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Electrospun poly(lactic acid) nanofiber mats for controlled transdermal delivery of essential oil from Zingiber cassumunar Roxb

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Abstract

A controlled release system of Plai (Zingiber cassumunar Roxb.) oil based on electrospun poly(lactic acid) (PLA) nanofiber mat was successfully developed. The physicochemical properties of the nanofibers loaded with select amounts of oil (15%, 20%, and 30% wt) were characterized using various techniques, including a morphological study using scanning electron microscopy (SEM), structural determination using Fourier transform infrared spectrometry (FTIR) and x-ray diffraction (XRD), as well as thermal properties study using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The loading content and the entrapment efficiency of Plai oil within the fiber mats were evaluated and were found to be remarkably high, ensuring that PLA was an appropriate material for Plai oil loading. The ability of the nanofiber mats to release (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) was also examined and the fiber mats showed controlled release characteristics. As the nanofiber mats have particularly high specific surface area with fully accessible and interconnected pore structures, a liquid medium with active ingredients will not be trapped in blind pores but can be fully released out of the fiber matrix. Furthermore, in vitro skin permeation of the active compound as well as a skin irritation were assessed using reconstructed human epidermis (EpiSkin™). It was found that DMPBD could efficiently penetrate through the skin model. Moreover, the nanofiber mats containing Plai oil also showed no skin irritation, indicating them as promising prototypes for medical applications.

1. Introduction

Herbal medicine has long been used to treat and prevent illnesses in humans. There has been substantial growth in use of herbal medicines as complementary or alternative medicines worldwide in the last decade. Zingiber cassumunar Roxb., is a well-known medicinal plant typically found in the tropical parts of Asia. It is known in Thailand as Plai. Traditionally, the extract and the essential oil from the rhizome of Plai have been used in topical preparations to treat pain and inflammation in muscles and joints [1–3]. Phenylbutanoid compounds are the main active ingredients in Plai oil and Plai extract, responsible for the anti-inflammatory activity [4–6].
Over the past few decades, development of effective drug delivery systems has become a major focus area in biomedical research [7–9]. A broad range of drug delivery systems have been established to increase solubility, stability and encapsulation efficiency of a drug, and also to control the drug release behavior to match the therapeutic needs. Among the various drug delivery systems, polymers have received much attention because they can be efficiently tailored for desired drug release profile and can also improve bioavailability of the drug [10–12]. Additionally, they also offer a wide variety of surface functionalities with surface properties ranging from hydrophobic to hydrophilic. As a result, they can certainly be applied with a broad range of drugs. Moreover, polymers can also be easily shaped to various forms (e.g., solid particle, vesicle, film, membrane, scaffold, fiber) in different sizes (micro-/nano-) and with properties suited for a diverse range of purposes and applications.

Due to concerns related to global plastic waste management, biodegradable polymers have been extensively applied as promising alternatives to the non-degradable ones. They are typically degraded after use or upon disposal, avoiding accumulation in the environment. They can be classified based on their sources into natural, semisynthetic and synthetic polymers. Biodegradable polymers [13] have been widely applied in the biomedical field not only because they can be degraded biologically but also because most of them are found to be biocompatible. Poly(lactic acid) is a synthetic biodegradable polymer produced from renewable and sustainable resources. Because it is biocompatible and biodegradable, it has become a material of choice in biomedical applications [14–16]. This also includes the use of PLA for long-term controlled release of active ingredients [17–22].

A transdermal medication is intended for external use to have localized action near or at the site of application [23]. In comparison to ordinary topical preparations, transdermal formulations are specifically designed to penetrate through the deeper skin layer and to treat the target beneath. Transdermal formulations for pain treatment are generally considered safe due to having fewer side effects than oral formulations. The commercial formulations include gels, creams, sprays and patches. Patches are a type of transdermal delivery systems that have recently become widely used. They are typically placed on the skin to deliver drugs or active compounds into the systemic circulation in a predetermined and controlled manner. They can be categorized into two main types, as either drug-in-matrix or drug-in-reservoir systems. A reservoir patch stores the drug in a solution or gel and the drug transportation is controlled by a porous membrane. In the case of matrix based system, the drug is dispersed or blended to a polymer and the chosen polymeric matrix will control the rate of delivery.

