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Original Research

Physicochemical Properties Comparison between Polyvinyl Alcohol/Chitosan/Trigona Honey Electrospun Nanofibers and Films for Wound Healing

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ABSTRACT

Infection and prolonged inflammation are among the main threats to effective wound healing. Hence, this study aimed to develop multifunctional medicated wound dressings in the form of electrospun nanofibers and films containing polyvinyl alcohol, chitosan, and *Trigona* honey (PVA/CS/TH), and compare their physicochemical properties. Two control samples without honey (PVA/CS) were also fabricated for comparison. Electrospinning and solvent casting techniques were used to fabricate electrospun nanofibers and films, respectively. The effects of different fabrication techniques as well as integration of TH into the polymer matrix were examined through surface morphology, elemental, wettability, dissolubility, and water vapour transmission rate (WVTR) analyses. The PVA/CS-1 and PVA/CS/TH-1 nanofibers showed interconnecting pores while the PVA/CS-2 and PVA/CS/TH-2 films exhibited smooth and non-homogeneous surfaces, respectively. Larger fiber diameters were observed for the PVA/CS-1 nanofibers. Inclusion of TH resulted in beaded fibers, reduced fiber diameter, and increased porosity of the PVA/CS/TH-1 nanofibers. Wettability analysis revealed that the PVA/CS-1 nanofibers (65.83° ± 5.90) were more hydrophilic than the PVA/CS-2 films (93.57° ± 4.19) and TH addition further reduced the water contact angle of both nanofibers and films. From the WVTR analysis, both nanofibers and PVA/CS-2 films exhibited higher WVTR exceeding 279 g/m^2/24h which represents the WVTR value in the first-degree burns. This study suggested that PVA/CS/TH-1 nanofibers have superior physicochemical properties as multifunctional medicated wound dressings than the other samples.

INTRODUCTION

Skin is continuously exposed to the external environment and is therefore susceptible to a range of injuries (Ahmadabad et al., 2025). Wound healing is a highly complex, natural physiological reaction that takes place in four phases namely hemostasis, inflammation, proliferation, and remodeling following tissue injury. A dysregulation to these phases for instance, longer duration of certain phases, lead to impaired tissue repair (Borbolla-Jiménez et al., 2023). Modern wound dressings are dissimilar to the conventional dressings, particularly addressing various functionality for better wound healing such as provide protection from external elements and microorganisms, prevent dehydration, alleviate pain, control

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water absorption, provide mechanical support and so forth (Ahmadabad et al., 2025). In addition, medicated dressings containing bioactive molecules, including natural compounds are among the promising therapeutic approaches of wound healing (Gallo et al., 2024).

Polyvinyl alcohol (PVA) is a synthetic polymer. It is a suitable material for wound dressings due to its non-toxicity, water-solubility, biodegradability, and biocompatibility. PVA has shown a good film-forming ability attributed by its high composition of hydroxyl groups which can form intermolecular hydrogen bonding (Choo et al., 2016). Chitosan (CS) is a natural polymer or also called biopolymers derived from chitin of crustacean shells. It exhibits interesting properties for use as a wound dressing material due to its non-toxicity, biocompatibility, biodegradability, antibacterial activity, hemostatic property, etc. (Choo et al., 2016; Ahmadabad et al., 2025). Biopolymers have limitations such as lack of mechanical properties and prone to contamination by microorganisms while the synthetic polymers normally show low adherence to the wound site, exhibit less permeability and absorption as well as less biocompatibility which may cause immune rejection. Blending of different polymers could provide better properties to the wound dressings (Borbolla-Jiménez et al., 2023). Trigona honey (TH) is locally known as kelulut honey. In Malaysia, this type of honey is produced by diverse species of stingless bee known as Trigona bees including Trigona itama, Trigona apicalis, Trigona laeviceps, and Trigona thorasica (Mohamad et al., 2019). TH has been widely studied in the past years due to its medicinal values including antimicrobial, antibiofilm, anti-inflammatory, antioxidant, anticancer, and proliferative properties (Mohamad et al., 2019; Salim et al., 2019; Badrulhisham et al., 2020; Aburayyan et al., 2024). Addition of natural compounds such as plant extracts, polyphenols, curcumin, and essential oils, antibiotics, and nanoparticles could enhance wound healing (Borbolla-Jiménez et al., 2023).
Electrospinning is a common technique that has been used to produce nanofibers due to its simple operation and can be upgraded to commercial scale (Samraj et al., 2021). Electrospun nanofibers which may consist of nano- and micro-sized fibers could mimic the natural extracellular matrix components such as collagen and elastin fibers, and therefore can serve as a platform for cells adherence, proliferation, and differentiation (Kaniuk et al., 2023). Interestingly, electrospun nanofibers demonstrate attractive properties such as high porosity, extremely high specific surface area, controllable pore size, light weight, excellent mechanical properties, flexibility in surface functionalities, etc. (Abduljabbar and Farooq, 2022). Solvent casting method, on the other hand, is a reliable and highly preferred method to produce films due to low cost, easiness, provide adequate thickness uniformity, and tunable to obtain desired properties. Polymeric films offered several advantages including ease of application, non-invasive, flexible, transparent, good adherence, and gas exchange, which are beneficial for wound healing (Borbolla-Jiménez et al., 2023).

