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Tannic acid/poly(vinyl alcohol)-modified chitosan/bacterial cellulose composite membranes with enhanced surface hydrophilicity and wet-state stability for anti-adhesion applications

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Abstract

Balancing wet-state mechanical stability with a hydrophilic and low-adhesion surface remains an important challenge in the design of postoperative anti-adhesion barriers. A chitosan/bacterial cellulose (CS/BC) composite membrane containing 10 wt% bacterial cellulose (CB10) was selected as the substrate and further modified with tannic acid/poly(vinyl alcohol) (TA/PVA) to improve surface hydrophilicity while maintaining wet-state structural performance. The coating time was varied to regulate the surface and physicochemical properties of the membranes. Increasing coating time progressively decreased the water contact angle, coefficient of friction, and swelling ratio, while causing only a modest reduction in wet-state tensile strength. Among the coated membranes, the membrane prepared with an 18 h coating time (CBTP18) exhibited a favorable balance of properties, with a water contact angle of 37°, a coefficient of friction of approximately 0.042, a swelling ratio of 4.4%, and a wet-state tensile strength of 33.8 MPa. The membrane retained approximately 71% of its initial mass after 35 days in phosphate-buffered saline and exhibited favorable hemocompatibility and cytocompatibility. Fibroblast adhesion decreased from 38.8% for CB10 to 18.1% for CBTP18. In a mouse abdominal wall–cecum adhesion model, postoperative adhesions occurred in four of six untreated animals, whereas no visible adhesion was observed in any of the six animals treated with CBTP18. Overall, the substrate–coating strategy combined wet-state mechanical stability with enhanced surface hydrophilicity and reduced fibroblast attachment, supporting further investigation of TA/PVA-modified CS/BC membranes for postoperative anti-adhesion applications.

Keywords: Chitosan; Bacterial cellulose; Tannic acid; Poly(vinyl alcohol); Surface modification; Anti-adhesion

Introduction

Postoperative tissue adhesion is a common complication following abdominal and pelvic surgery and may lead to chronic pain, intestinal obstruction, infertility, and difficulties during subsequent operations [1, 2]. Adhesion formation is closely associated with persistent fibrin deposition, fibroblast infiltration, and collagen accumulation after tissue injury [3]. Physical barriers such as membranes and hydrogels have therefore been widely investigated to separate injured tissue surfaces during the early stage of healing [4, 5]. Several barrier materials have entered clinical use, yet their effectiveness remains variable. A recent systematic review of 45 randomized controlled trials involving 4,120 patients reported inconsistent outcomes among different types of adhesion barriers [6]. In addition, insufficient mechanical stability under wet physiological conditions, premature loss of barrier integrity, excessive swelling, and poor conformity to irregular tissue surfaces may compromise barrier performance after implantation [7-9]. For membrane-based barriers in particular, sufficient wet-state structural stability is essential to maintain continuous separation of injured tissues during the early healing period. Beyond physical separation, the surface properties of the membrane can also influence protein adsorption and subsequent cell attachment [10-12]. Therefore, an effective postoperative anti-adhesion membrane should not only maintain adequate structural integrity under physiological conditions but also possess surface characteristics that minimize undesirable fibroblast adhesion.

Chitosan (CS) is a naturally derived polysaccharide widely investigated in biomedical applications because of its biocompatibility, biodegradability, antimicrobial activity, and abundant functional groups available for chemical modification [13-15]. Its potential as a physical barrier against postoperative adhesion has also been demonstrated. Zhang et al. [16] prepared a chitosan nanofibrous membrane by needleless electrospinning, which exhibited a tensile strength of 27.45 ± 6.30 MPa and significantly reduced peritoneal adhesion in vivo. However, the wet-state mechanical stability of chitosan membranes can be insufficient depending on their composition and fabrication method, potentially leading to deformation or loss of structural integrity after implantation and thereby compromising sustained physical separation of injured tissues. One strategy to address this limitation is to incorporate a reinforcing component such as bacterial cellulose (BC), which possesses a highly organized nanofibrous network, favorable mechanical properties, and good biocompatibility [17-19]. The mechanical reinforcing capability of BC has been demonstrated in polysaccharide-based composites. Wan et al. [20] incorporated BC nanofibers into glycerol-plasticized starch and reported that, at BC contents of 7.8, 15.1, and 22.0 wt%, the tensile strengths of the resulting composites reached 2.03, 2.18, and 2.37 times that of unreinforced starch, respectively. This reinforcing effect has also been observed in film-forming polysaccharide matrices. Wang et al. [21] incorporated nano-BC into agar films and found that increasing the BC content from 0 to 10 wt% increased the tensile strength from 22.10 to 44.51 MPa, with intermolecular interactions between BC and agar contributing to the reinforced structure. Given the good film-forming ability and abundant reactive groups of CS, incorporation of the mechanically robust BC nanofibrous network into a CS matrix may therefore provide a rational strategy for improving membrane structural stability while retaining the chemical functionality required for subsequent surface modification.

However, adequate wet-state mechanical stability alone may not be sufficient to ensure effective anti-adhesion performance, because the membrane surface remains in direct contact with injured tissues during healing. Surface hydrophilicity and interfacial friction are particularly relevant, as

they can influence protein–material interactions and subsequent fibroblast attachment [10, 11, 22]. Poly(vinyl alcohol) (PVA) is a hydrophilic and biocompatible polymer with abundant hydroxyl groups, making it suitable for constructing water-compatible polymer interfaces [23–25]. Weis et al. [26] demonstrated that PVA membranes and hydrogels significantly reduced both the number and severity of adhesions in a rabbit abdominal sidewall model. Tannic acid (TA), a naturally occurring polyphenol rich in phenolic hydroxyl groups, can form multiple hydrogen bonds with PVA and thereby strengthen intermolecular association within the polymer network [27, 28]. Li et al. [29] reported that incorporation of TA into PVA hydrogels enhanced intermolecular interactions and reduced the coefficient of friction to approximately 0.045 under aqueous conditions. In addition, phenolic groups in TA can undergo oxidation upon exposure to air to form quinone species capable of reacting with amino groups in CS through possible Schiff-base chemistry [30, 31]. These characteristics provide a rationale for using TA/PVA as a surface coating to regulate the hydrophilicity and frictional characteristics of CS/BC membranes while facilitating interactions with the CS-containing substrate.

In this study, CS/BC composite membranes with different BC contents were first prepared and evaluated to identify a substrate with a suitable balance between wet-state strength and deformability. A TA/PVA coating was subsequently introduced onto the selected CS/BC membrane by immersion coating. As illustrated in Fig. 1, intermolecular hydrogen bonding is proposed to contribute to the formation of both the CS/BC substrate and the TA/PVA coating, while possible Schiff-base interactions between oxidized TA-derived quinone groups and amino groups of CS may further contribute to coating–substrate association. Coating time was then used as the principal processing parameter to regulate coating formation and surface characteristics. The effects of coating time on surface morphology, hydrophilicity, friction, swelling behavior, wet-state mechanical properties, and long-term wet-state stability were systematically investigated. Hemocompatibility, cytocompatibility, and L929 fibroblast adhesion were further evaluated, followed by in vivo evaluation of the optimized membrane using a mouse abdominal wall–cecum adhesion model. Overall, this study presents a substrate–coating strategy for constructing a composite membrane that integrates wet-state structural stability with a hydrophilic and low-cell-adhesion surface, showing potential for preventing postoperative tissue adhesion.

