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Functional evaluation of a novel vitreous substitute using polyethylene glycol sols injected into a foldable capsular vitreous body

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Abstract: Polyethylene glycol (PEG) is a short-term (41 days) potential vitreous substitute and is too short for an ideal vitreous substitute. Previously, a foldable capsular vitreous body (FCVB) was designed to mimic vitreous function. The aim of this study is to evaluate whether PEG injected into FCVB can serve as a long-term vitreous substitute. *In vitro* study, a concentration of 5% (w/v) PEG sols showed natural-like mechanical and optical properties in terms of pH, density, light transmittance, refractive index, interfacial tension, viscosity, rheology, and cytotoxicity. Then *in vivo* tests, 30 rabbits received standard pars plana vitrectomy, of which 12 eyes were implanted with PEG injected into FCVB, nine eyes were injected with PEG sols alone, and nine others were injected with balance salt solution as control. A clinical evaluation of the anterior segment, fundus, and intraocular pressure was measured pre- and postoperatively up to 180 days, which showed that FCVBs had good retina supporting function, except for a higher incidence of cataracts. Gross pathology, hematoxylin and eosin, and terminal deoxynucleotidyl transferase dUTP nick end labeling staining analysis also showed that FCVBs had good biocompatibility, and that all quadrants of the capsular wall fitted well with the retina. This study demonstrated that PEG injected into FCVB can serve as a long-term vitreous substitute and has potential clinical use. J Biomed Mater Res Part A: 101A: 2538–2547, 2013.

Key Words: vitreous substitute, polyethylene glycol, biocompatibility, retention time, cytotoxicity

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INTRODUCTION

The vitreous body is a transparent gelatinoid structure occupying four-fifths of the volume in the eye. It consists of about 99% water and 1% inorganic salts, organic lipids, and hyaluronan.[1] The function of the vitreous body involves optical and mechanical performances, supporting the adjacent structures and acting as an important part of the blood–ocular barrier, which inhibited cell proliferation, inflammation, and neovascularization.[2] Throughout the world, vitreoretinal pathologies remain the second leading cause of extremely poor vision or blindness after cataracts.[3] Vitreous substitute is necessary to tamponade the reattached retina after vitrectomy for complicated retinal detachment.[4]

Currently, gases, perfluorocarbon liquids,[4,5] silicone oil (SO),[5–7] and heavy SO[8,9] are predominately applied clinically. However, these vitreous tamponades have several major disadvantages, including short residence time, elevation of the intraocular pressure (IOP), cataracts, emulsification, keratopathy, and secondary glaucoma. Natural polymers, such as hyaluronic acid (HA),[10] collagens,[11] and "hylan",[12] showed quick absorption in the vitreous cavity. It also showed poor tamponade effect. Recently, synthetic hydrogels, such as poly(1-vinyl-2-pyrrolidone),[13,14] polyacrylamide,[15] and poly(vinyl alcohol),[16,17] seemed to better mimic the property of natural vitreous. However, the use of these synthetic hydrogels was still limited because of IOP elevation, gel opacification, and potential retina cytotoxicity due to residual monomer polymers. Among the wide variety of vitreous substitutes, polyethylene glycol (PEG)-based hydrogels were considered potential vitreous substitutes.

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These substitutes were approved by the Food and Drug Administration for a wide range of applications—injectable hydrogels, intravitreal drug delivery, repairing of scleral incisions, and sealing retinal detachments.[18–20] Pritchard et al. indicated that PEG sol was a potential vitreous substitute as it had properties similar to natural vitreous. It also had excellent biocompatibility, except for its upregulation of glial fibrillary acidic protein. However, the sols disappeared over a period of 41 days,[20] which was too short for an ideal vitreous substitute.

How to prolong the retention time in the vitreous cavity is a great scientific issue. The risk of retina redetachment decreases steeply with increasing time after vitreous tamponade removal. Vitreous substitutes need to play the compression effect for at least 3–5 months and a longer period is needed for complex retinal detachment.[21] Unfortunately, most synthetic polymers—PEG included—reported a short residence time of only 4–6 weeks, which was unsuitable for long-term vitreous substitutes.

In our previous study, a foldable capsular vitreous body (FCVB) injected with media of saline or SO was designed to mimic vitreous function and to prolong the duration time of the media due to a physical barrier of FCVB.[22–28] During a 6-month implantation in rabbit eyes, FCVB filled with SO avoided the complications induced by using SO alone. These complications included emulsification and migration into the neighboring structure.[27] In human eyes, FCVB filled with SO was shown to be effective and safe in three eyes as a vitreous substitute over a 12-month implantation.[28] Considering the drawbacks that SO injected with FCVB, including elevation of IOP, cataracts, especially the ocular refraction change, we want to search for a more ideal vitreous substitute. PEG sol was a potential vitreous substitute as it had good optical characteristics similar to natural vitreous, and excellent biocompatibility, so in this study we aims to evaluate whether PEG injected into FCVB can prolong the duration time of the PEG and serve as a long-term vitreous substitute after a long period (180 days) of implantation.

MATERIALS AND METHODS

Preparation of PEG sols

PEG (Sigma Aldrich, St. Louis, MO) was prepared in sterile phosphate-buffered saline (PBS) with molecular weights of 400 kDa. Around 2.5, 5, and 7.5% (w/v) solution of PEG sols were evaluated, respectively.

Characterization of PEG sols

The pH level was measured with using digital pH meters (Thermo Fisher Scientific, Waltham, MA). Light transmittance was measured using a UV/VIS spectrophotometer (DU800; BeckmanCoulter, Brea, CA) in a wavelength of visible range. PBS was used as a blank. The refractive index was obtained with an Abbe refractometer (WYA-2W, Lumsail Industrial, Shanghai, China). The samples were prepared and measured in a wavelength of visible range. The density was measured using the pycnometer method according to Chinese Pharmacopoeia (2005 edition).[29] The interfacial tension was measured with the use of an angle measuring instrument (OCA20, Dataphysics, San Jose, CA). The viscosity of the materials was measured using ARG2 rheometer (TA Instruments, New Castle, DE) accompanied with a 40 mm diameter 2° cone and plate at a gap of 0.052 mm. All measurements were performed at 37°C. The measurements were performed with the steady-state flow analysis. The viscosity value was measured over time by varying the shear rates steadily from 0.1 to 100/s.

