Treating Bacterial Keratitis during Sleep Stage through Placing Hydrogel Beads with Sustained Tobramycin/Baicalin Delivery Capability at the Palpebra Inferior

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S1 Investigation of the optimum initial feeding composition of the PCBT hydrogel bead

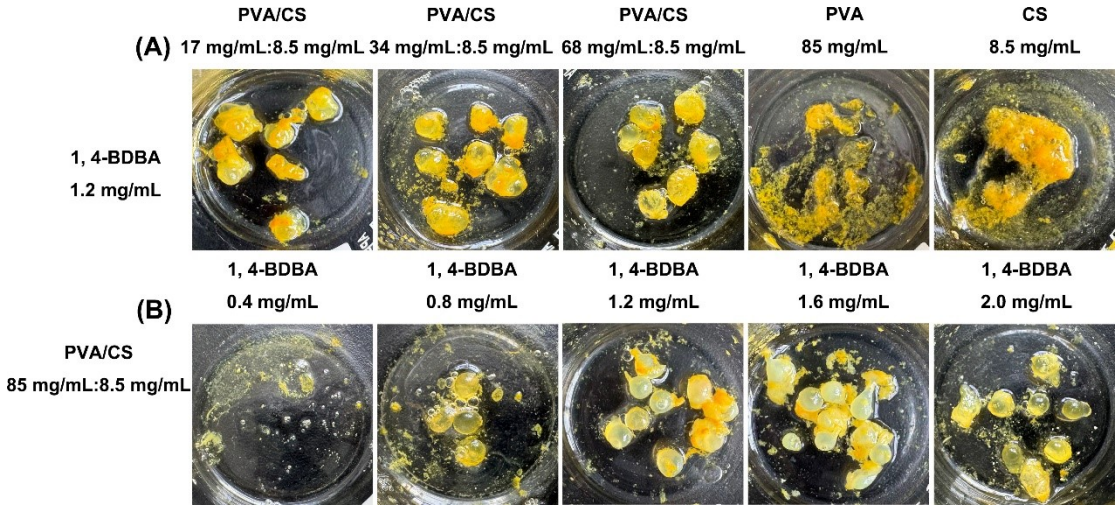

Fig. S1 Digital images of the PCBT hydrogel beads with varying feeding composition.: (A) the concentration of 1,4-BDBA was kept at 1.2 mg/mL while the PVA/CS ratio changed; (B) the PVA/CS ratio was fixed at 85 mg/ml:8.5 mg/mL while the concentration of 1,4-BDBA changed.

To figure out the optimum initial feeding composition of the PCBT hydrogel bead, the structural integrity/stability of the PCBT hydrogel beads with varying feeding compositions were investigated in detail. Through extrusion assays, the optimum concentration of CS was determined to be 85 mg/mL while the concentration of PVA should be maintained at less than 8.5 mg/mL. Only under these circumstances, the mixed solutions of PVA and CS could be easily extruded from the syringe needle and form uniform and stable liquid drop. In addition, we also investigated the optimum PVA/CS ratio and 1,4-BDBA concentration through observing the structural integrity/stability of the hydrogel beads. Obviously, only if dripping the PVA/CS solution into the 1,4-BDBA/BA solution could form the PCBT hydrogel beads (**Fig. S1A**). If the PVA/CS ratio was less than 85 mg/mL: 8.5 mg/mL (1,4-BDBA 1.2 mg/mL), a large number of floccules remained on the surface of the as-prepared PCBT hydrogel beads (**Fig. S1A**). Moreover, if the concentration of 1,4-BDBA was less than 1.2 mg/mL (PVA:CS = 85 mg/mL: 8.5 mg/mL), no uniform and stable PCBT hydrogel beads could be formed (**Fig. S1B**).

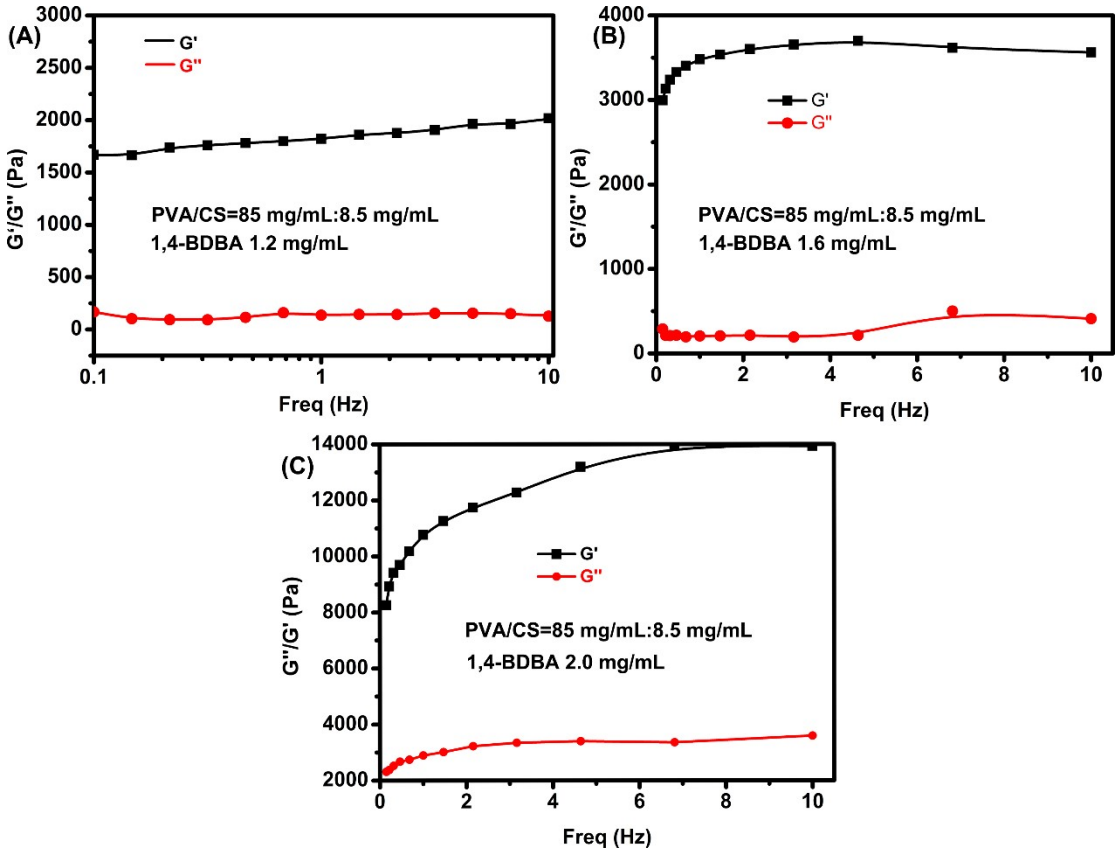

Fig. S2 Oscillatory shear rheology of the PCBT hydrogel beads with varying feeding composition. (A) PVA/CS=85 mg/mL:8.5 mg/mL, 1,4-BDBA 1.2 mg/mL; (B) PVA/CS=85 mg/mL:8.5 mg/mL, 1,4-BDBA 1.6 mg/mL; (C) PVA/CS=85 mg/mL:8.5 mg/mL, 1,4-BDBA 2.0 mg/mL.

In addition, when the PVA/CS ratio was kept at 85 mg/mL: 8.5 mg/mL, the elasticity (G') of PCBT hydrogel bead was increased with the increase of the 1,4-BDBA concentration (**Fig. S2**). Thus, to alleviate the foreign body sensation, the concentration of 1,4-BDBA should be kept at 1.2 mg/mL.

Collectively, the optimum concentrations for PVA and CS were 85 mg/mL and 8.5 mg/mL, respectively. The optimum 1, 4-BDBA concentration was 1.2 mg/mL.

