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Electrospun centella asiatica leaf extract–loaded poly (vinyl alcohol)/ gelatin fiber mats as potential wound dressings

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Abstract

The electrospun poly(vinyl alcohol) (PVA)/gelatin (GEL) fibers containing *Centella asiatica* leaf extract (CA) with different ratios of PVA/GEL and different contents of CA were fabricated. The ratios of PVA/GEL/CA affected the kinematic viscosities of solution and thereby affected the morphology and size of fibers. Only the electrospun fiber mats at PVA/GEL ratios of 9/1 and 8/2 containing 5% CA (PVA/GEL/CA 9/1/5 and 8/2/5) were selected to investigate their potential for use as wound dressings. The release behaviors of CA from these fiber mats were investigated at 37°C in phosphate buffer (pH 7.4) solution using the total immersion and the diffusion using modified Franz cell methods. For both release methods, the burst release of CA at the initial time followed by a gradual release until reaching a plateau was noticed. The PVA/GEL/CA 8/2/5 provided a greater release of CA than the PVA/GEL/CA 9/1/5 fiber mats. The slower rate and smaller amounts of CA released from the diffusion method than those from the total immersion method were observed. The degree of water swelling of the fiber mats was evaluated. Lastly, the PVA/GEL/CA 9/1/5 and 8/2/5 fiber mats exhibited antioxidant activity as determined by 1,1–diphenyl–2–picrylhydrazyl (DPPH) assay and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, which revealed the potential for use as wound dressing materials.

Keywords: Centella Asiatica, poly(vinyl alcohol), gelatin, wound dressing, electrospinning

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1. Introduction

Electrospinning is the process to produce ultrafine fibers in a range of micrometers down to nanometers from either polymer solutions or polymer melt. An emitting electrode of the high power supply charges the polymer solution or melts until the electrostatic field strength reaches a critical value, in which the pendant drop destabilizes into a conical shape (i.e., the Taylor cone) [1]. Beyond a critical value, a charged polymer jet is ejected from the cone and travelled to the collector by the electrostatic force. The thinning down of the charged jet during its flight to the collector is due to the Coulombic repulsion force.

Electrospun fibers have been proposed for use in a number of applications, for example, air and water filtration, sensors, catalysis, optical devices, tissue engineering, carriers for drug delivery systems, and wound dressings. The outstanding aspects of the ultrafine electrospun fibers for use as wound dressings are their interconnected porous structure with a high surface-to-volume ratio that provides the drug molecules to diffuse out continuously. The sustained release of drugs can be achieved using the electrospun fibers as carriers [2–4].

Among various types of materials used as the carriers in wound dressings, hydrogels are of the most interesting according to their ability in absorbing water or fluid but not dissolving. Poly(vinyl alcohol) (PVA) incorporated with drugs or bioactive molecules is widely used as promising wound dressings [5–7]. Crosslinking of PVA could be performed by using boric acid [8], diisocyanate [9], glyoxal [10], and glutaraldehyde [11] to improve the physical stability during usage and to control the degree of weight loss and swelling.

Several proteins or partially hydrolyzed proteins, for example, silk fibroin, elastin, and gelatin were integrated into wound dressings to improve cell attachment, cell proliferation, and the biocompatibility of the materials. Among these polypeptide substances, gelatin was chosen to incorporate into the electrospun PVA nanofibers in the present work. Gelatin is widely utilized in biomedical applications, for example, tissue engineering, targeted drug delivery, and wound dressings according to its non-toxicity, biocompatibility, biodegradability, and resemblant structure to collagen, an extracellular matrix of tissues [12, 13]. The biodegradability, viability, and proliferation of cells cultured on the alginate/gelatin scaffolds increased with an increase in the gelatin content [14].

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Electrospun gelatin/polyurethane nanofibers for use in wound healing applications exhibited higher cell proliferation when the gelatin content was increased [15].

Centella asiatica (CA) plant is widely found in moist and warm regions including Asia, Africa, Australia, Central America, and South America [16]. It is used as a local medicine for the treatment of many diseases or symptoms, for example, skin diseases, renal stones, asthma, and gastrointestinal diseases [17]. The major compounds in CA plant are triterpenes, asiatic acid, madecassic acid, and their derived triterpene ester glycosides including asiaticoside, and madecassoside [17]. CA extracts possess several pharmacological properties such as anti–ulcer, anti–inflammatory, antioxidant, and anti–microbial [17–19]. Many reports revealed that CA facilitates the wound healing process by stimulating collagen synthesis, enhancing cell proliferation, and reducing oxidation in the wound [18]. Wang L. et al. fabricated CA loaded–gelatin/chitosan hydrogel for use as wound dressings which exhibited antibacterial properties and good biocompatibility [19]. Yao C.H. et al. demonstrated that the electrospun gelatin nanofibers containing CA extract enhanced the recovery rate of wound healing in rats compared with gauze and neat gelatin membrane [20].

