# Peptide Hydrogel as an Intraocular Drug Delivery System for Inhibition of Postoperative Scarring Formation

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**ABSTRACT** A biocompatible hydrogel self-assembled from a peptide comprised of a peptide backbone containing Arg-Gly-Asp (RGD) sequence and a hydrophobic *N*-fluorenyl-9-methoxycarbonyl (FMOC) tail was designed and prepared to load antiproliferative model drug (5-fluorouracil, 5-Fu). After administrating this 5-Fu-loaded peptide hydrogel in the filtering surgery of rabbit eyes, because of the sustained release of 5-Fu from the hydrogel to inhibit the scleral flap fibrosis efficiently, the pathology and immunohistochemistry demonstrate that the filtration fistula is patent without postoperative scarring formation, resulting in the significantly low intraocular pressure (IOP) of the rabbit eyes within postoperative 28 days. In a comparison with the conventional 5-Fu exposure, the strategy demonstrated here presents several advantages including providing convenience and preventing the toxicity of 5-Fu to the surrounding ocular tissues efficiently, suggesting a feasibility of this peptide hydrogel as a potential implanted drug delivery system for the inhibition of postoperative scarring formation.

**KEYWORDS:** peptide hydrogel • drug delivery system • inhibition of scarring formation.

## INTRODUCTION

Glaucoma is an eye disease that can cause vision loss or blindness. For either open-angle glaucoma or angle-closure glaucoma, one of the main risk factors is the increased intraocular pressure (IOP), which can put pressure on the optical disk and result in injuring the optic nerve and ultimately causing vision loss (1–3). Currently, filtering surgery is a standard treatment for glaucoma, which involves generating a filtration fistula to allow the escape of aqueous humor from the anterior chamber into the subconjunctival space. However, the success rate of glaucoma-filtering surgery is generally limited by the formation of postoperative scarring (1). Scarring most commonly occurs at the level of the episclera, leading to the scleral flap fibrosis and eventual filtration failure. Antiproliferative 5-fluorouracil (5-Fu) is usually used subconjunctivally to prevent scleral flap fibrosis. It is known that 5-Fu is a chemotherapeutic agent that specifically mediates its antiproliferative effect by antagonizing pyrimidine metabolism to induce thymidine deficiency and decreased DNA synthesis (4, 5). In clinical practice, the postoperative 1−2 weeks are the critical period for the appearance of inflammatory and fibrotic reaction to result in the formation of scarring. 5-Fu is thus frequently administered via subconjunctival injection during this period. The frequent 5-Fu injections are uncomfortable for patient. Furthermore, the surrounding ocular tissues are easily exposed to the direct 5-Fu injection (6) and thus the corresponding toxicity can not be ignored because 5-Fu has been reported to exhibit toxicity on the conjunctival, corneal epithelium (7) and sometimes threaten vision (8).

The toxicity of 5-Fu injection could be reduced and even eliminated by using a subconjunctivally implanted polymeric drug delivery system because it can provide a localized and sustained release over an extended period. In the past few decades, a number of implanted polymeric drug delivery systems including viscous and semisolid materials have been developed to deliver ophthalmic drugs (9–12). The viscous poly(ortho ester) (POE) is the most representative one, which could be localized within eye and used as a controlled release device after a simple injection (10, 13). Up to now, even though numerous implanted polymeric drug delivery systems have been developed, critical challenges still remain, especially the biocompatibility. Because all these polymers are more like foreign body to have a potential to form a fibrous capsules around implants (13, 14). The future therapeutic strategy in ophthalmology should have the ability to integrate the well-biocompatible implant into clinical practice.

In recent years, arising from the abundant examples of protein self-assembly existing in nature, hydrogels formed via the self-assembly of peptides have attracted considerable

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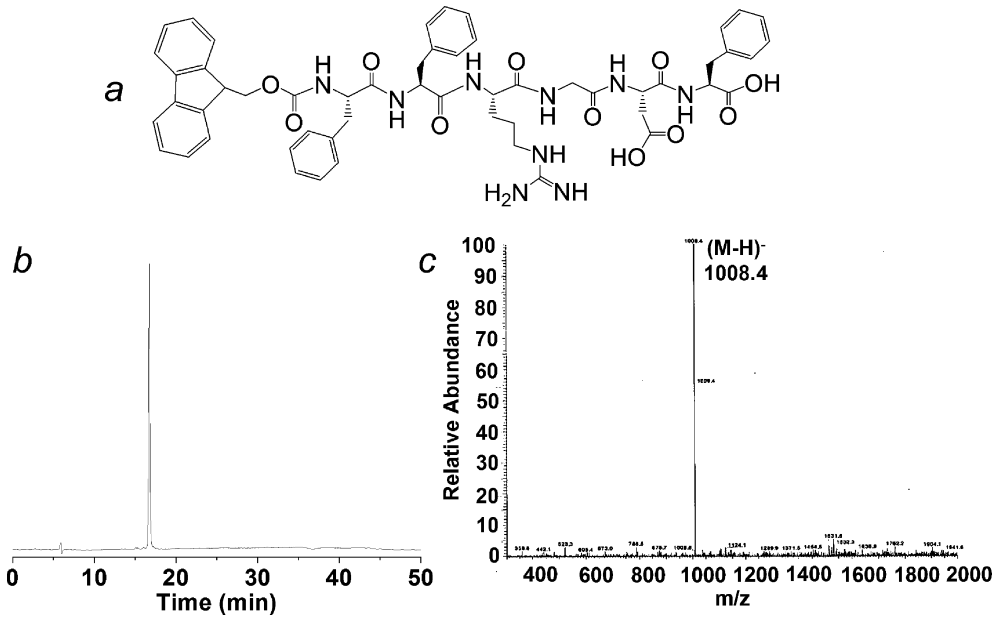


**FIGURE 1. (a) Molecular structure of the peptide FMOC-Phe-Phe-Arg-Gly-Asp-Phe (FMOC-FFRGDF); (b) HPLC profile of the peptide; (c) ESI-MS profile of the peptide.**

attention (15–18). Unlike the conventional hydrogels made of covalently cross-linked polymers, the peptides are able to grow from homogeneous solution into fibroid structure and the gelation is attributed to the entanglement of fibers or the formation of network structures and entrapment of solvent molecules via surface tension (19–22). Because of the potential advantages over the conventional hydrogels including inherent biodegradability, well biocompatibility, and similarity with the natural extracellular matrix (ECM), peptide hydrogels represent a kind of very important biocompatible materials that can meet various biomedical applications (23–26).