Rheological measurements

Rheological measurements were performed using ARES-RFS rheometer (TA Instruments, New Castle, DE). The samples were placed onto a 50-mm diameter parallel plate with a gap of 0.053 mm. All biophysical measurements were performed at 37°C.

Strain sweep test: A DStmSwp test was performed to determine the strain amplitude at which linear viscoelasticity was valid. The viscoelastic values were measured against the strain γ amplitude from 0.1 to 100% at a fixed oscillation frequency.

Dynamic rheology analysis: Dynamic rheology analysis was used as the criteria for potential vitreous substitutes. A DFreqSwp test was performed with a shear amplitude of $\gamma = 1.0\%$ by varying the frequency range over 0.01–10 Hz. The mechanical properties were studied by analyzing the storage (G') and the loss (G'') modulus and the resulting G' and G''; were plotted versus the frequency of oscillatory stress.

Cytotoxicity assay

Mouse fibroblast L929 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 50 U/mL penicillin, and 50 mg/mL streptomycin. The cells were incubated at 37°C with 5% CO_2. The experiment group included 2.5, 5, and 7.5% (w/v) PEG sols and a combination of FCVB that were injected with different concentrations of PEG sols. These groups were defined as 2.5, 5, 7.5, 2.5FC, 5FC, and 7.5FC, respectively. The samples were then put on the adhered cells through a coculture insert with pores of $\varphi = 8$ μm (Corning, NY). The experiment and control groups were incubated at the same conditions. The suspended cells were seeded in contact with samples in a 96-well culture plate at a density of 1×10^3 cells per well. Cytotoxicity was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma Aldrich, St. Louis, MO) at the following intervals: 24, 48, and 72 h. At least three replicates were performed for this test.

In vivo animal evaluation

FCVB fabrication. The rabbit FCVB (Vesber biotechnology, Guangzhou, China) was fabricated using an injection forming technology with tailor-made modified liquid silicone rubber (Dow Corning Company, Midland, MI). The shape was manipulated according to the rabbit vitreous parameters, the details of which were described in our previous study.[24]

Animal preparation and surgical procedure. All experimental procedures were approved by the Hospital Animal Research Review Committee and performed in accordance with the Association for Research in Vision and Ophthalmology Statement. A total of 30 New Zealand albino rabbits, each weighing 2.0–3.0 kg, underwent pars plana vitrectomy (PPV) and were implanted with various vitreous substitutes. The vitreous substitutes divided into three groups, PEG injected into FCVB (FCVB group, $n = 12$), PEG sols alone (PEG group, $n = 9$), and balanced salt solution (BSS) served as control (BSS group, $n = 9$). All the right eyes were assigned as study eyes, whereas the contralateral eyes served as control.

The rabbits were generally anesthetized using a combination of intramuscular injection of ketamine hydrochloride (30 mg/kg) and chlorpromazine hydrochloride (15 mg/kg). The pupils were dilated with 0.5% tropicamide. A standard three-port PPV was performed with Alcon Accurus vitrectomy unit and the vitreous was removed as much as possible. If obvious surgical complications occurred, such as the retina or the lens being touched, the animals were eliminated from the study. After the vitrectomy, the FCVB was folded into three petals and implanted into the vitreous cavity through the lengthened 3.0 mm sclera incision. About 1.0 mL of PEG sol was then injected into the capsule through a tube-valve structure. Finally, the silicone tube-valve was subsequently fixed under the conjunctiva (Fig. 1). Meanwhile, in the PEG or BSS group, about 1.0–1.2 mL of PEG or BSS was injected directly into the vitreous cavity after fluid-air exchange. The surgery was concluded with the subconjunctival injection of gentamycin and dexamethasone and with the application of compound tobramycin and atropine ointment, which was done for at least 2 weeks.

Follow-up examinations. Assessment was focused on conjunctival reaction, corneal and lens opacity, anterior chamber exudation, and the fundus on days 3, 7, 14, 30, 60, 90, and 180 postoperatively, with slit lamp biomicroscopy (SL-D7; Topcon Co., Japan) and ophthalmoscopy (TRL-50DX; Topcon Co., Japan). The IOP was measured using tonopen (Tonopen avia; Reichert Co.) preoperatively at days 3, 7, 14, 30, 60, 90, and 180 postoperatively. B-scan ultrasonography (CineScan, 10 MHz probe; BVI, France) was performed to determine the mechanical retina supporting function of the vitreous substitutes at days 30, 90, and 180 postoperatively.

Gross appearance and histopathological examinations. The rabbits were euthanized postoperatively at day 30 (FCVB group, $n = 3$; PEG group, $n = 3$; BSS group, $n = 3$) or day 90 (FCVB group, $n = 3$; PEG group, $n = 3$; BSS group, $n = 3$) or day 180 (FCVB group, $n = 6$; PEG group, $n = 3$; BSS group, $n = 3$). Immediately after enucleation, vitreous tamponades were removed for gross pathology and biophysical analysis. Then, the eyeballs were fixed in 10% neutral paraformaldehyde for 72 h and embedded in paraffin. The slice sections were cut at 5 μm thick and processed into hematoxylin and eosin (H&E) staining by standard techniques.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was performed using the *In Situ* Cell Death Detection Kit (Roche Applied Science, Germany). Sections were deparaffinized and rehydrated through graded concentrations of alcohol. Protein was then digested by treating the tissue slides with proteinase K at room temperature for 15 min. The slides were rinsed in PBS, followed by the quenching of the endogenous peroxidase activity with 3% hydrogen peroxide for 10 min. Each specimen was applied with 50 μL of dUTP/TdT mixture and then incubated in a humidified chamber at 37°C for 1 h. Afterwards, it was rinsed three times with PBS and mounted with antifade. Corresponding negative (without terminal transferase) and positive (with DNaseI treated) control sections were also prepared. The sections were observed under fluorescence microscopy (Carl Zeiss Microimaging GmbH, Hamburg, Germany).

Statistical analysis

Statistical analysis was performed with a statistical package (SPSS for Windows, ver. 13.0; SPSS, NC). Data was expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed using the ANOVA in different groups and time point preoperatively and postoperatively. If significance was identified, *post hoc* analysis with Tukey's honestly significant difference was used to confirm the significant changes. Statistical significance was set at $p < 0.05$.