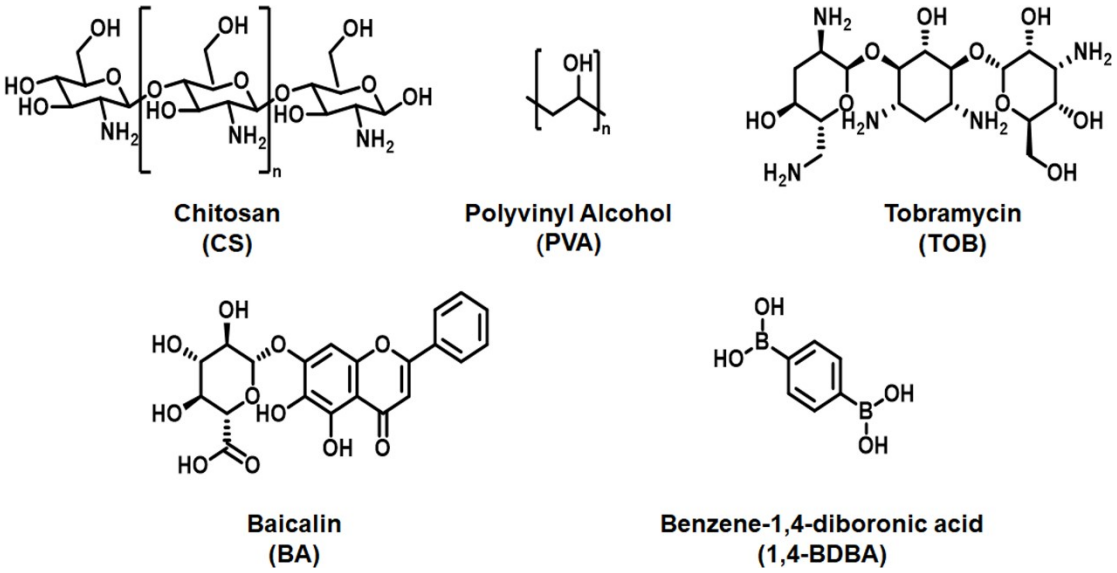

Fig. S3 The chemical structure of the main components of the PCBT hydrogel bead

S2 Plotting of the cumulative releasing curves of TOB/BA from the PCBT hydrogel beads

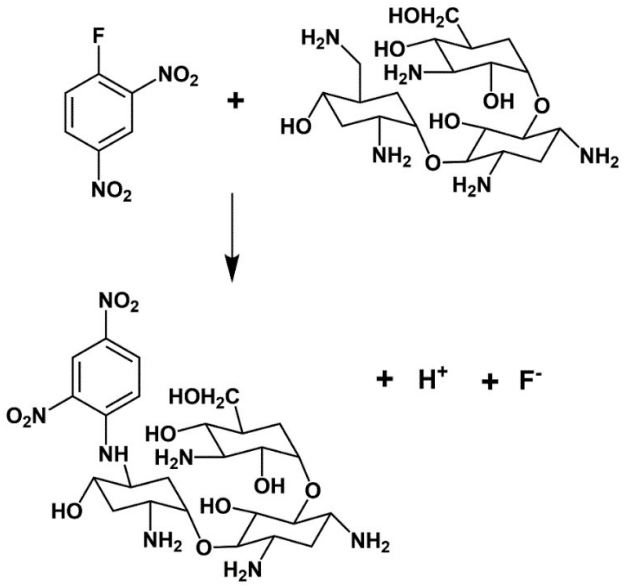

Fig. S4 Schematic diagram of the reaction between 2,4-dinitrofluorobenzene and TOB

(1) TOB releasing curves

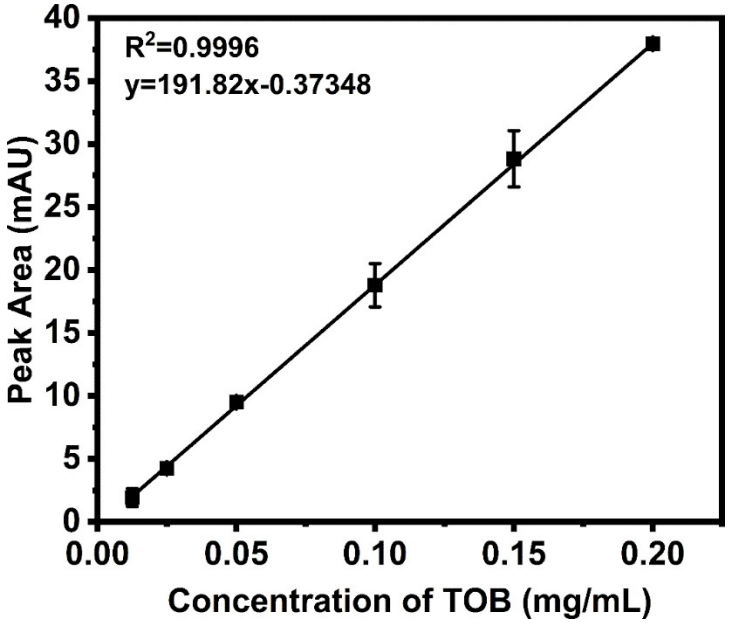

Fig. S5 Standard curve of Tob plotted through HPLC

Tob has no UV-vis absorbance or fluorescence emission, so conventional UV-vis spectrum, HPLC, and fluorescence spectrum couldn't be utilized for quantification of tobramycin. In this regard, tobramycin could be firstly converted into a compound with UV-vis absorbance. Then, the derivative was subjected to UV-vis or HPLC tests. Commonly used derivatives include 2,4-dinitrofluorobenzene (DNFB), phthalaldehyde (OPA), 2,4,6-trinitrobenzenesulfonic acid (TNBS), and isothiocyanate-1-neryl ester (NITC). In this study, DNFB was applied. DNFB can undergo nucleophilic reaction with the amino groups of tob molecules. The process of the reaction was illustrated in the **Fig. S4**, and the resultant product exhibited UV-vis absorbance at 365 nm. Adding trimethylol aminomethane could enhance the derivatization process. The specific procedures were as follows: 5 mL 2,4-dinitrofluorobenzene solution and 5 mL trimethylol aminomethane solution were successively added into 2 mL leaching solution. The resultant mixture was agitated for 30 min and then heated at 60 ℃ for around 50 minutes. After the solution was cooled to room temperature (25 ℃), 5 mL reaction solution was diluted with 5 mL of acetonitrile and then filtered with a 0.45 μm filter membrane. The resultant solution was finally subjected to HPLC tests. The amount of release TOB was determined

using a standard curve method (**Fig. S5**).

The HPLC conditions for TOB tests were as follows: Pepax C18 column (150 mm × 4.6 mm, 5 μm); mobile phase: 0.25% tris(hydroxymethyl)aminomethane: 0.5 mol/L sulfuric acid: acetonitrile (34:1:65); detection wavelength: 365 nm; column temperature: 30°C; flow rate: 1.0 mL/min; injection volume: 20 μL.

(2) RA releasing curves

The HPLC conditions for BA tests were as follows: Pepax C18 column (250 mm × 4.6 mm, 5 μm); mobile phase: acetonitrile: 0.1% phosphoric acid (27:73); detection wavelength: 354 nm; column temperature: 30°C; flow rate: 1.0 mL/min; injection volume: 10 μL. The amount of released BA was determined using a standard curve method (**Fig. S6**).

