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Preparation and characterization of carboxymethyl cellulose/polyethylene glycol films containing bromelain/curcumin: In vitro evaluation of wound healing activity

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Abstract

In this study, a wound-healing membrane was fabricated based on carboxymethyl cellulose (CMC) and polyethylene glycol (PEG) containing curcumin (Cur) and bromelain (Br). Citric acid (CA) was used as a cross-linking agent. This membrane showed an ideal degree of swelling, which was significantly dependent on the concentration and duration of cross-linked CA. The chemical characterization showed the CA cross-linker mechanism was more associated with chemical reactions with CMC carboxyl groups, and PEG hydroxyl groups played an important role in forming a hybrid polymer network. It greatly enhanced mechanical and adhesive properties, so the stress strength was improved from 29.4 to 38.52 Mpa. The hydrophilicity of the membrane surface according to the water contact angle assay showed the membrane surface is suitable for adhesion, growth, migration, and proliferation of skin cells. The drug delivery assay demonstrated that the Br and Cur were released during 48 h, but the Br followed the burst release in comparison with Cur. Antibacterial properties showed that CMC/PEG-Cur/Br have ideal antibacterial properties for preventing the growth of bacteria. In summary, the engineered CMC/PEG containing Cur/Br films with desired cell viability properties and antibacterial activity can potentially improve and accelerate skin regeneration for chronic wound healing.

Highlights

- Hydrogel film was prepared based on carboxymethyl cellulose (CMC), polyethylene glycol (PEG), and citric acid as cross-linking agent.
- CMC/PEG film were induced controlling curcumin (Cur) and bromelain (Br) release for wound healing process.
- CMC/PEG film revealed a wide range of physiochemical, mechanical, adhesion, and biological properties behavior.
- Incorporation of Cur and Br promoted in vitro biocompatibility, L-929 cells attachments, and cell migration.

KEYWORDS

antibacterial properties, bromelain, carboxymethyl cellulose, curcumin, drug delivery, polyethylene glycol, wound dressing

1 | INTRODUCTION

As the largest human tissue, the skin serves a crucial role as a barrier against infection.[1] Due to an incomplete physical barrier and exposed subcutaneous tissue, the skin tissue is vulnerable to bacterial infection, which can impede the healing process and pose significant health threats to patients, ultimately leading to an increased economic burden.[2,3] The healing of infected integumentary tissue is a complex physiological process that involves multiple mechanisms, including antibacterial functions, regulation of inflammation, and angiogenesis.[4,5] Nevertheless, a multifunctional strategy that can produce synergistic effects has yet to be developed.[6,7] Hemostasis, inflammation, proliferation, and remodeling are all steps in the natural healing process.[8] In addition, the timing of wound healing is critical for preventing abnormal tissue regeneration.[9] Consequently, several studies were conducted involving the use of natural polymers like chitin,[10] chitosan,[11] alginate,[12] Carboxymethyl cellulose,[13] and pectin[14] to develop wound dressings. As well as preventing bacterial infections, wound dressings maintain a moist environment in the wound, absorb excessive exudate, and accelerate tissue regeneration.[4] Therefore, developing novel wound dressings with the potential to speed up cell proliferation and shorten the anti-inflammatory phase is imperative.[15]

Cellulose and its derivatives are commonly used as natural substitutes to natural polymers in a variety of fields of study, including wound dressing, delivery of pharmaceuticals, and tissue engineering.[16] In nature, the carbohydrate polymer cellulose is widespread.[17] The advantages of these materials include biocompatibility, biodegradability, solubility in water, resources, cost-effectiveness, and environmental friendliness. For a period of time, CMC has been used as an organic material in hydrogels due to its high carboxymethyl group concentration on the cellulose backbone. Tissue engineering, delivery of drugs, wound dressing, and plant breeding are some of the applications of CMC.[18,19]

Polyethylene glycol (PEG) is an amphiphilic and water-soluble polyether, biocompatible, and not immunogenic.[20] Furthermore, PEG is relatively inexpensive. PEG has hydroxyl groups that can be easily combined with various functional groups at the chain terminal.[21] PEG-based hydrogels have been thoroughly investigated for delivering drugs. In addition, hydrophobic interactions between PEG ethylene groups and hydrophobic drugs as well as hydrogen bonding between CMC and PEG can help with long-term control and drug release.[22,23]

According to recent research studies, chronic inflammation and bacterial infection are the primary causes of delayed wound healing and angiogenesis at the wound site.[4] Turmeric (Curcuma longa) is commonly used as a herbal product in Asia to treat disorders such as rheumatism, anorexia, diabetic ulcers, and sinusitis.[24,25] Curcumin (Cur) is a major bioactive compound in Curcuma longa that is anticarcinogenic, anti-inflammatory, antioxidant, anticoagulant, antimutagenic, and anti-infectious.[26] It is supposed to have wonderful wound-healing properties. Curcumin enhances the formation of granulation tissues, collagen deposition, tissue remodeling, and wound contraction.[27] On the other side, bromelain (Br) is a proteolytic enzyme derived from pineapple stems and the mechanism of its action is generally anti-inflammatory, antimicrobial, and anticoagulant. This enzyme destroys unstable or weak and damaged tissues such as collagen, elastin, and fibrin formed on the wound, which is an excellent culture medium for pathogens, hydrolyzes by proteolysis, and stimulates angiogenesis and induces growth factors.[28,29] Moreover, previous studies revealed the role of Br in enzymatic debridement and its efficacy in chronic wound treatment.[30–32]

This study aimed to fabricate a multifunctional wound dressing containing Cur/Br with antibacterial activities. Cur/Br was incorporated into CMC/PEG membranes to promote the wound healing process. CMC/PEG with and without drugs was characterized based on their physicochemical and mechanical properties. Samples were evaluated for their cell migration, antibacterial response, and cell viability and adhesion activity.

