Supplementary Information

Instant adhesion of amyloid-like nanofilms with wet surfaces

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Supplementary Methods and Figures

Materials
Human lactoferrin (HLF) was purchased from BioRuler, INC. Lysozyme from egg white, immunoglobulin G (IgG), cyclosporin A (CsA) and hyaluronic acid (HA, 1,000-1,500 kDa) were purchased from Shanghai Yuanye Science and Technology Co., Ltd. Bovine serum albumin (BSA), human lysozyme, N, N'-Methylenebisacrylamide (BIS), ammonium persulfate (APS) and agarose were purchased from Sigma-Aldrich. Human serum albumin (HSA) and Congo red were purchased from Solarbio. Coomassie brilliant blue R250 was purchased from Macklin. Tris- (2-carboxyethyl) phosphine (TCEP), acrylic acid (AA) and acrylamide (AAm) were purchased from TCI. Polyvinyl alcohol 1799 (PVA 1799) and hydroxyethyl methacrylate (HEMA) were purchased from Aladdin. HA-FITC was modified by Xi’an Qiyue Biotechnology Co. LTD. Care solution of contact lenses (CLs) was purchased from BauschLomb. CLs was EASY DAY (Hydron, USA).

Characterization
All SEM observations were carried out by a FE-SEM (SU8020, Hitachi) at an acceleration voltage of 1 kV, and all the samples were not coated with gold. AFM was used to assess the surface topography and the thickness of the nanofilm on a Dimension Icon atomic force microscope (Bruker) in ScanAsyst mode. ATR-FTIR spectra were obtained between 400 and 4000 cm\(^{-1}\) with a resolution of 1 cm\(^{-1}\) using a PE-Frontier (PerkinElmer). Thermogravimetry analysis was conducted by a thermogravimetric analyzer (Q50 TGA, TA) at 10 °C/min in nitrogen atmosphere. XPS spectra were obtained by AXIS ULTRA from Kratos Analytical Ltd., the binding energies were calibrated by setting C\(_{1s}\) peak at 284.6 eV. The aggregation of unfolded protein was performed by DLS on an Anton Paar LITESIZER 500. The UV-Vis spectra were obtained by U3900/3900H (Hitachi). Raman spectra were analyzed by Renishaw inVia-Reflex, equipped with a 785 nm excitation laser, and the scanned Raman shift ranged from 100 to 3200 cm\(^{-1}\). Anti-protein adsorption property of nanofilm was evaluated by quartz crystal microbalance with dissipation monitoring (QCM-D) of Q-Sense Explorer (Biolin Scientific). Water contact angle (WCA) was measured on an OCA 20 contact angle goniometer (Dataphysics, Germany).

Fabrication of different hydrogels. Agarose hydrogel, 1 g agarose powders were dispersed in 100 mL Milli-Q water, and heated until the solution boiling. The agarose solution was poured into sterile petri dish, cured at room temperature. PVA hydrogel, 14 g PVA powders were dispersed in 100 mL Milli-Q water, and dissolved at 100 °C for 5 h, followed by defoaming with a centrifugal mixer. The PVA solution was poured into sterile petri dish, frozen at -20 °C for 8 h, and thawed at 25 °C for 3 h. Multiple cycles of freeze-thaw (cycle number = 1-5) could be done, but unless otherwise specified, we performed three freeze-thaw cycles. PAM precursor solution (20 wt% AAm, 0.025 wt% BIS, 0.05 wt% APS) was poured into the sterile petri dish and removed oxygen by vacuum oven then incubated at 60 °C for 3 h to obtain the PAM.
hydrogel. PAA precursor solution (40 wt% AA, 0.2 wt% BIS, and 0.05 wt% APS) was poured into the glass petri dish and then incubated at 80 °C for 8 h to obtain the PAA hydrogel. PHEMA precursor solution (20 wt% HEMA, 0.025 wt% BIS, and 0.05 wt% APS) was poured into the glass petri dish and removed oxygen by vacuum oven then incubated at 45 °C for 48 h, 60 °C for 10 h, 80 °C for 12 h and 100 °C for 5 h to obtain the PHEMA hydrogel. The hydrogels were immersed in Milli-Q water to reach the equilibrium state for the following experiment.

The molecular dynamics (MD) simulation of HLF unfolding. HLF is an 80 kDa glycosylated protein of 692 amino acids, so the PACE CG force field,\(^1\) a hybrid force field that couples united-atom protein models with coarse-grained (CG) solvent and inorganic salt, is used in the simulation of HLF unfolding. The advantages of this force field are that the atomic-level force field can ensure the accuracy of protein unfolding, while the coarse-grained force field greatly accelerates the simulation process.

The X-ray diffraction structure of HLF (PDB: 2BJJ) was used for this study. The reduced form of HLF was modeled without creating of disulfide bond. The simulation box is carried out through the online modeling website CHARMM GUI (https://charmm-gui.org/). The size of the simulation box is 10.8×10.8×10.8 nm\(^3\), the system temperature is 310K, and the system pressure is a standard atmospheric pressure. In addition to HLF, there are 13128 CG water molecules (1 CG water molecule represents 4 real water molecules) in the simulated box. The net charge of the system was neutralized with addition of 11 Cl\(^-\) ions. The balance of the HLF unfolding was confirmed by checking the root mean square deviation (RMSD) after 2.8 \(\mu\)s simulation. The MD simulation is carried out using the NAMD, and snapshots are rendered by the UCSF Chimera, an extensible molecular modeling system.

The replica exchange MD (REMD) simulation of the assembly of unfolded HLF at air/water interface. HLF is comprised of a simple polypeptide chain folded into two symmetrical lobes (the N-lobe and C-lobe), which are highly homologous with one another (33-41% homology). The two lobes are connected via a hinge region containing parts of an \(\alpha\)-helix between amino acids 333 and 343 in HLF, which confers flexibility to the molecule. The polypeptide chain includes amino acids 1-332, comprising the N-lobe, and 344-692, comprising the C-lobe, and it is made up of \(\alpha\)-helix and \(\beta\)-sheet structures that create two domains within each lobe (domains I and II).\(^2\) The results of the simulation of HLF unfolding indicated that the two lobes of HLF were similarly, but independently, unfolded. In order to greatly accelerate the simulation process, the N-lobe of unfolded HLF including amino acids 1-335 was selected to simulate the assembly of unfolded HLF at air/water interface.