This study aimed to fabricate electrospun nanofibers and solvent casting films containing PVA and CS with and without TH and investigate the physicochemical properties of the nanofibers and films for potential use as wound dressings.

MATERIALS AND METHOD

Materials

Chitosan (medium molecular weight and 75-85% deacetylated) and PVA (molecular weight of 89,000-98,000 and 99% hydrolyzed) were purchased from Sigma-Aldrich, United States. Analytical grade glacial acetic acid was supplied by RCl Labscan, Thailand. Trigona honey was directly purchased from a beekeeper in Kelantan, Malaysia.

Preparation of PVA/CS and PVA/CS/TH solutions

The PVA/CS solution was prepared by mixing 10% w/v of PVA dissolved in ultrapure water and 2% w/v of CS dissolved in 1% acetic acid at the ratio of 7:3 for 1 h under constant stirring at 50 °C. For the preparation of PVA/CS/TH solution, the 10% w/v of PVA (9.3 mL) and 2% w/v of CS (4 mL) were stirred for 1 h at 50 °C and later the polymer solution was mixed with 6.7 mL of 10% v/v TH and stirred again for 30 min at room temperature.

Fabrication of PVA/CS and PVA/CS/TH nanofibers and films

Fabrication of PVA/CS-1 and PVA/CS/TH-1 nanofibers was performed using electrospinning technique. The prepared solution was loaded in a 5 mL plastic syringe. The electrospinning was performed at the voltage of 20 kV with the distance between the collector and the tip of the nozzle maintained at 10 cm and the flow rate was kept constant at 0.6 mL/h. A flat collector plate covered with an aluminium sheet was used to collect the nanofiber sheet.

The PVA/CS-2 and PVA/CS/TH-2 were prepared by solvent casting technique. A total volume of 20 mL of PVA/CS and PVA/CS/TH solutions were poured into respective petri dishes and left dried in an oven at 50 °C for 44 h. The films were then neutralized by immersing the films into 10 %w/v of sodium hydroxide solution for 45 min. The films were rinsed several times with distilled water after the neutralization step. All films were air-dried and kept at room temperature until further use.

Surface morphology and elemental analyses

All samples were gold coated and analyzed using a scanning electron microscope (SEM, Hitachi TM3000) to study their surface morphology and elemental composition. The SEM was operated with accelerating voltage of 15kV. The SEM images were analyzed using an ImageJ software and histogram of fiber diameter distribution was plotted using an Origin software. The elemental analysis was examined using an Energy Dispersive X-ray Spectroscopy (EDX). Only one out of three replicates of each sample were analyzed for the morphology and elemental analyses.

Wettability analyses

The contact angle was measured using a VCA Optima contact angle (Optima, AST Products, USA). Images of water droplet on nanofibers and films were taken at 0 min. The contact angle was calculated from mean and standard deviation of triplicates data.

