*บทความวิจัย*

# การผลิตวัสดุปิดแผลยับยั้งเชื้อจุลชีพจากไฮโดรเจลชนิดเจลาตินและซิลค์เซริซินผสมน้ำมันหอมระเหยไทม์

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## บทคัดย่อ

ไฮโดรเจลจากพอลิเมอร์เหมาะสมแก่การพัฒนาเป็นวัสดุปิดแผล เนื่องจากไฮโดรเจลสามารถคุมปริมาณของเหลวและความชื้นบริเวณบาดแผล แสดงโครงร่างแหคล้ายเนื้อเยื่อและมีความเข้าได้กับร่างกาย ในงานวิจัยนี้ จึงมุ่งเน้นการผลิตฟิล์มไฮโดรเจลจากพอลิเมอร์ผสมชนิดเจลาตินและซิลค์เซริซินผสมน้ำมันหอมระเหยไทม์ และหล่อขึ้นรูปด้วยวิธีการใช้ตัวทำละลาย และเชื่อมสายโซ่พอลิเมอร์ด้วยสารกลูตารอลดีไฮด์ เพื่อเพิ่มการทนน้ำและสมบัติทางกลของไฮโดรเจล จากการศึกษาสัณฐานวิทยา สมบัติเชิงกล การดูดซึมน้ำ และพฤติกรรมการปลดปล่อยน้ำมันหอมระเหยไทม์ของไฮโดรเจล พบว่าเมื่อผสมน้ำมันไทม์ พื้นผิวของไฮโดรเจลมีความหยาบเกิดขึ้น ไฮโดรเจลมีความนุ่มและยืดหยุ่นได้มากขึ้น และสามารถดูดซึมน้ำได้ปริมาณสูง ไฮโดรเจลให้การปลดปล่อยแบบเร็วในชั่วโมงแรกของการทดสอบ ตามด้วยการปลดปล่อยแบบคงที่ในระยะเวลาที่นานขึ้น ในการทดสอบการต้านเชื้อจุลชีพด้วยวิธี disk agar diffusion ยืนยันได้ว่าไฮโดรเจลผสมน้ำมันไทม์สามารถยับยั้งการเจริญเติบโตของเชื้อจุลชีพชนิด *Staphylococcus aureus* และ *Staphylococcus epidermidis* ได้ดี จึงสามารถสรุปได้ว่าไฮโดรเจลจากพอลิเมอร์ชนิดเจลาตินและซิลค์เซริซินผสมน้ำมันหอมระเหยไทม์มีความเหมาะสมแก่การนำมาประยุกต์ใช้เป็นวัสดุปิดแผล


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# Antibacterial Hydrogels of Thyme Essential Oil-incorporated Gelatin/Silk Sericin as Wound Dressings

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## ABSTRACT

Polymer-based hydrogels are excellent materials for developing wound dressings, since the hydrogels enable to maintain wettability and moist in the wound, and show tissue-like structure and biocompatibility. In this study, the hydrogel films of gelatin and silk sericin blends were prepared by using simple solvent-casting method with loading thyme essential oil. Crosslinking with glutaraldehyde was used to enhance water resistance and the mechanical stability of the hydrogels. Surface morphology, chemical composition, the mechanical properties, swelling ratio and release behavior were investigated. SEM images of the hydrogels showed rough surface with the presence of thyme essential oil. Incorporation of thyme essential oil improved softness and flexibility of the hydrogels. Thyme essential oil-loaded hydrogels showed high water absorption. The release characteristics of the hydrogels exhibited initial burst release at the first hour of immersion, followed by maintained release. Using agar diffusion method, the hydrogels with loading essential oil inhibited bacterial growth against *Staphylococcus aureus* and *Staphylococcus epidermidis.* Therefore, antibacterial gelatin/silk sericin hydrogel films with loaded thyme essential oil are an effective candidate for use as wound dressings.

**Keywords:** Wound dressings, hydrogels, gelatin, silk sericin, thyme essential oil, antibacterial activity

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## Introduction

Wound dressings are necessary for the treatment of burns, chronic ulcers and decubitus ulcers, since they enable to retain a moist environment at the wound interface, allow gas permeability, protect the wound against dust and prevent wound from bacterial infection [1, 2]. Wound dressings are thus used to provide optimal healing conditions and regulate wound healing [3]. In addition, wound dressings are required for rapid wound closure and reduced scar formation [4]. Among several forms of dressings, including foams, electrospun fibers, membranes and hydrogels, hydrogel dressings are gained high interest in wound healing because of several advantages of maintained moist environment, good biocompatibility, the ability of absorbing wound exudates and no adherence with wound tissue [5]. Moreover, the hydrogel dressings are able to cool the wound site, leading to pain relief for patients [4]. Natural polymers, such as alginate, chitosan, collagen, gelatin and hyaluronic acid, have been widely developed to create hydrogel dressings due to they possess non-toxic, non-allergenic, biocompatible and biodegradable properties [1, 5]. In addition, natural polymer-based hydrogels possess excellent swelling ability in aqueous solution and have hydrophilic property beneficial for drug release [6].