2 | MATERIALS AND METHODS

2.1 | Materials

Sigma-Aldrich (USA) provided sodium carboxymethyl cellulose (CMC, average molar mass Mw = 100 kDa and low viscosity) and citric acid (CA, 99.5%, HOC(COOH)$(CH_2COOH)_2$). Merck (Germany) provided the PEG

(Mw = 2000 Da) and bromelain (Br, Mw = 5-U-FIP/mg). Riedel-dehaen (Germany) provided the curcumin (Mw = 368.39 Da). Bioidea of Iran supplied the phosphate buffered saline (PBS). The solutions were prepared using Nalgon (Brazil) filter paper with a pore size of 4 m, deionized water (DI water, Millipore Simplicity™), and the operations were carried out at room temperature (RT, 25 ± 5°C).

2.2 | Synthesis of CMC/PEG and CMC/PEG/Br/Cur hydrogel films

CMC:PEG solutions were obtained by combining 100 mL of DI water with 1.8 g of CMC and 0.2 g of PEG (i.e., 90% w/w of CMC and 10% w/w of PEG) and stirring at room temperature until the polymers were completely dissolved. Following dissolution, 10% w/w% of the cross-linking agent CA were added while being stirred for 20 min. After that, 0.25 mg of Cur and 0.025 mg of Br were added to PEG/CMC hydrogel solution. 10 mL of each solution was then added to a cast and dried for 24 h at 45 ± 3°C. The samples were kept at 65 ± 5°C (slow evaporation technique) for 24 h to allow the cross-linking process to complete. A sample without CA was also achieved and dried at the same temperatures for comparison.

2.3 | Chemical characterization of CMC/PEG hydrogel films (Fourier transform infrared spectroscopy)

The Fourier transform infrared spectroscopy (FTIR) analysis has been used to determine the chemical structure of the hydrogel films with and without drugs. PEG/CMC, PEG/CMC/Cur, and PEG/CMC/Cur/Br samples have been studied in the 4000–400 cm^{-1} wave number range.

2.4 | Physical evaluation of hydrogel films

The hydrophilicity of hydrogel samples (cross-linked CMC/PEG, CMC/PEG/CUR, CMC/PEG/Br, CMC/PEG/Cur/Br, and non-cross-linked CMC/PEG) was determined by measuring the water contact angle (a 6 μL droplet) with the sample's surface at room temperature ($n = 3$).

Cross-linked and non-cross-linked samples ($n = 3$) were weighted (W_1) and then soaked in PBS solutions at 37°C for 1, 2, 24, and 48 h to examine the impact of cross-linking on the mass swelling ratio (MSR) of the hydrogels. After weighing the wet hydrogels (W_2), the swelling ratio was calculated using Equation (1).

$$\text{Swelling ratio}\,(\%) = (W_2 - W_1)/W_1 \times 100 \quad (1)$$

Furthermore, the hydrogels were weighed (W_1) and immersed in a 5 mL solution of PBS in order to determine the degradation rate of the samples ($n = 3$). The hydrogels were dried and weighed after 1, 3, 7, 14, and 21 days of incubation at 37°C (W_2). The degradation ratio was then calculated using Equation (2).

$$\text{Degree of degradation}\,(\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

2.5 | In vitro drugs release

Cross-linked hydrogels contained Br and Cur (0.1 g) soaked in PBS solution (pH = 7.5)[33] for using in in vitro release assays. UV–Vis spectroscopy was used to determine the amount of Br and Cur released from the produced hydrogel at 280 and 452 nm, respectively, and the amount of drugs released was calculated from the calibration plot.[34,35] The drug-loaded hydrogel samples were shaken at 37°C and 100 rpm to release entrapped drugs into PBS (pH 7.5) in order to calculate the percentage of drug release. To maintain the release medium volume, 200 μL of buffer solution and 200 μL of fresh PBS solution were removed at predetermined intervals. Equation (3) was used to measure the amount of drugs released in each test, and it was done in triplicate.

$$\text{Accumulation of drug release}\,(\%) = \frac{\text{Amount of drugs released}}{\text{Amounts of drug loaded in films}} \times 100 \quad (3)$$

2.6 | Mechanical evaluation of hydrogel films

The mechanical properties of hydrogel samples were characterized using a tensile tester (Hounsfield H25KS, load cell 500 N, United Kingdom). For tensile testing, the samples with (30 mm × 10 mm × 0.5 mm) dimension ($n = 5$) were cut, according to the previous studies.[36] The strain rate was adjusted at 1 mm/min. Consequently, the stress–strain curves, tensile strength, percentage of elongation-at-break, and elastic modulus were obtained.

2.7 | Adhesive strength evaluation

A shear assay was performed using cow's skin, by ASTM F2458–05 standards, to measure the adhesive properties of the CMC/PEG and CMC/PEG-Cur/Br. Briefly, cow

skin was cut into small squares with a surface area of 1 cm^2. Two pieces of sheet metal were glued to the outer surfaces of the hair-removed skin. On the interior surfaces of both skin pieces, 500 μL of gel CMC/PEG and CMC/PEG-Cur/Br solution containing CA was applied. Finally, the samples were elongated at 1 mm/min until the separation.[37] Stress–strain curves were used to establish the adhesive strength of CMC/PEG and CMC/PEG-Cur/Br films hydrogels.

2.8 | Evaluation of antibacterial properties of CMC/PEG with drugs hydrogel films

Gram-negative *Escherichia coli* (ATCC 27853) and Gram-positive *Staphylococcus aureus* (ATCC 25923) bacteria were purchased from Pasteur Institute of Tehran, Iran and used to test the antibacterial properties of films. According to our previous study, nutrient agar medium was prepared and sterilized.[36] Over the nutrient agar medium, the overnight cultured medium containing bacteria (100 μL) was spread using a glass L-rod. The hydrogel samples were washed with a PBS solution three times and sterilized under UV-ray for 20 min. CMC/PEG and CMC/PEG containing Cur/Br hydrogels were placed in a nutrient Hinton agar and incubated at standard conditions for 24 h at 37°C. To measure the clearance zones around the discs, the plates were monitored. The test was done in triplicate.

