Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Development of Blend Polymer Films for Wound Dressing Containing the Chlorhexidine-Loaded Nanoparticles for Controlled Drug Release

Orranat Eiangmee[1], Jatupat Maikaew[1], Naruebodee Srisang[1], Supreeda Tambunlertchai[2], Siriwan Srisang[1*]

[1]Department of Engineering, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand
[2]National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand
Email: siriwan.sr @kmitl.ac.th

The objective of this research is to develop a blend of polymer films from polyvinyl alcohol (PVA) and carboxymethyl cellulose (CMC) by using the crosslinking agent as an acetic acid. Then, the mixture was added: the healing agent is a hyaluronic acid (HA), and the antimicrobial agent is a chlorhexidine (CHX)-loaded micelle. The sample of blend polymer films was investigated in part of water absorption, tensile strength, and drug release. Results showed that the water absorption increased when adding the HA, as presented in every mixture in the water absorption range, at 300-550%. An excellent water absorption was demonstrated in the concentration condition of CHX and HA at 1% by v/v (mixture A). This mixture condition is compared with the addition of CHX-loaded micelles at the same concentration at 1% by v/v (mixture X). The tensile strength of mixture A and X were 264.5□18.1 and 474.3□50.7 kPa, respectively. Results in mixture X provided the reduction of burst CHX release from 80% to 40% within 12 hours. It indicated that the CHX-loaded micelle helps to reduce the burst release of CHX in the film sample and against the S.aureus. Hence, this formulation of the development of the combination of biopolymer, carboxymethyl cellulose, healing agent, and antimicrobial agent could be successfully further developed for wound dressing application and tissue engineering.

Keywords: polyvinyl alcohol, carboxymethyl cellulose, chlorhexidine, hyaluronic acid, controlled release.

1. Introduction

Various injuries can cause the wounds on the skin, whether from the big or small accidents such as bruises, car crash wounds, wounds from sharp objects, surgical wounds, and the pressure sore in the bedridden patient. All the wound types should protect the bacterial or

microbial infections which could help the wound for the better wound healing (Kalaycıoğlu et al., 2020; Zhang et al., 2021). Nowadays, the numerous types of the wound dressings present the new designing strategies such as loading the antiseptic agent, drug, antimicrobial agent, and healing agent for healthcare supporting the patients. All above agents such as silver nanoparticle, zinc nanoparticle, chlorhexidine, vancomycin, gentamicin, chitosan and hyaluronic acid could use to enhance in the wound dressing application (Meedecha et al., 2024; Yuan et al., 2022; Zhang et al., 2021). The development of blend polymer films for wound dressing application has been used the biopolymer which promotes the compatible properties, low toxicity, and applicable in the human body. The mostly biopolymers, for example, polycaprolactone, poly (lactic acid), poly(L-lactide- co-glycolide), polyvinyl alcohol, cellulose and some block copolymers are used as the matrix of the films for production the wound dressing and wound healing application. Moreover, the advance to develop the wound dressing and healing has been continually generated which designs the functional to prevent the bacteria infection, excellent healing by loading the drug for prolonged and controlled the drug release (Habibi et al., 2023; Pourseif et al., 2023). Polyvinyl alcohol (PVA) has been widely used to produce the polymer films due to it presents the excellent with flexible, translucent, non-toxic, biocompatibility, and biodegradable(Jin, 2022; Liu et al., 2023). Carboxymethyl cellulose (CMC), a cellulose demonstrates the high potential for using in the pharmaceutical industry because it has the excellent properties in term of biocompatibility, low cost, biodegradation, abundant resources, hydrophilic, and availability (Farshi et al., 2022; Rostamitabar, Ghahramani, Seide, Jockenhoevel, & Ghazanfari, 2022). CMC is highly the hydrophilic and it can form likely the gel structure during the liquid absorption which suitable for healing wound. Chlorhexidine (CHX) is the antibacterial agent which can prevent both gram-positive, and gram-negative bacteria including fungi by the mechanism of CHX acts to the bacterial cell membrane, cell surface, and acting into the bacteria cell (Srisang & Nasongkla, 2020; Srisang et al., 2020).