Gelatin and silk sericin are of natural proteins that have been extensively used in biomedical applications of tissue engineering, drug delivery and wound healing. Gelatin, hydrolyzed product from collagen, provides excellent biodegradability, biocompatibility and cellular interaction [7]. It is non-cytotoxicity, easy to process, inexpensive and has a relatively low antigenicity compared to collagen [8]. Gelatin contains arginine-glycine-aspartic acid (RGD) sequence which promotes cell adhesion, proliferation and differentiation [9]. Especially, gelatin is a great material for hydrogel formation because of its unique gel-forming properties [10]. Gelatin-based hydrogels have transparency, moist and capacity to absorb excess exudates, rendering an interest toward the applications in wound dressings [9]. Silk sericin is a biocompatible protein derived from silkworm cocoon. Sericin, a glue protein, covers the core of fibroin in the silk fiber [11, 12]. Silk sericin is composed of highly hydrophilic polar side groups, like hydroxyl, carboxyl and amino groups, thus it is easy for blending sericin with other biopolymers [11]. Literature has reported that silk sericin provides inherent antioxidant, bioadhesive and bioactive properties [11, 13]. In addition, it has been confirmed that sericin enhanced adhesion and proliferation of human skin fibroblasts, and activated production of collagen in wounds [13].

In order to avoid wound infection, wound dressings should possess good antibacterial activity. Especially, the antibacterial wound dressings will contribute to the worse bacterial resistance phenomenon. Thus wound dressings are typically loaded antibiotic drugs in order to inhibit infection at wound site, such as vancomycin, terbinafine, doxorubicin and moxifloxacin

[14-17]. Apart from antibiotics, essential oils from natural origin are interesting alternative in biomedicine and pharmaceutical applications due to their antimicrobial and antioxidant activity. In particular, essential oil extracted through steam distillation of fresh flowers and leaves of thyme *(Thymus vulgaris)* is known as a human medicine with exhibiting antioxidant and antimicrobial properties against a broad spectrum of gram-negative or gram-positive bacteria [18]. Thyme essential oil contains more than 60 active compounds, especially the phenols thymol, rosmarinic acid and carvacrol thymol and carvacrol [18, 19]. Thyme essential oil has been proven to inhibit the growth of bacteria within and outside the body like bacterial infections in the genitals and urethra, intestines, and respiratory system as well as the external exposure of wounds. Therefore, oil of thyme is extensively used in processed food to prevent the growth of food-borne bacteria and extend the shelf life of processed foods [20]. Simultaneously, thyme essential oil is very attractive bioactive compound for wound healing.

The aim of this work was to simply fabricate gelatin/silk sericin hydrogel films with an incorporation of thyme essential oil. Relevant physico-chemical properties and the releasing function of the hydrogel films were investigated as well as the evaluation of antibacterial property.

## Materials and Methods

1. Extraction of silk sericin

The silk sericin was extracted from *Bombyx mori (B. Mori)* cocoon by autoclave method of degumming (Mandal et al) [11]. Briefly, 20 g of the cocoons that cut in pieces was immersed in 0.02 M $Na_2CO_3$ solution for 50 ml. The solution was then heated in autoclaving for 2 h. The extracted solution was dialyzed (12-kDa membrane, Sprectra/Por®, USA) against water for 2 days to remove salt ions. The extract silk sericin was further lyophilized to obtain the silk sericin powder.

2. Preparation gelatin/silk sericin hydrogels

Gelatin solution at concentration of 5 %w/v was prepared by dissolving gelatin (type A from porcine skin, Sigma, USA) in deionized water. The mixture was kept at room temperature for 30 min to hydrate gelatin powder and then was stirred at 60°C until obtained homogeneous solution. The extracted silk sericin powder was dissolved in deionized water at concentration of 5 %w/v. The sericin solution was stirred at room temperature until homogeneous solution was obtained. Equal volume of sericin and gelatin solutions were mixed to achieve blending ratio at 1:1. The films were cast on petri-dishes, dried overnight and peeled off from the dishes. The films were crosslinked by immersion in glutaraldehyde (GA) at the different concentrations of 1, 2.5, and 5 %v/v in deionized water for 24 h. Each GA solution of

10 ml was used for one gram of polymer film. After that, the crosslinked films were removed, washed three times by deionized water and dried at room temperature for 24 h. In the preparation of thyme essential oil (TEO; Sigma-Aldrich, USA); incorporated hydrogels, variation of TEO contents was added into the polymer blend solution at the amount of 1 and 1.5 %v/v of the solution. Then, the film preparing process was followed the previous method described in the case of G/SS hydrogel films.

3. Morphology analysis

The surface morphology of fabricated hydrogels was observed by using scanning electron microscope (SEM; Quanta 250 microscope, Japan). Dried hydrogels were attached to stubs using a two-sided adhesive tape, then they were coated with gold using a gold coater (Jeol, JFC-1200) and examined using an acceleration voltage of 10 kV.

