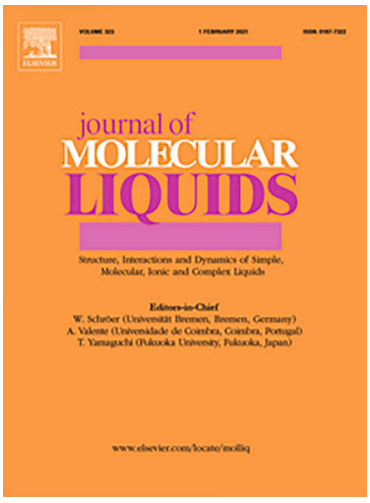

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Ionic liquid microcapsules for the topical delivery of pterostilbene: Enhanced transdermal delivery and anti-wrinkle and skin brightening effects

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Abstract: Pterostilbene (PTB) is a hydrophobic compound derived from plants in the *Astragalus* family. PTB possesses remarkable skin brightening and antioxidant properties. However, delivery methods for PTB require enhancement, and this can be achieved by formulating suitable carriers. In this study, we prepared ionic liquid microcapsules containing PTB by using a microencapsulation technique based on a matrine and cocinic acid ([Mat][Coc]) ionic liquid. The particle size of the microcapsules was 154.5 ± 3.42 nm, and the encapsulation efficiency was as high as 99.7%. Further, the PTB-loaded ionic liquid microcapsules had good biocompatibility, enhanced skin retention (7.7-times higher than that of free PTB), and improved skin penetration without damaging the skin structure. Clinical testing with human subjects revealed that the microcapsule emulsion reduced fine lines and brightened skin tone. Therefore, PTB-loaded ionic liquid microcapsules have promise for use in cosmetic formulations and applications in transdermal drug delivery and efficacy enhancement.

Keywords: microencapsulation technique; ionic liquid microcapsules; transdermal drug delivery; antioxidant; anti-wrinkle

1. Introduction

Nature provides a rich and diverse source of compounds that can be utilized for a wide range of therapies. Some natural active ingredients have significant advantages for preventing and treating skin diseases and enhancing skin activity.[1-3] However, these active substances usually require modification to improve their efficacy and specificity.[4, 5] In addition, there is a trend toward using natural active substances in cosmetics because such compounds have a wide range of activities that make them suitable for use in treating skin problems.[6] Moreover, compared with synthetic compounds, natural compounds can cause fewer side effects, have higher biosafety, and elicit greater public acceptance.[7]

Pterostilbene (PTB) (IUPAC: 4-[(1*E*)-2-(3,5-dimethoxyphenyl)ethenyl]phenol) is a natural plant defense compound. Many studies have shown that PTB has a beneficial effect on melanin synthesis [8] and anti-inflammatory properties [9] and can repair sun damage [10] and treat skin cancers, [11] as well as other conditions. In addition, it is an antioxidant.[12, 13] Resveratrol is a widely known plant ingredient with anti-aging and antioxidant function, and it is a key functional ingredient in wine. PTB is a structural analog of resveratrol and has similar antioxidant and anti-aging effects [14] but more advantages. [15] First, PTB has higher lipophilicity, better transdermal passage, and easier absorption and utilization by the body. Secondly, PTB has the best peroxisome proliferator-activated receptor (PPARα) induction among the stilbenes [16] and is more effective in scavenging oxygen free radicals and inhibiting the growth of colon cancer and melanoma cells. PTB also has excellent antibacterial activity and can be used to prevent and treat skin inflammation and other skin problems.[17] However, there are still many challenges facing the application of PTB. Similar to other naturally derived active substances, these compounds have poor biological and chemical stabilities. In addition, on exposure to light and air, the hydroxyl group of the compound is easily oxidized, resulting in a loss of biological activity. Further, irritation and poor water solubility affect the bioavailability of PTB, which must be delivered to specific targets using high-load carriers.[18] In recent years, researchers have used various methods to improve the stability and water solubility of PTB to exploit its potential. For example, Coimbra et al.[19] enhanced the water solubility by phosphorylating PTB and then encapsulating it in nanoparticles to reduce the contact and reaction between PTB and the external environment; however, the system was not stable. In addition, Bethune et al. [18] increased the water solubility of PTB by six times by co-crystallizing it with piperazine or glutaric acid, but the presence of monomers was detected after dissolution in water for several hours, probably because of the decomposition of the co-crystals. Therefore, despite the various attempts, the practical application of PTB remains limited.