The N-lobe of unfolded HLF was put in a simulation box with a size of 7.75×9.07×7.23 nm\(^3\) at a distance of 1.2 nm from the boundary of the box, and then 15170 water molecules were added to fill the simulation box. After that 13 Na\(^+\) ions were added to maintain electrical neutrality of the system. In order to simulate the assembly of the protein at the air/water interface, the simulation box was extended to 11 nm on the z-axis, so that two air/water interfaces were formed at both ends of the z-
axis. The GROMOS 54a7 force field³ was used in the simulation of unfolded protein assembly at air/water interface. The simulations were carried out at constant capacity, using the GROMACS. The pressure was calculated with a molecular virial and held constant by weak coupling⁴ to an external pressure bath with a coupling time of 0.5 ps. All bond lengths were constrained using the SHAKE algorithm,⁵ allowing a time step of 2 fs. The temperature of the replica exchange is estimated on the website of http://virtualchemistry.org/remd-temperature-generator/. The number of replicas is 32, and the temperature of each replica are 333.00, 334.22, 335.45, 336.68, 337.91, 339.15, 340.39, 341.63, 342.88, 344.13, 345.39, 346.65, 347.91, 349.18, 350.45, 351.73, 353.00, 354.29, 355.57, 356.86, 358.16, 359.45, 360.76, 362.06, 363.37, 364.69, 366.01, 367.33, 368.66, 369.99, 371.32, 372.00 K. The trajectory is stored every 20 ps. The simulation time of a single replica is 25 ns, and time of all replica is 810 ns. The balance of the assembly of unfolded HLF at air/water interface was confirmed by checking the RMSD after 25 ns simulation. The snapshots are rendered by the visual molecular dynamics (VMD) program and UCSF Chimera.

The MD simulation of the adhesion of the assembly of unfolded HLF at air/water interface with PHEMA hydrogel.

The preparation of PHEMA hydrogel. 50 polymer molecules with the degree of polymerization set to 15 and 6911 water molecules were added into the box with a size of 7.75×9.07×5.00 nm³ (In order to dock with the protein box in the second step, the size of the box in the x and y directions must be the same as that in the simulation of the assembly of unfolded HLF at air/water interface). The mass fraction of the polymer is about 44%. Before the simulation, we also extended the z-axis of the simulation box to 8.0 nm to form an air/hydrogel interface. The simulation was carried out at 300 K and constant capacity. The balance of the polymer molecules on the interface was confirmed by checking the RMSD after 190 ns simulation.

The adhesion of the assembly of unfolded HLF at air/water interface with PHEMA hydrogel. The balanced hydrogel surface was docked with the unfolded HLF assembled at the air/water interface in the z-axis direction. In order to observe the interaction process of these two interfaces, a vacuum of about 5 Å is maintained between the hydrogel interface and the protein interface. A vacuum of 2 nm at both ends was left to prevent the protein and hydrogel from interacting in the non-interface direction of the z-axis after long-term simulation, and the final size of the entire simulation box is 7.75×9.07×17.00 nm³. In order to fully investigate the interaction between the protein and the hydrogel, the potential parameters for the protein and the hydrogel are taken from the CHARMM36 force. The molecular force field of the polymer is generated from the online website CHARMM GUI, and the force field of the protein is generated using GROMACS pdb2gmx. The simulation parameters are set according to the parameters of the CHARMM force field on the official website of GROMACS https://manual.gromacs.org/documentation/2018/user-guide/force-fields.html. The system temperature is 300 K. The Nose-Hoover temperature coupling is used in this simulation, and the simulation is still performed
under constant capacity conditions. The trajectory is stored every 20 ps. The balance of the adhesion was confirmed by checking the RMSD of protein and hydrogel after 80 ns simulation. The hydrogen bond was analyzed by the hbond tool of GROMACS and a typical 0.35 nm cutoff distance of X-Acceptor and 30° cutoff angle of Hydrogen-Donor-Acceptor was used to calculate the hydrogen bond. Contact residues statistics are the residues within 4.0 Å between the residues of protein and polymer of hydrogel. The snapshots are rendered by the VMD program and UCSF Chimera.

**Friction between the eyelid and the eyeball.** In an earlier study by Shani Pillar et al, the authors found a mean total upper lid force of 53.6 g in healthy subjects.\(^6\) We assume that the resistance of the upper eyelid lifting is only from the friction \((f)\) between it and the eyeball (ignoring the gravity of the upper eyelid itself), so the friction between the eyelid and the eyeball can be calculated by the following equation:

\[
f = m \times g
\]

where \(m\) is the mean total upper lid force in healthy subjects, \(g\) is gravitational acceleration.

**Anti-lacrimal proteins adsorption of functionalized CL.** Functionalized CL and commercial CL were incubated in 5 mL protein solutions, i.e., 2.7 mg/ml HLF, 0.3 mg/ml IgG, 1.3 mg/ml HSA and 2.5 mg/ml human lysozyme, respectively,\(^7\) at 37 °C for 12 h. After that, samples were rinsed gently with Milli-Q water and then subjected to ultrasonication in 3 mL polyoxyethylene ether (POE) solution (0.5% wt) at 40 kHz for 2 h. In order to eliminate the interference of functionalized PTHLF film itself, the functionalized CL ultrasonicated in 3 mL POE solution at 40 kHz for 2 h was used as a control. The mass of adsorption protein was performed by the Micro bicinchorinic acid (BCA) protein assay. 0.020 ml POE solution (i.e., solution containing the eluted proteins from the samples by POE) was pipetted into 96-well plates, respectively, and 0.200 ml the Micro BCA working reagent was added into each well. The well plate was covered and incubated at 37 °C for 2 h. After cooling the plate to room temperature, absorbance of the protein solutions in each well was measured by a BioTek synergy neo2 at 562 nm. The absorbance obtained was analyzed based on the standard curve plotted for each lacrimal protein solution. In order to improve the quality of the test conducted, 3-5 repetitions were performed for each sample.

**The average loading density of CsA in functionalized nanofilm.** Since the methanol is the good solvent for CsA, the amount of CsA contained in the functionalized nanofilm was carried out by immersing functionalized nanofilm-coated glass (1.8×1.8 cm\(^2\)) in 10 ml methanol and shaking at 37 °C with a speed of 100 rpm. During the release experiments, 2 ml released solution were pipetted out constantly in the 12 h intervals, and then added 2 ml fresh methanol into release samples. The PTHLF nanofilm-coated glass that was identical with the samples with the exception that they did not contain any drug as the blank sample. We suppose that the CsA in the functionalized nanofilm can be completely dissolved in methanol, then the equilibrium cumulative release amount is the mass of CsA encapsulated in the nanofilm. The absorbance of CsA was
measured in the wavelength range from 200 to 220 nm, with a UV-Vis spectrophotometer (U3900/3900H, Hitachi), rather than at a single wavelength to ensure that the experimental methods did not lead to drug degradation which will manifest as changes in the absorption spectrum. The experiment was carried out at least quintuplicate.