In our previous study, a new peptide with a sequence of Phe-Phe-Arg-Gly-Asp-Phe and a hydrophobic *N*-fluorenyl-9-methoxycarbony (FMOC) tail (Figure 1a) was reported (27). It was found that this peptide can self-assemble into transparent hydrogel that exhibited well biocompatibility in rabbit eyes. In this study, this peptide hydrogel was further developed as an implanted carrier to deliver antiproliferative model drug (5-Fu) in rabbit eyes for the inhibition of postoperative scarring formation.

## MATERIALS AND METHODS

**Materials.** The peptide with a purity of 99.1% used was previously synthesized on the 2-chlorotrityl chloride resin employing a standard FMOC solid phase peptide synthesis (SPPS) technique (27). Sodium hyaluronate (1.4 wt-%) and hematoxylin-eosin were purchased from Shanghai Jianhua Fine Biological Products Co. (China) and used as received. All other reagents and solvents were of analytical grade and used directly.

**Animals.** Japanese albino rabbits weighing between 2.5 and 3.5 kg were used in this study (Laboratory Animal Center, Tongji Medical College, Huazhong University of Science and Technology, China). The experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

**Preparation of Peptide Hydrogel.** The peptide (15 mg) was dissolved in ultra purified water (1 mL) to form 1.5-wt% aqueous solution. After placing at room temperature for several minutes, a well-defined hydrogel was formed via the supramolecular self-assembly of the peptide. The 5-Fu-loaded peptide hydrogel was similarly prepared by dissolving 15 mg peptide and 0.75 mg 5-Fu (5-wt% based on the amount of peptide used) in 1 mL ultra purified water.

**Characterization of Peptide Hydrogel.** Rheology experiments were performed on ARES-RFS III rheometer (TA Instruments, USA) to examine the viscoelastic property of the peptide hydrogel. Prior to performing the rheology measurement, the peptide aqueous solution was prepared as described above and transferred to the rheometer. After stabilization for 3 min, a time sweep was performed to study the viscoelastic property of the hydrogel. To observe the interior morphology of the peptide hydrogel, we diluted the hydrogel in distilled water and applied a small volume of diluted hydrogel solution to a copper grid with Formvar film and dried it for the observation on a JEM-100CXII transmission electron microscope (TEM). Fourier transform infrared spectroscopy (FT-IR), circular dichroism (CD), and fluorescence spectroscopy were employed to study the self-assembly mechanism of the peptide. FT-IR spectrum of the peptide hydrogel was collected on a Perkin-Elmer spectrophotometer by placing hydrogel sample in a $CaF_2$ cell. To obtain CD information of the peptide hydrogel, the hydrogel was fixed in a 0.5 mm quartz cell and analyzed on a Jasco J-810 spectropolarimeter with 4 s accumulations every 1 nm and averaged over 3 acquisitions. The fluorescence emission spectrum of the peptide hydrogel was recorded on a LS55 luminescence spectrometry (Perkin-Elmer) with excitation at 265 nm and emission data range between 300 and 700 nm.

**In vitro Drug Release.** The 5-Fu-loaded peptide hydrogel was prepared as described above in a cylindrical glass vial with only the top surface exposed for the drug release. Thereafter, 1 mL of phosphate buff solution (PBS, pH7.4) was added to the top of the hydrogel. At a predetermined time interval, the total volume of PBS was removed and 1 mL fresh PBS was added after each sampling. Each time interval was performed in triplicate and the experiment was carried out at physiological temperature (37 °C) for 1 week. The amount of 5-Fu released from the hydrogel was measured by using a UV spectrophotometer (Perkin-Elmer Lambda Bio 40 UV/vis spectrometer, USA) at 265 nm. The cumulative drug release was calculated as: Cumulative amount released (%) = $(M_t/M_\infty)100$, where $M_t$

is the amount of 5-Fu released from the hydrogel at time $t$ and $M_\infty$ is the amount of 5-Fu loaded in the hydrogel.

**Filtering Surgery.** General anesthesia was performed through intramuscular injection of 50 mg/kg ketamine and 15 mg/kg xylazine. And the local anesthesia was carried out using 1-wt % dicaine.

Prior to the filtering surgery, the peptide hydrogel or 5-Fu-loaded hydrogel was prepared after filtering the solution using a 250 nm filtration membrane to remove bacteria. After the gelation, the hydrogel was further sterilized via UV irradiation for 30 min before the study. The filtering surgery of four groups of rabbit eyes was performed under a dissecting microscope with coaxial-light illumintion and a variable magnification of $(2 \pm 10)\times$ (Topcon, Japan). A lid speculum was inserted to expose the globe and a limbus-based conjunctival flap was fashioned. And the sclera was simultaneously exposed. Thereafter, a limbus-based half-thickness scleral flap ($4 \times 4$ mm$^2$) was dissected by using a 45° supersharp blade until clear cornea was observed. At this time, one group of rabbit eyes were exposed to 5-Fu. In detail, a $4 \times 1$ mm$^2$ dry section of a sponge was soaked in 5-Fu aqueous solution with a concentration of 50 mg/mL. And the sponge was then placed between the conjunctiva and the sclera over the planned filtration site for 5 min. Thereafter, the corneal epithelium was protected by using a wet sponge over the cornea. And the treated site was subsequently irrigated with 20 mL of balanced salt solution. After the 5-Fu exposure to the rabbit eyes in this group, a 3-mm limbal incision was made with a 45° supersharp blade that entered the anterior chamber of all the rabbit eyes in four groups, followed by a filtering surgery, where excision of a $3 \times 1$ mm$^2$ sclera and cornea was performed over the limbus under the scleral flap. And a peripheral iridectomy was also performed. The scleral flap was subsequently closed by using two 10−0 nylon sutures (Alcon Laboratories, Inc., USA). At this time, a 0.9 mm needle (20 gauge) was inserted in the subconjunctival space of the rabbit eyes of another two groups. And 200 $\mu$L of the peptide hydrogel and 5-Fu-loaded hydrogel were respectively injected adjacent to the filtering surgery sites of the rabbit eyes in these two groups. The conjunctiva was then closed by using continuous 8−0 Vicryl sutures (Ethicon, Inc., USA) and topical cyclomycin ophthalmic ointment was applied. Herein, the four groups of rabbit eyes used in this study were identified as the eyes underwent filtering surgery alone (filtering surgery), intraoperatively exposed to 5-Fu (5-Fu), intraoperatively received the peptide hydrogel (gel), and the 5-Fu-loaded hydrogel (5-Fu-loaded gel), respectively. In each group, the right eyes of six rabbits were used for filtering surgery and the left eyes were employed as control.