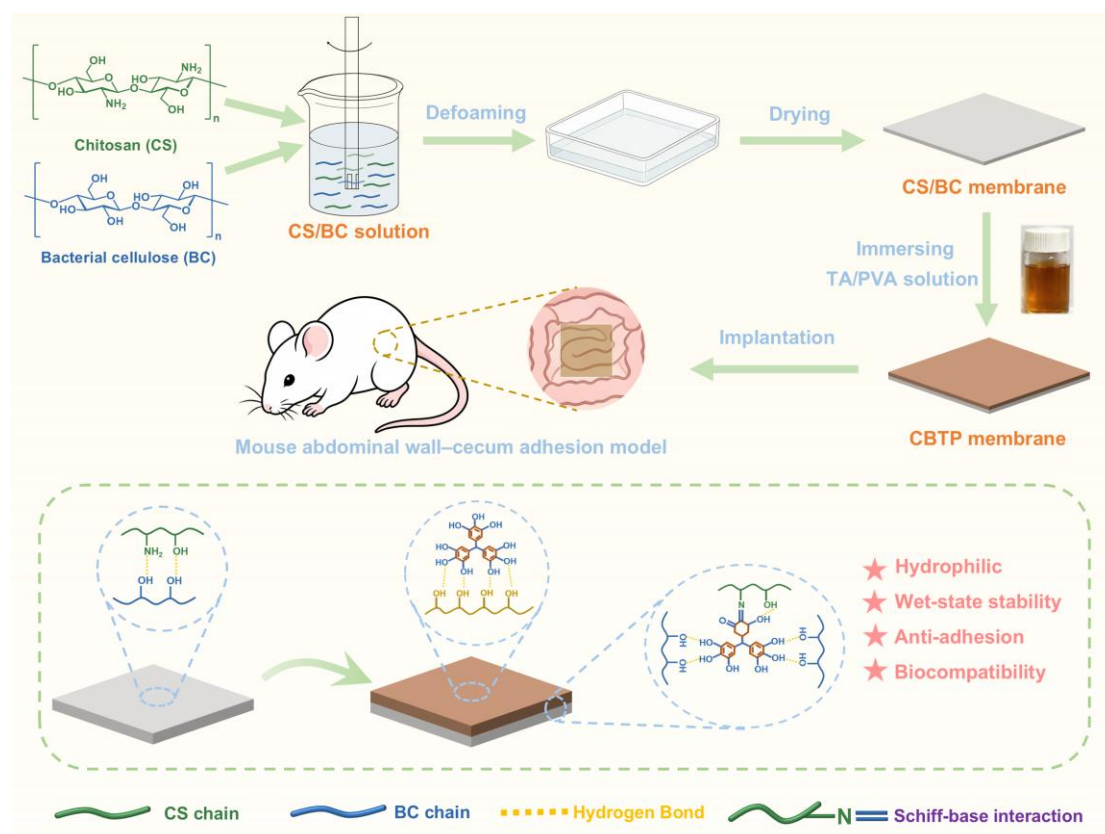


Fig. 1 Schematic illustration of the fabrication of TA/PVA-coated CS/BC composite membrane (CBTP) and intermolecular interactions among the membrane components

Materials and methods

Materials

Chitosan (CS, $M_w = 300$ kDa) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Bacterial cellulose (BC, BR) was obtained from Guilin Qihong Technology Co., Ltd. (Guilin, China). Tannic acid (TA, 99%) and poly(vinyl alcohol) (PVA, 1799 type) were purchased from Beijing Kaiguo Technology Co., Ltd. (Beijing, China) and Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China), respectively. Glacial acetic acid, sodium hydroxide (NaOH), and absolute ethanol were obtained from Beijing Bailingwei Technology Co., Ltd. (Beijing, China). Phosphate-buffered saline (PBS, pH 7.4) was purchased from Haikelong Biotechnology Co., Ltd. (Beijing, China). All reagents were of analytical grade and used as received without further purification.

Preparation of CBTP membranes

CS/BC composite membranes were prepared by a solution-casting method. Briefly, 1.0 g of chitosan was dissolved in 99.0 g of 1 wt% acetic acid solution under magnetic stirring for 3 h. CS and BC were then mixed at mass ratios of 97:3, 95:5, 93:7, 90:10, 85:15, 80:20, and 75:25. The suspensions were diluted with deionized water to a total polymer concentration of 0.5 wt%, homogenized at 20,000 rpm for 5 min, and degassed under vacuum. Subsequently, 100 mL of each suspension was cast into Petri dishes and dried at 50 °C for 24 h. The dried membranes were immersed in 1 mol L⁻¹ NaOH solution to neutralize residual acetic acid, washed repeatedly with

deionized water until neutral, and dried at room temperature. The composite membranes were denoted as CB3, CB5, CB7, CB10, CB15, CB20, and CB25 according to the BC content. Pure CS and BC membranes were prepared using the same procedure. Based on their physicochemical and mechanical properties, CB10 was selected as the substrate for subsequent coating modification.

A TA/PVA coating was then introduced onto CB10 membranes by an immersion-assisted coating process. To prepare the coating solution, 3.0 g of TA and 3.0 g of PVA were dissolved in 94.0 g of 50 wt% aqueous ethanol under stirring at 90 °C for 6 h. CB10 membranes were immersed in the coating solution and maintained at 50 °C for 6, 12, 18, or 24 h under continuous stirring. After coating, the membranes were removed and dried at room temperature. The resulting membranes were denoted as CBTP6, CBTP12, CBTP18, and CBTP24 according to the coating time.

Structural characterization of CBTP membranes

The chemical structures of the membranes were characterized by Fourier transform infrared spectroscopy (FTIR; Nicolet iS50, Thermo Fisher Scientific, USA) in attenuated total reflectance (ATR) mode over the wavenumber range of 4000–400 cm^{-1} . The surface and cross-sectional morphologies of the membranes were observed using scanning electron microscopy (SEM; Sigma 360, Carl Zeiss, Germany). Before SEM observation, dried membrane samples were cut into appropriate sizes, mounted on conductive adhesive tape, and sputter-coated with gold. X-ray photoelectron spectroscopy (XPS; ESCALAB 250Xi, Thermo Fisher Scientific, USA) was used to analyze the elemental composition and surface chemical states of the membranes.

Surface hydrophilicity test

The surface hydrophilicity of the membranes was evaluated by static water contact angle measurements using a contact angle analyzer (CA200, China) at room temperature. Membrane samples were fixed on glass slides, and a 4 μL droplet of deionized water was deposited onto the membrane surface. The contact angle was recorded after droplet stabilization, and measurements were performed at three randomly selected positions on each sample. The average value was calculated as the final contact angle. To evaluate the stability of surface hydrophilicity under aqueous conditions, the membranes were immersed in PBS (pH 7.4) at 37 °C for 7, 14, and 28 days. After incubation, the samples were gently rinsed with deionized water, and the water contact angle was measured using the same procedure.

Coefficient of friction test

The frictional properties of the membranes were evaluated using a multifunctional friction coefficient tester (HKE-3720H, China) under conditions at room temperature. The membrane samples were securely mounted on the testing platform and tested against a glass counterface under a normal load of 2 N. The sliding speed and sliding distance were set to 300 mm min^{-1} and 30 mm, respectively. Each sample was subjected to 100 consecutive sliding cycles, during which the dynamic coefficient of friction was continuously recorded. Three replicate tests were performed for each group, and the mean dynamic coefficient of friction was calculated. The coefficient of friction was used to characterize the frictional behavior of the TA/PVA-modified membrane surfaces under repeated sliding.

Swelling ratio test

The swelling behavior of the membranes was evaluated under simulated physiological conditions. Membrane samples were cut into 20 mm × 20 mm specimens, and their initial volumes in the dry state were measured and recorded as V_0 . The samples were then completely immersed in PBS (pH 7.4) and incubated at 37 °C for 24 h. After incubation, the samples were removed from the solution, and excess liquid on the surface was gently removed using filter paper. The swollen volume was subsequently measured and recorded as V_1 . At least three independent specimens were tested for each group. The volumetric swelling ratio can be expressed as equation (1):

$$\text{Swelling ratio (\%)} = \frac{V_1 - V_0}{V_0} \times 100\% \quad (1)$$

Tensile property test

Tensile property of the membranes was measured using a universal testing machine (HT-101S, China). The membranes were cut into dumbbell-shaped specimens, and specimen thickness was measured before testing. Tensile tests were performed at room temperature at a crosshead speed of 50 mm min⁻¹, and tensile strength and elongation at break were recorded. For wet-state measurements, the specimens were immersed in PBS (pH 7.4) for 24 h, gently blotted to remove excess surface liquid, and immediately tested. At least three independent specimens were analyzed for each group.

Thermogravimetric analysis

The thermal stability of the membranes was evaluated by thermogravimetric analysis (TGA). Approximately 10 mg of each membrane sample was placed in an alumina crucible and heated from room temperature to 600 °C under a nitrogen atmosphere at a heating rate of 10 °C min⁻¹. The changes in sample mass as a function of temperature were recorded to characterize the thermal degradation behavior of the membranes.