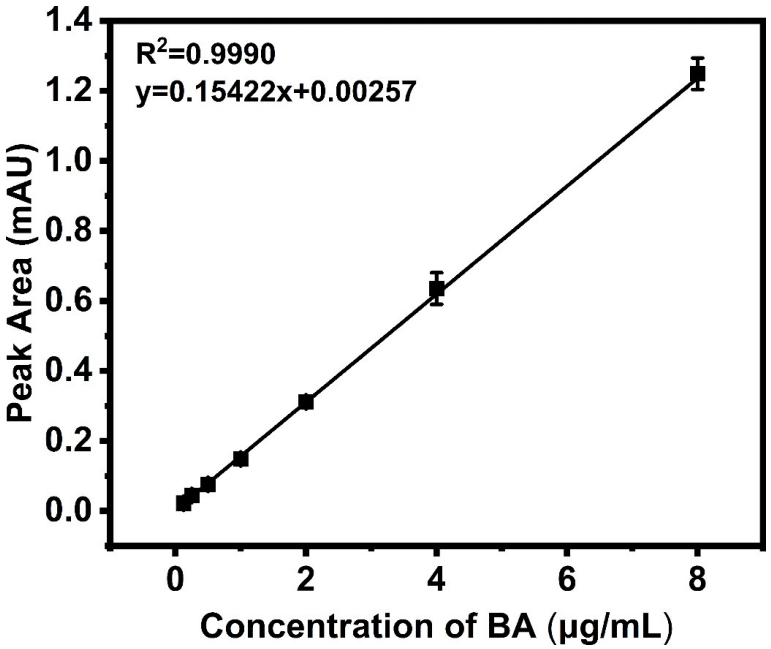

Fig. S6 Standard curve of BA plotted through HPLC

S3 Mathematical fitting of drug releasing curves

The Zero-order model, First-order model, Higuchi model, Hixson-Crowell model, Korsemeyer-peppas model, Kopcha model and Weibull model were utilized to fit the drug releasing curves and then explain the releasing mechanism.

(1) Zero-order model

$$Q_t = Q_0 + K_0 t$$

Where Q_t is the amount of drug dissolved at time t, Q_0 is the initial amount of drug in the solution (most times, Q_0=0) and K_0 is the zero-order release constant expressed in unit of concentration/time.

Application: This relationship is utilized to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.

(2) First-order model

$$\log(C) = \log(C_0) - kt/2.303$$

Where C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time.

Application: This relationship is used to describe the drug dissolution in pharmaceutical dosages forms such as those containing water-soluble drugs in porous matrices.

(3) Higuchi model

$$Q_t = Q_0 + k_H t^{\frac{1}{2}}$$

Where Q_t is the cumulative amount of drug release at time t, Q_0 is the initial amount of drug, k_H is the Higuchi constant.

Application: This relationship is used to describe the drug release from an insoluble matrix based on Fickian diffusion. It also can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs.

(4) Hixson-Crowell model

$$W_0^{1/3} - W_t^{\frac{1}{3}} = \kappa t$$

Where W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and κ is a constant incorporating the surface-volume relation.

Application: This expression applies to pharmaceutical dosage form such as tablets, where the dissolution occurs in planes that are parallel to the drug surface if the tablet dimensions diminish proportionally, in such a manner that the initial geometrical form keeps constant all the time.

(5) Korsmeyer-Peppas model

$$Q_t = K_{kp} t^n$$

Where Q_t is the cumulative amount of drug release at time t, K_{kp} is the Korsmeyer-Peppas constant, n is the release exponent which defines the drug release mechanism.

Application: This relationship is used to describe drug release from the polymeric system. When the Korsmeyer-Peppas model is used to thin films, the release index n ($n = 0.5$ or $n \leq 0.5$) conforms to Fickian diffusion, while the values of n ($0.5 < n < 1$) is related to non-Fickian release, meaning that the drug release follows both erosion and diffusion mechanisms. And $n = 1$ corresponds to the zero-order release which defines the drug release is independent of time.

(6) Kopcha model

$$Q_t = At^{1/2} + Bt$$

Where A is the diffusion constant and B is the erosional exponent.

Application: If the ratio of A/B is high (A is much greater than B), implying the drug release will follow the diffusion mechanism; and if A/B is low (B is much greater than A), meaning that the polymer erosion or relaxation plays a dominant role in drug release.

(7) Weibull model

$$M = M_0[1 - e^{-\frac{(t-T)^b}{a}}]$$

Where M is the amount of drug dissolved as a function of time t. M_0 is total amount of drug being released. T accounts for the lag time measured as a result of the dissolution process. Parameter a denotes a scale parameter that describes the time dependence, while b describes the shape of the dissolution curve progression.

Application: The Weibull model is more useful for comparing the release profiles of matrix type drug delivery.

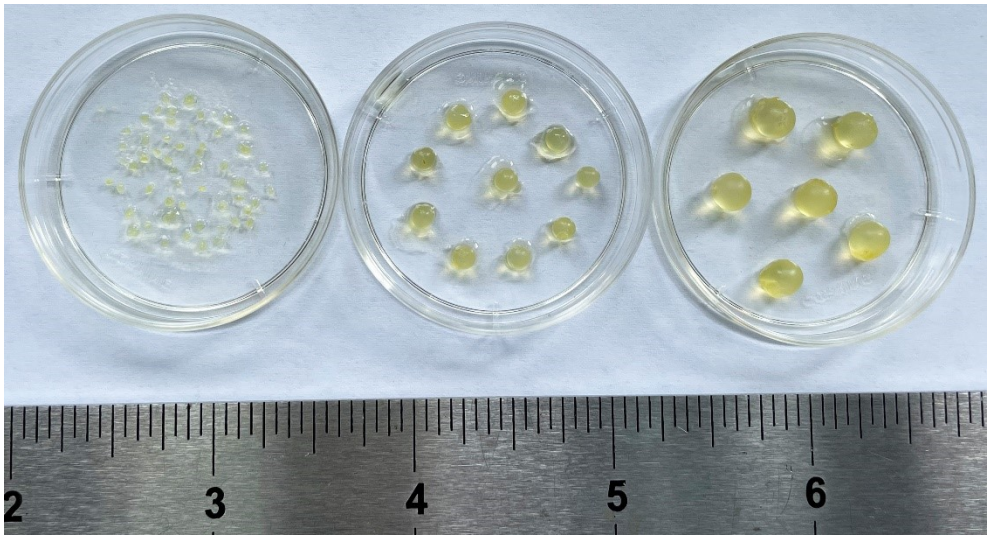

Fig. S7 Digital photographs of as-prepared PCBT hydrogel beads

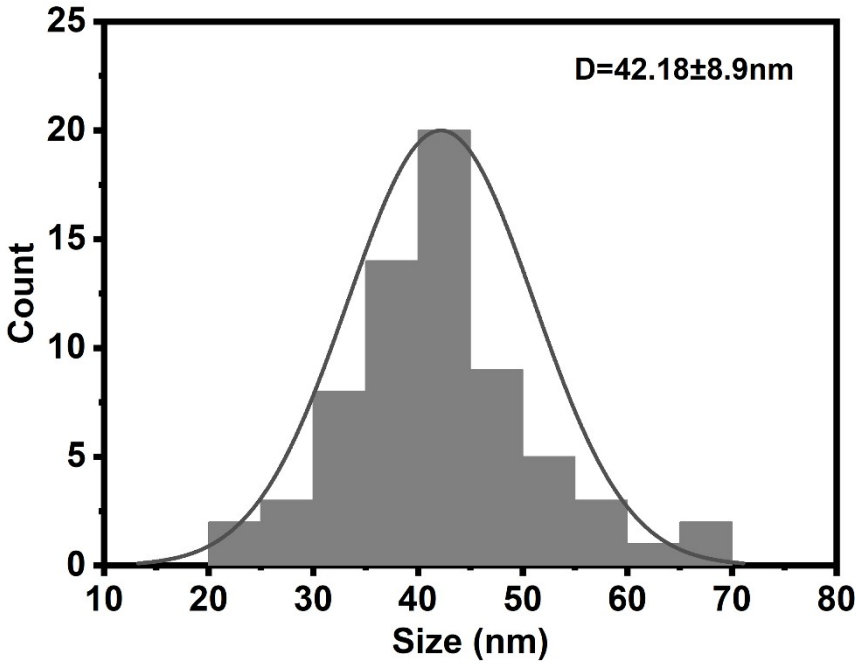

Fig. S8 Size and size distribution of the pores on the surface of the lyophilized PCBT hydrogel beads