**Clinical Follow-Up.** Rabbit eyes in four groups were examined at regular intervals up to 28 days after the filtering surgery. A slit-lamp examination was performed to assess the filtering bleb status and the overall inflammatory state of the surgical eyes. Survival analysis was carried out to examine the bleb failure and Log-rank test was employed to investigate the overall survival difference among the rabbit eyes in four groups. Conjunctival hyperemia was scored according to the reported method (13): grade 0, normal vessels; grade 1, definitely injected vessels; grade 2, diffuse crimson red, individual vessels not easily discernible; and grade 3, diffuse beefy red. Corneal edema was also scored as follows: grade 0, normal cornea; grade 1, slight corneal edema presented at the surgical site; grade 2, diffuse corneal edema extending to half the surface of the cornea; and grade 3, opaque cornea with neovascularization. Statistical analysis was employed by using a nonparametric Mann−Whitney test. $P < 0.05$ was considered significant. In addition, intraocular pressure (IOP) of the surgical eyes was also measured at the same time intervals using an applanation tonometer (Tono-Pen XL, Mentor, USA), with the rabbits under local anesthesia via the injection of dicaine 1 wt %. Statistical analysis was carried out using student's $t$ test. $P < 0.05$ was considered significant.

**Pathology and Immunohistochemistry.** Rabbits were sacrificed at different time ranging from 7 to 28 days after filtering surgery by an overdose of pentobarbital. The eyes were subsequently enucleated and stored in 4% paraformaldehyde overnight for conventional optical microscopy. After embedding the eyes in paraffin blocks, 4 $\mu$m sections in the conjunctival, iridocorneal angle, and the site of the bleb were obtained. These microtome sections were stained with hematoxylin-eosin for general histological analysis. Simultaneously, Masson trichrome stain was also performed to examine the status of collagen deposition. Additional tissue sections were used for $\alpha$-patent muscle actin ($\alpha$-SMA) immunohistochemistry to identify the distribution of fibroblasts. Every sample was treated simultaneously to reduce variation among fixation, embedding, and section procedures.

## RESULTS AND DISCUSSION

**Characterization of Peptide Hydrogel.** It is known that RGD peptide sequence can support fibroblast attachment and thus inhibit fibroblast adhesion to fibronectin as well as transferring of fibroblast (28, 29). Because the failure of glaucoma-filtering surgery is generally attributed to the proliferation of fibroblast and ultimate formation of postoperative scarring, using the antiproliferative drug-loaded hydrogel formed from a peptide containing RGD sequence in glaucoma-filtering surgery can efficiently inhibit the fibroblast adhesion to fibronectin and transferring of fibroblast around the surgical site. Simultaneously, the localized and sustained release of antiproliferative drug can also prevent the proliferation of fibroblast. On the basis of this purpose, a peptide containing RGD sequence was designed and the molecular structure of this peptide is shown in Figure 1a. There is a hexapeptide backbone with a sequence of Phe-Phe-Arg-Gly-Asp-Phe. The incorporation of Phe residues in the peptide backbone is to adjust the hydrophilicity/hydrophobicity of the peptide to make sure the success of hydrogel formation via the supramolecular self-assembly of the peptide. Besides, a hydrophobic FMOC functional group also attaches to the peptide backbone as a tail to provide the $\pi$-stacking interaction during the supramolecular self-assembly. The HPLC profile of this peptide in Figure 1b indicates a high purity of the peptide (99.1%). The corresponding ESI-MS data in Figure 1c demonstrate the validity of the peptide structure.

After the peptide is dissolved in ultra purified water, a three-dimensional hydrogel can be formed within several minutes (around 10 min) (Figure 2a). From the time sweep of this formed hydrogel (Figure 2b), the storage modulus (G′) is higher than loss modulus (G″), indicating a gel characteristic rheological behavior of the solid-like material. The interior morphology of the peptide hydrogel was examined by transmission electron microscope (TEM). Long nanofibers with the width around 20 nm are formed in the hydrogel (Figure 2a). In order to understand the molecular arrangement in the formed nanofibers, FT-IR, circular dichroism (CD), and fluorescence spectroscopy were performed. From the FT-IR spectrum of the peptide hydrogel, the absorbance maxima of amide I frequency is at ~1635 cm$^{-1}$ (see Figure S1 in the Supporting Information), a typical band of $\beta$-sheet