In the present work, the electrospun CA leaf extract-loaded PVA/gelatin fiber mats with different ratios of PVA and gelatin and different contents of CA were fabricated. The morphology and size of fibers were examined. The potential for use of the electrospun PVA/gelatin/CA fiber mats as materials for topical and transdermal delivery of CA was investigated. The release behaviors of CA therefrom were studied using the total immersion and the diffusion methods in the phosphate buffer (pH 7.4) solution at 37°C. The degree of water swelling of the fiber mats was evaluated. Lastly, the antioxidant activity and the antibacterial activity of these fiber mats were determined.

2. Experiment

2.1 Materials

Centella asiatica (CA) leaves were brought from the local market in Pathumthani, Thailand. Poly(vinyl alcohol) (PVA; degree of hydrolysis: 86.0–89.0%, MW: 85,000–124,000 g/mol) and gelatin (GEL) powder were purchased from SD Fine Chemicals (India). Glutaraldehyde (25% aqueous solution) was purchased from Acros Organics (USA). Ethanol, disodium hydrogen phosphate (Na_2HPO_4), and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Carlo Erba (Italy). 1,1–diphenyl–2–picrylhydrazyl (DPPH) was purchased from Sigma Aldrich (USA).

2.2 Extraction of Centella Asiatica Leaf

CA leaves were collected, washed with water, and dried at room temperature for 5 h. They were cut into small pieces and immersed in ethanol at a solid:liquid ratio of 10 g:20 mL. The mixture was shaken in a closed container at room temperature for 24 h. The filtrate was collected from vacuum filtration. The liquid extract of CA was placed in a rotary evaporator to remove the solvent at 40°C for 30 min. The obtained slurry was further freeze–dried. Lastly, the CA extract was obtained in a solid form and kept in a desiccator.

2.3 Electrospinning of CA Extract-loaded PVA/GEL Fiber Mats

Aqueous solutions of PVA and GEL were prepared at 12% and 8% w/v at 80°C and 45°C, respectively. PVA and GEL solutions were mixed at the ratios of 9/1, 8/2, 7/3, and 6/4 w/w and stirred until the homogeneous solutions were obtained. The obtained solutions were designated as the PVA/GEL 9/1, the PVA/GEL 8/2, the PVA/GEL 7/3, and the PVA/GEL 6/4, respectively. For each of the PVA/GEL solutions, the CA extract was subsequently added at different concentrations of 5, 10, and 20% w/w. The pristine PVA/GEL 9/1 solution without the addition of CA and the CA extract–loaded PVA/GEL solutions were designated as the PVA/GEL/CA 9/1/0, the PVA/GEL/CA 9/1/5, the PVA/GEL/CA 9/1/10, and the PVA/GEL/CA 9/1/20, respectively. The other solutions were named in a similar manner. Kinematic viscosities of the solutions were measured using a Cannon-Fenske Routine viscometer (a constant kinematic viscosity of 2.351 cSt/s at 40°C).

Each of the PVA/GEL/CA solutions was electrospun into a non-woven nanofiber mat. The solution was placed in a plastic syringe connected with a stainless-steel needle used as a nozzle. The diameter of the nozzle was 0.91 mm. A high voltage of either 15 kV was applied to the solution using a Gamma High Voltage Research ES30P-5W power supply. The rotating collector which was covered with aluminum foil was used to collect the electrospun fibers at 100 rpm. The distance between the collector and the nozzle was 15 cm. The flow rate of the solution was kept constant at 0.5 mL/h using a syringe pump (SP-8800 AMPall, Korea). The electrospun PVA/GEL/CA fiber mat with the thickness of about 60 ± 10 μm was obtained after 10h of electrospinning. The selected PVA/GEL/CA fiber mats were chosen to investigate in the further study including the release behavior of CA therefrom, the degree of water swelling, the antioxidant activity, and the antibacterial activity. The PVA/GEL/CA fiber mats were crosslinked by a glutaraldehyde vapor treatment. The fiber mats were placed in a closed container saturated with the vapor of glutaraldehyde, and were kept in an oven at 40°C for 10h. Later, the fiber mats were left in a ventilated area for 24h at room temperature to allow complete evaporation of glutaraldehyde.

The morphology of the electrospun fibers before and after crosslinking was investigated using a JSM–5410LV JEOL scanning electron microscope (SEM).

The samples were coated with a thin layer of gold using a Polaron SC7640 coater prior to observing under the SEM.

