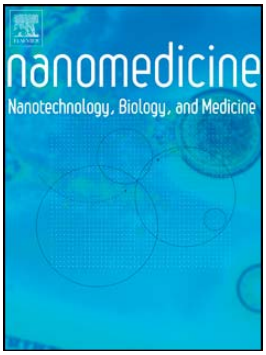

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Self-assembly of a ibuprofen–peptide conjugate to suppress ocular inflammation

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Abstract

In present study, we designed and synthesized a hydrogelator comprised of ibuprofen (IPF) and GFFY peptide linked through a cleavable ester bond. We found that the synthesized hydrogelator could spontaneously self-assemble into a hydrogel under a heating–cooling process. When the hydrogel was acted upon by an esterase, IPF was released in a sustained manner. Moreover, the hydrogel had significantly elevated anti-inflammatory efficacy, compared with IPF, in RAW264.7 macrophages. The hydrogel showed good cytocompatibility, as well as excellent ocular biocompatibility when instilled topically. *In vivo* results further demonstrated that the hydrogel had therapeutic efficacy comparable to that of a current treatment, sodium diclofenac (DIC) eyedrops, in suppressing ocular inflammation in lipopolysaccharide (LPS) induced uveitis. Our findings illustrated, for the first time, an effective approach for developing supramolecular assemblies as anti-inflammatory ophthalmic therapeutics for eye disorders.

Key words: Self-assembled hydrogel, anti-inflammatory, ocular drug delivery, endotoxin induced uveitis

BACKGROUND

Low molecular weight hydrogels formed by therapeutic agents and their derivatives have recently received considerable attention because of their high drug payloads, as well as their sustained and responsive drug release properties.[1-10] In particular, supramolecular hydrogels derived from drug–peptide conjugates are emerging as new candidates for drug delivery because of their excellent biocompatibility, biodegradability and thixotropic properties.[11-19] A number of agents, including anti-tumor (*e.g.*, paclitaxel, camptothecin and curcumin), anti-bacterial (*e.g.*, vancomycin) and anti-inflammatory (*e.g.*, dexamethasone and triamcinolone) drugs, coupled with peptides, were successfully shown to act as hydrogelators, forming supramolecular hydrogels for drug delivery.[20-29] More recently, Xu *et al*. described a hydrogelator, composed of a D-amino acid and a non-steroidal anti-inflammatory drug (NSAID), which had enhanced selectivity as a cyclooxygenase-2 (COX-2) inhibitor.[3] In addition, we recently demonstrated that the covalent conjugation of ibuprofen (IPF) with a simple peptide sequence, through an amide bond, produced a hydrogel-forming substance. Unfortunately, the conjugate had much lower pharmacological activity than the parent drug.[30] This undesired shortcoming significantly limited the *in vivo* applications of the conjugate. Therefore, it would be beneficial to design a drug–peptide conjugate that not only would self-assemble to form a hydrogel, but would also retain the biological activity of the native drug.

Considering the exceptional anti-inflammatory activity of native IPF, we choose to release this drug from the hydrogel via an enzymatic reaction. Drawing from previous studies reporting enzymatic disassembly of supramolecular hydrogelators,[1, 4, 21] we designed and synthesized a hydrogelator composed of IPF and a GFFY peptide, linked through a cleavable ester bond. Introduction of the aromatic compound hydroxybenzoic acid (HYD) as an enzyme cleavable linkage not only facilitated self-assembly of the compound, forming a hydrogel, through π–π stacking and

hydrophobic interactions, but also enabled restoration of biologically active IPF by esterase catalyzed hydrolysis. We further investigated the intraocular biocompatibility and *in vivo* anti-inflammatory efficacy of the resulting hydrogel in a rabbit model.

METHODS

Materials

HYD and IPF were from J&K Scientific (Shanghai, China). Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Diclofenac sodium eyedrops (0.1 wt% DIC; Shenyang Xingqi Pharmaceutical Co., Ltd, Shenyang, China) was from the Eye Hospital of Wenzhou Medical University. N-Fmoc-protected amino acids and O-Benzotriazole-N,N,N,N-tetramethyl-uronium-hexafluorophosphate (HBTU) were from GL Biochem (Shanghai, China) Ltd. All other chemicals were analytical grade.

Synthesis and characterization of IPF–HYD

Briefly, IPF (1.0 g, 4.85 mmol) was first dissolved into $SOCl_2$ (15 mL) and refluxed for 3 h. Next, unreacted $SOCl_2$ was removed under vacuum to yield a yellow liquid, containing the acyl chloride. Then, hydroxybenzoic acid (HYD; 0.67 g, 4.85 mmol) and pyridine (5 mL) were added to the acyl chloride in dry acetone (10 mL) at 0°C and the solution stirred at room temperature for 5 h. Finally, the mixture was filtered and the organic solvent removed by evaporation. The residue was purified by flash chromatography on silica gel, eluted with ethyl acetate:petroleum ether 1:3–1:2 (v/v) to obtain the corresponding product at 75% yield. The final product was characterized by ^{1}H-NMR.