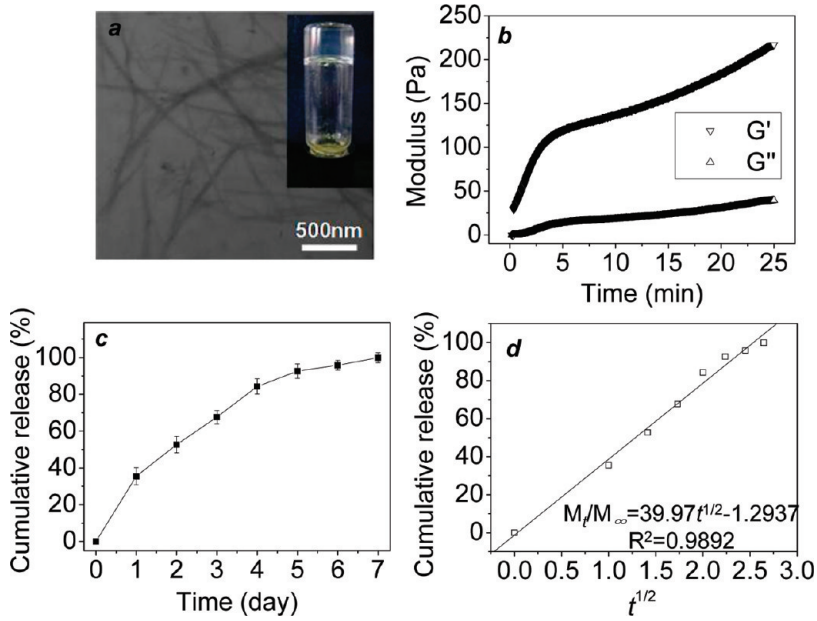


**FIGURE 2. (a) Optical and TEM images of the peptide hydrogel; (b) Oscillatory rheology of the peptide hydrogel; (c) Cumulative release of 5-Fu from the 5-Fu-loaded peptide hydrogel at physiological temperature (37 °C); (d) 5-Fu release kinetics of the peptide hydrogel on a $t^{1/2}$ scale.**

like superstructure formed via intermolecular hydrogen bonding interactions (30–32). And the relatively weak peak at ~1687 $cm^{-1}$ is indicative of antiparallel alignment of the intermolecular hydrogen bonds among peptide backbones (32). From the CD spectrum of the peptide hydrogel (see Figure S2 in the Supporting Information), the negative band at ~223 nm (peptide n−$\pi$* transition), which is a typical CD signal of polypeptide with a $\beta$-sheet conformation (30–32), suggests that the peptide backbones of the peptide molecules form $\beta$-sheet like superstructure via the supramolecular self-assembly. The negative broad band ranging from 250 to 315 nm can also be observed in the CD spectrum, which mainly corresponds to the $\pi$−$\pi$* transition of the terminal FMOC tails. The emission spectrum of the peptide hydrogel provides the detailed environment of FMOC tails in the nanofibers (see Figure S3 in the Supporting Information). In comparison with the emission spectrum of the peptide solution (~320 nm), there is a small peak at ~330 nm (10 nm red shift) in the emission spectrum of the peptide hydrogel, implying the $\pi$-stacking of FMOC tails via antiparallel fashion in the formed nanofibers (30, 32). Herein, a broad peak centered at ~475 nm in the emission spectrum of the peptide hydrogel suggests that more than two FMOC tails stack efficiently in the nanofibers via $\pi$−$\pi$ interaction, similar to the case of $\pi$-stacked polyfluorenes (33). Combining the information from FT-IR, CD, and emission spectra, the features of the supramolecular self-assembly of the peptide can be identified as follows (see Figure S4 in the Supporting Information). The peptide backbones of the peptide molecules carry out $\beta$-sheet like arrangement via hydrogen bonding interaction. And FMOC tails are thus positioned to $\pi$-stack through antiparallel fashion, resulting in the formation of nanofibers. And the gelation of the peptide thus appears on the basis of the entanglement of nanofibers (network structure formation) and entrapment of solvent molecules via surface tension (19–22, 32).

**In vitro Drug Release.** One of the great benefits to use a self-assembling system for therapeutic delivery is that the therapeutic drugs can be incorporated during the formation of materials. This strategy allows precise control over the concentration of the incorporated drugs (34). In this study, after the formation of the 5-Fu-loaded peptide hydrogel, the drug release property of the peptide hydrogel was investigated at physiological temperature (37 °C) and the corresponding release profile is exhibited in Figure 2c. About 21.7% incorporated 5-Fu is released from the hydrogel at the first day. And the cumulative drug release increases to ~44.2% at the third day. After the sustained release for 7 days, almost the total loaded 5-Fu is released from the hydrogel. It should be noted that the volume of the peptide hydrogel remains constant and no swelling or shrinking is observed upon the addition of buffer solution to the top of the hydrogels. Also, during the whole release process, there is no detectable change in size of the peptide hydrogel (around 1.5 cm in diameter and 1 cm in height), and no evidence of surface erosion and degradation (the weight of the hydrogel keeping around 1.2 g). Therefore, the release of incorporated 5-Fu in this peptide hydrogel is only diffusion-controlled and dependent on the interaction between the drug and the hydrogel network, which is demonstrated by the 5-Fu release kinetics of the peptide hydrogel on a $t^{1/2}$ scale. As shown in Figure 2*d*, the 5-Fu release kinetics of the peptide hydrogel meets the classic drug release law suggested by Peppas et al. ($M_t/M_\infty = kt^{1/2}$, diffusion-controlled drug release) (35). Importantly, from the data in Figure 2c, it is found that 5-Fu is gradually released from the peptide hydrogel and no burst release can be observed. This unique property indicates that the peptide hydrogel prepared in this