RESULTS

Characteristic property of PEG sols

The pH, density, interfacial tension, and refractive index of 2.5, 5, and 7.5% (w/v) of PEG sols were listed in Table I, the pH ranged from 7.12 to 7.23; density ranged from 1.044 to 1.059; interfacial tension ranged from 62.107 to 62.357 dyn/cm; refractive index ranged from 1.334 to 1.343 n_D. Light transmittance ranged from 86.51 to 60.01% with increasing concentration, which revealed that PEG sols did not have complete transparency and 7.5% (w/v) was absolutely not suitable for the demand of the refractive medium. With the increased concentration of PEG sols, the viscosity also increased, ranged from 235.3 to 8047.1 cP [Fig. 2(E)]. PEG sols were very similar to natural vitreous, whereas the different concentrations of PEG sols had slight differences. A concentration of 5% (w/v) of the PEG sols showed that pH at 7.12, density at 1.049, interfacial tension at 62.107 dyn/cm, refractive index at 1.336 n_D, light transmittance at 80.48%, and viscosity at 1787.9 cP, whose properties were most similar to natural vitreous, and was chosen as the vitreous substitute in *in vivo* tests.

Reological property of PEG sols

The strain sweep test showed a linear viscoelasticity at shear strain $\gamma = 1.0\%$ [Fig. 2(A)]. The mechanical properties were analyzed according to the storage (G') and the loss (G'') modulus. G' gave the elastic or solid-like component,

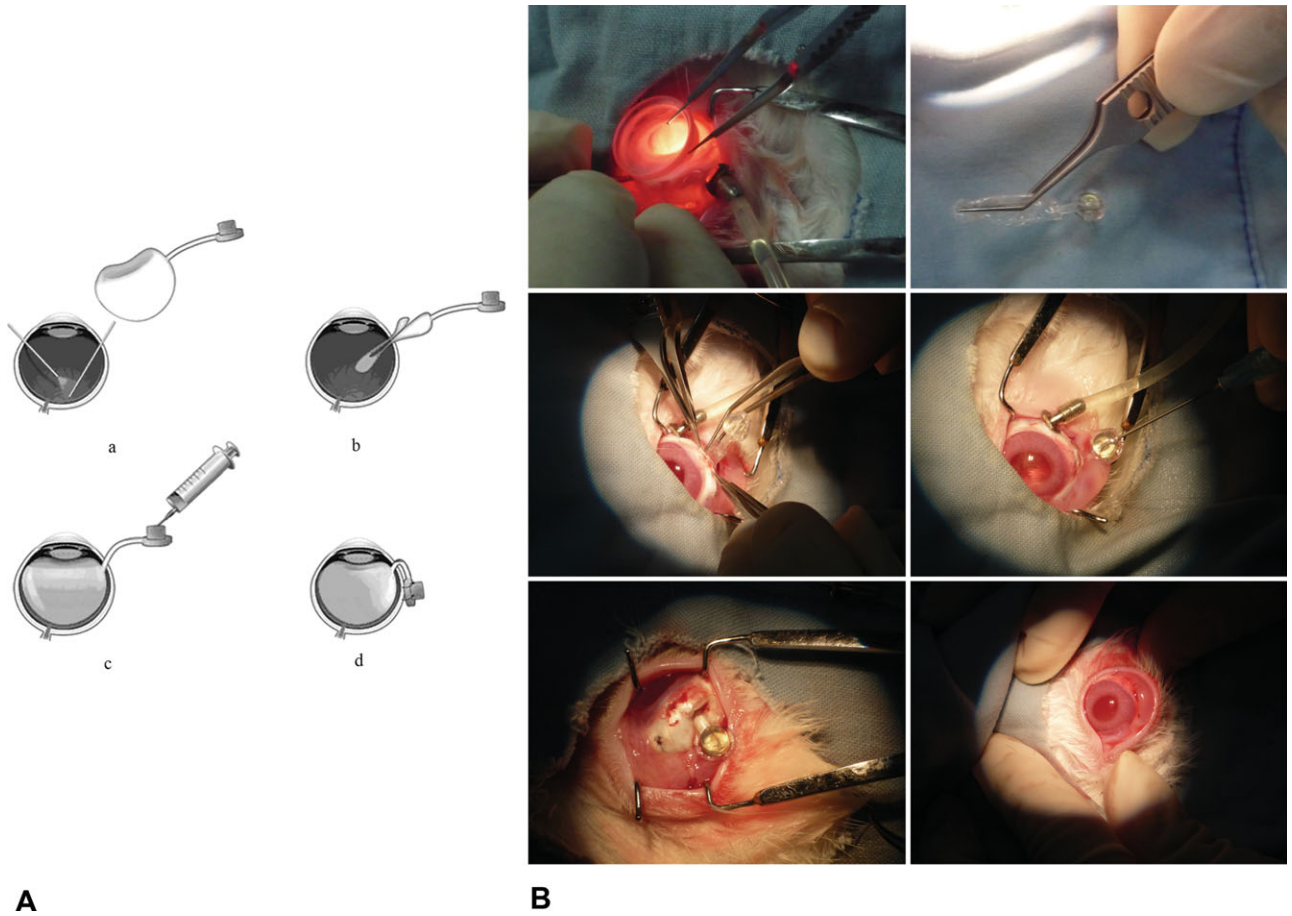

FIGURE 1. Illustration of FCVB implantation process. (A) The ideograph of the surgical process. (B) Animal surgical procedure. FCVB was folded and implanted into the vitreous cavity during PPV surgery, then about 1.0 mL PEG sols in a syringe was injected into the capsule through a silicone tube-valve system, and the capsule was inflated to support the retina. Finally, the valve was subsequently fixed under the conjunctiva. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

whereas G'' gave the viscous or liquid-like component. With the increased concentration of PEG sols, viscoelasticity also increased. PEG sols showed an elastic modulus of 0.491–28.989Pa, and a loss modulus of 2.053–46.792 Pa. The PEG sols showed a slightly higher G'' than G' [Fig. 2(C)], which indicated that the property of PEG sols behaved more likely as a viscoelastic liquid. Contrasted with HA (Medical Hyaluronan Gel; Iviz, Shangdong, China) and SO (Oxane 5700; Bauch & Lomb, Rochester, NY), PEG sols were much similar to HA, especially the 7.5% (w/v) sols [Fig. 2(D)]. The relative contribution to the elastic or viscous natures was quantified by loss tangent, which was estimated by recording the dissipation factor. This parameter was defined as tan δ, tan $\delta = G''/G'$. The definition of δ was as follows: if tan $\delta > 1$ indicated a liquid behavior, then tan $\delta < 1$ indicated a gel or solid state, else tan $\delta = 1$ was considered a threshold. With the increasing concentration of PEG sols, loss tangent decreased [Fig. 2(B)], revealing that 5 and 7.5% PEG sols had a less liquid-like behavior. The tests indicated that 5 and 7.5% (w/v) of the PEG sols showed similar rheological property to natural vitreous body.