Dissolution analyses

Dissolution test was conducted by adding water dropwise on each sample which has been cut 1.5 cm x 1.5 cm and placed in petri dish. The dissolution properties of samples were recorded by continuous shooting mode, where digital photos were captured at the predetermined time intervals including before contact with water (0 s), 5 s, 30 s and 60 s after contact with water (Ponrasu et al., 2021).

Water vapour transmission rate (WVTR) analyses

WVTR of samples was analyzed according to Wang et al. (2015) with modifications. A volumetric flask containing 10 mL of distilled water was fully covered with a sample that were previously cut. The weight of the volumetric flask containing 10 mL of distilled water was recorded as W0. The flasks covered with samples were placed in a desiccator that contained silica gel, which was used to absorb water. The weight of the flasks was measured for every 24 h for 3 days. WVTR was measured by dividing the difference of measured weight with the cross-section area of the flask using the formula:

$$\text{WVTR (g/m2/day)} = (W0 - Wt)/(tA)$$

Where W0 = weight of system at time 0; Wt = weight of system at time t; t = measurement time; A = cross-section area of volumetric flask. Results were presented as mean and standard deviation of triplicates data.

RESULT AND DISCUSSION

Surface morphology and elemental composition

The SEM images of all samples are shown in Figure 1. It can be seen from Figure 1(a) and (b) that both PVA/CS-1 and PVA/CS/TH-1 nanofibers were composed of fibers with both small and larger diameters. It can also be observed that inclusion of TH in the PVA/CS polymer matrix decreased the PVA/CS/TH-1 nanofiber diameter and increased its porosity (Figure 1b). The nanofiber diameter distribution for PVA/CS/TH-1 nanofiber was recorded ranging from 78 - 873 nm whereas PVA/CS-1 nanofiber diameter ranged from 78 - 1498 nm. The presence of pores in the nanofibers will facilitate the exchange or mass transfer of oxygen, water, and nutrients at the wound sites as well as facilitate cellular respiration (Sapru et al., 2018; Zhang et al., 2024). Decrease in the fiber diameter of PVA/CS/TH-1 nanofiber could be attributed to the decreasing viscosity of the polymer matrix upon addition of TH which appeared as a watery honey due to its higher water content (Turaga et al., 2016; Noiset et al., 2025). A dissimilar result was observed in the past study which reported an increase in the fiber diameter of PVA nanofibers upon addition of highly-viscous honey (Turaga et al., 2016).

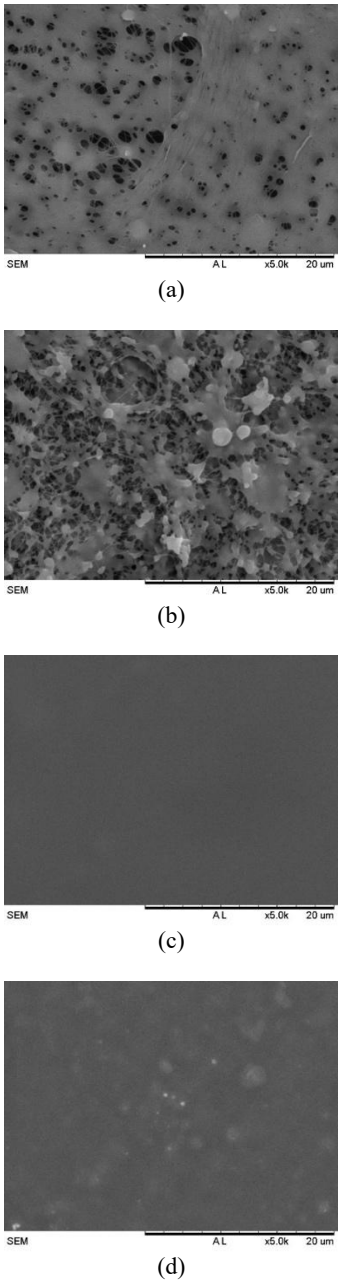


Fig. 1 SEM images of: (a) PVA/CS-1 nanofiber, (b) PVA/CS/TH-1 nanofiber, (c) PVA/CS-2 film and (d) PVA/CS/TH-2 film.

