

Supporting Information

Reversible antibiotics loading and pH-responsive release from polymer brushes on contact lens for therapy and prevention of corneal infections

*Yishun Guo^{a,c} #, Siyuan Qian^a #, Lu Wang^a #, Jiahong Zeng^a, Renjie Miao^a, Yongchun Meng^{a, *}, Yingying Jin^a, Hao Chen^{a, *}, and Bailiang Wang^{a, c*}*

^a Research Group of Advanced Ophthalmic Bionic Interface Materials & Drug Delivery, School of Ophthalmology & Optometry, Eye Hospital, Wenzhou Medical University, Wenzhou, 325027, China

^b Central Laboratory, Yantai Afliated Hospital of Binzhou Medical University, 717 Jinbu Street, Yantai, Shandong 264100, China

^c State Key Laboratory of Ophthalmology, Optometry and Visual Science, Wenzhou Medical University, Wenzhou, 325027, China

* Corresponding authors. Fax: +86 577 88017524.

E-mail: yongchun.meng@hotmail.com (Y.C. Meng); Chenhao823@mail.eye.ac.cn (H. Chen);

blwang@wmu.edu.cn (B. L. Wang)

#These authors contributed equally to this work and should be considered co-first authors.

Materials and methods

Materials

Silicone hydrogel CL was bought from Bausch and Lomb LTD. ACPA, poly(ethyleneimine) (branched PEI, Mw: 25 kDa), Van, 4,4'-Azobis(4-cyanovaleric acid) (V501), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (RAFT agent), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and N-hydroxysulfosuccinimide (NHSS) were purchased from Sigma-

Aldrich. Polydimethylsiloxane (PDMS) Sylgard184 was purchased from Dow Corning and cured through crosslinking according to the manufacturer's instructions. The bacteria including *Staphylococcus epidermidis* (*S. epidermidis*, ATCC 12228), and *S. aureus* (ATCC 6538) were kindly provided by Prof. Jian Ji (Zhejiang University, Hangzhou, China). Dulbecco's modified Eagle Medium (DMEM), fetal bovine serum (FBS), bacterial LIVE/DEAD staining kit and Cell Counting Kit-8 (CCK-8) were purchased from Life Technology (Carlsbad, CA, USA).

Preparation of PCVB onto CL

Both CL and model substrates including silicon wafers and PDMS were used to coat the PCVB coating for easy testing of biological and material science functions. CL and substrates were first aminated with PEI solution (5 mg/m, 30 min) to form a positively charged surface. To immobilize the RAFT agent on the aminolyzed CL, EDC (10 mmol), NHSS (20 mmol), and RAFT agent (0.0234 mmol) were successively added into 2-(N-Morpholino)ethanesulfonic acid buffer (0.1 M, pH 5.5) with magnetic stirring for 12 h. The P (ACPA) brush coatings were synthesized by RAFT polymerization in ACPA monomer solution in ethanol with different concentration. For Van loading into the brushes, the CL was immersed into the Van solution in pH 7.4 PBS based on formation of boric acid dynamic chemical bonds. The thickness of the self-assembled coatings on silicon wafer was measured by spectroscopic ellipsometry (M-2000 DITM, J.A. Woollam). The surface topography was measured by scanning electron microscope (SEM) (SiRion100). Surface wettability of the coatings was measured by drop shape analysis (KRÜ SS, DSA10-MK2).

Release of Van from the PCVB-CL

The antibiotics release from the PCVB-CL in different medium (pH 7.4, 6.8, 5.0 and 4.0 PBS) was applied to examine the pH responsive property. The concentration of Van was measured through a spectroscopy method as described in our previous works^{1, 2}. In brief, Van solution shows spectral absorption at 280 nm using a spectrophotometer (Spectronic Instruments, Rochester, NY), which was used to calculate the Van concentration comparing with calibration curve.

***In vitro* antibacterial tests of the PCVB-CL**

Three kinds of *in vitro* antibacterial tests including shake-flask culture method, zone inhibition test and bacterial LIVE/DEAD stain method were applied to evaluate antimicrobial tests of CL after antibacterial films modification. In these experiments, *S. aureus* and *S. epidermidis* were used as model bacteria. The CL was incubated with bacteria solution, the survival ratio of bacteria during incubation was quantified through the classic dilution plate counting method. The survival curve of the bacteria shows the sterilization rate of the antimicrobial coating on CL. In the zone inhibition test, 0.2 mL of 1.0×10^6 colony forming units (CFU)/mL bacteria solution was evenly coated onto nutrient agar in Petri dishes. Then, the antibacterial films modified CL was gently attached to the surface and cultivated for 24 h to measure the zone of inhibition (ZOI) formed around the CL. The released antibiotics could kill the contacted bacteria indicating the scope of action and bactericidal performance of antibacterial coating. The living and death of bacteria attached onto the CL surface was also examined through the LIVE/DEAD BacLight bacterial viability kit (L-7012, Invitrogen) according to the kit protocol. The images of bacteria on the

surface were taken under the fluorescence microscope investigation (Zeiss, Germany).