Cytotoxicity tests

No changes in cell morphology were observed in all groups cocultured with different materials, such as in the 2.5, 5, and 7.5 (w/v) in PEG groups and in the 2.5FC, 5FC, and 7.5FC combined with FCVB and PEG groups at 24, 48, and 72 h. It displayed a growing trend of precipitates in cell medium with the increasing concentration in 2.5, 5, and 7.5 PEG group (Supporting Information Fig. S1(A)]. Conversely,

TABLE I. The Biophysical Properties of PEG Sols

Group (%)	pH	Density	Interfacial Tension Versus H_2O (dyn/cm)	Refractive Index, n_D	Light Transmittance (%)	Viscosity (cP)	G' (Pa)	G'' (Pa)
2.5	7.23	1.044	62.307	1.334	86.51	235.3	0.491	2.053
5	7.12	1.049	62.107	1.336	80.48	1787.9	6.713	14.801
7.5	7.18	1.059	62.357	1.343	60.01	8047.1	28.989	46.792

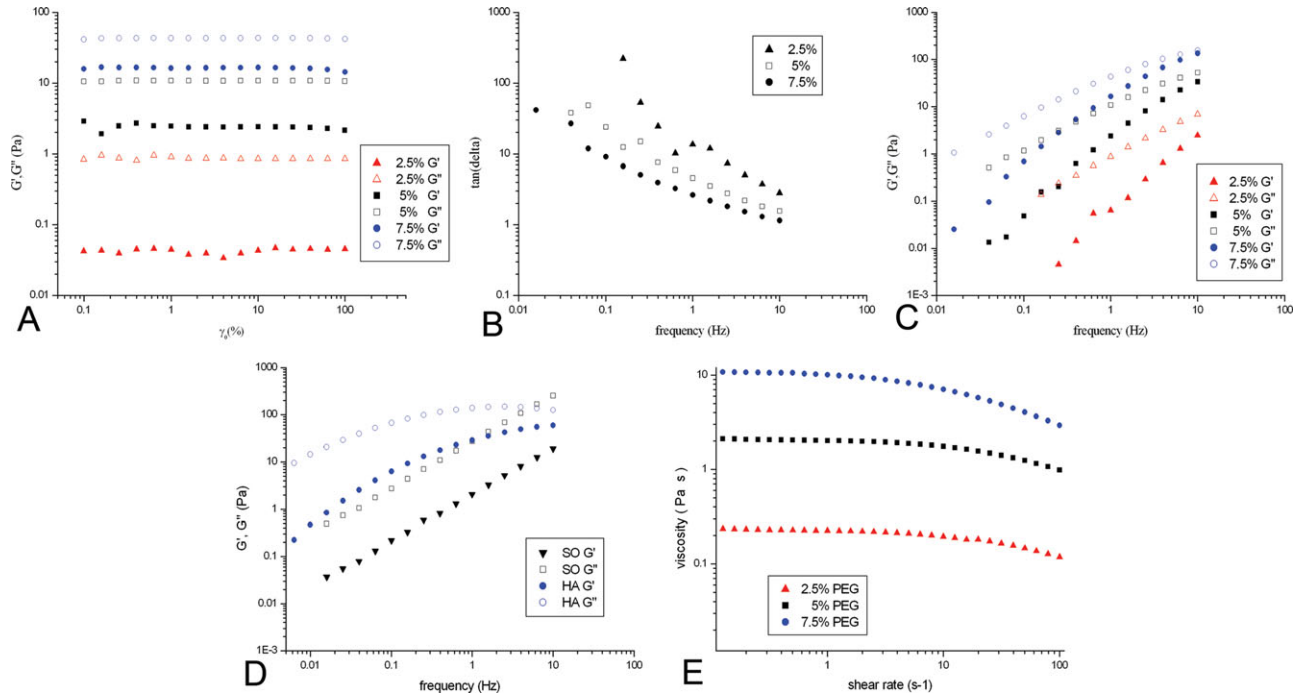

FIGURE 2. The rheological analysis of PEG sols. (A) Strain sweep test plotted logarithmically against a shear strain amplitude γ (0.1–100%). (B) Loss tangent plotted logarithmically against frequency (0.01–10 Hz) at 37°C. (C) Storage (G') and loss (G'') modules plotted logarithmically against frequency (0.01–10 Hz) at 37°C for PEG sols. (D) Storage (G') and loss (G'') modules plotted logarithmically against frequency (0.01–10 Hz) at 37°C for SO and HA. (E) Viscosity plotted logarithmically against the shear rate (0.1–100/s) at 37°C for PEG sols. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

it showed little precipitate in the FCVB groups and no significant difference was shown in different concentrations. Cytotoxicity was evaluated by MTT assay and OD values did not show a significant difference among material extracts versus negative control [$p>0.05$; Supporting Information Fig. S1(B)].

Follow-up clinical examinations

Slit lamp and fundoscopic examinations. There was a slight conjunctival hyperemia by day 7 in all groups (Fig. 3). No fibrin formation and posterior synechia were observed. The conjunctiva, cornea, and anterior chamber presented no defect, with the exception of the lens subluxation ($n = 2$) in the FCVB-implanted eyes. The incidence of cataract varied in different groups at days 30, 90, and 180, respectively. In the FCVB-implanted eyes, the incidence of cataract was 25% (2/12), 66.7% (6/9), and 100% (4/4, $n = 2$ for lensectomy). In the PEG-implanted eyes, the incidence of cataract was 11.1% (1/9), 33.3% (2/6), and 33.3% (1/3). In the BSS-implanted eyes, the incidence of cataract was 11.1% (1/9) during the entire observation process.