Synthesis of IPF–HYD–GFFY hydrogelator

Briefly, the IPF–HYD–GFFY hydrogelator was synthesized by classic solid-phase peptide synthesis using 2-chlorotrityl chloride resin and N-Fmoc-protected amino acids. The 2-chlorotrityl chloride resin was first swelled in dry dichloromethane (DCM) for 20 min and then the first amino acid was loaded onto the resin, as a solution of Fmoc-protected amino acid (1.5 equiv) and N,N-diisopropylethylamine (DIEA; 2 equivalents) in DMF, for 2 h. After washing the column 5 times with DCM,

the unreactive sites of the resin were blocked for 10 min with a blocking solution (DCM:MeOH:DIEA = 17:2:1). The Fmoc protecting group was then removed by adding 20% piperidine in DMF, followed by coupling the Fmoc-protected amino acid (2 equivalents) to the free amino group on the resin using DIEA (2 equivalents) and HBTU (1 equivalents) as the coupling agent, in DMF, for 2 h. These two steps were repeated to elongate the peptide chain. Finally, IPF–HYD was coupled to the peptide by adding DIEA (2 equivalents) and HBTU (1 equivalents), in DMF, for 2 h. The resulting IPF–HYD–GFFY hydrogelator was cleaved from the resin with washing solution (TFA: TIS: H_2O = 95:2.5:2.5; v/v/v). The crude product was purified by reversed phase HPLC, characterized by ^{1}H-NMR (**Fig. 1**) and MS, and lyophilized for further use.

Formation of the IPF–HYD–GFFY hydrogel

Briefly, a calculated weight of the IPF–HYD–GFFY hydrogelator was first suspended into phosphate buffered saline (PBS, pH 7.4) followed by the addition of a molar equivalent of Na_2CO_3, in aqueous solution (1 mM). This produced a transparent solution after heating at 80°C. The IPF–HYD–GFFY supramolecular hydrogel formed spontaneously after cooling this solution to room temperature.

Characterization of the IPF–HYD–GFFY hydrogel

Transmission electron microscope (TEM)

The microstructure of the IPF–HYD–GFFY supramolecular hydrogel was observed by TEM. The IPF–HYD–GFFY hydrogel samples were placed onto a grid and stained with 0.5 wt% phosphotungstic acid for TEM observation.

Rheology

A rheology test was performed on a TA AR2000 rheometer (New Castle, Delaware, USA) using a 40 mm cone-plate. For the dynamic time sweep, the solution of hydrogelator (0.5 mL; 0.5 wt% IPF–HYD–GFFY) was directly transferred to the rheometer and the storage modules (G') as well as loss modules (G''), as a function of time, were monitored at 37°C, with a frequency of 1 Hz and strain of 1%. Dynamic

frequency sweep was performed in the region of 0.1–100 rad/s at a strain of 1% and dynamic strain sweep was performed at a frequency of 1 Hz.

Circular dichroism (CD) spectroscopy

The CD experiment was performed using 0.01 cm quartz cells in a J-810 CD spectrometer fitted with a Peltier temperature controller. CD spectra of the supramolecular hydrogel were recorded between 180 and 260 nm with a 1 nm data pitch.

***In vitro* release experiment**

Briefly, 0.3 mL 0.5 wt% IPF–HYD–GFFY supramolecular hydrogel was pre-formed in 5 mL glass bottles and these were placed in an air bath (37°C). Next, 1 mL PBS or PBS containing 20 U/mL porcine liver esterase (pH 7.4) was added for an *in vitro* release experiment. At predetermined time points, aliquots of the release medium were collected for quantitative determination of the released IPF by high performance liquid chromatography (HPLC) and another 1 mL freshly prepared PBS (with or without esterase) was added. HPLC analysis was performed on a reversed phase C18 column (4.6×150 mm, 5μm, ZORBAX Eclipse XDB-C18). The mobile phase was composed of methanol and 0.1 mol/L monobasic potassium phosphate (adjusted to pH 3.5 with phosphoric acid) (75/25; v/v) at flow rate of 1.0 mL/min, with peaks in the eluent detected by a diode array detector (DAD) at 270 nm.

***In vitro* cytotoxicity assay**

Cytotoxicity of the IPF–HYD–GFFY supramolecular hydrogel was evaluated *in vitro* using the MTT assay. The mouse fibroblast cell line (L-929 cells) and human corneal epithelial cell line (HCEC cells) are widely used to screen chemical toxicity of compounds. Therefore, cytotoxicity of the IPF–HYD–GFFY supramolecular hydrogel for both L-929 and HCEC cells was assessed. Briefly, the L-929 or HCEC cells were seeded in a 96-wells plate at a density of 1×10^4 cells/well with 100 μL cell culture medium (RPMI1640 containing 10% FBS) and incubated overnight. Then, 100 μL medium containing different IPF–HYD–GFFY hydrogel concentrations, from

0–1000 μM, was added and, after 24 h incubation, cell viability was detected by the MTT assay.