Long-term wet-state stability test

The long-term wet-state stability of the membranes was evaluated by monitoring their mass retention during incubation in PBS. Membrane samples were cut into 20 mm × 20 mm specimens, dried to a constant mass, and weighed to obtain the initial dry mass (M_0). The samples were completely immersed in PBS (pH 7.4) and incubated in a thermostatic shaker at 37 °C and 40 rpm for 35 days. The PBS solution was refreshed weekly to maintain stable incubation conditions. At predetermined time points (1, 2, 3, 5, 7, 14, 21, 28, and 35 days), the samples were collected, gently rinsed with deionized water to remove residual salts, dried to a constant mass, and weighed (M_n). The mass retention was calculated as equation (2):

$$\text{Mass retention (\%)} = \frac{M_n - M_0}{M_0} \times 100\% \quad (2)$$

Biocompatibility test

(1) Hemolysis assay

The hemocompatibility of the membranes was assessed by a hemolysis assay using anticoagulated rat blood. After centrifugation at 2000 rpm for 10 min, the erythrocytes were collected and washed repeatedly with normal saline. The washed erythrocytes were then diluted with normal saline to prepare a 4% (v/v) suspension. Membrane specimens of 10 mm × 10 mm were placed in 48-well

plates, and each specimen was incubated with 500 μL of erythrocyte suspension and 500 μL of normal saline at 37 $^{\circ}\text{C}$ for 1 h. For the negative and positive controls, 500 μL of erythrocyte suspension was mixed with an equal volume of normal saline or 2% (v/v) Triton X-100 solution, respectively. After incubation, the suspensions were centrifuged at 1500 rpm for 10 min, and the absorbance of the supernatant was measured at 540 nm using a microplate reader (SpectraMax iD3, USA). The hemolysis ratio was calculated as equation (3):

$$\text{Hemolysis ratio (\%)} = \frac{OD_{\text{sample}} - OD_{\text{negative}}}{OD_{\text{positive}} - OD_{\text{negative}}} \times 100\% \quad (3)$$

where OD_{sample} is the absorbance of sample group, OD_{negative} is the absorbance of negative control group and OD_{positive} is the absorbance of positive control group.

(2) Cytocompatibility assay

The cytocompatibility of the membranes was evaluated using L929 mouse fibroblasts and a Cell Counting Kit-8 (CCK-8) assay. Membrane specimens of 10 mm $\times$ 10 mm were sterilized by ultraviolet irradiation and incubated in 1 mL of complete culture medium at 37 $^{\circ}\text{C}$ for 24 h to prepare membrane extracts. The extracts were collected and sterilized by filtration before use. L929 fibroblasts were seeded into 96-well plates at a density of 1×10^5 cells mL^{-1} , with 100 μL of cell suspension added to each well, and cultured for 24 h. The culture medium was then replaced with 100 μL of membrane extract, followed by incubation for 72 h. Cells maintained in fresh complete medium served as the control, while wells containing culture medium without cells were used as blanks. After 72 h of exposure, the test solutions were removed and replaced with 100 μL of fresh medium containing 10% (v/v) CCK-8 reagent, followed by incubation for 2 h at 37 $^{\circ}\text{C}$. The absorbance was subsequently measured at 450 nm using a microplate reader (SpectraMax iD3, USA). Cell viability was calculated as equation (4):

$$\text{Relative cell viability (\%)} = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100\% \quad (4)$$

where OD_{sample} is the absorbance of sample group, OD_{control} is the absorbance of untreated cell control group and OD_{blank} is the absorbance of the blank group containing medium and CCK-8 without cells.

In vitro fibroblast adhesion assay

Fibroblast attachment has also been used as an in vitro indicator for evaluating the anti-adhesive surface characteristics of barrier materials [22]. The interaction between L929 fibroblasts and membrane surfaces was assessed by quantifying the cells retained on the membranes after incubation. Briefly, membrane discs (10 mm in diameter) were sterilized by ultraviolet irradiation and placed in 24-well plates. L929 fibroblasts were prepared in complete culture medium at a concentration of 1×10^5 cells mL^{-1} and introduced onto the membrane surfaces. After incubation for 72 h at 37 $^{\circ}\text{C}$ under 5% CO_2 , the culture medium was renewed every 48 h. Cell-free wells and wells containing fibroblasts cultured without membranes were designated as blank and control groups, respectively. At the end of incubation, the membranes were gently rinsed with sterile medium to eliminate loosely attached cells. The remaining cells on the membranes were quantified using the CCK-8 assay by adding fresh medium containing 10% (v/v) CCK-8 reagent and incubating for 2 h. The absorbance was recorded at 450 nm using a microplate reader (SpectraMax iD3, USA). The relative cell adhesion ratio was calculated as equation (5):

$$\text{Relative cell adhesion ratio (\%)} = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100\% \quad (5)$$

In vivo anti-adhesion evaluation

All animal experiments were conducted in accordance with the guidelines of the Ethics Committee of the Chinese Academy of Sciences and approved by the same committee. The in vivo anti-adhesion potential and local tissue response of the optimized membrane were evaluated using a mouse abdominal wall–cecum adhesion model. Similar abdominal wall–cecum injury models have been widely used for evaluating postoperative anti-adhesion barriers in vivo [32–34]. Twelve healthy mice were randomly assigned to two groups ($n = 6$ per group): the control group without membrane implantation and the CBTP18 group implanted with the optimized membrane. Circular CBTP18 membrane specimens (15 mm in diameter) were sterilized by ultraviolet irradiation and immersed in sterile normal saline before implantation. After anesthesia, a surgical injury model was established by creating a petechial bleeding area on the cecal surface through gentle abrasion, followed by generating a corresponding injury area on the opposing abdominal wall (approximately 10 mm $\times$ 10 mm). In the CBTP18 group, the membrane was placed between the injured surfaces and fixed with one suture to prevent displacement. The control group underwent the same injury procedure without membrane implantation. The animals were monitored daily after surgery. Fifteen days after implantation, the abdominal cavity was reopened, and the formation of adhesions, membrane integrity, and interaction between the implanted membrane and surrounding tissues were visually examined. The severity of adhesion formation was evaluated according to the following grading criteria: 0, no adhesion; 1, mild adhesion that could be easily separated by blunt dissection; 2, moderate adhesion requiring partial sharp dissection; and 3, severe adhesion requiring extensive sharp dissection and accompanied by tissue damage or bleeding [35]. Following macroscopic evaluation, the tissues surrounding the implantation sites were harvested for histological analysis. After fixation in paraformaldehyde, the samples were processed, sectioned, and stained with hematoxylin and eosin (H&E). The stained sections were examined microscopically to evaluate tissue morphology and local inflammatory responses.

Statistical analysis

All quantitative data were expressed as the mean $\pm$ standard deviation (SD) from at least three independent experiments ($n \geq 3$), unless otherwise specified. Statistical analyses were performed using GraphPad Prism 11 software. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used for comparisons among multiple groups where applicable. A value of $p < 0.05$ was considered statistically significant. Statistical significance was denoted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Results and discussion

Optimization of the CS/BC substrate

CS/BC composite membranes with different BC contents were first evaluated to identify a suitable substrate for subsequent TA/PVA modification. Supplementary FTIR, XRD, and SEM analyses showed composition-dependent structural and morphological changes following the incorporation of BC (Fig. S1 and S2). Increasing BC content markedly enhanced the wet-state tensile strength while progressively reducing the elongation at break (Fig. S3a, b). The wet-state tensile strength

increased from 6.8 ± 0.4 MPa for pure CS to 36.6 ± 1.1 MPa for CB10, whereas the elongation at break decreased from $85\% \pm 1\%$ to $30\% \pm 1\%$. Further increasing the BC content increased the tensile strength to 46.8 ± 1.0 MPa for CB20, but this was accompanied by a further decrease in elongation at break to $20\% \pm 1\%$. Meanwhile, water absorption and swelling decreased markedly as the BC content increased up to 10 wt%, whereas further increases in BC content produced only limited additional changes in these properties (Fig. S3c, d). SEM observations also showed increasingly dense fibrous structures at higher BC contents (Fig. S2). Taken together, these results indicate that the incorporation of BC improved the wet-state mechanical strength and dimensional stability of the CS membrane, while excessive BC incorporation was accompanied by reduced deformability. Therefore, CB10 was selected as the substrate for subsequent surface modification based on its balanced wet-state strength, deformability, and dimensional stability.

