

Citric Acid-Crosslinked PVA/CMC Composite Membranes with Enhanced Wet-State Stability for Prevention of Postoperative Pericardial Adhesions

Ying Liu

Fengji Wang

Kaidi Ding

Jing Zeng

Jingbo Chen

Xuejia Ding

dingxj@mail.buct.edu.cn

Beijing University of Chemical Technology <https://orcid.org/0000-0003-2201-2208>

Research Article

Keywords: poly(vinyl alcohol), carboxymethyl cellulose, citric acid crosslinking, wet-state stability, mechanical enhancement, pericardial adhesion

Posted Date: September 30th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11016147/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Citric Acid-Crosslinked PVA/CMC Composite Membranes with Enhanced Wet-State Stability for Prevention of Postoperative Pericardial Adhesions

Ying Liu¹ · Fengji Wang² · Kaidi Ding³ · Jing Zeng² · Jingbo Chen¹ · Xuejia Ding²

¹ School of Materials Science & Engineering, Zhengzhou University, Zhengzhou, China.

² Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, Beijing, China.

³ National Institutes for Drug Control, Beijing, China.

Correspondence:

Xuejia Ding: dingxj@mail.buct.edu.cn

Abstract

Maintaining sufficient barrier integrity under wet physiological conditions remains a key challenge for membranes used to prevent postoperative pericardial adhesions. Citric acid content was investigated as a crosslinking parameter to regulate the wet-state stability of PVA/CMC (PCC) composite membranes. Increasing CA content progressively reduced water absorption, volumetric swelling, and mass loss, while substantially improving wet mechanical performance. Among the formulations, PCC15 exhibited the best overall performance, with a swelling ratio of 93%, a wet tensile strength of 6.7 MPa, and an elongation at break of 190%. After 30 days in PBS, PCC15 retained a wet tensile strength of 5.7 MPa, demonstrating sustained mechanical integrity under prolonged wet conditions. All PCC membranes showed hemolysis ratios below 5% and cell viabilities above 70%, with lower fibroblast attachment than the tissue-culture plate control. In a rabbit pericardial adhesion model, PCC15 markedly reduced heart-to-sternum adhesion and collagen deposition without obvious local inflammation. Overall, increasing CA crosslinking effectively improved the wet-state stability of PCC membranes while maintaining favorable flexibility and biocompatibility, supporting their potential as physical barriers for postoperative pericardial adhesion prevention.

Keywords poly(vinyl alcohol) · carboxymethyl cellulose · citric acid crosslinking · wet-state stability · mechanical enhancement · pericardial adhesion.

Introduction

Postoperative pericardial adhesions are a frequent consequence of cardiac surgery and can substantially increase the difficulty and risk of subsequent reoperations [1]. Dense adhesions between the pericardium, sternum, and surrounding tissues obscure anatomical planes and may increase the risk of cardiac or vascular injury and bleeding during repeat sternotomy [2]. Among the strategies developed to reduce postoperative adhesion formation, physical barrier membranes remain attractive because they temporarily separate injured tissue surfaces during postoperative healing [3, 4]. For pericardial applications, however, effective barrier function requires more than

simple tissue separation. The membrane must remain flexible and mechanically intact on the continuously moving cardiac surface while being exposed to blood, exudate, and other physiological fluids [5, 6]. Therefore, maintaining dimensional and mechanical integrity during prolonged exposure to physiological fluids is particularly important for pericardial anti-adhesion membranes.

Poly(vinyl alcohol) (PVA) is a widely used synthetic polymer in biomedical materials because of its good biocompatibility, hydrophilicity, film-forming ability, and tunable mechanical properties [7]. Its abundant hydroxyl groups also provide multiple sites for intermolecular interactions and chemical modification, making PVA suitable for the fabrication of flexible polymeric membranes. Carboxymethyl cellulose (CMC), a water-soluble cellulose derivative, has also been extensively investigated for biomedical applications owing to its favorable biocompatibility, biodegradability, and hydrophilicity [8, 9]. In particular, CMC-containing materials have shown considerable potential as physical barriers for postoperative adhesion prevention. Combining PVA with CMC can integrate the film-forming and mechanical characteristics of PVA with the hydrophilic and anti-adhesion properties of CMC [10, 11]. Accordingly, PVA/CMC-based membranes and hydrogels have been investigated for adhesion prevention in several experimental models [12-14]. These studies have established the feasibility of PVA/CMC systems as anti-adhesion barriers. However, achieving long-term wet-state stability while maintaining sufficient mechanical flexibility remains challenging, particularly for barriers subjected to continuous cardiac motion. This challenge is largely attributed to the intrinsic hydrophilicity of PVA and CMC, which makes their performance highly sensitive to the surrounding aqueous environment. Excessive water uptake may induce pronounced swelling and polymer-chain relaxation, accompanied by loss of soluble components and deterioration of mechanical strength [15]. Such changes can compromise the structural integrity and effective barrier function of the membrane during postoperative healing [16].

Crosslinking is an effective strategy for improving the aqueous stability and mechanical integrity of hydrophilic polymer networks [17]. Conventional chemical crosslinkers such as glutaraldehyde can efficiently form stable covalent networks, but concerns regarding residual aldehyde groups and associated cytotoxicity have limited their use in biomedical materials [18]. In contrast, citric acid (CA) is a naturally occurring polycarboxylic acid with relatively low toxicity and multiple carboxyl groups that can react with hydroxyl-containing polymers through esterification, making it an attractive alternative crosslinker for hydrophilic polymer systems [19]. CA-mediated crosslinking has been shown to improve water resistance and mechanical stability in various hydrophilic polymer systems [20, 21]. More importantly, PVA/CMC-based materials have been reported for biomedical and related applications [22, 23], supporting the feasibility of this polymer combination. However, the effect of CA content on prolonged wet-state performance has received comparatively limited attention, particularly with respect to the combined evolution of swelling, material loss, surface integrity, and wet mechanical properties in a pericardial barrier system. For pericardial anti-adhesion membranes, the key requirement is not merely increased crosslinking, but the maintenance of an appropriate balance between swelling resistance, flexibility, and mechanical integrity during prolonged exposure to physiological fluids. Therefore, systematic evaluation of CA-dependent long-term wet-state stability is important for determining whether such membranes can maintain effective barrier function in the dynamic cardiac environment.

In this study, glycerol-plasticized, citric acid-crosslinked PVA/CMC composite membranes (PCC)

were developed with particular emphasis on their long-term wet-state stability (Fig. 1). After preliminary optimization of glycerol content to provide sufficient membrane flexibility, CA content was systematically varied as the principal crosslinking parameter. The relationship between CA content and water absorption, swelling, material loss, wet mechanical properties, and morphological integrity after prolonged PBS incubation was investigated. The formulation exhibiting the most favorable balance between wet mechanical strength, flexibility, and structural stability was further evaluated in a rabbit pericardial adhesion model as a functional validation. Overall, this study provides a strategy for regulating the wet-state stability of hydrophilic PVA/CMC membranes through CA-mediated crosslinking, enabling the development of flexible and durable barrier materials for postoperative adhesion prevention.

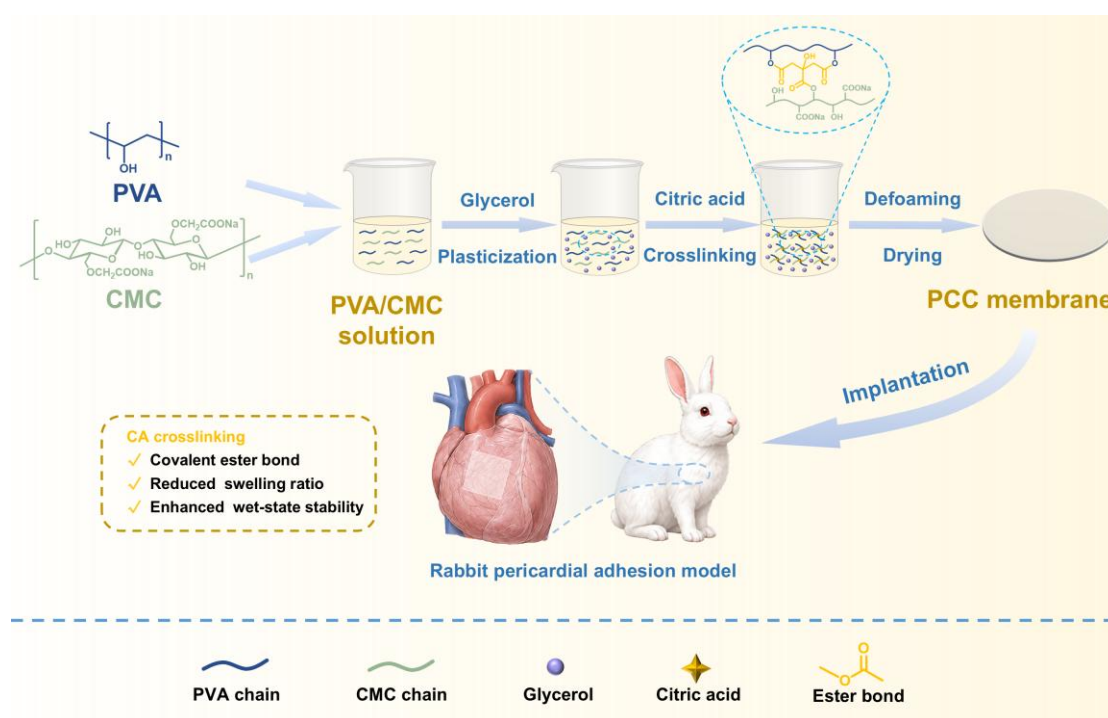


Fig. 1 Schematic illustration of the preparation and intended pericardial anti-adhesion application of PCC membrane.