**In vitro drug release test.** *In vitro* drug release test was carried out by immersing a functionalized CL in 5 ml freshly prepared simulated tear fluid (STF, composition: NaCl 0.67 g, NaHCO₃ 0.20 g, CaCl₂·2H₂O 0.008 g and Milli-Q water to 100.0 g) and shaking at 37 °C with a speed of 100 rpm. The STF contained 0.5 wt% POE to assess sink conditions. During the release experiment, 2 ml released solution were pipetted out constantly in the scheduled time intervals, and then added 2 ml fresh STF into release samples. The CsA content was determined by the method as mentioned before.

**Animals and Cells**

Sprague-Dawley rats (SD rats) (male, 4-6 weeks, 120-140 g) were commercial rats obtained from Shanghai Jiesijie Experimental Animal Co., Ltd., China. All the experimental animals involved were kept in the Experimental Animal Center of Wenzhou Medical University in accordance with the “Code of Conduct for the Care and Use of Laboratory Animals”. This experiment was approved by the Experimental Animal Ethics Review Committee of Wenzhou Medical University, and all operations were approved by the Laboratory Animal Center. All the cells involved in this study were purchased from the American Model Culture Collection (ATCC), including human corneal epithelial cells (HCECs) and L929. After resuscitation and passage, the stable cell line obtained was cultured at 37°C in a constant humidity cell incubator with 5% CO₂.

**In vitro biological safety test.** The *in vitro* biological safety of PTHLF nanofilm and functionalized nanofilm was evaluated by cell viability/cytotoxicity test. Firstly, cell slides were modified with PTHLF nanofilm, PTHLF/HA nanofilm, and functionalized PTHLF nanofilm at different drug loading dosage of CsA (21, 22, 25, 34 and 49 μg). Then the modified cell slides were incubated with HCECs for 2, 4, 7, and 10 h, and then co-incubated with CCK-8 detection reagent for another 2-4 h. The optical density (OD) value of each group at 450 nm was measured by the BioTek synergy neo2. In addition, the modified cell slides were also incubated with L929 for 10 h to detect cell viability/cytotoxicity. The cell viability calculation formula is as follows:

\[
\text{Cell viability (\%)} = \frac{\text{OD}_{\text{treated cells}} - \text{OD}_{\text{background}}}{\text{OD}_{\text{untreated cells}} - \text{OD}_{\text{background}}} \times 100\%
\]

The functionalized nanofilms at different drug loading dosage (0, 21, 22, 25, 34 and 49 μg) were used in live-dead cell staining test. After each group was incubated with HCECs for 2, 4, 7, and 10 h, the live-dead cell staining reagent was added and co-incubated for another 10-15 min. Then the cell culture fluid was washed away, and the survival status of the cells was directly observed by fluorescence microscope.
**In vivo safety test.** The *in vivo* biocompatibility of the PTHLF nanofilm was carried out on the eyes of healthy SD rats. Recent studies have found that long-term wearing of commercial CL will increase the occurrence of corneal hypoxia, dryness, infection, etc.. In clinical application, the wearing time of CL often does not exceed 8 h per day. During *in vivo* experiment, the wearing time of CL was 6 h per day. Combined with the results of cytotoxicity test, we selected three groups of functionalized CL with higher drug loading dosage (25, 34, 49 μg) for *in vivo* safety experiment. The pristine CL was used to be the control group. SD rats with healthy cornea were selected to wear different groups of CL, and there were 6 SD rats in each group. The ocular surface of SD rats was observed with a slit lamp and the corneal epithelium was observed by staining with fluorescein sodium at 0, 1, 3, 5, and 7 days after various treatments. Scoring was performed according to the clinical ocular surface inflammation scoring standard (Table S2). After 7 days of intervention, the SD rats were euthanized, and the removed eyeballs were immersed into fixative solution for 24 h, after that cornea was separated from eyeball. The cornea was dehydrated, and then embedded in paraffin. Then corneal tissue sections were prepared for Hematoxylin-eosin (H&E) staining and terminal deoxynucleotidyl transferase (TdT) deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) staining to observe the pathological changes of the cornea.

The CL worn by SD rats was 5 mm in diameter. The sterilized CL was cut into 5 mm diameter discs with an autoclaved corneal trephine (5 mm diameter).

**The animal model with dry eye syndrome (DES).** SD rats around 4 weeks old were reared adaptively in constant temperature and humidity environment for 2 weeks, then ocular surfaces of SD rats were observed with slit lamp. The SD rats with healthy ocular surface were selected for creation DES model. Firstly, 5 μl 0.2 wt% benzalkonium chloride was dropped onto the ocular surface of the SD rats twice a day with an interval of 10 h. After 14 days, the morphological changes of corneal epithelium were observed by staining with fluorescein sodium. Scoring was performed based on internationally recognized standards (Table S3). SD rats with a score greater than 8 was considered to be a successful DES model.

**In vivo intervention test.** DES SD rats wore different CL, including PTHLF/HA nanofilm-coated CL group and functionalized CL with 21 μg CsA group, for 6 h a day. In addition, DES rats without any treatment and with the treatment of Restasis (0.05 wt% CsA, 5 μl each time, twice a day) groups were used as the control groups. Slit lamp bright field observation, corneal fluorescein sodium staining, ocular surface inflammation score and tear secretion volume detection were performed on the ocular surface of the rats in different groups after 0, 1, 3, 5, and 7 days of intervention. Tear film breakup time (TBUT) detection were performed on the ocular surface of the rats in different groups after 7 days of intervention. After 7 days of intervention, the SD rats were euthanized, and the eyeballs were removed. Some of eyeballs were immersed into the fixative solution for 24 h, and then the cornea and conjunctiva were separated from the eyeball. The cornea and conjunctiva were dehydrated, and then embedded in paraffin, respectively. Then cornea tissue sections with a thickness of 5 μm were
prepared for H&E staining and TUNEL staining to observe the pathological changes of cornea. The conjunctiva tissue sections were prepared for H&E staining to observe the changes of goblet cells. The corneas separated from other eyeballs were stored at -80 °C and used to analyze the expression of inflammatory factors by RT-qPCR.

**Tear content measurement.** The tear volume of SD rats was measured by the modified tear test strip. Firstly, the fluorescein sodium tear test strip (Tianjin JingMing New Technological Development Co., Ltd) was cut along the midline and split it into two test strips with a width of 2-3 mm. The anterior segment of the test strip turned over along the fluorescein sodium indicator line, and then the test strip was placed into the outer side of the lower eyelid about 1/3 of SD rats. Then the test strip was taken out after 20 s, and the distance of the fluorescein sodium moved forward was recorded. Each eye was tested for 3 times.