Nanofiber mats have proven themselves as one of the most promising materials for controlled release, and possess several distinctive characteristics including large specific surface area by volume, high porosity as a result of randomly oriented non-woven fibers, light weight, and good mechanical properties [23–26]. Moreover, the interconnected pore structure allows the permeation of air and water, enabling thorough and effective release of active substances in contrast to conventional bulk film patches. As reported by Tungprapa et al [27] cellulose acetate based electrospun nanofiber mats exhibited greater release than corresponding as-cast films. This could be attributed to the fact that the electrospun nanofibers exhibited much higher volume specific surface than the cast films. This was also demonstrated by Seif et al [28] finding that the release of lysozyme from PVA nanofiber mats was higher than from cast films with the same composition. Furthermore, Shen et al [29] also demonstrated that PVA nanofiber mats showed greater release amount and higher release rate of prazosin hydrochloride than corresponding as-cast films.

Because of such high surface to volume ratio and excellent controlled release characteristics of electrospun nanofiber mats in comparison to conventional as-cast films [27–29], we chose to prepare a tentative controlled transdermal delivery system of Plai oil based on electrospun nanofiber mat. Although electrospun nanofiber mats loaded with Plai oil have been already reported, they were prepared based on the use of water soluble polymer, namely polyvinylpyrrolidone (PVP) [30]. Since PVP is hydrophilic, incorporation of Plai oil into the polymer matrix is rather limited. Additionally, the Plai oil loaded electrospun PVP fiber mats also showed a relatively short shelf life only up to 1 week and then the fibers were fused together. This is certainly due to the hygroscopic behavior of PVP. To address those problems, Tonglairoum et al have blended PVP with 2-hydroxypropyl-ß-cyclodextrin (HP/ßCD). It was found that the stability of the Plai oil loaded HP/ßCD/PVP nanofiber mats against moisture and humidity was relatively improved. However, the Plai oil could be loaded into the PVP/HP/ßCD nanofiber mats only up to maximum of 20%. Furthermore, the fiber mats also showed a fast release of Plai oil and the maximum cumulative release of 70%–80% within 4 h. To improve the Plai oil loading capability and to prolong a release of Plai oil, polymers with less hydrophilic nature might be a better choice. Therefore, within this work PLA was used as a matrix for Plai oil loading. The PLA nanofiber mats loaded with different amounts of Plai oil were prepared by electrospinning as illustrated in scheme 1. The effects of polymer concentrations and oil loadings on the fiber morphologies were carefully evaluated. The physicochemical properties of the fiber mats were thoroughly characterized using various techniques. Studies of chemical stability, in vitro release and in vitro skin permeation of the bioactive substance, as well as skin irritation
of the essential oil loaded nanofiber mats were also carried out, in order to assess the possibility of using the proposed system for transdermal applications.

2. Materials and methods

2.1. Materials

PLA (Mw ≈ 100,000) was obtained from PTT Research and Technology Institute (Thailand). Plai oil was purchased from Thai China Flavours & Fragrances Industry Co., Ltd (Thailand). Reconstructed human epidermis small model (0.38 cm²), large model (1.08 cm²), maintenance and assay media were purchased from EpiSkin™ (France). All salts and organic solvents were of analytical grade or higher and were obtained from Merck. Ultrapure water from a water purification system was used throughout this work.

2.2. Determination of DMPBD content in Plai oil

Plai oil was analyzed using a gas chromatograph interfaced with a mass spectrometer (GC/MS) from Shimadzu (QP2020, Japan). A capillary SH-Rxi-5Sil MS column (30 m; 0.25 mm ID; 0.25 μm film thickness, Shimadzu, Japan) was used throughout the analysis. Plai oil was dissolved in hexane and 1 μl of the sample was injected with a split ratio of 1:1. The temperature of the injection port was set at 200 °C. The oven temperature was ramped up from 60 to 150 °C at the heating rate of 5 °C min⁻¹, then from 150 to 180 °C at a heating rate of 10 °C min⁻¹. After that, the temperature was held at 180 °C for 5 min. The electron ionization (EI) mode was used and a mass scan covered the range of 45–400 amu. Identification of compounds was performed using the mass spectral library and quantitative analysis was carried out using DMPBD as a marker.

Isolation of DMPBD from Plai oil was carried out by silica gel column using hexane-ethyl acetate (95:5 v/v) as the mobile phase. The solvent was removed under reduced pressure to afford DMPBD (500 mg, 98% yield) as a colorless oil. DMPBD was stored under N2 atmosphere at −20 °C with light protection. ¹H and ¹³C NMR spectra were recorded in CDCl₃ on a Bruker AvanceLL-300 (USA). A high resolution mass spectrometry (HRMS) spectrum was obtained on a MicroTOF mass spectrometer (Bruker Daltonics, Germany).