PVA and CMC are environmentally friendly as biological materials that can use to produce the wound dressing (Jin, 2022; Saraiva et al., 2023). Various research presents the suitable application for using the PVA, CMC as a composition to produce the biomaterial in the tissue engineering, medical devices by simply forming and 3D-printing (Harmanci et al., 2022; Rezaei, Poursamar, Naeimi, Taheri, & Rafienia, 2024). Due to the PVA and CMC have hardly mixed each other, it is necessary to use the crosslink agent for the conducting the bonding which provide the homogenously of the polymer films such as citric acid, acetic acid, and poly (ethylene glycol) diacrylate. Moreover, the using the nanoparticles such as micelles, nanospheres, and liposomes has been shown the application for controlled the drug release in the medical devices.

Hence, in this work aims to develop the blends polymer films by mixing the PVA, CMC by crosslink agent which adding the hyaluronic acid (HA) and loading the CHX, and CHX-micelles for wound dressing application. Results from this research will indicate the PVA/CMC combined with the HA and CHX-micelles as a polymer film which prepared by a simple crosslinking method have the potential use in wound healing application and tissue engineering application.

2. Materials and methods

2.1 Materials

Polyvinyl alcohol (PVA) molecular weight of 89- 98 kDa and poly (ethylene glycol)-block-poly (ε-caprolactone) (PEG-b-PCL) were purchased from Sigma-Aldrich (Damstadt, Germany). Carboxymethyl cellulose sodium salt (CMC) was purchased from Tokyo Chemical Industry Co., LTD (Japan). Acetic acid and chlorhexidine gluconate (CHX) were purchased from S. Tong Chemicals Co., Ltd (Bangkok, Thailand). Hyaluronic acid (HA) was purchased from S.N.P. scientific Co., LTD (Bangkok, Thailand).

2.2 Preparation the PVA/CMC polymer films

The mixture ratios of PVA, CMC, acetic acid, CHX and HA were shown in Table 1 and Table 2. The steps of forming the wound dressing sample were initial the mixing the PVA and CMC at 90:10 (%w/w) in the 1% of acetic acid solution by stirring on the hot plate at room temperature and 400 rpm for 3 hr. Then the previous mixture was added the CHX and HA as following the Table 1 which it continually stirred for 3 hr until the mixture showed the homogenously and poured into the petri dish. All samples were used the freeze-thaw method at -20 ºC for 24 hr, then take it out and place it at room temperature for 24 hr for 6 cycles.

2.3 Water absorption test

The wound dressing samples were tested the water absorption (WA) according to the standard ASTM D570. The samples were cut at the size 20 x 20 mm. Then samples were dried at 70 ºC for 5 hr, which obtained the dried weighed (W_0) of the samples. The samples were then immersed in deionized water (DI) for 48 hr, which obtained the wet weighed (W_1). The samples test was repeated in triplicate. Then the percentage of water absorption was calculated by following the equation (1):

$$\%WA = \frac{W_1 - W_0}{W_0} \times 100 \quad (1)$$

%WA is percentage of water absorption

W_1 is Wet weight.

W_0 is Dry weight.

2.4 Tensile strength test

The wound dressing samples were tested for the strength by the Stable Micro Systems version TA-XT plus and the test was following the standard ASTM D882. The samples were prepared the width at 15 mm and the length at 80 mm. The tension speed was 50 mm/min, and the load cell was 5 N. The samples test was repeated in triplicate.

2.5 In vitro release of CHX-loaded the PVA/CMC

polymer films

For the release study, CHX-loaded PVA/CMC polymer films were submerged in 5 mL of PBS at pH 7.4 and then incubated in a shaking incubator (Hanchen, model ES-60 E, UK) at

37 ◦C and 90 rpm. The sample was collected at 0, 3, 6, 12, 24, 48, and 72 hr.

2.6 Antibacterial properties of CHX-loaded the

PVA/CMC polymer films

The antibacterial properties of PVA/CMC polymer films were tested against Staphylococcus aureus ATCC 25923 using the disk diffusion method. The microorganism solution

was adjusted to 1 $\times 10^6$ CFU/mL. The sample was cut into a disk with a diameter of 5 mm, then placed in the middle of the plate, and incubated at 37 ◦C for 24 hr. The inhibition zone was measured. The samples test was repeated in triplicate.

Table 1 The mixture conditions of the PVA/CMC polymer films without the CHX-micelles.