In fundoscopic examinations, the eyes in the FCVB-implanted group presented clarity for about 90 days. However, the fundus gradually blurred accompanied by cataracts. In the PEG- or BSS-implanted group, the eyes maintained clarity during the observation period up to 180 days (Fig. 4). The retina and the optic nerve appeared to be normal. No vitreous hemorrhage, membrane formation, retina detachment and chorioretinal atrophy were observed.

Intraocular pressure. The fluctuation of the IOPs showed a downward trend at day 3 postoperatively, and returned to baseline within 14 days [Fig. 5(A)]. Hypotonia following vitreoretinal surgery was usually related to the inflammation of the ciliary body and to the leakage of the production of the aqueous humor. No statistical significance was found among the three groups preoperatively and at days 3, 7, 14, 90, and 180 postoperatively ($p > 0.05$). However, significance was noticed at days 30 and 60 postoperatively ($p = 0.000$, 0.025, respectively).

B-scan ultrasonography. No retinal detachment was observed during the entire observation process in all groups. In the FCVB-implanted eyes, some scattered and mild enhanced echoes, with a similar capsule-like morphology, were observed. The presence of an epiretinal membrane in the vitreous cavity [Fig. 5(C)], which appeared to be the posterior wall of FCVB, indicated that the capsular wall of the FCVB was in good contact with the retina.

Pathologic examinations. In gross examination, the capsular wall of the FCVB perfectly fitted with the vitreous cavity in the rabbit eyes and all quadrants of the capsular wall fitted well with the retina [Fig. 5(B)]. In the FCVB-implanted eyes, PEG sols appeared homogeneous and transparent on the inside, except for the precipitates that existed in the inferior and posterior wall of the capsule. Meanwhile, in the PEG-implanted eyes, it was difficult to distinguish the PEG sols and the vitreous body. The viscosity could reflect the

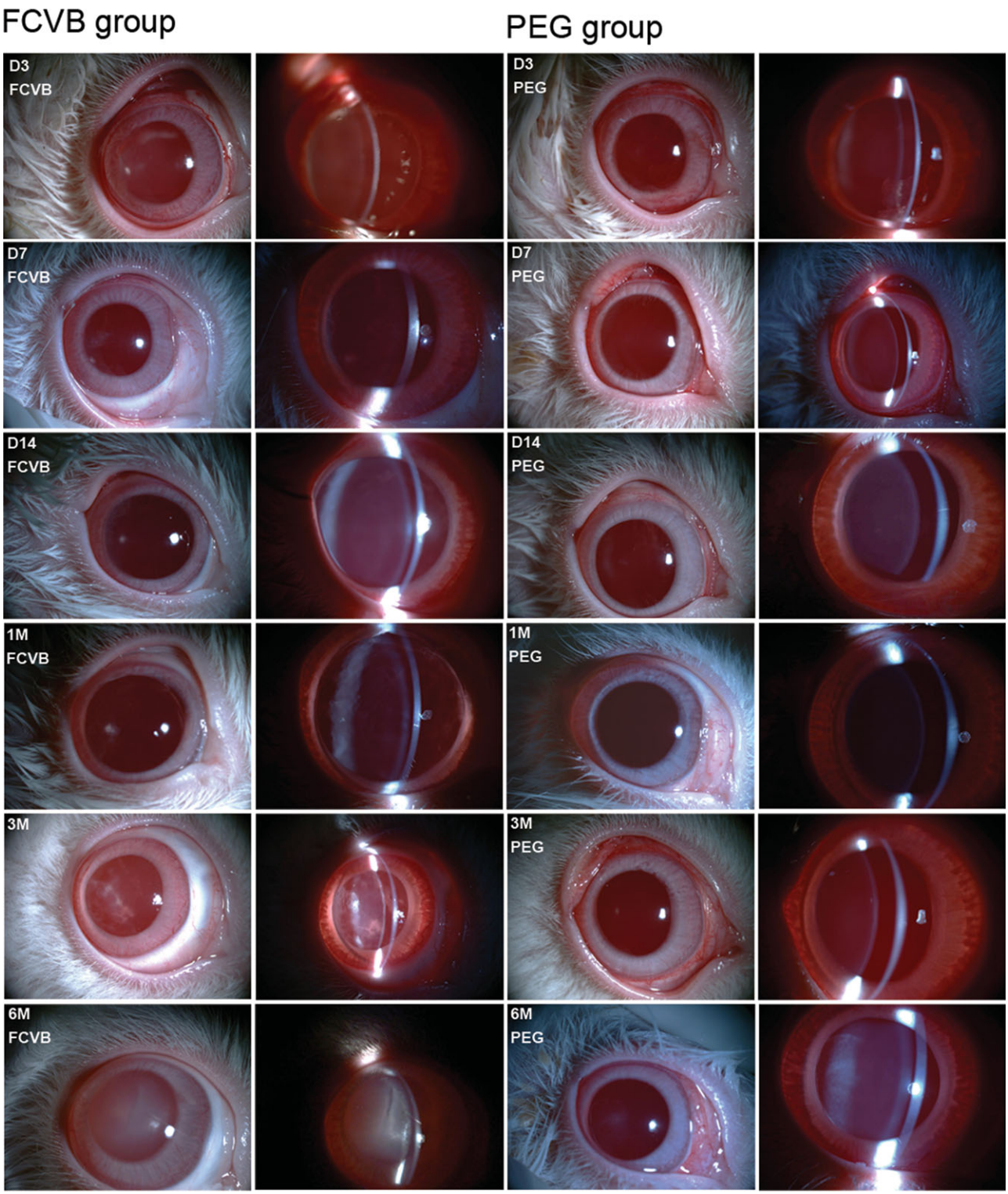

FIGURE 3. Anterior ocular segment evaluation. Images of slit lamp examinations were taken at days 3, 7, 14, 30, 90, and 180 postoperatively in FCVB-implanted eyes and in PEG-implanted eyes. The conjunctiva, cornea, and anterior chamber presented no defect, with the exception of a higher incidence of cataract in FCVB groups during the observation of 180 days. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

amount of polymers. *In vitro* analysis indicated that viscosity in PEG-implanted eyes at days 30, 90, and 180 was at 17.7, 5.8, and 3.9% of the original value, respectively (Supporting Information Table S1). In the FCVB-implanted eyes, viscosity was 72.0, 65.7, and 56.8% of the original value. The results revealed that the combination of the FCVB could prolong the retention time of the PEG sols in the vitreous cavity.

