

ORIGINAL ARTICLE

Effect of Tualang Honey Concentration on the Morphology of Electrospun PVA Nanofibers for Wound Healing Application

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ABSTRACT

Introduction: Tualang Honey (TH) is known for its antimicrobial and anti-inflammatory properties, making it a potential candidate for wound healing applications. Chronic wounds present a significant challenge in medical treatment due to their extended healing durations and elevated risk of infection. **Materials and Methods:** In this study, the potential of electrospun Polyvinyl Alcohol (PVA) nanofibers incorporated with Tualang Honey for wound healing was investigated. PVA nanofibers containing various concentrations of Tualang Honey (0%, 2%, 4%, and 6%) were prepared using the electrospinning method. The morphological and chemical characteristics of the synthesized nanofibers were evaluated. The water contact angle (WCA) of the nanofibers was also measured to assess hydrophilicity. **Results:** Scanning Electron Microscopy (SEM) revealed changes in fiber morphology with increasing honey concentrations, while Fourier Transform Infrared Spectroscopy (FTIR) confirmed the incorporation of honey within the nanofibers. WCA measurements indicated improved hydrophilicity with the addition of honey, particularly at higher concentrations. Optimal results were observed with 4% honey concentration, which demonstrated uniform fiber formation and enhanced hydrophilicity. **Conclusion:** The findings suggest that PVA/Tualang Honey nanofibers possess great potential for wound healing applications, merging the mechanical properties of PVA with the therapeutic benefits of honey.

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INTRODUCTION

Wound healing is a multifaceted physiological process that encompasses a broad spectrum of therapeutic strategies addressing both acute and chronic wounds (1). Acute wounds typically have the potential to heal, leading to a full restoration of the skin. In contrast, chronic wounds can experience delayed or halted healing processes, often resulting in prolonged durations and a higher likelihood of recurrence (2). Such chronic conditions can arise from various factors, including diseases like diabetes and external contamination (3-5). Historically, wound dressings were primarily

designed to cover wounds, preventing dehydration and infections. However, traditional passive dressings often posed challenges, such as causing new injuries during replacement and being less effective against bacterial contamination (6). This led to a paradigm shift towards the development of smart wound dressings aimed at aiding the body in healing wounds more rapidly, reducing scarring, pain, and discomfort (7).

Electrospinning has emerged as a promising technique for fabricating smart wound dressings with fibrous structures, offering properties like fluid absorption, nutrient and gas exchange, and bacterial contamination barriers (8,9). The electrospinning process involves applying a high-intensity electric field to a polymeric solution, resulting in the formation of ultrafine fibers collected on a target. The properties of these fibers can be tailored by adjusting the electrospinning parameters

and environmental conditions (10). Among the materials explored for electrospinning, natural polymers, including Polyvinyl alcohol (PVA), a synthetic polymer, have shown significant promise due to their biocompatibility, hydrophilicity, and support for cell adhesion and proliferation (11). PVA is particularly noted for its biodegradability, non-toxicity, and favorable physical properties (1,12-14).

Honey, a natural product with a rich history in wound care, offers antimicrobial, anti-inflammatory, and wound healing properties (15). Tualang Honey, derived from *Apis Dorsata* bees in Malaysia, has gained attention for its rich bioactive components and promising applications in the field of wound care (16). This wild multifloral honey is known for its potent properties and potential to support wound healing processes. However, the direct application of honey to wounds poses challenges, such as flow out of the wound bed, leading to inconvenience.

In consideration of the unique structural advantages provided by electrospun nanofibers and the beneficial properties of Tualang Honey in wound care applications, this study focuses on the fabrication of Tualang Honey/PVA nanofibrous membranes through electrospinning (17,18). The morphology and chemical composition of the nanofibers were thoroughly examined using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and water contact angle (WCA), respectively. This approach aims to address the current research gap by elucidating how varying concentrations of Tualang Honey affect the structural characteristics and potential therapeutic benefits of the resulting nanofibers.

