Development of Multilayer Nanofiber Wound Dressings Enhanced with Bio-Selected Stingless Bee and Manuka Honey via Electrospinning

Chotchanit Nakprasit [1] and Onnalin Sungwiset [1]
Putcharaporn Wichasan[1]and Wirapong Kantamala[1]
[1]*Princess Chulabhorn Science High School Mukdahan ,281 Moo 6, Ban Nong Hoi, Bang Sai Yai Sub-district, Mueang Mukdahan District, Mukdahan 49000, Thailand*
**office@pccm.ac.th*

Abstract

Chronic diabetic wounds remain a critical public health challenge, as conventional dressings such as gauze tend to adhere to regenerating tissue, causing re-injury upon removal, and fail to maintain proper moisture balance. This study aimed to develop a multilayer nanofiber wound dressing incorporating the bioactivity of stingless bee honey, a locally sourced bioresource, in comparison with Manuka honey, a widely used medical-grade honey, via electrospinning. Preliminary bioactivity screening using DPPH, protein denaturation, and antiglycation assays showed that stingless bee honey exhibited higher antioxidant activity, while Manuka honey showed superior anti-inflammatory and anti-glycation effects. However, stingless bee honey retained its bioactivity more stably when blended with polyvinyl alcohol (PVA), and was therefore selected for fiber fabrication. The optimal condition, 10% wt honey in PVA at a flow rate of 0.3 mL/hr, produced continuous, bead-free fibers with an average diameter of 456.57 ± 25.64 nm. This active layer was further developed into a bilayer structure by overspinning with polycaprolactone (PCL) and integrating a medical-grade bordered adhesive. The bilayer structure significantly enhanced water resistance, reducing permeability from 25.39% to 0%, increased tensile strength to 5.75 MPa, nearly doubled elongation at break, and sustained over 60% bioactivity for up to 7 days. The prototype caused no damage to simulated skin and withstood more than 50 flexion cycles without detachment. This innovation demonstrates strong potential as an accessible, cost-effective smart wound dressing to advance chronic wound care for diabetic patients.

keywords : nanofiber wound dressing, stingless bee honey, Manuka honey, electrospinning, diabetic chronic wound.

Introduction

Non-communicable diseases (NCDs), particularly diabetes mellitus, are a major cause of **chronic wounds**. Hyperglycemia promotes the accumulation of reactive oxygen species, persistent inflammation, and the formation of Advanced Glycation End-products (AGEs), which damage tissue structure. These

conditions cause wounds to remain stalled in the healing process and become highly susceptible to severe infection, potentially leading to limb loss.

Conventional wound dressings, such as gauze, have two major limitations: (1) their coarse fiber structure adheres to newly regenerated tissue, causing re-injury upon dressing changes, and (2) they fail to adequately regulate moisture balance or provide an effective barrier against pathogens a critical risk factor for immunocompromised patients.

Electrospinning technology has therefore been applied, as it produces nanoscale fibers with a structure resembling the body's natural extracellular matrix (ECM), promoting cell adhesion and proliferation. Its high specific surface area also enables efficient loading and controlled release of bioactive compounds.

This study therefore proposes the development of a multilayer, medically-engineered nanofiber wound dressing, incorporating stingless bee honey a locally sourced bioresource in comparison with Manuka honey, a honey type widely used in medical-grade products. Both are combined with polyvinyl alcohol (PVA) to form an active layer, reinforced with a polycaprolactone (PCL) layer to regulate release kinetics and enhance mechanical strength, and finally assembled with a bordered medical-grade adhesive system. The resulting prototype represents an accessible and effective innovation with the potential to advance the standard of care for chronic wounds in diabetic patients.

Methodology

1.Preliminary bioactivity screening. Honey solutions were prepared at six concentrations 25, 50, 100, 150, 200 and 1000 ppm and PVA-honey blends were prepared at four concentrations 10, 15, 25 and 30% wt. Both sets were evaluated for three bioactivities:

1. antioxidant activity by DPPH radical-scavenging assay, with absorbance measured at a wavelength of 503 nm after 30-min incubation in the dark.