2.4 Release of CA from the PVA/GEL/CA Fiber Mats

2.4.1 Preparation of the phosphate buffer (pH 7.4)

For the proposed application of the PVA/GEL/CA fiber mats as wound dressings, the phosphate buffer solution (pH 7.4) was used as a releasing medium in order to simulate the physiological condition of the wound. For the preparation of 1 L of the phosphate buffer (pH 7.4), 20.2 g of $Na_2HPO_4.7H_2O$ and 3.4 g of $NaH_2PO_4.H_2O$ were dissolved in distilled water. The final volume of the solution was adjusted to 1 L. Few drops of sodium hydroxide solution or hydrochloric acid could be added to adjust the pH to 7.4. The release characteristics of CA from the PVA/GEL/CA fiber mats were investigated by the total immersion method and the diffusion method at 37°C using the phosphate buffer solution as a releasing medium.

2.4.2 Release assay by the total immersion method

The PVA/GEL/CA fiber mat was cut into a square shape of 2x2 cm^2 and immersed in 40 mL of the phosphate buffer solution at 37°C. The solution was slowly stirred using a magnetic stirrer during the releasing time ranging from 0 - 8h. At each specified time point, 1.0 mL of the releasing medium was withdrawn and diluted with the buffer solution before measuring for the absorbance by the UV-vis spectrophotometer at 208 nm. The same amount (i.e. 1.0 mL) of fresh phosphate buffer solution was refilled into the release bottles in order to keep the constant volume. The amounts of CA released were quantified from the absorbance of the releasing solution against the pre–determined standard curve of the CA solution in the phosphate buffer solution. The cumulative percentage of the CA released was calculated according to the equation (1):

$$\text{Cumulative CA release (\%)} = \frac{C_t}{C_{total}} \times 100 \qquad (1)$$

where C_t is the cumulative weight of CA released at time t and Ctotal is the initial weight of the CA loaded in the PVA/GEL/CA fiber mats. The experiments were carried out in triplicate. In order to determine Ctotal, the actual drug content (i.e., the actual amount of CA extract presented in the fiber mats) was quantified. The fiber mat was cut into a square piece of 2x2 cm^2 and was completely dissolved by continuously stirred in 20 mL of distilled water at 80°C for 3 h. The actual amount of CA extract was quantified from its absorbance at 208 nm using the UV–vis spectrophotometer.

2.4.3 Release assay by the diffusion method

The PVA/GEL/CA fiber mat was placed on top of the modified Franz diffusion cell which was fully filled with the phosphate buffer (pH 7.4) solution. The diameter of the Franz diffusion cell which exposed to the tested fiber mat was 13 mm. The total volume of solution in the Franz cell was 9.0 mL. The experiments were performed at 37°C and the solutions were slowly stirred using a magnetic stirrer during the releasing time ranging from 0 - 8h. At each specified time point, 0.3 mL of the releasing medium was withdrawn and diluted with the buffer solution before measuring for the absorbance by the UV–vis spectrophotometer at 208 nm. The same amount (i.e. 0.3 mL) of fresh phosphate buffer solution was refilled into the Franz diffusion cell. Similar to the total immersion method, the cumulative percentage of the CA released was calculated according to equation (1). The experiments were carried out in triplicate.

2.5 Water swelling of the PVA/GEL/CA fiber mats

The degree of water swelling of the PVA/GEL/CA fiber mats was determined after immersion in the phosphate buffer (pH 7.4) solution at 37°C for 2 and 4h. The tested fiber mat was cut into a square shape of 2x2 cm^2 and immersed in 40 mL of the phosphate buffer solution. The percentage of water swelling was calculated according to the equation (2):

$$\text{Water swelling (\%)} = \left(\frac{M - M_i}{M_i}\right) \times 100 \qquad (2)$$

where M_i is the initial dry weight of the fiber mat and M is the weight of the fiber mat after immersion in the phosphate buffer solution at a specified time point.

2.6 Antioxidant activity of the PVA/GEL/CA fiber mats

The antioxidant activity of the PVA/GEL/CA fiber mats was evaluated by the radical scavenging DPPH assay. The PVA/GEL/CA fiber mat was cut into a square shape of 2x2 cm^2 and immersed in 40 mL of the phosphate buffer solution at 37°C. At 30, 60, and 120 min of immersion time, 1.0 mL of the releasing media were withdrawn and mixed with 3.0 mL of 0.5 mM DPPH solution in methanol. The mixture was kept in darkness for 30 min and was measured for absorbance at 517 nm by the UV–vis spectrophotometer. The pristine 0.5 mM DPPH solution was also stored in the same condition for being as a control. The antioxidant activity was calculated according to the equation (3);

$$\text{Antioxidant activity (\%)} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \qquad (3)$$

where $A_{control}$ and A_{sample} are the absorbances at 517 nm of the DPPH solution without and with the presence of the as–released CA solution, respectively. The experiments were carried out in triplicate

2.7 *Antibacterial activity of the PVA/GEL/CA fiber mats*

The antibacterial activity of the PVA/GEL/CA fiber mats against *Staphylococcus aureus (S. aureus:* ATCC 25923) and *Escherichia coli* (*E. coli:* ATCC 25922) bacteria was evaluated by the agar disc diffusion method. The circular filter papers saturated with either deionized water or ethanol were used as the negative and positive control, respectively. The tested fiber mat was cut into a circular disc of 6 mm diameter. The agar disc was incubated at 37°C for 24 h. Lastly, the diameter of inhibition zone, included the diameter of the disc, was measured.