**TBUT detection.** 1 μl 1 wt% fluorescein sodium was dropped into the lower eyelids of SD rats. The upper and lower eyelids of SD rats was gently closed three times. Then the time when the first dark spot appears on the cornea, which is the tear film rupture time, was observed by the cobalt blue light of slit lamp (Chongqing Kanghua Ruiming Technology Co., Ltd). Each eye was tested for 3 times.

**H&E staining.** H&E staining made the morphology of the cell/tissue clearly visible. The sections were stained with hematoxylin and eosin according to standard H&E staining method. The changes of corneal epithelium and conjunctival goblet cells were observed by the microscope.

**TUNEL staining.** TUNEL staining was a method that can detect the apoptotic state of cells by marking the broken DNA of apoptotic cells. The sections were stained according to the manual of Apoptosis Detection Kit (Shanghai YEASEN Biotech Co. Ltd). A positive control group was set at the same time. The sections were observed by the fluorescence microscope (Leica DM4B), the apoptotic cells will be marked with a green fluorescent.

**RT-qPCR analysis.** RT-qPCR analysis technology was performed to verify the expression of inflammatory factors in corneal tissue, including IL-1β and IL-6. According to the manufacturer’s recommendations, total RNA was extracted from a single whole cornea, and quantified by spectrophotometry (260 nm). Then, 1 μg RNA was reverse transcribed into cDNA using PrimeScriptTM RT reagent Kit (TaKaRa, Japan). After that, real time-PCR analysis of IL-1β and IL-6 were performed by SYBR Green PCR Master Mix (TaKaRa, Japan). The primers were listed in Table S4. Assays were performed in duplicate and repeated three times using different samples from different experiments. The final qPCR data of IL-1β and IL-6 were processed using 2ΔΔct method against GAPDH for normalization.
The improved bioavailability of CsA by functionalized CL. According to the inflammation score (Figure 6C), fluorescein sodium staining score (Figure 6D) and Schirmer tear test (Figure 6E) of the DES model rats with intervention treatment, the therapeutic efficacy of functionalized CL for 5 days is almost equivalent to that of Restasis for 7 days. The improved bioavailability of CsA by functionalized CL calculation formula is as follows:

\[
\text{The improved bioavailability (\%) } = 1 - \frac{m_{\text{Functionalized CL}}}{m_{\text{Restasis}}}
\]

where \( m_{\text{Functionalized CL}} \) is the mass of CsA about functionalized CL for 5 days of intervention, \( m_{\text{Restasis}} \) is the mass of CsA about control group Restasis for 7 days of intervention.

During in vivo intervention experiment, the mass of CsA about control group of Restasis for 7 days (0.05% CsA, 5 μl each time and twice a day) is 35 μg. A functionalized CL containing 21 μg of CsA was selected for in vivo intervention experiment, which drug loading density is 6.4774 μg/cm² (Figure 4E). The CL worn by SD-rats was about 5 mm in diameter \( (S = 0.0625\pi \text{ cm}^2) \) with 1.2712 μg of CsA. The mass of CsA about functionalized CL for 5 days of intervention is 6.3560 μg.
Figure S1: The FE-SEM images of the PTHLF nanofilms formed at air/water interface by using TCEP solutions at different pH. The nanofilm prepared by incubating the mixture of 7 mg/ml of HLF (in Milli-Q water) and 50 mM TCEP (in Milli-Q water) in which pH is (A) 5.05, (B) 6.06, (C) 6.98, (D) 7.98, (E) 9.11, (F) 10.02, (G) 11.12 and (H) 12.03 for 12 h at room temperature.

Figure S2: (A) The DLS size distribution of the protein aggregate formed in the mixture of HLF and TCEP aqueous solution at different HLF concentration as a function of reaction time. (B) The zeta potential of the protein aggregate formed in the mixture of HLF and TCEP aqueous solution as a function of reaction time.
**Figure S3:** The killing efficiency of native HLF and PTHLF toward *S. aureus*, with the use of blank (*S. aureus* suspension in PBS buffer without the addition of any other chemical reagents) and TCEP solution (*S. aureus* suspension in PBS buffer with TCEP added) as the control groups.

**Figure S4:** AFM characterization of the PTHLF nanofilm coated on silicon wafer at different incubation time. AFM images of the nanofilm formed by incubating the mixture of 7 mg/ml HLF and 50 mM TCEP (pH=6.98) for (A) 1, (B) 2, (C) 3, (D) 4, (E) 5, (F) 6, (G) 7 h at room temperature. (H) The relationship between the nanofilm root mean square (RMS) and incubation time. (I) Thickness of the film controlled by the incubation time.
Figure S5: AFM characterization of the PTHLF nanofilm coated on silicon wafer at different pH of TCEP. AFM images of the nanofilm fabricated by incubating the mixture of 7 mg/ml HLF and 50 mM TCEP which pH is (A) 6.06, (B) 6.98, (C) 7.98, (D) 9.11 for 2 h at room temperature. (E) The relationship between the nanofilm thickness and pH of TCEP. (F) The relationship between the nanofilm root mean square (RMS) and pH of TCEP.

Figure S6: (A) The change of the ANS fluorescence intensity at 475 nm as function of the reaction time at different pH during the phase transition of HLF. (B) The change of the ThT fluorescence intensity at 484 nm as function of the reaction time at different pH during the phase transition of HLF. (C) Fluorescence spectra of the ThT solution and the film dyed by ThT.
Figure S7: (A) Deconvolution of the ATR-FTIR amide I peak of the native HLF and PTHLF film. (B) Far-UV CD spectra of the native HLF and PTHLF film.

Figure S8: (A) XPS characterization of the PTHLF nano film. (B) Deconvolution of high-resolution C1s XPS spectrum of the film surface. The high-resolution XPS of C1s indicated a variety of chemical structures existing on the nanofilm surface, mainly including aliphatic carbon (C-H/C-C), amines (C-N), ethers/hydroxyls (C-O), thiols (C-S) and amides (O=C-N).
Figure S9: (A) Deconvolution analysis of the Raman spectra of native HLF. (B) Deconvolution analysis of the Raman spectra of the PTHLF. (C) Propensity diagram of the typical amino acid residues in the native HLF and PTHLF films. The peak position for each kind of structure was shown in Table S1.

Figure S10: Deconvolution of high-resolution XPS C1s of native HLF.
Figure S11: The MD simulation of HLF unfolding triggered by the breakage of S-S bonds. Hydrophobicity according to Moon et al.\textsuperscript{12} (mfHydrophobicity) of the (A) native HLF surface and (B) unfolded HLF surface after MD simulation for 2.8 \( \mu \)s. Colours range from blue to orange red represents the most hydrophilic to the most hydrophobic. (C) The root mean square deviation (RMSD) of HLF without S-S bonds from the initial structure as a function of time for the simulations. (D) The change of solvent-accessible surface areas (SASA) of 20 amino acid residues in the unfolded HLF after MD simulation for 2.8 \( \mu \)s compared to native HLF.