Ionic liquids (ILs) are supramolecular compounds and can provide protection for biomacromolecules and can facilitate their passage through the stratum corneum (SC) of the skin to cells to achieve therapeutic effects.[20] Matrine coconut oil ionic liquid can achieve the effect of auxiliary transdermal penetration and system synergism.[21] In addition, microcapsules are special liposomes formed of nonionic surfactants instead of phospholipids, and have good biocompatibility.[22, 23] Specifically, they can easily penetrate the pores and SC cell spaces and transport active ingredients to the dermis and basal layer. Therefore, the use

of microencapsulation technology to improve the stability, water solubility, and bioavailability of drugs has become a research hotspot. [24]

In this study, we used a supramolecular solvent for microencapsulation, and successfully prepared a PTB-loaded ionic liquid microcapsule (denoted Mat-IL-MicCap@PTB), which effectively resolved the poor stability and low bioavailability of PTB. Further, transdermal experiments showed that the microcapsules improved the permeation and retention of the active component. In vitro experiments confirmed that the microcapsules reduced the irritation and side effects of PTB, and they not only achieved a lasting antioxidant effect but also inhibited the production of melanin, attenuated fine lines, and enhanced skin elasticity and health. Therefore, Mat-IL-MicCap@PTB is a feasible carrier for transdermal delivery and synergistic skin care, providing an effective strategy for improving the transdermal delivery of various dermatological drugs.

2 Experimental

2.1 Materials

PTB (97% purity) and glycerol (99% purity) were purchased from Aladdin and used as received. Caprylic capric triglyceride (GTCC, ≥ 98%), L-α-Lecithin (98%), and vitamin E acetate (96%) were obtained from Macklin and used as received. Polyglycerol-10-laurate was supplied by Beijing Wokai Biotechnology Co., Ltd. Matrine (Mat; 98%) and cocinic acid (Coc; 98%) were purchased from Maya Reagents Co., Ltd. Biochemical reagents used in the experiments were purchased from Beyotime Biotechnology. Other common organic solvents were also commercially available.

2.2 Preparation methods

2.2.1 Preparation of [Mat][Coc] IL

The [Mat][Coc] IL was prepared following a previously reported method.[25] Briefly, equal moles of matrine and cocinic acid were dissolved in 10 mL of anhydrous ethanol. The cocinic acid-ethanol solution was then slowly added to the matrine-ethanol solution. The mixture was allowed to react overnight at room temperature under a nitrogen atmosphere. Finally, [Mat][Coc] was obtained by rotary evaporation.

2.2.2 Preparation of Mat-IL-MicCap@PTB

Caprylic/capric triglyceride (10.0 wt%), soybean lecithin (3.0 wt%), polyglycerol-10-laurate (5.0 wt%), and vitamin E acetate (0.1 wt%) were stirred and mixed at 70 °C in an oil bath. PTB was then dissolved in [Mat][Coc] IL (10.0 wt%) and added to the above solution as the oil phase. Next, 10.0 wt% glycerol and 61.4 wt% purified water were used as the aqueous phase.

The oil phase was slowly added to the aqueous phase and emulsified at high speed for 2 min at 12000 rpm to mix the components fully. The PTB-loaded ionic liquid microcapsules were homogenized five times at 700 bar by a high pressure microfluidizer (NanoGenizer 30K, Genizer, USA) and are denoted Mat-IL-MicCap@PTB. Microencapsulation could not be achieved without high-pressure homogenization, and the thus-formed product is denoted "Free PTB."

2.3 Characterization of Mat-IL-MicCap@PTB

The mean particle size and zeta potential were determined using dynamic light scattering (DLS; Zetasizer Nano ZS system, Malvern Instruments Ltd., Malvern, UK). Measurements were performed at 25 ± 0.1 °C and a scattering angle of 173° with a laser wavelength of 633 nm. Each sample was filtered through a Millipore Mill ex-LG filter with a pore size of 0.45 μm to remove dust and contaminants and allowed to reach equilibrium for 10 min before particle size measurements were carried out. The morphology of Mat-IL-MicCap@PTB was observed using a high-resolution transmission electron microscopy (TEM; FEI Tecnai G2 F30).