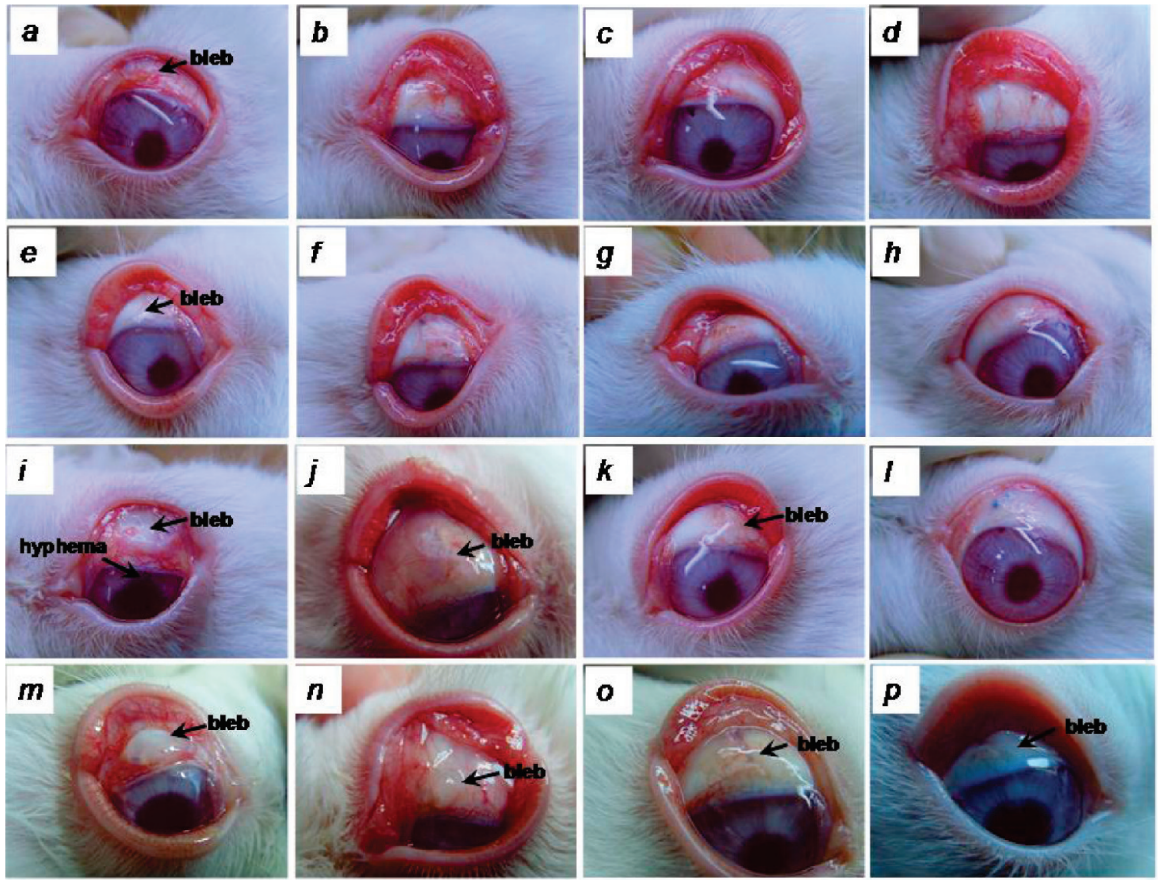


**FIGURE 3. (a−d): Photo images of the rabbit eyes that underwent filtering surgery alone at postoperative (a) 7, (b) 14, (c) 21, and (d) 28 days, respectively; (e−h) photo images of the rabbit eyes that received the peptide hydrogel at postoperative (e) 7, (f) 14, (g) 21, and (h) 28 days, respectively; (i−l) photo images of the rabbit eyes that exposed to 5-Fu intraoperatively at postoperative (i) 7, (j) 14, (k) 21, and (l) 28 days, respectively; (m−p) photo images of the rabbit eyes that received the 5-Fu-loaded peptide hydrogel at postoperative (m) 7, (n) 14, (o) 21, and (p) 28 days, respectively.**

study has a potential for use as a valuable drug delivery system for the application in biomedical fields.

**Filtering Surgery and Clinical Evaluation.** Our previous in vivo biocompatibility assay demonstrated that the peptide hydrogel had a good biocompatibility and can be used as scaffold in biomedical fields (27). The drug release profile described above indicates a favorable drug delivery property of the peptide hydrogel. All these results suggest a great potential to employ this peptide hydrogel as an implanted drug delivery system in ophthalmology. Here, the peptide hydrogel was used to load antiproliferative model drug (5-Fu) and then administrated in the filtering surgery of rabbit eyes. And another three groups (eyes that underwent filtering surgery alone; eyes intraoperatively exposed to 5-Fu; and eyes that intraoperatively received peptide hydrogel) were used as control. The direct observation of the rabbit eyes is shown in Figure 3. There are no postoperative complications of wound dehiscence, wound leaks, hyphema, and endophthalmitis in the rabbit eyes that underwent filtering surgery alone or received the peptide hydrogel or the 5-Fu-loaded hydrogel. However, hyphema can be observed in the rabbit eyes that were exposed to 5-Fu intraoperatively (Figure 3i) at postoperative 7 days, indicating the presence of potential toxicity triggered by direct exposure of the normal ocular tissues to 5-Fu (7, 8). The score of conjunctival hyperemia of the rabbit eyes was evaluated. On the basis of our experiments, there is triggered hyperemia for all the rabbit eyes in four groups at postoperative 1 day. At postoperative 3 days, the conjunctival hyperemia triggered by the intraoperative injection of the hydrogel, intraoperative exposure of 5-Fu, and intraoperative injection of 5-Fu-loaded hydrogel has a score of $\sim 0.83 \pm 0.41$, $\sim 1.17 \pm 0.41$ and $\sim 1.33 \pm 0.52$, respectively, which is higher than that of the eyes underwent filtering surgery alone ($\sim 0.67 \pm 0.55$) ($P = 0.685, 0.752, 0.596$). However, the conjunctival hyperemia decreases significantly at postoperative 7 days and can disappear at approximately postoperative 2 weeks. Besides the evaluation of the conjunctival hyperemia, the corneal edema of the rabbit eyes was also assessed and presented in Figure 4. As for the rabbit eyes that underwent filtering surgery alone, a slight corneal edema is triggered at the surgical site and can resolve after 1 week, which is similar with that of the rabbit eyes received the peptide hydrogel intraoperatively. However, 5-Fu employed as intraoperative exposure triggers more severe edema, extending to half cornea of three eyes on the basis of our experiments. Moreover, this corneal edema is constantly encountered until postoperative 21 days. The frequency and the severity of corneal edema of the rabbit eyes exposed to 5-Fu intraoperatively is significantly higher than that of the eyes

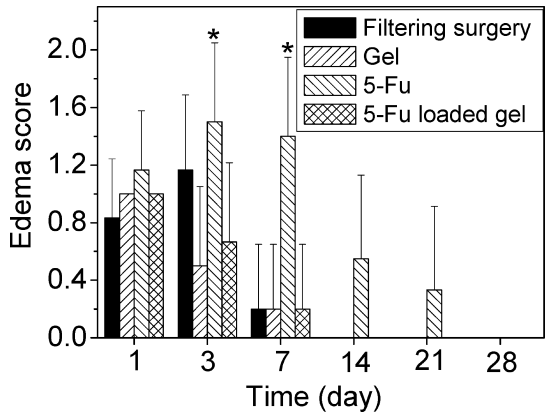


FIGURE 4. Evaluation of edema score of the rabbit eyes underwent filtering surgery alone (filtering surgery), received the peptide hydrogel (gel), exposed to 5-Fu (5-Fu), and received the 5-Fu-loaded peptide hydrogel (5-Fu-loaded gel), respectively. *Significant difference from Filtering surgery group ($P < 0.05$).