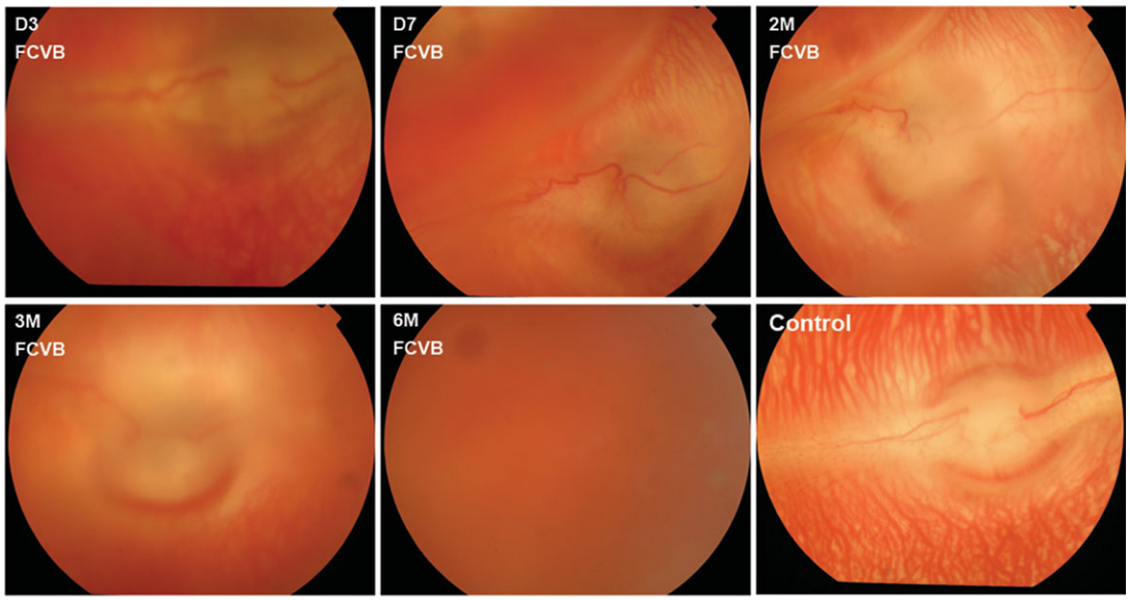

FIGURE 4. Evaluation of ocular posterior segment. Images of fundoscopic examinations were taken at days 3, 7, 60, 90, and 180 postoperatively in FCVB-implanted eyes and the control eyes. No vitreous hemorrhage, membrane formation, retina detachment and chorioretinal atrophy were observed. The fundus in FCVB-implanted eyes were not clear untill day 7, and the fundus presented clarity from days 7 to 90, then the fundus gradually blurred accompanied by cataracts. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The morphology of the cornea, ciliary body, anterior chamber, and choroid was normal, except for the thickness of the corneal stroma and of the fiber derangement in some specimens in the FCVB-implanted eyes (Fig. 6). The retina in the FCVB-implanted eyes showed a good integrity during the early stage (30 days), then the retina became disordered during the middle stage (90 days), with thinning of the ganglion cells layer and of the outer plexiform layer. The retina showed a sign of deformation during the late stage (180 days) with macrophages and vacuolations, hyperplasia, and organization of nerve fiber layers. In the PEG-implanted eyes, the structure of the retina was similar with that of the FCVB-implanted eyes during the early and middle stage.

The apoptosis activity was evaluated using TUNEL staining. The cornea and ciliary body showed no positive cells in all study eyes and control eyes (Supporting Information Fig. S2). The retina showed few weakly positive cells at the inner nuclear layer and outer nuclear layer in the FCVB-implanted eyes and PEG alone implanted eyes. Positive control specimen showed significant positive cells at the cornea, ciliary body, and in all layers of the retina, whereas the negative control specimen did not.

DISCUSSION

In this study, the optical, mechanical, and biophysical properties of the PEG sols were investigated and the function of the FCVB combined with PEG after a long period (180 days) of implantation was evaluated. It was found that FCVB injected with media of PEG sols as vitreous substitutes could serve as a long-term vitreous substitute and as a good refractive medium as well.

An ideal vitreous substitute should mimic the biophysical properties of the natural vitreous. The ocular performance of the natural vitreous included light transmittance of around 80–95%, pH of 7.0–7.4, and a refractive index of about 1.3345–1.3348. The biophysical properties of the natural vitreous included an elastic modulus of 4.2–4.7 Pa and a loss modulus of 1.9–3.7 Pa.[2,30,31] A concentration of 5% (w/v) of the PEG sols showed that pH at 7.12, density at 1.049, interfacial tension at 62.107 dyn/cm, refractive index at 1.336 n_D, light transmittance at 80.48%, viscosity at 1787.9 cP, elastic modulus of 6.713 Pa, and a loss modulus of 14.801 Pa. *In vitro* study showed that a solution of 5% (w/v) PEG sols was an attractive candidate for vitreous substitutes. Clinical examinations showed that FCVB injected with PEG sols own a similar optical property to the natural vitreous. The gross examination, on the other hand, showed that the vitreous remained clear in the FCVB-implanted eyes until the enucleation. However, some aggregates formed after long term of disposition in the inferior posterior wall of the FCVB capsule in gross pathology, which may be due to the purity of the PEG material. The lamellar amount of precipitates was not on the vision axis; therefore, it has little impact on the vision.

An ideal vitreous substitute should also be inert, nonbiodegradable, and easy to manipulate (easy to store, sterilize, and able to be injected through a small-gauge needle).[2,30,31] The cytotoxicity and animal clinical examinations showed that both PEG sols alone and FCVB injected with PEG *in vitro* and *in vivo* were relatively safe. Clinical and pathological examinations showed that FCVB injected with PEG sols had good biocompatibility in the rabbit eyes as no corneal opacity, intraocular inflammation, vitreous hemorrhage, and retina detachment were observed, with the exception of a higher incidence of cataracts and lens subluxation. The application of the FCVB can prolong the retention time of