2.4. Stability of Mat-IL-MicCap@PTB

Mat-IL-MicCap@PTB was stored at 20, 30, 45, 4, and -25 °C for 90 days. The changes in appearance at different time points were recorded by photography to investigate emulsion stability. Particle size changes at different time points were detected using a laser particle size analyzer (Litesizer™ 500, Anton Paar). High-performance liquid chromatography (HPLC, Ultimate 3000, Dionex) was used to detect changes in the PTB content at different time points to investigate the containment stability.

2.5 Determination of encapsulation efficiency

Mat-IL-MicCap@PTB was subjected to sonication in a water bath for 15 min. Free PTB was separated from the PTB-loaded microcapsules by centrifugation at 5000 rpm for 10 min. To lyse the microcapsules and measure the amount of encapsulated drug, the top phase (PTB-loaded microcapsule) was diluted 20 times with methanol, and the dissolution and drug loading of the microcapsules were determined after ultrasonication for 30 min. A standard curve of PTB in methanol was obtained using a UV-Vis spectrophotometer (Carry 5000, Agilent) at 318 nm.

Drug encapsulation efficiency (EE) was calculated using the following formula:

$$EE(\%) = \frac{Mass\ of\ PTB\ in\ MicCap}{Mass\ of\ PTB\ in\ feed} \times 100\%.$$

2.6 Cytotoxicity assays in vitro

The cytotoxicities of Free PTB and Mat-IL-MicCap@PTB were evaluated based on the relative viability of fibroblasts and human melanoma cells. Cells in the logarithmic growth

phase were seeded into a 96-well cell culture plate (8×10^3 cells/well) and incubated at 37 °C with 5% CO_2 for 24 h. Then, 200 μL of culture medium containing 10% phosphate buffered saline (PBS) was added to each well in the control group, 200 μL of culture medium containing 10% Active Ingredient (DMSO) was added for the positive control group, and 200 μL of culture medium containing the corresponding concentration of the sample was added to each well in the sample group. In the blank group, no cells were seeded, and only 200 μL of cell culture medium was added. After incubation at 37 °C for 24 h, 0.5 mg mL^{-1} MTT solution was added to each well, and incubation was continued for 2 h. Absorbance was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Gen5). The cell relative viability (%) was calculated as follows:

$$Cell\ relative\ viability\ (\%) = \frac{\mathrm{SampleOD_{490\ nm}} - \mathrm{ZeroingOD_{490\ nm}}}{\mathrm{ControlOD_{490\ nm}} - \mathrm{ZeroingOD_{490\ nm}}} \times 100\%.$$

2.7 In vitro transdermal diffusion tests

In vitro transdermal experiments were performed using Franz diffusion cells. The undamaged skin of healthy piglets was selected under a dissecting microscope, and six pieces of skin of the same size were cut, washed once with PBS, and the surface moisture was dried with filter paper. The skin was fixed on a Franz diffusion cell with the SC facing the donor chamber and the dermis facing the receptor chamber. Next, 6.5 mL of PBS was added to the receptor chamber, and air bubbles between the dermis and the receptor solution were removed. The instrument was turned on in advance, adjusted to a water bath temperature of 32 ± 1 °C with a stirring speed of 300 rpm, and 1 mL of the sample was added to the donor chamber, sealed with sealing film and covered with tin foil to prevent liquid evaporation. The effective permeation area was 0.36 πcm^2. The skin was sampled and extracted at 0.5, 1, 4, and 8 h. After filtration though a 0.22-μm organic membrane, the skin was analyzed by HPLC and the intradermal retention was calculated as follows:

$$Q_S\left(\mu g\ cm^{-2}\right) = C_{sn} \times \frac{V_S}{A_S} + \sum_{i=1}^{n-1} C_{Si} \times \frac{S}{A_S},$$

where Q_s is the cumulative drug permeation per unit area (μg cm^{-2}), C_{sn} is the measured drug concentration (μg mL^{-1}) in the receptor fluid at n sampling intervals, V_s is the volume of the receptor chamber, $\sum_{i=1}^{n-1} C_{Si}$ is the cumulative drug concentration in the receptor fluid, S is the sampling volume, and A_s is the effective diffusion area.

Finally, the skin was rinsed repeatedly with PBS, and pathological examination was performed 24 h later. Part of the skin was taken for immunohistochemical staining, frozen sectioning, and confocal laser scanning microscopy (CLSM, Nikon, Japan).