¹H-NMR (300 MHz, CDCl₃): δ ppm 3.86 (s, 3H), 3.89 (s, 3H), 5.12 (dd, 1H, J = 9.8, 1.1 Hz), 5.29 (dd, 1H, J = 17.4, 1.6 Hz), 6.40–6.56 (m, 2H), 6.60–6.73 (m, 1H), 6.80 (d, 1H, J = 8.0 Hz), 6.89–6.98 (m, 2H); ¹³C-NMR (75 MHz, CDCl₃): δ ppm 55.7, 55.8, 108.6, 111.1, 116.5, 119.7, 127.8, 130.1, 132.6, 137.2, 148.9, 149.0; HRMS (+ESI): M/Ζ = 213.0888 [M + Na]⁺ (calculated: M/Ζ = 213.0891).

2.3. Preparation of Plai oil loaded electrospun fiber mats

PLA solution was prepared in a binary solvent mixture of DCM and DMSO in the ratio 8:2 (v/v). The effects of polymer concentration (8%, 10% or 12% w/v) on electrospinning were examined. Plai oil at different concentrations (15%, 20% and 30% wt to PLA) was added and then thoroughly mixed into the as-prepared polymer solutions. The solution was then charged into a 10 ml syringe equipped with a blunt 20 gauge dispensing needle. The electrospinning solution was delivered by the NE-4000 programmable syringe pump (New Era Pump Systems Inc., USA) at the dispensing rate of 500 μl h⁻¹. The electrospinning was carried out at 20 kV (ES30P-5W, GAMMA, USA) and the electrospun fiber was collected on a metallic rotating cylindrical drum. The distance between the collector and the needle tip was kept at 15 cm. The fiber mats were stored in sealed plastic boxes at 4°C until further characterization.
2.4. Characterization of nanofiber mats

2.4.1. Morphology analysis
Samples were attached to sample holder stubs with two-sided adhesive tape and then sputter coated with gold (Polaron Range SC7620, Quorum Technology Ltd, UK). The morphology of nanofibers was examined with a scanning electron microscope (Quanta 450, FEI, the Netherlands) at 15 kV accelerating voltage. The distribution and average of fiber diameters were evaluated by analysis of 100 fibers from the SEM micrographs, using ImageJ software (NIH).

2.4.2. Entrapment efficiency and Plai oil loading content in nanofiber mat
Determination of DMPBD in the fiber mats was carried out by immersing each fiber mat in 20 ml hexane and stirring for 6 h. Then, the solution was sampled and analyzed by GC/MS. The amount of DMPBD was used as a marker to determine the Plai oil loading content (LC) and the entrapment efficiency (EE) in the fiber mat. The percentage of Plai oil loading content and entrapment efficiency were obtained using equations (1) and (2), respectively:

\[
LC\% = \left( \frac{w_t}{m} \right) \times 100
\]

where \( w_t \) is the amount of Plai oil calculated based on the amount of DMPBD extracted from the fiber mat
\n\[
EE\% = \left( \frac{w_f}{w_t} \right) \times 100
\]

where \( w_f \) is the amount of Plai oil calculated based on the amount of DMPBD extracted from the fiber mat

2.4.3. XRD analysis
The crystalline phases of the pristine and Plai oil loaded fiber mats were characterized using an x-ray diffractometer (Bruker D8 ADVANCE, Germany). The XRD was carried out at an applied current of 30 mA and an accelerating voltage of 40 kV using CuKα radiation (\( \lambda = 1.5406 \text{ Å} \)). The diffraction data were recorded from 5° to 40° at a scan rate of 5° min⁻¹.

2.4.4. FTIR analysis
The fiber mats were characterized using a Fourier transform infrared spectrometer (Perkin-Elmer Spectrum One FTIR, USA) in transmission mode. The spectra were recorded between 4000 and 650 cm⁻¹ from a total of 64 scans at a resolution of 4 cm⁻¹.

2.4.5. Thermal analysis
Differential scanning calorimetry (DSC; 204-F1, Netzsch-Gerätebau GmbH, Germany) and thermogravimetric analysis (TGA 8000, Perkin Elmer, USA) were used to investigate thermal properties of the fiber mats. TGA was conducted under nitrogen flushing from 30 °C to 600 °C with a heating rate of 10 °C min⁻¹. DSC was performed under nitrogen flushing (30 ml min⁻¹) in the temperature range from −85 °C to 300 °C with a heating rate of 10 °C min⁻¹. The degree of crystallinity \( (X_c) \) was calculated using equation (3) [22]:

\[
X_c(\%) = \left[ \frac{\Delta H_f}{\Delta H_f^0 \times W_f} \right] \times 100
\]

where \( \Delta H_f \) is enthalpy of fusion of the sample
\( \Delta H_f^0 \) is enthalpy of fusion of 100% crystalline PLA measured at equilibrium melting point (\( T_m^e \)) (93.7 J g⁻¹) [31]
\( W_f \) is weight fraction of polymer in the sample