MATERIALS AND METHODS

Materials

The materials used in this study included Polyvinyl alcohol (PVA) and Tualang honey. The PVA, a synthetic polymer known for its biodegradability, non-toxicity, and favorable physical properties, was sourced from Sigma-Aldrich. The Tualang honey was sourced from certified local suppliers in Pontian. Its authenticity and quality were verified through a certificate of analysis accredited by the Malaysian Agriculture Research and Development Institute (MARDI). The physico-chemical properties of the Tualang honey used in this study include a pH of 3.5, moisture content of 17%, and high levels of antioxidants and antimicrobial agents. These properties significantly influence the characteristics of the nanofibers produced. Distilled water was used as the solvent for preparing the solutions.

Preparation of PVA/Tualang Honey Nanofibers

To prepare the solution for electrospinning, 1.2 grams of PVA powder was measured using an electronic balance. Deionized water, chosen for its minimal mineral and ion content, was heated to 80°C on a hotplate. Ten milliliters

of this hot water were transferred to a beaker and stirred. The PVA powder was incrementally added to the water until the solution became clear and transparent. To ensure a bubble-free solution, stirring was continued even after the heating was turned off.

For the PVA/Tualang honey solution, the PVA solution was first prepared as described above. Once the solution was clear, the temperature was gradually reduced, and specific honey concentrations (0.2 mL, 0.4 mL, and 0.6 mL) were added. The mixture was stirred for approximately 1 to 1.5 hours at room temperature until homogeneity was achieved. The resulting PVA/Tualang honey solution was then immediately subjected to the electrospinning process.

Electrospinning Process

The prepared PVA/Tualang honey solutions were loaded into 5 mL syringes fitted with metallic needles. These syringes were then placed in a syringe pump (Fig. 1), operating under specific parameters as outlined in Table I. The collector surface of the setup was covered with aluminum foil to efficiently gather the electrospun nanofibers.

Different flow rates were used for the varying honey concentrations to compensate for the changes in solution viscosity caused by the honey. This adjustment ensured the nanofibers maintained a uniform diameter and minimized the formation of unwanted particles (beads), aligning with the findings from previous studies (20, 21).

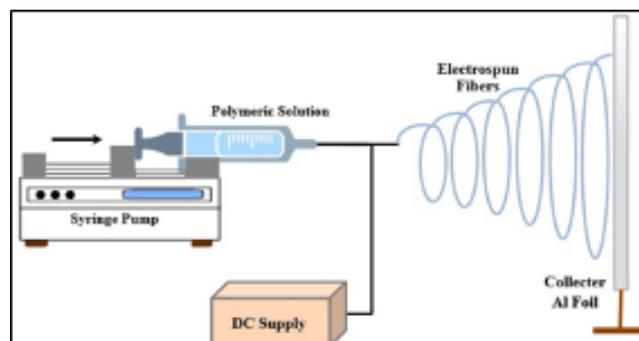


Fig. 1: Schematic diagram of electrospinning process to form nanofiber.

Table I: Electrospinning parameters and compositions for PVA/Tualang honey (TH) nanofiber samples

Samples	Amount of Water Used	PVA Concentration (% w/v)	TH Concentration (% v/v)	Feed Rate (mL/hr)	Voltage (kV)	Collector to Needle Distance (cm)
1	10 mL	12%	0%	0.7	20 kV	15 cm
2	10 mL	12%	2%	1.0	20 kV	15 cm
3	10 mL	12%	4%	1.3	20 kV	15 cm
4	10 mL	12%	6%	1.5	20 kV	15 cm

Electrospun PVA/Tualang Honey Nanofiber Characterization

Scanning Electron Microscopy (SEM)

The surface morphology of the PVA/Tualang honey nanofiber composite mats was analyzed using a scanning electron microscope (SEM, Hitachi S-7400, Japan). The nanofiber diameter distribution was characterized using ImageJ software and SEM images. Samples were sputter-coated with a thin layer of gold nanoparticles to enhance conductivity and resolution during SEM analysis. Scanning was performed at magnifications of x3.00k and x5.00k SE.