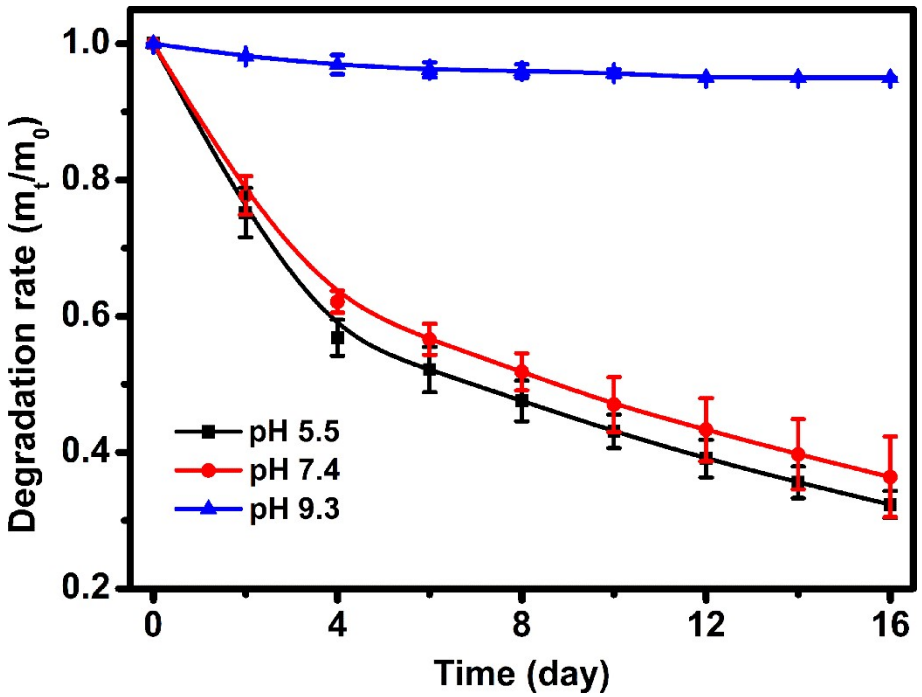

Fig. S9 *In vitro* degradation behaviors of the PCBT hydrogel beads in PBS with different pH values

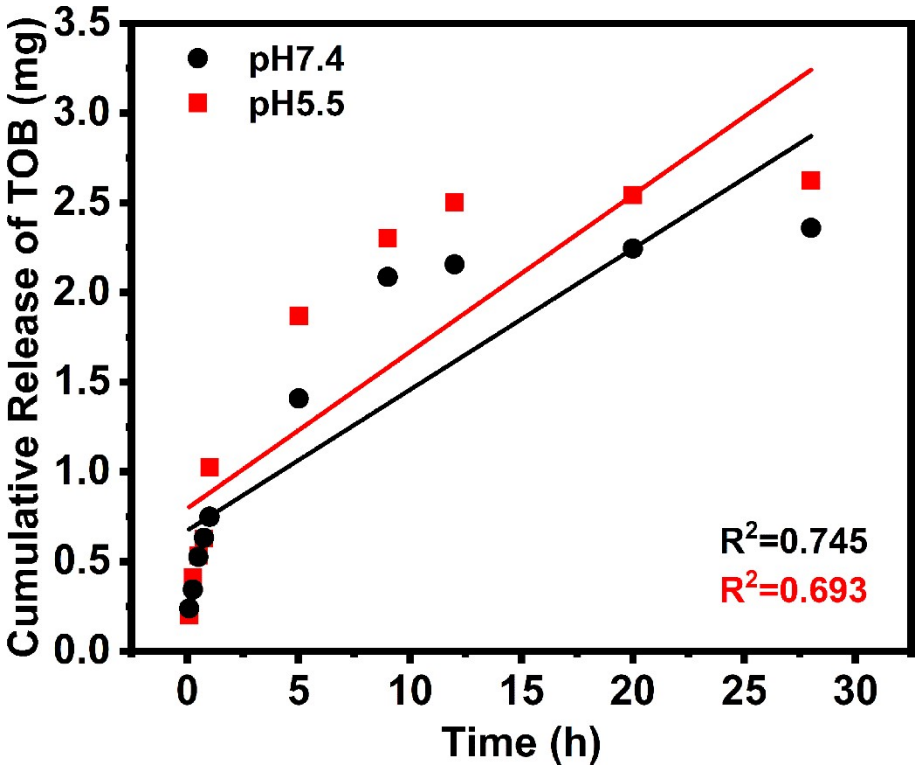

Fig. S10 Cumulative release curves of TOB from the PCBT hydrogel beads fitted using Zero-order model

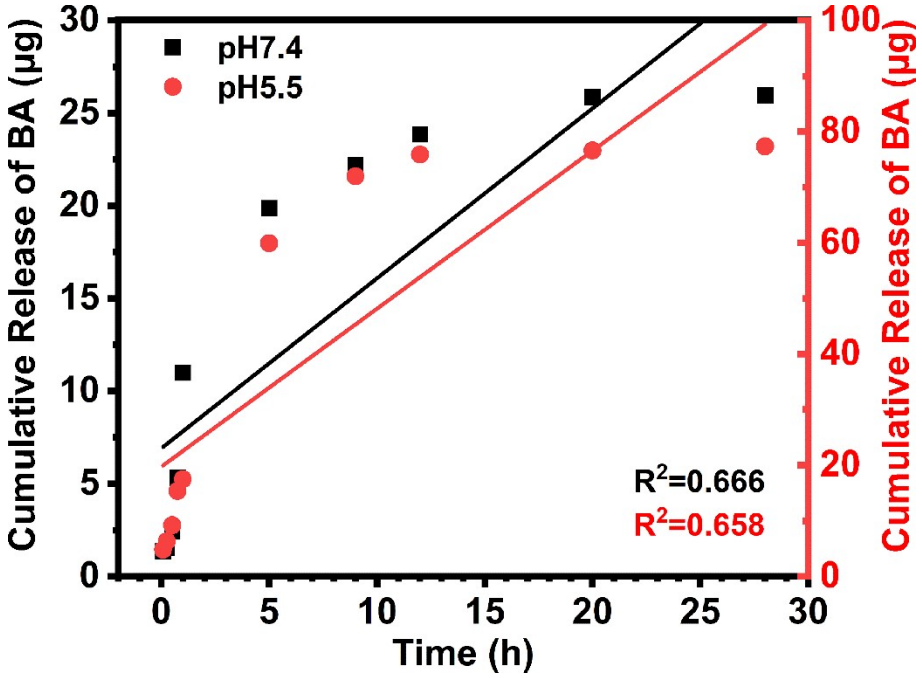

Fig. S11 Cumulative release curves of BA from the PCBT hydrogel beads fitted using Zero-order model