**Supplementary Discussion:** The RMSD refers to the minimum RMSD between the simulated structure and native HLF (2BJJ). Its definition is as the formula below:

\[ RMSD = \left[ \frac{1}{N} \sum_{i=1}^{N} m_i \left( r_i(t_1) - r_i(t_2) \right)^2 \right]^{1/2} \]

where \( \sum_{i=1}^{N} m_i \), \( r_i(t) \) is the location of atom \( i \) at \( t \) moment. RMSD is usually used to analyze the conformational differences of proteins under different environmental conditions. The small fluctuation of RMSD means that the simulation system is in equilibrium and the protein structure is in a stable state, which state was used for further analysis.
Figure S12: The REMD simulation of the assembly of unfolded HLF at air/water interface. The representative snapshots of unfolded HLF (A) before and (B) after MD simulation. (C) The RMSD of the unfolded HLF from the initial structure (i.e., the unfolded HLF assembled at the air/water interface at 0 ns) as a function of time for the simulations of assembly at air/water interface. (D) The conformational change after the unfolded HLF assembled at air/water interface. (E) and (F) The change of SASA of hydrophobic and hydrophilic amino acid residues in the unfolded HLF as a function of time for the simulation. (G) The surface of the unfolded HLF at air/water interface. Colours range from blue to orange red represents the most hydrophilic to the most hydrophobic. (H) The contents of each amino acid on the unfolded HLF surface at air/water interface.

Supplementary Discussion: Based on the SASA of hydrophobic and hydrophilic amino acid residues in the unfolded HLF at 0 ns, the hydrophobic amino acids on the unfolded HLF surface in the water was 39.60% (E). While after the unfolded protein assembly at air/water interface, the hydrophobic amino acids on the unfolded HLF surface at air/water interface was increased to 46.53% (F), which is consistent with the XPS spectroscopy results of the C1s signal of the aliphatic carbon (C-H/C-C) groups. These results mean that the hydrophobic residues in the unfolded HLF were tended to expose at the air/water interface.
Figure S13: The representative snapshots of the MD simulation of PTHLF (bottom) adhesion on PHEMA hydrogel surface (top) at (A) 0 ns, (B) 0.02 ns and (C) 80 ns. (D) The RMSD of the unfolded HLF from the initial structure (i.e., the unfolded HLF assembled at the air/water interface at 0 ns) as a function of simulation time. (E) The van der Waals force (hydrophobic) and electrostatic interaction energy of PTHLF interacting with PHEMA hydrogel as a function of time. (F) The number of hydrogen bonds formed between PTHLF and PHEMA hydrogel. (G) The number of residues contacting with PHEMA hydrogel at 80 ns.
Figure S14: The surface topography and cross-section of the freeze-dried nanofilm-coated (A) PHEMA, (B) PAA, (C) PVA, (D) AG and (E) PAM hydrogels.

**Supplementary Discussion:** As shown in FE-SEM images, the nanofilm stably adhered on all the hydrogels regardless of the roughness of the hydrogel surface, which shows that the nanofilm is adaptive at microscopic level during the adhesive process so that a conformal coating could be formed on hydrogels with different surface morphology.
Figure S15: The ATR-FTIR spectra of the PTHLF nanofilm and different hydrogels (A) PHEMA, (B) PAA, (C) PVA, (D) AG and (E) PAM without and with modification by PTHLF nanofilm. All samples were freeze-dried prior to ATR-FTIR experiment. The red and blue curves in E3 have been enlarged in the same proportion.

Supplementary Discussion: The PTHLF nanofilm consists of typical hydrogen-donor (N-H and O-H) and hydrogen-acceptor (C=O) functionalities and all of the above hydrogels have the hydrogen-donor (O-H or N-H), and some also have hydrogen acceptor (C=O). This result indicated that it was very easy to form hydrogen bonding between PTHLF nanofilm and hydrogel. All the FTIR spectra of PTHLF nanofilm-
coated hydrogels showed amide II and I bands for PTHLF nanofilm in the 1500-1580 cm\(^{-1}\) (N-H bending and C-N stretching modes) and 1600-1700 cm\(^{-1}\) region (mainly of the C=O stretching vibration and minor contribution of N-H stretching), respectively, which reflected the successful coating of PTHLF nanofilm on hydrogels (A1-E1; A2-E2). Moreover, the peak of the -OH/-NH\(_2\) groups (3200-3600 cm\(^{-1}\)) of the PTHLF nanofilm-coated hydrogels (A3-E3) was significantly red-shifted compared to pristine hydrogels. This result implied that hydrogen bonding was formed between the PTHLF nanofilm and hydrogels, which then averaged the electron cloud density to result in a redshift of the bands of -OH/-NH\(_2\) groups from the PTHLF nanofilm and hydrogels.

**Figure S16:** (A) Photographs of PTHLF nanofilm-coated different hydrogels. (B) Photographs of PTHLF nanofilm-coated different hydrogels immersed into Milli-Q water. (C) The water contact angle of different hydrogels. (D) The water contact angle of PTHLF nanofilm-coated different hydrogels.

**Supplementary Method:** Before modifying hydrogels, the nanofilm was dyed by 0.1 wt% Congo red with the method in Figure S17. Upon the adhesion completed, the nanofilm-coated hydrogels suffered from rinsing with Milli-Q water and the nanofilm still stably adhered onto hydrogels, which can be proven in Movies S2-S5.
Figure S17: (A) The PTHLF nanofilm floating on Milli-Q water. (B) The PTHLF nanofilm (stained by Congo red) floating on Milli-Q water. (C) The PTHLF nanofilm (stained by Coomassie brilliant blue) floating on Milli-Q water.

Supplementary Method: The nanofilm, without any intrinsic color, displayed excellent optical transparency as high as ~100% between 300 and 800 nm, thereby can be a unique class of stealth coating. In order to make the process of modification more intuitive, the floating nanofilm was dyed by Congo red or Coomassie brilliant blue. For this purpose, the nanofilm was transferred on 0.1 wt% Congo red or Coomassie brilliant blue aqueous solution surface at room temperature for 3 min, then transferred on Milli-Q water surface for rinsing the physically adsorbed dyes. After that, the nanofilm was transferred on Milli-Q water surface for following inspection. The PTHLF nanofilm was fabricated by incubating the mixture of HLF solution (7 mg/ml in Milli-Q water) and TCEP solution (50 mM in Milli-Q water, pH=7) in equal volume 1:1 for 2 h at room temperature.