Fourier Transform-Infrared Spectroscopy (FT-IR)

FT-IR analysis was conducted using a Thermo Scientific Nicolet iS10 infrared spectrometer, operated with the Thermo Scientific Nicolet OMNIC software package. Spectral data were collected in absorbance mode for quantitative determination of peak areas, allowing for the identification of the molecular structure and bonding composition of the material. The nanofiber's chemical structure was examined and measured between 400 and 4000 cm^{-1} in wavelength.

Wettability Analysis via Water Contact Angle

The Water Contact Angle (WCA) was used to determine the hydrophilicity of PVA and PVA/Tualang honey nanofiber mats using a VCA Optime contact angle meter (AST Products, Inc.) as depicted in Fig. 2. Nanofiber samples were cut into small square mats and placed on a flat surface. A 0.004 mL drop of deionized water was deposited on each mat. The camera and light were activated, and the platform was aligned. Contact angles were measured immediately and recorded up to the 5th second to avoid evaporation errors. Each sample was measured six times, and the average contact angle was calculated. A live video feed captured by the camera enabled precise measurement through image analysis software.

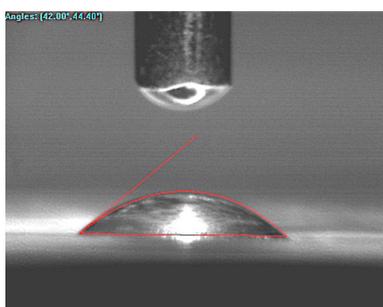


Fig. 2: An example of contact angle measurement using the VCA Optima Contact Angle Analyzer.

RESULTS

Morphological Analysis of Electrospun PVA/Tualang Honey Nanofibers:

The SEM analysis was employed in determining the optimal concentration and parameters for producing

high-quality nanofibers. The SEM images depicted distinct morphologies for each sample, with Fig. 3(a-b) representing Sample 1, pristine PVA, and Fig. 3(c-d), Fig. 3(e-f), and Fig. 3(g-h) incorporating 2%, 4%, and 6% Tualang honey, respectively. The pristine PVA nanofibers exhibited a smooth and uniform surface, as shown in Fig. 2b. However, as the concentration of Tualang honey increased, the fibers began to display variations in their structure. Specifically, the nanofibers with 2% and 4% honey (Samples 2 and 3) were relatively smooth, but with 6% honey (Sample 4), the fibers became more branched and exhibited a distinct morphology, as depicted in Fig. 3d, Fig. 3f, and Fig. 3h. The distribution of nanofiber diameters, as shown in Figure 4, predominantly falls within the range of 100-800 nm. This range is consistent with the typical definitions of nanofibers (50–500nm) and is suitable for wound healing applications due to the high surface area to volume ratio, which enhances cell attachment and proliferation. Although some fibers approach the upper limit of the nanometer scale, they remain effective within the targeted application scope. Interestingly, the incorporation of honey into the PVA polymer led to a decrease in the nanofiber diameter. This reduction might be attributed to changes in the conductivity of the honey solution, which is closely related to its concentration. A higher solution conductivity implies that the jet carries more charge, resulting in increased elongation force and decreased fiber diameters (21).