***In vitro* anti-inflammatory activity test**

RAW264.7 cells were seeded into 35 mm culture dishes overnight, and then different amounts of IPF or IPF–HYD–GFFY supramolecular hydrogel were added for a 24 h incubation. After that, LPS was added to the cells at a final concentration of 0.5 μg/mL. After 16 h incubation, total cellular proteins were extracted using RIPA lysis buffer, separated by 10% SDS-PAGE and transferred to a PVDF membrane. The membrane was blocked in PBST with 5% skimmed milk and incubated with primary antibody overnight at 4°C. Then, the membrane was washed and incubated with the appropriate HRP-linked secondary antibody for 2 h at room temperature. Protein bands were visualized with a gel imaging system (ChemiDoc™ Imaging Systems, Bio-Rad, Hercules, CA, USA) using enhanced chemiluminescence (ECL) substrates from CWBIO (Beijing, China). Primary antibodies used were: iNOS (1:2000, Cell Signaling Technology, Beverly, MA, USA), COX-2 (1:2000, Cell Signaling Technology) and GAPDH (1:2000, Santa Cruz Biotechnology, Heidelberg, Germany). Quantitative analysis of protein levels was performed using Image J Software (National Institutes of Health, Bethesda, MD, USA). Protein levels were normalized to that of GAPDH and these values were standardized to those in samples from corresponding groups treated only with LPS, without hydrogel or IPF.

Intraocular biocompatibility test

Animals were provided by Wenzhou Medical University Laboratory Animal Center. Japanese big-eared white rabbits (weight, 2.0–2.5 kg; age, 6–8 weeks) were raised under a 12 h light and 12 h dark cycle and were supplied with food and water ad libitum. All animal experiments were approved by the Ethical Committee of Wenzhou Medical University. Six rabbits were used for the intraocular biocompatibility test. In this test, the right eyes were instilled with 50 μL 0.3 wt% IPF–HYD–GFFY supramolecular hydrogel 4 times per day for 3 successive days and the left eyes were instilled with normal saline (NS) as a control. On 1, 3 and 7 days post instillation, the

clinical signs, including hyperemia, conjunctiva edema and anterior chamber abnormalities, were monitored by a slit-lamp microscopy. In addition, fluorescein staining was used to check the integrity of the corneal epithelium. The corneal thickness, as well as corneal endothelial cell density, were measured by the Optovue iVue (Optovue Inc., Fremont, CA, USA) and a noncontact specular microscope (Topcon SP-3000P, Topcon Corporation, Tokyo, Japan), respectively, during the entire course of the experiment. Histological observation by H&E staining was employed to visualize morphological and microstructural changes in corneal tissues.

Endotoxin-induced uveitis (EIU) model and treatment

Similar to a previous study[31], the EIU model was induced by direct intravitreal injection of 100 ng LPS in the rabbit eye with a 1 mL insulin syringe (30G). Twenty-four rabbits were randomly divided into three groups (n = 8). After induction by LPS, the right eyes were immediately instilled with either 0.3 wt% IPF–HYD–GFFY supramolecular hydrogel (0.0735 wt% IPF) or the clinical product 0.1 wt% DIC eyedrops, each at 4 times per day. The left eyes were untreated and served as controls. Twenty-four hours later, the clinical signs of an inflammatory response were evaluated, in a blinded manner, by two experienced investigators. After this evaluation, the rabbits were immediately sacrificed and aqueous humor samples were collected for quantitative analysis of cell infiltration, performed using a hemocytometer under a light microscope with 100× magnification. In addition, measurement of leakage, based on tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) levels, was assessed by ELISA. The whole eyeballs were excised and immersed into 10% formalin for fixation overnight, followed by paraffin embedding. The eyes were then sectioned (5 μm) and stained with hematoxylin and eosin (H&E) for histopathological observations.

Statistical analysis

The data were subjected to one-way analysis of variance using the Origin 7.5 software package (Northampton, MA, USA). Statistical significance was defined at a probability level ($p< 0.05$).

RESULTS

Preparation and characterization of hydrogel

As a follow-up to previous studies, we successfully constructed a hydrogelator composed of IPF and GFFY peptide linked by a cleavable ester bond (**Fig. S1–S2**). We then tested its gelation ability. As shown in **Fig. 2A**, a transparent hydrogel formed spontaneously under a heating–cooling process with a minimum gelation concentration (MGC) of 0.25 wt%. The TEM image showed that the hydrogel had a typical filamentous structure with filament diameters of 15–30 nm (**Fig. 2A**). The rheological test (**Figs. 2B and S3**) indicated that the elastic network, formed by the nanofibrils, made the hydrogel relatively mechanically strong. CD spectroscopy, revealing the secondary structure of peptides packing in the fibrils of the hydrogel, exhibited a negative band at 208 nm and positive bands in the vicinity of 188 nm, corresponding to the characteristic spectrum of a β-sheet structure (**Fig. 2C**).[32] This result agreed with the finding by Yang *et al*., who demonstrated that the low molecular weight hydrogel derived from GFFY peptide, with different capping groups at the N-terminus, exhibited a typical β-sheet structure.[33]

In vitro release study

We monitored the release profile of IPF from the hydrogel in PBS (pH 7.4) and PBS containing 20 U/ml esterase at 37°C (**Fig. S4**). As shown in **Fig. 2D**, the hydrogel displayed a constant release of IPF in esterase solution during a period of 24 h, but released minimal amounts of IPF in PBS lacking esterase. This confirmed that IPF release from the hydrogel was enzymatically controlled.