Figure 1a was in accordance to the previous study that pointed out the presence of fiber fusion, film layers and aggregates appeared more often at high PVA concentration (Mata et al., 2022). The formation of superaggregate having a width of several micrometers was seen in many PVA fibers that connected with one another due to the complex association and dissociation of fibril bundles and/or fibrils (Wang et al., 2024). The beaded fibers as seen from Figure 1b could be due to lack of polymer chain entanglement, a similar observation reported by the past study who demonstrated that low polymer concentration led to beaded fibers. The study also reported that ultrafine fibers were obtained when they increased the polymer concentration (Ponrasu et al., 2021).

It is important to note that the presence of interconnected pores in the PVA/CS-1 and PVA/CS/TH-1 nanofibers structures could support cell migration while increasing porosity could mimic the

natural tissue extracellular matrix and thus may promote cell infiltration, proliferation, and differentiation of specific cells (Kaniuk et al., 2023; Zhang et al., 2024). Besides, the high surface area of the electrospun nanofibers could carry functional additives such as the Trigona honey, which has various therapeutic properties that can promote faster recovery of wound tissue and prevent infection (Zhang et al., 2024).

In contrast, the PVA/CS-2 film (Figure 1c) exhibited smooth surface and homogeneous morphology while the PVA/CS/TH-2 film showed nonhomogeneous morphology with prominent granular structures (Figure 1d). The similar observation was also reported by a past study who demonstrated a relatively smooth surface of chitosan/PVA based hydrogel films at high chitosan concentration, indicating a homogenous blend with good interfacial adhesion following evenly distributed chitosan in the PVA matrix and the availability of more chitosan to bind with PVA (Chopra et al., 2022). The interaction between the amino and hydroxyl groups of CS and hydroxyl groups of PVA formed hydrogen bonds that resulted in the homogenous dispersion of the blend matrix (Choo et al., 2016).

Table 1 denotes the elemental composition of all samples. It was evident from the results that addition of TH in the blend matrix reduced the carbon composition and increased in oxygen composition. The oxygen atoms in honey could have been attributed to its composition which are mainly consists of water, sugars, organic acids and polyphenols (Misran et al., 2025).

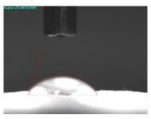
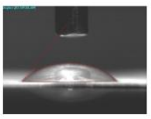
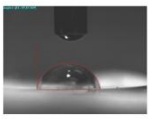
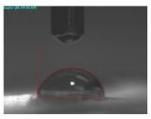
Table 1 Chemical composition of nanofibers and films determined by EDX.

Element	PVA/CS-1 (weight %)	PVA/CS/TH-1 (weight %)	PVA/CS-2 (weight %)	PVA/CS/TH-2 (weight %)
Carbon	70.92	54.94	67.04	55.78
Oxygen	29.08	45.06	32.96	44.22
Total	100.00	100.00	100.00	100.00

Wettability

Table 2 presents the wettability results of the samples where the interaction of the nanofibers and films with water was investigated by contact angle analysis. It can be observed from Table 2 that nanofibers exhibited lower water contact angle (WCA) values than the films. Three samples exhibited surface hydrophilicity with WCA values lower than 90° while PVA/CS-2 films exceeded 90° which indicated its hydrophobic surface. PVA/CS/TH-1 nanofibers showed further reduction in WCA values mainly due to the integration of TH into the nanofiber's structure. Similarly, addition of TH to form the films resulted in reduced contact angle and hydrophobicity of PVA/CS/TH-2 films (86.00° ± 5.09), the values which were lower than the PVA/CS-2 films (93.57° ± 4.19).

Table 2 Contact angle measurements of nanofibers and films.

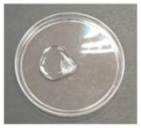
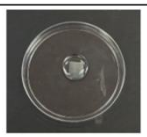
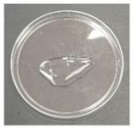
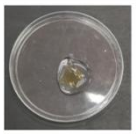
PVA/CS-1	PVA/CS/TH-1	PVA/CS-2	PVA/CS/TH-2
			