Chemical characterization of CBTP membranes

The formation of the TA/PVA coating on the CS/BC membrane was first investigated by FTIR spectroscopy. As shown in Fig. 2, the CB10 membrane exhibited the characteristic absorption bands of the CS/BC substrate. After TA/PVA modification, additional absorption bands appeared at approximately 1714 cm^{-1} and 1607 cm^{-1} , which were attributed to carbonyl and aromatic skeletal vibrations associated with TA, respectively. Changes were also observed around 1440 cm^{-1} , which may arise from overlapping contributions of aromatic-ring vibrations of TA and CH_2 bending vibrations of PVA and the polysaccharide matrix. These spectral changes were consistently observed in the coated membranes, supporting the successful introduction of TA/PVA components onto the CS/BC substrate. Compared with CB10, the coated membranes exhibited enhanced absorption in the broad $3200\text{--}3600\text{ cm}^{-1}$ region, which was associated with the abundant hydroxyl groups introduced by TA and PVA and with changes in the hydrogen-bonding environment after coating modification [27, 28]. In addition, the C–O-related absorption band of CB10 shifted from approximately 1066 cm^{-1} toward around 1024 cm^{-1} after coating, suggesting changes in the local chemical environment arising from intermolecular interactions among TA, PVA, and the CS/BC substrate.

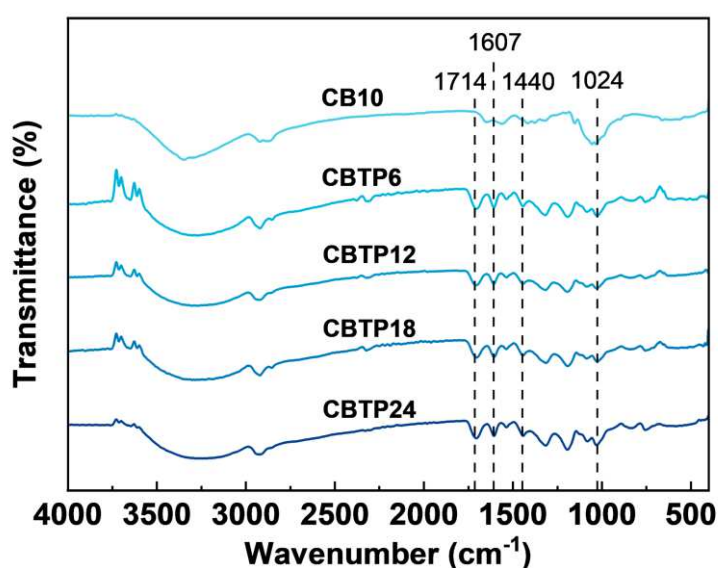


Fig. 2 FTIR spectra of CB10 and CBTP membranes prepared at different coating times

The surface chemical states of the membranes were further analyzed by XPS. Since the coated membranes exhibited similar XPS characteristics, CBTP18 was selected as a representative sample for detailed analysis (Fig. 3). For CB10, the high-resolution C 1s spectrum was deconvoluted into three peaks at binding energies of approximately 284.8, 286.3, and 288.6 eV, assigned to C–C, C–O/C–N, and C=O bonds, respectively. The N 1s spectrum of CB10 exhibited a predominant peak at approximately 400.0 eV, corresponding to C–N bonds. Following TA/PVA modification, the high-resolution C 1s spectrum of CBTP18 showed peaks at similar binding energies, while changes in their relative intensities indicated an altered surface chemical environment. More notably, the N 1s spectrum of CBTP18 could be deconvoluted into two peaks at approximately 400.0 and 402.0 eV, assigned to C–N and C=N-related bonds, respectively. The appearance of the new C=N peak after modification suggests possible Schiff-base formation between quinone groups generated by oxidation of TA and amino groups of CS [30, 31]. Together with the FTIR results, the XPS analysis confirms the successful formation of the TA/PVA coating on the CS/BC membrane and supports interactions between the coating and substrate.

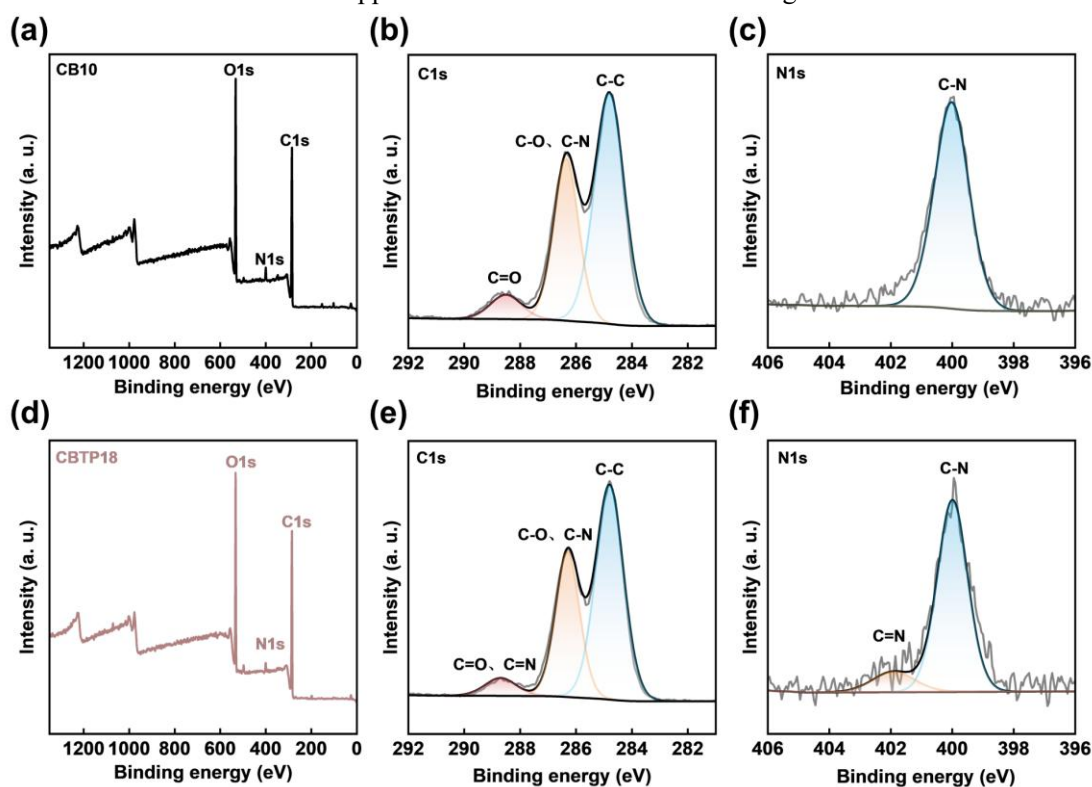


Fig. 3 XPS analysis of CB10 and CBTP18 membranes. (a, d) Survey spectra; (b, e) high-resolution C 1s spectra; and (c, f) high-resolution N 1s spectra

Surface and cross-sectional morphology of CBTP membranes

The surface and cross-sectional morphologies of the membranes were examined by scanning electron microscopy (SEM) to evaluate the formation and integrity of the TA/PVA coating. As shown in Fig. 4a–e, the unmodified CB10 membrane exhibited a relatively rough surface with visible fibrous structures, which were mainly associated with the BC nanofibrous network within the CS/BC matrix. After TA/PVA modification, the characteristic fibrous features were gradually covered, and the membrane surfaces became smoother and more homogeneous. No obvious

cracks, pores, or surface defects were observed on the coated membranes, indicating the formation of a continuous TA/PVA coating layer on the CS/BC membrane. The cross-sectional SEM images further confirmed the successful construction of the surface coating (Fig. 4f–j). Compared with the unmodified CB10 membrane, all CBTP membranes exhibited a distinct surface layer with a thickness of several micrometers. The coating layer appeared to be well integrated with the underlying CS/BC substrate, with no apparent interfacial gaps or delamination. Notably, the coating thickness was relatively small compared with the overall membrane thickness, suggesting that TA/PVA modification mainly occurred at the membrane surface without significantly altering the bulk architecture of the CS/BC membrane. The continuous and well-integrated coating structure observed by SEM was consistent with the FTIR results, further confirming the successful formation of the TA/PVA layer on the CS/BC membrane. The intimate contact between the coating and substrate may be attributed to the multiple intermolecular interactions among CS, BC, TA, and PVA, which contributed to the stable attachment of the coating layer. Overall, SEM observations showed that TA/PVA modification produced a continuous surface coating that remained well integrated with the underlying CS/BC substrate without substantially altering the bulk membrane structure.