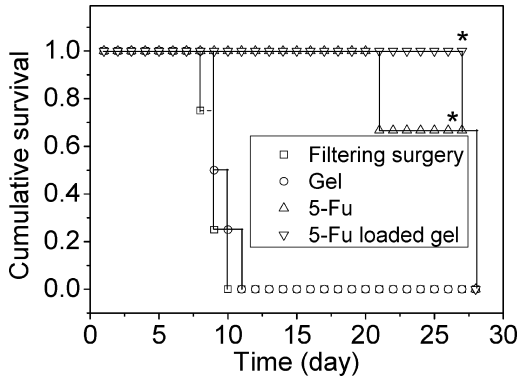


FIGURE 5. Kaplan−Meier plot of the duration of blebs of the rabbit eyes underwent filtering surgery alone (filtering surgery), received the peptide hydrogel (gel), exposed to 5-Fu (5-Fu), and received the 5-Fu-loaded peptide hydrogel (5-Fu-loaded gel), respectively. *Significant difference from filtering surgery group ($P < 0.05$).

underwent filtering surgery alone at postoperative 3 and 7 days ($P = 0.03$ and 0.016, respectively). In this study, although the rabbit eyes received 5-Fu-loaded hydrogel also present corneal edema, it can localize at the surgical site and decrease significantly at postoperative 3 days, and even resolve after 1 week. And there is no significant difference compared to the rabbit eyes underwent filtering surgery alone ($P = 0.786$).

It is known that the main clinical symptom of glaucoma is the increased intraocular pressure (IOP). The glaucoma-filtering surgery aims to generate a filtration fistula to allow the escape of aqueous humor from the anterior chamber into the subconjunctival space. Subsequently, IOP is lowered and a bleb could be thus formed at the surgical site (1–3). In other words, the appearance of bleb and lowered IOP are vital to the success of glaucoma-filtering surgery. In this study, the bleb and IOP of the rabbit eyes were examined during the clinical follow-up. The bleb survival curve of the rabbit eyes is exhibited in Figure 5. In the case where the rabbit eyes underwent filtering surgery alone, all the blebs collapse at postoperative 10 days and the median survival time of bleb is calculated as 9 days. With respect to the rabbit eyes that received the peptide hydrogel intraoperatively, the blebs present slightly delayed persistence in comparison

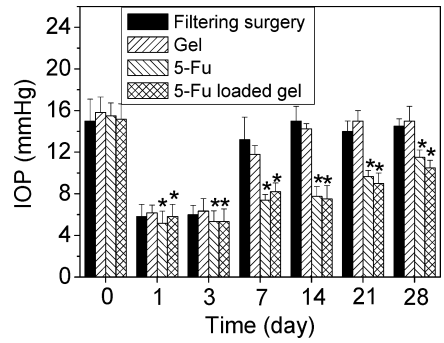


FIGURE 6. Postoperative mean IOP of the rabbit eyes underwent filtering surgery alone (filtering surgery), received the peptide hydrogel (gel), exposed to 5-Fu (5-Fu), and received the 5-Fu-loaded peptide hydrogel (5-Fu-loaded gel), respectively. *Significant difference from Filtering surgery group ($P < 0.05$).

with that of the eyes that underwent filtering surgery alone and the median survival time of bleb is 10 days. But there is no significant difference between the eyes in these two groups ($P = 0.2539$), implying that the intraoperative injection of the peptide hydrogel alone can not have significant influence on the bleb survival. For the rabbit eyes exposed to 5-Fu intraoperatively, the blebs are more persistent in comparison with that of the eyes underwent filtering surgery alone ($P = 0.0069$). All the blebs can survive at least postoperative 21 days and some blebs survive until postoperative 28 days. The median survival time of bleb is determined as 26 days. The similar profile is also presented for the rabbit eyes received 5-Fu-loaded hydrogel intraoperatively. All the blebs survive until postoperative 27 days and the median survival time of bleb is 28 days. The mean IOP of the rabbit eyes is measured and presented in Figure 6. The mean IOP of the rabbit eyes underwent filtering surgery alone is lower within postoperative 3 days. But it almost returns to preoperative level at postoperative 7 days. The mean IOP of the rabbit eyes that received the peptide hydrogel intraoperatively presents the similar profile. However, the mean IOP of the rabbit eyes that intraoperatively received 5-Fu-loaded hydrogel is significantly lower within postoperative 28 days ($P = 0.03$), which is similar as the eyes intraoperatively exposed to 5-Fu ($P = 0.436$). From the results of the survival time of bleb and mean IOP, the rabbit eyes received 5-Fu either as exposure or loaded in the hydrogel present more persistent bleb survival time and lower mean IOP in comparison with that of the rabbit eyes underwent filtering surgery alone. All these results imply that the presence of 5-Fu inhibits the scleral flap fibrosis efficiently and filtration fistula is thus patent for the rabbit eyes in these groups, which will be demonstrated by the following results of pathology and immunohistochemistry analysis.