2 . anti-inflammatory activity by BSA protein-denaturation inhibition assay, with absorbance measured at 311 nm following heat induced denaturation (37 °C for 20 min, then 70 °C for 5 min)

3. anti-glycation activity, with absorbance measured at 300 nm after incubating BSA with fructose (50 °C, 48 h) in the presence of sodium azide.

Percentage inhibition was calculated as %Inhibition = [(Ac − (As − Abs))/Ac] × 100, where Ac, As and Abs denote the absorbance of the control, sample and blank, respectively. IC50 values were derived from linear regression of %inhibition versus concentration. All assays were performed in triplicate (n = 3).

2 . Fabrication of the honey-loaded active layer. Based on the screening results, PVA (10% w/v in deionized water, prepared at 80 °C) was blended with stingless bee honey or Manuka honey at 10, 15, 25 and 30% wt and electrospun using a high-voltage supply of 18 kV, a 21G needle, a tip-to-collector distance of 15 cm, and flow rates of 0.3 and 0.5 mL/h. Fiber morphology was examined by scanning electron microscopy (SEM) at 5000×

magnification, and the average fiber diameter was compared between honey types using an independent-samples t-test (α = 0.05). The condition yielding continuous, bead-free fibers with the narrowest diameter distribution was selected as the optimal active layer.

3.Development of the bilayer structure.

The selected active layer (PVA + 10% wt stingless bee honey, 0.3 mL/h) was used as a substrate for direct over-spinning of PCL (20G needle, 0.3 mL/h, 18 kV, 15 cm) to obtain a two-layer nanofiber composite (Active layer + PCL). A PCL-only mat was also fabricated and tested for antioxidant, anti-inflammatory and anti-glycation activity to confirm the biological inertness of the protective layer.

4.Physical, mechanical and release characterization.

1. Thickness was measured with a vernier caliper (1×1 cm, n = 3).

2. Water resistance was determined from the weight change of a 3×3 cm sample after a 200-µL water droplet was applied for up to 60 min.

3. Swelling ratio was calculated from the wet-to-dry weight change after immersion in PBS (pH 7.4, 37 °C) for up to 30 min. Tensile strength.

4. Elongation at break were measured on 3×3 cm strips using a mechanical force gauge.

5. Burst-release kinetics were assessed by DPPH, protein-denaturation and anti-glycation assays at 30, 60 and 90 min, and sustained-release/bioactivity retention was followed on days 1, 3, 5 and 7 for both single-layer and bilayer mats.

5.Prototype assembly and functional testing.

The bilayer mat (3×3 cm) was framed with medical-grade adhesive tape applied only along a 2-mm border (bordered adhesive), leaving the central pad adhesive-free. The prototype was evaluated for adhesion strength on acrylic and simulated skin (dry and moist) using the mechanical force gauge; skin trauma and adhesive residue after peel-off, scored on a 0-3 scale by three independent raters; and flexibility, by cyclic bending (50 cycles) over a simulated joint, recording the cycle at which edge lifting and total detachment first occurred. All quantitative data are reported as mean ± SD (n = 3) and compared by independent t-test, with $p < 0.05$ considered statistically significant.

Result and Discussion

1.Preliminary screening revealed that stingless bee honey exhibited markedly higher antioxidant activity than Manuka honey, whereas Manuka honey showed superior anti-inflammatory and anti-glycation activity (Table 1). At 1000 ppm, stingless bee honey reached 89.22% DPPH inhibition (IC50 = 132.38 ppm), compared with 47.15% for Manuka honey, for which an IC50 could not be determined (n.d.) within the tested concentration range. Conversely, Manuka honey showed higher anti-inflammatory (85.29% vs. 66.18%) and anti-glycation activity (89.50% vs. 80.10%) than stingless bee honey at the same concentration.