2.9 | Cell culture

The interaction of fibroblast cells with PEG/CMC and PEG/CMC/CUR/BR hydrogel samples was examined using the MTT assay. Iran National Cell Bank provided the L929 fibroblast cell line. First, 7 mm-diameter hydrogel samples were created, treated with 70% ethanol, and sterilized for 20 min under UV light. At 37°C and 5% CO_2, fibroblast cells were cultured in dulbecco's modified eagle medium (DMEM) with 10 v/v% fetal bovine serum (FBS) and 1 v/v% streptomycin/penicillin. As a consequence, tissue culture plate (TCP) (negative control) and samples were seeded with fibroblast cells (10^4 cells/well), which were subsequently cultured for 5 days with the medium used for culture replaced every other day.

The incubation medium was discarded on the first, third, and fifth days, and the cell-seeded samples were incubated with 50 μL of MTT solution (5 mg/mL) for 4 h. Following dissolution of the dark blue formazan crystals in dimethyl sulfoxide (DMSO), 50 μL of each sample's dissolved formazan solution was then added to a 96-well plate, and the optical density of each sample was measured against DMSO at 490 nm using the microplate reader (BioRad 680 Instruments, USA). The relative cell survival has been calculated using the equation below[38]:

$$\text{Relative cell survival}\,(\%\ \text{to control}) = \frac{X_S - X_d}{X_t - X_d} \quad (4)$$

where X_S, X_d, and X_t were absorbance of the sample, DMSO was the blank sample, and TCP was the control group, respectively.

After 3 days of cell seeding, the samples were rinsed with PBS and fixed with 1.5% glutaraldehyde for 4 h at 4°C in order to study the morphology and adhesion of the L929 cells on hydrogel samples. The cells were then air-dried after being dehydrated with ethanol at various doses. The samples were coated in gold and examined using scanning electron microscopy (SEM, Philips, XL30) before SEM imaging.[39]

For evaluation of cells migration, 5×10^4 cells/well of L929 fibroblast cells were seeded into 12 well plates as a model system and incubated for 24 h to provide a cell monolayer. According to previous studies,[40] an in vitro wound healing assay was done using a 100 μL sterile pipette tip on the middle of the confluent surface and the films with a diameter of 20 mm, placed on both sides of the scratch and confluence cells surface. In vitro wound closure was imaged by an inverted microscope (Olympus, Japan) during 0, 12, and 24 h. Also, the cell-seeded TCP was used as the control. After 24 h, the wound closure was evaluated by ImageJ Software (2020, MRI wound healing tool).

3 | RESULTS AND DISCUSSION

3.1 | Chemical characterization (FTIR)

The FTIR analysis determined the functional groups of CMC/PEG with the cross-linking agent and CMC/PEG-Cur/Br. As shown in Figure 1, the FTIR results indicated that numerous peaks in the range of 600–3000 cm^{-1}, which could be defined as various chemical groups, such as C—O at 1300 cm^{-1}, carboxylic bond at 1600 cm^{-1}, —COOH groups at 1730 cm^{-1}, CH functional groups at 2786 cm^{-1} demonstrating the presence of CMC.[41–43] Additionally, the FTIR spectrum of the samples containing PEG exhibited new peaks at 1100 cm^{-1} (C—O—C bond), 1240 cm^{-1} (C—O), and 1360 cm^{-1} (C—H) that demonstrated the presence of PEG.[44] After cross-linking CMC/PEG samples, the presence of the board peak of C═O (at 1724 cm^{-1}) and reduce intensity during 3200 and 3600 cm^{-1} of hydroxyl band was often due to chemical interaction between hydroxyl and ester groups.[22]

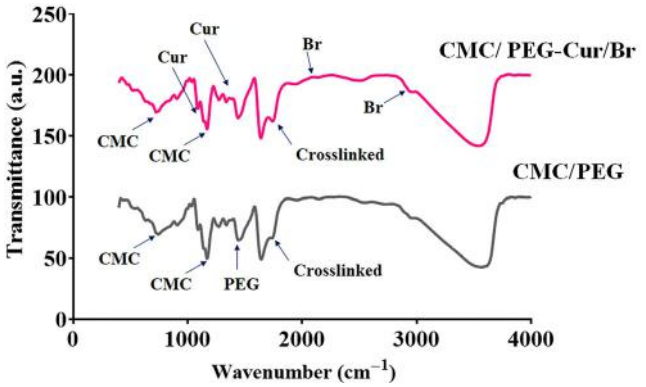

FIGURE 1 Fourier transform infrared spectra of carboxymethyl cellulose/polyethylene glycol (CMC/PEG) and CMC/PEG containing curcumin/bromelain (Cur/Br).

Additionally, By incorporating Br into the membranes, some functional groups was apeared in the range of 1200-2900 cm^{-1}, such as C—N stretching at 1510 cm^{-1}, C═O at 1654 cm^{-1}, and C═O at 1634 cm^{-1}.[29,45,46] After adding Cur to samples the sharp peak showed at 1277 cm^{-1}, and 1071 cm^{-1} that related to C—O tensile vibrations and C—O—C tensile vibrations, respectively.[47,48]

3.2 | Physical evaluation of hydrogel films

3.2.1 | Water wettability

According to water contact angle assay results (Figure 2A), the non-cross-linked-CMC/PEG membrane was the most hydrophilic sample (~28.2 ± 4°) compared with other groups. By adding CA to CMC/PEG the water contact angle increased to 40.7 ± 1.8° which is due to the consumption of hydroxyl and carboxyl chemical groups of PEG and CMC by CA carboxyl group in a cross-linking process that reduces the interaction of the polymer with water molecules. Similar changes of CA effect in cellulose-based film were explored by previous researchers.[49] For example, Wu et al.[50] evaluated the effect of CA of potato starch/chitosan composite films and demonstrated that by adding CA the water contact angle increased from 34.47° to 56.43° (with addition of 15% CA), indicating that the surface hydrophobicity of potato starch/chitosan films was markedly improved. This could be related to the formation of hydrophobic ester groups between citric acid and the polysaccharides, leading to a decrease in the number of polar groups. The water contact angle of the CMC/PEG/Br and cross-linked CMC/PEG hydrogel samples did not differ significantly ($p > 0.05$) while it increased to 66.32 ± 6° in the CMC/PEG/Cur sample because of the hydrophobic nature of the Cur. In the CMC/PEG containing Cur/Br, the contact angle was measured at 56.03 ± 4° which can be due to the superposition effect of hydroxyl functional groups of the Br and the hydrophobic nature of the Cur.[51,52] Perumal et al.[53] demonstrated that the contact angle between 40 and 60° can be ideal for repairing skin-damaged tissues through optimize the skin cell adhesion and proliferation.