In vitro cytotoxicity assay

Because cell compatibility is a very import property for new biomaterials being developed for tissue engineering and drug delivery applications, we examined cytotoxicity of the hydrogel for L-929 and HCEC cells, after a 24 h incubation. The hydrogel showed negligible cytotoxicity for both cell types at

concentrations up to 800 μM (**Fig. S5**). Thus, it might be suitable for potential drug delivery applications.

***In vitro* anti-inflammatory activity**

To evaluate the pharmacological activity of IPF in the hydrogel, we examined changes in levels of COX-2 and iNOS in RAW264.7 cells treated with 0.5 μg/mL LPS (**Fig. 3**). Incubation of cells with either native IPF or the hydrogel alone produced only trace amounts of COX-2 and iNOS, while significant levels of COX-2 and iNOS were released after treatment with 0.5 μg/mL LPS alone. In the LPS-stimulated cells, increased concentrations of IPF or hydrogel in the medium significantly supressed expression of COX-2 and iNOS, in a dose-dependent manner (**Fig. 3**). More important, the highest dose of hydrogel (500 μM) inhibited both COX-2 and iNOS expression more effectively than did the native drug. This indicated that the hydrogel had enhanced pharmacological activity over IPF alone. These observations suggested that the hydrogel we designed, without compromising the biological activity of IPF, is a potentially promising candidate for anti-inflammatory therapy.

Ocular biocompatibility

To evaluate the *in vivo* biocompatibility of the hydrogel, we examined irritation in rabbit eyes after successive treatment with normal saline and hydrogel for 3 days. The eyes treated with both normal saline and hydrogel exhibited a normal clinical appearance, with no apparent changes in corneal transparency or damage to corneal epithelium, as shown by green fluorescence staining (**Fig. 4A**). Corneal thickness and mean endothelial cell counts were not significantly changed during the entire period of the experiment (**Fig. 4B**). Consistent with the clinical observations, histological observations showed a normal corneal architecture and thickness and no inflammatory cell infiltration or edema after hydrogel treatment (**Fig. 4C**).

***In vivo* anti-inflammatory efficacy**

Beside the *in vitro* pharmacological activity and *in vivo* biocompatibility assessments, we further evaluated the *in vivo* therapeutic efficacy of the hydrogel in LPS-induced uveitis, with the clinically product diclofenac (0.1 wt% DIC) eyedrops as a reference treatment. **Fig. 5A** shows representative photographs of the eyes, at 24 h after various treatments. The figure clearly illustrates that the eyes treated with only normal saline exhibited a severe inflammatory response, with abundant exudation in the anterior chamber.[34-36] In contrast, the eyes treated with either 0.1 wt% DIC eye drops or the hydrogel formulation had significantly attenuated inflammation in the anterior chamber, with minimal exudation. Inflammatory cell counts in the aqueous humor suggested that the cellular infiltration in fellow eyes from each group were significantly elevated, to (102 ± 33) × 10^5 and (94 ± 34) × 10^5 cells/mL, respectively (**Fig. 5B**). This indicated a breakdown of the blood–aqueous barrier, enabling influx of inflammatory cells into the anterior chamber[34, 35]. In eyes treated with 0.1 wt% DIC eye drops or the hydrogel, cellular infiltration in the aqueous humor was significantly decreased to (41 ± 11) × 10^5 and (40 ± 17) × 10^5 cells/mL, respectively ($p < 0.05$). This result strongly suggested that the hydrogel exhibited a therapeutic efficacy comparable to that of the clinical product, DIC eye drops, in suppressing the inflammatory response. We next assessed IL-6 and TNF-α levels in the aqueous humor (**Figs. 5C and D**). Aqueous humor levels of the cytokines IL-6 and TNF-α in eyes treated with 0.1 wt% DIC eye drops or the hydrogel were much lower than in the fellow eyes ($p < 0.05$). Consistent with the clinical observations, histological analysis demonstrated that treatment with either 0.1 wt% DIC eye drops or the hydrogel formulation attenuated neutrophil infiltration and protein exudation in the anterior chamber, compared with in corresponding control, untreated, eyes (**Fig. S6**). Based on these results, we propose that IPF–HYD–GFFY supramolecular hydrogel is a potential therapeutic alternative for ocular inflammation.