4. Analysis of chemical composition

Chemical components of the hydrogels were identified by using Attenuated total reflectance/Fourier transform infrared spectroscopy (ATR-FTIR; Perkin-Elmer Spectrum One FT-IR). The dried hydrogel samples were analyzed under transmission mode, taking 64 scans per experiment with a resolution of 4 $cm^{-1}$.

5. Tensile test

Tensile test of hydrogels was performed by using Universal testing machine (Instron 5966, USA). Rectangular specimens (n=8) with a dimension of 15 mm width, 60 mm length and 1 mm thickness were prepared. Then the specimen was tested at dry state until failure with the cross-head speed of 5 mm/min under a load of 10 kN. The mechanical values including Young's modulus, ultimate tensile strength and elongation at break were determined and reported as average ± standard deviation (SD).

6. Water absorption investigation

Dried G/SS-based hydrogel disks (n=5) with a diameter of 15 mm and the thickness of 1 mm were weighed (W) and were immersed in 20 ml of phosphate buffered saline (PBS, pH 7.4 at 37°C) at different time point for 30 h. The samples were blotted with filter paper, weighed ($W_t$). Water absorption (%) was calculated by using following equation:

$$\% \text{ Water absorption} = [(W_t - W_i)/W_i] \times 100$$

where $W_t$ and $W_i$ are the weight of the swollen sample at each time point and the weight of dry sample, respectively.

7. Release characteristic and release kinetics study

In order to investigate the release characteristic of TEO-embedded G/SS hydrogels, the hydrogel disks (n=5) with a diameter of 15 mm and with thickness of 1 mm were immersed into the 20 ml of PBS solution (pH 7.4 at 37°C) as the releasing medium for various time

intervals. The samples were placed in an orbital shaker at 37°C and 120 rpm for 20 h. At each time point, 1 ml PBS solution was taken. At the same time, 1 ml fresh PBS solution was added. The absorbance of the PBS solution was measured at 273 nm by using UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). The obtain data were shown in the percentage of TEO cumulative release normalized by the actual weights of the TEO-loaded G/SS hydrogel specimens and plotted as immersion time.

The release kinetics of TEO from G/SS hydrogels were determined by using Ritger-Peppas model, which is suitable to describe drug release from polymeric systems [21]. In order to estimate the mechanism of release, initial 60% release data were fitted in following equation

$$M_t/M_\infty = Kt^n$$

where $M_t/M_\infty$ is fraction of TEO release at time t, K is rate constant and n is release exponent, which characterizes the release mechanism. The n value was determined by the slope of the plot of logarithm of $M_t/M_\infty$ as a function of logarithm of time.

9. Evaluation of antibacterial activity

The antibacterial activity of the TEO-loaded G/SS hydrogels was investigated based on the disk diffusion method against two pathogenic bacteria. *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) were chosen for this study due to their frequent involvement in wound infections. The strains were grown overnight on agar plates at 37°C prior to use. The bacteria suspension at a density $10^6$ CFU/ml was spread over an agar plate. The specimens were cut in circular disks with a diameter of 15 mm. The hydrogels without TEO was used as a control. Sample disks were pre-treated under UV light for 30 min prior to test for released oil-induced bacterial inhibition. Each specimen was adhered to the agar plate and incubated at 37°C for 24 h. After that, the diameter of an inhibition zones around the sample disks were determined. The experiments were determined in triplicate (n=3).

10. Statistical analysis

The data were reported as the mean ± the standard derivation. A one-way ANOVA was used to compare the means of different data sets and a significant was accepted at a 0.05 confidence level.

## Results

1. Surface morphology

SEM analysis was used for morphological observation of gelatin/silk sericin with loading thyme essential oil (G/SS+TEO)-based hydrogel films shown in Fig. 1. After crosslinked with glutaraldehyde (GA) at various concentrations, G/SS hydrogels without the presence of TEO had a smooth and homogeneous surface with free of cracks (Fig. 1(a, d, g)). It is probably

due to compatible blending of two protein-based polymers including gelatin and silk sericin. Addition of TEO into the films led to rough surface as obviously observed in the hydrogel films with the presence of 1.5% TEO (Fig. 1(c, f, i)). Increasing surface roughness by addition of essential oil was previously reported in several studies [22-25]. However, an incorporation of 1 %v/v TEO (Fig. 1(b, e, h)) did not change morphological structure of the hydrogel films compared to the hydrogels without the presence of TEO. In addition, droplets were distributed on the surface of the films with the presence of 1.5 %v/v TEO, as well as an observation of few micropores on the surface, after crosslinked with 1, 2.5 and 5 %v/v GA (Fig. 1 (c, f, i)). It is believed that the droplet-like phase was caused by migration of oil drops along the film surface and subsequently volatilization during water evaporation. The occurrence of micropores might be a result of leakage of aggregated oil droplets during crosslinking process by immersion in glutaraldehyde (GA) solution. Surprisingly, an increase of GA concentration used from 1% to 2.5 %v/v, the surface morphology of G/SS hydrogels with 1.5 %v/v TEO was altered from dispersed droplets to co-continuous phase (Fig. 1(c versus f)), while a dense morphology was observed after crosslinked with 5 %v/v GA (Fig. 1(i)). It is possible that high GA content led the crosslinking rate of polymer to be fast, initiated from the surface of the films. As a result, the leakage of oil was inhibited, forming a dense surface. Thus, it can be suggested that the effect of TEO added was significant at the high level of 1.5 %v/v TEO, whist concentration of GA crosslinking agent affected the microstructure of the film surface.