Materials and Methods

Materials

Poly(vinyl alcohol) (PVA, M_w 90000) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Carboxymethyl cellulose (CMC, M_w 90000) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Citric acid (CA, 99.5%) was supplied by Energy Chemical Co., Ltd. (Shanghai, China). Glycerol (AR) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Phosphate-buffered saline (PBS, pH 7.4) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). All chemicals were used as received.

Preparation of PCC membranes

PVA solution was prepared by dissolving 4.0 g of PVA in 46.0 g of deionized water at 90 °C under continuous stirring. Separately, 1.0 g of CMC was dissolved in 99.0 g of deionized water at room temperature. After cooling the PVA solution to room temperature, the two solutions were thoroughly mixed to obtain a homogeneous PVA/CMC solution. Glycerol (2.0 g, 40 wt% relative to the total dry mass of PVA and CMC) was added as a plasticizer. The glycerol content was selected based on preliminary optimization of the mechanical properties and morphology of the membranes (Fig. S1-S3). CA was then added at 0, 0.15, 0.30, 0.45, 0.60, and 0.75 g, corresponding to 0, 3, 6, 9, 12, and 15 wt% relative to the total dry polymer mass, respectively. The mixed solutions were stirred until homogeneous and allowed to stand at room temperature for 24 h to remove entrapped air bubbles.

The mixed solutions were poured into circular plastic molds (15 cm in diameter) and dried at 55 °C for 12 h. The membranes were subsequently heat-treated at 80 °C for 2 h to promote CA crosslinking. The membranes containing 0, 3, 6, 9, 12, and 15 wt% CA were designated as PCC0, PCC3, PCC6, PCC9, PCC12, and PCC15, respectively. All membranes were equilibrated at room temperature for 24 h before further characterization.

Characterization of PCC membranes

The chemical structures of the PCC 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 morphologies of the membranes were observed using scanning electron microscopy (SEM; Sigma 360, Carl Zeiss, Germany). For SEM observation, dried membrane samples were cut into 5 mm $\times$ 5 mm pieces, mounted on conductive adhesive tape, and sputter-coated with gold before imaging.

Water absorption ratio test

Membrane samples (20 mm $\times$ 20 mm) were weighed to obtain the initial mass (M_0) and then immersed in deionized water for 2 h until equilibrium swelling was reached. The samples were removed, gently blotted with filter paper to remove surface water, and weighed (M_2). Each sample was measured at least three times, and the average value was reported. The water absorption ratio was calculated as formula (1):

$$\text{Water absorption ratio (\%)} = \frac{M_2 - M_0}{M_0} \times 100\% \quad (1)$$

Swelling ratio test

Phosphate-buffered saline (PBS, pH 7.4) was used to simulate physiological conditions. Membrane samples (20 mm × 20 mm) were measured for their initial dimensions and thickness to calculate the initial volume (V_0). The samples were then immersed in PBS and incubated at 37 °C for 12 h. After incubation, the samples were removed from PBS, gently blotted with filter paper to remove excess surface liquid, and immediately remeasured to calculate the swollen volume (V_1). The swelling ratio was calculated as formula (2):

$$\text{Swelling ratio (\%)} = \frac{V_1 - V_0}{V_0} \times 100\% \quad (2)$$

Water contact angle test

The surface wettability of the membranes was evaluated by static water contact angle measurements using a contact angle meter (CA200, China) at room temperature. Membrane samples (20 mm × 20 mm) were fixed onto glass slides using double-sided adhesive tape. A droplet of deionized water (3 μL) was gently placed on the membrane surface, and the contact angle was recorded. For each sample, measurements were performed at three different locations, and the average value was calculated.

Mechanical property test

The mechanical properties of the membranes were evaluated using a universal testing machine (HT-101S, China). For dry-state testing, the specimens were tested at room temperature. For wet-state testing, the specimens were immersed in PBS for 2 h. After immersion, the specimens were removed from PBS, gently blotted with filter paper to remove excess surface liquid, and immediately subjected to tensile testing. All tests were conducted at a crosshead speed of 50 mm/min. Tensile strength and elongation at break were recorded. Three independent specimens were tested for each group.

Thermal stability test

Thermal stability of the membranes was evaluated by thermogravimetric analysis (TGA). Approximately 5~10 mg of each sample was placed in a platinum pan and heated from room temperature to 600 °C at a heating rate of 10 °C/min under a nitrogen atmosphere. The nitrogen flow rate was maintained at 10 mL/min throughout the experiment. The weight loss behavior and thermal decomposition characteristics were analyzed based on the obtained thermogravimetric curves.

Long-term wet-state stability test

Membrane samples (20 mm × 20 mm) were accurately weighed (M_0) and placed in well plates, followed by the addition of PBS to fully immerse the samples. The samples were incubated in a shaking incubator at 37 °C with a shaking speed of 100 rpm. The PBS solution was refreshed weekly to maintain a stable environment. At predetermined time points (1, 3, 5, 7, 14, 21, 28, and 30 days), the samples were removed, rinsed with deionized water to eliminate surface salts, and dried to constant weight (M_3). Each group was tested in triplicate. The mass loss was calculated as formula (3):

$$\text{Mass loss (\%)} = \frac{M_0 - M_3}{M_0} \times 100\% \quad (3)$$

To evaluate morphological changes during prolonged PBS incubation, membrane samples were collected after 30 days, gently rinsed with deionized water to remove residual salts, freeze-dried, and examined by SEM. To evaluate the retention of wet mechanical properties after prolonged incubation, tensile specimens from each group were maintained under the same conditions. After 30 days, the specimens were removed from PBS, gently blotted with filter paper to remove excess surface liquid, and immediately subjected to tensile testing under wet conditions. Three independent specimens were tested for each group.

Hemolysis and cytocompatibility assays

(1) Hemolysis assay

The hemolysis assay was performed according to a previously reported method with slight modifications [24]. Briefly, commercially obtained anticoagulated rat whole blood was centrifuged at 2000 rpm for 10 min at 4 °C to isolate red blood cells (RBCs). The RBCs were washed three times with physiological saline and diluted with saline to obtain a 4% (v/v) RBC suspension. PCC membrane samples (10 mm × 10 mm) were placed in 48-well plates, followed by the addition of 500 µL of physiological saline and 500 µL of RBC suspension. Physiological saline and 1% Triton X-100 were used as the negative and positive controls, respectively. After incubation at 37 °C for 1 h, the suspensions were centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was measured at 540 nm. The hemolysis ratio was calculated as formula (4):

$$\text{Hemolysis ratio (\%)} = \frac{OD_{\text{sample}} - OD_{\text{negative}}}{OD_{\text{positive}} - OD_{\text{negative}}} \times 100\% \quad (4)$$

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 PCC membranes was evaluated using an extract-based CCK-8 assay with L929 fibroblasts. Membrane samples (10 mm × 10 mm) were sterilized by UV irradiation for 2 h on each side and incubated in 1 mL of complete culture medium at 37 °C for 24 h. The resulting extracts were collected and filtered through a sterile membrane. L929 cells were seeded in 96-well plates at a density of 1×10^5 cells mL⁻¹ (100 µL per well) and cultured for 24 h. The culture medium was then replaced with 100 µL of membrane extract, while fresh complete medium was used for the control group. After 72 h of incubation, the medium was replaced with fresh medium containing 10% (v/v) CCK-8 reagent and incubated for an additional 2 h. Absorbance was measured at 450 nm, and relative cell viability was calculated as formula (5):

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

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.