Figure S18: The thermogravimetric (TG) characterization of the PTHLF nanofilm. The TG curve indicated that the degradation temperature of film was around 200 °C under nitrogen atmosphere. Before TG test, the nanofilm was freeze-dried for 24 h.
Figure S19: (A) The schematic cartoon to show the stability test of nanofilm-coated hydrogel under extreme conditions. The photographs of PTHLF nanofilm-coated hydrogel suffering from a variety of extreme conditions such as (B) various organic solvents, (C) extreme pH, (D) ultrasonication at 40 kHz for 30 min in Milli-Q water and (E) ultrasonication at 40 kHz for 30 min in 0.5 wt% polyoxyethylene ether (POE) aqueous solution. The nanofilm in (E) was dyed by 0.1 wt% Congo red for 3 min before modifying hydrogel.
Figure S20: Measurement method of peeling strength between the nanofilm (after Congo red staining) and hydrogel. (A) The DCAT 21 apparatus for the peeling strength measurement. (B) The photo of copper plate with a contact (peeling) area being controlled at 0.25 cm². (C) The typical photo of copper plate with the nanofilm peeled from the nanofilm-coated hydrogel (inset: the peeled nanofilm on scotch tapes). The typical photo of the nanofilm-coated hydrogel before (D) and after (E) peeling the nanofilm from the nanofilm-coated hydrogel. (F) Microscopic image of the nanofilm-coated hydrogel after peeling the nanofilm from the nanofilm-coated hydrogel.
Figure S21: The typical macroscopic photo (A) and microscopic image (B) of the nanofilm-coated hydrogel after the nanofilm was peeled from the nanofilm-coated hydrogel. The typical macroscopic fluorescent photo (C) and microscopic fluorescent image (D) of the nanofilm-coated hydrogel after the nanofilm was peeled from nanofilm-coated hydrogel. The typical macroscopic photo (E) and microscopic image (F) of the peeled nanofilm on scotch tapes. The typical macroscopic fluorescent photo (G) and microscopic fluorescent image (H) of the peeled nanofilm on scotch tapes.

Supplementary Discussion: In order to ensure the accuracy of the peel strength and verify that there was no tissue adhesive Histoacryl penetrate into the hydrogel to interact with the hydrogel during the peeling experiment, Histoacryl mixed with 0.1% of ThT dye (dissolved in ethanol) was used to interact with the nanofilm-coated hydrogel. After the peeling experiment was completed, macroscopic fluorescent imaging (the sample under 488 nm laser irradiation) and laser scanning confocal microscopy (LSCM) were performed on the nanofilm-coated hydrogel and the peeled nanofilm. The result showed that there was no fluorescence of the nanofilm-coated hydrogel after the peeling test, indicating that the Histoacryl did not penetrate into the hydrogel and but just interacted with the nanofilm during the peeling test. So, the peeling strength measured by the method shown in Figure S20 is the adhesion strength between the hydrogel and the nanofilm.
Figure S22: Representative curves of peeling force after PTHLF nanofilm adhering onto AG hydrogel for different time (A) 2 min, (B) 10 min, (C) 30 min, (D) 1 h, (E) 5 h, (F) 24 h, (G) 48 h, (H) 72 h and (I) 120 h.

Figure S23: Representative curves of peeling force between PTHLF nanofilm and (A) PHEMA, (B) PAA, (C) PVA, (D) AG and (E) PAM hydrogels. The measurement was carried out after the PTHLF nanofilm adhered onto hydrogels for 24 h.
**Figure S24:** The stability of the nanofilm-coated PHEMA hydrogel. (A) The schematic diagram of setup for controlling the flow of water. (B) The image of the nanofilm-coated PHEMA hydrogel. (C) The photograph of the hydrogel fixed on Petri dish. (D) The photograph of the nanofilm-coated PHEMA hydrogel flushed with water flow. The images of the nanofilm-coated PHEMA hydrogel flushed with water flow for (E) 6, (F) 12, (G) 18, (H) 24 h. (I) There is no obvious change of water contact angle of the nanofilm-coated PHEMA before and after flushing with water flow. For easy visualization, the nanofilm was dyed with Congo red.

**Figure S25:** The images of the nanofilm-coated hydrogels before (A) and after (B) freeze-drying.

**Supplementary Discussion:** In order to further elucidate the adhesion mechanism of the nanofilm to hydrogel, the nanofilm-coated hydrogels were freeze-dried. The nanofilm stably adhered to different hydrogels at the macroscopic level after freeze-drying, which indicated that the nanofilm penetrated the hydration layer to interact with the polymer network, rather than bonded with a monolayer of water molecules. For easy visualization, the nanofilm was dyed with Congo red.
Figure S26: The equilibrium water content in different hydrogels. The hydrogels were immersed into water to reach the equilibrium state and followed by removing the free water of hydrogel surface with a filter paper and then weighing the initial weight ($W_0$) of hydrogel. After that, the hydrogels were dried in an oven at 65 °C for 4 h until the weight ($W_t$) did not change ($n=5$). The equilibrium water content of hydrogel was calculated by the formula as following:

$$\text{water content} = \frac{W_0 - W_t}{W_0} \times 100\%$$

Supplementary Discussion: The hydrogel is an aggregate of water and polymers, with the water being the majority of constituent, and the polymers being the minority constituent. Some or all of hydrogel form a sparse and strong network, with the mesh size at the order of nanometer/micrometer being much larger than a water molecule. The water molecules in the hydrogel are in the liquid state, changing neighbors readily, and transmitting force negligibly, so strong adhesion of the coating to hydrogel must rely on the minority constituent of the hydrogel, which is the primary polymer network.
Figure S27: The adhesion of the phase-transitioned protein nanofilms to AG hydrogel. (A) The contact angle of phase-transitioned protein nanofilm-coated AG hydrogel. (B) Peeling strength between AG hydrogel and phase-transitioned protein nanofilm. Representative curves of peeling force between (C) PTHLF, (D) PTB and (E) PTL nanofilm and AG hydrogel. The measurement was carried out after the nanofilm adhering onto AG hydrogel for 24 h.
Figure S28: Photographs of various tissues including (A) skin (pig) (B) stomach (chicken) (C) muscle (chicken) coated with PTHLF nanofilm after immersion into Milli-Q water. (D) The change of water contact angle of PTHLF nanofilm-coated various tissues. (E) The peeling strength between various tissues and the nanofilm. Values represent the mean and standard deviation (n=3-7).

Figure S29: Representative curves of peeling force between PTHLF nanofilm and various tissues (A) skin, (B) stomach and (C) muscle. The measurement was carried out after the PTHLF nanofilm adhering onto tissues for 24 h.
Figure S30: Young’s moduli of hydrogels without or with modification by the PTHLF nanofilm.