2. Chemical composition

ATR-FTIR analysis was performed to identify the blending between gelatin and silk sericin, and the interaction with glutaraldehyde crosslinking agent. Fig. 2(a) demonstrates IR spectrum of G/SS blended films crosslinked with 5 %v/v GA compared to the spectra of pure SS and G/SS films without crosslinking. The characteristic absorption bands of pure SS included amide I (C=O stretching vibration) and amide II (N-H bending) at 1627 $cm^{-1}$ (peak d) and 1523 $cm^{-1}$ (peak e), respectively [26, 27]. The peak of amide III was appeared at 1236 $cm^{-1}$ (peak f) due to the interaction between C-N stretching and N–H bending [26, 27]. Absorption band of N-H stretching existed at 3750 $cm^{-1}$ and O-H stretching band occurred at 3266 $cm^{-1}$. With the presence of gelatin blend, the characteristic peaks of protein attributed to amide I, II and III (peaks d, e, f) were shown in the spectrum of G/SS. But their intensity increased compared with the intensity of these peaks in the spectrum of pure SS, indicating the integration of both protein-based polymers. In addition, the characteristic peak at 3276 $cm^{-1}$ (peak a) was attributed to N-H stretching of gelatin. In the spectrum of G/SS/5%GA, a sharp peak at 1450 $cm^{-1}$ ($\Delta$) was observed in the crosslinked G/SS hydrogels. This peak was assigned to aldimine (CH=N) absorption [26], which confirms successful crosslinking of proteins. In addition, the doublet peak

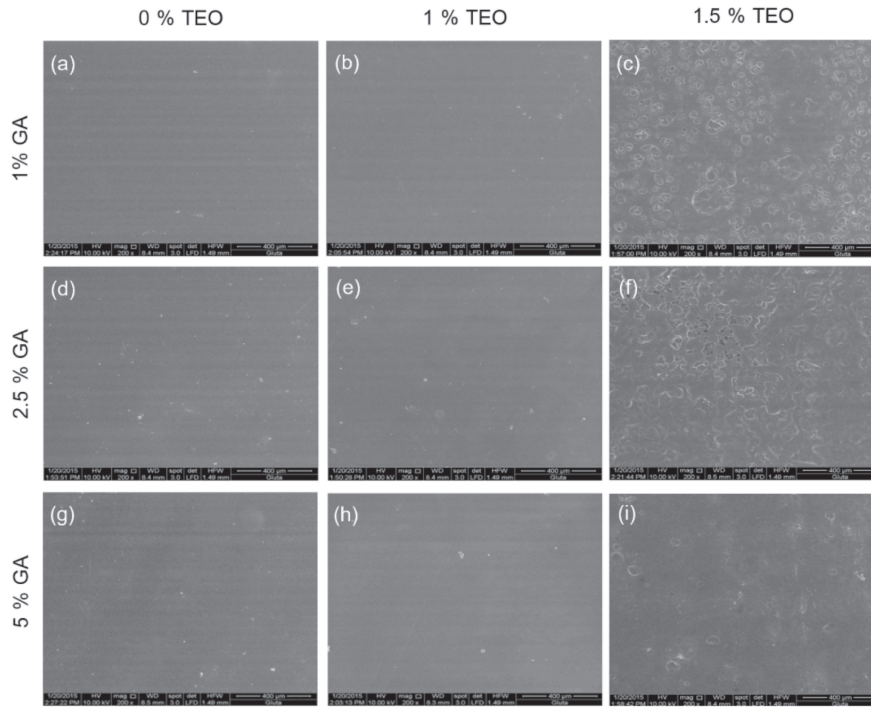


**Figure 1** Scanning electron micrographs showing surface morphology of thyme essential oil-loaded gelatin/silk sericin (G/SS) hydrogel films using various amount of thyme essential oil (0, 1 and 1.5 %v/v TEO) and glutaraldehyde (1, 2.5 and 5 %v/v GA) crosslinking agent.

at 2947 and 2878 $cm^{-1}$ (peaks b, c) corresponding to C-H stretching in the spectrum of crosslinked hydrogels showed sharper peak with higher intensity compared to the spectrum of uncrosslinked hydrogels, due to the incorporation of C-H bonds in GA. The result confirms progression of crosslinking proteins with glutaraldehyde.