Fibroblast attachment assay

The attachment behavior of L929 fibroblasts on the PCC membranes was evaluated using a CCK-8 assay, following a previously reported protocol [25]. PCC membranes were cut into

circular specimens (15 mm in diameter), sterilized by UV irradiation for 2 h on each side, and fixed at the bottom of 24-well plates using sample holders. L929 fibroblasts were seeded onto the membrane surfaces at a cell density of 1×10^5 cells mL⁻¹ and cultured at 37 °C in a humidified atmosphere containing 5% CO₂ for 72 h. The culture medium was replaced every 48 h. Tissue-culture wells without membranes were used as the control. After 72 h, the culture medium was removed and the membranes were gently washed to remove non-attached cells. Fresh culture medium containing 10% (v/v) CCK-8 reagent was then added, followed by incubation for 2 h. The absorbance was measured at 450 nm, and relative cell attachment ratio was calculated as formula (6):

$$\text{Relative cell attachment ratio (\%)} = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100\% \quad (6)$$

where OD_{sample} is the absorbance of cells cultured on the PCC membranes, OD_{control} is the absorbance of cells cultured directly in the tissue-culture wells without membranes, and OD_{blank} is the absorbance of the cell-free culture medium containing CCK-8 reagent.

In vivo pericardial anti-adhesion evaluation

A rabbit pericardial adhesion model was established to evaluate the in vivo anti-adhesion performance of PCC15. New Zealand White rabbits were randomly assigned to the control and PCC15 groups, with four animals in each group. The rabbits were anesthetized with sodium pentobarbital administered intravenously. After removal of the chest hair and disinfection of the surgical area with povidone-iodine, a median sternotomy was performed to expose the heart. A pericardial defect of approximately 15 mm × 15 mm was created, and the exposed epicardial surface was gently abraded with sterile gauze in multiple directions until petechial bleeding was observed. In the PCC15 group, a PCC15 membrane (approximately 20 mm × 20 mm) was sutured over the pericardial defect to separate the injured cardiac surface from the sternum and surrounding tissues. Animals in the control group underwent the same procedure without membrane implantation. The thoracic cavity was subsequently closed and the incision was sutured. The animals were monitored throughout the postoperative period and euthanized 30 days after surgery. The thoracic cavity was reopened through the original incision, and adhesion formation was macroscopically evaluated using the following scoring system: 0, no adhesion; 1, mild adhesion that could be easily separated by blunt dissection; 2, moderate adhesion partially requiring sharp dissection; and 3, severe adhesion requiring sharp dissection and associated with bleeding. For histological evaluation, tissue samples from the cardiac surface and surrounding adhesion area were fixed in 10% neutral formalin, dehydrated through a graded ethanol series, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome to evaluate tissue morphology, inflammatory response, and collagen deposition.

Statistical analysis

Quantitative data are presented as the mean ± standard deviation (SD), unless otherwise specified. Statistical analyses were performed using GraphPad Prism 10. Comparisons among multiple groups were performed using one-way ANOVA followed by Tukey's multiple-comparison test. Macroscopic adhesion scores between the two groups were compared using the Mann–Whitney U test. Statistical significance is indicated by asterisks: *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

and $p < 0.001$, respectively. Results with $p < 0.05$ were considered statistically significant.

Results and discussion

FTIR analysis of PCC membranes

The FTIR spectra of PCC membranes with different CA contents are shown in Fig. 2. For PCC0, the broad absorption band centered at approximately 3275 cm^{-1} was assigned to O–H stretching vibrations of PVA and CMC, while the band at approximately 2938 cm^{-1} was attributed to C–H stretching of $-\text{CH}_2$ groups. The characteristic band at approximately 1597 cm^{-1} was assigned to the asymmetric stretching vibration of $-\text{COO}^-$ groups in CMC [26]. After the incorporation of CA, clear changes were observed in the carboxyl-related absorption regions. With increasing CA content, the intensity of the $-\text{COO}^-$ band at approximately 1597 cm^{-1} gradually decreased, which can be attributed to protonation of $-\text{COO}^-$ groups in CMC under the acidic conditions introduced by CA [27]. Meanwhile, the bands at approximately 1715 and 1237 cm^{-1} became more pronounced [28]. These bands are associated with C=O and C–O stretching vibrations of carboxylic acid and ester functionalities, reflecting changes related to both $-\text{COO}^-$ protonation and ester formation. Overall, the decrease in the $-\text{COO}^-$ band together with the strengthening of the C=O and C–O bands is consistent with the esterification reaction involved in CA crosslinking of the PCC membranes [29].

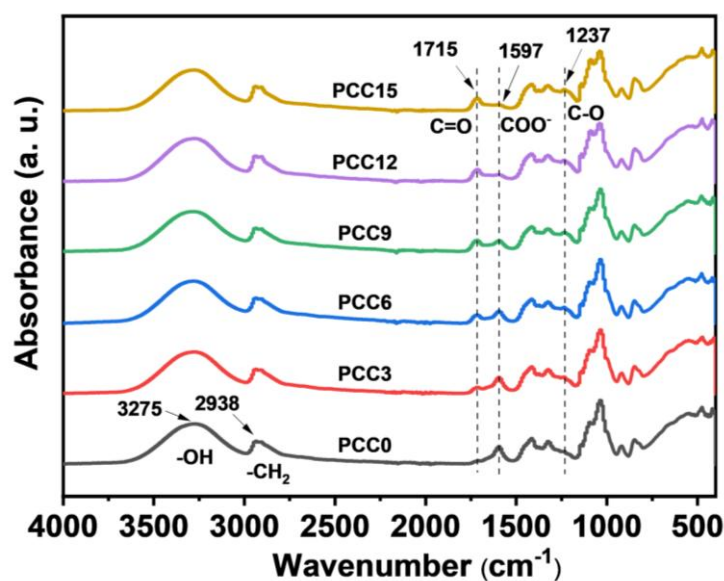


Fig. 2 FTIR spectra of PCC membranes with different CA contents.

Surface morphology of PCC membranes

The surface morphologies of PCC membranes with different CA contents were examined by SEM. As shown in Fig. 3a–f, PCC0 exhibited a relatively smooth, dense, and homogeneous surface without obvious cracks, large pores, or bubble defects. No distinct phase-separated domains were observed on the membrane surface. After the incorporation of CA, PCC3–PCC15 maintained similar surface morphologies. Increasing the CA content did not produce obvious changes in surface roughness or induce visible cracks, pores, or phase separation. These observations indicate that CA incorporation and subsequent crosslinking did not markedly alter the overall surface morphology of the PCC membranes.

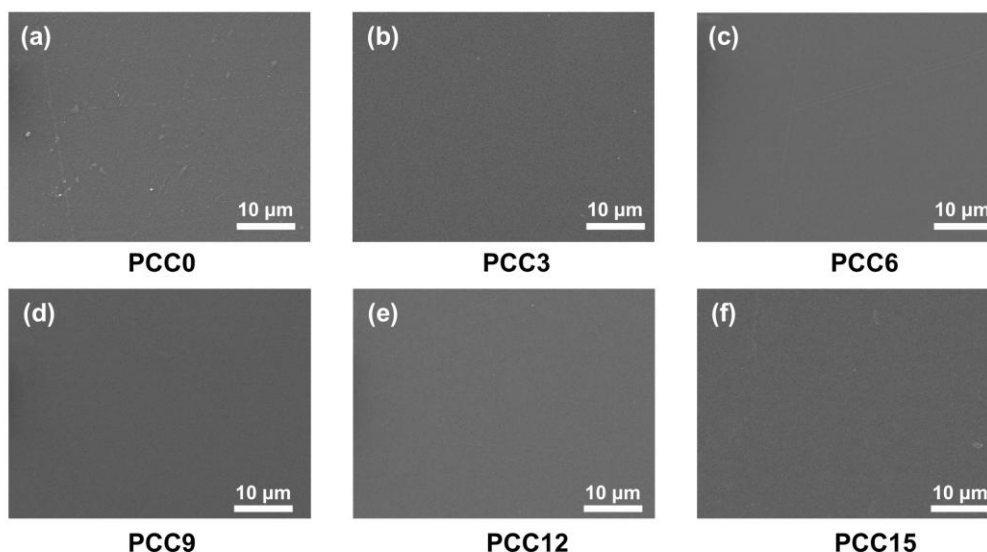


Fig. 3 SEM images of PCC membranes with different CA contents: (a) PCC0, (b) PCC3, (c) PCC6, (d) PCC9, (e) PCC12, and (f) PCC15. Scale bars = 10 μm .