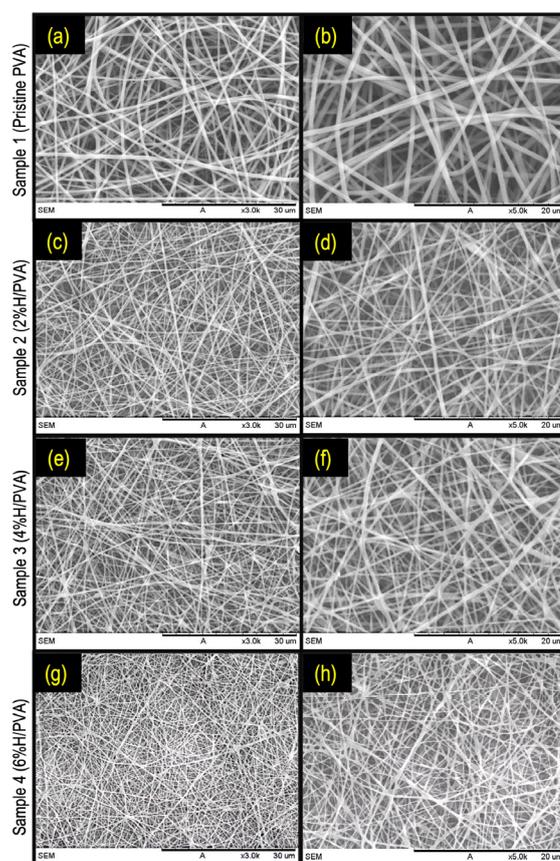


Fig. 3: SEM images at magnifications x3.0k and x5.0k of electrospun nanofibers: (a-b) Pristine PVA, (c-d) 2% honey/PVA, (e-f) 4% honey/PVA, and (g-h) 6% honey/PVA.

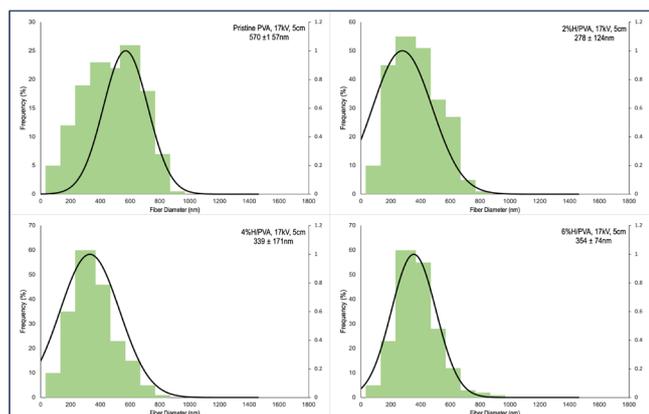


Fig. 4: Distribution of electrospun PVA fiber diameters with increasing Tualang honey concentration.

FTIR Analysis of Electrospun PVA/Tualang Honey Nanofibers

Fourier Transform-Infrared Spectroscopy (FTIR) was utilized to ascertain the presence of functional groups within the PVA/honey nanofibers. The primary compositions of honey, notably hydrated glucose and fructose, along with other carbohydrates, manifested as hydrogen-bonded hydroxyl groups in the FTIR spectrum. As reported by Sarkar et al. (18), distinctive peaks for honey were observed at 668, 814, 950, 1042,

1168, 1373, 1454, 2963, and 3420 cm^{-1} . The absorption bands at ~ 1040 and 1134 cm^{-1} can be attributed to the stretching of C-O and C-C, respectively. Peaks at ~ 1370 and 1454 cm^{-1} arise from O-C-H, C-O-H, and C-C-H bending, while those at ~ 1630 and 1690 cm^{-1} correspond to the C=C and C=O stretches of esters and carboxylic acids.

In this study, FTIR results, as shown in Fig. 5, reveal that upon the integration of honey into PVA, there was a noticeable increase in absorption peaks at the hydroxyl group, indicative of the presence of carbohydrate molecules. Additional peaks were identified at 1722 (C=O stretching), 1509 (N-O stretching), 1445 (C-H bending), 1330 (O-H bending), 1229 (C-C stretching), 1143 (C-O stretching secondary alcohols), 1092 (C-O stretching), 906, 741, and 561 (C-H bending for sugar molecules). For the PVA nanofibers, characteristic peaks were observed at 3261 cm^{-1} (O-H stretching), 2908 cm^{-1} (C-H stretching), 1327 cm^{-1} (C-O-H bending), 1089 cm^{-1} (C-O stretching), and 835 cm^{-1} (C-C bending). These findings are consistent with previously reported PVA characteristic peaks. Moreover, a distinct band at 1142 cm^{-1} (C-O stretching) was exclusively observed in nanofibers incorporated with honey.