In Fig. 2(b), ATR-FTIR analysis was further used to elucidate the possible alternation after the incorporation of thyme essential oil in the G/SS hydrogels. When compared to the spectrum of crosslinked G/SS hydrogels, similar results were observed in the spectra of TEO-loaded hydrogels. The characteristic absorption peaks of gelatin and sericin were preserved. It means that the presence of TEO did not affect the characteristic peaks of polymer matrix, as evidenced by no peak deviation and no additional peak after the addition of TEO. The result confirms no interaction between oil components and polymer matrix (gelatin and sericin). Although, intensity of those characteristic peaks was reduced by an increase of TEO content. It might be due to the addition of TEO interfered the interaction between gelatin and silk sericin such as hydrogen bonding, since the oil was entrapped inside the polymer network. This phenomenon probably affects the mechanical properties of the TEO-loaded hydrogels as well.

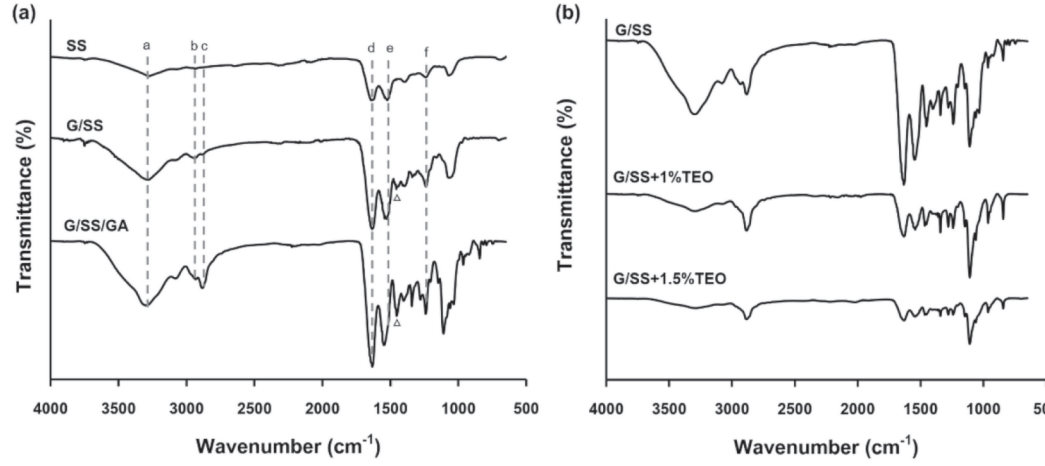


**Figure 2** (a) ATR-FTIR spectra of gelatin/silk sericin hydrogel films after crosslinked with 5% glutaraldehyde (G/SS/GA) compared with the spectra of pure silk sericin (SS) hydrogel films and gelatin/silk sericin (G/SS) blended hydrogel films and (b) ATR-FTIR spectra of thyme essential oil-loaded crosslinked gelatin/silk sericin (G/SS+TEO) hydrogels using various amount of thyme essential oil, including 1 and 1.5 %v/v TEO, compared with the spectrum of the G/SS hydrogels without the presence of TEO

3. Mechanical properties

The mechanical properties of the hydrogel films were tested under tension and the obtained stress-strain curves are presented in Fig. 3(a). The mechanical values of the hydrogel films are reported in Fig. 3(b-d) in terms of Young's modulus, ultimate tensile strength and elongation at break. The stress-strain curves were shown in a typical stress-strain behavior of polymer hydrogels [28]. Effect of incorporated TEO in the hydrogels was significant on the mechanical properties in terms of elastic modulus, tensile strength and elongation at fracture. It is obvious that the curve of representative G/SS hydrogels exhibited the behavior of brittle materials (Fig. 3(a)). Young's modulus of G/SS hydrogels were 2.3 ± 0.1 MPa, Fig. 3(b), while the elongation at break was 158 ± 23% (Fig. 3(d)). In contrast with TEO-loaded G/SS hydrogel films, increased amount of TEO led to the reduction of stiffness and tensile strength of the films, as indicated by the decreased Young's modulus (Fig. 3(b)) and the declined strength values prior to break (Fig. 3(c)). On other hand, the G/SS+1%TEO and G/SS+1.5%TEO hydrogel films were able to be elongated more than the hydrogels without the presence of TEO (see Fig. 3(a) and 3(d)). It might be an advantage of entrapped thyme essential oil in the hydrogels. Dispersed oil drops were well incorporated in the polymer network and thus acted as a plasticizer in the hydrogels, leading to enhanced softness of the hydrogels.