Physicochemical properties of PCC membranes

The physicochemical properties of the PCC membranes were strongly influenced by CA content. As shown in Fig. 4a, the water absorption ratio decreased markedly from approximately $1255\% \pm 69\%$ for PCC0 to $69\% \pm 1\%$ for PCC15 with increasing CA content. Similarly, the swelling ratio in PBS decreased from approximately $475\% \pm 26\%$ for PCC0 to $93\% \pm 2\%$ for PCC15 (Fig. 4b). The high water absorption and swelling of PCC0 can be attributed to the strong hydrophilicity of PVA and CMC and the absence of CA-mediated crosslinking, which allowed extensive hydration, polymer-chain relaxation, and dimensional expansion. With increasing CA content, the progressively constrained polymer network restricted water penetration and chain mobility, thereby reducing both water uptake and volumetric swelling. These trends are consistent with the FTIR results indicating CA-mediated esterification and demonstrate that increasing CA content effectively enhanced the resistance of the PCC membranes to hydration-induced swelling.

Water contact angle measurements further revealed changes in the surface wettability of the membranes. As shown in Fig. 4c, the water contact angle gradually decreased from $51.7^\circ \pm 4.6^\circ$ for PCC0 to $24.5^\circ \pm 3.2^\circ$ for PCC15, indicating increased surface wettability with increasing CA content. Although this trend appears opposite to the reductions in water absorption and swelling, these observations reflect different aspects of membrane–water interactions. The water contact angle primarily characterizes the initial wettability of the membrane surface, whereas water absorption and swelling are governed by water penetration into and expansion of the bulk polymer network. The increased surface wettability of CA-containing membranes may be associated with changes in the distribution of polar groups at the membrane surface, including residual carboxyl groups introduced by CA. Therefore, despite the increased surface wettability, CA-mediated crosslinking effectively restricted bulk water uptake and dimensional expansion of the PCC membranes.

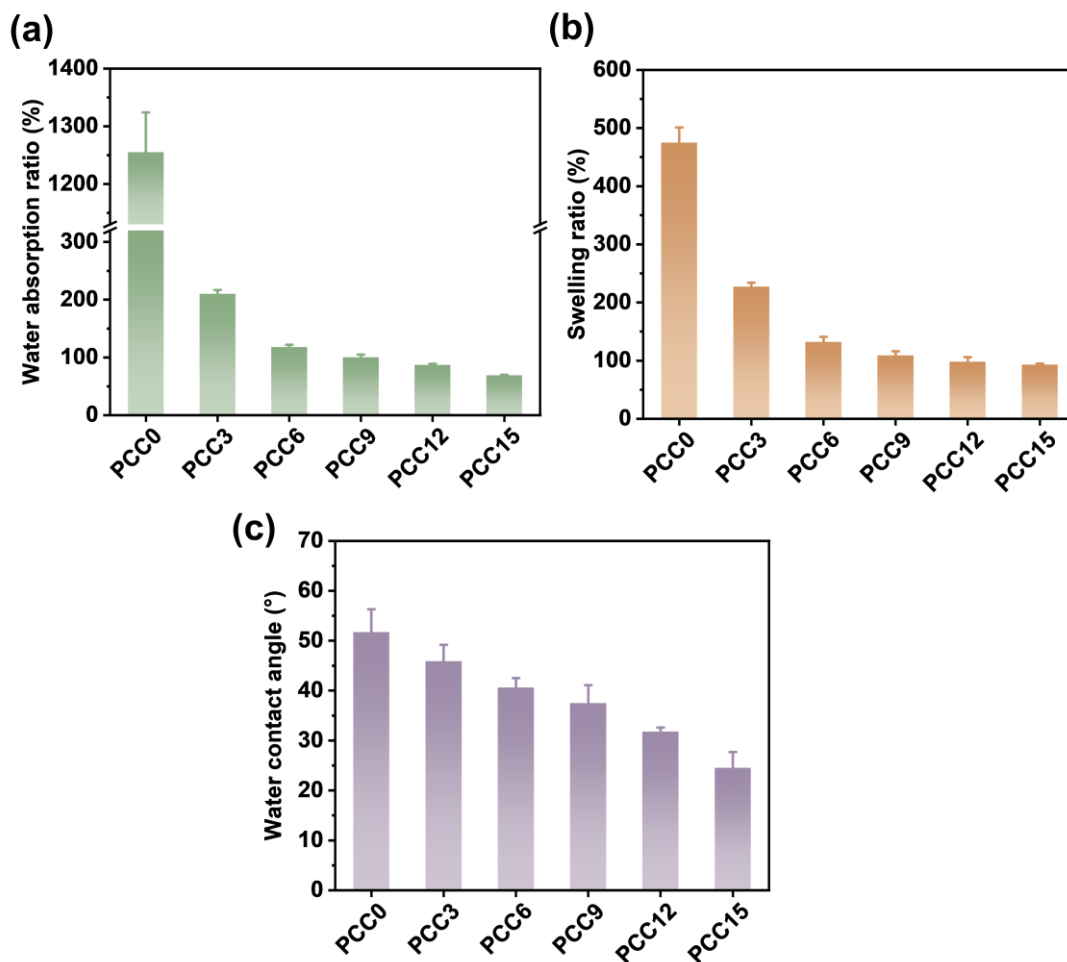


Fig. 4 Physicochemical properties of PCC membranes with different CA contents: (a) water absorption ratio, (b) swelling ratio, and (c) water contact angle.

Mechanical properties of PCC membranes

The mechanical properties of PCC membranes were evaluated under both dry and wet conditions. As shown in Fig. 5a, the tensile strength increased with increasing CA content. The dry-state tensile strength increased from 30.3 ± 0.5 MPa for PCC0 to 39.4 ± 1.6 MPa for PCC15, while the wet-state tensile strength increased more markedly from 1.5 ± 0.3 to 6.7 ± 0.2 MPa. The increase in tensile strength is consistent with the formation of a strengthened crosslinked network after CA incorporation. Crosslinking restricts the relative movement and slippage of PVA and CMC chains under tensile loading, thereby increasing the resistance of the membranes to deformation [30]. The effect of CA crosslinking was particularly evident under wet conditions. All PCC membranes exhibited lower tensile strength after PBS immersion than in the dry state, reflecting the pronounced effect of water on the PVA/CMC matrix. Water penetration weakens intermolecular interactions and increases polymer-chain mobility, leading to a marked reduction in mechanical strength [31]. This effect was most pronounced for PCC0, which lacked sufficient crosslinking to constrain chain movement after hydration. With increasing CA content, the strengthened crosslinked network increasingly restricted polymer-chain mobility and separation in the wet state, enabling the membranes to retain higher tensile strength under wet conditions. This trend is consistent with the reduced water absorption and swelling described above and further

demonstrates the improvement in wet-state stability achieved by CA crosslinking. As shown in Fig. 5b, the elongation at break showed the opposite trend. The dry-state elongation decreased from 99% $\pm$ 5% for PCC0 to 57% $\pm$ 2% for PCC15, while the wet-state elongation decreased from 250% $\pm$ 19% to 190% $\pm$ 10%. This decrease can also be attributed to the increased restriction of polymer-chain mobility after crosslinking, which limits chain rearrangement and extension during tensile deformation [32]. Despite the reduction in elongation, PCC15 achieved a wet tensile strength of 6.7 MPa while maintaining an elongation at break of 190%, indicating a favorable balance between mechanical strength and flexibility under wet conditions.

To place the wet mechanical performance of PCC15 in a clinically relevant context, comparison was made with representative values reported for native human pericardium and a commercial cardiac adhesion barrier. Native human pericardium has been reported to exhibit an ultimate tensile strength of 6.4 MPa with a fracture strain of 33.4% [33], while the bioresorbable cardiac adhesion barrier REPEL-CV was designed with a minimum hydrated tensile strength of approximately 6.2 MPa [34]. Notably, PCC15 exhibited a comparable wet tensile strength of 6.7 MPa while maintaining a substantially higher elongation at break of 190%. These results indicate that PCC15 combines sufficient wet mechanical strength with high extensibility, which may improve its ability to accommodate repetitive cardiac motion.

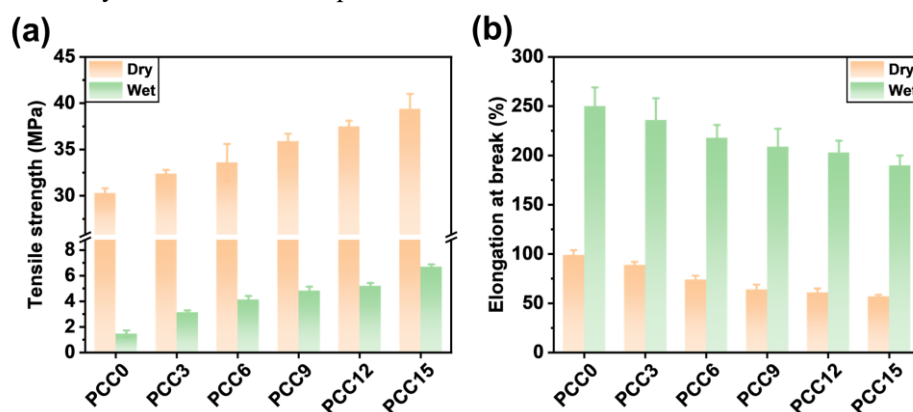


Fig. 5 Mechanical properties of PCC membranes with different CA contents under dry and wet conditions: (a) tensile strength and (b) elongation at break.