2.4.6. Physical and chemical stability of oil loaded fiber mats
Long-term physical and chemical stability of the essential oil loaded fiber mats was studied. All samples were stored in glass vials with crimped gas-tight seals at 4, 30 and 40 °C for 1, 3 and 6 months. To evaluate chemical stability of entrapped DMPBD, it was extracted from the fiber mats and the percentage of DMPBD remaining was evaluated using GC/MS. Fiber morphologies observed in SEM images were used to evaluate the physical stability of the fibers during long-term storage.
2.5. In vitro release study of DMPBD

In vitro release of DMPBD from the oil loaded electrospun fibers was tested using static Franz diffusion cells (diffusion area 2.54 cm², volume 12 ml). The temperature of the cells was kept at 32 ± 0.5°C to mimic skin surface temperature. The Plai oil loaded nanofiber mats (ø 1.6 cm) were mounted onto a regenerated cellulose membrane (Spectra/Ports® MWCO 6–8 kDa, Spectrum Laboratories, Inc., USA) and placed between the receptor and donor compartments. The receptor medium was a binary mixture of phosphate buffer (pH 7.4) and ethanol (80:20 v/v) and was stirred constantly throughout the experiment. At the predetermined times of 0.17, 0.33, 0.5, 0.67, 0.83, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h, 1 ml of receptor medium was collected and immediately recharged with fresh receptor medium of equal amount. The samples were extracted with hexane (500 μl) while vortexed for 2 min. The upper organic layer was then collected. The extraction was repeated twice and the combined organic layer was evaporated under reduced pressure. The residue was then dissolved in 200 μl of hexane and the DMPBD content was subsequently analyzed by GC/MS. The in vitro release studies were carried out in triplicate and the cumulative amount of DMPBD released was calculated.

Release kinetics was evaluated using the Higuchi’s model [32], which is proper for a matrix-based system. The cumulative release data were fitted with equation (4):

\[ Q = K_H t^{1/2} \]  

where \( Q \) is the cumulative amount of active compound released at time \( t \) and \( K_H \) is the Higuchi constant.

2.6. In vitro skin permeation study of DMPBD

Upon arrival, each well of EpiSkin large model cultured for 13 days was detached from the nutrient gel and mounted into a sterile 12-well plate filled with 2 ml of maintenance medium per well. EpiSkin samples were afterwards incubated at 37°C and 98% RH with 5% CO₂. After 24 h, the maintenance medium was displaced with the assay medium and the samples were further incubated at the same condition for another 24 h. Afterwards, EpiSkin samples were transferred to the new 12-well plate filled with a receptor medium (5% ethanol in PBS) and incubated for 2 h prior to the permeation test. The nanofiber mats loaded with different amounts of oil (15%, 20% and 30%) were then placed onto the donor phase of the skin samples and were incubated at 37°C and 98% RH with 5% CO₂. At the predetermined times of 2, 4, 8, 12, 24 h, 200 μl of a solution in the receptor compartment was collected and directly recharged with the same volume of fresh receptor medium. The samples were extracted by adding hexane (200 μl) and vortexing for 2 min. The upper organic layer was then collected. The extraction was repeated twice and the combined organic layer was evaporated under reduced pressure. The residue was then dissolved in 200 μl of hexane and the DMPBD content was subsequently analyzed by GC/MS. The tests were carried out in triplicate and the cumulative amount of DMPBD permeated through RhE was calculated.

After 24 h incubation, the skin samples were washed with 15 ml of PBS twice and dried with sterile cotton buds. The skin samples were then removed from the transwell, cut into small pieces, extracted with 2 ml of methanol and sonicated for 15 min. The extraction was carried out three times. Then, the combined methanol extract was evaporated under reduced pressure and the residue was subsequently dissolved in 100 μl of hexane. The DMPBD content was then analyzed by GC/MS.

2.7. In vitro skin irritation testing

The in vitro skin irritation test was carried out according to OECD Test Guideline 439 (OECD, 2019) with modification in the exposure time of test sample (from 15 min to 24 h) to mimic the real application. Briefly, the EpiSkin™ kit (small model) was removed from the nutrient gel and mounted into a sterile 12-well plate filled with 2 ml of maintenance medium per well. The EpiSkin was then incubated at 37°C and 98% RH with 5% CO₂. After 24 h, the nanofiber mats loaded with 15% and 30% Plai oil were mounted onto the skin samples and were incubated at 37°C and 98% RH with 5% CO₂. After 24 h incubation, the skin samples were washed with 15 ml of PBS twice and subsequently mounted to new 12-well plate filled with 2 ml of maintenance medium. Then, the samples were incubated at 37°C and 98% RH with 5% CO₂ for a period of 42 ± 1 h. After that, the skin samples were mounted to a new 12-well plate filled with 2 ml of MTT solution (0.3 mg ml⁻¹) and incubated for another 3 h. The formazan crystals were extracted from the skin samples by acidic isopropanol and the optical density (OD) of the obtained solutions was then measured at 570 nm. The % tissue viability was then calculated using equation (5):

\[ \% \text{ tissue viability} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{negative control}}} \times 100 \]  