3. Results and Discussions

3.1 *Electrospinning of the PVA/GEL/CA fiber mats*

Prior to electrospinning, the as-prepared solutions of the CA extract–loaded PVA/GEL at various ratios were measured for their kinematic viscosities. The values of kinematic viscosities are presented in Table 1. The morphology of the electrospun PVA/GEL/CA fibers was observed by using the SEM. The selected SEM images and the average diameters of the electrospun PVA/GEL/CA fibers which were measured using ImageJ software [21] are shown in Table 1. For the PVA/GEL solutions at a ratio of 9/1, the viscosities of solutions increased with increasing the CA content. Consequently, the average fiber diameters were increased. The viscosity of the solution is one of the important properties that governed the morphology and the size of electrospun fibers. The higher viscosity of the solution leads to the higher viscoelastic force which resists the stretching of the fiber jets from the electrostatic attraction and the Coulombic repulsion forces [22]. Therefore, the size of the fiber is generally increased when the viscosity of the polymer solution increased. The smooth surface with the round cross-sectional shape of fibers was evidenced in the case of the PVA/GEL/CA 9/1/0 and 9/1/5. However, the fibers of PVA/GEL/CA 9/1/10 were fused together. More fused fibers which revealed the film–like morphology was observed in the case of the PVA/GEL/CA 9/1/20. It is known that the fusion of the electrospun fibers is mainly caused by the inefficient evaporation rate of solvent from the fiber jets or the too slow solidification rate of the fiber jets. Too high amounts of CA extract in the as-prepared solutions (i.e., PVA/GEL/CA 9/1/10 and 9/1/20) led to the presence of fused fibers. The possible reason could be that some excess amounts of CA extract could not mix well with PVA and GEL and therefore existed on the surface of the as–spun fibers which hindered the evaporation of the solvent (i.e., water) or solidification of fibers.

For the PVA/GEL solutions at a ratio of 8/2, the viscosities of solutions increased with increasing the CA content. Therefore, the higher average fiber diameters were observed as a similar trend as those in the case of the PVA/GEL of 9/1 ratio. Also, the PVA/GEL/CA 8/2/20 fibers were fused together in some areas and wide distribution of the fiber diameters was observed.

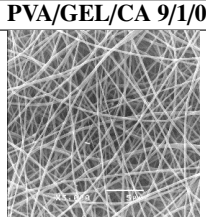
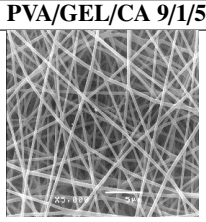
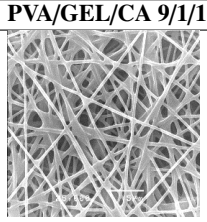
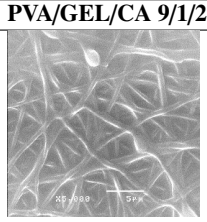
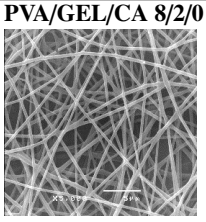
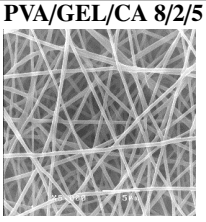
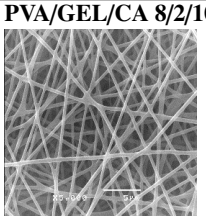
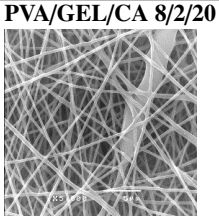
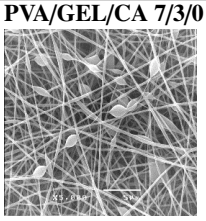
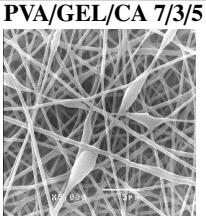
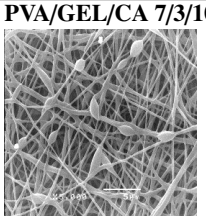
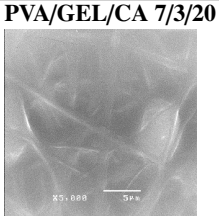
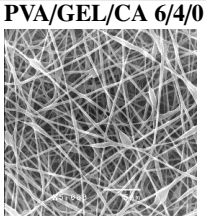
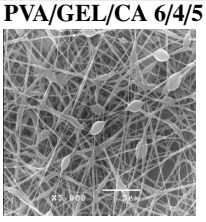
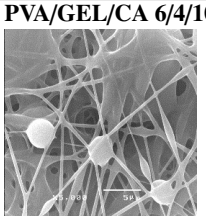
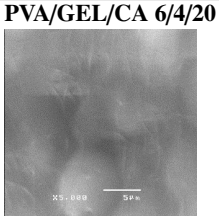
For both ratios of the PVA/GEL solutions at 9/1 and 8/2, the fibers without beads were produced. However, the beaded fibers were obtained from the PVA/GEL solutions at the ratios of 7/3 and 6/4 for all conditions with various CA contents. The presence of beads was mainly caused by the substantial low viscosities of the solutions (i.e., about 55–82 cSt for the ratio 7/3 and about 17–29 cSt for the ratio of 6/4) in which the viscoelastic force was not sufficient to withstand the electrostatic and the Coulombic forces [22]. Again, some parts of fibers were fused together when the CA content was at 10% for both the PVA/GEL ratios of 7/3 and 6/4. Eventually, the fibers were completely fused until the film–like morphology was obtained when the CA content was up to 20%. Based on these results, the PVA/GEL solutions at ratios of 7/3 and 6/4 were not suitable to fabricate into the fibers for use in the further studies because the obtained fibers were not uniformed. In addition, for the PVA/GEL solutions at ratios of 9/1 and 8/2, the CA contents at 10 and 20% were considerably too high in which the fused fibers were obtained. Therefore, only the PVA/GEL/CA 9/1/5 and 8/2/5 were used in the further studies including the release of CA, the antioxidant, and the antibacterial activities of the fiber mats for the proposed application in wound healing.