DISCUSSION

In the past three decades, diphenylalanine (FF) has been widely investigated as one of the simplest and most common recognition motifs for molecular self-assembly[14, 20, 29, 30]. Generally, efficient hydrogelation of FF-based oligopeptides require substitution at the N-terminus with many aromatic groups. This can facilitate self-assembly of the oligopeptides to form supramolecular hydrogels, via π–π stacking and hydrophobic interactions[4, 5, 33]. More recently, Xu *et al*. introduced a variety of prodrug supramolecular hydrogels produced by conjugation of NSAIDs to the N-termini of FF-based peptides, using amide linkages. However, such conjugates had significantly lower pharmacological activity than the native drugs[3]. Herein, we introduced an enzymatically cleavable linkage, HYD, to construct a hydrogelator composed of IPF and a GFFY peptide for topical treatment of anterior uveitis (**Fig. S2**). In contrast to conventional physical encapsulation approaches, this supramolecular hydrogel not only can enable restoration of the biological activity of the drug by esterase catalyzed hydrolysis, but can also significantly alleviate concerns associated with drug–carrier issues. Results of the *in vitro* anti-inflammatory test indicated that the supramolecular hydrogel exhibited greater anti-inflammatory efficacy than the parent drug, as demonstrated by decreased COX-2 and iNOS levels. A similar phenomenon was widely demonstrated in self-assembled hydrogels containing anti-cancer drugs. In these, the self-assembled nanostructure of the drugs facilitated their uptake by cancer cells and, hence, increased their pharmacological efficacy[3, 5, 20, 29]. Topically instilled in the rabbit eye, the supramolecular hydrogel displayed satisfactory ocular biocompatibility, without causing any side effects. Taking into the consideration that rabbit eyes are considered more susceptible to foreign substances than those of humans, this suggested that the hydrogel would be suitable for further clinical applications.

To date, many therapeutic strategies, including steroidal drugs and NSAIDs, have been used for anterior uveitis[31, 35, 37, 38]. Although they have lower anti-inflammatory activities than steroidal drugs, the NSAIDs also have lower

risks of the severe side effects associated with steroids (*e.g*., cataracts and glaucoma). IPF, as one type of NSAID, has lower anti-inflammatory efficacy than DIC, which has significantly limited its usefulness for treating anterior uveitis. Among the limitations of eyedrop formulations are rapid drug clearance and poor bioavailability after topical instillation, necessitating frequent administration to maintain effective therapeutic drug concentrations. This not only produces variable drug concentration profiles, but also increases the risk of potential toxic side effects. Prodrug supramolecular hydrogels derived from drug–peptides, acting as "drug self-delivery" systems, might be alternative, more promising, ocular drug delivery systems because they would not involve drug carriers or prolonged drug retention at the corneal surface. In our study, we compared the therapeutic efficacy of 0.3 wt% IFP–HYD–GFFY hydrogel (0.0735 wt% IPF) with that of the clinical product 0.1 wt% DIC eyedrops in the uveitis model. Based on our findings, treatment with either 0.1 wt% DIC eyedrops or the supramolecular hydrogel significantly attenuated the inflammatory response (*e.g*., redness, exudation) in the anterior chamber **(Fig. 5A)**. A number of studies indicated that breakdown of the blood–aqueous humor barrier is the major cause of initiation of inflammatory symptoms in EIU[31, 34, 39]. Recruitment of leukocytes from the peripheral blood to the aqueous humor was significantly inhibited by treatment with 0.1 wt% DIC eyedrops or the hydrogel (**Fig. 5B**). Inflammatory mediators, including nitric oxide (NO), TNF-α, interleukin-1 (IL-1) and IL-6, play very important roles in progression of uveitis[38-40]. Both the 0.1 wt% DIC eyedrops and hydrogel treatments decreased IL-6 and TNF-α production dramatically (**Figs. 5C and D**). These results confirmed that the therapeutic efficacy of 0.1 wt% DIC eyedrops and the hydrogel likely resulted from downregulation of inflammatory cytokines.

In summary, we designed and synthesized a hydrogelator composed of IPF and a GFFY peptide linked by a cleavable ester bond. Under a heating–cooling protocol, a hydrogel formed spontaneously via intermolecular noncovalent interactions (e.g., π–π stacking and hydrophobic interactions). The resulting

hydrogel was cytocompatible and had excellent ocular biocompatibility *in vivo*. Although native IPF has lower anti-inflammatory efficacy than DIC, the 0.3 wt% IFP–HYD–GFFY hydrogel (0.0735 wt% IPF) had comparable therapeutic efficacy to that of the clinical product 0.1 wt% DIC eyedrops in an LPS-induced rabbit uveitis model. Both treatments acted by decreasing IL-6 and TNF-α levels in the aqueous humor. Overall, our findings identify a strategy for constructing an NSAID supramolecular hydrogel as a potential therapeutic alternative for ocular inflammation.

