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The Crosslinking Agent Application Combined with Loading Healing and Antibacterial Agents in Wound Dressing for Agents-Release Control

Siriwan Srisang,Naruebodee Srisang*, Jatupat Maikaew, Orranatt Eiangmee

Department of Engineering, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon 86160, Thailand
Email: naruebodee.sr@kmitl.ac.th

The research aims to investigate wound dressing (WD) production from polyvinyl alcohol (PVA) and carboxymethyl cellulose (CMC) using a crosslinking agent (polyethylene glycol diacrylate, PEGDA). Moreover, the antibacterial agent (chlorhexidine gluconate, CHG) and healing agent (Hyaluronic acid, HA) are added to improve the WD functionalities. The PVA : CMC ratio is used at 90 : 10 % w/v and mixed with the PEGDA concentration at 10% w/v. The CHG concentrations are varied at 1, 3, and 5% w/v combined with the HA (0 and 1% w/v). The CHG was provided in nonmicelles and micelles forms. The WD properties are examined with the appearance aspect, water absorption (WA), tensile strength, and CHG release. Results showed all mixtures could be formed into the WD sheet. The WD sheets were opaque when the HA and CHG micelles were added to the mixture. The WD made from CHG 3% had the maximum WA, and the usage of HA and CHG micelles in the mixture caused the increased WA. The CHG micelles 3% and HA 1% provided the maximum value of tensile strength (≈ 320 kPa), and it demonstrated the prolonged CHG release for 48 h (≈ 22%), which implied continuous healing.

Keywords: Wound Dressing, Micelles, Water Absorption, Tensile Strength.

1. Introduction

Wound dressing (WD) is an essential material for wound healing, which can be produced from various techniques and biomaterials. Sethuram and Thomas [1] studied the fabrication of highly efficacious electrospun nanofibers using a eugenol microemulsion reinforced on a Chitosan/PVA (biopolymeric) matrix; they showed greater antibacterial efficiency and hemocompatibility for wound healing. Solvent casting and electrospun techniques have been widely used due to their biocompatibility, but solvent casting was more accessible [2]. These different techniques affected the WD attributes [3].

Nowadays, WD is produced from the natural substance (CMC) for friendliness with the environment [4] and includes the drug-loaded micelles within WD for drug-release control [5, 6]. A suitable crosslinking agent is necessary for the homogeneous bond connection, especially, the connection between the PVA and CMC substances which had different chains. The proper crosslinking agent usage can improve mechanical properties due to the bond connection. Shin, Lee [7] reported the WD properties after treatment with the different crosslink using cyclic freezing/thawing and subsequent γ-ray irradiation; they found that the irradiated WD hydrogels have high compressive strength (42.5 kPa) and cell viability (184%) when compared with those without irradiated. Farid, Kamoun [8] found that increased citric acid concentration for crosslinking increased mechanical strength. The drug-loaded micelles were used to improve the drug release in WD; the nanotechnology can encapsulate the drug into a nanocarrier to control and prolong the drug release to the target site and sustained-release application. Zhao, Liu [9] explored the curcumin and rifampicin-loaded micelles-hydrogel and found that it significantly reduces the skin barrier regeneration period; this result indicates its potential for drug delivery to target and prolonged drug release in chronic wound infections. Bai, Jia [10] demonstrated that the release of drug-loaded micelles was less than 10% after 1 day. Dadashzadeh, Imani [11] investigated Aloe vera-loaded noisome incorporated in the hybrid alginate/gelatin hydrogel, which showed an extended-release of 20% after 7 days. The crosslinking agent and drug-loaded method significantly impacted on the forming and WD qualities o and resulted in the developed healing process in patients with chronic wounds [12].

Thus, this research aims to investigate the effect of CHG concentrations (1, 3, and 5% w/v) and HA concentrations (0 and 1% w/v) on WD properties. The WD was made from PVA and CMC using PEGDA as a crosslinking agent. The CHG was used in two forms, i.e., nonmicelles and micelles. The WD properties were examined with the appearance aspect, water absorption (WA), tensile strength, and CHG release.

2. Materials and Methods

2.1 Materials

Polyvinyl alcohol (PVA) (Average molecular weight ≈ 89,000 – 98,000 Da) and polyethylene glycol diacrylate (PEGDA) (Average molecular weight ≈ 700 Da) were purchased from Aldrich (St. Louis, MO). Carboxymethyl cellulose (CMC) (Average molecular weight ≈ 250,000 Da) was purchased from TCI (Japan). Hyaluronic acid (HA) was purchased from S.N.P. SCIENTIFIC CO., LTD (Bangkok, Thailand). Chlorhexidine gluconate (CHG) obtained from S. Tong Chemicals Co., Ltd (Bangkok, Thailand).

