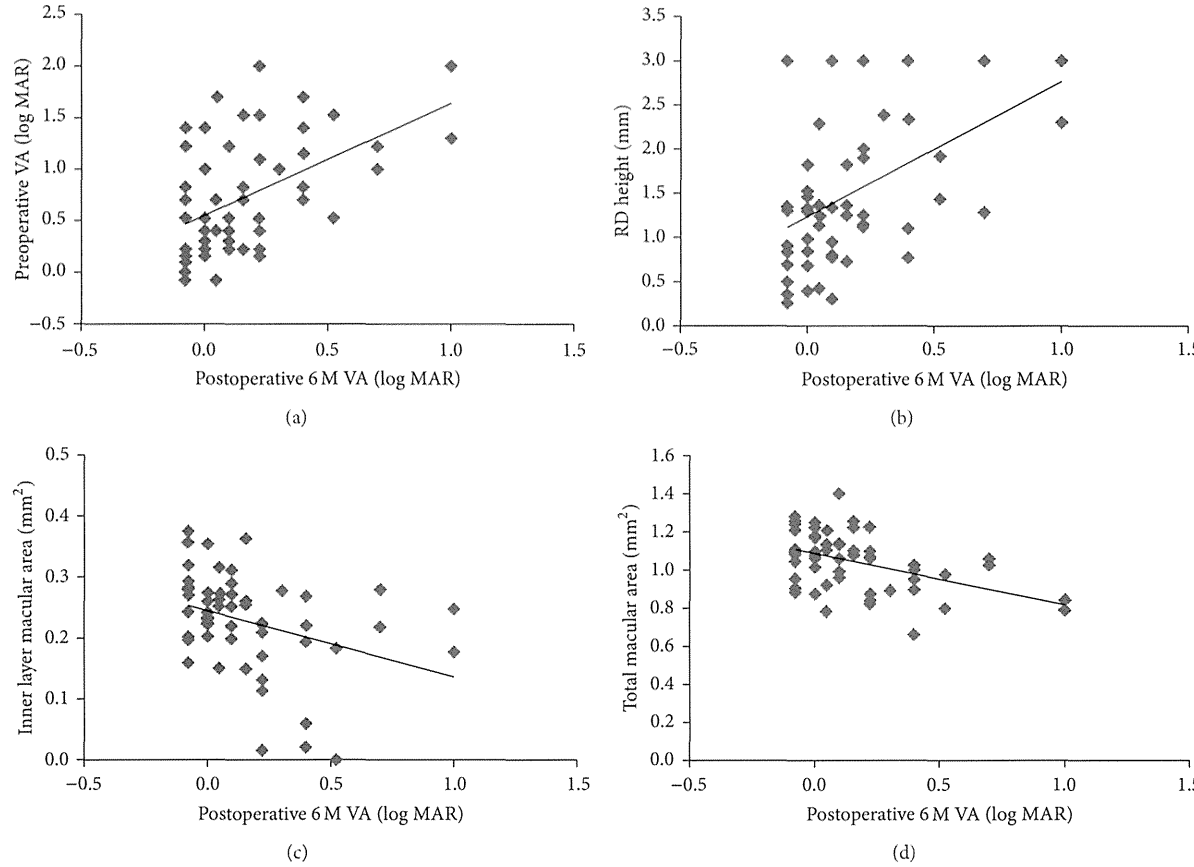

FIGURE 2: Correlation of preoperative clinical findings to 6-month postoperative best-corrected visual acuity (BCVA). There was a positive correlation between preoperative and 6-month postoperative BCVA ((a); $r = 0.48$, $P < 0.001$). There was also a positive correlation between retinal detachment height and 6-month postoperative BCVA ((b); $r = 0.47$, $P < 0.001$). There was a negative correlation between the cross-sectional area of the inner macular layer and 6-month postoperative BCVA ((c); $r = -0.43$, $P = 0.001$). There was also a negative correlation between total macular cross-sectional area and 6-month postoperative BCVA ((d); $r = -0.44$, $P < 0.001$).

TABLE 2: Multiple regression analysis for independent factors contributing to 6 M postoperative VA.

Dependent	Variable Independent		β	P value
Postoperative VA	Age		0.041	0.784
	Duration of macular detachment		0.869	0.024
	Preoperative VA		0.188	0.249
	Preoperative OCT findings	RD height	0.212	0.203
		Total macular area	−0.511	0.041
		Outer layer macular area	0.334	0.180
		Middle layer macular area	0.267	0.156

VA = visual acuity, OCT = optical coherent tomography, RD = retinal detachment, and β = standard partial regression coefficient.

speed of existing 3D OCT devices, making it impossible to evaluate foveal volume in these eyes. To overcome this technical difficulty, we used two-dimensional OCT to measure the cross-sectional area of the macular layer in a 2 mm circle centered on the fovea and investigated its potential as an indicator of final BCVA. We developed this measurement parameter after observing that the detached section of the macula is not straight or flat in OCT images but instead lies obliquely across the image plane. Conventionally measuring cross-sectional area in a square or rectangle horizontal to the choroid would thus tend to overestimate foveal thickness. By contrast, measurements of cross-sectional area in a 2 mm circle centered on the fovea (which is about 1 mm in diameter) should be reliable regardless of the orientation or position

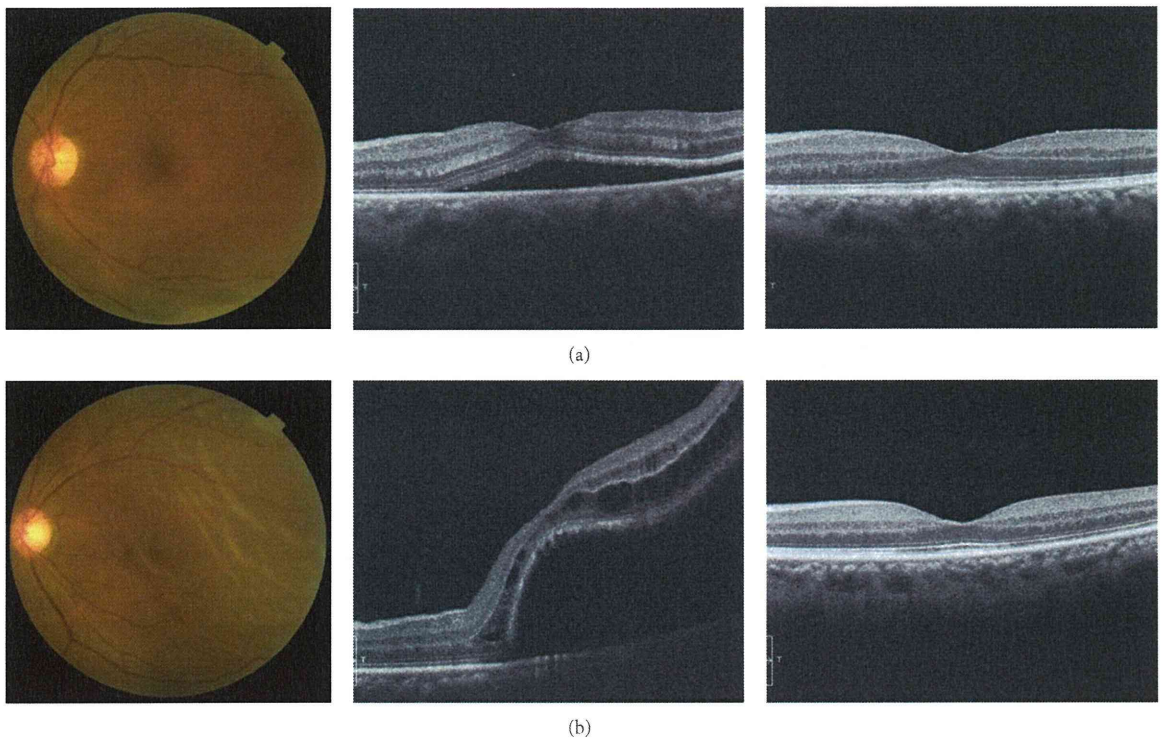

FIGURE 3: Representative eyes with good and poor visual outcomes after surgery for macula-off rhegmatogenous retinal detachment. (a) 65-year-old woman (preoperative decimal visual acuity: 0.7) with a good visual outcome (postoperative decimal visual acuity: 1.2). (b) 60-year-old woman (preoperative decimal visual acuity: 0.3) with a poor visual outcome (postoperative decimal visual acuity: 0.3). Preoperative photographs of the fundus, preoperative optical coherence tomography (OCT) images, and postoperative OCT images are shown on the left, center, and right, respectively. Preoperative foveal area was relatively thinner in the case with a poor outcome than in the case with a good outcome.

of the detachment. A circular area larger than 2 mm would begin to lose reliability, as it would be more influenced by intraretinal edema, bending, or severe undulation of the detachment. Thus, we believe that it is most reasonable to adopt the cross-sectional area of the macular layer within 2 mm of the fovea as an indicator of macular health.