2.8 Oxygen radical absorption capacity

2.8.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH (2 mg) was accurately weighed and dissolved in 50 mL ethanol to produce a 0.1 mM DPPH ethanol solution, which was stored away from light. Mat-IL-MicCap@PTB was diluted to a series of concentrations using ethanol, and the sample solution was prepared for use. A sample tube (*T*), sample background (*T*0), DPPH tube (*C*), and solvent background (*C*0) were prepared, and three replicates were prepared for each sample. To each sample tube (*T*) and sample background (*T*0), 1 mL of sample solution of the same concentration was added. All test tubes were filled with 2 mL of ethanol and mixed thoroughly. Next, DPPH ethanol solution (1 mL) was added to the sample (*T*) and DPPH (*C*) tubes, and ethanol was used to make up the sample (*T*0) and solvent (*C*0) backgrounds. The tubes were gently shaken and allowed to stand at room temperature for 5 min. Subsequently, each reaction solution was transferred to a 1 cm colorimetric dish, and the absorbance at 517 nm was measured using a UV spectrophotometer. The DPPH radical scavenging rate and median inhibitory concentration (IC50) were calculated using the following formula:

$$DPPH\ radical\ scavenging\ rate\ (\%) = \left(1 - \frac{T - T_0}{C - C_0}\right) \times 100\%.$$

2.8.2 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay

Potassium persulfate (6.62 mg) was dissolved in distilled water in a 10-mL volumetric flask to prepare a 2.45-mM stock solution. ABTS (38.41 mg) was dissolved in distilled water in a 10-mL volumetric flask to prepare a 7-mM stock solution. The ABTS+ working solution was prepared by mixing equal volumes of the two stock solutions and incubating them at room temperature and in the dark for 12 h. Before use, the ABTS+ working solution was diluted with PBS buffer to obtain an absorbance at 734 nm (A_{734}) of 0.70 ± 0.02. Sample tubes (*A*s), background tubes (*A*b), and blank tubes (*A*0) were prepared and three replicates were used for each sample. The same concentration of sample solution (0.2 mL) was added to *A*s and *A*b, and PBS buffer (0.2 mL) was added to *A*0. ABTS+ working solution (0.8 mL) was added to *A*s and *A*0, and PBS buffer (0.8 mL) was added to *A*b. The samples were incubated in the dark for 6 min. The reaction solution in each tube was transferred to a 1-cm colorimetric dish, and the absorbance at 734 nm was measured using a UV spectrophotometer. The ABTS+ radical scavenging rate and IC50 were calculated using the following formula:

$$ABTS^{+}\ radical\ scavenging\ rate\ (\%) = \left(1 - \frac{A_s - A_b}{A_0}\right) \times 100\%.$$

2.9 Skin brightening

2.9.1 Cellular melanin synthesis inhibition assay

The brightening effects of Free PTB and Mat-IL-MicCap@PTB were determined by measuring the melanin inhibition rate in human melanoma cells. Cells in the logarithmic growth phase were seeded into a 96-well plate (2×10^5 cells/well) and incubated at 37 °C with 5% CO_2 for 24 h. Untreated cells were used as blank controls, and three replicates were used

for each sample. The supernatant was then discarded, 1 M NaOH containing 10% DMSO was added to each well (1 mL), and the plate was incubated in an oven at 80 ℃ for 1 h. After cooling to room temperature, the solution from each well (200 μL) was transferred to a new 96-well plate, and 1 M NaOH containing 10% DMSO was used as blank control. The absorbance was read at 405 nm, and the relative inhibition rate of melanin synthesis was calculated using the following formula:

$$Cell\ melanin\ inhibition\ rate\ (\%) = \left(1 - \frac{SampleOD_{490\ nm} - ZeroingOD_{490\ nm}}{ControlOD_{490\ nm} - ZeroingOD_{490\ nm}}\right) \times 100\%.$$

2.9.2 Tyrosinase inhibition assay

The brightening ability was tested by evaluating the inhibitory effects of Free PTB and Mat-IL-MicCap@PTB on tyrosinase activity. Sample tubes (*T*), sample background tubes (*T*0), enzyme reaction tubes (*C*), and solvent background tubes (C_0) were prepared, with three replicates for each sample tube and enzyme reaction tube. The same concentration of sample solution (1 mL) was added to *T* and *T*0, and disodium bicarbonate-citric acid buffer (1 mL) was added to *C* and *C*0. Tyrosinase solution (0.5 mL) was added to *T* and *C*, and disodium bicarbonate-citric acid buffer (0.5 mL) was added to *T*0 and *C*0. The samples were mixed well with tyrosinase and incubated in a water bath at 37 ℃ for 10 min. Then, 0.5 mL of L-DOPA solution was added to each tube and the reaction was continued for another 10 min. The reaction solution in each tube was transferred to a 1-cm colorimetric dish, and the absorbance at 490 nm was measured using a UV spectrophotometer. The tyrosinase inhibition rate and IC_{50} were calculated using the following formula:

$$Tyrosinase\ inhibition\ rate\ (\%) = \left(1 - \frac{T - T_0}{C - C_0}\right) \times 100\%.$$