Furthermore, crosslinking of the PVA chains is necessary in order to improve the stability or physical properties of the fiber mats. Figure 1 shows the SEM images of the PVA/GEL/CA 9/1/5 and 8/2/5 before and after treatment with glutaraldehyde vapor at 40°C for 10 h. Crosslinking of PVA involves the formation of acetal groups between two hydroxyl groups of PVA. After crosslinking, most of the fibers fused together. However, the fiber–like morphology still remained.

3.2 *Electrospinning of the PVA/GEL/CA fiber mats*

The water swelling behaviors of the PVA/GEL/CA 9/1/5 and 8/2/5 fiber mats were investigated after submersion in the phosphate buffer solution (pH 7.4) at 37°C for 2h and 4h (see Figure 2). The percentages of water swelling of the PVA/GEL/CA 9/1/5 and 8/2/5 at 2h of submersion were about 375% and 569%, respectively. At 4h of submersion, these values were about 516% and 633%, respectively. It was found that the degree of water swelling increased with increasing time of submersion. Additionally, at either 2h or 4h, the PVA/GEL/CA 8/2/5 exhibited a higher degree of water swelling than the PVA/GEL/CA 9/1/5. Similar observations were also reported that the hybrid hydrogels fabricated from PVA and GEL for use in osteoarthritis surgery had a higher swelling ability when the amount of GEL increased since the molecules of GEL are more extendable and can hold more water compared to the PVA chains [23].

Table 1. The SEM images of the electrospun PVA/GEL/CA fibers, the average fiber diameters (FD), and the viscosities of the as–prepared solutions (μ).

Samples	**PVA/GEL/CA 9/1/0**	**PVA/GEL/CA 9/1/5**	**PVA/GEL/CA 9/1/10**	**PVA/GEL/CA 9/1/20**
SEM images				
FD (nm)	218.94 ± 28.12	305.62 ±30.46	326.60 ± 41.81	N/A
μ (cSt)	153.44 ± 1.29	189.98 ± 1.43	220.06 ± 1.06	228.01 ± 3.81
Samples	**PVA/GEL/CA 8/2/0**	**PVA/GEL/CA 8/2/5**	**PVA/GEL/CA 8/2/10**	**PVA/GEL/CA 8/2/20**
SEM images				
FD (nm)	301.67 ± 55.61	334.52 ± 51.24	335.20 ± 47.82	336.00 ± 61.05
μ (cSt)	120.92 ± 1.03	153.07 ± 1.35	162.07 ± 1.83	166.80 ± 1.51
Samples	**PVA/GEL/CA 7/3/0**	**PVA/GEL/CA 7/3/5**	**PVA/GEL/CA 7/3/10**	**PVA/GEL/CA 7/3/20**
SEM images				
FD (nm)	185.42 ± 30.76	319.71 ± 57.26	301.72 ± 53.87	N/A
μ (cSt)	55.19 ± 1.52	61.24 ± 1.12	69.06 ± 1.02	81.60 ± 1.64
Samples	**PVA/GEL/CA 6/4/0**	**PVA/GEL/CA 6/4/5**	**PVA/GEL/CA 6/4/10**	**PVA/GEL/CA 6/4/20**
SEM images				
FD (nm)	226.30 ± 55.41	202.05 ± 71.32	N/A	N/A
μ (cSt)	17.04 ± 1.22	17.19 ± 0.50	18.13 ± 0.68	29.43 ± 0.47