3.2.2 | In vitro swelling ratio

Figure 2B shows the swelling ratio of the samples. There is no significant difference between the CMC/PEG samples exposed to the cross-linking process for 24 and 48 h but for the samples with an 18 h cross-linking time, the swelling ratio showed a dramatic increase (about 1.5 times more than the other samples).

These results show that the cross-linking process is not completed within 18 h, and there is some vacant space in the bulk of the hydrogel and between the polymeric chains that caused water penetration in the polymer network. But after 24 h, the cross-linking process is completed resulting in the involvement of hydrophilic functional groups, and the creation of strong covalent bonds, which leads to the entanglement of the polymer networks and reduces the vacant spaces between the chains. Therefore, the water absorption capability decreases. However, results showed that the CMC/PEG samples with a 24 h cross-linked process are still in the range of super absorbent dressing. According to the obtained results, increasing the cross-linking time from 24 to 48 h does not make a significant difference in increasing covalent bonding, so in the continuation of the study, the 24 h cross-link process time was selected. Also, the results showed that the water absorption decreased with the addition of Cur, which is due to the hydrophobic nature of Cur. This result is consistent with the previous findings of the samples' hydrophilicity evaluations. Moreover, by adding Br as a hydrophilic drug, the interaction of water with the polymer is enhanced due to the increase of hydroxyl functional groups, which leads to a rise in the water absorption capacity of the hydrogel network and an increase in swelling. Another research also confirms the increase of scaffold water absorption by adding Br.[29]

3.2.3 | In vitro degradation behavior

The results of cross-linked CMC/PEG hydrogel with different cross-linking process times are presented in Figure 2C. The non-cross-linked CMC/PEG membrane was completely degraded during the first hour (not presented). There was no significant difference in the weight

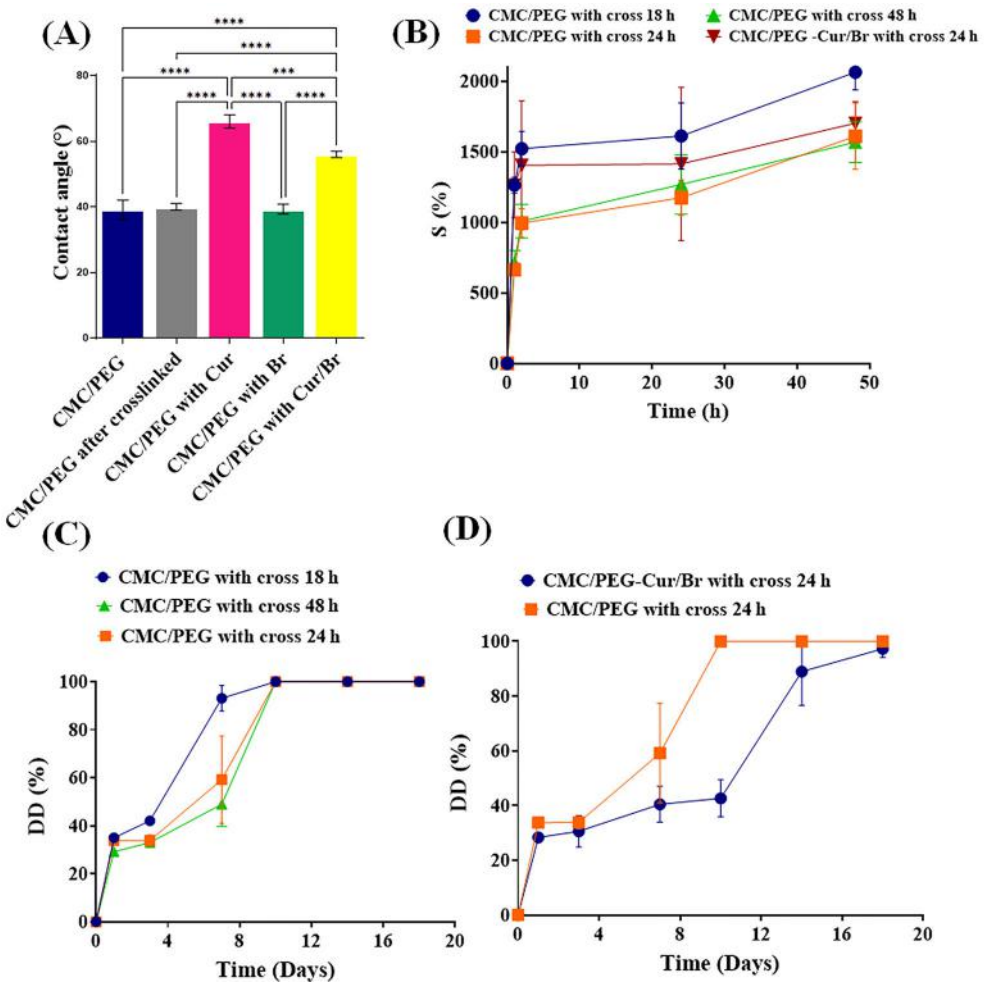

FIGURE 2 Physiological stability of carboxymethyl cellulose/polyethylene glycol (CMC/PEG) and CMC/PEG containing curcumin/bromelain (Cur/Br): (A) water contact angle, (B) mass swelling ratio in the phosphate buffered saline (PBS) solution, (C) degradation of the hydrogels during 18 days of incubation in PBS for samples without drug with different cross-linked times, and (D) degradation of the hydrogels during 18 days of incubation in PBS for samples with 24 h cross-linked process. All the values are expressed as means ± standard deviation for $n = 3$ specimens.