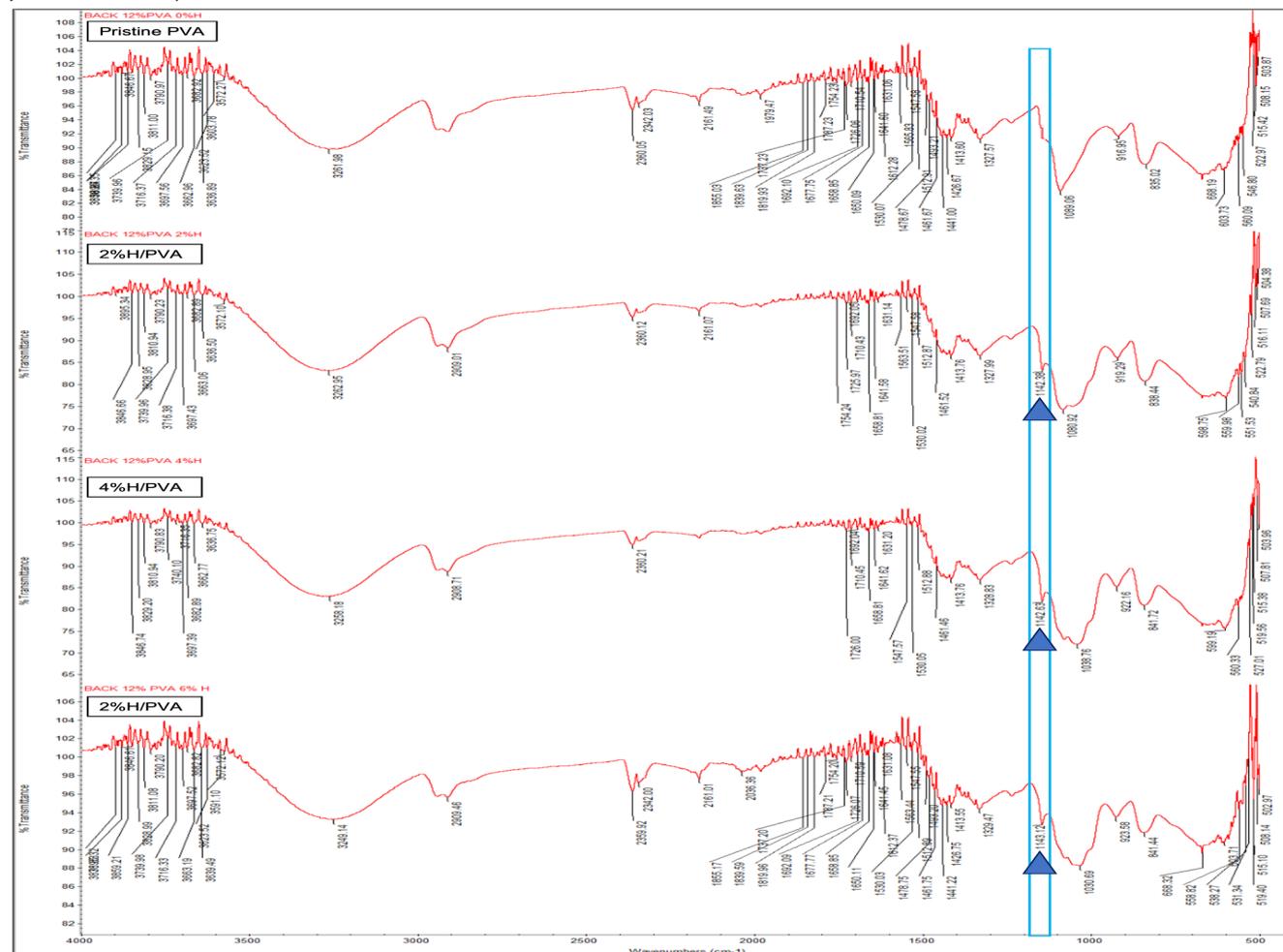


Fig. 5: FTIR spectra of PVA nanofibers: Pristine PVA, 2% honey/PVA, 4% honey/PVA, and 6% honey/PVA.