**Pathology and Immunohistochemistry.** The appearance of bleb and lowered IOP in the clinical follow-up is attributed to the formation of a filtration fistula after the glaucoma-filtering surgery. However, the filtration fistula is generally closed due to the proliferation of fibroblasts and formation of postoperative scarring, resulting in the collapsed bleb and the eventual failure of filtration (1). In this study, to investigate whether the filtration fistula and bleb

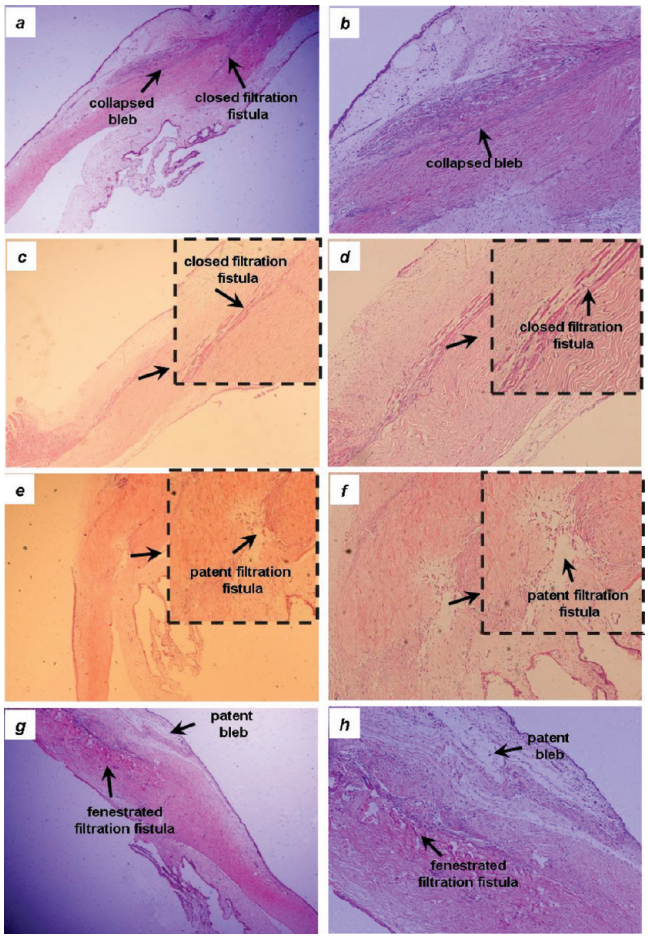


**FIGURE 7. (a, b) Histological section through the iridocorneal angle of the rabbit eyes underwent filtering surgery alone at postoperative 7 days. Hematoxylin-eosin magnification: (a) 40×, (b) 100×. (c, d) Histological section through the iridocorneal angle of the rabbit eyes received the peptide hydrogel at postoperative 7 days. Hematoxylin-eosin magnification: (c) 40×, (d) 100×. (e, f) Histological section through the iridocorneal angle of the rabbit eyes exposed to 5-Fu at postoperative 28 days. Hematoxylin-eosin magnification, (e) 40×, (f) 100×. (g, h) Histological section through the iridocorneal angle of the rabbit eyes received the 5-Fu-loaded peptide hydrogel at postoperative 28 days. Hematoxylin-eosin magnification: (g) 40×, (h) 100×.**

are closed or not, we sacrificed the rabbits through injection of an overdose of phenobarbitone at different times ranging from 7 to 28 days. And the eyes were subsequently enucleated and studied histologically. As shown in Figure 7, bleb is collapsed at postoperative 7 days for the rabbit eyes underwent filtering surgery alone, which is attributed to the bulk filling by granulation tissue and fibroblasts (Figure 7a). Moreover, the filtration fistula is less fenestrated, with formation of scarring tissue intermingled with fibroblasts, some of which are active with prominent mitosis (Figure 7a,b). For the eyes that intraoperatively received the peptide hydrogel, there is no acute inflammatory reaction within postoperative 7 days, once demonstrating our previous report that this peptide hydrogel presented good biocompatibility in rabbit eyes. However, some fibrotic fibroblasts and scarring tissue could be also observed in scleral flap and episclera of the eyes (Figure 7c,d). As for the eyes that intraoperatively received 5-Fu either as exposure or loaded in hydrogel, because of the presence of antiproliferative 5-Fu

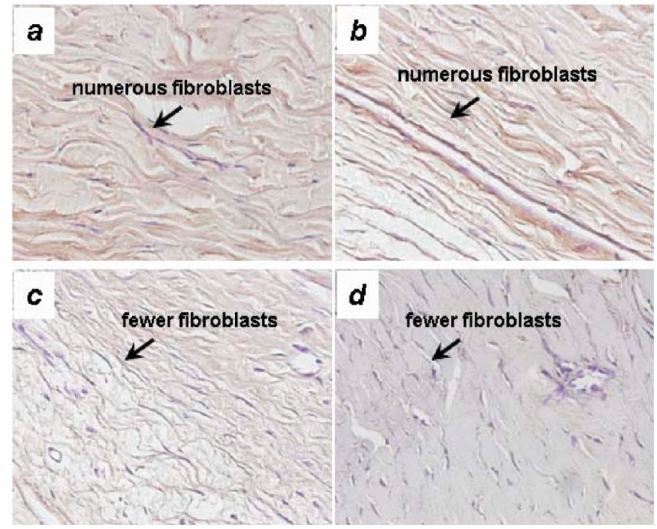


**FIGURE 8. (a–d) α-SMA immunostaining of blebs of the rabbit eyes that (a) underwent filtering surgery alone, (b) received the peptide hydrogel, (c) were exposed to 5-Fu, and (d) received the 5-Fu-loaded peptide hydrogel at postoperative 14 days. α-SMA staining; magnification 400×.**

to inhibit the scleral flap fibrosis efficiently, there are no signs of fibrosis of the filtration fistula until the end of experiments (Figure 7e–h). Especially for the eyes that received 5-Fu-loaded hydrogel, the scleral flap and episclera are absent from any fibrotic subconjunctival fibroblasts and the filtration fistula is fenestrated, with irregular wide spaces. Besides, the conjunctival filtration fistula is efficient, visible as a small cavity surrounded by fibrin and cells (Figure 7g,h). However, the eyes intraoperatively exposed to 5-Fu present slight modifications of the iris (Figure 7e,f).