2.10 Anti-wrinkle and firming/lifting efficacy tests

The anti-wrinkle and firm/lifting effects of Free PTB and Mat-IL-MicCap@PTB were evaluated by measuring the type-I collagen and matrix metalloproteinase-1 (MMP-1) contents of fibroblast cells. Cells in the logarithmic growth phase were seeded into a 96-well plate (8×10^4 cells/well) and incubated at 37 °C with 5% CO_2 for 24 h. In the blank and negative control groups, each well received 1 mL of cell culture medium. For the positive control group, each well received 1 mL of culture medium containing 100 μg mL^{-1} vitamin C and 7 μg mL^{-1} vitamin E. For the sample group, each well received 1 mL of culture medium containing the corresponding concentration of the test substance. After 24 h of treatment, the negative control, positive control, and sample groups were exposed to a total dose of 9 J cm^{-2} UVA radiation, whereas the blank control group was kept in the same environment (UVA radiation dose, 0 J cm^{-2}). After another 24 h of incubation, the cell culture supernatant was collected and stored in Eppendorf tubes at -80 ℃. The type-I collagen and MMP-1 levels were measured and analyzed according to the ELISA kit manufacturer's instructions.

2.11 Clinical trials

Our clinical trial was approved by the ethics committee of Shenzhen Shinesky Biotechnology (project number 2022302JC01). The study was titled “Cosmetic anti-wrinkle firming experiment” and followed a clinical study protocol that was approved by the ethics committee. All necessary permissions were obtained.

2.11.1 Subjects

Thirty-one subjects aged 18–60 years who were not sensitive to commonly used daily chemical products and had not participated in other clinical research projects in the last three months were recruited.

2.11.2 Restrictions

During the test period, any other impact test on the tested area was prohibited. The use of other products that could affect the test site was prohibited. The use of products such as bath lotions, soaps, shampoos, and other daily necessities were maintained as before the start of the test, and no changes were allowed.

2.11.3 Test method

Spot test equipment and the closed patch test method were used. Mat-IL-MicCap@PTB (0.020–0.025 g) was placed in the spot test equipment, which was applied to the curved side of the subject’s forearm with hypersensitive tape. The patch was removed after 24 h, and the skin reaction was observed after 0.5, 24, and 48 h. Results are based on the skin reaction classification standard in the Cosmetic Technical Standard (2015 edition).

2.12 Statistical analysis

Results are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the significance of the differences. Statistical significance was set at $0.01 \leq p < 0.05$, $0.001 \leq p < 0.01$, and $p < 0.001$.