\( \text{OD}_{\text{sample}} \) and \( \text{OD}_{\text{negative control}} \) are the optical densities of the sample and negative control solutions, respectively.
2.8. Statistical analysis
All the experimental data are reported as mean ± standard derivation (SD). One-way analysis of variance (one-way ANOVA) was used to compare the means by factor levels, and p-values less than 0.05 were considered significant.

3. Results and discussion

3.1. Determination of DMPBD content in essential oil
The volatile compounds in Plai essential oil were analyzed using GC/MS. The chromatogram showed the presence of several compounds. The identities of each compound were verified from their fragmentation patterns by comparison to a mass spectral library, and are listed in table S1 is available online at stacks.iop.org/MRX/7/055305/mmedia. DMPBD, a phenybutanoid compound, is the main active ingredient found in Plai oil, and it exhibits potential anti-inflammatory activity [5, 33]. The DMPBD content was found to be 8.57%, which is in the range typically found in Plai oil from hydro-distillation [34]. Since DMPBD shows strong anti-inflammatory activity, it was purified and used as a marker for further in vitro release and skin permeation studies. The NMR spectrum of DMPBD is shown in figure S1.

3.2. Preparation and characterization of oil loaded electrospun fiber mats
The oil loaded fiber mats were successfully fabricated using electrospinning. The effects of the polymer concentration (i.e., 8%, 10% or 12% w/v) and Plai oil loading (i.e., 15%, 20% and 30%) on the fiber size and morphology were examined using SEM. As seen in figure 1, the mean diameters of the nanofibers prepared with 8% (w/v) PLA were noticeably smaller than those with 10% and 12%, respectively. This suggests that the fiber diameter depends directly on the polymer concentration. Considering the morphology, the fibers obtained using 8 and 10% PLA concentrations (figures 1(a)–(f)) showed high to moderate bead-on-string fiber structures at all Plai oil loading amounts. In contrast, at 12% of PLA uniform nanofibers with bead-free morphology were observed (figures 1(g)–(i)). This clearly reveals that polymer concentration affected fiber morphology. At very low concentration of the polymer, the polymer solution does not have enough entanglements to produce a stable jet leading to the development of particles instead of fibers (data not shown). The formation of those particles is as a consequence of the disintegration of polymer jet into droplets when the surface tension exceeds the viscoelastic force of the low viscous polymer solution [20]. When the polymer concentration is slightly beyond the entanglement concentration, bead-on-string fibers are created. Once the polymer concentration is high enough (typically 2–2.5 times of the entanglement concentration) to provide sufficient entanglement, bead-free fibers are generated [35]. Therefore, 12% (w/v) PLA was considered the optimal concentration and was used to prepare the Plai oil loaded fiber mats for further studies.

At the same PLA concentration but different initial Plai oil loading amounts, the corresponding nanofibers showed no apparent differences in fiber size, revealing that the loading amount of Plai oil exhibited no obvious effect on the fiber size. At 12% of PLA, the morphology of the electrospun fibers is similar for all the Plai oil loading amounts. On the contrary, at 8 and 10% of PLA more bead-on-string morphology was observed as the Plai oil loading amount increased. This is possibly due to the decrease in solution viscosity when the Plai oil loading amount increased.

The total amounts of DMPBD extracted from the fiber mats were quantified using GC/MS and were used to calculate the Plai oil loading content and the entrapment efficiency in the electrospun fibers. The resulting loading content and the entrapment efficiency are summarized in table 1. At all initial amounts of Plai oil loaded into the polymer solutions (15%, 20% and 30%), the loading contents found in the fiber mats only showed slight differences in comparison to their initial values. This implies that electrospinning does not make any chemical changes to the active component of the Plai oil, making it a suitable platform for nanofiber fabrication. The loading amount of Plai oil in this work could be as high as 29.95% with the calculated entrapment efficiency of up to 99.83% which is mainly due to good miscibility of PLA with Plai oil. This is in contrast to a previous report [30] where the Plai oil could be loaded into electrospun PVP fiber mats only up to 20% loading, even in the presence of 2-hydroxypropyl-β-cyclodextrin (HPβCD), revealing that PLA is a promising material for Plai oil loading.