Thermal stability of PCC membranes

The thermal stability of the individual components and PCC membranes was evaluated by thermogravimetric analysis. As shown in Fig. 6a, PVA and CMC exhibited multistage thermal decomposition, whereas CA underwent its major thermal decomposition around 230 °C. For PVA, the initial mass loss was mainly associated with moisture evaporation and dehydration, followed by decomposition of the polymer backbone at higher temperatures [35]. For CMC, the initial mass loss was mainly attributed to moisture evaporation, followed by decomposition of the carboxymethyl groups and polysaccharide backbone [36].

The PCC membranes exhibited more complex multistage mass-loss behavior because of their multicomponent composition (Fig. 6b). The initial mass loss below approximately 120 °C was mainly attributed to the removal of free and bound water. The subsequent region between approximately 120 and 260 °C was associated with dehydration and the initial thermal decomposition of the membrane components. The mass loss between approximately 260 and 375 °C was mainly associated with glycerol evaporation and thermal decomposition of the

polymer matrix, followed by further decomposition and carbonization at higher temperatures. At the same extent of mass loss, the decomposition temperature generally increased with increasing CA content, indicating improved thermal stability of the PCC membranes. This improvement can be attributed to the strengthened crosslinked structure formed by CA, which restricts polymer-chain mobility and increases the energy required for thermal decomposition.

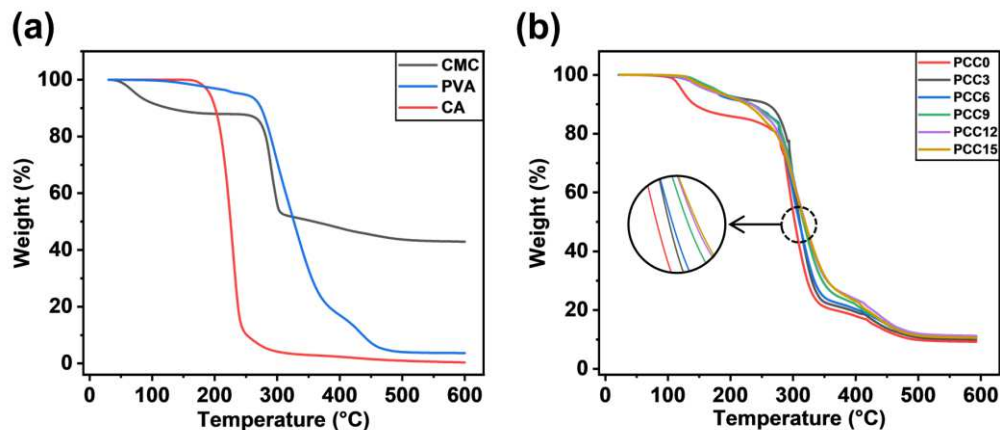


Fig. 6 Thermogravimetric curves of (a) PVA, CMC, CA and (b) PCC membranes with different CA contents.

Long-term wet-state stability of PCC membranes

The long-term wet-state stability of PCC membranes was evaluated by monitoring mass loss during 30 days of PBS incubation and by examining surface morphology and wet mechanical properties after 30 days. As shown in Fig. 7a, all PCC membranes exhibited pronounced mass loss during the initial stage of incubation. After 1 day, the mass loss decreased from $39.3\% \pm 1.8\%$ for PCC0 to $30.4\% \pm 1.8\%$ for PCC15 with increasing CA content. The substantial initial mass loss was mainly attributed to the dissolution and release of soluble components that were not effectively retained within the membrane network [37]. The lower mass loss at higher CA contents indicates that CA crosslinking improved the resistance of the membranes to material loss during PBS incubation. After 30 days, the mass loss similarly decreased from $45.4\% \pm 0.7\%$ for PCC0 to $34.1\% \pm 1.1\%$ for PCC15 as the CA content increased. This trend is consistent with the reduced swelling observed at higher CA contents and further demonstrates the improved resistance of the membranes to aqueous-induced material loss. The strengthened crosslinked structure reduced material loss and enabled the membranes to maintain greater structural stability during prolonged PBS incubation.

After 30 days of incubation in PBS, pronounced differences in wet mechanical properties were observed between PCC0 and PCC15 (Fig. 7b). PCC0 exhibited a wet tensile strength of 1.2 ± 0.1 MPa and an elongation at break of $286\% \pm 11\%$, whereas PCC15 maintained a substantially higher wet tensile strength of 5.7 ± 0.2 MPa with an elongation at break of $206\% \pm 6\%$. These results indicate that CA crosslinking markedly improved the wet mechanical integrity of the PVA/CMC membranes after prolonged PBS incubation while maintaining high extensibility. SEM observations after 30 days further revealed differences in the structural integrity of the PCC membranes (Fig. 7c-h). Compared with their initially smooth surfaces, the incubated membranes exhibited varying degrees of surface roughness, erosion, and pore formation. These morphological changes became less pronounced with increasing CA content, and membranes containing higher

amounts of CA retained relatively more continuous and intact surfaces. Taken together, the mechanical and morphological results demonstrate that CA crosslinking effectively improved the long-term wet-state stability of the PCC membranes.

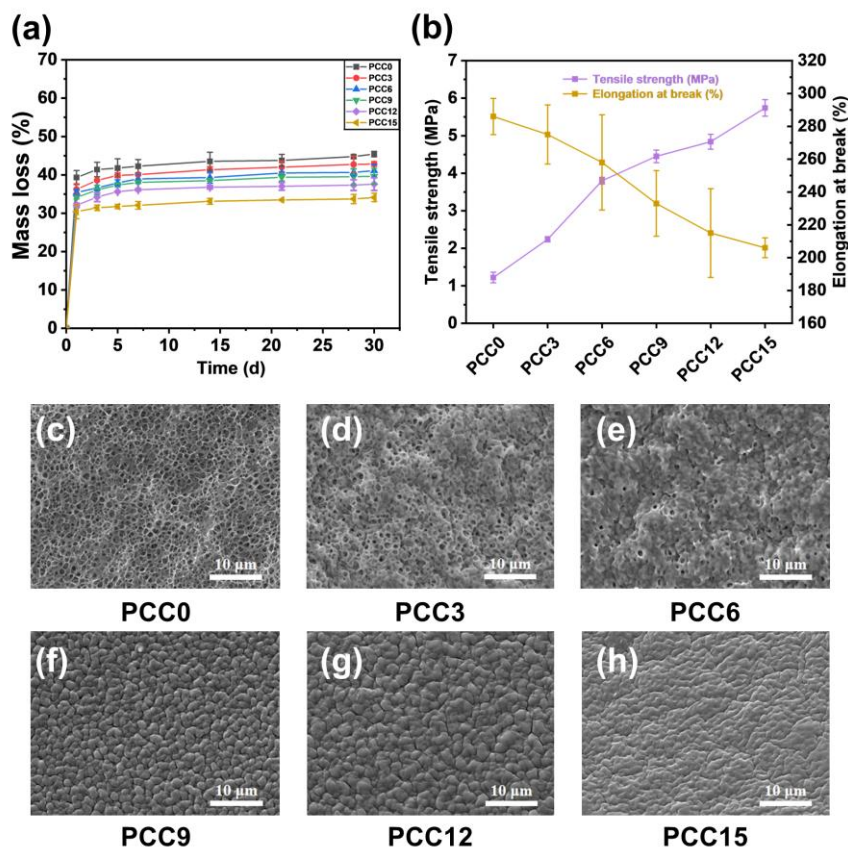


Fig. 7 Long-term wet-state stability of PCC membranes during PBS incubation: (a) mass loss during 30 days of incubation; (b) wet tensile strength and elongation at break of PCC membranes after 30 days of incubation; and (c–h) SEM images of PCC0, PCC3, PCC6, PCC9, PCC12, and PCC15 after 30 days of PBS incubation. Scale bars = 10 μ m.