Figure S31: The stability of PTHLF nanofilm-coated CL evaluated by immersing it into 10 ml care solution for different time. The nanofilm still stably adhered onto CL after one year storage in care solution. The CL care solution was changed once per day.
**Figure S32:** Nitrogen adsorption-desorption isotherms (A) and corresponding pore size distribution (B) of the functionalized PTHLF nanofilm. (C) The molecular structure of CsA depicted by Material studio. The unit of the molecule size shown in the image is angstrom (Å).

**Figure S33:** Moisture retention of the functionalized PTHLF nanofilm-coated AG hydrogel. The introduction of HA into the functionalized PTHLF nanofilm attenuated water loss during the dehydration process.

**Supplementary Method:** The dehydration tests were carried out at room temperature with low humidity (23 °C and 25 % humidity) for 7 h.
Figure S34: The LSCM images of functionalized nanofilm at different CsA loading dosage (A) 0, (B) 13, (C) 21, (D) 22, (E) 25 and (F) 34 μg after culturing in the *S. aureus* liquid for 6 h. Clean surfaces without significant bacteria adhesion are not dependent on the CsA dosage, but contributed by the introduction of HA into the functionalized PTHLF nanofilm that attenuated non-specific adsorption of microbes on the nanofilm.

Supplementary Method: The strain was cultured on a 37 °C constant temperature shaker (60 rpm) for 12 h after dilution in the MHB (24 g/L, dissolved in Milli-Q water, 121 °C high pressure 20 min to sterilization). The original bacterial solution was diluted with the MHB to a concentration of $10^5$ cell/mL, and then the samples were immersed into the *S. aureus* solution. The samples were washed with Milli-Q water after culturing at 37 °C for 6 h, and then stained with SYTO® 9 for the observation through LSCM.
The anti-nonspecific adsorption of proteins by the functionalized nanofilm. The representative QCM curves of functionalized nanofilm with different average loading density of HA (A) 0.33, (B) 0.40, (C) 0.55 μg/cm² for adsorption of lacrimal protein such as HLF, human IgG, HSA and human lysozyme. (D) The protein adsorption mass of the functionalized PTHLF nanofilm with different average loading density of HA.

**Supplementary Method:** The functionalized PTHLF nanofilm-coated QCM Au chip was prepared by the same method as the PTHLF nanofilm modifying the hydrogels. The concentration of HLF, IgG, HSA and lysozyme are 2.7 mg/ml, 0.3 mg/ml, 1.3 mg/ml and 2.5 mg/ml, respectively.
Figure S36: The FE-SEM images of functionalized nanofilms at different CsA loading dosage (A) 0, (B) 13, (C) 21, (D) 22, (E) 25, (F) 34 and (G) 49 μg.
Figure S37: The comparison between the encapsulation of CsA-FITC in the PTHLF film and the simple adsorption of CsA-FITC on the PTHLF film surface. The microscopic fluorescent (A, C, E) and optical (B, D, F) images for the PTHLF film without any entrapment or surface adsorption of CsA-FITC (A, B), simply adsorbing CsA-FITC (C, D) and entrapping CsA-FITC (E, F) in the nanofilm. (G) The corresponding fluorescence intensity curves for the pristine PTHLF film, the film simply adsorbing CsA-FITC on its surface and the film entrapping CsA-FITC. The PTHLF film was obtained by incubating the mixture of HLF solution (7 mg/ml in Milli-Q water) and TCEP solution (50 mM in Milli-Q water) in equal volume 1:1 for 2 h at room temperature. The simple adsorption of CsA-FITC on the film surface was achieved by immersion of the PTHLF film in CsA-FITC solution (0.375 mg/ml in aqueous ethanol, 50% by volume) for 12 h. The PTHLF film with entrapment of CsA-FITC was obtained by incubating the mixture of HLF solution (7 mg/ml in Milli-Q water), CsA-FITC solution (7 mg/ml in aqueous ethanol, 50% by volume) and TCEP solution (50 mM in Milli-Q water) in volume 10:10:1 for 12 h at room temperature.
Figure S38: The comparison between the encapsulation of HA-FITC in the PTHLF film and the simple adsorption of HA-FITC on the PTHLF film surface. The microscopic fluorescent (A, C, E) and optical (B, D, F) images for the PTHLF film without any entrapment or surface adsorption of HA-FITC (A, B), simply adsorbing HA-FITC (C, D) and entrapping HA-FITC (E, F) in the nanofilm. (G) The corresponding fluorescence intensity curves for the pristine PTHLF film, the film simply adsorbing HA-FITC on its surface and the film entrapping HA-FITC. The PTHLF film was obtained by incubating the mixture of HLF solution (7 mg/ml in Milli-Q water) and TCEP solution (50 mM in Milli-Q water) in equal volume 1:1 for 2 h at room temperature. The simple adsorption of HA-FITC on the film surface was achieved by the immersion of the PTHLF film in HA-FITC solution (3 mg/ml in Milli-Q water) for 12 h. The PTHLF film with entrapment of HA-FITC was obtained by incubating the mixture of HLF solution (10.5 mg/ml in Milli-Q water), HA-FITC solution (9 mg/ml in Milli-Q water) and TCEP solution (75 mM in Milli-Q water) in equal volume 1:1:1 for 12 h at room temperature.
Figure S39: The loading density of HA in PTHLF nanofilm. (A) The standard curve of fluorescence intensity in function with concentration of HA-FITC. (B) The fluorescence curve of functionalized nanofilm at different initial feeding concentration of HA-FITC. (C) The average area density of HA in the PTHLF nanofilm at different initial feeding concentration of HA-FITC.

Supplementary Method: Before the fluorescence test, ten layers of HA-FITC-loaded nanofilm (1.8×1.8 cm\(^2\)) were dissolved by 1 mL of 1 wt% SDS aqueous solution. The HA-FITC also dissolved by 1 wt% SDS aqueous solution when drawing the standard curve. The average density of HA was calculated by the followed formulation:

\[
\rho = \frac{F_{SDS} - 139.4833}{61.2647} \times \frac{V_{SDS}}{S_{film}}
\]

where \(\rho\) is the average density of HA, \(F_{SDS}\) is the fluorescence intensity of HA-FITC loaded nanofilm dissolved in the SDS solution, \(V_{SDS}\) is the volume of the SDS solution, \(S_{film}\) is the area of the nanofilm dissolved in the SDS solution.

Figure S40: The loading mass of CsA in commercial CL (Hydron EASY DAY) and functionalized CL with different feeding concentration of CsA.

Supplementary Method: The encapsulation of CsA in commercial CL by immersion of CL in 1 ml of CsA solution, which containing 3 mg HA, with different concentration (controlled by different volume of 7.5 mg/ml CsA dissolved in aqueous ethanol, 50% by volume) for 12 h. The modification of CL was fabricated by adhesion of functionalized nanofilm onto one side or two sides of CL. The method to prepare the functionalized nanofilm was in Materials and Methods section in the manuscript.
**Figure S41:** The fitted curve of cumulative percentage release of CsA from the functionalized PTHLF nanofilm with different encapsulated mass of CsA (A) 49, (B) 34, (C) 25, (D) 22, (E) 21, and (F) 13 μg at different time. Values represent the mean and standard deviation (n=5).