Condition	PVA: CMC (%w/w)	Acetic acid (%v/v)	free CHX (%v/v)	HA (%w/v)
A	90:10	1	1	-
B			3	
C			5	
D			1	1%
E			3	
F			5	

Table 2 The mixture conditions of the PVA/CMC polymer films without the CHX-micelles.

Condition	PVA: CMC (%w/w)	Acetic acid (%v/v)	CHX-micelles (%v/v)	HA (%w/v)
W	90:10	1	1	-
X				1

3. Results and discussion

3.1 Preparation and characterization of the PVA/CMC polymer films

The PVA/CMC polymer films with using the acetic acid as a crosslink agent, adding the free CHX and without HA showed in the Figure 1A-1C. Results showed the milky color in all sample and not homogenously texture of the samples which comparison with adding the HA as shown in Figure 1D-1F. These samples in mixtures D, E, F showed more homogenously texture.

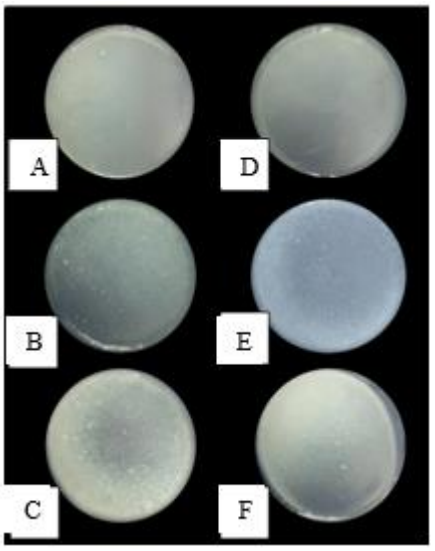

Figure 1. The PVA/CMC polymer films in each mixture and adding the free CHX.

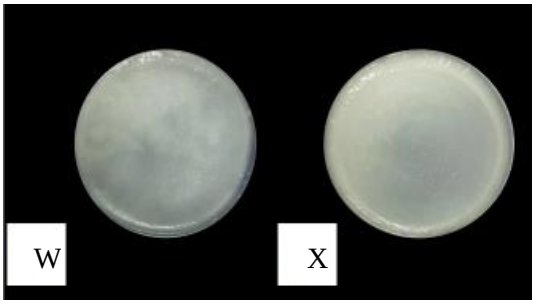

Figure 2. The PVA/CMC polymer films in each mixture and loading the CHX-micelles.

3.2 Water absorption of the PVA/CMC polymer films

The PVA/CMC polymer films samples were evaluated as following in the section 2.3. Results of the water absorption in all samples showed in the Figure 3. All samples presented the water absorption in range 300-550% that indicated the good properties for used in the wound dressing application. The degree wound vary depending on the ratio between PVA and CMC, type of crosslink agent and the percentage of crosslink agent used (Meedecha et al., 2024). Hence, all mixtures passed the water absorption properties. Moreover, the mixtures from the PVA/CMC polymer films in mixture A and mixture X were selected further to investigate the mechanical properties in term of tensile strength as shown in Figure 4.

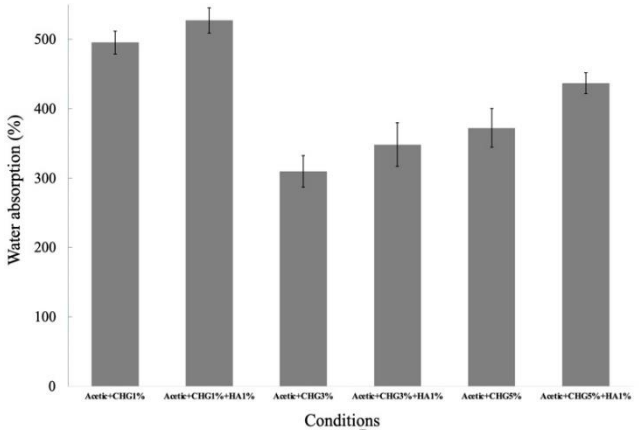

Figure 3. The water absorption of the PVA/CMC polymer films.