To further examine the status of filtration fistula and bleb, we performed immunohistochemistry because the fibroblast phenotype can be characterized by the expression of the α-patent muscle actin (α-SMA) in immunohistochemistry (36). In the case of the rabbit eyes that underwent filtering surgery alone or intraoperatively received the peptide hydrogel, α-SMA immunostaining could be found conspicuously at postoperative 14 days, indicating the formation of numerous fibroblasts in the subconjunctival space and episcleral tissues (Figure 8a,b). In contrast, the rabbit eyes that received 5-Fu, either as exposure or loaded in hydrogel, have relatively fewer fibroblasts at postoperative 14 days (Figure 8c,d). It is known that fibroblasts can secret collagen which induces the formation of postoperative scarring and this collagen could be identified by Masson trichrome stain. From the images of Masson trichrome stain in Figure 9, the rabbit eyes underwent filtering surgery alone or intraoperatively received the peptide hydrogel have linear and dense collagen fibers secreted by fibroblasts deposit inside the collapsed bleb and scleral surface at postoperative 14 days (Figure 9a,c). However, within the same time frame, for the rabbit eyes intraoperatively exposed to 5-Fu or received 5-Fu-loaded hydrogel, the filtration fistula remains prominent and randomized collagen deposition is presented subconjunctivally (Figure 9e,g). Furthermore, at postoperative 28 days, the bleb is still filled with dispersed and randomized collagen (Figure 9f,h). But the bleb and filtration fistula of

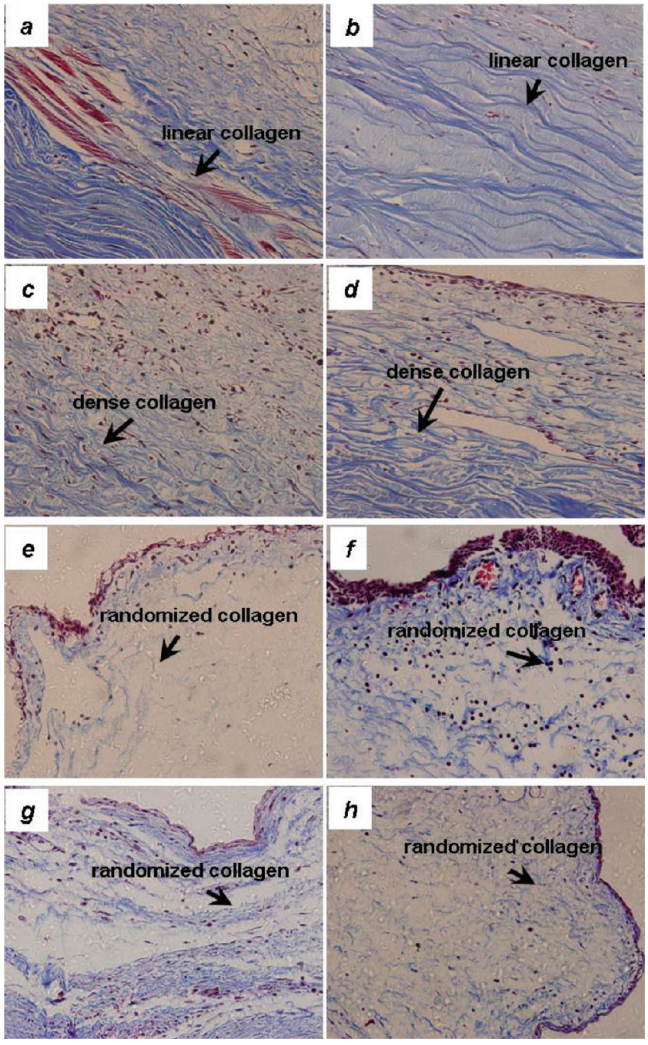


**FIGURE 9. (a, b) Masson trichrome stain of the blebs of the rabbit eyes that underwent filtering surgery alone at postoperative (a) 14 and (b) 28 days, respectively. (c, d) Masson trichrome stain of the blebs of the rabbit eyes received the peptide hydrogel at postoperative (c) 14 and (d) 28 days, respectively. (e, f) Masson trichrome stain of the blebs of the rabbit eyes exposed to 5-Fu at postoperative (e) 14 and (f) 28 days, respectively. (g, h) Masson trichrome stain of the blebs of the rabbit eyes received the 5-Fu-loaded peptide hydrogel at postoperative (g) 14 and (h) 28 days, respectively. Magnification, 400×.**

the rabbit eyes underwent filtering surgery alone or intraoperatively received the peptide hydrogel are filled with dense collagen deposition at this moment (Figure 9b,d).

Combining the results of clinical evaluation, pathology, and immunohistochemistry, the postoperative 1−2 weeks are the critical period for the appearance of inflammatory and fibrotic reactions that result in scarring, even though the total loaded 5-Fu is almost released from the peptide hydrogel within 7 days as demonstrated in the release study; it can also prevent fibrotic reaction from occuring efficiently. Therefore, there is no appearance of scleral flap fibrosis during the next follow-up period. As a result, the filtration fistula that formed after the filtering surgery is always patent and the bleb is efficient, resulting in lowered IOP of rabbit eyes within postoperative 28 days. Importantly, in comparison with the conventional 5-Fu exposure, because of the localized and sustained release of 5-Fu from the hydrogel, the corresponding toxicity to surrounding ocular tissues can be efficiently avoided. As a result, no acute inflammatory reaction is present during the clinical follow-up. It is known that the rabbit model is different from that of humans for glaucoma-filtering surgery; fibrin formation and cellular proliferation are much more rapid and intense than that of humans. In general, a filtering surgery lasts well longer than 1 year in humans, whereas in rabbits it lasts less than 1 week without adjunct antimitotic agents and less than 1 month in the presence of antiproliferative drug (37, 38). Therefore, the efficiency of the 5-Fu-loaded hydrogel demonstrated in this study can be expected to be more favorable in human eyes for the inhibition of postoperative scarring formation.

## CONCLUSIONS

A biocompatible hydrogel self-assembled from a peptide comprising a peptide backbone containing RGD sequence and a hydrophobic FMOC tail was prepared as an ocular implant to load antiproliferative model drug (5-Fu) and then administrated in the filtering surgery of rabbit eyes. Because of the localized and sustained release of 5-Fu from the peptide hydrogel, the toxicity to the surrounding ocular tissues can be avoided. Simultaneously, the presence of 5-Fu can also efficiently inhibit the scleral flap fibrosis. As a result, the filtration fistula of the rabbit eyes is thus patent and the mean IOP is significantly lower within postoperative 28 days. In comparison with the conventional 5-Fu exposure, all these advantages suggest this strategy as a potential alternative for the inhibition of postoperative scarring formation.

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**Supporting Information Available:** FT-IR, CD and emission spectra of the peptide hydrogel, schematic illustration of the supramolecular self-assembly of the peptide (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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