3 Results and discussion

3.1 Characterization of Mat-IL-MicCap@PTB

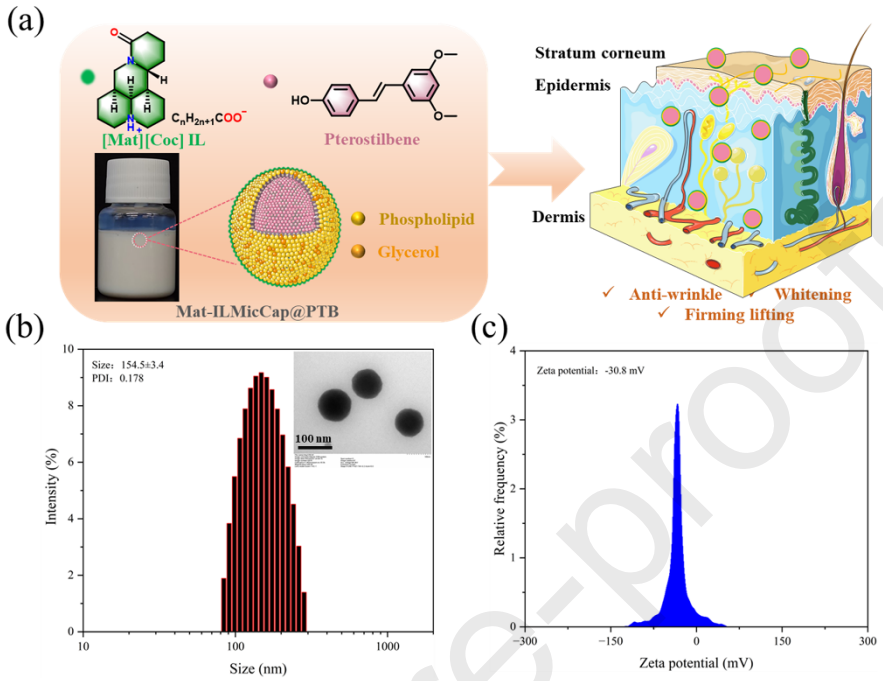

Figure 1. (a) Schematic of a supramolecular microcapsule for loading and delivering PTB to the deep layers of the skin. Particle size (b) and zeta potential (c) of Mat-IL-MicCap@PTB determined by DLS.

The structure and function of Mat-IL-MicCap@PTB are shown schematically in Figure 1a. Microcapsules are closed vesicles composed of phospholipids and glycerol having a bilayer structure. The role of the hydrophilic [Mat][Coc] IL is to open the tight junctions in the SC by enhancing the fluidization of proteins and lipids, thus promoting paracellular transport and enhancing permeability.[26] Crucially, because of its isolation from the environment, the microencapsulated PTB is stabilized and off-target stimulation to the body is prevented, thus achieving highly targeted sustained release in the skin. Figure 1b shows that the particle size and polydispersity index (PDI) of Mat-IL-MicCap@PTB, as measured by DLS, were 154.5 ± 3.4 nm and 0.178, respectively, indicating that the obtained microcapsule had a uniform particle size distribution without aggregation into large particles. These results suggest that the microcapsules prepared by our method are suitable for transdermal drug delivery. In addition, the microcapsules were observed using TEM and found to have a spherical morphology.

The zeta potential is an important index for measuring the physical stability of dispersed systems. A higher absolute zeta potential indicates a more stable system. As shown in Figure 1c, the zeta potential of Mat-IL-MicCap@PTB was -38.6 mV, indicating strong electrostatic repulsion and, thus, good stability in aqueous solution. A standard graph of the MAT-IL-MicCap@PTB based on absorbance is shown in Figure S1. The encapsulation efficiency was

found to be 99.7 ± 0.3% (n = 3).

