding	Clear, conjunctiva ok Temporal detachment, otherwise attached. Some precipitate centrally. IOP 0	No injection. Cornea clear. No cataract. Clear vitreous. Total nasally. Clear vitreous, funnel- shaped RD. IOP 0 (palp)	No external Inflammation. Minimal cataract Total retinal detachment. IOP normal	Total RD, highest inferiorly. Seems inflamed here. No gel	Total retinal Detachment with destruction of the retina Choroidal inflammation and dissolved RPE	Up-regulated, Müller Cells disorganized
No. 2, S400	0.9 ml	Minimal conjunctiva injection. Cornea clear. Moderate cataract. Minimal vitreous hemorrhage. No precipitate. IOP ~20	No injection. Cornea clear. Lens clear anteriorly, posterior cataract. RD in the periphery inferiorly, centrally ok. Some precipitate on the posterior lens capsule. IOP 10	Minimal cataract. Vitreous clear. Retina attached and normal. IOP ok	Normal dissection. No gel	Retina normal. Epi- and intra-retinal clusters of brownish large cells (leucocytes?)	Up-regulated generally, normal Müller morphology
No. 3, BSS	Not examined	Not examined	No injection. Cornea clear. Lens and vitreous clear. IOP 10 (palp)	Clear, retina ok, IOP ok	OK	Normal	Minimal up-regulation
No. 4, BSS	Not examined	Not examined	No injection. Cornea clear. Mild cataract corresponding to the right sclerotomy. Vitreous clear. IOP 10 (palp)	Clear, retina ok, IOP ok	Minimal retinal folds, artefact?	Normal	Minimal up-regulation
No. 5, S300	1.3 ml	Minimal injection. Precipitate in the inferior vitreous. Photo taken. Minimal cataract. IOP ~10	No injection. Cornea clear. Mild cataract. Vitreous clear. Some precipitate inferiorly. Retina ok. IOP 10 (palp)	Minimal cataract, vitreous clear without precipitate. Retina ok, IOP ok	Some retinal folding. No gel	Minimal central detachment (fold?). Retina looks ok, but inflammatory cells subretinally and large cells in the ONL are present in some sections	Some elongation of Müller cell nuclei
No. 6, S400	1.5 ml (some backflow)	Minimal injection. Precipitate in the inferior vitreous. Photo taken. Minimal cataract. IOP ~15	No injection. Lens clear. Some precipitate inferiorly RD superiorly by the myelinated streak and inferiorly. Centrally ok. IOP 0-5	No cataract. Vitreous clear but slightly brownish with precipitate inferiorly. One retinal fold superiorly, otherwise ok. IOP ok	Total RD, artefact from dissection? No gel, Retina missing superiorly	Total RD, swollen choroid. Outer/inner segments missing. Developed a low detachment in the post-operative period	Up-regulated generally, normal Müller morphology
No. 7, S400	1.2 ml	Minimal injection. Precipitate in the vitreous inferiorly Minimal cataract. IOP ~10	No injection. Cornea clear. No cataract. Clear vitreous. Retina ok. Some precipitate inferiorly. IOP 10	Clear, retina ok, IOP ok	Normal, no gel	Normal	Up-regulated generally, normal Müller morphology
No. 8, S400	1.0 ml	Minimal injection Precipitate in the inferior vitreous Photo	No injection. Cornea clear. No cataract. Clear vitreous. Retina ok. Some precipitate inferiorly. IOP 10	No cataract, vitreous with minimal precipitate	Some precipitate inferiorly,	Normal.	Up-regulated generally, normal Müller morphology

Case No. and vitreous substitute	Injection volume Surgical notes	Day 6	Day 20	Day 41	Dissection	H&E	GFAP
		taken. Minimal cataract. IOP ~0 taken. Minimal cataract. IOP ~0		inferiorly, retina ok, IOP ok	otherwise normal. No gel		

H&E, hematoxylin and eosin stained histologic sections; GFAP, glial fibrillary acidic protein immunofluorescence labeled sections.

Table 3

Pre- and post-operative (day 41) ERG data.

Eye No. and vitreous substitute	Blue light		White light		30 Hz flicker		Summary
	Pre-op	Post-op	Pre-op	Post-op	Pre-op	Post-op	
No. 1, S300	89.8	0.0	61.2	0.0	20.8	0.0	All modalities reduced to 0
No. 2, S400	72.9	78.1	50.8	69.0	10.4	8.16	No change
No. 3, BSS	76.8	53.4	50.1	50.1	20.8	12.3	Reduced rods and cones
No. 4, BSS	39.1	89.8	27.3	31.3	10.0	3.9	Reduced cones, increased rods
No. 5, S300	56.0	0.0	59.9	0.0	26.3	0.0	All modalities reduced to 0
No. 6, S400	91.1	0.0	65.8	0.0	19.7	0.0	All modalities reduced to 0
No. 7, S400	40.4	77.0	46.9	84.6	12.7	12.2	Increased rods and combined
No. 8, S400	56.0	52.1	65.8	57.3	12.5	15.4	No change

Values of the wave amplitudes (μV) for rod ERG (blue light), combined ERG (white light), and dark-adapted single flash cone ERG (30 Hz flicker) are given.