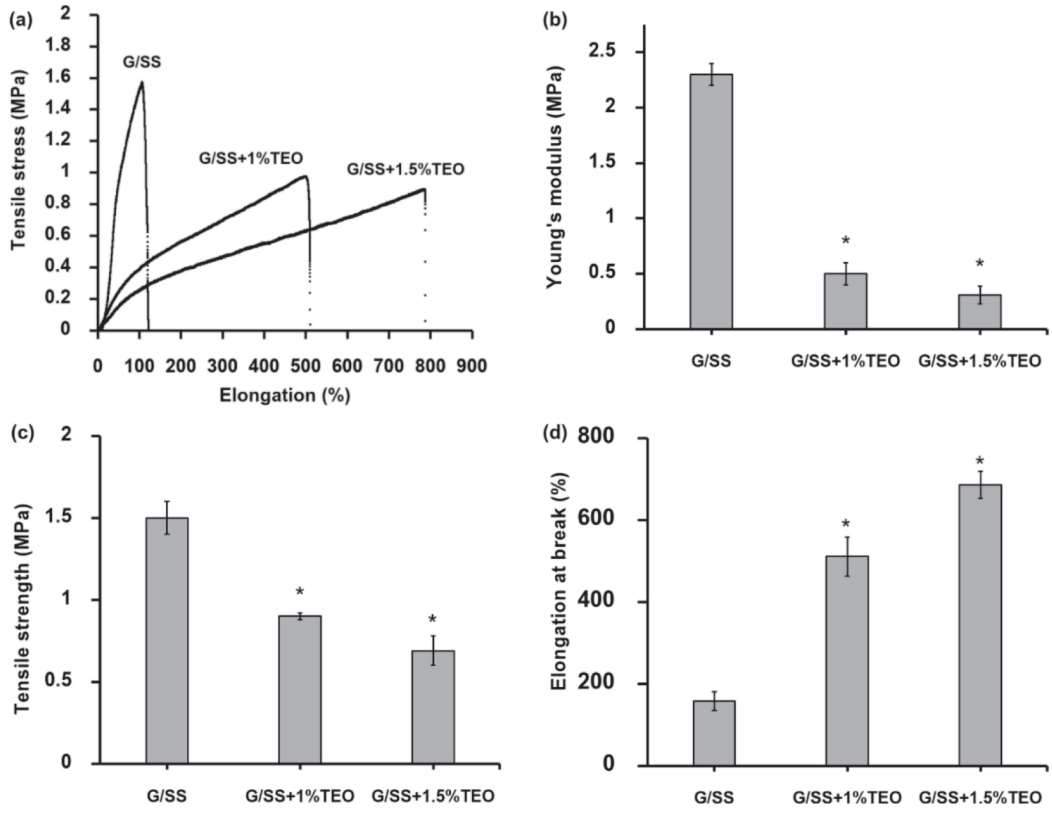


**Figure 3** (a) Representative tensile stress-strain curves of G/SS hydrogels without loading TEO (G/SS), and with loaded several contents of thyme essential oil (G/SS+1%TEO and G/SS+1.5%TEO), (b) Young's modulus, (c) Tensile strength and (d) elongation at break of the prepared G/SS hydrogels with several contents of thyme essential oil. The values are presented as mean ± SD (n = 8) with significant statistical difference under one-way ANOVA test (*$p \leq 0.05$)

4. Water absorption

Ability to absorb aqueous solution of the prepared G/SS hydrogels with the load of thyme essential oil was evaluated by immersion in phosphate buffered saline (PBS) solution. In Fig. 4, both G/SS+1%TEO and G/SS+1.5%TEO hydrogels did not show significant difference of water absorption behavior. At initial stage of investigation, both types of the hydrogels enabled to gain large amount of PBS solution reaching around 115% after immersion for 1 h. At longer time, their water absorption was gradually increased until reached 6 h of immersion in PBS. After that, the absorption ability of G/SS+1%TEO hydrogels maintained up to 200 % till the end of investigation (30 h). An equilibrium point of G/SS+1%TEO hydrogels was approximately at 6 h. This finding suggests the structural stability of the hydrogels till the

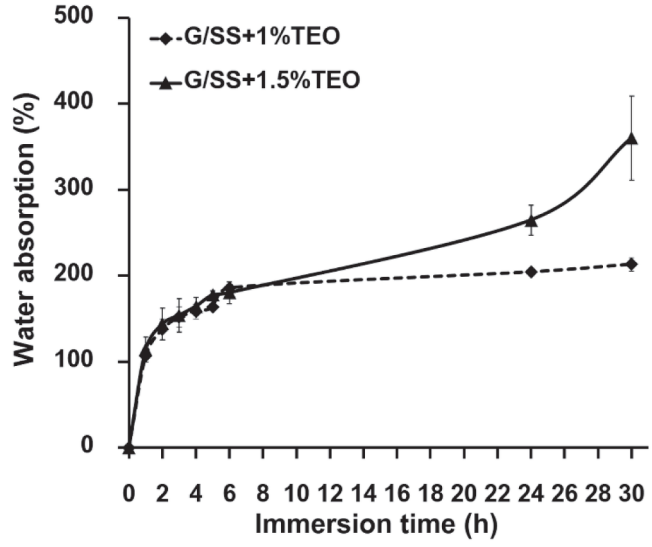


**Figure 4** Water absorption of G/SS+1%TEO and G/SS+1.5% TEO hydrogels after crosslinked with 5 %v/v glutaraldehyde

end of investigation. In comparison with the result of the G/SS+1.5%TEO hydrogels, the water absorption ability of adding 1.5 %v/v TEO was continuously increased up to 300% after 30 h of immersion in PBS solution. This phenomenon is probably due to a plasticizing effect of embedded TEO in the hydrogels on the structural stability. Since the incorporation of TEO led to decreased stiffness and increased flexibility of the hydrogels, water molecules would be able to penetrate easily into hydrogel network compared to the stiff hydrogels.