Biocompatibility of PCC membranes

The hemocompatibility of PCC membranes was evaluated by a red blood cell hemolysis assay. As shown in Fig. 8a, the hemolysis ratios of all PCC membranes remained below 5%, suggesting acceptable hemocompatibility under the tested conditions [38]. No pronounced increase in hemolysis ratio was observed with increasing CA content, indicating that CA crosslinking did not adversely affect the hemocompatibility of the membranes. The cytocompatibility of the PCC membranes was further evaluated using L929 fibroblasts cultured with membrane extracts. After 72 h of incubation, cell viability remained above 70% for all PCC groups (Fig. 8b), indicating no pronounced cytotoxicity under the tested conditions. These results indicate that the PCC membranes exhibited favorable preliminary hemocompatibility and cytocompatibility.

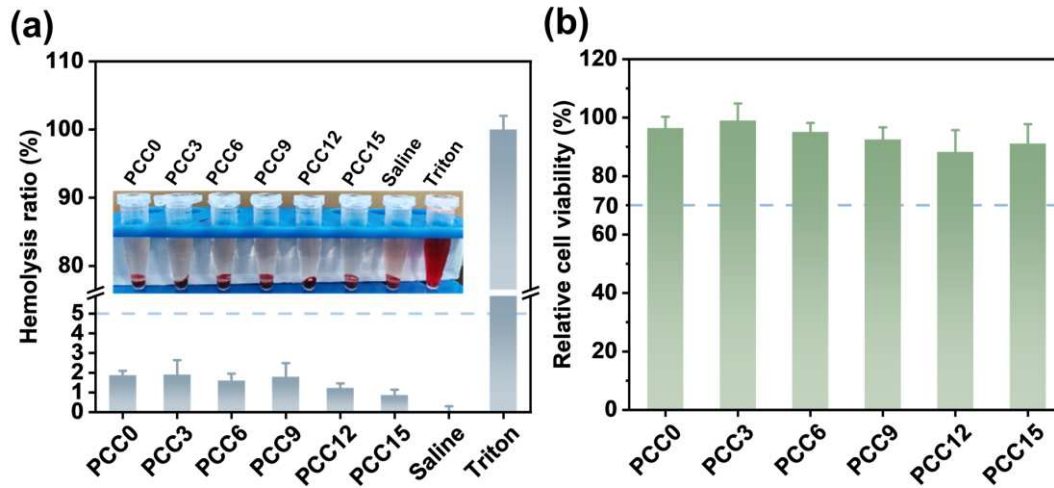


Fig. 8 In vitro biocompatibility of PCC membranes. (a) hemolysis ratio, and (b) relative cell viability of PCC membranes.

Fibroblast attachment behavior of PCC membranes

Fibroblast attachment to the PCC membranes was evaluated by culturing L929 cells directly on the membrane surfaces for 72 h. As shown in Fig. 9a, the CCK-8 absorbance values of all PCC membrane groups were lower than that of the tissue-culture plate control. The relative fibroblast attachment ratios showed the same trend, with all PCC groups exhibiting lower values than the control group (Fig. 9b), indicating reduced fibroblast attachment to the PCC membrane surfaces. The relative fibroblast attachment showed a slight increase with increasing CA content, accompanied by the progressive increase in surface wettability observed from the water contact angle measurements. Nevertheless, fibroblast attachment remained lower than that of the tissue-culture plate control for all PCC membranes. These results indicate that the PCC membranes maintained relatively low fibroblast attachment across the investigated CA contents.

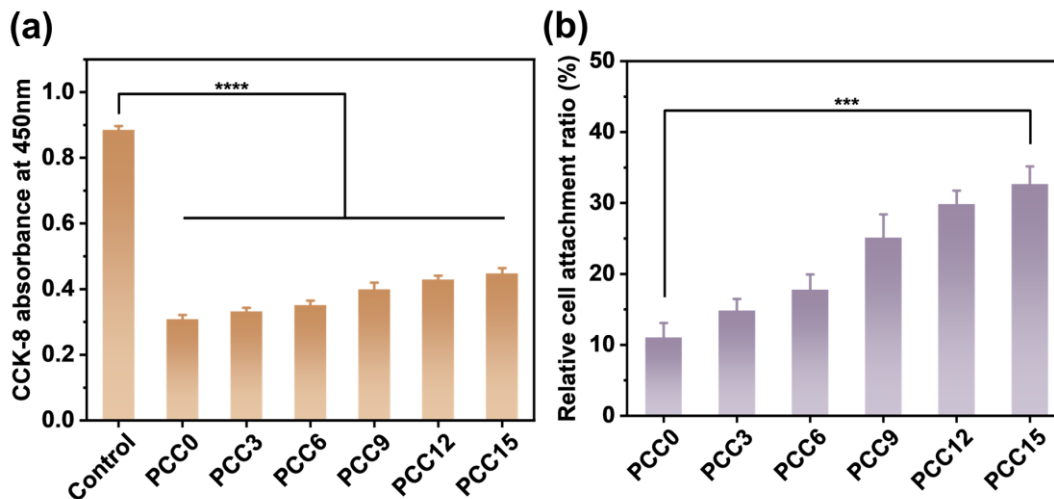


Fig. 9 L929 fibroblast response on PCC membranes after 72 h of culture: (a) CCK-8 absorbance at 450 nm and (b) relative cell attachment ratio.

In vivo pericardial anti-adhesion performance of PCC15 membrane

Based on its favorable wet-state stability, mechanical properties, and biocompatibility, PCC15 was

selected for further evaluation in a rabbit pericardial adhesion model. The surgical procedure is shown in Fig. 10a. During the 30-day observation period, animals in both groups remained in good general condition, with no severe infection or other obvious adverse reactions. At 30 days after surgery, severe adhesions between the heart and sternum were observed in the untreated control group and generally required sharp dissection for separation. In contrast, no severe adhesions were observed in the PCC15 group, and the mild adhesions could be readily separated by blunt dissection. The implanted membrane could also be easily detached from the cardiac surface (Fig. 10b). The adhesion scores were consistent with these macroscopic observations. In the control group, one animal had an adhesion score of 2 and three had a score of 3, whereas in the PCC15 group, three animals had a score of 0 and one had a score of 1 (Table 1). These results indicate that PCC15 markedly reduced the severity of postoperative pericardial adhesions.

Histological examination further supported the macroscopic findings (Fig. 10c). H&E staining showed dense adhesion between the cardiac surface and surrounding tissues in the control group, accompanied by an apparent inflammatory response in the adhesion region. In contrast, no obvious dense adhesion or pronounced local inflammatory response was observed in the PCC15 group. Masson's trichrome staining revealed extensive collagen deposition in the control group, whereas substantially less collagen deposition was observed in the epicardial region of the PCC15 group. The reduced collagen deposition was consistent with the lower adhesion severity observed macroscopically. Overall, PCC15 effectively reduced postoperative pericardial adhesion formation and collagen deposition without inducing an obvious local inflammatory response.

Table 1. Distribution of macroscopic pericardial adhesion scores at 30 days after surgery.

Group	n	Score 0, n	Score 1, n	Score 2, n	Score 3, n
Control	4	0	0	1	3
PCC15	4	3	1	0	0