Cytotoxicity Assays.

Human corneoscleral tissues not qualified for clinical use from 20- to 50-year-old donors were obtained from the Wenzhou University Eye Bank in Zhejiang Province, China. The human corneal epithelial cells (HCECs) were cultured from limbal explants using the same method. The PHCECs were cultured in the supplemented hormonal epidermal medium (SHEM) supplemented with 5% FBS, 10 ng/mL human epidermal growth factor, 5 µg/mL insulin, 50 µg/mL gentamicin, 1.25 µg/mL amphotericinb, 0.5% DMSO, 0.5 µg/mL hydrocortisone, and 30 ng/mL cholera toxin, and the medium was replaced every other day. Confluent cells were digested using 0.25% trypsin-0.02% EDTA, followed by centrifugation (1000g for 3 min) to harvest the cells. Subsequently, the single cell suspension was used for cell number calculation using a haemocytometer. After confluence, cells were digested and resuspended for cultivation on the materials. The HCECs were seeded onto the specimens at a density of 1.0×10^4 cells per sample by using a 96-well tissue culture plate as the holder. Cultivation was conducted for 24 h. Then, cell counting kit-8 assays (CCK-8) was used for the viability and morphology studies of cells grown on the resultant coatings¹[1][1][1][1][1]¹(Wang et al., 2016a)^[1a]¹¹.

A CCK-8 (Beyotime, China) assay was employed in this experiment to quantitatively evaluate the cell viability³. After HCECs were inoculated on the coating-modified dishes for 24 h, the original medium was replaced with 100 µL of 10% FBS DMEM/F12 (1: 1) mixed medium containing 10 µL of CCK-8. It was incubated at 37 °C for 2 h to form water soluble formazan. Then 100 µL aliquots of the above formazan solution were taken from each sample and added to one well of a 96-well plate. Six parallel replicates were prepared. The absorbance at 450 nm (calibrated wave) was determined using a microplate reader (Multiskan MK33, Thermo Electron Corporation, China).

Animal Experiments of Antibacterial Activity

The study was approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University. Experiments were carried out Sprague Dawley (SD) rats (obtained from the Animal Administration Center of Wenzhou Medical University). The rats were treated in accordance with guidelines set forth by the Association for Research in Vision and Ophthalmology.

To build uniform corneal bacterial infection model, rats were anesthetized using isoflurane delivered by facemask. Under sterile conditions, Tweezers were used to hold the eyeball in the conjunctiva and fascia outside the puncture point or the ipsilateral corneal margin. About 2/3 of the corneal epithelium was scraped use a razor in the center of the cornea. Then a drip of *S. aureus* solution (10 µL) with a concentration of 10^8 CFU/mL was dripped on to the cornea. The eyelids were sutured for 24 hours before the observation of the bacterial infections under a slit lamp. With similar corneal bacterial infections, 24 rats were selected and divided into four groups: pristine CL, P(ACPA)-CL, PCVB-CL and free Van respectively. Specially, the pristine or antibacterial coating modified CL was fixed on the rat eyes for 10 hours at a time every day for 3 days. In the free Van group, the same total amount Van as that in the PCVB-CL was administered to the eye surface for two times (each time 10 µL). All instruments were sterilized prior to surgery and stored in sealed

Petri dishes.

The corneal condition was observed with a slit lamp before and after the building of the bacterial infection model, and on the first and third days during the treatment. The treatment was continued for 3 days before clinical observation was performed. The number of bacteria in the ocular surface secretions was counted through agar plate counting after the model was built and being treated for 3 days. At day 3, all rats were euthanized by intramuscular injection of an excessive amount of anesthetic, and the eyeballs were removed for corneal tissue histological analysis and inflammation degree evaluation. The sections were examined for histology, and the inflammatory cells were observed under a microscope to evaluate the inflammation. According to the general scoring standard of corneal infection experiment, no corneal opacity was recorded as 0, slight corneal opacity and covering the anterior segment of the cornea as 1, and dense opacity of part or all of the pupil as 2 points, corneal dense opacity covering all anterior eyes was scored as 3 points, corneal perforation as 4 points. The eyeballs were placed in the fixative solution for at least 24 h. After being fixed with paraffin embedding, the eyeballs were cut into tissue sections (5 mm thick). Then, the tissues were mounted onto slides for hematoxylin and eosin (H&E) staining. The pictures were taken under optical microscope.

[1] B.L. Wang, Y.M. Han, Q.K. Lin, H.H. Liu, C.H. Shen, K.H. Nan, H. Chen, *Journal of Materials Chemistry B*, 2016, **4**, 1853-1861.

[2] B.L. Wang, Z. Ye, Y.H. Tang, H.H. Liu, Q.K. Lin, H. Chen, K.H. Nan, *Journal of Physical Chemistry C*, 2016, **120**, 6145-6155.

[3] L. Sun, W. Jiang, H. Zhang, Y. Guo, W. Chen, Y. Jin, H. Chen, K. Du, H. Dai, J. Ji, B. Wang, *ACS Appl Mater Interfaces*, 2019, **11**, 2302-2316.