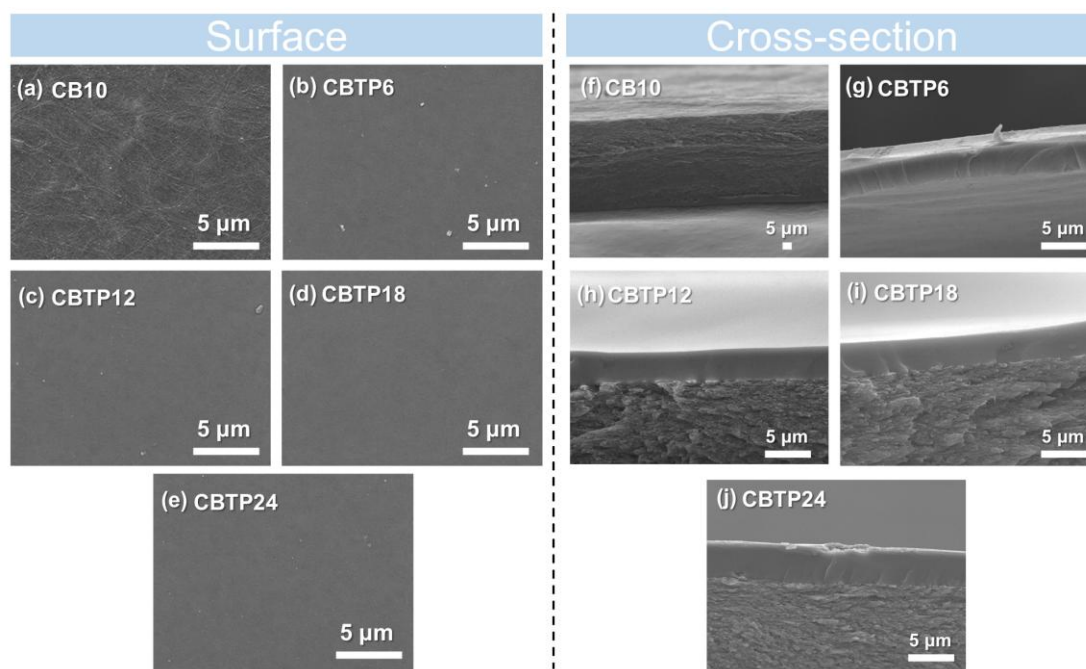


Fig. 4 Surface and cross-sectional SEM images of CB10 and CBTP membranes. (a–e) Surface morphologies and (f–j) cross-sectional morphologies of CB10, CBTP6, CBTP12, CBTP18, and CBTP24 membranes, respectively. Scale bar = 5 μm

Physicochemical properties of CBTP membranes

TA/PVA coating markedly altered the surface properties of CB10. As shown in Fig. 5a, the water contact angle decreased from $105.5^\circ \pm 0.6^\circ$ for CB10 to $37.4^\circ \pm 1.0^\circ$ for CBTP18, with only a slight further reduction to $34.5^\circ \pm 1.0^\circ$ after extending the coating time to 24 h. This enhanced hydrophilicity was attributed to the hydroxyl-rich TA/PVA coating. A similar coating-time-dependent trend was observed for the coefficient of friction (Fig. 5b), which decreased from 0.133 ± 0.001 for CB10 to 0.042 ± 0.001 for CBTP18 and showed little further

reduction for CBTP24 (0.041 ± 0.003). The marked decrease in the coefficient of friction indicates that TA/PVA modification substantially altered the surface frictional characteristics of the CS/BC membrane. The reduction became limited beyond approximately 18 h of coating, suggesting that the effect of coating time on friction approached a plateau. This behavior may be related to the smoother and more homogeneous surface formed after TA/PVA coating. The swelling ratio decreased after coating modification, from $7.6\% \pm 0.1\%$ for CB10 to $4.4\% \pm 0.1\%$ for CBTP18 and $2.9\% \pm 0.3\%$ for CBTP24 (Fig. 5c). The suppressed swelling may be related to the compact coating structure and enhanced intermolecular interactions that restricted polymer-chain mobility [37, 38].

Importantly, the improved surface properties were achieved without substantial deterioration of wet-state mechanical performance (Fig. 5d). CB10 exhibited a tensile strength of 36.6 ± 1.1 MPa and elongation at break of $30\% \pm 1\%$, while CBTP18 retained values of 33.8 ± 1.1 MPa and $29\% \pm 1\%$, corresponding to approximately 92% and 95% of the original tensile strength and elongation, respectively. Even after 24 h of coating, the membrane maintained a tensile strength of 33.3 ± 0.9 MPa. Overall, increasing coating time progressively enhanced surface hydrophilicity and reduced friction while largely maintaining the wet-state mechanical performance of the CS/BC substrate. Since the improvements in contact angle and friction coefficient became limited beyond 18 h, whereas CBTP18 retained slightly better mechanical properties than CBTP24, CBTP18 was selected for subsequent biological evaluation.

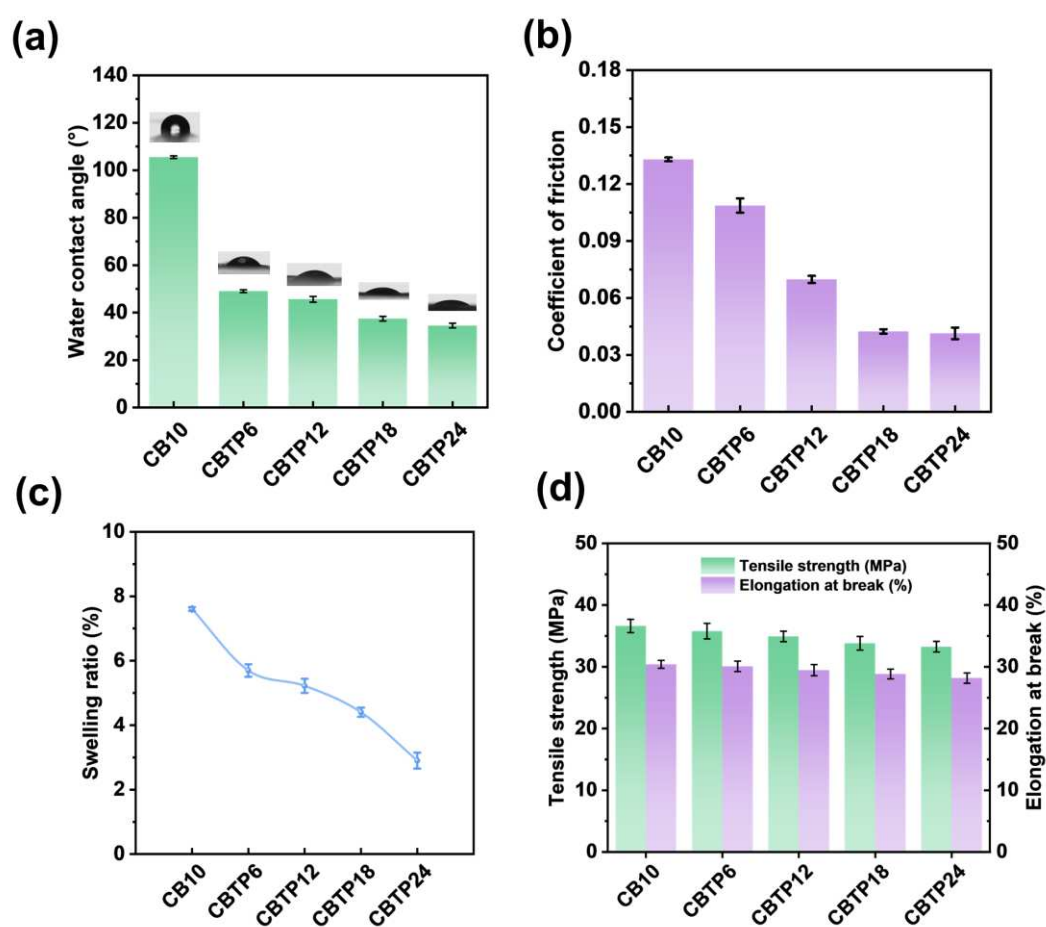


Fig. 5 Physicochemical properties of CB10 and CBTP membranes. (a) Water contact angle; (b) coefficient of friction; (c) swelling ratio; and (d) wet-state tensile strength and elongation at break