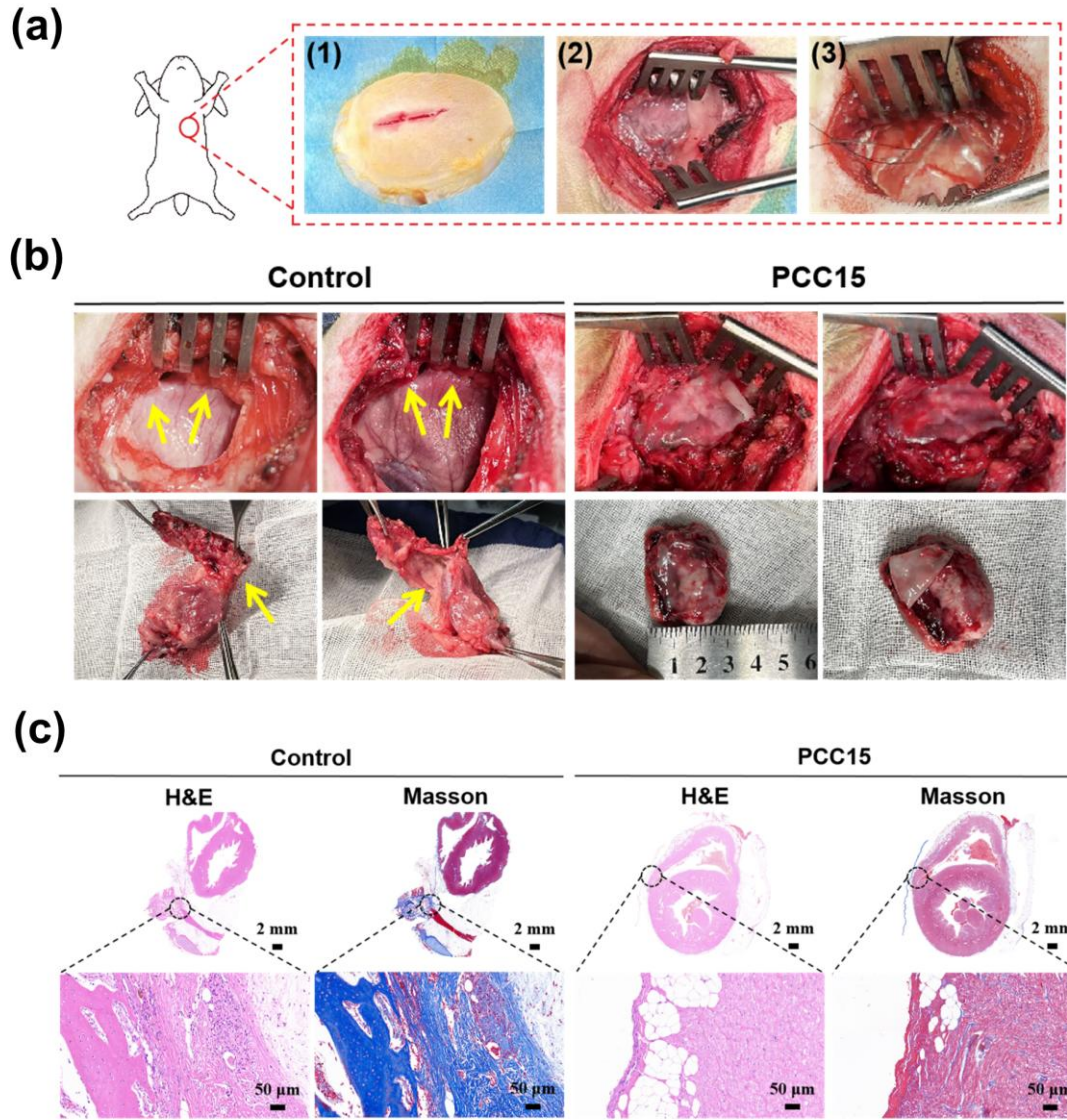


Fig. 10 In vivo pericardial anti-adhesion performance of PCC15 in rabbits: (a) schematic and representative images of the surgical procedure; (b) representative gross observations at 30 days after surgery; and (c) H&E and Masson's trichrome staining of tissues surrounding the heart. Yellow arrows indicate adhesion sites. Scale bars are 2 mm for overview images and 50 μ m for magnified images.

Conclusion

In this study, citric acid-crosslinked PVA/CMC composite membranes were prepared to investigate the effect of CA content on their wet-state stability and anti-adhesion performance. Increasing CA content strengthened the crosslinked structure, leading to higher tensile strength, together with reduced water absorption, swelling, and mass loss during PBS incubation. Among the formulations, PCC15 achieved the most favorable balance between wet mechanical strength, flexibility, and structural stability during prolonged PBS incubation. Moreover, the PCC membranes exhibited favorable hemocompatibility and cytocompatibility, together with relatively low fibroblast attachment. In the rabbit pericardial adhesion model, PCC15 effectively reduced

adhesion formation and collagen deposition without an obvious local inflammatory response, supporting its potential as a physical barrier for the prevention of postoperative pericardial adhesions.

Supporting Information The online version contains supplementary material.

Funding This research was supported by the National Key Research and Development Program of China (No.2020YFC1107300), and Biomedical Transformation of Beijing University of Chemical Technology-China-Japan Friendship Hospital Engineering Research Center Joint Fund (No. XK2023-24).

Data Availability The data used for the research 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.

References

- [1] Hill MA, Walkowiak OA, Head WT, Kwon JH, Kavarana MN, Rajab TK (2022) A review of animal models for post-operative pericardial adhesions. *Front Surg* 9:966410. <https://doi.org/10.3389/fsurg.2022.966410>
- [2] Shi K, Li T, Hu X, Chen W, Yu Y, Bei Z, Yuan L, Tong Q, Liu J, Fan Q, Qian Y, Qian Z (2025) Injectable and sprayable thermoresponsive hydrogel with fouling-resistance as an effective barrier to prevent postoperative cardiac adhesions. *Adv Sci* 12:2500731. <https://doi.org/10.1002/advs.202500731>
- [3] Wang X, Liu Z, Sandoval-Salaiza DA, Afewerki S, Jimenez-Rodriguez MG, Sanchez-Melgar L, Güemes-Aguilar G, Gonzalez-Sanchez DG, Noble O, Lerma C, Parra-Saldivar R, Lemos DR, Llamas-Esperon GA, Shi J, Li L, Lobo AO, Fuentes-Baldemar AA, Bonventre JV, Dong N, Ruiz-Esparza GU (2021) Nanostructured non-Newtonian drug delivery barrier prevents postoperative intrapericardial adhesions. *ACS Appl Mater Interfaces* 13:29231–29246. <https://doi.org/10.1021/acsami.0c20084>
- [4] Akhlaghi S, Ebrahimnia M, Shokouhi Niaki D, Solhi M, Rabbani S, Haeri A (2023) Recent advances in the preventative strategies for postoperative adhesions using biomaterial-based membranes and micro/nano-drug delivery systems. *J Drug Deliv Sci Technol* 85:104539. <https://doi.org/10.1016/j.jddst.2023.104539>
- [5] Metem V, Thonglam J, Juncheed K, Khangkhamano M, Kwanyuang A, Meesane J (2024) Tissue-mimicking composite barrier membranes to prevent abdominal adhesion formation after surgery. *J Mech Behav Biomed Mater* 152:106417. <https://doi.org/10.1016/j.jmbbm.2024.106417>
- [6] Fujita M, Policastro GM, Burdick A, Lam HT, Ungerleider JL, Braden RL, Huang D, Osborn KG, Omens JH, Madani MM, Christman KL (2021) Preventing post-surgical cardiac adhesions with a

- catechol-functionalized oxime hydrogel. *Nat Commun* 12:3764. <https://doi.org/10.1038/s41467-021-24104-w>
- [7] Bercea M (2024) Recent advances in poly(vinyl alcohol)-based hydrogels. *Polymers* 16:2021. <https://doi.org/10.3390/polym16142021>
- [8] Zennifer A, Senthilvelan P, Sethuraman S, Sundaramurthi D (2021) Key advances of carboxymethyl cellulose in tissue engineering & 3D bioprinting applications. *Carbohydr Polym* 256:117561. <https://doi.org/10.1016/j.carbpol.2020.117561>
- [9] Pourmadadi M, Rahmani E, Shamsabadipour A, Samadi A, Esmaceli J, Arshad R, Rahdar A, Tavangarian F, Pandey S (2023) Novel carboxymethyl cellulose based nanocomposite: a promising biomaterial for biomedical applications. *Process Biochem* 130:211–226. <https://doi.org/10.1016/j.procbio.2023.03.033>
- [10] Capanema NSV, Mansur AAP, Carvalho IC, Carvalho SM, Mansur HS (2023) Bioengineered water-responsive carboxymethyl cellulose/poly(vinyl alcohol) hydrogel hybrids for wound dressing and skin tissue engineering applications. *Gels* 9:166. <https://doi.org/10.3390/gels9020166>
- [11] Farid E, Kamoun EA, Taha TH, El-Dissouky A, Khalil TE (2022) PVA/CMC/attapulgit clay composite hydrogel membranes for biomedical applications: factors affecting hydrogel membranes crosslinking and bio-evaluation tests. *J Polym Environ* 30:4675–4689. <https://doi.org/10.1007/s10924-022-02538-7>
- [12] Hu C, Tang F, Wu Q, Guo B, Long WA, Ruan Y, Li L (2021) Novel trilaminar polymeric antiadhesion membrane prevents postoperative pericardial adhesion. *Ann Thorac Surg* 111:184–189. <https://doi.org/10.1016/j.athoracsurg.2020.03.011>
- [13] Jaenigen BM, Weis C, Odermatt EK, Hopt UT, Obermaier R (2009) The new adhesion prophylaxis membrane A-Part—from in vitro testing to first in vivo results. *J Biomed Mater Res B Appl Biomater* 89B:293–299. <https://doi.org/10.1002/jbm.b.31215>
- [14] Müller SA, Weis C, Odermatt EK, Knaebel HP, Wente MN (2011) A hydrogel for adhesion prevention: characterization and efficacy study in a rabbit uterus model. *Eur J Obstet Gynecol Reprod Biol* 158:67–71. <https://doi.org/10.1016/j.ejogrb.2010.11.008>
- [15] Thungphotrakul N, Prapainainar P (2024) Development of polyvinyl alcohol/carboxymethylcellulose-based bio-packaging film with citric acid crosslinking and clove essential oil encapsulated chitosan nanoparticle Pickering emulsion. *Int J Biol Macromol* 282:137223. <https://doi.org/10.1016/j.ijbiomac.2024.137223>
- [16] Wang X, Fadlilah FN, Yang Q, Hong Y, Wu D, Peng M, Peng X, Wu J, Luo Y (2024) A biodegradable shape memory polyurethane film as a postoperative anti-adhesion barrier for minimally invasive surgery. *Acta Biomater* 189:311–322. <https://doi.org/10.1016/j.actbio.2024.09.018>
- [17] Yammine P, El Safadi A, Kassab R, El-Nakat H, Obeid PJ, Nasr Z, Tannous T, Sari-Chmayssem N, Mansour A, Chmayssem A (2025) Types of crosslinkers and their applications in biomaterials and biomembranes. *Chemistry* 7:61. <https://doi.org/10.3390/chemistry7020061>
- [18] Shi J, Lian H, Huang Y, Zhao D, Wang H, Wang C, Li J, Ke L (2020) In vitro genotoxicity evaluation and metabolic study of residual glutaraldehyde in animal-derived biomaterials. *Regen Biomater* 7:619–625. <https://doi.org/10.1093/rb/rbaa041>
- [19] Salihu R, Abd Razak SI, Ahmad Zawawi N, Rafiq Abdul Kadir M, Izzah Ismail N, Jusoh N, Riduan Mohamad M, Hasraf Mat Nayan N (2021) Citric acid: a green cross-linker of biomaterials for biomedical applications. *Eur Polym J* 146:110271. <https://doi.org/10.1016/j.eurpolymj.2021.110271>
- [20] Reddy N, Yang Y (2010) Citric acid cross-linking of starch films. *Food Chem* 118:702–711.