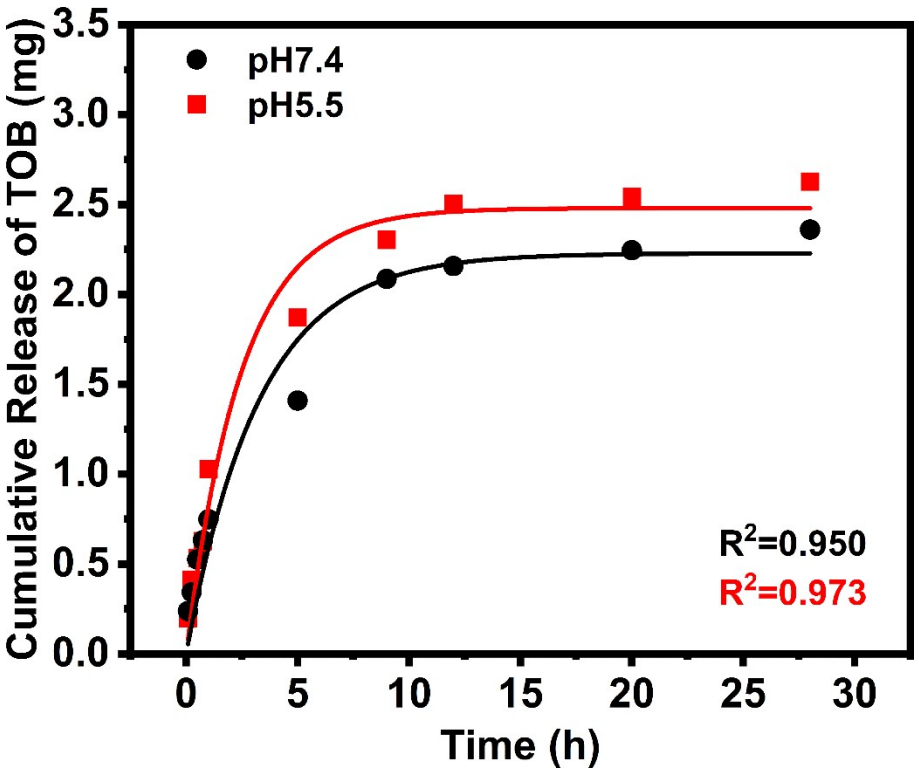

Fig. S12 Cumulative release curves of TOB from the PCBT hydrogel beads fitted using First-order model

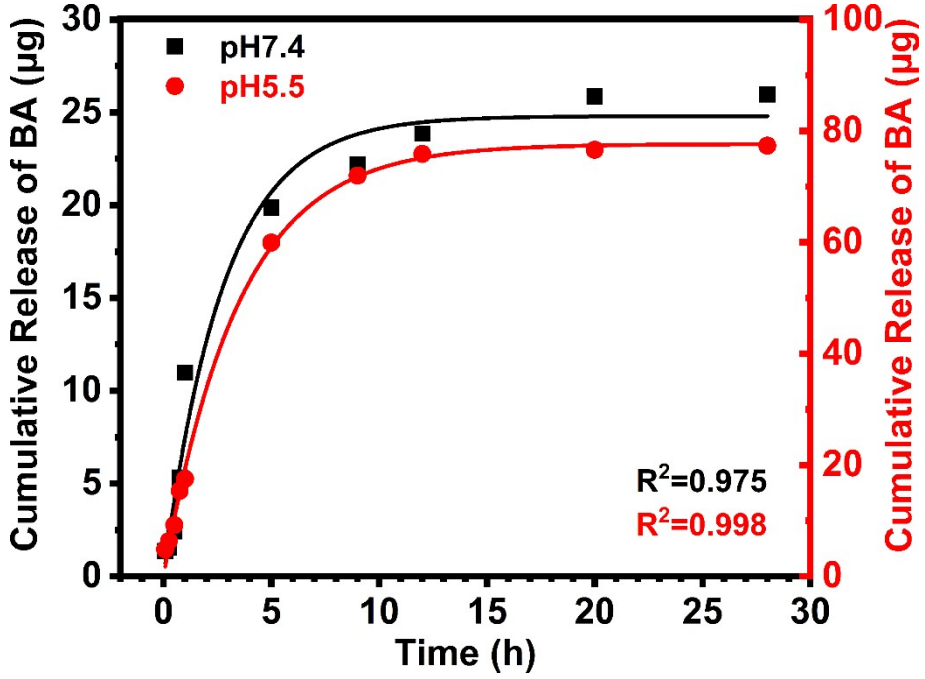

Fig. S13 Cumulative release curves of BA from the PCBT hydrogel beads fitted using First-order model

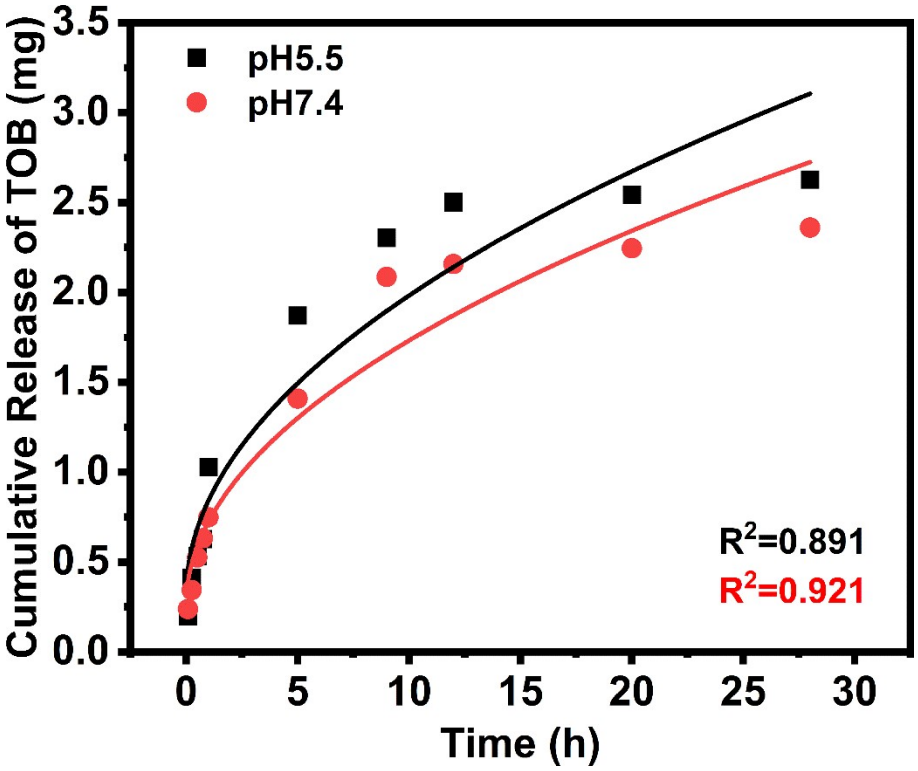

Fig. S14 Cumulative release curves of TOB from the PCBT hydrogel beads fitted using Higuchi model

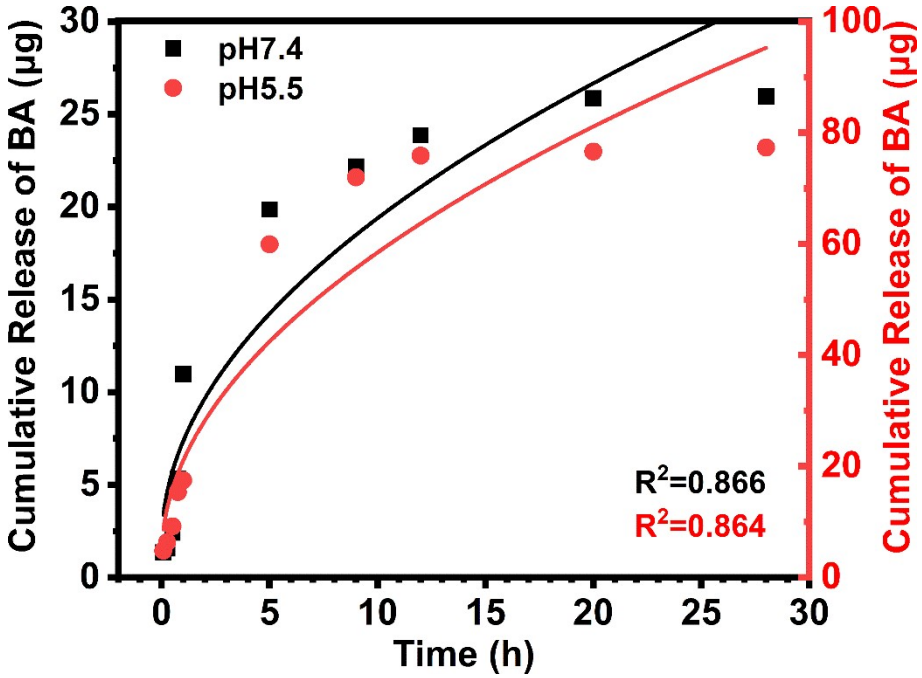

Fig. S15 Cumulative release curves of BA from the PCBT hydrogel beads fitted using Higuchi model