2.2 Wound dressing preparation

The WD preparation from PVA and CMC adapted from the method of Meedecha, Srisang [13] and the crosslinking agent was PEGDA at the concentration of 10% w/v. The PVA : CMC ratio was 90 : 10 % w/v which was mixed at room temperature and stirred at 400 rpm for 3 h. The different concentrations of CHG (1, 3, and 5% w/v) and HA (0 and 1% w/v) were added to the WD. The CHG was formed in the nonmicelles and micelles by the CHG was loaded into micelles using the solvent evaporation method [14]. After that, the solution was poured into a Petri dish to undergo six consecutive freeze/thawing cycles, each with 24 hours of freezing at

–20 ºC and 24 hours of thawing at room temperature [15]. The WD thickness was less than 1 mm. The WD aspect was observed.

2.3 Water absorption test

The water absorption (WA) was tested as corresponded with the methods of Farazin, Zhang [16]. The WD was dried at 70 ºC for 5 h and weighed (W_0). Afterward, the WD was placed inside the Petri dish containing 10 mL of DI water for 48 h and weighed (W_1). The experiment was repeated at least three times, and WA was calculated by (1):

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

2.4 Tensile strength test

Tensile strength was measured by the Stable Micro Systems tester, TA-XT plus version, and the testing method was used as corresponded with the research of Ou, Dong [17]. The specimen was cut as follows with ASTM D882. The WD was pulled at the speed rate of 50 mm/min with a load cell of 1 kN.

2.5 In vitro release of CHG from WD

The drug release is separated into 2 parts: burst and sustained releases. The first stage is burst release, which is the initial time point and often uses high concentrations at the target site. In the second stage, the drug is controlled and prolonged in release, which will significantly damage the nanocarrier (encapsulated drug) due to the drug's release from inside the nanocarrier or micelles [18]. In the CHG-release testing, the WD samples in each condition were cut with the size of 1cm x 1 cm and were immersed in Phosphate-buffered saline (PBS) solution 0.01 M (pH 7.4). Each sample about 3 mL in a micro-tube was taken at predetermined times (1, 15, 30, 60, 1440, and 2880 min) to measure the CHX release. The sample from each time was filtered through a 0.22 µm membrane and then analyzed by a UV-visible spectrophotometer with a wavelength of 270 nm [14].

2.6 Statistical analysis

WA and tensile strength were measured in three duplications, and WA was analyzed through a one-way ANOVA with Tukey's HSD method using SPSS (V.29. SPSS: An IBM Company).

3. Results and Discussion

3.1 Wound dressing aspect

Fig. 1 shows the WD from the PVA : CMC ratio at 90 : 10 % w/v using the crosslinking agent of PEGDA (10% w/v) combined with the addition of CHG (nonmicelles) at the concentration of 1, 3, and 5% w/v and HA 1% w/v. All conditions could be formed into the WD sheet like the general WD. The increased CHG concentration from 1 to 5% w/v caused the elevated turbidity of WD. In the same way, the WD aspect was opaquer when the HA was added to the mixture in every CHG concentration. The enhanced CHG concentration from 3% to 5% w/v led to a change in color from white into mild yellow. Andermann, Buhler [19] reported that the yellow could be formulated from the chlorhexidine reaction with other substances. These

results indicated the efficient crosslinking agent application for WD production, as accorded with the many research [20]. These works reported successful WD production in the form of WD hydrogels or composites. Hemmatgir, Koupaei [21] demonstrated that PEGDA could be used as a crosslinking agent for the PVA and tragacanth gum, as confirmed by their FTIR results, which exhibited the PEGDA chains. Bialik-Wąs, Pluta [22] investigated the sodium alginate/PVA/Aloe vera hydrogels crosslinked with PEGDA, which found that PEGDA crosslinking improved hydrogels properties for WD production. The crosslinking agent of PEGDA could produce polymeric WD and improve its properties such as mechanical strength, hydrophilic, and swelling. Furthermore, the nanocarriers as drug-loaded micelles also improved adhesion and WD formation [23]. These results were not still enough needed additional study to analyze the bonding characteristic that took place from PEGDA.