<https://doi.org/10.1016/j.foodchem.2009.05.050>

- [21] Wu H, Lei Y, Lu J, Zhu R, Xiao D, Jiao C, Xia R, Zhang Z, Shen G, Liu Y, Li S, Li M (2019) Effect of citric acid induced crosslinking on the structure and properties of potato starch/chitosan composite films. *Food Hydrocoll* 97:105208. <https://doi.org/10.1016/j.foodhyd.2019.105208>
- [22] Li R, Mei X, Jin W, Yang Z, Liu Y, Li Z, Xu X, Wang P (2025) Perfect balance between the water absorption and mechanical properties of carboxymethyl cellulose/polyvinyl alcohol electrospun nanofibre pads through citric acid cross-linking. *Food Prod Process Nutr* 7:62. <https://doi.org/10.1186/s43014-025-00341-0>
- [23] Mansur AAP, Rodrigues MA, Capanema NSV, Carvalho SM, Gomes DA, Mansur HS (2023) Functionalized bioadhesion-enhanced carboxymethyl cellulose/polyvinyl alcohol hybrid hydrogels for chronic wound dressing applications. *RSC Adv* 13:13156–13168. <https://doi.org/10.1039/D3RA01519J>
- [24] Capanema NSV, Mansur AAP, Carvalho SM, Martins T, Gonçalves MS, Andrade RS, Dorneles EMS, Lima LCD, de Alvarenga ÉLFC, da Fonseca EVB, de Sá MA, Lage AP, Lobato ZIP, Mansur HS (2023) Nanosilver-functionalized hybrid hydrogels of carboxymethyl cellulose/poly(vinyl alcohol) with antibacterial activity for prevention and therapy of infections of diabetic chronic wounds. *Polymers* 15:4542. <https://doi.org/10.3390/polym15234542>
- [25] Zhang Z, Liu L, Wang H, Xie W, Zhai W, Wen L, Zhang B, Liu K, Zhang X, Liu S, Huang L, Li D, Zhou Y (2025) LIPUS responsive dopamine-modified PVDF piezoelectric nanofiber membrane for full-thickness skin wound healing. *Int J Nanomedicine* 20:5693–5707. <https://doi.org/10.2147/IJN.S496921>
- [26] Santos MB, Souza JAS, Cândido VS, Del Nero J, Paschoal Junior W, Maia AAB, Viegas BM, Alves Junior S, Silva Jr CAB, Paula MVS (2026) Corn starch/carboxymethyl cellulose films: influence of citric acid on water susceptibility, morphological and tensile properties. *ACS Omega* 11:5479–5488. <https://doi.org/10.1021/acsomega.5c08949>
- [27] Mansur AAP, Carvalho SM, Brito RMdM, Capanema NSV, Duval IdB, Cardozo ME, Rihs JBR, Lemos GGM, Lima LCD, Reys MPd, Rodrigues APH, Oliveira LCA, Sá MA, Cassali GD, Bueno LL, Fujiwara RT, Lobato ZIP, Mansur HS (2024) Arginine-biofunctionalized ternary hydrogel scaffolds of carboxymethyl cellulose–chitosan–polyvinyl alcohol to deliver cell therapy for wound healing. *Gels* 10:679. <https://doi.org/10.3390/gels10110679>
- [28] Pratinthong K, Punyodom W, Jantrawut P, Jantanasakulwong K, Tongdeesoontorn W, Sriyai M, Panyathip R, Thanakkasaranee S, Worajittiphon P, Tanadchangsang N, Rachtanapun P (2024) Modification of a carboxymethyl cellulose/poly(vinyl alcohol) hydrogel film with citric acid and glutaraldehyde crosslink agents to enhance the anti-inflammatory effectiveness of triamcinolone acetone in wound healing. *Polymers* 16:1798. <https://doi.org/10.3390/polym16131798>
- [29] Ghorpade VS, Dias RJ, Mali KK, Mulla SI (2019) Citric acid crosslinked carboxymethylcellulose-polyvinyl alcohol hydrogel films for extended release of water soluble basic drugs. *J Drug Deliv Sci Technol* 52:421–430. <https://doi.org/10.1016/j.jddst.2019.05.013>
- [30] Huang SM, Liu SM, Tseng HY, Chen WC (2023) Effect of citric acid on swelling resistance and physicochemical properties of post-crosslinked electrospun polyvinyl alcohol fibrous membrane. *Polymers* 15:1738. <https://doi.org/10.3390/polym15071738>
- [31] Nataraj D, Reddy R, Reddy N (2020) Crosslinking electrospun poly(vinyl alcohol) fibers with citric acid to impart aqueous stability for medical applications. *Eur Polym J* 124:109484. <https://doi.org/10.1016/j.eurpolymj.2020.109484>
- [32] Khin MN, Ahammed S, Aziz T, Al-Asmari F, Sameeh MY, Cui H, Lin L (2024) Characterization

of composite film containing polyvinyl alcohol cross-linked with dialdehyde cellulose using citric acid as a catalyst for sustainable packaging. *Packag Technol Sci* 37:975–987. <https://doi.org/10.1002/pts.2841>

[33] Mirsadraee S, Wilcox HE, Korossis SA, Kearney JN, Watterson KG, Fisher J, Ingham E (2006) Development and characterization of an acellular human pericardial matrix for tissue engineering. *Tissue Eng* 12:763–773. <https://doi.org/10.1089/ten.2006.12.763>

[34] Haensig M, Mohr FW, Rastan AJ (2011) Bioresorbable adhesion barrier for reducing the severity of postoperative cardiac adhesions: Focus on REPEL-CV®. *Med Devices (Auckl)* 4:17–25. <https://doi.org/10.2147/MDER.S7957>

[35] Hayashi T, Kinashi K, Sakai W, Tsutsumi N, Fujii A, Inada S, Yamamoto H (2021) Spin-trapping analysis for thermal degradation of poly(vinyl alcohol). *Polymer* 217:123416. <https://doi.org/10.1016/j.polymer.2021.123416>

[36] Farias MCS, Costa WRP, Nóbrega KC, Romualdo VB, Costa ACA, Nascimento RCAM, Amorim LV (2025) Thermal degradation of carboxymethyl cellulose (CMC) in saline solution for applications in petroleum industry fluids. *Polymers* 17:2085. <https://doi.org/10.3390/polym17152085>

[37] James RMS, H'ng PS, Mohd Yusof N (2026) Preparation and characterization of polyvinyl alcohol/carboxymethyl cellulose films with citric acid and activated carbon for active food packaging. *BioResources* 21:6163–6178. <https://doi.org/10.15376/biores.21.3.6163-6178>

[38] Zhou R, Huang J, Zhang W, Wang W, Peng W, Chen J, Yu C, Bo R, Liu M, Li J (2024) Multifunctional hydrogel based on polyvinyl alcohol/chitosan/metal polyphenols for facilitating acute and infected wound healing. *Mater Today Bio* 29:101315. <https://doi.org/10.1016/j.mtbio.2024.101315>

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupportingInformation.pdf](#)