3.2 Stability of Mat-IL-MicCap@PTB

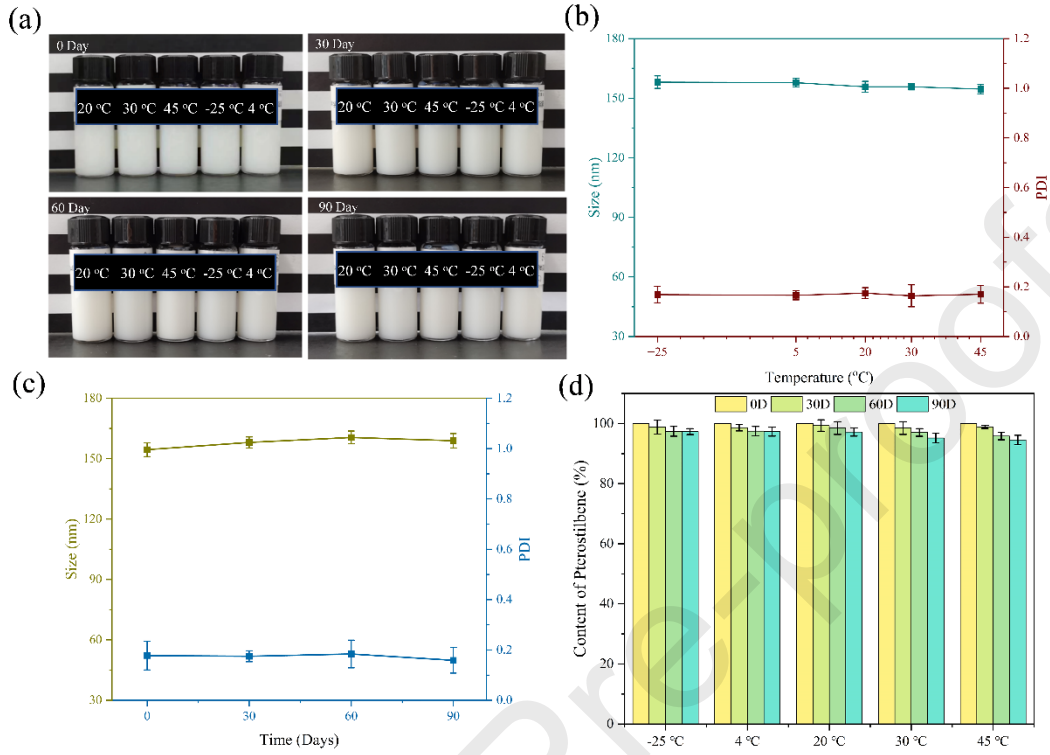

Figure 2. Stability of Mat-IL-MicCap@PTB. (a) Photos of Mat-IL-MicCap@PTB at different periods (0, 30, 60, and 90 days) and temperatures (20, 30, 45, -25, and 4 °C). (b, c) DLS-determined particle size of Mat-IL-MicCap@PTB with respect to temperature and storage period. (d) PTB content at different time points by HPLC. N = 3; mean ± SD.

During the storage of nanocarriers, particle aggregation may occur. This results in an increase in the average particle size of the system and can result in precipitation and delamination. Therefore, the stability of the Mat-IL-MicCap@PTB was evaluated at various temperatures (-4 to 45 °C) and periods (0–90 days). As shown in Figure 2, the Mat-IL-MicCap@PTB emulsion remained homogeneous, without signs of precipitation or other phenomena, over the whole test period (Figure 2a). In addition, the particle size and PDI of Mat-IL-MicCap@PTB at different temperatures and storage periods were investigated, as shown in Figures 2b and 3c. Notably, after storage at five different temperatures for three months, the particle size and PDI of the system did not change significantly, remaining approximately 158 nm and below 0.2, respectively, indicating that there was no particle agglomeration. This good stability is probably due to the formation of a dense emulsifier shell on the outside of the microcapsule, which makes it difficult for the encapsulated lipid particles to fuse. In addition, the presence of a large amount of glycerol reduces the probability of collision between particles, thus avoiding particle aggregation. Furthermore, we examined the change in the PTB content of the microcapsules (Figure 2d), finding that it did not change significantly after storage from -4 to 45 °C. In addition, the PTB content remained 95% after

90 days. These results confirm that Mat-IL-MicCap@PTB is highly stable and, thus, suitable for applications requiring high stability under a range of conditions.