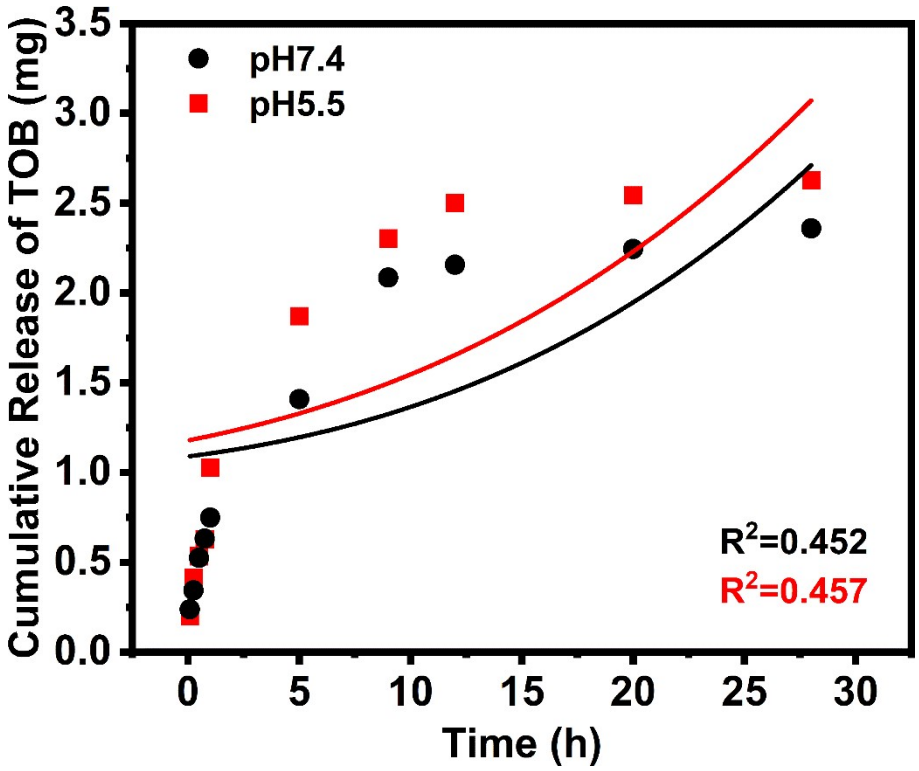

Fig. S16 Cumulative release curves of TOB from the PCBT hydrogel beads fitted using Hixson-Crowell model

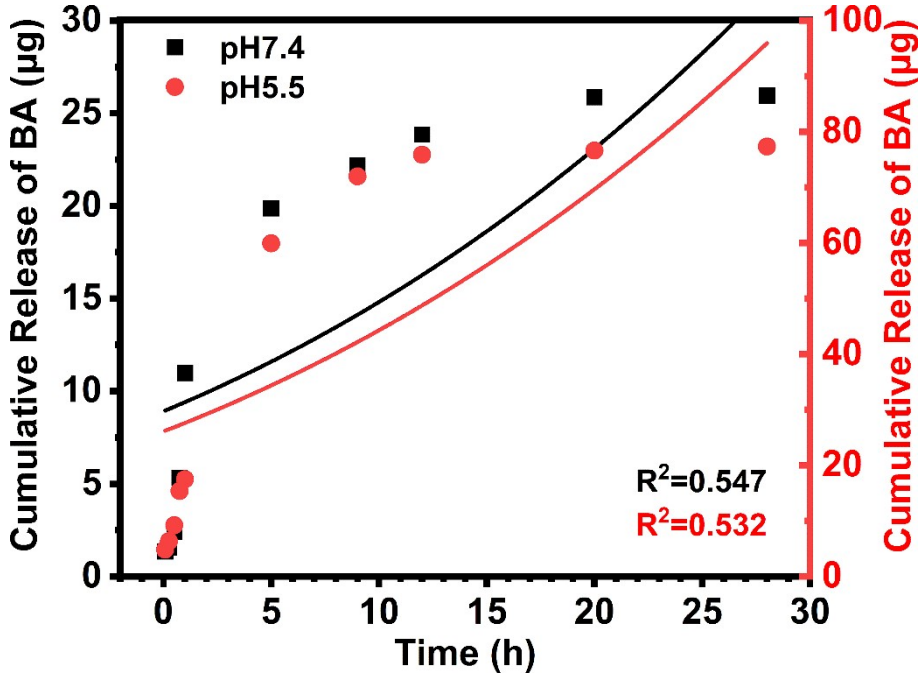

Fig. S17 Cumulative release curves of BA from the PCBT hydrogel beads fitted using Hixson-Crowell model

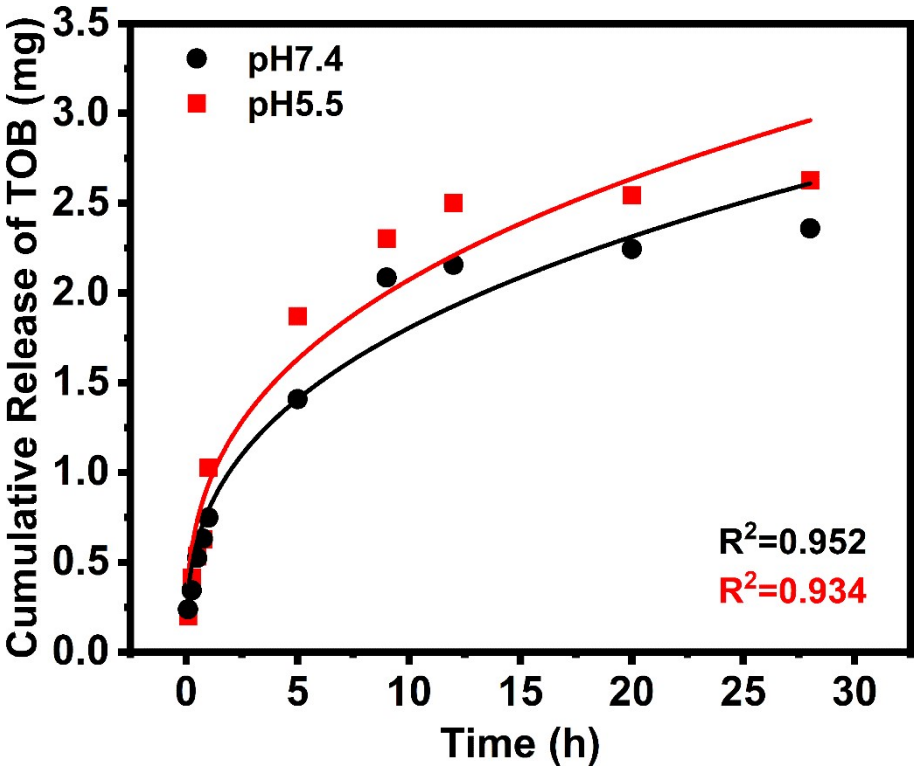

Fig. S18 Cumulative release curves of TOB from the PCBT hydrogel beads fitted using Korsmeyer-Peppas model