65.83° ± 5.90	55.30° ± 3.38	93.57° ± 4.19	86.00° ± 5.09

As shown in Figure 1c, the smooth and homogenous surfaces of PVA/CS-2 film could have lowered the interfacial free energy, resulted in poor wetting due to less water ability to penetrate the films and therefore increases the WCA (Pang et al., 2013). On top of that, less interaction between hydroxyl groups with water may also contribute to the reduction in hydrophilicity of the PVA/CS-2 films, possibly due to the interactions between hydroxyl groups of PVA with amino or hydroxyl groups of CS (Pineda-Castillo et al., 2018). On the contrary, the high porosity of the PVA/CS-1 and PVA/CS/TH-1 nanofibers allows water penetration and thus formed hydrophilic surfaces with lower WCA. The presence of TH in the nanofibers' structures enable more interaction between hydroxyl groups and water, increasing the hydrophilicity of the PVA/CS/TH-1 nanofibers.

Dissolution

Results of dissolution analysis are presented in Table 3. It is clearly shown that all samples did not dissolve within 60 s of testing. This is most likely be due to the intermolecular interactions between polymeric chains of PVA and CS instead of water interactions (Pineda-Castillo et al., 2018). These results were in accordance with the past study who pointed out that combination of PVA with chitosan generates a system with lower solubility degree than PVA alone, despite the higher levels of solubility of PVA and the presence of amino groups in CS which can partially attach themselves to water molecules (Pineda-Castillo et al., 2018).

Table 3 Dissolution of nanofibers and films at 60 s.

PVA/CS-1	PVA/CS/TH-1	PVA/CS-2	PVA/CS/TH-2
			

Water vapour transmission rate (WVTR)

It can be observed from Table 4 that WVTR of nanofibers were much higher than the films. The PVA/CS-1 nanofibers showed the greatest transmission rate followed by PVA/CS/TH-1, PVA/CS-2 and PVA/CS/TH-2 for the three consecutive days. The high WVTR offered by the nanofibers are linked to the presence of pores and higher surface area that allows passage of water molecules through the spaces between the nanofibers (Zhang et al., 2024). Inclusion of TH into the nanofibers and films' structures further reduced water loss into the environment, thus it can be deduced that the presence of TH in the nanofibers and films could maintain a moist environment which is also vital for cells functionality (Nuutila and Eriksson, 2021). Specifically, cells require a liquid medium for transporting growth factors and other signaling molecules to enhance their communication during wound healing. Moist environment also promotes rapid migration of keratinocytes over the wound surface which takes place less effectively in a dry environment, promotes collagen synthesis by the fibroblasts as well as supports break down of dead (necrotic) tissue by the endogenous enzymes (Nuutila and Eriksson, 2021).

Table 4 WVTR values of nanofibers and films.

Hours	PVA/CS-1 ($g/m^2/day$)	PVA/CS/TH-1 ($g/m^2/day$)	PVA/CS-2 ($g/m^2/day$)	PVA/CS/TH-2 ($g/m^2/day$)
24 h	385.96 ± 15.19	315.79 ± 0.00	301.08 ± 0.87	263.16 ± 0.84
48 h	368.42 ± 0.00	289.47 ± 0.00	311.83 ± 40.59	263.16 ± 0.00
72 h	362.57 ± 10.13	315.79 ± 0.00	290.32 ± 32.26	260.23 ± 5.06

The low WVTR values seen in this study indicated that the nanofibers and films permitted less water loss (Nuutila and Eriksson, 2021), which is not within the recommended range of 2000-2500 g/m2/day for optimal wound healing (Millotti et al., 2025). However, this study proposed that the PVA/CS-1 and PVA/CS/TH-1 nanofibers as well as PVA/CS-2 films could be used as wound dressings for treating first-degree burns with WVTR values of 279 g/m2/day (Millotti et al., 2025).

CONCLUSION

In brief, PVA/CS-1 and PVA/CS/TH-1 nanofibers exhibited superior physicochemical properties than the PVA/CS-2 and PVA/CS/TH-2 films for potential use as wound dressings, contributed by high porosity, high hydrophilicity, and high WVTR values. Addition of TH to the PVA/CS matrix further improved the properties of both nanofibers and films, and this study proposed that PVA/CS/TH-1 nanofiber could be used as a multifunctional medicated wound dressing that can enhance wound healing due to the excellent therapeutic properties of Trigona honey.

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