Wettability Analysis of Electrospun PVA/Tualang Honey Nanofibers:

The wettability of the PVA/Tualang honey nanofibers was evaluated through water contact angle (WCA) measurements. A single water drop was placed on each nanofiber mat with different honey concentrations to measure the contact angle. The results are summarized in Table II. The contact angle for pristine PVA was 42.6°, indicating a relatively hydrophobic surface. As the concentration of Tualang honey increased, the contact angle measurements showed a trend towards increased wettability. The PVA nanofibers with 2% Tualang honey had a contact angle of 24.1°, while those with 4% and 6% honey exhibited contact angles of 23.6° and 21.5°, respectively.

Table II: Contact angles of PVA/Tualang honey nanofiber samples

Samples	Contact Angle (°)	Standard Deviation (°)
PVA	42.6	2.42
PVA + 2% TH	24.1	1.97
PVA + 4% TH	23.6	4.35
PVA + 6% TH	21.5	4.67

DISCUSSION

The choice of 12% PVA for this study was influenced by previous research, which highlighted its potential for electrospinning when combined with honey (1, 18). The SEM results revealed that all the nanofibers, irrespective of the honey concentration, were smooth, homogeneous, dense, and bead-free. This bead-free nature is crucial as beads can compromise the mechanical properties and functionality of the nanofibers (20). The bead-free nature might be attributed to the meticulous mixing process employed. Comparing the results with the findings of Sarkar et al. (18), it was observed that the structure of the nanofibers remained largely consistent across different honey concentrations, with the exception of the highest concentration (6%). At this concentration, the nanofibers appeared more branched, possibly due to increased solution viscosity. The formation of a Taylor cone during the electrospinning process is indicative of a successful procedure (21). In this study, the Taylor cone was not observed at lower feed rates, suggesting that the solution's high concentration might have hindered its formation. Adjusting the feed rate is crucial to ensure the smooth formation of nanofibers (20).

Moreover, The FTIR results provide compelling evidence of the successful incorporation of Tualang honey into the PVA nanofibers. The observed shifts and emergence of new peaks indicate the physical mixing of honey's constituents with the PVA polymer matrix (24, 18). This physical interaction could potentially enhance the therapeutic properties of the nanofibers, making them suitable for various biomedical applications. The presence of additional peaks in the PVA/honey nanofibers, especially at 1142 cm⁻¹, underscores

the successful integration of honey's molecular components. The increased absorption at the hydroxyl group, coupled with the appearance of new peaks, suggests a potential interaction between the PVA and honey molecules, possibly due to hydrogen bonding or other intermolecular forces (25).

The PVA nanofibers exhibited a contact angle of 42.6°, indicating a relatively hydrophobic surface. This hydrophobic nature is due to the inherent properties of PVA, which, despite its potential for hydrogen bonding, can present a hydrophobic surface under certain conditions. The hydrophobic property can limit cell adhesion, making pristine PVA less ideal for wound healing applications where high cell adhesion is desired.

However, the addition of Tualang honey to the mats resulted in a significant decrease in contact angle measurements, indicating an increase in the wettability of the mats. This increased hydrophilicity can be attributed to the composition of Tualang honey, which includes various organic compounds such as phenolic acids, flavonoids, and proteins. The results also suggest improved wettability, which can enhance cell adhesion. This is crucial for wound healing applications, as good cell adhesion promotes tissue regeneration and repair (26). The contact angles for PVA with 2%, 4%, and 6% Tualang honey indicate that even at the highest concentration tested, the nanofibers maintain significantly improved wettability compared to pristine PVA.

CONCLUSION

The field of wound care has seen notable progress, with the goal of creating the best wound dressing. Our study on PVA/Tualang Honey nanofibers shows significant potential in this area. SEM results revealed how varying concentrations of Tualang Honey affect fiber morphology, while FTIR confirmed the integration of honey within the nanofibers. Water Contact Angle (WCA) measurements indicated improved hydrophilicity with the addition of honey, particularly at higher concentrations. Optimal results were observed with 4% honey concentration, demonstrating uniform fiber formation and enhanced therapeutic properties. However, broader clinical application requires further investigation. Future studies should evaluate the biocompatibility, therapeutic effects, and antimicrobial properties of the nanofibers, as well as their stability under different conditions. For PVA/Tualang Honey nanofibers to be widely adopted in wound care, a thorough assessment of their properties and effectiveness is essential.

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