loss and degradation rate of the samples subjected to a 24 and 48 h cross-linking process while it was faster for the samples with an 18 h cross-linking process. The results following the swelling assays confirm that 24 h is enough time to completely cross-linked CMC/PEG hydrogel. This finding is very important for controlling the release of drugs. Therefore, in the next step, the degradation study was performed for the CMC/PEG containing Cur/Br by the 24-h cross-linking process. Figure 2D shows the effect of the presence of drugs in the cross-linked hydrogel on the degradation process. The sample containing drugs experienced a slower degradation rate. It could be due to the hydrophobic nature of Cur, which delays the diffusion of water into the hydrogel structure. Also, the results of the contact angle assay showed that in the hydrogel containing both drugs, the presence of Br with hydrophilic functional groups reduced the hydrophobic effect of Cur, however, it still has a greater contact angle (more hydrophobicity) compared with the samples without drugs. According to Figure 2D a 3-stage weight loss pattern was observed. At the initial stage of degradation, the hydrogel followed the continuous degradation and this was caused by the swelling water molecules penetration in the polymer structure. As time increased, in the second stage, the longer chains in the polymer structure break to the shorter chains through the hydrolysis process. In the third stage, the exit of smaller chains from the bulk of the hydrogel led to the intensification of weight loss.[54] All of the cross-linked CMC/PEG samples maintained their integrity well until the 5th day and most of them were completely degraded by day 12. The degrading behavior shown in this study may be ideal for wound healing hydrogels. The degradation of wound dressing is important for the drug's efficacy because it affects drug dispensing patterns and facilitates cell diffusion, nourishment flow, and integration with host tissue.[55]

3.3 | Drug release

The cross-linked CMC/PEG/Cur/Br hydrogel was immersed in PBS medium and cumulative drug release was monitored for up to 48 h. As can be seen from

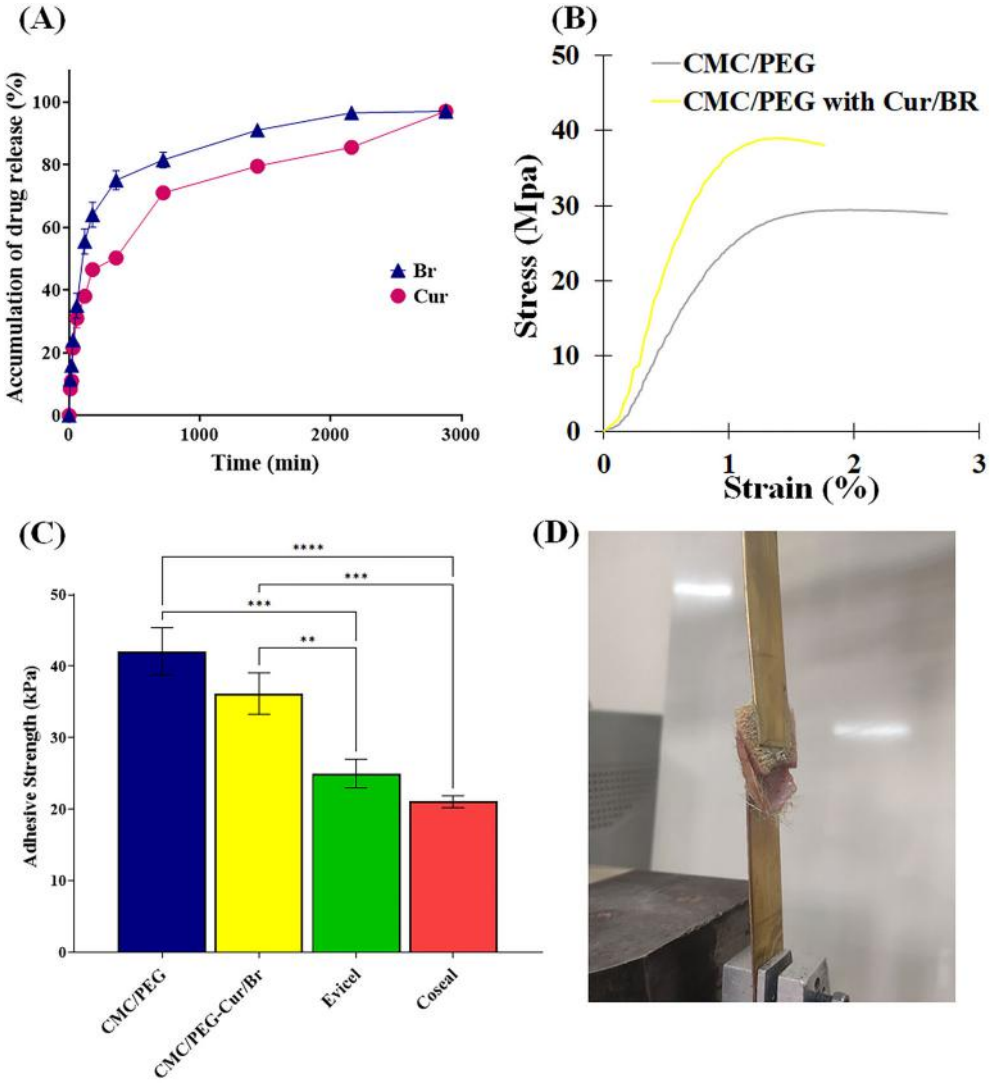

FIGURE 3 (A) Evaluation of curcumin (Cur) and bromelain (Br) release from carboxymethyl cellulose/polyethylene glycol (CMC/PEG) containing Cur/Br in the phosphate buffered saline solution at 37°C for 24 h. All the values are expressed as means ± standard deviation for $n = 3$ specimens, (B) stress–strain curve of CMC/PEG and CMC/PEG containing Cur/Br, (C, D) the average adhesive strength of CMC/PEG, CMC/PEG-Cur/Br, and commercial samples.