5. *In vitro* release of thyme essential oil

The release characteristics of G/SS hydrogels with loading 1 and 1.5 %v/v TEO and crosslinking with 1 and 5 %v/v GA are presented in Fig. 5(a). Four different samples exhibited an initial burst release followed by maintained release rate at longer time of immersion. The burst release of the hydrogels was found after immersed for 60 min, which is induced by the release of TEO at the surface of the hydrogels. Afterward, the release of TEO maintained in all cases until the end of investigation. The release trend was in agreement with the behavior of water absorption (see Fig. 4) that absorption ability retained constantly after reaching equilibrium state in longer time of immersion. The hydrogels with an incorporation of higher amount of TEO (1.5 %v/v) after crosslinked either with 1 or with 5 %v/v GA showed larger amount of TEO release than the released amount from the 1 %v/v TEO-loaded hydrogels. G/SS+1.5%TEO/1%GA hydrogels provided the release reaching around 45% and the TEO release of 20% was found in the hydrogels of G/SS+1.5%TEO/1%GA after immersion of 300 min. Similar to the case of used 5 %v/v GA, G/SS+1.5%TEO and G/SS+1%TEO hydrogels exhibited the release

of approximately 35 and 10%, respectively. In addition to amount of TEO loading, the concentration of crosslinking agent clearly showed an influence in the release behavior. Higher GA concentration used (5% v/v) with both G/SS+1%TEO and G/SS+1.5%TEO hydrogels exhibited lower release of TEO, in comparison with the use of 1 %v/v GA. This result can be explained that higher degree of crosslinking showing better mechanical and structural stability of the hydrogels hindered penetration of water molecules, becoming an obstacle for oil diffusion. In summary, the amount of TEO loading and crosslinking degree played an important role in releasing ability of the G/SS hydrogels.

The release profiles in Fig. 5(a) were extended to the study of release kinetics in order to investigate the release mechanism of TEO from the G/SS hydrogels. Values of kinetic exponent (n) indicating the release mechanism and regression coefficient ($R^2$) were reported in Fig. 5(b). When n value is less than 0.5, it indicates diffusion mechanism of the release. The value of n is higher than 1.0 means the release mechanism is corresponding to case II transport. If the n value is in the range of 0.5−1.0, it indicates an overlapping of these two previous transports [29]. In Fig. 5(b), the n values of G/SS+1%TEO with 1 and 5 %v/v GA crosslinking were 0.51 and 0.85, respectively. While, the G/SS+1.5%TEO hydrogels crosslinked with 1 and 5 %v/v GA showed the n values of 0.56 and 0.21, respectively. From the results, the release mechanism of most hydrogel samples was controlled by a combination of TEO diffusion and polymer matrix dissolution in the aqueous medium [30]. Exceptionally, the release of G/SS+1.5%TEO/5%GA hydrogels was driven by the mechanism of diffusion. It occurs through the diffusion of water molecules from medium solution into polymer matrix, leading to diffusion of TEO out of the matrix into the medium. While the mechanism of case II transport occurs by penetration of the aqueous medium, swelling and polymer chains disentanglement and relaxation, respectively [30]. The difference in release mechanisms found in the present study may influence by the factors of oil and GA contents. Amount of embedded oil and degree of crosslinking impacted on the polymer entanglement and relaxation, and consequently affected the release ability.

6. Antibacterial activity

The antibacterial property of TEO-loaded G/SS hydrogels against *S. aureus* and *S. epidermidis* was tested according to the disk diffusion method. The disks of G/SS hydrogels without TEO served as control. Bacterial inhibition zones around the sample disks were shown in Fig. 6. After 24 h of incubation, both hydrogel samples including G/SS+1%TEO and G/SS+1.5%TEO hydrogels could inhibit the growth of *S. aureus* and *S. epidermidis* showing the inhibition zones of 20 ± 2 and 22 ± 2 mm, and 24 ± 2 and 28 ± 3 mm, respectively Meanwhile, no inhibition zone was observed around the control disk. The antibacterial activity of 1.5 %v/v