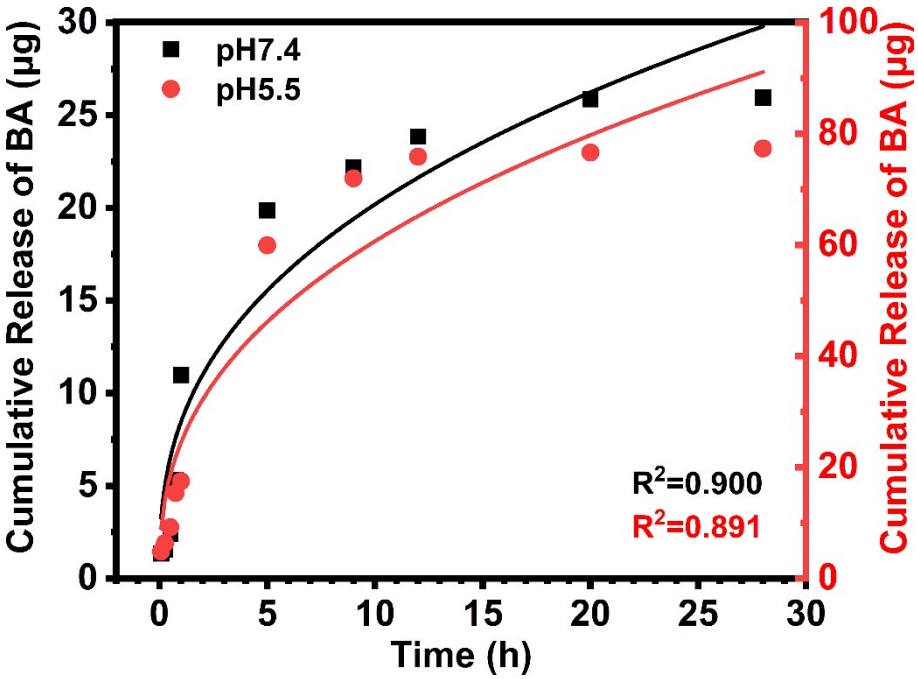

Fig. S19 Cumulative release curves of BA from the PCBT hydrogel beads fitted using Korsmeyer-Peppas model

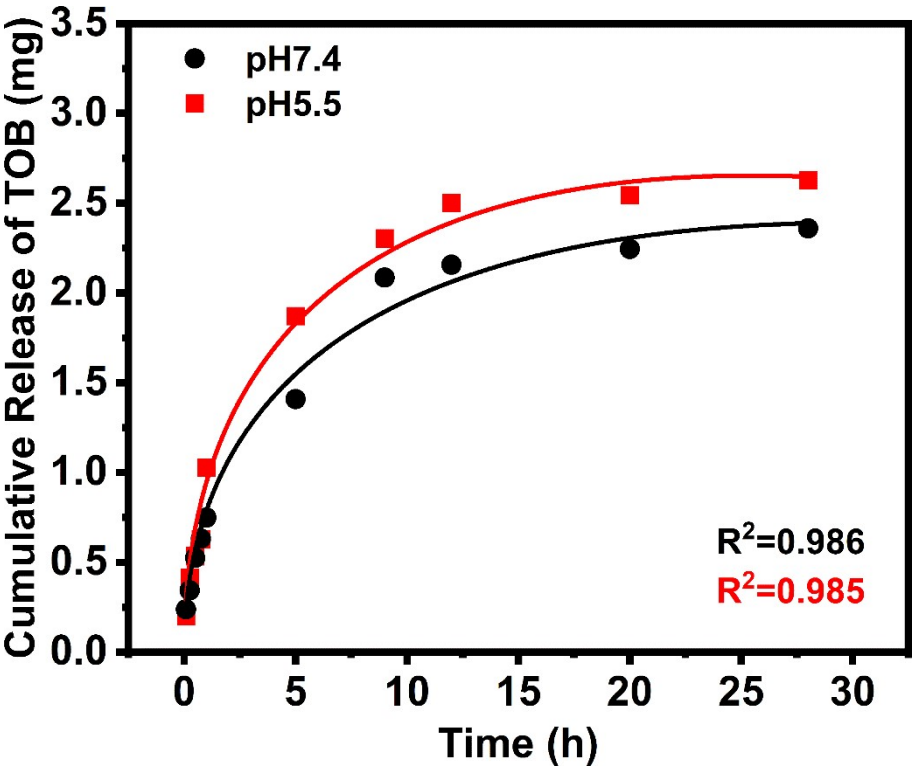

Fig. S20 Cumulative release curves of TOB from the PCBT hydrogel beads fitted using Kopcha model

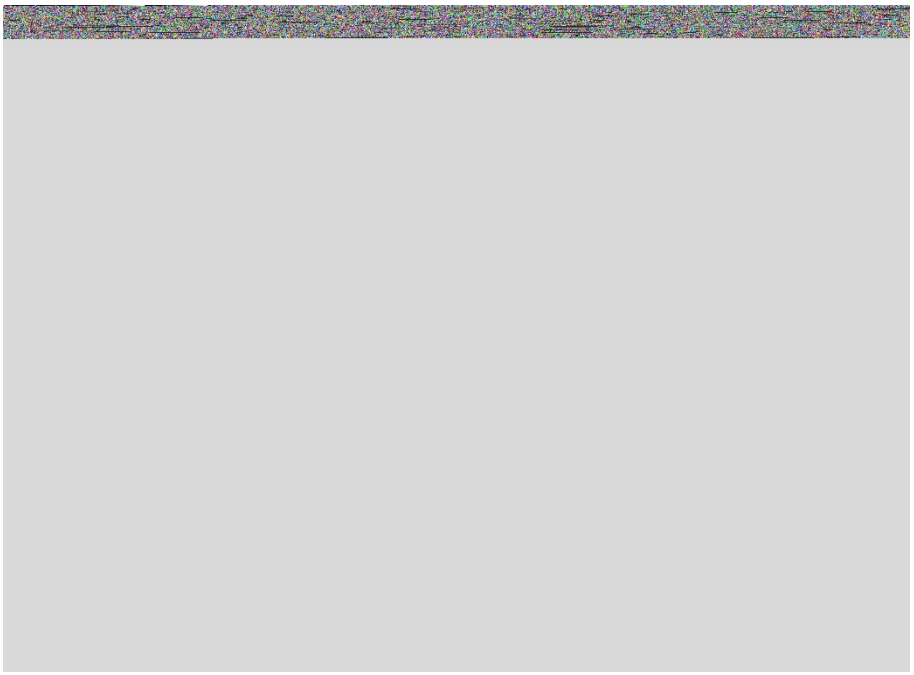

Fig. S21 Cumulative release curves of BA from the PCBT hydrogel beads fitted using Kopcha model