Figure 3A, about 50% of Cur release in the first 12 h while it was about 80% for Br. The faster release of Br is related to the more hydrophilic nature and smaller size of the chemical structure of its molecule. After 48 h, almost all of the drugs were released from the hydrogel. This drug release pattern is related to the degradation process, drug retention in the cross-linked hydrogel network, and its hydrophilicity. Referring to Figure 2, it can be seen that a fast degradation rate occurred in the first 48 h leading to the 40% weight loss which is related to the dissolution of the amorphous region of the cross-linked hydrogel. It not only causes the release of the existing drug in this region but facilitates water penetration into the hydrogel bulk and accelerates the release of the drug. On the other hand, proper cross-linking of the hydrogel structure partially controlled the release of Br in the early hours. Shoba et al.[56] reported an 80% release of Br from PVA/PCL/Br core-shell fibers during 5 h. Also, Bayat et al.[29] observed a 90% release of Br from chitosan particles during 4 h. In the present research, 60% of Br was released during 4 h and reached about 80% during 12 h.

Previous studies have shown that releasing Cur (as an antioxidant drug) during 48 h can accelerate the wound healing process. Previous research also indicates that the ideal wound dressing releases an antibacterial agent in the early hours to prevent bacterial growth and infection of the wound.[57,58]

3.4 | Mechanical evaluation of hydrogel films

Stress-strain behavior of the CMC/PEG samples with and without drugs is presented in Figure 3B. The results demonstrated that the ultimate strength of hydrogel with drugs (38.52 ± 3.4 MPa) was significantly higher than the sample without drugs (29.4 ± 2.1 MPa). Also, adding drugs to CMC/PEG hydrogel increased its elastic modulus while its elongation decreased. It seems interaction

between the functional groups of Cur and Br with the functional groups of the hydrogel causes a hydrogen bounding and acts as an extra cross-linking in the structure enhancing the hydrogel's strength and stiffness.[59] Divakaran et al. also reported that adding Cur to polyurethane hydrogels improved compressive strength because the drug partially filled the network vacancy and formed new bonds in the hydrogel/drug network.[60]

3.5 | Adhesive properties of CMC/PEG

To evaluate the suitability of CMC/PEG based as a wound dressing, we measured the adhesive strength of the gel based hydrogel films by using cow skin (Figure 3C,D). Figure 3C shows the bond strength of his PEG/CMC and PEG/CMC-Cur/Br compared to Evicel and Coseal commercial wound dressings. The results showed that the adhesive strength of PEG/CMC based with or without drugs were significantly higher than both commercial wound dressings in the range of 35–43 kPa.

The suitable adhesive strength characteristics of the CMC/PEG and CMC/PEG-Cur/Br could be related to hydrogel-tissue interlocking,[61] hydrogen bonding between free hydroxyl, and carboxyl groups.[62]

3.6 | Evaluation of antibacterial properties

Microorganisms have easy access to open wounds. Wound dressings with antibacterial activity at the wound site are preferred to prevent their attraction to the wound side and infections. As a result, the potential of hydrogels to suppress bacterial growth was evaluated by adding and controlling the release of antibacterial medications and agents.[63,64] Figure 4A,B presented the antibacterial properties of CMC/PEG and CMC/PEG containing Cur/Br, against both *S. aureus* and *E. coli* bacteria using the zone-inhibition approach. It was observed that CMC/PEG-Cur/Br samples showed a superposition effect against both bacteria strains, and the inhibition zone against *E. coli* and *S. aureus* was 35 mm and 26 mm, respectively. Infection is a challenge in skin wound healing, which may postpone the regeneration process of damaged tissues.[65] Moreover, antioxidation activity of the CMC/PEG/CUR/BR hydrogel could inhibit bacterial biofilm formation, and prevent bacterial attachment to host receptors.[66]

Previous research revealed that Gram-positive bacteria are more susceptible to Cur than Gram-negative ones.[14] In our investigation, *S. aureus* had substantially broader inhabitation zones than *E. coli* for the same dosages of curcumin-containing hydrogels. Furthermore, because *S. aureus* is the most common bacteria on skin and in infected wounds, the hydrogels developed in this work may be efficient against infection when used as wound dressing material.

In the present study, the potential antibacterial effect increased due to the presence of Br. According to previous studies, Br can break the bonds of bacterial protein that can inhibit the growth of bacteria.[65] Br also inhibits the development of certain bacteria by inhibiting bacterial attachment to particular glycoprotein receptors on the surface. Furthermore, Br suppresses the formation of enterotoxin by *E. coli* and prevents *E. coli*-caused diarrhea.[67] Br is antibacterial against both Gram-positive and