Table 1 Bioactivity of stingless bee and Manuka honey (1000 ppm, n = 3)

Assay	Stingless bee (%)	Manuka (%)
Antioxidant (DPPH)	89.22	47.15
IC50 (ppm)	132.38	n.d.
Anti-inflammatory	66.18	85.29
Anti-glycation	80.10	89.50

2.Optimization of the electrospinning process showed that both honey type and concentration significantly affected fiber morphology. A flow rate of 0.3 mL/h consistently produced more uniform, narrower fibers than 0.5 mL/h. At the lowest honey loading (10% wt), PVA blended with stingless bee honey yielded continuous, bead-free fibers with an average diameter of 456.6 ± 25.6 nm, comparable to Manuka honey (464.6 ± 50.0 nm; $p = 0.552$) but with a narrower size distribution, indicating better spinning quality. Raising the honey content to 25-30% wt markedly increased fiber diameter and produced fused, irregular structures, especially for Manuka honey (up to 1,152 ± 1,459 nm at 30% wt, 0.5 mL/h). Because stingless bee honey also retained bioactivity more stably once blended with PVA (86.94% anti-glycation inhibition at 30% wt vs. 74.70% for Manuka honey), the 10% wt stingless bee honey/PVA formulation at 0.3 mL/h was selected as the optimal active layer.

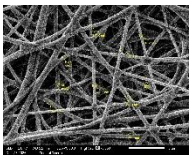

Fig. 1 Electrospun stingless bee honey 10% wt

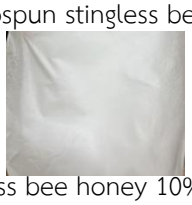

Fig. 2 stingless bee honey 10% wt (Mats)

3.The PCL layer alone showed negligible bioactivity (< 1% inhibition in all three assays), confirming its biological inertness. Bilayer mats (Active + PCL) retained bioactivity close to that of the active layer alone (75.7% vs. 77.1% DPPH inhibition), with no statistically significant loss (independent t-test, $p > 0.05$), indicating that PCL over-spinning did not impede the release of the encapsulated honey. During the initial burst phase (30-90 min), the bilayer released bioactive compounds more slowly than the single-layer mat (release rate 0.057 vs. 0.072 %/min), and this trend continued over 7 days of storage, with the bilayer retaining over 60% of its antioxidant, anti-inflammatory and anti-glycation activity throughout, compared with a sharper decline for the single-layer mat (Fig. 1). These results support a membrane-controlled diffusion mechanism, in which the hydrophobic PCL layer regulates water ingress and slows the outward diffusion of honey-derived compounds.

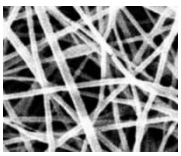

Fig. 3 Electrospun of Bilayer (Active + PCL).

Fig. 4 Bilayer mats (Active + PCL).

4.The bilayer structure also markedly improved the physical and mechanical performance of the dressing (Table 2). Water permeability decreased from 25.39% to 0% (complete water resistance), tensile strength increased from 3.46 to 5.75 MPa (+66%), and

elongation at break nearly doubled (100% to 197.5%), while the swelling ratio decreased from 266.4% to 45.4%, indicating improved control of fluid uptake. All differences between the single- and bilayer mats were statistically significant ($p < 0.05$).

Table 2 Physical and mechanical properties of single-layer (Active) vs. bilayer (Active+PCL) nanofiber dressings (n = 3)

Property	Active	Active+PCL
Water resistance(%)	25.39	0.00
Swelling ratio (%)	266.39	45.45
Elongation at break (%)	100.0	197.5
Tensile strength (MPa)	3.46	5.75

5.The bordered-adhesive prototype achieved higher adhesion strength than the single-layer design on both dry (0.640 vs. 0.422 N/cm) and moist skin (0.396 vs. 0.250 N/cm), while causing minimal skin trauma (score 0.67 ± 0.47 on dry skin, 0.00 ± 0.00 on moist skin) and leaving little to no adhesive residue. Under cyclic flexion, the single-layer mat began to lift after only 15 ± 3 cycles, whereas the bilayer mat withstood 42 ± 5 cycles, and the complete bordered-adhesive prototype endured more than 50 cycles without any detachment. This is attributed to the adhesive-free central pad, which allows the nanofiber mat to expand and contract freely with the skin, thereby distributing mechanical stress away from the wound bed — a key requirement for dressings applied over joints or frequently moving areas in diabetic patients.