The microstructure of the nanofibers was characterized using x-ray diffraction (XRD) technique. As depicted in figure 2, the XRD pattern of pristine PLA fiber exhibited typical broad peaks at 2-theta angles around 15° and 30°, indicating that the PLA fiber has an amorphous structure. As the Plai oil loading increased, the diffraction patterns of the Plai oil loaded fibers remained unchanged, revealing that blending Plai oil into PLA did not affect crystallinity of the electrospun fibers.

The compatibility of PLA/Plai oil blend was confirmed using FTIR. As depicted in figure 3, the neat PLA fiber shows typical C=O stretching and bending peaks at 1755 cm⁻¹ and 1267 cm⁻¹, respectively. It also shows
signals of C–O–C stretching at 1184 and 1088 cm$^{-1}$, C–H bending at 1455 cm$^{-1}$ and C–H stretching at 2996 and 2946 cm$^{-1}$. By comparison of the spectra, the incorporation of Plai oil with PLA did not affect peak positions of the pristine PLA fiber, indicating that the loaded Plai oil did not cause any chemical changes to or have any strong interactions with the PLA. This favors the release of Plai oil from the fiber mat. As seen in table S1, the majority of compounds present in Plai oil (∼85%) are volatile monoterpenes which normally exhibit characteristic peaks of C–H bending and C–H stretching. These peaks are typically in the same region of those C–H bending and stretching of PLA. Along with the solvent evaporation during the spinning process, those volatile monoterpenes partly vaporized. As a result, only a slight increase in peaks intensity in the region of C–H bending and stretching was observed as the Plai oil loading content increased. Contrarily, the weakly volatile aromatic compounds such as DMPBD still remain in the fibers. This can be evident from the significantly high %EE and %LC of Plai oil calculated based on the amount of DMPBD extracted from the fiber mats and the

| Fiber mats          | Plai oil loading content (%) | Entrapment efficiency (%) |
|---------------------|------------------------------|----------------------------|
| PLA + 15% Plai oil  | 14.87 ± 1.94                 | 99.14 ± 2.94               |
| PLA + 20% Plai oil  | 19.94 ± 0.67                 | 99.70 ± 3.36               |
| PLA + 30% Plai oil  | 29.95 ± 1.25                 | 99.83 ± 4.16               |

Figure 1. SEM images of electrospun nanofibers prepared with different PLA concentrations and Plai oil loadings: (a) 15% (w/w) Plai oil in 8% PLA, (b) 20% (w/w) Plai oil in 8% PLA, (c) 30% (w/w) Plai oil in 8% PLA, (d) 15% (w/w) Plai oil in 10% PLA, (e) 20% (w/w) Plai oil in 10% PLA, (f) 30% (w/w) Plai oil in 10% PLA, (g) 15% (w/w) Plai oil in 12% PLA, (h) 20% (w/w) Plai oil in 12% PLA, (i) 30% (w/w) Plai oil in 12% PLA. The insets show the fiber diameter histograms of the electrospun nanofibers.
development of peak between 1755 cm\(^{-1}\) and 1455 cm\(^{-1}\) from the aromatic skeleton vibration at around 1515 cm\(^{-1}\) [36].

Thermal properties of the fiber mats were characterized using differential scanning calorimetry (DSC). The glass transition temperature (\(T_g\)), crystallization temperature (\(T_c\)), melting temperature (\(T_m\)) and degree of crystallinity (\(X_c\)) of the samples were summarized in table S2. As seen in figure 4, the neat PLA fiber shows \(T_g\), \(T_c\) and \(T_m\) at 62.3, 92.5 and 152.5 °C, respectively. Upon the addition of \(Plai\) oil, the glass transition temperature of the fibers decreases with \(Plai\) oil loading level, mainly due to the plasticizing effect of \(Plai\) oil on PLA. As a plasticizer, \(Plai\) oil can increase the free volume between the PLA chains resulting in higher chain mobility at lower temperature. The decrease in \(T_g\) also allows crystallization to start at lower temperature. Therefore, the crystallization temperature of the \(Plai\) oil loaded PLA fibers becomes lower. This is possibly due to the essential oil enhancing chain mobility of the polymer, thereby improving the crystallization kinetics in the blend [36]. The plasticizing effect of \(Plai\) oil also causes a slight decrease in melting temperature of the fiber. As shown in table S2, the degree of crystallinity of the \(Plai\) oil loaded PLA fibers is higher than that of the neat PLA fiber mat. Furthermore, the percentage of crystallinity also increases with \(Plai\) oil loading level. This indicates that the crystallization of PLA becomes easier due to the increased chain mobility caused by the \(Plai\) oil. This similar

Figure 2. XRD patterns of neat PLA and oil loaded PLA nanofiber mats.