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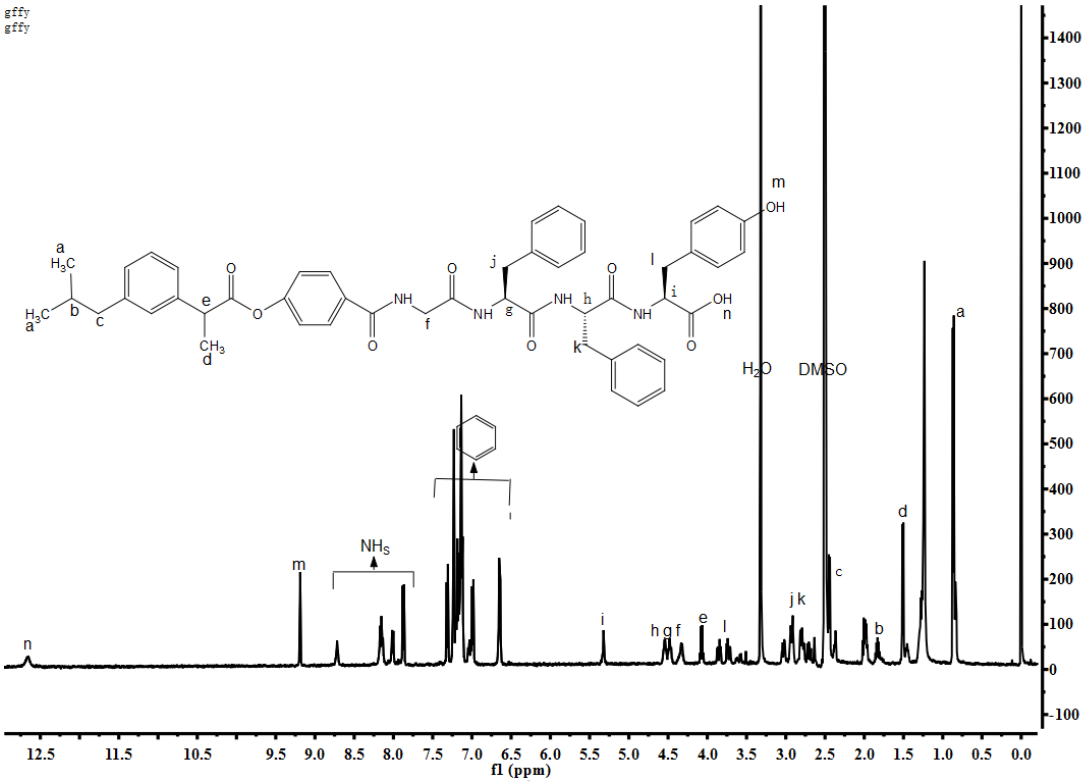