3.5 Thermal stability of CBTP membranes

The thermal stability of CBTP membranes was evaluated by thermogravimetric analysis (TGA). As shown in Fig. 6, all membranes exhibited similar multistage weight-loss profiles. The initial weight loss below approximately 150 °C was mainly attributed to the evaporation of physically adsorbed and bound water within the hydrophilic polymer network. The second stage corresponded to the major thermal decomposition of the membrane components. For CB10, the main decomposition process started at approximately 246 °C, with the maximum decomposition rate occurring at approximately 273 °C, which was associated with the degradation of the CS/BC polysaccharide framework. Compared with CB10, the CBTP membranes exhibited an earlier onset of weight loss at approximately 184 °C, while their maximum decomposition temperatures remained comparable (approximately 280 °C). The earlier weight loss was likely associated with the thermal degradation of the introduced TA/PVA components. However, the comparable maximum decomposition temperature suggested that the main thermal stability of the CS/BC framework was maintained after coating modification. No obvious difference was observed among CBTP6, CBTP12, CBTP18, and CBTP24, indicating that coating time had limited influence on the overall thermal degradation behavior. At higher temperatures, the further weight loss above approximately 350 °C was mainly associated with the gradual decomposition and carbonization of residual polymer structures. The onset temperatures of this stage were approximately 352 °C for CB10 and 345 °C for CBTP membranes. The residual mass decreased from approximately 56.5% for CB10 to 47.3% for CBTP membranes, reflecting differences in composition and carbonization behavior after TA/PVA incorporation. Overall, the TA/PVA coating resulted in a slight shift in the thermal degradation behavior of the CS/BC membrane while maintaining its intrinsic thermal stability.

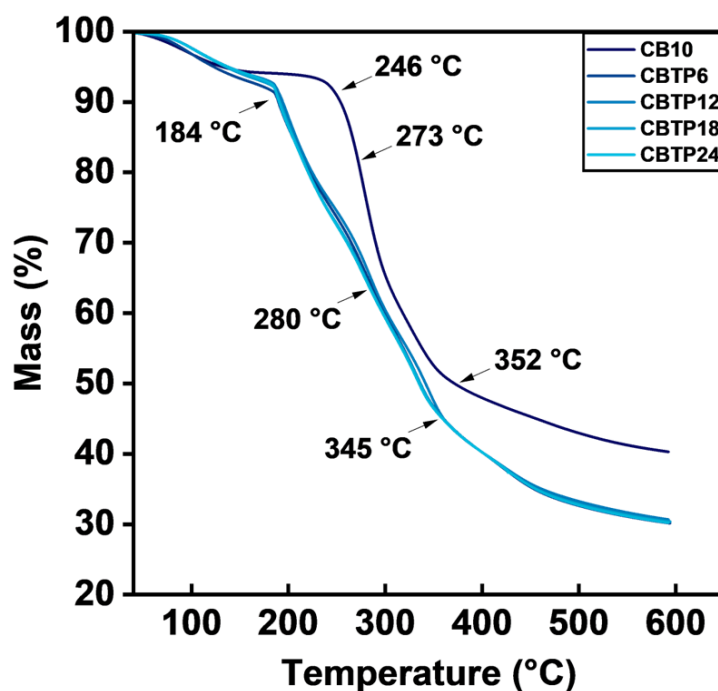


Fig. 6 TGA curves of CB10 and CBTP membranes prepared at different coating times

Stability of surface hydrophilicity after PBS incubation

The stability of the TA/PVA coating under prolonged aqueous exposure was evaluated by monitoring changes in water contact angle during PBS incubation. As shown in Fig. 7, all CBTP membranes maintained substantially lower contact angles than the unmodified CB10 membrane throughout the 28-day incubation period, although small fluctuations were observed over time. For example, the water contact angle of CBTP6 increased from $49.0^\circ \pm 0.6^\circ$ initially to $52.3^\circ \pm 0.6^\circ$ after 28 days. CBTP18 showed an even smaller change, from $37.4^\circ \pm 1.0^\circ$ to $39.3^\circ \pm 0.4^\circ$, indicating good retention of surface hydrophilicity during prolonged immersion. CBTP24 remained highly hydrophilic despite a moderate increase in contact angle from $34.6^\circ \pm 1.0^\circ$ to $39.1^\circ \pm 1.0^\circ$. Overall, the changes in contact angle were limited, suggesting that the hydrophilic character introduced by the TA/PVA coating was largely maintained under aqueous conditions. The slight variation in contact angle may be related to hydration-induced rearrangement of the surface components and changes in intermolecular interactions within the coating during PBS exposure. Water molecules can interact with the hydroxyl-rich TA/PVA layer and partially alter the organization of the surface without eliminating its hydrophilic character. Therefore, the contact-angle results provide indirect evidence that the modified surface remained functionally stable during long-term immersion. Among the coated membranes, CBTP18 exhibited the smallest overall change in water contact angle over 28 days. Together with its favorable lubrication, swelling behavior, and wet-state mechanical properties, this result further supports the selection of CBTP18 for subsequent biological evaluation.

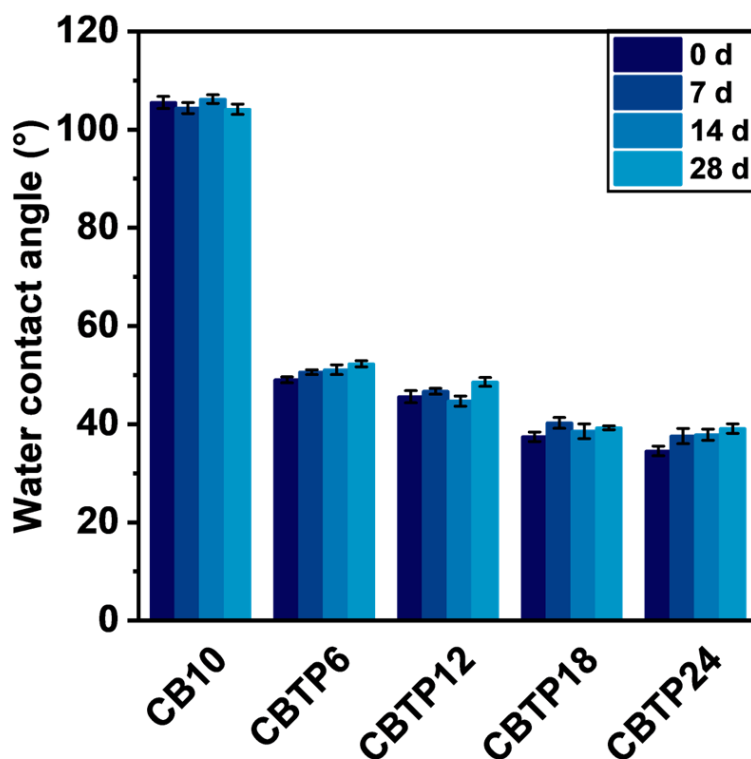


Fig. 7 Changes in water contact angles of CB10 and CBTP membranes after immersion in PBS at 37 °C for 0, 7, 14, and 28 days