Figure 3. FTIR spectrum of neat PLA and oil loaded PLA nanofiber mats.
effect was also observed in candeia oil loaded PLA fiber mat [37] and PLA/essential oil (i.e., bergamot, lemongrass, rosemary, or clove oil) blends [38].

Thermal stability of the fiber blends was studied using thermogravimetric analysis (TGA). As shown in figure 5, the pristine PLA fiber shows a single step weight loss with the onset temperature ($T_o$) and the maximum rate of decomposition temperature ($T_{max}$) at 320 and 366 °C, respectively. In contrast to the pristine polymer, the fibers loaded with Plai oil show two steps of thermal weight loss. The small change in the first weight loss step corresponds to gradual evaporation as well as degradation of the Plai oil, and is found to be in the range 4.5%–5.1%. However, the weight loss in this step does not correlate to the Plai oil loading amounts, possibly resulting from partial vaporization of highly volatile organic compounds in Plai oil during the electrospinning process as mentioned earlier. The second step is related to the decomposition of PLA with $T_{max}$ slightly shifted to 362 °C–365 °C depending on the loading level of Plai oil. The final residue at the end of heating from pristine fiber and from the fiber blends is less than 1%, indicating practically complete decomposition of the polymer.

3.2.1. Physical and chemical stability of oil loaded fiber mats
Chemical stability of the oil loaded fiber mats was tested in terms of the stability of the active compound, DMPBD, encapsulated within the fiber. For this purpose, DMPBD was extracted from the fiber mats stored at
different storage temperatures for various times, and then the remaining DMPBD contents were quantified using GC/MS. As shown in table 2, after storing the fibers at 4 °C, the percentage of the remaining DMPBD content found in all the samples was still higher than 98% of the initial, even after a 6-month storage. As the storage temperature was increased to 30 °C (40 °C), the DMPBD content gradually decreased and was less than 90% after 6 (3) months of storage. This temperature-dependent effect is in accordance with previous work [39] where Plai oil was loaded into an emulgel. Therefore, the Plai oil loaded fiber mats should be stored refrigerated at 4 °C.

Physical stability of the fiber was evaluated in terms of fiber morphology after long-term storage. As depicted in figure S2, the morphologies of the Plai oil loaded fibers showed no drastic changes upon 6 months of storage at any storage temperature tested here, indicating that the fiber mats were physically stable for at least 6 months.

3.3. In vitro release of DMPBD
Cumulative in vitro release of DMPBD from the fiber mats loaded with different amounts of oil (15, 20 and 30% w/w) was calculated. It was observed that DMPBD showed an initial fast release arising from desorption of weakly bound DMPBD adhering to the considerably large surface of nanofibers, followed by a sustained slower release from DMPBD entrapped inside the nanofibers. As depicted in figure 6(a), DMPBD was gradually released and reached a plateau after 24 h. As the oil content in the nanofiber mats increased, the cumulative release of DMPBD also increased. Moreover, the percentage of DMPDB released is shown in figure 6(b). In all cases, the cumulative amounts of DMPBD released from the PLA fiber mats reached ~80% after 12 h. This is in contrast to a previous study where Plai oil loaded HP/βCD/PVP fiber blend [30] showed a burst release and the maximum cumulative release already reached 70%–80% within the first 4 h. As such, this clearly reveals that the PLA fiber mats showed slower release of Plai oil compared to the HP/βCD/PVP fiber blend. Since the shape of PLA fibers is similar to that of the HP/βCD/PVP fiber blend, a major factor affecting the release behavior of both fibers is a type of polymer. PVP is a water soluble polymer which is more hydrophilic in nature than PLA. As a result, the miscibility of PVP and Plai oil is rather limited. From this incompatibility, a faster release of Plai oil

| Fiber mats | Temperature (°C) | Remaining DMPBD content (%) |
|------------|----------------|-----------------------------|
| PLA + 15% Plai oil | 4 | 99.76 | 99.13 | 98.51 |
| | 30 | 97.89 | 94.24 | 89.12 |
| | 40 | 93.31 | 87.01 | 75.31 |
| PLA + 20% Plai oil | 4 | 99.45 | 99.01 | 98.74 |
| | 30 | 97.21 | 93.98 | 89.61 |
| | 40 | 93.45 | 86.76 | 76.12 |
| PLA + 30% Plai oil | 4 | 99.21 | 98.92 | 98.32 |
| | 30 | 96.32 | 93.41 | 88.91 |
| | 40 | 92.53 | 85.21 | 73.23 |

Figure 6. In vitro release of DMPBD from Plai oil loaded fiber mats: (a) cumulative release (μg mg⁻¹) and (b) cumulative release (%).