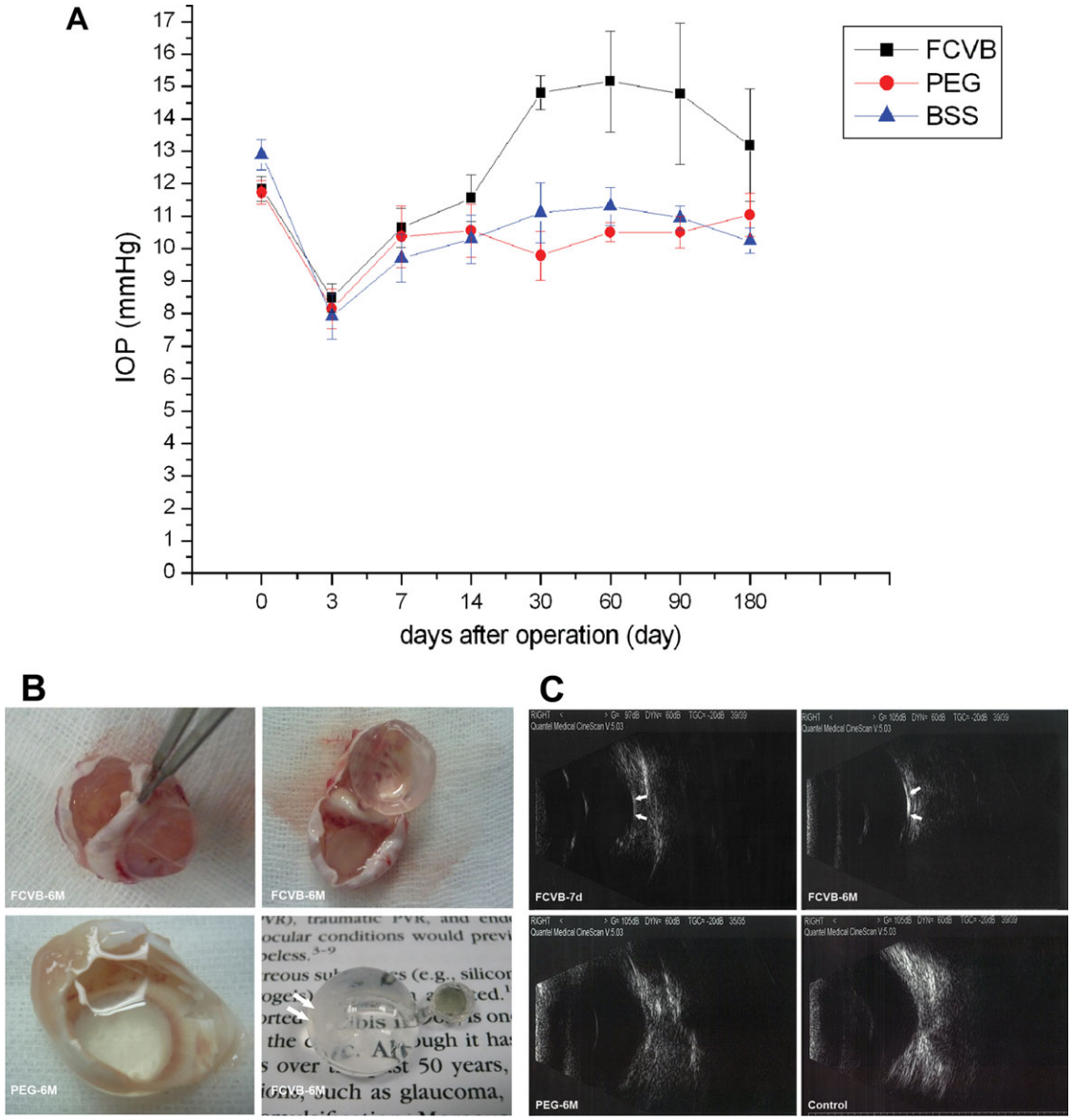

FIGURE 5. Evaluation the retina-support function of FCVB injected with PEG sols. (A) Fluctuation of intraocular pressure (IOP). No statistically difference was found among the FCVB, PEG and BSS groups preoperatively and at 3, 7, 14, 90, and 180 days postoperatively ($p = 0.118$, 0.771, 0.378, 0.667, 0.440, 0.086, and 0.413, respectively). However, the significant differences were noted at 30 and 60 days postoperatively ($p = 0.000$, 0.025, respectively). The FCVB-implanted eyes showed a higher IOP, even reaching an abnormal hypertonia. (B) B-scan ultrasonography. In the FCVB-implanted eyes, B-scan ultrasonograph showed some scattered and mild enhanced echoes, with a similar capsule-like morphology, which indicated that FCVB had a good retina supporting function. (C) Gross appearance of ocular specimens in FCVB-implanted and PEG-implanted eyes at 180 days. In FCVB-implanted eyes, the appearance of PEG sols inside was homogeneous and transparent, except for the precipitates existing in the inferior and posterior wall of the capsule. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the PEG sols. The PEG sols inside still had a viscoelastic property (56.8%) after a long period (180 days) of implantation. On the contrary, in the PEG-implanted eyes, the sols almost disappeared (17.7%) at day 30 postoperatively. The surgical procedures included a standard three-port PPV without the process of fluid-air exchange, reducing the difficulty of the operation skills. The PEG sols could easily be injected through a syringe into the capsule of the FCVB. Also, it was easy to remove the FCVB and PEG sols completely when required.

An ideal vitreous substitute for long-term implantation has not been found so far. As such, we were inspired by the capsule-like implants containing the fluid substitute used in breast augmentation surgery and in the process, invented the FCVB. In our previous study, FCVB injected with media of BSS or SO[22,25] was designed to mimic vitreous function, prolong the duration time of the media, and to avoid the complications caused by vitreous substitutes, which have been mentioned above, such as emulsification and migration into the neighboring structure.[27] Considering that SO