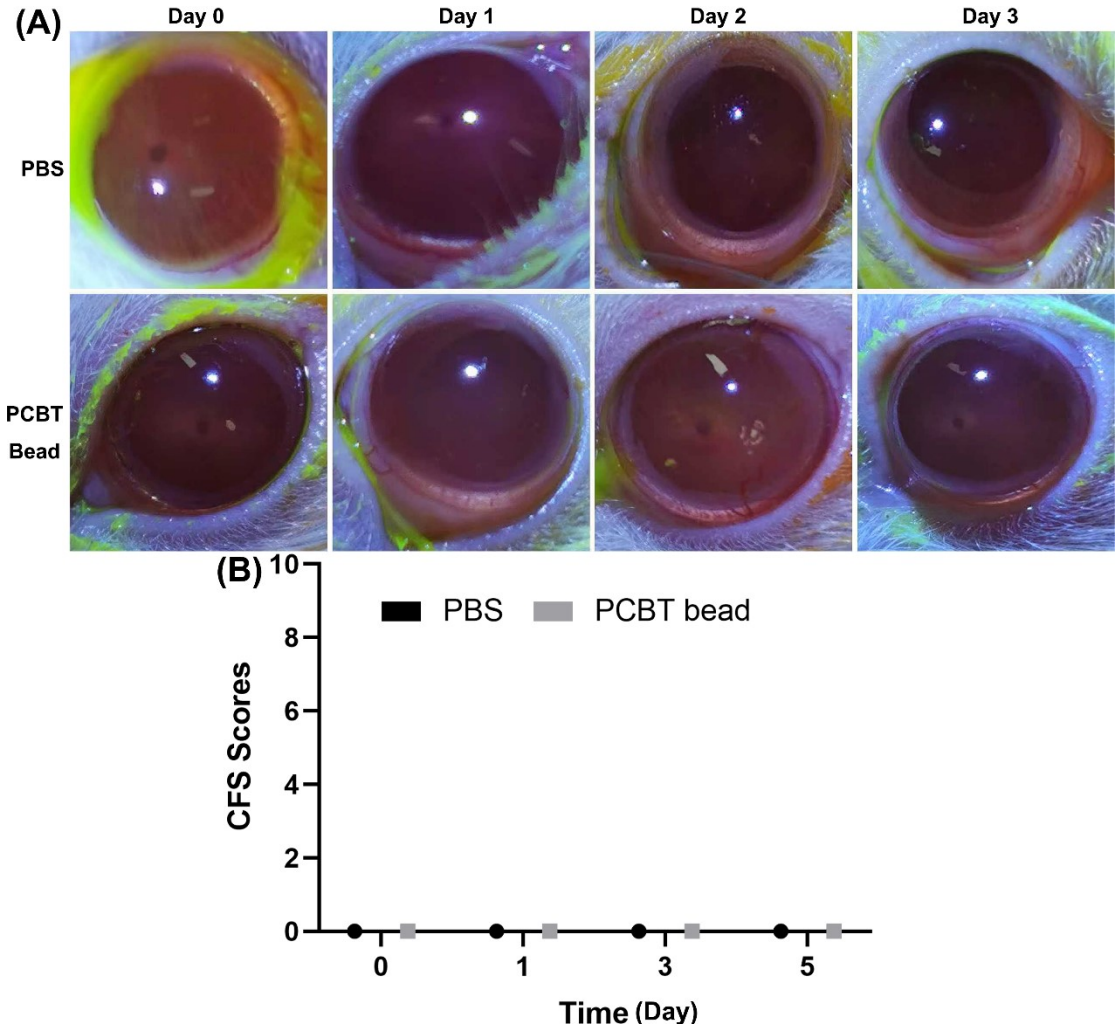

Fig. S22 (A) Representative images of the Draize test and (B) corresponding scoring

Table. S1 Visual scoring system for rabbit BK

Symptom		Score
Density of corneal opacity	Slight cloudiness, outline of iris and pupil discernable	1
	Cloudy, but outline of iris and pupil remain visible	2
	Cloudy, opacity not uniform	3
	Uniform opacity	4
Conjunctiva	White, without congestion	0
	Small patch bleeding	1
	Congestion,but outline of blood vessels	2
	Seriously congested	3
	Mixed congestion,the blood vessels are indistinguishable	4
Eyelids	Without red and swollen	0
	Mild red and swollen	1
	Red and swollen	2
Eye discharge	None	0
	Mild	1
	Severe	2
lachrymal secretion	None	0
	Mild	1
	Severe	2
Photophoby	None	0
	Squint or blink a lot.	1
	Fear to open eyes	2

Symbols: (0-4) nil, (5-7) mild, (8-12) moderate and (13-16) severe.

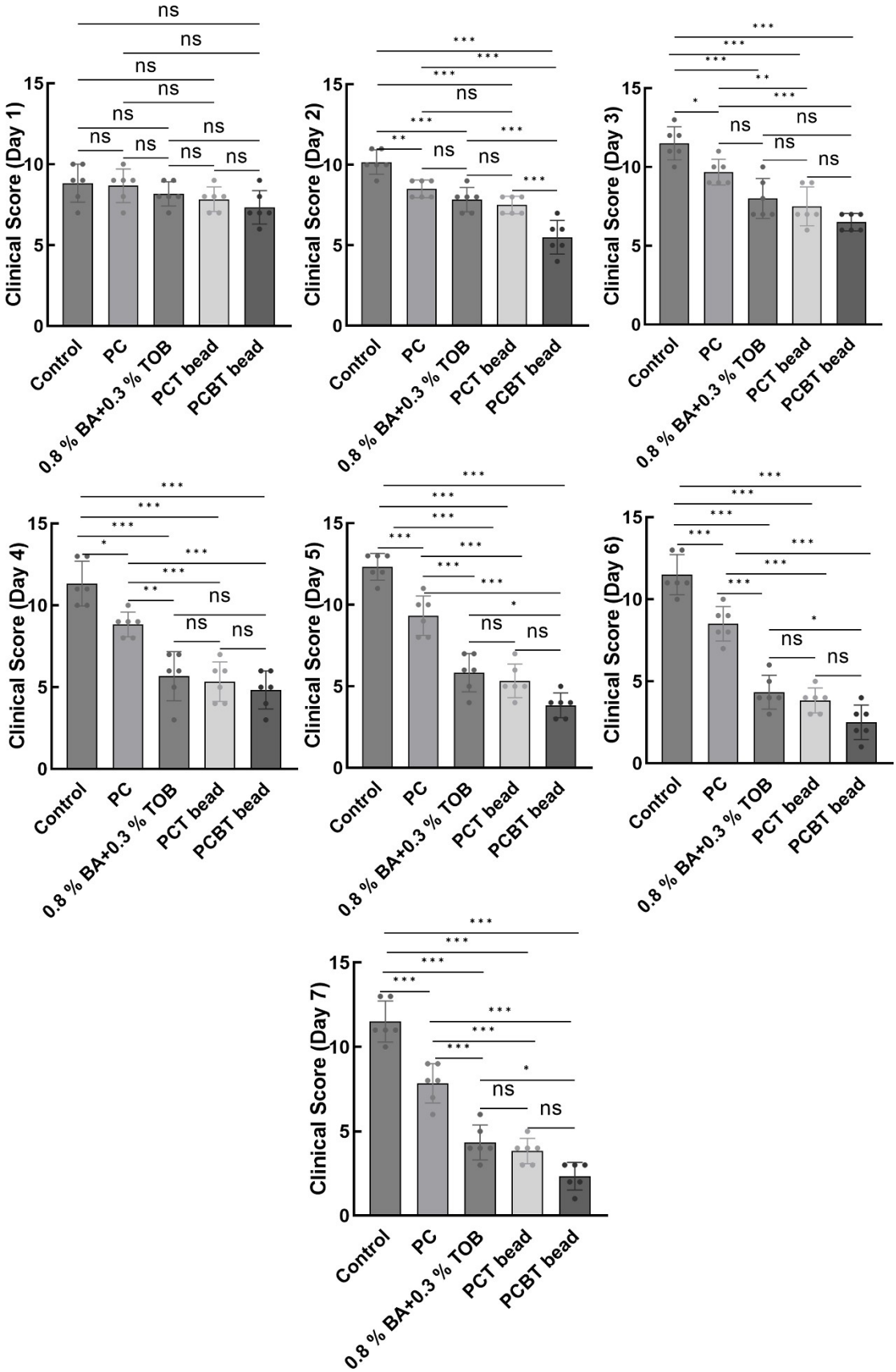

Fig.S23 Significant differences of clinical scoring between different groups at days 1, 2, 3, 4, 5, 6, and 7. (ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.005)