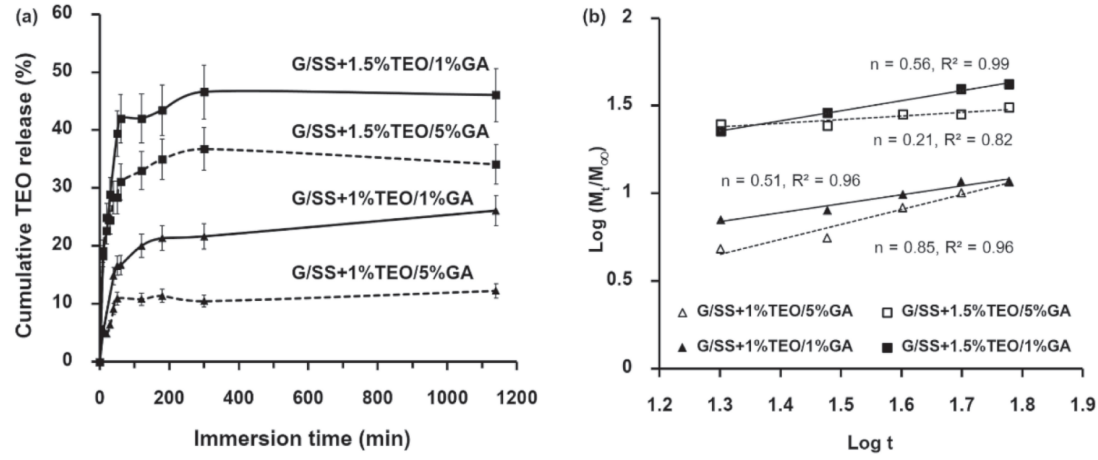


**Figure 5** (a) Release behavior of thyme essential oil from G/SS+1%TEO and G/SS+1.5%TEO hydrogels, both were crosslinked by using 1 and 5 %v/v glutaraldehyde, after immersed in phosphate buffered saline (PBS, pH 7.4, 37°C) for 1200 min and (b) Ritger and Peppas model parameters for TEO release from G/SS+1%TEO and G/SS+1.5% TEO hydrogels, both were crosslinked by using 1 and 5 %v/v glutaraldehyde (n is kinetic exponent and $R^2$ is regression coefficient)

TEO-loaded G/SS hydrogels was significantly different from that of 1 %v/v TEO-loaded G/SS hydrogels. The result indicates that the release dose of TEO from the hydrogels influenced the growth inhibition of both bacteria. Thus the G/SS hydrogels acted as an effective device offering functions for loading and releasing thyme essential oil, and providing an advantage of antibacterial property against bacterial growth. These beneficial features of the TEO-loaded G/SS hydrogels confirms their potential to be used as a wound dressing.

## Conclusion and Discussion

An application for thyme essential oil-incorporated gelatin/silk sericin hydrogel films with antibacterial function as a wound dressing is proposed in the present work. The physico-chemical properties including surface morphology, chemical composition, the mechanical properties and water absorption of the prepared hydrogels were investigated. It was found that the incorporation of thyme essential oil and crosslinking degree significantly affected surface morphology of the gelatin/silk sericin hydrogels. High content of thyme essential oil up to 5 %v/v led to increased surface roughness of the hydrogels, while high concentration of glutaraldehyde crosslinking agent altered the surface morphology from droplets to co-continuous phase. ATR-FTIR analysis confirmed the existence of gelatin and silk sericin blending polymers and successful process of crosslinking the proteins with glutaraldehyde. The incorporated thyme

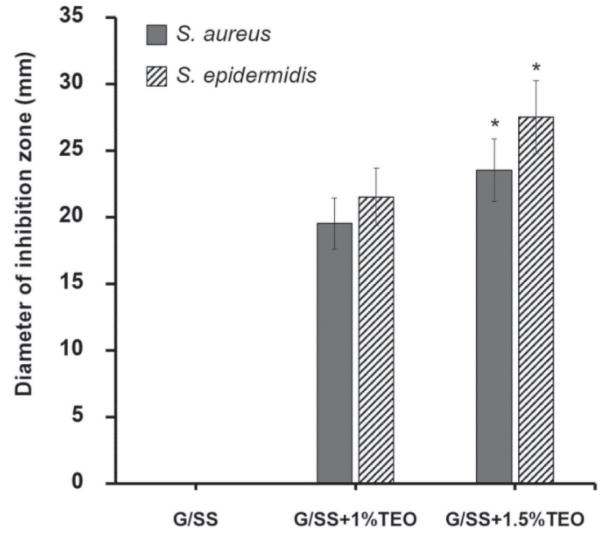


**Figure 6** Antibacterial activity of G/SS, G/SS+1%TEO and G/SS+1.5% TEO hydrogels that was investigated by disk-diffusion method and the results were indicated by diameter of inhibition zone around sample disks. The values are presented as mean ± SD (n = 3) with significant statistical difference under one-way ANOVA test (*$p \leq 0.05$)

essential oil acted as a plasticizer in the polymer blend and subsequently Young's modulus and tensile strength of the hydrogels was decreased while the elongation at break was improved. The hydrogels were able to absorb large amount of water reaching around 300%. In terms of release behavior, the oil-loaded hydrogels provided the release profile in biphasic manner, including initial burst release after 1 h of immersion in phosphate buffered saline and maintained release at longer time of immersion (30 h). Amount of essential oil and concentration of glutaraldehyde showed an important impact on the release characteristics. Increased oil content resulted in high oil release and increased glutaraldehyde concentration inhibit the oil release. This phenomenon was suggested by the reason of enhanced polymer interaction and increased entanglement, leading to retard of water diffusion and oil release. Finally, thyme essential oil-loaded hydrogels could inhibit bacteria growth against *S. aureus* and *S. epidermidis.* In particular of the hydrogels with loading 1.5 %v/v thyme essential oil, they provided significant activity to inhibit the growth of bacteria, indicated by the highest diameter of inhibition zone among the others. In conclusion, the essential thyme oil-incorporated gelatin/silk sericin crosslinked hydrogels would be a great candidate to be used as a wound dressing.

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