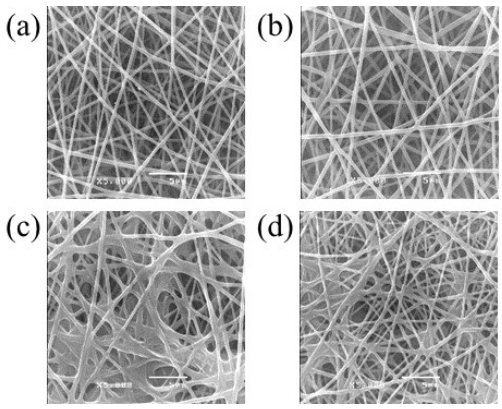


Figure 1: SEM images of the electrospun PVA/GEL/CA fibers before crosslinking at ratios of (a) 9/1/5, (b) 8/2/5 and after crosslinking with glutaraldehyde vapor at ratios of (c) 9/1/5, and (d) 8/2/5.

3.3 Release of CA from the PVA/GEL/CA fiber mats

The release behaviors of CA from the PVA/GEL/CA 9/1/5 and 8/2/5 fiber mats were investigated by the total immersion method and the diffusion method at 37°C during 0–8h using the phosphate buffer solution (pH 7.4) as the releasing

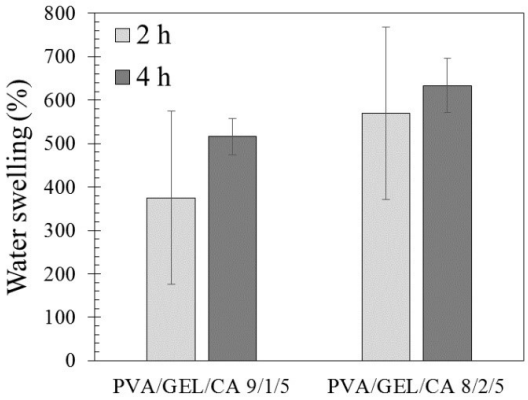


Figure 2: Water swelling of the PVA/GEL/CA 9/1/5 and 8/2/5 at 2 and 4h of immersion in phosphate buffer (pH 7.4).

medium.

3.3.1 Total immersion method

Figure 3(a) shows the percentages of the cumulative release of CA therefrom by the total immersion method at 37°C in the phosphate buffer solution (pH 7.4). The burst release was observed at the initial 10 min of release. Later, the gradual release until reaching the plateau amounts was noticed. The maximum amounts of CA released from the PVA/GEL/CA 9/1/5 and 8/2/5 at 480 min (8 h) were about 84.4% and 90.0%, respectively. The higher amounts of CA released from the PVA/GEL/CA 8/2/5 than those from the PVA/GEL/CA 9/1/5 were evidenced. A number of factors affect the rate and the amount of a substance released into media, for example, the temperature [24], the solubility of substance in media (i.e., hydrophilicity and lipophilicity), the degree of weight loss of the matrix, the degree of swelling of the matrix [25] and pH of releasing media [26]. It was found that the release behaviors correlated with the degree of water swelling in Figure 2 in which the PVA/GEL/CA 8/2/5 had a higher swelling ratio than the PVA/GEL/CA 9/1/5. The greater swelling ability of the matrix indicates that it can hold more water or be highly solvated by water and therefore allowing the drug molecules to diffuse out conveniently [26]. Isoglu et al. investigated the release of CA from the electrospun poly(D, L–lactide–co–glycolide) (PLGA)/poly(3–hydroxybutyrate–co–3–hydroxy valerate) (PHBV) fibers [27]. CA was rapidly released from the electrospun PLGA/PCL membrane in the first 5h, followed by a slow release. In this work, the release of CA from the PVA/GEL/CA fiber mats showed the burst release in the first hour which was faster than that observed from their work. The higher hydrophilicity of PVA and GEL than those of PLGA and PHBV could be one of the factors affecting the greater degree of water swelling, and therefore faster release of CA. However, the release of CA from the PLGA/PCL fibers reached a plateau at about 10h which was close to this work (i.e., 8h).