This study showed that RD height at the fovea, a measurement parameter used in a number of earlier studies, was associated with postoperative BCVA in a single regression analysis, confirming earlier reports [11, 12]. This is an understandable result, as when the distance between the retinal pigment epithelium and the photoreceptors increases, the foveal cones receive less oxygenation and nutrition from the choroid and photoreceptor cell degeneration increases. However, multiple regression analysis revealed that it was not an independent factor predicting 6-month postoperative BCVA ($P = 0.203$). The cause of this discrepancy is unclear but may have been related to the instability of RD height, particularly in bullous RRD and particularly in older eyes, because the detached macula can more easily shift its position in the vitreous. It is difficult to accurately and reproducibly evaluate RD height in such eyes, leading us to speculate that preoperative RD height cannot be considered a reliable predictor of postoperative visual function in eyes with macula-off RRD.

Limitations of this study included a relatively short follow-up time of 6 months, a relatively small sample size (about 60), and the omission of postoperative functional findings from standard automated perimetry or focal electroretinography. Additionally, although bullous RRD eyes are often seen in the clinic, the method described here cannot be used to predict postoperative outcomes in cases when OCT scans do not show the macula. Furthermore, to prevent bias in the results, it was necessary to omit the inner macular layer in the cross-sectional image from our multiple regression analysis, because the total and inner layer values were not independent, both being OCT findings and being closely correlated with 6-month postoperative BCVA. At first, we hypothesized that visual outcome would be associated with the area of the outer macular layer, as this contains the outer nuclear layer and the photoreceptor cells, but this hypothesis was not borne out by the data. It is unclear why this was so, but it may have been related to the susceptibility of the outer layer to intraretinal edema or undulation, which makes it difficult to obtain accurate measurements. Nevertheless, we believe our results show that simple OCT measurement of total cross-sectional area within 2 mm of the fovea is currently the most useful and objective way to predict postoperative visual outcomes in eyes with macula-off RRD, at least until technology to quickly evaluate

macular volume in three dimensions becomes available for use in eyes with detached maculas. The usefulness of the measurement method described here would also be greatly enhanced by an OCT program to automatically measure cross-sectional macular area in eyes with macula-off RRD.

In conclusion, OCT measurement of preoperative total cross-sectional area of the macular layer within 2 mm of the fovea is a useful and objective way to predict postoperative visual outcomes in eyes with macula-off RRD and was closely correlated with 6-month postoperative BCVA. Further investigation is needed to measure the macular volume and determine its relationship with visual outcomes, which could lead to the development of a new automatic OCT program.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

The principal investigator, Dr. Noriyuki Suzuki, and the coinvestigator, Dr. Naoko Aizawa, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the analysis. Involved in the design and conduct of the study were Hiroshi Kunikata and Toru Nakazawa; collection, management, analysis, and interpretation of the data Noriyuki Suzuki, Hiroshi Kunikata, and Naoko Aizawa; drafting of the paper Hiroshi Kunikata; and review or approval of the paper Hiroshi Kunikata, Toshiaki Abe, and Toru Nakazawa. All authors read and approved the final paper.

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Clinical Study

25-Gauge Microincision Vitrectomy to Treat Vitreoretinal Disease in Glaucomatous Eyes after Trabeculectomy

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Purpose. To determine the feasibility of using 25-gauge microincision vitrectomy surgery (25GMIVS) to treat vitreoretinal disease in glaucomatous eyes which have previously undergone trabeculectomy (TLE). *Methods.* A consecutive, interventional case series. We performed 25GMIVS in 15 glaucomatous eyes that had undergone TLE. Follow-up period was 11.5 months. *Results.* 25GMIVS was successfully used and led to improvement in visual acuity ($P < 0.01$). We performed 25GMIVS for proliferative diabetic retinopathy with neovascular glaucoma in 53% of eyes (8 of 15). Although 3 eyes needed further TLE following 25GMIVS, final IOP was below 21 mmHg in all eyes except one eye (93%) and was comparable to pre-25GMIVS IOP ($P = 0.20$) without an increase in the number of glaucoma medications ($P = 0.14$). *Conclusions.* 25GMIVS is a feasible treatment for vitreoretinal disease in eyes with preexisting TLE, effective in both significantly improving BCVA and preserving the filtering bleb, while not excluding further glaucoma surgery.

1. Introduction

Trabeculectomy (TLE) is a procedure most often performed when drug-based therapies for glaucoma have been ineffective. It can effectively reduce intraocular pressure (IOP) over the long term [1–3], but can lead to problems if further severe retinal diseases requiring vitrectomy arise. This is particularly the case for conventional 20-gauge par planar vitrectomy (20GPPV), because that procedure requires suturing and a conjunctival incision, which can disrupt the ocular surface and lead to impairment of the filtering bleb [4]. Furthermore, 20GPPV can make future or unanticipated filtering surgery more difficult as it causes conjunctival-scleral adhesion in multiple quadrants. It would thus be desirable to establish an alternative vitrectomy technique that has a lower risk of causing filtering bleb failure and does not exclude further glaucoma surgery.

Twenty-five-gauge microincision vitrectomy surgery (25GMIVS) was first reported in 2002, and this procedure is now commonly used worldwide [5–15]. One of the advantages of this technique is that intraoperative suturing is not needed, which reduces postoperative ocular pain and discomfort in patients. Furthermore, 25GMIVS allows earlier postoperative visual improvement than 20GPPV and does not induce significant changes in the corneal topography or optical quality of the cornea [16–20]. Although 25GMIVS does have limitations [21–23], we believe that because it is sutureless, it is the best choice to treat retinal disease in eyes with preexisting TLE and can best preserve the filtering bleb. However, to the best of our knowledge, using the PubMed search system, there are no reports discussing or evaluating the use of 25GMIVS to treat retinal diseases in glaucomatous eyes after TLE, except one case report of familial amyloid polyneuropathy (FAP) [24].

Thus, the purpose of this study was to determine the feasibility of using 25GMIVS to treat vitreoretinal disease in glaucomatous eyes that have undergone TLE.

2. Patients and Methods

2.1. Participants. This was a retrospective, consecutive, interventional case series performed at a single center. Fifteen consecutive post-TLE eyes of 15 patients with retinal diseases who underwent 25GMIVS were studied. The inclusion criterion was any retinal vitreous disease causing visual dysfunction in eyes that had previously undergone TLE, (meaning the eyes already had a filtering bleb). The exclusion criteria were prior scleral buckling, prior trauma, and a follow-up period of less than 3 months. The preoperative demographics and postoperative courses of the patients are shown in Table 1. All of the surgeries were performed at the Surgical Retina Service of Tohoku University Hospital from October 2008 to May 2012. All 25GMIVS procedures were performed by a single surgeon (H.K.). After the purpose and procedures of the operation were explained, informed consent was obtained from all patients. This study conformed to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the School of Medicine, Tohoku University.