Long-term wet-state stability of CBTP membranes

The long-term wet-state stability of CBTP membranes was evaluated by monitoring mass retention during incubation in PBS at 37 °C for 35 days. As shown in Fig. 8, all membranes exhibited a gradual decrease in mass retention with increasing incubation time. A relatively faster mass loss occurred during the initial stage, followed by a slower decrease during prolonged immersion. During the first 7 days of immersion, CBTP18 retained $85.4\% \pm 0.3\%$ of its initial mass, while CB10 retained $89.2\% \pm 0.4\%$. The relatively greater early mass loss of the CBTP membranes may be related to the initial interaction between the hydrophilic TA/PVA coating and the aqueous environment during PBS immersion. With prolonged immersion, the mass retention of CBTP18 gradually decreased to $70.7 \pm 0.6\%$ after 35 days, compared with $76.4\% \pm 0.8\%$ for CB10. The subsequent slower decrease in mass retention indicated that the membranes gradually approached a relatively stable state during prolonged PBS exposure. Notably, all CBTP membranes exhibited similar overall mass-retention trends despite differences in coating time. After 35 days, the mass retention values of CBTP6, CBTP12, CBTP18, and CBTP24 were $71.9\% \pm 0.6\%$, $69.3\% \pm 0.5\%$, $70.7\% \pm 0.6\%$, and $71.5\% \pm 0.61\%$, respectively. Although slightly lower than that of CB10, all CBTP membranes retained approximately 70% of their initial mass after five weeks of immersion. The narrow variation among the CBTP groups further indicated that extending the coating time from 6 to 24 h slightly affected their long-term mass-retention behavior. Overall, the CBTP membranes maintained substantial mass retention during prolonged PBS exposure, indicating that TA/PVA modification did not result in rapid loss of membrane integrity under aqueous conditions. Considering its balanced surface properties, wet-state mechanical performance, and long-term stability, CBTP18 was selected for subsequent biological evaluation.

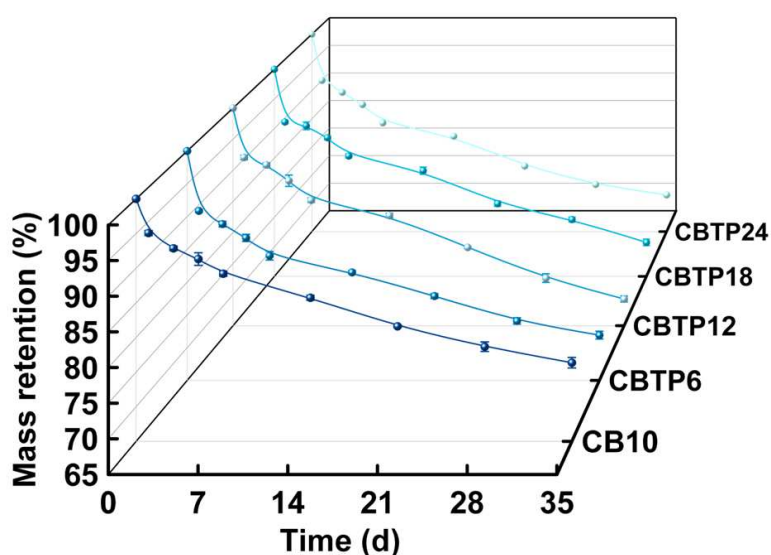


Fig. 8 Mass retention of CB10 and CBTP membranes after incubation in PBS for 35 days

In vitro biocompatibility of CBTP membranes

The in vitro biocompatibility of CBTP membranes was evaluated by hemolysis and CCK-8 assays to assess their hemocompatibility and cytocompatibility, respectively. As shown in Fig. 9a, all CBTP membranes exhibited hemolysis ratios below 5%, indicating good hemocompatibility under the experimental conditions. No obvious increase in hemolysis was observed after TA/PVA modification, suggesting that the coating did not adversely affect the blood compatibility of the CS/BC substrate. The cytocompatibility of the membranes was further assessed using L929 fibroblasts cultured with membrane extracts. As shown in Fig. 9b, all CBTP groups maintained relative cell viabilities above 70% after 72 h of incubation. These results demonstrate that TA/PVA-modified membranes exhibited favorable cytocompatibility and did not induce apparent adverse effects on cell survival under the tested conditions. Overall, the low hemolysis ratios and favorable cell viability indicate that TA/PVA modification did not adversely affect the biocompatibility of the CS/BC membrane. Together with the improved surface properties and mechanical stability, these results support the potential application of CBTP membranes as postoperative anti-adhesion barrier materials.

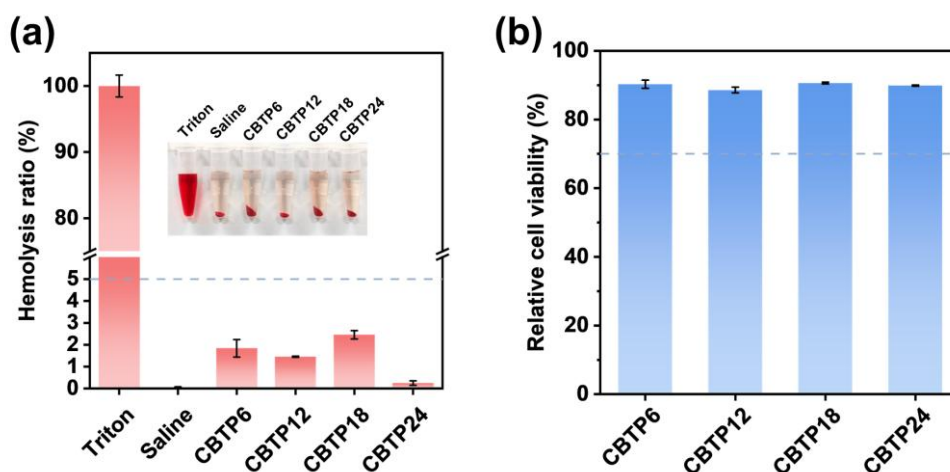


Fig. 9 In vitro biocompatibility of CBTP membranes: (a) hemolysis ratio and (b) relative cell viability of CBTP membranes

In vitro fibroblast adhesion evaluation of CBTP membranes

The adhesion behavior of L929 fibroblasts on the membrane surfaces was evaluated to investigate whether TA/PVA coating could reduce fibroblast attachment to the CS/BC membrane. As shown in Fig. 10a, the amount of cells retained on the membrane surfaces gradually decreased with increasing coating time, as indicated by the reduced CCK-8 absorbance. A similar trend was observed for the relative fibroblast adhesion ratio (Fig. 10b). CB10 exhibited an adhesion ratio of $38.8\% \pm 2.4\%$, whereas the values decreased to $18.1\% \pm 2.3\%$ for CBTP18 and $14.4\% \pm 1.9\%$ for CBTP24. Compared with CB10, CBTP18 showed an approximately 53% reduction in fibroblast adhesion. The decreased fibroblast attachment was consistent with the enhanced surface hydrophilicity and reduced friction after TA/PVA modification. A more hydrophilic surface may weaken the stable interaction between fibroblasts and the membrane interface, thereby reducing cell retention on the material surface [22]. Since all CBTP membranes exhibited favorable cytocompatibility in the extract-based CCK-8 assay, the reduced fibroblast adhesion was mainly associated with the altered surface characteristics rather than impaired cell viability. Although CBTP24 exhibited the lowest fibroblast adhesion ratio, the further reduction compared with

CBTP18 was relatively limited. Considering the comprehensive performance of the membranes, CBTP18 provided a better balance among surface hydrophilicity, lubrication, swelling resistance, wet-state mechanical properties, long-term aqueous stability, and biocompatibility. Therefore, CBTP18 was selected for subsequent *in vivo* anti-adhesion evaluation. Overall, the TA/PVA coating effectively reduced fibroblast attachment to the CS/BC membrane, demonstrating its potential as a surface modification strategy for postoperative anti-adhesion barriers.