Table 2. Percentage of DMPBD remaining in the fiber mats stored by storage temperature and time.
from PVP fibers can be expected. Furthermore, a higher water uptake ability of PVP also undoubtedly accelerated the release of *Plai* oil from the PVP fibers. On the contrary, a more hydrophobic nature of PLA retarded its hydrolytic degradation, thereby slowing down the release of *Plai* oil from the PLA fiber mats [21]. After 48 h, DMPBD released from the PLA fiber mats loaded with 15, 20 and 30% oil was found to be 97.26 ± 5.27, 101.48 ± 6.29 and 102.09 ± 1.36%, respectively. This evidently suggested that PLA is a suitable material for the extended release of *Plai* oil.

To further elaborate the release mechanism of DMPBD from the fiber mats, the release profiles were fitted with Higuchi’s model. The coefficient of determination ($R^2$) indicating goodness of fit was in the range 0.9911–0.9933 in all cases, so the in vitro release kinetics of DMPBD were very well fit with Higuchi’s model. This also indicates that the release of DMPBD from the nanofiber mats follows the diffusion mechanism.

### 3.4. In vitro skin permeation of DMPBD

The *in vitro* skin permeation of DMPBD was studied using reconstructed human epidermis from EpiSkin™. The permeation of DMPBD was reported as the fraction (percentage) found in the receptor medium of the initial amount of DMPBD in the test samples. The cumulative amount of DMPBD penetrated through the skin at 2, 4, 8, 12 and 24 h was plotted against the time (figure 7). The cumulative amount of DMPBD permeated through the skin model after 24 h of incubation was found to be 46.51 ± 5.41%, 52.47 ± 4.55% and 54.51 ± 3.54% for the fibers loaded with 15%, 20% and 30% oil, respectively. To quantify the remaining amount of DMPBD in the skin sample, the skin tissue was removed from the test chamber and extracted with methanol. After extraction, the remaining amounts of DMPBD were found to be 35.85 ± 17.63%, 36.39 ± 12.36% and 38.25 ± 12.11% for the fibers loaded with 15%, 20% and 30% oil, respectively. This means that approximately 50% of DMPBD could penetrate through the skin tissue. However, a certain amount of DMPBD accumulated in the lipophilic environment of the skin, possibly due to the hydrophobicity of DMPBD.

### 3.5. In vitro skin irritation testing

*In vitro* skin irritation test was carried out using reconstructed human epidermis provided by EpiSkin™. After 24 h exposure of the test sample to the skin model, the % tissue viability was determined. In this test, a sample is regarded as a non-irritant when the % tissue viability obtained using MTT assay is higher than 50%; and as an irritant when the % viability is less than or equal to 50%. In the study, PBS was used as a negative control. It provided an OD of 1.123 ± 0.031 which was in the acceptance range (0.6–1.5) as recommended in the OECD 439 test guideline. 5% SDS was on the other hand used as a positive control and the result showed that the tissue viability was 14.04 ± 0.43%. In the case of the test samples, the nanofiber mats loaded with 15% and 30% *Plai* oil were tested for their compatibility with the skin model. After 24-hour exposure to the fiber mats, the viability was higher than 50% namely 111.57 ± 3.79% and 90.93 ± 8.16%, respectively. This indicates that the fiber mats prepared are biocompatible with human skin at all the studied *Plai* oil loading levels, and have potential for transdermal applications.
4. Conclusions

In this work, PLA based nanofiber mats were loaded with Plai oil by mixing it in before electrospinning. Under appropriate spinning conditions, the obtained nanofibers exhibited smooth and bead-free morphologies with diameter in the nanometer range. The resulting loading content and entrapment efficiency were also exceptionally high, suggesting that PLA is a promising material for Plai oil loading. Entrapment of oil in the nanofiber gave an extended duration of oil release. In addition, such entrapment could improve stability of the oil as it is protected from atmospheric oxygen and light, which reduces the degradation rate. As the volume specific surface area of the nanofibers is particularly high, and there are no blind pores, these fibers can release active ingredients to an impregnating fluid easily. Further, in vitro skin permeation and skin irritation tests also revealed that the active ingredient could successfully penetrate through the epidermis, and the fiber mats did not irritate the skin tissue. This suggests that the fiber mats demonstrated in this study have potential for transdermal applications.

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Conflicts of interest

The authors declare no conflict of interest.

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