2.2. Surgical Procedures. All surgeries were performed under retrobulbar anesthesia using the oblique sclerotomy technique and were performed using the Accurus Vitrectomy System (Alcon Laboratories; Fort Worth, Texas, USA). First, an infusion cannula was inserted through the inferotemporal sclera followed by the insertion of two cannulas through superotemporal and superonasal sites. The insertion point of the cannulas was shifted as necessary to avoid disturbing the conjunctiva adjacent to the filtering bleb. Next, a 2.4 mm superotemporal corneal incision was made, followed by phacoemulsification, aspiration (PEA), and intraocular lens (IOL) implantation before the vitrectomy, if the eye had a cataract. After resecting the vitreal core, 4 mg of triamcinolone acetonide (TA; Kenacort-A, Bristol-Meyers Squibb, Tokyo, Japan) was injected into the vitreous cavity to determine if a posterior vitreous detachment (PVD) was present. If a PVD was not present, we created one with a 25-gauge cutter. After shaving the peripheral gel, the proliferative membrane was removed, and fluid air exchange and endophotocoagulation were performed if needed. The exact surgical procedures varied according to the type of vitreoretinal disease. Additional TLE was also performed under retrobulbar anesthesia, with a fornix-based conjunctival flap. A half-thickness 4.0 by 4.0 mm rectangular scleral flap was made in the superior area. Mitomycin C (MMC) was used with a concentration of 0.04% and an exposure time of 5 minutes. The area was irrigated thoroughly with 200 mL of balanced salt solution. Trabeculectomy was then performed, followed by peripheral iridectomy. The scleral flap and conjunctiva were closed with a 10-0 nylon suture. Postoperatively, antibiotics and corticosteroids were injected subconjunctivally.

2.3. Measurements of Clinical Findings. We evaluated best-corrected visual acuity (BCVA), IOP, number of glaucoma medications, intraoperative subconjunctival hemorrhage, intraoperative suturing at the sclerotomy site, and additional TLE (after 25GMIVS). BCVA was measured using the Landolt C visual acuity chart, and the decimal BCVA was converted to logarithm of the minimal angle of resolution (LogMAR) units for statistical analysis. Success of the 25GMIVS procedure was defined as improvement or maintenance of BCVA and maintenance of IOP $\leq$21 mmHg with the use of topical glaucoma medication, with no need for additional TLE. The procedure was recorded as a failure if there was a decrease in BCVA, additional TLE was required, or IOP could not be maintained $\leq$21 mmHg with the use of topical glaucoma medication.

2.4. Statistical Analysis. The data are presented as the mean $\pm$ standard deviation. The significance of the difference between the pre-25GMIVS BCVA and final BCVA in logMAR units was determined by the single tailed paired t-test. For the statistical analysis, "count fingers" visual acuity was set as 2.0 logMAR units, and "hand motion" acuity was set as 3.0 logMAR units. The significance of the difference in IOP before TLE, after TLE, and after 25GMIVS was determined by the Friedman test, and the significance of the difference between the IOP after TLE and after 25GMIVS was determined by the Scheffe's test. The significance of the difference in the pre-25GMIVS number of glaucoma medications and the final number was also determined by the two tailed paired t-test. A P value of less than 0.05 was considered to be statistically significant.

3. Results

A summary of the patients' characteristics and pre-25GMIVS course is shown in Table 1. There were 8 men and 7 women with a mean age of 57.4 $\pm$ 13.2 years. The type of glaucoma originally requiring TLE included neovascular glaucoma (NVG, 8 eyes; 53%), open angle glaucoma (2 eyes; 13%), malignant glaucoma (2 eyes; 13%), traumatic glaucoma (1 eye), uveitis-associated secondary glaucoma (1 eye), and developmental glaucoma (1 eye). Vitreoretinal diseases in eyes with preexisting-TLE treated with 25GMIVS in our study included proliferative diabetic retinopathy (PDR, 7 eyes; 47%) (Figure 1), malignant glaucoma (2 eyes; 13%), rhegmatogenous retinal detachment (1 eye) (Figure 2), branch retinal vein occlusion (1 eye), macular hole (1 eye), dislocated intraocular lens (1 eye), endophthalmitis (1 eye), and choroidal hemorrhage (1 eye). The mean period between the original TLE procedure and 25GMIVS was 25.3 $\pm$ 29.7 months, with a range of 0.3 to 105 months. All blebs were located in the upper quadrants. Prior to TLE, vitrectomy had been performed in 3 eyes (20%) with PDR. Before 25GMIVS, intravitreal injection of bevacizumab (IVB) was performed in 4 eyes (27%) with NVG, but after 25GMIVS it was not necessary. There were 9 eyes (60%) with pseudophakia before 25GMIVS.

Table 1: Characteristics and pre-25-gauge microincision vitrectomy course of 15 glaucomatous eyes.

Patient no./sex/age, yrs	Eye	Type of glaucoma	Pre-25GMIVS retinal disease	Period of 25GMIVS after TLE (M)	Site of bleb	Pre-TLE vitrectomy	Pre-25GMIVS IVB	Pre-25GMIVS pseudophakia
1/M/45	L	Trauma	RRD	4	Upper temporal	N	N	Y
2/M/58	L	NVG	BRVO/VH	30	Upper nasal	N	N	N
3/M/62	R	NVG	PDR/VH	48	Upper temporal	N	Y	Y
4/F/60	L	NVG	PDR/TRD	36	Upper nasal	N	Y	N
5/M/68	L	NVG	PDR/VH	10	Upper nasal	Y	Y	Y
6/F/62	L	Uveitis	MH	55	Upper nasal	N	N	Y
7/F/44	R	NVG	PDR/VH/CD	0.5	Upper	N	Y	Y
8/F/83	R	Malignant glaucoma	Malignant glaucoma	1	Upper nasal	N	N	Y
9/F/33	L	Developmental glaucoma	Lens luxation	105	Upper nasal	N	N	N
10/M/52	R	POAG	Endophthalmitis	0.3	Upper temporal	N	N	N
11/M/59	R	POAG	ERM	50	Upper temporal and nasal	N	N	N
12/M/61	R	NVG	PDR/VH	25	Upper temporal	Y	N	Y
13/F/56	R	NVG	PDR	7	Upper temporal	Y	N	Y
14/F/77	L	Malignant glaucoma	Choroidal hemorrhage	1	Upper temporal	N	N	Y
15/M/41	R	NVG	PDR/VH	6	Upper nasal	N	N	N
Mean 57.4		NVG 53%	PDR 47%	25.3		20%	27%	60%

25GMIVS: 25-gauge microincision vitrectomy surgery; TLE: trabeculectomy; IVB: intravitreal injections of bevacizumab; NVG: neovascular glaucoma; TRD: tractional retinal detachment; RRD: rhegmatogenous retinal detachment; BRVO: branch retinal vein occlusion; PDR: proliferative diabetic retinopathy; VH: vitreous hemorrhage; MH: macular hole; ERM: epiretinal membrane; POAG: primary open angle glaucoma; CD: choroidal detachment.

A summary of the patients' characteristics and post-25GMIVS course is shown in Table 2. PEA, IOL, and 25GMIVS were performed together in 4 eyes (27%), and 25GMIVS was performed by itself in 11 eyes (73%). None of the eyes required suturing of the 25-gauge sclerotomy site at the end of the initial surgery except one (7%) that had undergone vitrectomy before TLE. The mean operative time was 38.3 ± 16.3 minutes. The mean decimal pre-25GMIVS BCVA and final BCVA were 0.05 and 0.3, respectively. Final BCVA in logMAR units was significantly better than the pre-25GMIVS BCVA ($P = 0.01$). Pre-TLE IOP, pre-25GMIVS IOP, and final IOP were 36.0, 11.9, and 15.7 mmHg, respectively. There were significant IOP differences pre-TLE, pre-25GMIVS, and post-25GMIVS ($P < 0.001$), but no significant difference between the pre-25GMIVS IOP and final IOP ($P = 0.20$). There was no difference in the pre-25GMIVS and final number of glaucoma medications ($P = 0.14$). Subconjunctival hemorrhage occurred in 5 eyes (33%); however, in 3 of these eyes, there was no hemorrhage invasion into the filtering bleb. Four eyes (27%) had intraocular pressure >20.0 mmHg after 25GMIVS, and 3 of these eyes needed additional TLE. It should be mentioned that this additional TLE was a technically simple procedure, because 25GMIVS had been performed without any sutures. Vitreoretinal diseases in all 15 eyes with preexisting TLE were successfully treated with 25GMIVS. We achieved success with 25GMIVS in 10 cases (67%) and did not observe surgical complications, such as bacterial endophthalmitis, associated with either TLE or 25GMIVS in any of the cases. The mean follow-up period was 11.5 ± 7.7 months with a range of 6 to 34 months.