Overall, these results indicate that combining a locally sourced, low-cost stingless bee honey active layer with a PCL protective layer yields a mechanically robust, water-resistant and atraumatic dressing capable of sustained bioactive release, consistent with previous reports on PCL/PVA bilayer and Manuka-honey-based wound dressings (Deshmukh et al., 2021; Tavares et al., 2025; Iranpour Mobarakeh et al., 2024), and supports its further development as an accessible smart dressing for chronic diabetic wound care.

Conclusions

This study successfully developed a multilayer nanofiber wound dressing incorporating the bioactivity of stingless bee and Manuka honey via electrospinning. Screening results showed that stingless bee honey exhibited higher antioxidant activity, while Manuka honey showed superior anti-inflammatory and anti-glycation activity, with all three bioactivities increasing consistently with honey concentration. Because stingless bee honey retained its bioactivity more stably within the PVA matrix, it was selected as the primary bioactive agent. The optimal fabrication condition was 10% wt stingless bee honey blended with PVA at a flow rate of 0.3 mL/h, yielding continuous fibers with an average diameter of 456.6 ± 25.6 nm, while higher concentrations and flow rates produced larger and more irregular fibers.

Incorporating a PCL layer, confirmed to be biologically inert on its own, shifted the release mechanism to membrane-controlled

diffusion, sustaining over 60% of bioactivity for up to 7 days while comprehensively improving physical properties achieving complete water resistance (from 25.39% to 100%), increasing tensile strength (3.46 ⟶ 5.75 MPa), and nearly doubling elongation.

When assembled with a bordered adhesive system, the resulting prototype exhibited strong adhesion, minimal skin damage, and withstood more than 50 flexion cycles without detachment. These findings confirm the potential of combining a locally sourced bioresource such as stingless bee honey with electrospinning technology and a multilayer architecture for further development into a medically engineered wound dressing for chronic wound care in diabetic patients.

.

Acknowledgments

The authors gratefully acknowledge Ms. Putcharaporn Wichasan and Mr. Wiraphong Kanthamala, project advisors, for their invaluable guidance and support throughout this research. The authors also thank Princess Chulabhorn Science High School Mukdahan for providing the facilities, laboratory space, and equipment used in this study, and extend their gratitude to their families for their encouragement throughout the project.

References

Deshmukh, G., Sahu, H., & Rath, G. (2021). Manuka honey: A promising wound dressing material for the chronic nonhealing discharging wounds: A retrospective study. *National Journal of Maxillofacial Surgery.* 12(2): 233-237. https://doi.org/10.4103/njms.NJMS_154_20

Esa, N. E. F., Ansari, M. N. M., Razak, S. I. A., Ismail, N. I., Jusoh, N., Zawawi, N. A., Jamaludin, M. I., Sagadevan, S., & Mat Nayan, N. H. (2022, May). A review on recent progress of stingless bee honey and its hydrogel-based compound for wound care management. *Molecules.* 27(10): 3080. https://doi.org/10.3390/molecules27103080

Iranpour Mobarakeh, A., Shahmoradi Ramsheh, A., Khanshan, A., Aghaei, S., & Mirbagheri, M. S. (2024). Fabrication and evaluation of a bi-layered electrospun PCL/PVA patch for wound healing: Release of vitamins and silver nanoparticle. *Heliyon.* 10: e33178. https://www.sciencedirect.com/science/article/pii/S2405844024092090

Tavares, T. D., Pinho, S. L. C., Delgado-Pujol, E. J., Begines, B., Alcudia, A., Silva, C., Antunes, J. C., & Felgueiras, H. P. (2025). Layer-by-layer electrospun PCL/PVA mats loaded with nisin Z for enhanced diabetic foot ulcer treatment. *International Journal of Pharmaceutics.* 685: 126208. https://doi.org/10.1016/j.ijpharm.2025.126208