Fig. 2 exhibits the WD from the PVA : CMC with the CHG and HA addition (micelles) at the concentration of 3 and 1% w/v, respectively. The CHG usage with micelles form resulted in more dimness within the WD than the nonmicelles form at the same CHG and HA concentrations as compared with Fig. 1C and 1D. The HA enhancement in the mixture caused the raised opacity likewise with the escalated HA in the CHG solution under nonmicelles form.

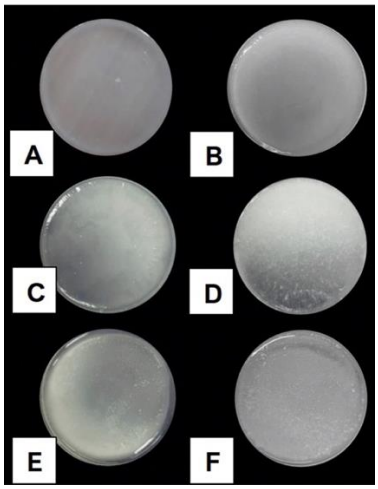

Fig. 1. Aspect of wound dressing from PVA and CMC using crosslink agent (PEGDA) combined with loading CHG (nonmicelles) : A) CHG 1% w/v, B) CHG 1% w/v and HA 1% w/v, C) CHG 3% w/v, D) CHG 3% w/v and HA 1% w/v, E) CHG 5% w/v, and F) CHG 5% w/v and HA 1% w/v.

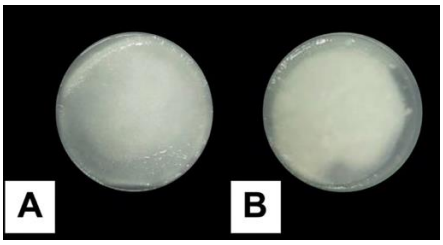

Fig. 2. Aspect of wound dressing from PVA and CMC using crosslink agent (PEGDA) combined with loading CHG (micelles) : A) CHG 3% w/v (micelles) and B) CHG 3% w/v

(micelles) and HA 1% w/v.

3.2 Water absorption

Fig. 3 demonstrates the WA property of WD made from the different conditions of CHG and HA concentrations and the CHG forms (micelles and nonmicelles). The maximum WA was 459% at the CHG and HA concentrations of 3 and 1% w/v, respectively. The WA insignificantly enlarged with the addition of HA and CHG concentration. The raised WA trend was caused by the intrinsic HA property, which had abundant hydrophilic functional groups [24]. Nonetheless, the WA reduced when the CHG concentration was elevated to 5% w/v; this result may arise from the ionic strength variation within the CHG medium and result in the abated WA [25]. The concentration of CHG 3% w/v and HA 1% w/v provided the desired attribute with the most WA; hence, these concentrations were selected to compare the WA between the micelles and nonmicelles forms.

Fig. 3 illustrates the WA remarkably augments in the WD made from the CHG with micelles form by the WA increases to 672 and 742% with the CHG 3% w/v and CHG 3% w/v mixed with HA 1% w/v, respectively. The expanded WA may be caused by the crosslinking agent of PEDGA, which was contained in the micelles form as hydrophilic functional groups and resulted in the WA enhancement [26]. The CHG with the micelles form could improve the more WA than the nonmicelles form (≈ 1.3-1.6 times). However, the WA did not mainly enhance after the additional HA in the mixture as same as in the nonomicelles form. The high WA was a pleasurable property for the WD because it supported the exudation absorption from the wound and kept moisture, which would help to accelerate wound healing [27]. Therefore, the concentration of CHG 3% w/v mixed with HA 1% w/v was chosen to compare the attributes of tensile strength and CHG release from the WD.

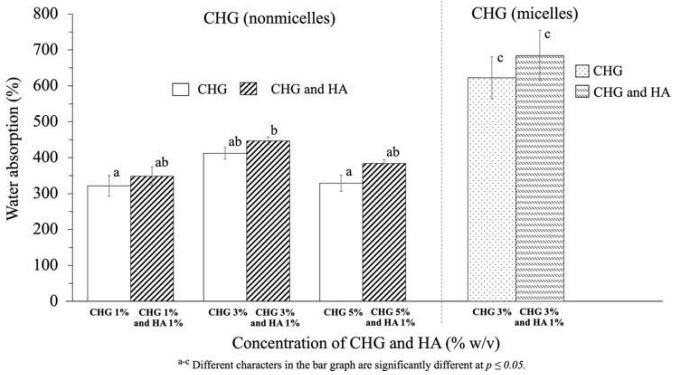

Fig. 3. Water absorption of wound dressing from PVA and CMC using crosslink agent (PEGDA) combined with loading CHG (nonmicelles and micelles) and HA.