3.3 Tensile strength of the PVA/CMC polymer films

A wound dressing sample should be strong enough, not to easily break out by touch, and more flexible for cover the wound. The tensile strength of polymer films of this study was investigated by the uniaxial tensile test and obtained the stress of samples. The PVA/CMC polymer films in mixture A and mixture X were investigated the strength of the materials as shown in Figure 4. The tensile strength of mixture A and X were 264.5±18.1 and 474.3±50.7 kPa, respectively. The strength of mixture A was lower than the mixture X due to the content of HA that added in mixture X. It helps to increase the bonding in the film matrix while in mixture not adding the HA. This character of HA was reported by (Mohammadi, Alihosseini, & Hosseini, 2020; Patrick Micheels, Didier Sarazin, & Christian Tran, 2016).

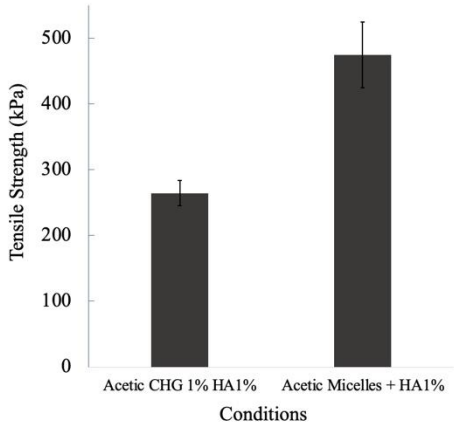

Figure 4. The tensile strength of polymer films in mixture A and mixture X.

3.4 In vitro CHX- released

The blend polymer films in mixture A and mixture X were investigated the CHX released as shown in Figure 5. Results showed the reduction of burst CHX release from 80% in mixture A to 40% in mixture X within 12 hours. It indicated that the CHX-micelles can helps the

prolonged and controlled the drug release (Srisang & Nasongkla, 2019a, 2019b, 2020; Srisang et al., 2020). The CHX released in mixture X can sustain more than 72 hr.This strategy can help the wound dressing to purpose in part of controlled the CH release and prevention the antibacterial infection.

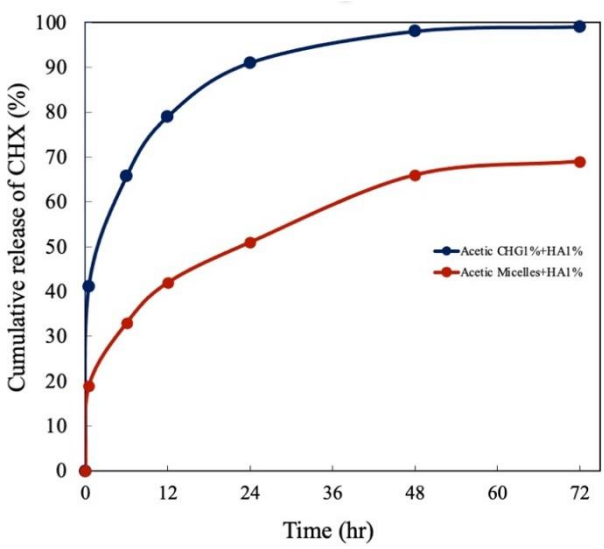

Figure 5. CHX-released of blend polymer films in mixture A and mixture X.

3.5 Antibacterial activity

Table 3 demonstrated the inhibition zone of the blend polymer film in mixture A and mixture X. Both mixture contained the CHX which can prevent the S.aureus by showing the inhibition zone.

Table 3 The inhibition zone of the blend polymer film.

Mixture condition	Inhibition zone (mm)
Mixture A	10.1 ± 0.5
Mixture X	11.2 ± 0.9

4. Conclusion

The development of the blend polymer films from polyvinyl alcohol and carboxymethyl cellulose by using the crosslinking method via combined with the hyaluronic acid and chlorhexidine micelles were successfully prepared. Results of the blend polymer films in part of water absorption, tensile strength, drug release, and antibacterial activity showed the potential to the wound dressing application. The water absorption and tensile strength increased when adding the HA including the CHX-loaded micelles can help to the reduction of burst CHX release. Moreover, the polymer film sample can prevent the S.aureus. Hence, this formulation of the development of the combination of biopolymer, carboxymethyl cellulose, healing agent, and antimicrobial agent could be further developed for wound dressing application, tissue engineering and other application in biomaterials field.

Acknowledgement

This research was supported by King's Mongkut Institute Technology Ladkrabang (REKRIS/FF65/06).

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