3.3.2 Diffusion method

The attempt to investigate the release characteristics of CA from the fiber mats in the most similar conditions as in the applications of wound dressings was performed by the diffusion method. Figure 3(b) shows the percentage of the cumulative release of CA from the PVA/GEL/CA 9/1/5 and 8/2/5 fiber mats by the diffusion method at 37°C in the phosphate buffer solution (pH 7.4). Similar to the results observed in the total immersion method, the burst release of CA at the initial time of release was presented. However, the slower and smaller amounts of CA released were noticed from the diffusion method. The amounts of CA released in the region of the burst release were only about 35–40%, whereas those from the total immersion method were about 67–70%. Later, the gradual release until reaching the plateau amounts was revealed. The maximum amounts of CA released from the PVA/GEL/CA 9/1/5 and 8/2/5 at 480 min (8 h) were about 74.3% and 80.4%, respectively. Also, the maximum amounts of CA released in the diffusion method were lower than those in the total immersion method.

Consistently, it can be noticed that the PVA/GEL/CA 8/2/5 fiber mats exhibited more amounts of CA released than the PVA/GEL/CA 9/1/5 which were similar to the results of the total immersion method. The greater swelling ability of the PVA/GEL/CA 8/2/5 fiber mats could be the reason that they can provide the greater amounts of CA released as discussed earlier.

3.4 Antioxidant activity of the PVA/GEL/CA fiber mats

Wound healing is the complex biological process to restore the structure and functional integrities of injured skin tissues. Antioxidants are the substances that can prevent or delay the production of free radical species. Antioxidants are asserted to help control wound oxidative stress and thereby accelerate wound healing [28]. The antioxidant activities of the

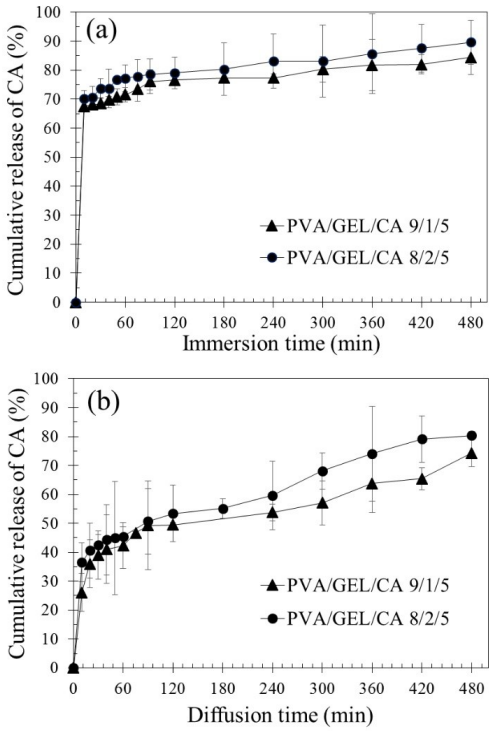


Figure 3: Cumulative release amounts of CA from the PVA/GEL/CA in phosphate buffer (pH 7.4) as determined by (a) total immersion method and (b) diffusion method.

PVA/GEL/CA 9/1/5 and 8/2/5 fiber mats were evaluated by the DPPH assay. The DPPH radical (DPPH·) is a stable free radical that can accept electron or hydrogen radical to become a non-radical molecule (DPPH-H). The transformation of the DPPH radicals into the non-radical molecules induced by CA was measured by the reduction in its absorbance at 517 nm according to Equation (3). Figure 4 presents the antioxidant activities of these fiber mats after immersion in the phosphate buffer solution (pH 7.4) for 30, 60, and 120 min. For both types of fiber mats, the antioxidant activity increased with increasing time of immersion according to the higher amounts of CA released as time increased. Interestingly, the antioxidant activities of the PVA/GEL/CA 8/2/5 were greater than those of the PVA/GEL/CA 9/1/5 fiber mat. The values at 120 min of the PVA/GEL/CA 9/1/5 and 8/2/5 fiber mats were 12.01 ± 1.05 and 13.66 ± 1.12%, respectively. Once more, the results of the antioxidant activity corresponded to the trends observed in the water swelling and the release amounts of CA that the PVA/GEL/CA 8/2/5 fiber mats possessed the greater values.