4. Discussion

We set out to evaluate the feasibility of using 25GMIVS to treat vitreoretinal disease in glaucomatous eyes that had undergone TLE. The most common vitreoretinal disease in eyes with preexisting TLE we treated with this technique was PDR complicated by NVG (in almost 50% of cases). In spite of the relatively high incidence of such a severe disease, mean BCVA improved significantly after 25GMIVS. Additionally, although 3 eyes needed further TLE following our 25GMIVS procedure, the final measurement of IOP was statistically

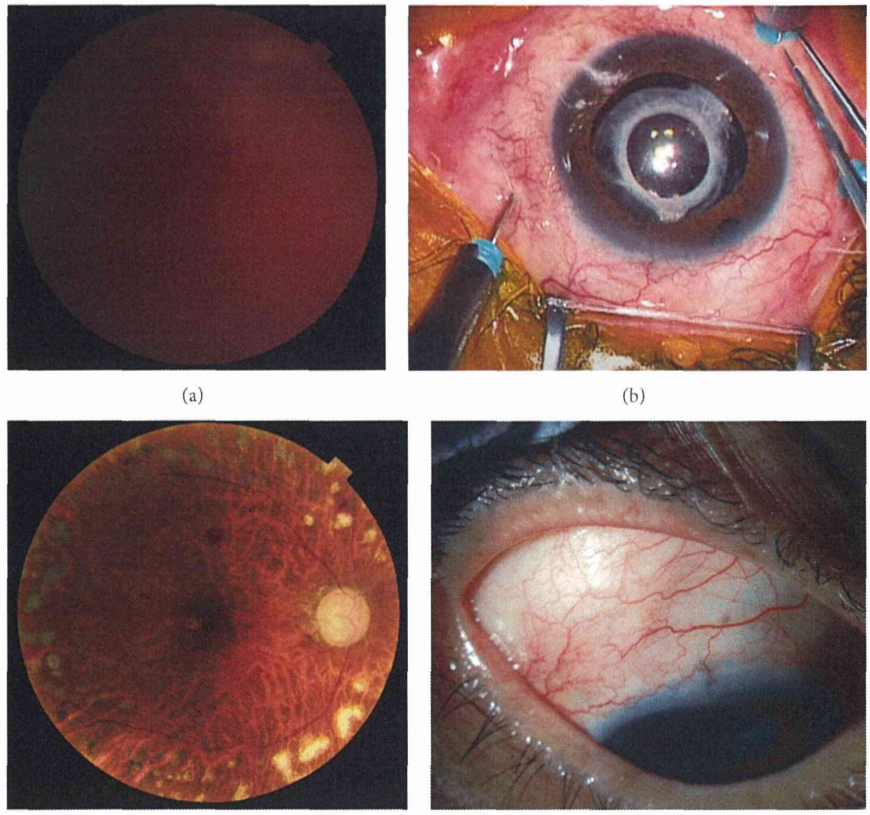

FIGURE 1: Representative example of proliferative diabetic retinopathy (PDR) complicated by neovascular glaucoma (NVG) (Patient 12; see Table 1). Fundus, anterior segment, and intraoperative photographs of the eye of a 61-year-old man with PDR/NVG. The eye underwent 25-gauge microincision vitrectomy surgery (25GMIVS) after trabeculectomy. (a) Preoperative photograph of the fundus. We could not visualize the posterior fundus due to vitreous hemorrhage (VH). (b) Intraoperative photograph of the anterior segment. 25GMIVS was being performed with 3 ports. The insertion placement of the cannulas was shifted to avoid disturbing the subconjunctival hemorrhage of the filtering bleb in the upper temporal region. (c) Postoperative photograph of the fundus. The VH has been removed and the retinal surface can be seen clearly. (d) One-day postoperative photograph of the anterior segment. There was no subconjunctival hemorrhage, including the filtering bleb, in the upper temporal region.

comparable to IOP before 25GMIVS, without an increase in the number of glaucoma medications. Furthermore, because of our use of sutureless 25GMIVS, the additional TLE procedure itself, following 25GMIVS, was not more difficult than a standard TLE procedure.

Our results confirm existing data that, in about one-third of cases, eyes will develop elevated pressure if they undergo vitrectomy after TLE [4]. About 30% of our case series had IOP ≥20.0 mmHg following 25GMIVS, and 3 cases needed additional TLE. In the 3 eyes with types of glaucoma other than NVG, IOP was maintained ≤21 mmHg after 25GMIVS and further TLE was not necessary. In almost 50% of post-TLE eyes we treated with 25GMIVS, however, PDR with NVG was present (Thompson et al; 13% of eyes had PDR) [4]. Our final result for IOP control could thus be considered reasonably successful, given that NVG is known to be generally refractory to conventional TLE with MMC. Specific prognostic factors for surgical failure have been reported to be young age, previous vitrectomy, and, when PDR is present, a fellow eye with NVG [25]. An alternative technique to effectively reduce elevated IOP in eyes with NVG is vitrectomy and complete pan-retinal photocoagulation combined with TLE [26, 27]. Our results, which also support a single existing case report on 25GMIVS for a FAP eye with a filtering bleb, show that 25GMIVS has the potential to become the treatment of choice for vitrectomy in glaucomatous eyes that have already undergone TLE [24]. Additionally, our study now shows that there is no hypotony (IOP < 5.0 mmHg) after 25GMIVS. Thompson et al. reported that IOP outcomes after 20GPPV were rather variable; one-third of eyes in that study developed hypotony, one-third developed elevated pressure, and one-third maintained bleb function [4]. The cause of this discrepancy with our results is unclear, but we speculate that one reason was the high retinal reattachment rate that results from 25GMIVS. The hypotonic eyes from the earlier report included some with persistent retinal detachments following vitrectomy [4]. Additionally, we also believe that the smaller gauge required less infusion