**Supplementary Discussion:** The cumulative release of CsA from the functionalized PTHLF nanofilm was characterized by an initial burst release in 0.5 h period, which was followed by a slow, near-linear release during next 23.5 h period. Linear release period can be divided into two stages with 10 h as the node, the release rate of CsA after 10 h (the blue fitting line) is slower than the release rate before 10 h (the red fitting line). A small percentage of CsA was released from functionalized nanofilm in an initial burst, which then followed by slow and stable release of CsA over a long period of time, and maintained an appropriate concentration of CsA for 24 h. This feature is basically in line with the ideal drug delivery system that need to deliver a relatively constant dose of drug during the wearing of therapeutic contact lenses. However, when the mass of CsA encapsulated in the functionalized nanofilm was too small (such as 13 μg), the CsA released by the functionalized nanomembrane within 4 h is almost zero (F), which cannot achieve clinical treatment effects.
Figure S42: The cumulative amount of CsA released from functionalized CL in 8 h which is the current recommended time of wearing CL.

Supplementary Method: Topical CsA eye drops (Restasis) is currently the only pharmacologic treatment approved by the US FDA specifically for DES. 0.05% CsA is delivered through 1-2 drops/time (assuming about 25 μL per drop) and 2-3 times/day of oil-in-water emulsion that deliver about 75 μg (assuming 2 drops/time and 3 times/day) of drug to the eye for the treatment of dry eye.\(^{13,14}\) Recently the bioavailability of CsA delivered through Restasis is determined at 1-2% (assuming the bioavailability is 2%),\(^{14}\) indicating that about 1.5 μg/day of CsA is delivered to cornea and conjunctiva through this treatment route. The increased residence time by CL may lead to a rise in drug bioavailability to possible 30-50% compared to 1-2% of eye drops,\(^{15}\) so the minimum drug release mass from CL should be 3 μg/day (assuming the bioavailability is 50%), the equation shows below:

\[
m = C_{CsA} \times n \times T \times \frac{B_{Eye\, drop}}{B_{CL}}
\]

where \(m\) is the required minimum drug release, \(C_{CsA}\) is the concentration of CsA in the Restasis, \(n\) is the number of drops per time, \(T\) is the delivery times one day, \(B_{Eye\, drop}\) is the bioavailability of eye drops and \(B_{CL}\) is the bioavailability of CL.
**Figure S43:** The fitted curve of cumulative percentage release of HA from the functionalized PTHLF nanofilm at different time. Values represent the mean and standard deviation (n=5).

**Figure S44:** In vitro biocompatibility evaluation. (A) Cell viability of HCECs co-incubated with bare PTHLF, PTHLF/HA, and functionalized PTHLF films at different CsA loading doses for 2, 4, 7, and 10 h. (B) Cell viability of L929 cells co-incubated with bare PTHLF, PTHLF/HA, and functionalized PTHLF films at different CsA loading doses for 10 h. (C) Fluorescent images of live (green)/dead (red) staining of HCECs after co-incubation with the functionalized PTHLF films at different CsA loading doses for 2, 4, 7, and 10 h.
Table S1: Vibrational modes of amino acid side chains and chemical bonds.\textsuperscript{16-22}

| Amino acid/chemical bond | Vibrational modes                  |
|--------------------------|------------------------------------|
| -S-S-                    | 507-550 cm\textsuperscript{-1}    |
| Trp                      | 568, 751-757, 871, 1340-1347, 1350, 1360, 1380-1390, 1546, 1560 cm\textsuperscript{-1} |
| Cys                      | 650-690, 760-790, 800 cm\textsuperscript{-1} |
| Tyr                      | 821-852, 1160, 1170-1175, 1257-1290, 1440, 1500-1505, 1520, 1600-1615 cm\textsuperscript{-1} |
| Phe                      | 621, 1000-1006, 1035, 1209 cm\textsuperscript{-1} |
| His                      | 1187, 1335, 1495, 1576 cm\textsuperscript{-1} |
| -COOH/COO\textsuperscript{-} | 1400, 1686-1710 cm\textsuperscript{-1} |
| NH\textsuperscript{2+}/NH\textsuperscript{3+} | 1118-1144, 1409, 1449 cm\textsuperscript{-1} |

Table S2: Anterior segment inflammation score.

| Structure | Score | Clinical feature |
|-----------|-------|------------------|
| Conjunctiva | 0      | normal           |
|           | 1      | mild edema       |
|           | 2      | moderate edema, mild to moderate hyperemia, slight infiltration |
|           | 3      | severe edema, severe congestion, severe infiltration |
|           | 0      | clearness        |
| Cornea    | 1      | local edema      |
|           | 2      | there is diffuse edema but the iris is visible |
|           | 3      | opaque, the iris is invisible |
|           | 0      | normal           |
| Atria     | 1      | a small amount of floating, a small amount of flocculent exudate |
|           | 2      | mass fiber exudation |
|           | 3      | empyema in anterior chamber |
|           | 0      | normal           |
| Iris      | 1      | mild hyperemia   |
|           | 2      | moderate hyperemia |
|           | 3      | severe hyperemia, adhesion, irregular pupils |

Note: the grading standard is formulated with reference to Peyman’s and other standards.
Table S3: Scoring standard of corneal fluorescein sodium staining.

| Structure | Score | Clinical feature                        |
|-----------|-------|-----------------------------------------|
| Cornea    | 0     | no staining                              |
|           | 1     | dot staining less than 30                |
|           | 2     | dot staining more than 30, but no diffuse staining |
|           | 3     | Diffuse staining but no plaque staining  |
|           | 4     | Large plaque staining                    |

Note: the grading standard is formulated with reference to Christophe Baudouin’s and other standards.

Table S4: Primer sequences used in RT-PCR.

| Gene (Rat) | Primer nucleotide sequence               |
|------------|-----------------------------------------|
| GAPDH      | Forward 5’-GAAGCTGGTCATCAACGGA-3’        |
|            | Reverse 5’-GAAGGGCGGGAGATGATGAC-3’      |
| IL-1β      | Forward 5’-ATGAGGACCCAAAGCACCTTC-3’     |
|            | Reverse 5’-AGCTCAGTGTCAGACAG-3’         |
| IL-6       | Forward 5’-CTGGTTCTTCTGGAGTTCCGTT-3’    |
|            | Reverse 5’-GGTCTTTGGTCTTACCTAG-3’       |

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