Figure 1

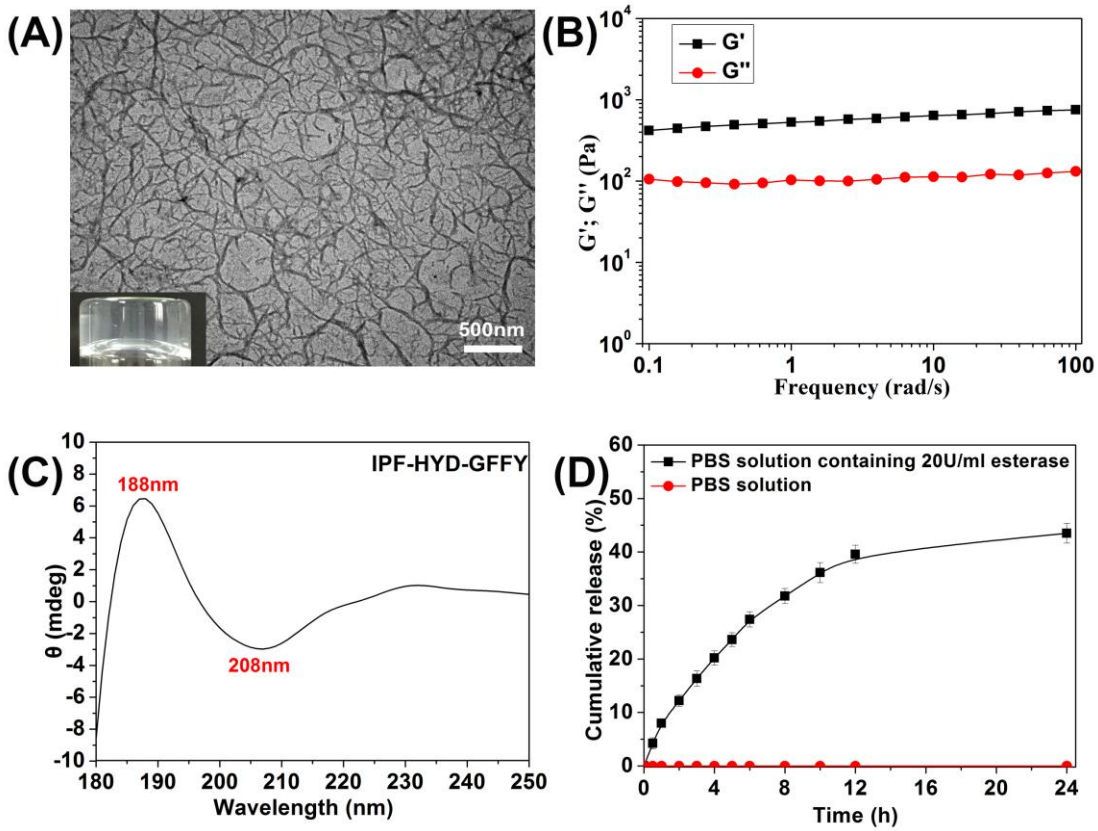

Figure 2

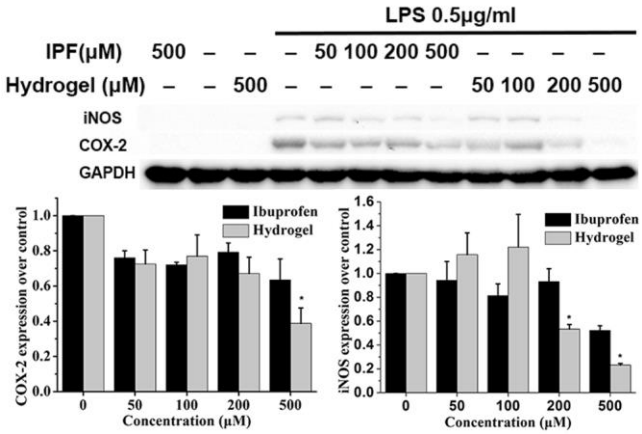

Figure 3

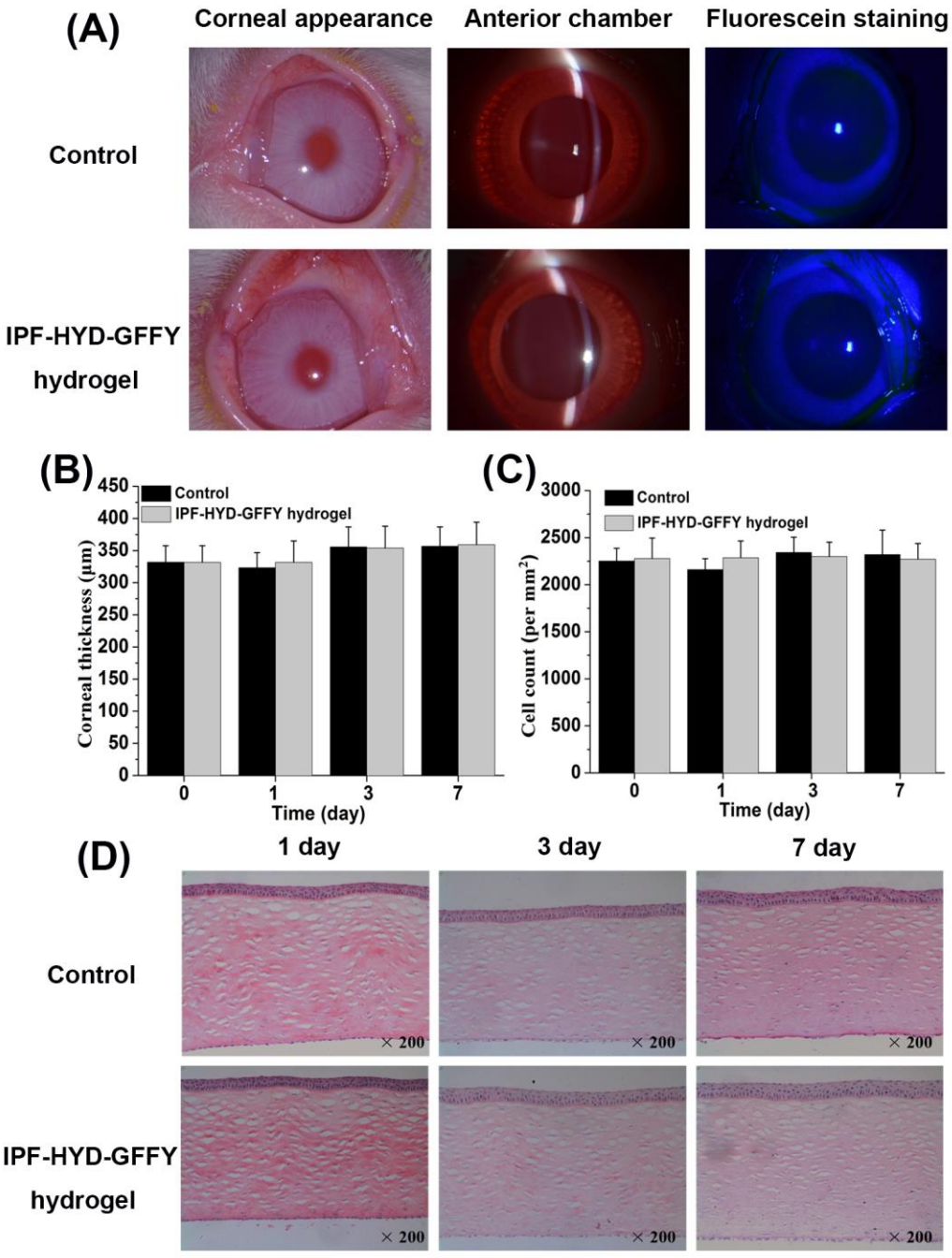
(A)
Corneal appearance
Anterior chamber
Fluorescein staining
Control
IPF-HYD-GFFY hydrogel
(B)
Control
IPF-HYD-GFFY hydrogel
Corneal thickness (μm)
Time (day)
(C)
Control
IPF-HYD-GFFY hydrogel
Cell count (per mm²)
Time (day)
(D)
1 day
3 day
7 day
Control
IPF-HYD-GFFY hydrogel
× 200