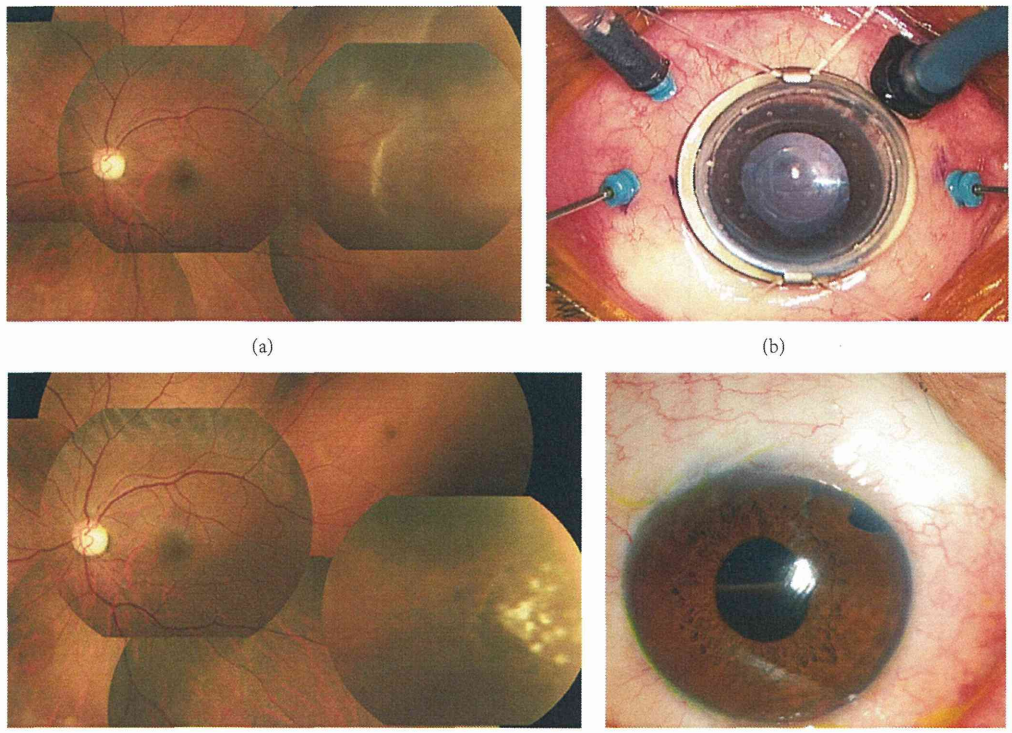

FIGURE 2: Representative example of rhegmatogenous retinal detachment (RRD) (Patient 1; see Table 1). Fundus, anterior segment, and intraoperative photographs of the eye of a 45-year-old man with RRD. The eye underwent 25-gauge microincision vitrectomy surgery (25GMIVS) after trabeculectomy. (a) Preoperative photograph of the fundus. There was focal retinal detachment with a peripheral retinal tear. (b) Intraoperative photograph of the anterior segment. 25GMIVS was being performed with 4 ports. The insertion placement of the cannulas was shifted to avoid disturbing the subconjunctival hemorrhage of the filtering bleb in the upper temporal region. (c) Postoperative photograph of the fundus. Retinal reattachment was achieved with 25GMIVS. The white retinal scars of endophotocoagulation can be seen. (d) One-day postoperative photograph of the anterior segment. There was no subconjunctival hemorrhage, including the filtering bleb, in the upper temporal region. An air-fluid level line of intraocular gas tamponade can be seen through the pupil.

of balanced solution during the procedure and had less of a negative intraoperative effect on the ciliary bodies, which have the important role of producing the intraocular aqueous humor.

We speculate that subconjunctival hemorrhage during 25GMIVS affects the preexisting filtering bleb. Many earlier reports have demonstrated the advantages and disadvantages of using autologous blood injection to treat overfiltering or leaking blebs after glaucoma surgery [28–33]. Thus, the sutureless nature of 25GMIVS, which prevents intraoperative subconjunctival hemorrhage from flowing into preexisting bleb, could be a great benefit for the treatment of vitreoretinal disease in eyes with preexisting TLE. We also believe that 25GMIVS can prevent conjunctival adhesion, thereby preserving an existing filtering bleb and clearing the way for additional glaucoma surgery. Subconjunctival hemorrhage after 25GMIVS occurred in about 30% of our cases, with consequent additional glaucoma surgery (about 20%). We thus find it highly advisable to avoid disrupting conjunctival vessels when creating a 25-gauge sclerotomy in glaucomatous eyes that have undergone TLE. The prognostic factors for surgical failure of TLE with MMC in vitrectomized eyes have been reported to be high preoperative IOP and NVG [34]. As stated above, we had 3 NVG patients undergo additional TLE following 25GMIVS, and we were able to achieve a final reduction in IOP in all 3 eyes. We believe that one reason for our success in such severe cases was the conjunctiva's good condition following the sutureless 25GMIVS procedure, in contrast with the earlier report on vitrectomy for NVG, in which a conventional 20GPPV procedure was used. The good condition of the conjunctiva after 25GMIVS might also make it possible to implant recently introduced glaucoma drainage devices, which can aid in the management of complicated glaucoma such as NVG. The need for suturing of a 25-gauge sclerotomy at the end of the 25GMIVS procedure in one vitrectomized eye (patient 5) due to high leakage leads us to believe that vitrectomy can be difficult to perform multiple times without any scleral suturing. In addition, it is difficult to perform IVB in vitrectomized eyes with NVG (patients 5, 12, and 13), because, as has already been demonstrated, injected bevacizumab is quickly washed away from a vitrectomized eye [35]. However, there is one report demonstrating that IVB before TLE might further improve the surgical success rate for NVG in previously vitrectomized eyes [36].

Table 2: Characteristics and post-25-gauge microincision vitrectomy course of 15 posttrabeculectomy eyes.

Patient no./ sex/age (y)	Decimal VA course		IOP (mmHg) course			Number of glaucoma medications		25GMIVS			Post-25GMIVS hyposphagma	Post-25GMIVS interventions	Followup (M)	Post-25GMIVS time before failure	Post-25GMIVS success
	Pre-25GMIVS	Final	Pre-TLE IOP	Pre-25GMIVS	Final	Pre-25GMIVS	Final	Combined cataract surgery	Port suturing	Operative time (min)					
1/M/45	1.2	1.2	29	9	9	0	0	N	N	41	N	N	12	—	Y
2/M/58	HM	0.8	35	15	15	0	2	Y	N	17	N	N	6	—	Y
3/M/62	CF	0.01	36	7	19	3	2	N	N	35	N	TLE	9	0.5	N
4/F/60	0.02	0.03	46	21	26	1	4	Y	N	56	Y	N	6	4	N
5/M/68	HM	1.2	37	11	10	0	0	N	Y	41	Y	N	8	—	Y
6/F/62	0.9	1.2	38	7	7	0	0	N	N	22	N	N	6	—	Y
7/F/44	CF	0.2	56	16	19	0	4	N	N	45	Y	TLE	13	8	N
8/F/83	1	1.2	30	30	13	3	0	N	N	25	N	N	12	—	Y
9/F/33	0.15	0.9	32	12	20	0	0	Y	N	74	N	N	9	—	Y
10/M/52	HM	0.7	16	7	16	3	4	N	N	26	Y	N	34	—	Y
11/M/59	0.6	0.6	19	10	15	0	2	Y	N	26	N	N	6	—	Y
12/M/61	HM	0.6	30	9	16	1	2	N	N	22	N	N	23	—	Y
13/F/56	HM	NLP	49	9	18	3	3	N	N	35	N	N	13	13	N
14/F/77	HM	0.08	32	10	17	3	3	N	N	59	Y	N	7	—	Y
15/M/41	0.03	HM	55	6	16	1	2	N	N	50	N	TLE	8	1	N
Mean 57.4	0.05	0.3	36.0 mmHg	11.9	15.7	1.2	1.9	27%	7%	38.3	33%	20%	11.5		67%

25GMIVS: 25-gauge microincision vitrectomy surgery; IOP: intraocular pressure; VA: visual acuity; IOP: intraocular pressure; TLE: trabeculectomy; HM: hand movement; CF: counting fingers; NLP: no light perception.

$P < 0.01$; Wilcoxon signed-ranks test; pre-25GMIVS VA versus final VA.

$P < 0.001$; Friedman test for 3 groups: pre-TLE IOP, pre-25GMIVS IOP and final IOP.

$P = 0.50$; Scheffe's test; pre-25GMIVS IOP versus final IOP.

$P = 0.67$; Wilcoxon signed-ranks test; pre-25GMIVS number of glaucoma medications versus final number of glaucoma medications.

There were limitations to our study, including the retrospective nature of the analysis, a short follow-up period, and a small number of patients. We did not discuss filtering bleb function in detail because our study included eyes with blebs whose function before 25GMIVS was doubtful (these eyes continued to need glaucoma medication after TLE). Furthermore, we did not compare 25GMIVS and 20GPPV. A comparative, prospective study in post-TLE eyes would provide valuable insights into the relative value of these techniques but is impossible due to ethical considerations, making an experimental analysis using an animal model perhaps the most useful approach for such a future study. Nevertheless, as post-TLE eyes that require glaucoma medication for IOP control before vitrectomy are commonly observed clinically and indeed comprised about 50% of the cases in our study, we believe that this is a valuable study of a useful treatment for post-TLE eyes with various retinal conditions. Further investigation is needed to evaluate postoperative visual quality and complications in the late postoperative period before a final determination can be made of the efficacy of this procedure.