3.3 Tensile strength

Fig. 4 demonstrates the tensile strength of WD which loads the CHG in micelles and non-micelles forms. The WD required high tensile strength to resist the damage from tears during usage [28]. The CHG with micelles form could ameliorate more tensile strength than the nonmicelles form approximately 1.3 times (77 kPa) due to the promoted adhesion force from micelles or nano-carriers [29, 30]. Gong, Wu [31] investigated curcumin-loaded micelles for

cutaneous wound healing, which showed higher tensile strength. Rungrod, Kapanya [28] found that the WD from hydrogel-loaded micelles had higher mechanical properties than without-loaded micelles, about 30 kPa. These researches proved that loading micelles in WD could boost the mechanical properties.

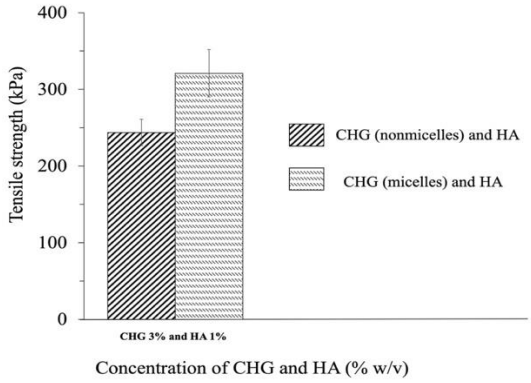

Fig. 4. Tensile strength of wound dressing from PVA and CMC using crosslink agent (PEGDA) combined with loading CHG (nonmicelles and micelles) and HA.

3.4 Release of CHG and CHG-loaded micelles from PVA/CMC wound dressing

Fig. 5 represents the CHG release from WD under the different conditions of CHG loading (micelles and nonmicelles). The CHG quantity from WD in both forms was rapidly released in the initial time (60 min); the nonmicelles form presented a higher burst release of 11% than the micelles form with the CHG discharge of 8%; these results came from the CHG was encapsulated with micelles resulting in the slower release [32]. The above CHG release affected the bacteria growth inhibition by the gram-positive bacteria could be hindered the growth at the CHG concentrations of 18.75 μg/mL [33]. The gradual drug release is a necessary property for WD to avoid excess drug usage to wounds and reduce side effects [34]. The CHG release percentages in both forms had similar level at 16% after 1,440 min (1 day) and 22% after 2,880 min (2 days). Gong, Wu [31] reported the release behaviors of curcumin-loaded micelles at 60% after 14 days. Piazzini, Landucci [35] presented that aripiprazole loaded micelles could prolong the release about 50% after 7 days while the nonmicelles release about 100% for 4 h. These literatures confirmed the more postponed drug release from micelles than the nonmicelles.

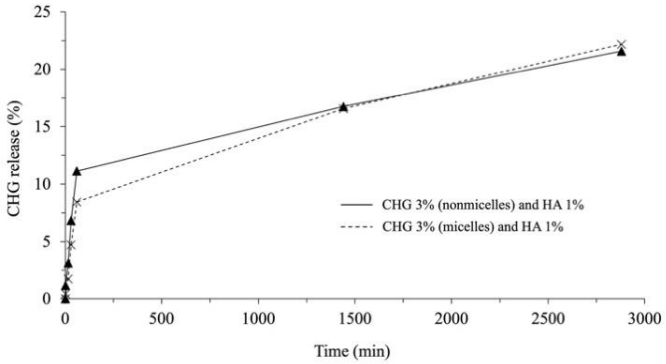

Fig. 5. CHG release of wound dressing from PVA and CMC using crosslink agent (PEGDA) combined with loading CHG (nonmicelles and micelles) and HA.

4. Conclusion

The environmentally friendly polymer of PVA and CMC could be formed into wound dressing via the crosslinking agent (PEGDA) combined with the antibacterial agent loading (CHG) and the healing agent loading (HA). The suitable condition for WD production was the CHG concentration of 3% w/v mixed with the HA concentration of 1% w/v by the CHG was loaded in micelles form. These conditions provided the proper WD attributes, i.e., the most water absorption (> 650%) and the acceptable tensile strength (≈ 320 kPa). Furthermore, the CHG release from micelles was more prolonged than the nonmicelles, which implied continuous wound healing.

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