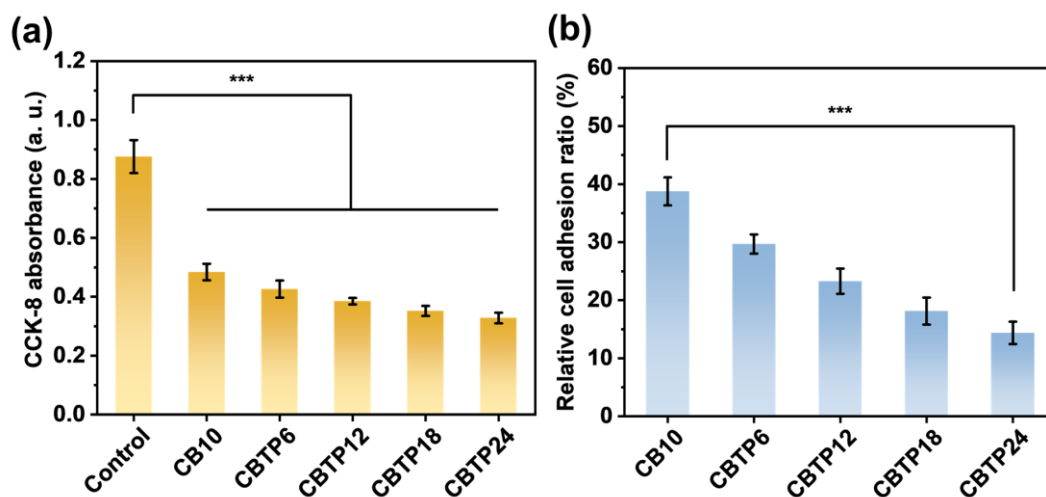


Fig. 10 L929 fibroblast response on CB10 and CBTP membranes after 72 h of culture: (a) CCK-8 absorbance at 450 nm and (b) relative cell adhesion ratio.

In vivo evaluation of the anti-adhesion performance of CBTP18

The *in vivo* anti-adhesion performance and local tissue response of the optimized CBTP18 membrane were evaluated using a mouse abdominal wall–cecum abrasion model. During the 15-day implantation period, all animals remained healthy, with normal activity and satisfactory wound healing, and no obvious adverse reactions were observed. At the end of the implantation period, the abdominal cavity was reopened to evaluate postoperative adhesion formation. As shown in Fig. 11a and b, adhesions of varying severity were observed in the untreated control group. Among the six control animals, two showed no adhesion (score 0), one developed mild adhesion (score 1), and three developed moderate adhesion (score 2) requiring partial sharp dissection. Thus, postoperative adhesions occurred in four of the six animals (66.7%) in the control group. In contrast, no visible adhesion was observed in any of the six animals treated with CBTP18, with all animals receiving an adhesion score of 0 (Table 1). These findings demonstrate a marked reduction in both the incidence and severity of postoperative adhesion following CBTP18 implantation. In addition to the reduced adhesion formation, the implanted CBTP18 membranes remained macroscopically intact without apparent rupture or deformation at the time of evaluation. This observation is consistent with the favorable wet-state mechanical properties and long-term aqueous stability demonstrated *in vitro*, and suggests that the membrane retained sufficient structural integrity to maintain physical separation of the injured tissue surfaces during the postoperative period. Histological examination was further performed to evaluate the local tissue response (Fig. 11c and d). Representative H&E-stained sections showed generally preserved tissue architecture in the CBTP18 group without obvious histopathological abnormalities around the implantation site. Inflammatory-cell infiltration appeared less pronounced in the

CBTP18-treated tissues than in the untreated control tissues. These findings, together with the absence of obvious adverse reactions during implantation, indicate favorable local tissue compatibility of the membrane under the tested conditions. The observed reduction in adhesion formation may be attributed to the complementary barrier and interfacial functions of the composite membrane. The BC-reinforced CS substrate provides wet-state mechanical stability and maintains physical separation between the injured tissue surfaces, whereas the TA/PVA-modified surface provides enhanced hydrophilicity, reduced interfacial friction, and decreased fibroblast attachment. These combined characteristics may reduce direct contact between injured tissues and unfavorable cell–material interactions during the early postoperative healing period, thereby contributing to the observed anti-adhesion effect [39, 40]. Overall, the *in vivo* findings, together with the physicochemical and *in vitro* biological results, support the potential of CBTP18 as a postoperative anti-adhesion barrier.

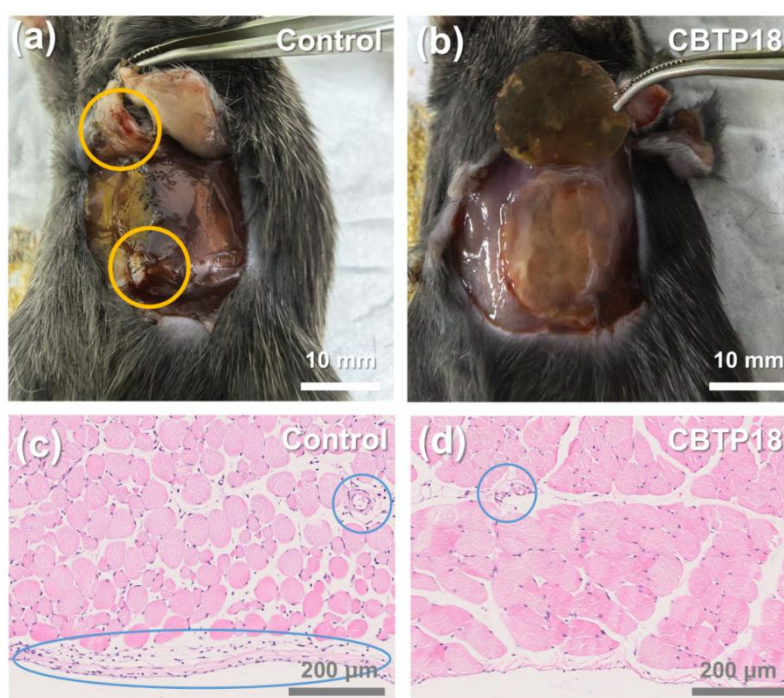


Fig. 11 *In vivo* anti-adhesion performance and local tissue response in a mouse abdominal wall–cecum adhesion model 15 days after surgery. (a, b) Representative macroscopic images of the control and CBTP18 group, respectively (Scale bar = 10 mm). Yellow circles indicate representative adhesion sites in the control group. (c, d) Representative H&E-stained sections of tissues from the control and CBTP18 group, respectively (Scale bar = 200 μm). Blue circles indicate representative regions of local tissue response

Table 1 Distribution of adhesion scores in the control and CBTP18 group 15 days after surgery

Group	n	Score 0, n	Score 1, n	Score 2, n	Score 3, n	Adhesion incidence
Control	6	2	1	3	0	66.7%
CBTP18	6	6	0	0	0	0

Note: Adhesion incidence was defined as the presence of any macroscopically detectable adhesion

(adhesion score ≥ 1).

Conclusion

In this study, a TA/PVA-modified CS/BC composite membrane was developed through a substrate-coating strategy. The incorporation of BC effectively reinforced the CS matrix, while the TA/PVA coating enhanced surface hydrophilicity and reduced interfacial friction without substantially compromising the wet-state mechanical properties and structural stability of the membrane. Among the coated membranes, CBTP18 achieved a favorable balance among surface properties, mechanical performance, swelling resistance, and long-term wet-state stability. The optimized membrane exhibited favorable hemocompatibility and cytocompatibility and significantly reduced fibroblast attachment compared with the unmodified CS/BC substrate. Furthermore, in a mouse abdominal wall-cecum adhesion model, postoperative adhesions occurred in four of six untreated animals, whereas no visible adhesion was observed in any of the six animals treated with CBTP18. These findings support the potential of CBTP18 as a postoperative anti-adhesion barrier and demonstrate that the substrate-coating strategy provides an effective approach for integrating wet-state structural stability with anti-adhesive interfacial properties in polymeric barrier membranes.

Supporting Information The online version contains supplementary material.

Author contributions Ying Liu: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing – original draft preparation. Yuhan Liu: Methodology, investigation, material preparation, characterization, and data analysis. Kaidi Ding: Investigation and data analysis. Jing Zeng: Experimental support and discussion of results. Jingbo Chen: Experimental support, scientific discussion, and writing – review and editing. Jiang Liu: Supervision, project administration, funding acquisition. Xuejia Ding: Conceptualization, supervision, project administration, funding acquisition, and writing – review and editing.

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Data Availability The data used for this study are available from the corresponding authors on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethical Approval All animal experiments were conducted in accordance with the guidelines of the Ethics Committee of the Chinese Academy of Sciences and approved by the same committee. All efforts were made to minimize animal suffering.

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