In conclusion, 25GMIVS is technique that can feasibly be used to treat vitreoretinal disease in glaucomatous eyes that have undergone TLE. Regardless of a high incidence of PDR with NVG in our case series, we were able to achieve good final results for BCVA and IOP with 25GMIVS, without increasing the number of glaucoma medications. Though there were a few cases that needed additional TLE following 25GMIVS, the additional TLE procedure was not more technically difficult than usual, because of the good condition of the conjunctiva after sutureless 25GMIVS. Our results showed that 25GMIVS was effective in preserving the filtering bleb and the other quadrant conjunctiva in eyes with glaucoma and did not exclude further surgical intervention for this disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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A Platform for Controlled Dual-Drug Delivery to the Retina: Protective Effects against Light-Induced Retinal Damage in Rats

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Retinal diseases such as glaucoma, age-related macular (AMD) degeneration, and retinitis pigmentosa (RP) are among the most common causes of visual impairment worldwide.[1] Retinal degenerations from progressive loss of photoreceptor or retinal ganglion cells are largely responsible for vision loss.[2–4] Multiple drugs have been used to treat patients with ocular diseases, such as glaucoma[5,6] and to suppress choroidal neovascularization in patients with AMD.[7] Generally, a better therapeutic efficacy to slow the progression of retinal degeneration can be obtained when using two or more, compared with therapy using a single drug.

One of the risk factor for the onset and/or progression of AMD is excessive light exposure, which leads to reactive oxygen species (ROS) generation and intracellular $Ca^{2\pm}$ influx,[8,9] resulting in photoreceptor degeneration.[10,11] Thus, in this study, edaravone (EDV), a potent-free radical scavenger,[11] and unoprostone isopropyl (UNO), a large conductance $Ca^{2\pm}$ activated $K^{\pm}$ channels activator,[12,13] were used as potential therapeutics and retinal neuroprotection by controlled transscleral co-delivery of the two drugs using a polymeric device was tested.

We previously developed a polymeric device that can release multiple compounds at independently controlled release rates.[14] The device comprises a microfabricated reservoir, a controlled-release cover, and formulations made of photopolymerized tri(ethyleneglycol)dimethacrylate (TEGDM) and poly(ethyleneglycol)dimethacrylate (PEGDM). The release rate of each compound is controlled by varying the PEGDM/TEGDM ratio in its formulation and in the cover. The device is designed to deliver drugs via the transsceleral route, which is less invasive compared to intravitreal injections[15] and more bioavailable compared to topical eye drops.[16,17] Thus, we investigated the fabrication of the device that contains different formulations of EDV and UNO and evaluated the protective effects of this device against light-induced retinal damage in rats.

First, we investigated the controlled release of EDV and UNO using this polymeric system. The drugs were pelletized with P60 (PEGDM/TEGDM prepolymer mixture ratios of 60%/40%), loaded in the reservoir, and sealed with covers having various PEGDM/TEGDM proportions. The release of both EDV (**Figure 1**a) and UNO (Figure 1b) can be tuned by changing the ratio of PEGDM/TEGDM in the cover, and the release rate was almost constant in the covered devices. The release of both EDV and UNO was dependent on the PEGDM/TEGDM ratio and the release rate decreased with a decreasing PEGDM ratio in the cover (Figure 1c,d). A pure TEGDM (P0) cover was impermeable to EDV and UNO. Both of the devices without a cover showed a rapid burst-like release. The release rates estimated from the gradient curve for P60-, P40-, P20-, and P0-covered EDV-DDSs were 6.28, 2.80, 0.82, and 0 μg per day, respectively. In turn, the release rates for P60-, P40-, P20-, and P0-covered UNO–DDSs were 1.10, 0.48, 0.16, and 0 μg per day, respectively. The ability to control the release of drugs from the device is based on the swelling of the PEGDM/TEGDM polymer.[14] The polymer made of short chains of TEGDM is likely to be compact, allowing no penetration of drugs. On the other hand, long chains of PEGDM may result in a greater tendency to swell, facilitating permeation of small molecules

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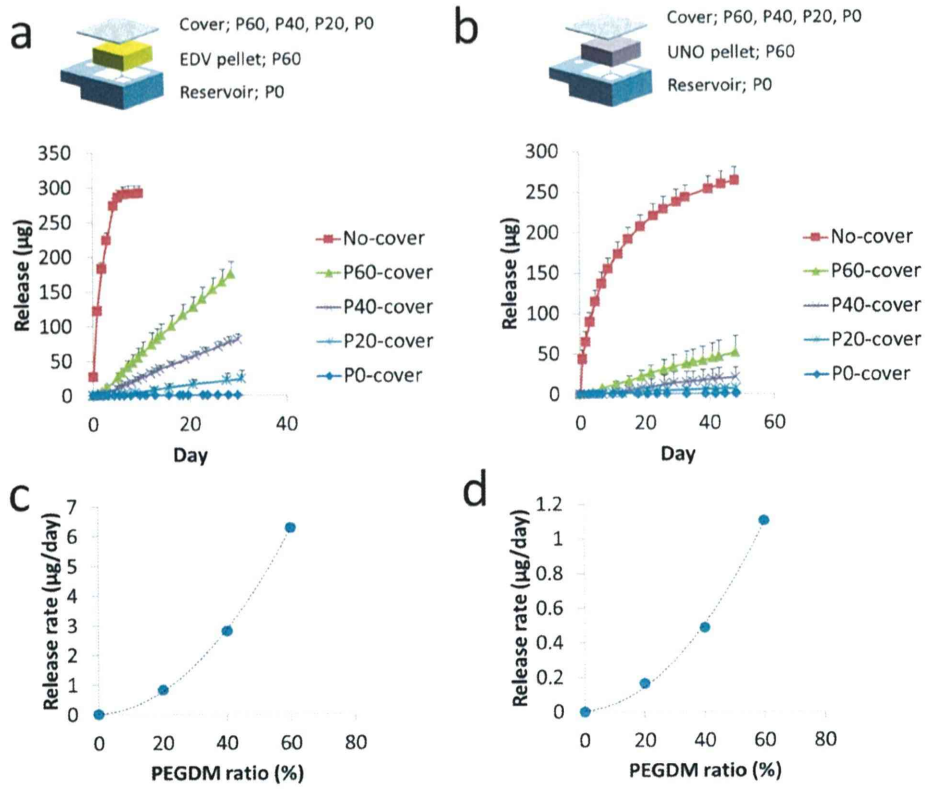

Figure 1. Release of a) EDV and b) UNO from devices sealed with various PEGDM/TEGDM covers. Correlation between PEGDM ratio in the cover and release rate of c) EDV and d) UNO. Release rate was estimated from the slope of the curve at the initial stable release period in (a) and (b). Values are mean ± SD.

through the cover. The release rate may be influenced by physical characteristics of the drug substance such as lipophilicity and water solubility.[18] In fact, hydrophobic UNO exhibited a lower release rate than hydrophilic EDV under the same device conditions (Figure 1).

The dual-drug release device was next applied to a light-induced retinal injury model and retinal function was evaluated using an electroretinogram (ERG) and compared to results obtained using single-drug release devices. The devices are designed for implantation on the rat sclera and they release drugs unidirectionally via a cover to the scleral side by encapsulating drugs with a TEGDM (P0) reservoir (**Figure 2**a). To evaluate the effects of the EDV/UNO-loaded device, PBS/PBS-, EDV/PBS-, UNO/PBS-, and EDV/UNO-loaded devices were fabricated as shown in Figure 2b. The appropriate combination of EDV- and UNO-formulations was evaluated using P40 and P60. The combination of EDV in P60 (E60)/UNO in P40 (U40) showed more effective protection as a function of ERG amplitudes than the other combinations of E60/UNO in P60 (U60), EDV in P40 (E40)/U60, and E40/U40 (Figure S1, Supporting Information). Therefore, an E60/U40 (EDV/UNO)-loaded device (Figure 2c) that could release EDV and UNO at concentrations of 3.24 and 0.79 μg per day, respectively (Figure 2d), was used to evaluate the effects of co-delivery of EDV and UNO via this device. The EDV/UNO-loaded device demonstrated higher ERG amplitudes than the single-drug-loaded devices and the PBS-loaded device (Figure 2e). Although single-drug administrations showed significantly higher amplitudes compared with the PBS-loaded device, multiple administrations resulted in b-waves having significantly higher amplitudes compared to those elicited by single administrations of EDV and UNO (Figure 2f,g).