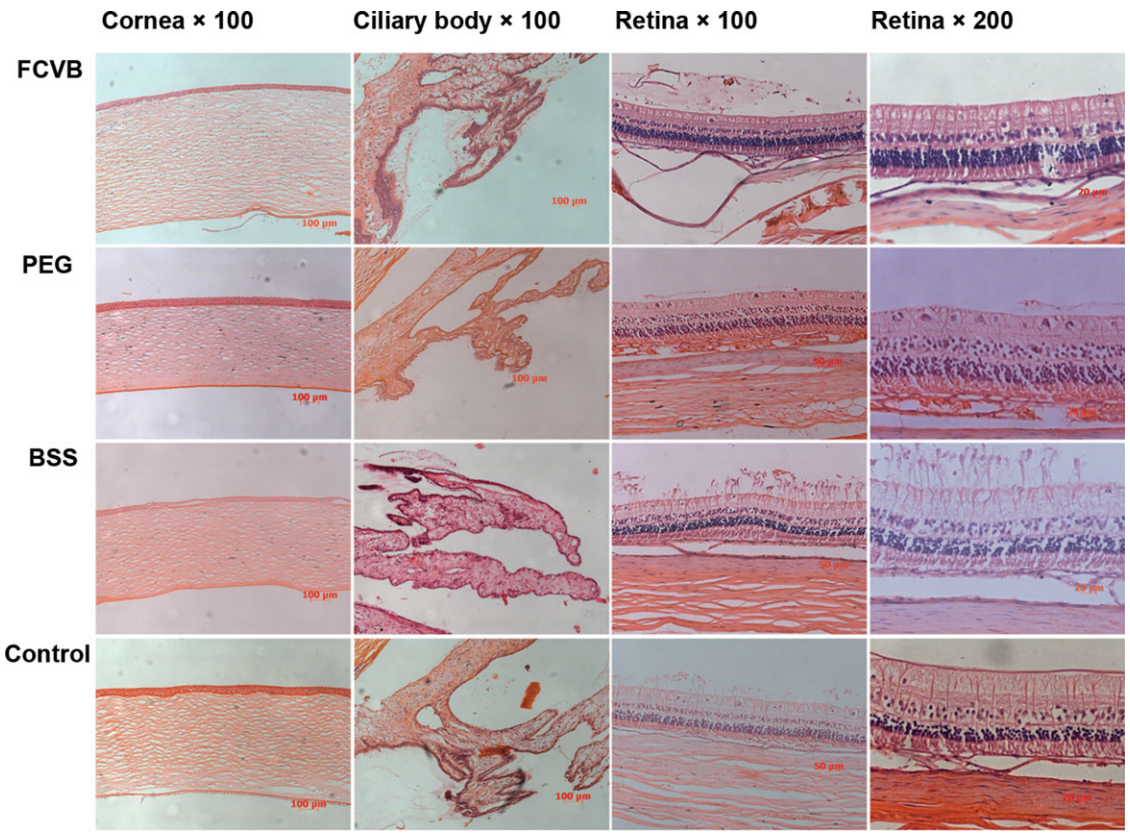

FIGURE 6. Light micrography of ocular specimens. The morphology of cornea, ciliary body, anterior chamber, and choroid was normal in FCVB-implanted eyes, PEG-implanted eyes, BSS-implanted eyes. The retina in the FCVB-implanted eyes showed a good integrity during the early stage (30 days), then the retina became disordered during the middle and late stage. In the PEG-implanted eyes, the structure of the retina was similar with that of the FCVB-implanted eyes during the early and middle stage. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

injected with FCVB can change ocular refraction, in this study, we evaluated the function of a novel approach, which used FCVB combined with PEG sols as a long-term (180 days) vitreous substitute.

In this study, the application of the FCVB has three main functions and advantages. These include (1) matching the shape of the vitreous cavity and effectively supporting the retina, (2) being biocompatible in the vitreous cavity, and (3) playing a support role for at least 3–5 months. Most importantly, FCVB provides better retina support and cellular barriers function *in vivo*. The fundus examinations showed that the capsule of FCVB fitted well with the shape of the vitreous cavity. Also, B-scan ultrasonography showed that FCVB enhanced echoes in the epiretinal membrane, prompting FCVB to support retinal attachment via a mechanical pressing method, and have an all-round retina supporting function. Gross examination also proved that. FCVB-implanted eyes showed a higher IOP at days 30 and 60 postoperatively. They even reached an abnormal hypertonia, which may have been due to the complications of cataract or lens subluxations. IOP then gradually decreased with the slow degradation of the PEG material.

We have also validated the feasibility and biocompatibility of FCVB in this study. Histopathology showed that FCVB combined with PEG sols was relatively safe in H&E and TUNEL tests. The cornea, ciliary body, and retina in the FCVB-implanted eyes showed no significant apoptosis activity. However, after long-term implantation, the possibility of retina toxicity appeared. It was hard to explain that toxicity may have been due to the mechanical oppression on the retina, which was likely caused by the complication of cataract or lens subluxation. We still need to evaluate the potential retina toxicity in subcellular structures by transmission electron microscopy. Furthermore, it was found that FCVB, when used as a physical barrier between the vitreous body and the retina could prolong retention time as well. Previously, PEG was reported as a short-term (41 days) vitreous substitute because of its excellent ocular characteristics and biocompatibility.[20] However, 41 days was too short to tamponade retina well and the retention time for an ideal vitreous is at least 3–5 months.[21] In this study, gross examination showed that FCVB can prolong the retention period of PEG sols. It was found that after a long period (180 days) of implantation, the PEG sols inside the FCVBs still had a viscoelastic property. The results were consistent with the hypothesis. FCVB served as a barrier that inhibited material migration from the vitreous cavity to the retina, prolonging the retention time in the vitreous cavity and relieving retina toxicity as well.

There were some limitations to our study. We were not able to get the electroretinogram signal to evaluate the

retina function in the FCVB-implanted eyes because of the insulation of the material and due to severe cataract as well. A higher incidence of cataracts and lens subluxation developed in the FCVB groups, which were possibly caused by inconspicuous damage to the lens during the FCVB implantation and inflation process, and by the materials as well. In addition, the influence of FCVB on the lens should be further evaluated. Based on our exploratory clinical trial at Zhongshan Ophthalmic Center for the treatment of severe retinal detachment, where 10/11 eyes were aphakia and 1/11 eye was phakia,[26] the influence to the lens could be considered secondary. As such, FCVB combined with PEG sols may be a good candidate for aphakia eyes.

CONCLUSIONS

This work presents a novel approach for finding vitreous substitutes using PEG sols injected into FCVB *in vivo* and *in vitro*. The media—5% (w/v) PEG sols—showed similar mechanical and optical properties to the natural vitreous. The application of FCVB could prolong the retention time of PEG sols and could provide a better support and cellular barrier function in the vitreous cavity. Our future plans will focus on the optimization of the retina supporting function of FCVB. We will try to simulate accurately with the mechanical-pressing method using computer software and we will search for an IOP balance point between the retina safety range and effective retina mechanical supporting pressure, lubricating the sac wall of the FCVB to reduce the potential toxicity of the retina as well.

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