Figure 4

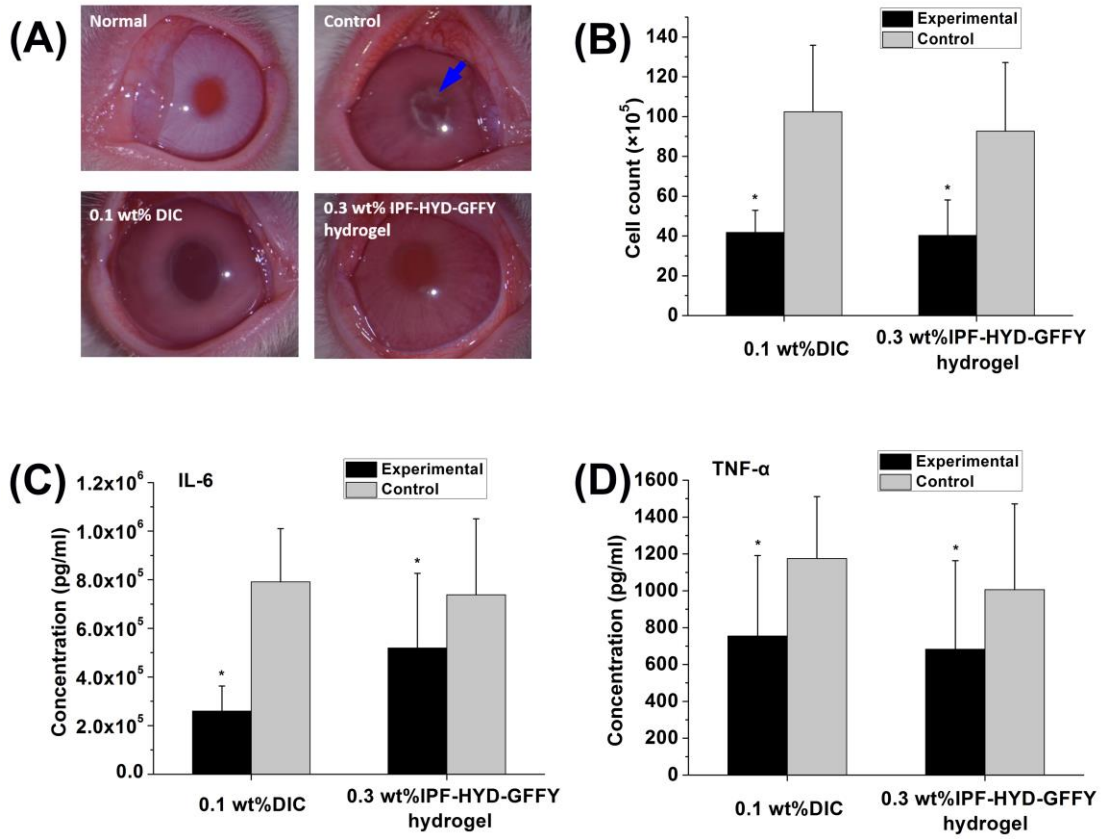

Figure 5

Figure captions

Fig. 1. ^{1}H-NMR spectrum of IPF–HYD–GFFY hydrogelator.

Fig. 2. (A) TEM image of the supramolecular hydrogel (insert image: 0.5 wt% IPF–HYD–GFFY hydrogel); **(B)** Dynamic strain sweep of the supramolecular hydrogel. 0.5 wt% IPF–HYD–GFFY hydrogelator (G': Storage modulus; G'': Loss modulus); **(C)** CD spectrum of the IPF–HYD–GFFY supramolecular hydrogel; **(D)** Cumulative release of IPF from the supramolecular hydrogel formed by 0.5 wt% IPF–HYD–GFFY hydrogelator in PBS and PBS containing 20 U/mL esterase.

Fig. 3. Effects of various concentrations of IPF and IPF–HYD–GFFY supramolecular hydrogel on LPS-induced levels of iNOS and COX-2 proteins in RAW264.7 cells.

Fig. 4. (A) Corneal appearance, anterior chamber abnormalities and fluorescence staining in eyes from the control and IPF–HYD–GFFY hydrogel groups on d 1 of treatment; **(B)** Corneal thickness in the control and IPF–HYD–GFFY hydrogel groups after 1, 3 and 7 d treatment; **(C)** Corneal endothelial cell counts in control and IPF–HYD–GFFY hydrogel groups after 1, 3 and 7 d treatment; **(D)** H&E histological analysis of corneal tissue from the control and IPF–HYD–GFFY hydrogel groups after 1, 3 and 7 d treatment.

Fig. 5. (A) Clinical signs in anterior chambers of normal eyes and eyes from the control group, 0.1 wt% DIC group and 0.3 wt% IPF–HYD–GFFY supramolecular hydrogel (0.0735 wt% IPF) group. The blue arrow indicates exudation; **(B)** Cell infiltration in control and experimental groups at 24 h post LPS induction; **(C)** Aqueous humor IL-6 levels in control and experimental groups at 24 h post LPS induction; **(D)** Aqueous humor TNF-α levels in control and experimental groups at 24 h post lipopolysaccharide induction.

Graphical Abstract

We have designed and synthesized a hydrogelator composed of ibuprofen (IPF) and GFFY peptide via a cleavable ester bond linkage. The proposed supramolecular hydrogel exhibits an enhanced anti-inflammatory activity in in RAW264.7 macrophage and displays comparable therapeutic efficacy with clinically used sodium diclofenac (DIC) eyedrops to suppress the ocular inflammation in lipopolysaccharide (LPS) induced uveitis. This work, for the first time, illustrates an effective approach for developing supramolecular assemblies as anti-inflammatory ophthalmic therapeutics for treating eye disorders.