Histological evaluation revealed that the outer nuclear layer (ONL) thickness was preserved in the transplanted superior retina in all of the drug-loaded device groups, in contrast to the PBS-device group (**Figure 3**a), and many points of the inferior retina through the phase of the optic disc were also preserved in the EDV/UNO-loaded device group (Figure 3b). TUNEL (Figure 3c) and quantitative analysis were used to study light-induced apoptotic cell death, and they indicated that the EDV/UNO-loaded device significantly reduced the number of TUNEL-positive cells compared with single-drug-loaded devices (Figure 3d). Western blots were performed 15 d after light injury (11 d after ERG), because phosphorylated p38 (p-p38) increased 15 d after light injury (Figure S2, Supporting Information). P-p38 was decreased in the drug-loaded device groups compared with the PBS-loaded device group and it was lowest in the EDV/UNO-loaded device group (Figure 3e). Statistically significant lower levels of p-p38 were observed in the EDV/UNO device group compared to the PBS-loaded device group. On the other hand, phosphorylated ERK1/2 (p-ERK1/2) was found to be lowest in the PBS-loaded device group, while it increased in the drug-loaded device groups and was highest in the EDV/UNO-loaded device group (Figure 3f). Statistically

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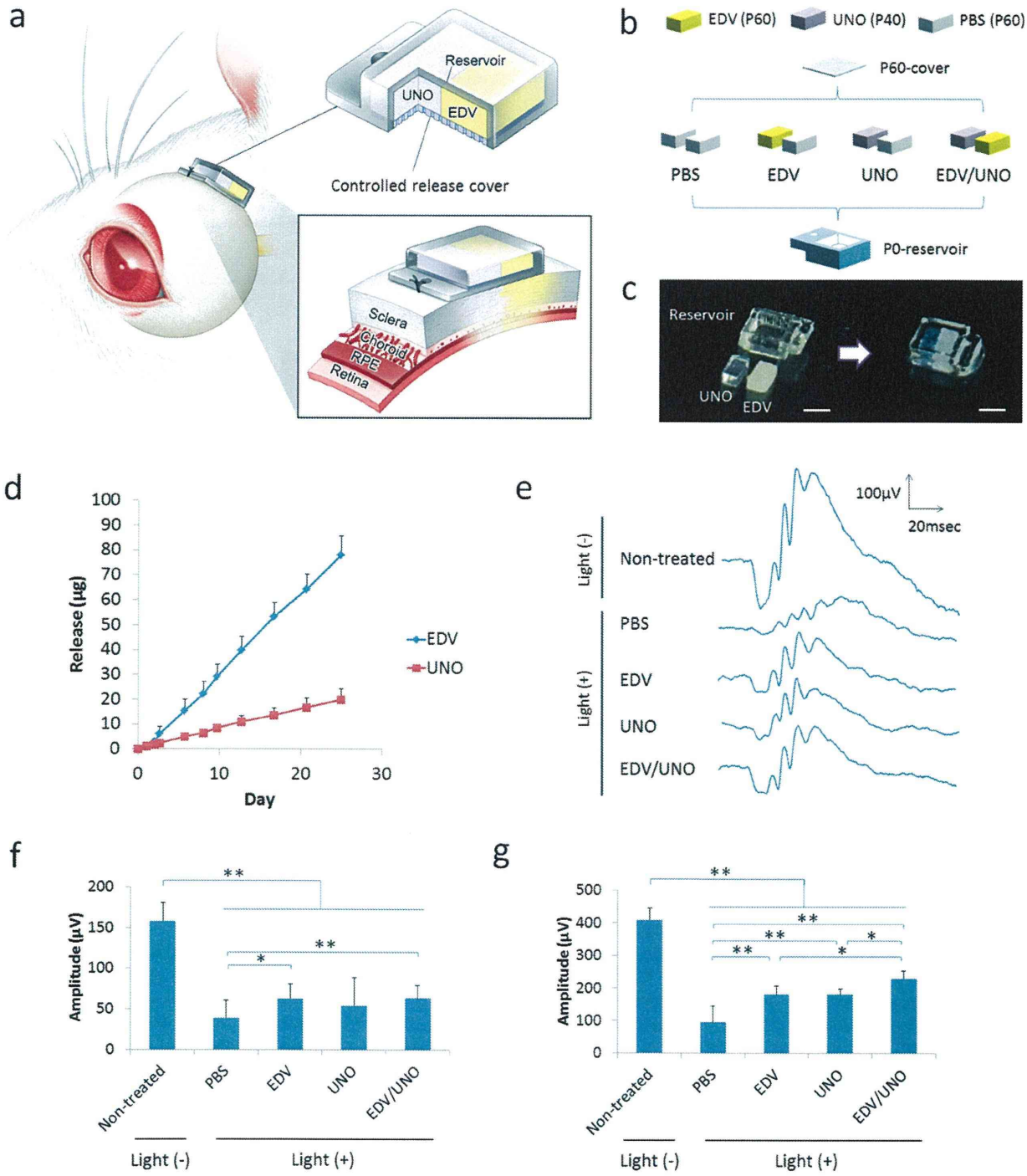

Figure 2. a) Schematic image of the device implantation on the rat sclera. b) Device conditions for in vivo study of synergistic protective effects of EDV and UNO. c) Photographs showing two kinds of pellets, including UNO and EDV, and a reservoir before assembling, and the device after assembling. d) Simultaneous in vitro release profiles of EDV and UNO from the device that combined EDV pelletized with P60 and UNO pelletized with P40, and covered with P60. Representative ERG spectra after light injury in rats e) and ERG amplitudes of a–f) and b-waves g) in the EDV/UNO-loaded device-treated group (EDV/UNO), the EDV/PBS-loaded device-treated group (EDV), the UNO/PBS-loaded device-treated group (UNO), and the PBS/PBS-loaded device-treated group (PBS). Values are mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ (one-way analysis of variance (ANOVA) with Tukey's test).

significant higher p-ERK1/2 expression was observed in the EDV/UNO-loaded device group compared to that of the PBS-loaded device group.

In the in vivo experiments, reduction of ONL thickness, expression of TUNEL-positive cells, and reduction of ERG amplitudes after light exposure were precluded in groups

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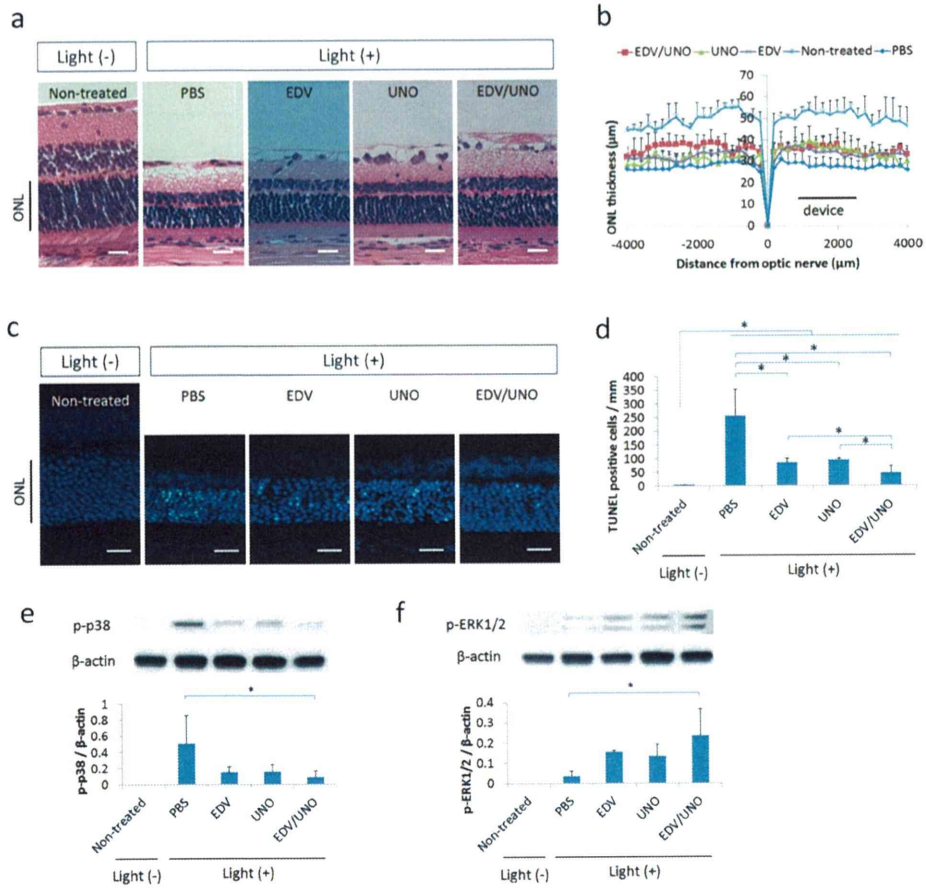