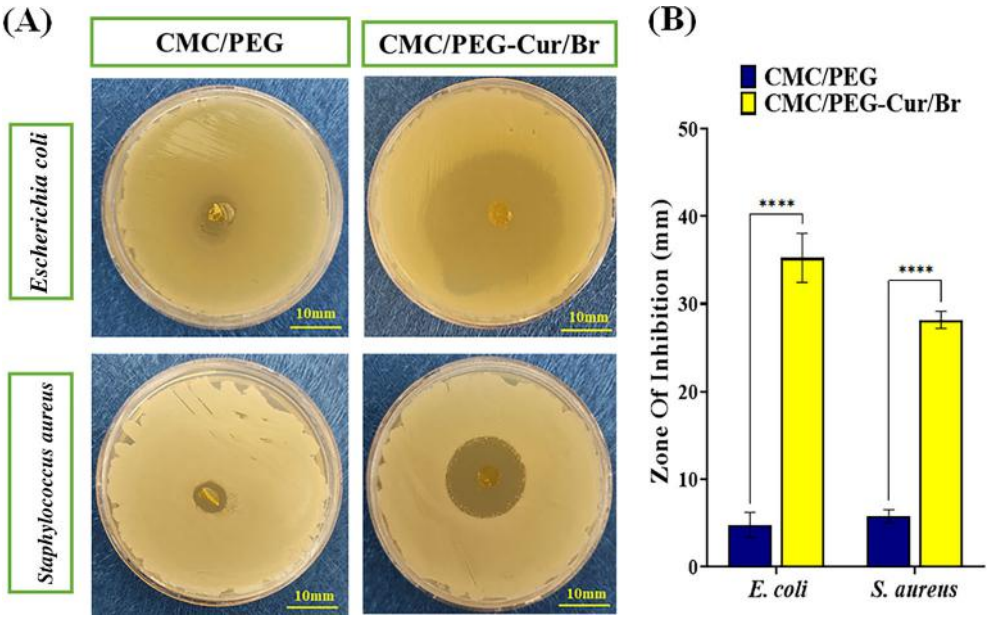

FIGURE 4 (A) Digital images of inhibition zone of samples and (B) quantitative evaluation of inhibition zone of different films. All the values are expressed as means ($n = 3$) ± standard deviation (*$p < 0.05$). CMC/PEG, carboxymethyl cellulose/polyethylene glycol; Cur/Br, curcumin/bromelain.

Gram-negative bacteria, including *E. coli* and *S. aureus*.[68] Furthermore, the synergistic usage of bromelain with antibiotics boosts the antibacterial action due to higher antibiotic absorption mediated by bromelain, resulting in better medication distribution in microorganisms.[69,70]

Similarly, Hasannasab et al.[71] fabricated an antibacterial and anti-inflammatory wound dressing for burn wound by adding bromelain and zinc oxide nanoparticles on silk fibroin nanofibers and demonstrated that release of Br at the wound site prevents bacterial colonization and wound infection.

3.7 | Cell culture

Cell viability of the L929 fibroblast cell line against the CMC/PEG, CMC/PEG-Cur, and CMC/PEG-Cur/Br hydrogel samples was evaluated using MTT assay, and the results are presented in Figure 5. It demonstrated that cross-linked CMC/PEG hydrogel with and without drugs have no cytotoxicity effects. Moreover, there was a significant difference in L929 proliferation of cells in the vicinity of the CMC/PEG/Cur/Br group after 5-day cell culture ($p < 0.05$).

The better cell performance in the CMC/PEG-Cur/Br hydrogel was related to the proper dosage of the drugs and their suitable hydrophilic surface. According to earlier studies, suitable hydrophilicity (contact angle of around 50) and mechanical properties mimic the surrounding soft tissue.[72,73]

Similarly, Capanema et al.[22] evaluated the cells interaction of CMC/PEG cross-linked with CA and demonstrated that these CMC-based hydrogels were cytocompatible considering the in vitro cell viability responses of ~95% toward human embryonic kidney cells (HEK293T) used as model cell line and can be suitable for wound healing application.

The SEM images of cells adhered to the CMC/PEG/Cur/Br hydrogel are shown in Figure 5B. The cells' significant adhesion, spreading, and anchoring confirmed that there were sufficient cell responses when in contact with CMC/PEG containing Cur/Br samples. In other words, cell adhesion exhibited a well-spread shape with obvious cellular pseudopodia in the Br-Cur-containing hydrogel samples. CMC/PEG-Br/Cur was shown to have no negative effect on initial cell adhesion. The cells gradually spread and covered the hydrogel sample.

In another study, Tao et al.[74] developed multifunctional near infrared laser-induced hydrogel based on

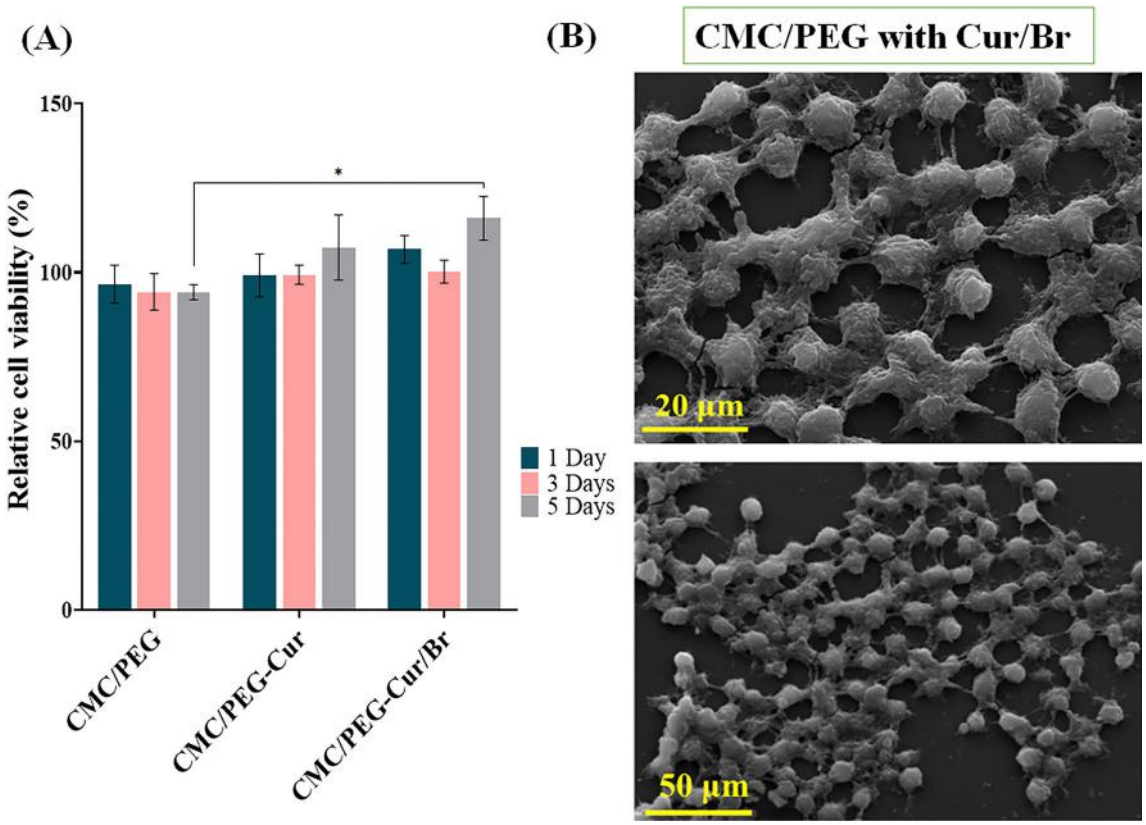

FIGURE 5 Cell cytotoxicity, cell adhesion, and cell migration evaluation: (A) L929 cell viability and (B) scanning electron microscopy images of L929 cell morphology on the carboxymethyl cellulose/polyethylene glycol (CMC/PEG) with curcumin/bromelain (Cur/Br) after 3 days.