3.5 Antibacterial activity of the PVA/GEL/CA fiber mats

The antibacterial property is one of the important properties of wound dressings. The antibacterial activities of the PVA/GEL/CA 9/1/5 and 8/2/5 fiber mats were investigated against *S. aureus* and *E. coli* by agar disc diffusion method. Table 2 shows the average diameter of inhibition zone observed from the bacterial culture disc. No inhibition zone was observed for a negative control (i.e., deionized water) for both types of bacteria. Ethanol as a positive control showed the average diameter of inhibition zone of 10.66 ± 0.58 and 10.34 ± 0.58 mm for *S. aureus* and *E. coli*. Interestingly, both types of the PVA/GEL/CA fiber mats exhibited the larger diameters of inhibition zone than that of a positive control for both bacteria. Contrary to the release behaviors, the PVA/GEL/CA 8/2/5 showed a smaller inhibition zone compared to the PVA/GEL/CA 9/1/5 fiber mats. However, its inhibition zone is still greater than a positive control. The higher contents of GEL in the electrospun ciprofloxacin–loaded alginate/PVA/GEL fibers [4] and in the tetracycline hydrochloride–encapsulated poly(lactic acid)/GEL hydrogel [29] also caused a smaller antibacterial properties against *S. aureus* and *E. coli*. The outstanding antibacterial property of the PVA/GEL/CA fiber mats is affirmed by this study which indicate the potential for use as the carriers for transdermal drug delivery and for wound healing applications. Mouro *et al*. fabricated the double–layered composite membranes composing of a layer of the electrospun chitosan–sodium tripolyphosphate and poly(vinyl alcohol) containing CA on top of a polycaprolactone layer [30]. These membranes exhibited the outstanding antibacterial activities against *S. aureus* (99.96%) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (99.94%) as tested by ASTM E2180–07 standard. The antibacterial activities of the

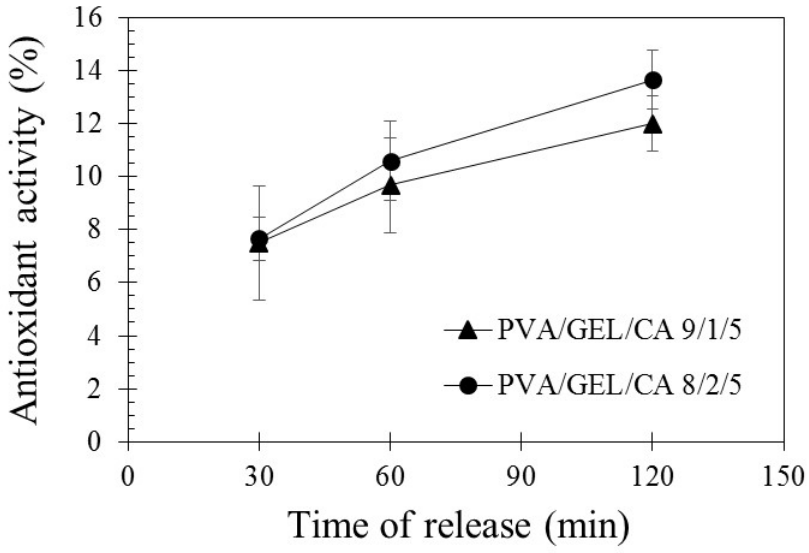


Figure 4: Antioxidant activities of the PVA/GEL/CA fiber mats at different immersion times.

Table 2. Antibacterial activities of the PVA/GEL/CA against *S. aureus* and *E. coli* as determined by the agar disc diffusion method. The diameter of the circular disc was 6 mm.

Samples	**Average diameter of inhibition zone (mm)**	
	S. aureus	***E. coli***
Negative control: deionized water	0.00 ± 0.00	0.00 ± 0.00
Positive control: ethanol	10.66 ± 0.58	10.34 ± 0.58
PVA/GEL/CA 9/1/5	14.34 ± 0.58	13.26 ± 0.22
PVA/GEL/CA 8/2/5	11.34 ± 0.58	12.00 ± 0.44

PVA/GEL/CA fiber mats herein are consistent well with the previous study.

4. Conclusions

In the present contribution, CA leaf extract which is widely known for its anti-inflammatory, antioxidant, and antibacterial was encapsulated in the electrospun PVA/GEL fiber mats. The as-prepared solutions with various ratios of PVA/GEL and various contents of CA were used in the electrospinning. The ratios of PVA/GEL/CA affected the kinematic viscosities of the solution and thereby affected the morphology and size of fibers. The potential for use of the selected electrospun PVA/GEL/CA fiber mats (i.e., 9/1/5 and 8/2/5) as wound dressing patches was explored. The release behaviors of CA therefrom were investigated at 37°C in phosphate buffer (pH 7.4) solution using the total immersion and the diffusion using modified Franz cell methods. For both release methods, the PVA/GEL/CA 8/2/5 provided the greater release of CA than the PVA/GEL/CA 9/1/5 fiber mats which corresponded with the values of water swelling. The burst release of CA at the initial time followed by a gradual release until reaching a plateau was observed for both types of release methods. However, the more sustained release was noticed in the case of the diffusion method. The antioxidant activity of the PVA/GEL/CA 8/2/5 was slightly higher than that of the PVA/GEL/CA 9/1/5 fiber mats which correlated with the release amounts of CA. Both types of fiber mats exhibited antibacterial activities against *S. aureus* and *E. coli* which revealed the potential for use as wound dressing materials.

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