Figure 3. a) Retinal cross-sections, b) ONL thickness, c) TUNEL staining, d) TUNEL-positive cell number and western blotting for e) phosphorylated p-38 and f) ERK1/2 of retinal specimens from rats treated with PBS/PBS-, EDV/PBS-, UNO/PBS-, and EDV/UNO-loaded devices 15 d after light exposure. Scale bars; 20 µm. Values are mean ± SD. $*P < 0.05$, $**P < 0.01$ (one-way analysis of variance (ANOVA) with Tukey's test).

treated with EDV/UNO-loaded devices, suggesting that co-delivery of EDV and UNO attenuates light-induced retinal damage both morphologically and functionally. The cytoprotective actions of EDV and UNO that were observed in the in vitro cell culture experiments (Figure S3, Supporting Information) might correlate with retinal neuroprotection. EDV could reduce oxidative damage by suppressing ROS generation,[11] which in turn would attenuate the increase of $[Ca^{2+}]_i$ as seen in the in vitro cell culture results (Figure S4, Supporting Information). UNO could prevent the intracellular Ca^{2+} increase,[12,13] resulting in an anti-apoptotic effect (Figure S5, Supporting Information). ROS leads to the activation of MAP kinase signaling, including p38 and ERK1/2, elevates cellular Ca^{2+} levels, and induces oxidative damage.[19] Thus, down-regulation of p-p38 by suppressing ROS generation might correlate with the rescue of the cells from apoptosis (Figure 3e).[20,21] ERK1/2 activation may act in some cases to promote cell survival while also participating in pathways leading to neuronal cell death.[22] Although the exact role of ERK1/2 after light injury is unclear, up-regulation of p-ERK1/2 by drug administrations might correlate with cell survival (Figure 3f).

Strict local delivery of the drugs through the device described herein would avoid the risk of side effects from systematic application. Additionally, prolonged sustained drug release would be suitable in treating chronic retinal diseases. Previous studies reporting multiple drug delivery systems, however, did not address independent release control and had a short drug release period.[23] Furthermore, release profiles for biodegradable systems are generally complex, and they may have associated burst effects.[24] In contrast, a non-biodegradable device containing a drug reservoir sealed with a controlled release cover allows for sustained release and reduces the sizes of the bursts.[25–28] It is theoretically possible that the device utilized for these experiments in a rat model system could effectively release EDV and UNO for 46 and 188 d, respectively (Figure 2d). In spite of the promising carriers reported previously, in vivo evidence for the potential of multi-drug delivery has not yet been provided to date. Consequently, to the best of our knowledge, we are the first to demonstrate evidence for retinal neuroprotective effects of controlled transscleral co-delivery of EDV and UNO in a retinal degeneration model.

Comparable studies using larger animals are planned to investigate differences in drug permeability and efficacy among

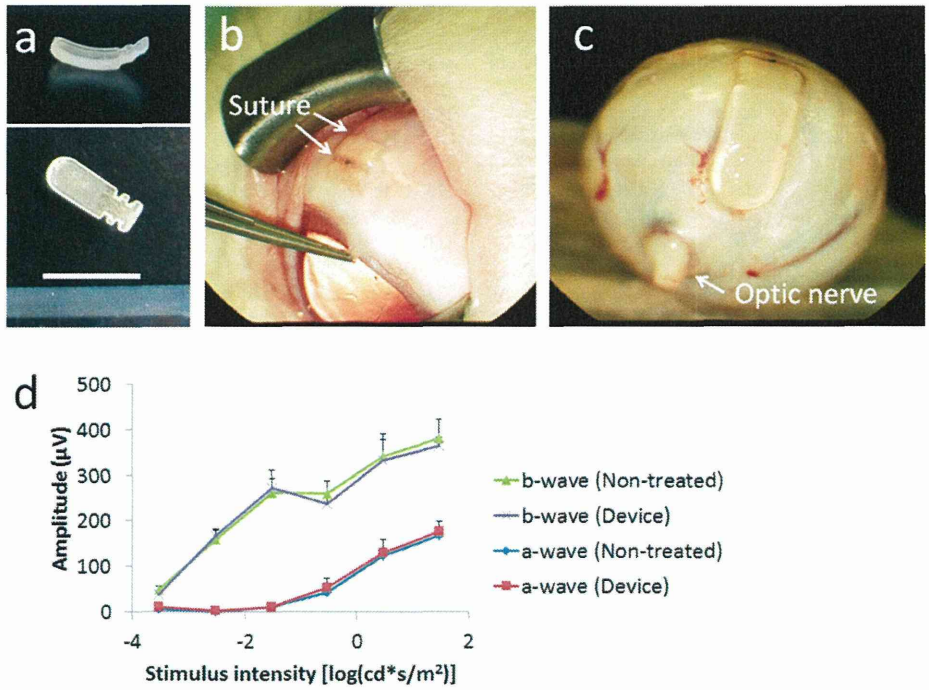

Figure 4. a) Prototype of the device used in the rabbit experiments. This device has a rounded shape that fits on the rabbit eyeball and is thin (thickness; 1.6 mm) to avoid discomfort after implantation. The reservoir has dimples to fix it on the sclera by suture, shown as arrows in b). c) The device, which has the reservoir located at one end, was placed so that the reservoir reached the posterior site near the optic nerve. d) ERG amplitudes 8 weeks after implantation. Scale bar: 10 mm. Values are mean ± SD.

different species using a prototype device designed for a rabbit model (**Figure 4**a). The device materials, PEGDM and TEGDM, can be easily molded into different substrate shapes by UV curing.[29,30] A microfabrication technique was used because the shape and volume of the reservoir can be easily modified by AutoCAD design (Figure S6, Supporting Information). The device has grooves for suture to fix it on the sclera (Figure 4b), and it is designed to fit the curve of the eyeball. The edge, where the drug reservoir is located, of the device could reach around the posterior segment of the eye, especially the macular area in the case of humans (Figure 4c). There was no difference in ERG amplitudes from device-implanted eyes versus non-treated eyes 8 weeks after implantation (Figure 4d), suggesting that the device could be used to safely administer drugs by the transscleral approach without disturbing intraocular tissues.

One of the limitations of this study is the lack of a specific stochastic search for drugs. Although EDV and UNO were selected based on previous reports of their neuroprotective effects against light-induced retinal damage following systemic administration,[10,11] many drug search algorithms have been used to select drug numbers and/or concentrations for multi-drug applications.[31,32] The limited span of the in vivo experiments, due to the study design (24 d from implantation to sacrifice), means that the duration of the effect and the appearance of side effects after long-term drug delivery remain to be determined.

In conclusion, a polymeric system that can simultaneously administer two drugs having distinct kinetics showed synergistic retinal neuroprotection against light injury in rats when compared with single-drug-loaded devices. The device can be designed to contain various drug formulations and dosages, and it can be microfabricated as various forms for implantation on the sclera of rats, rabbits, and potentially humans, allowing for a wide range of potential biomedical applications. Transscleral administration by our device would offer a safer therapeutic method than intravitreal injections or intraocular implants. Thus, our device is expected to be a promising candidate for sustained intraocular multi-drug delivery.

Experimental Section

Experimental details are described in the Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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