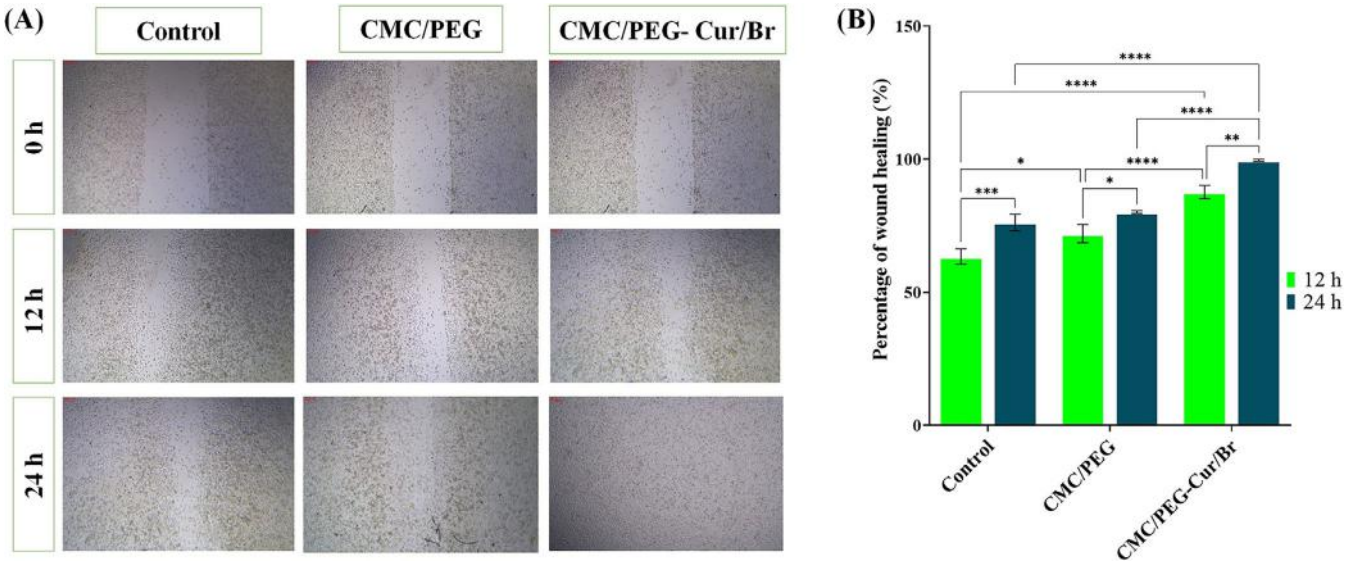

FIGURE 6 (A) The scratch wound assay images and (B) its analysis on L929 treated with carboxymethyl cellulose/polyethylene glycol (CMC/PEG) and CMC/PEG-curcumin/bromelain (Cur/Br) for 12 and 24 h. All the values are expressed as means ($n = 3$) ± standard deviation (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$).

poly (ethylene glycol)/Chitosan-Cur-loaded mesoporous polydopamine nanoparticles via Schiff/Michael addition reaction and their results showed by rapid release of Cur propitious for NIH-3 T3 fibroblast cells proliferation, exhibiting good biocompatibility, and cell attachment observed.

Similarly, Chen et al.[75] evaluated the biological response of bromelain immobilized eletrospun poly (ε-caprolactone) (PCL) fibers by using the dopamine-assisted and described that BrPDA-PCL fibers were biocompatible, allowing for effective cellular adhesion and proliferation. They exhibited superior biocompatibility relative to the hydrophilic nature of the PDA and Br for superior cellular adhesion, thus making them ideal for wound dressings application.

To investigate the potential of CMC/PEG-Cur/Br to stimulate cell migration, we conducted a scratch wound healing test. As shown in Figure 6A,B, CMC/PEG-Cur/Br significantly reduced the wound gap in a time-dependent manner.

Cur and Br delivery increased cell migration by 25% at 12 and 24 h compared to the control group. Similarly, Chittasupho et al.[76] revealed improved fibroblast migration in contact with Quercetin and curcumin wound dressing due to the release of curcumin. Improved formation and maturation of granulation tissue at the wound site could lead to the development of an extracellular matrix (ECM) for reepithelialization and blood vessel sprouting. It could benefit the early stages of wound healing.[77]

In another study, Xu et al.[78] evaluated the effect of Br in eschar dissolution and the immunoregulator effect of keratinase on burn wounds and demonstrated that by using Br the scratch was completely closed during 12 h.

4 | CONCLUSIONS

The goal of this research was to develop and fully evaluate biocompatible wound dressing membranes produced from a CMC derivative that has been chemically cross-linked by CA and modified with PEG. The outcomes showed that superabsorbent films (SAP) were developed with appropriate mechanical, adhesive, swelling, and degrading properties for skin wound healing. Using an in vitro MTT assessment, these wound dressings showed no cytotoxicity based on responses from over 95% of the cell viability responses (L929). Compared to the other sample, Cur/Br-containing CMC/PEG improved cell viability, adhesion, and migration while enhancing its antibacterial effects against both Gram-positive and Gram-negative bacteria. Chronic skin wounds have a long wound healing process with inflammations. It seems by using this hydrogel films structure with super absorbent capacity can lead to the creation a suitable environment for wound healing process. Also with the controlled release of an anti-inflammation and antibacterial drug such as curcumin and an antioxidant agent such as bromelain, it can accelerate the wound healing process and reduce inflammation responses.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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