3.3 Cytotoxicity of Free PTB and Mat-IL-MicCap@PTB

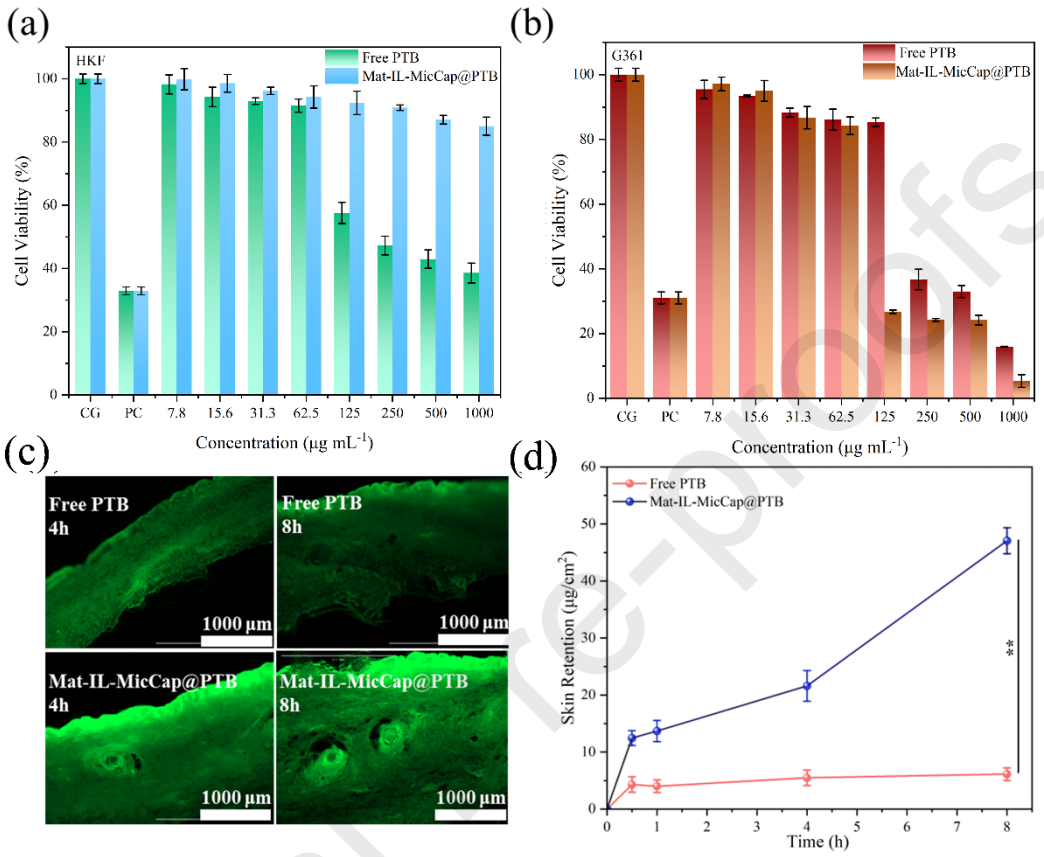

Figure 3. Cytotoxicity and transdermal delivery of Free PTB and Mat-IL-MicCap@PTB. (a, b) Cell viability of HKF and G361 cells after 24 h incubation. (c)CLSM images of Free PTB and Mat-IL-MicCap@PTB penetrating the skin after 8 h incubation. (d) Cumulative penetration of Free PTB and Mat-IL-MicCap@PTB ($N = 3$, mean ± SD; $^{**}p < 0.01$).

PTB has an inhibitory effect on many types of cancer cells. Therefore, the application of liposomes containing PTB as cosmetics requires careful consideration. Crucially, the product must have low cytotoxicity to human cells.

Therefore, we examined the cytotoxicity of Free PTB and Mat-IL-MicCap@PTB using human kidney fibroblast (HKF) and G361 cell lines as models. As shown in Figure 3a, Mat-IL-MicCap@PTB showed very low toxicity to HKF cells. Even at the highest concentration (1 mg mL^{-1}), > 85% cell viability was maintained, and cell viability was close to 100% at lower concentrations. These results suggest that Mat-IL-MicCap@PTB has almost no harmful effect on healthy cells. In addition, the proliferation of melanoma G361 cells was inhibited by 36.7% at a dose of 0.25 mg mL^{-1}. This may be attributed to the cytotoxic effect of the emulsifier. Nevertheless, the cell viability was nearly 100% at lower doses, indicating that the emulsifier's

toxicity was substantially reduced by dilution. This did not compromise the survival of G361 cells.

Notably, the inhibitory effect of Mat-IL-MicCap@PTB on G361 cells was significantly greater than that of Free PTB, which is consistent with reported results for lipid nanoparticles and nanostructured lipid carriers.[27-29] Through the nanoencapsulation of lipids, the contact between PTB and cells is increased, and the water solubility of pterostilbene is improved so that it can better adsorb onto cells, thus improving the bioavailability of PTB and killing cells or inhibiting their proliferation.

3.4 Skin penetration of Free PTB and Mat-IL-MicCap@PTB

The active ingredients of cosmetics must reach the deep layers of the skin rather than remaining on the skin surface or entering the blood stream. Therefore, the amount of the active substance retained in the skin is an important index.[30] In these experiments, the transdermal passage of the microcapsules at different doses was investigated under simulated physiological conditions using the Franz diffusion cell method.[31] The transdermal behavior and skin-targeting ability of PTB were analyzed by calculating the skin retention and degree of transdermal passage after application to the skin, and the bioavailability of PTB in the skin was, thus, predicted.

First, we validated the experimental method, as shown in Figure S2. As shown in Figure 3c, it was difficult for Free PTB to penetrate the SC after incubation in the skin for 4 h. In contrast, Mat-IL-MicCap@PTB fully reached the dermis within 8 h, and its transdermal efficiency was significantly better than that of Free PTB. These results indicate that the preparation of supramolecular microcapsules could improve the osmotic efficiency and bioavailability of PTB. The results of Figure 3d showed that skin retention of Mat-IL-MicCap@PTB was higher than that of Free PTB at different time points. After 8 h of reaction, the skin retention of Mat-IL-MicCap@PTB was 7.7-times higher than that of Free PTB. A key reason for the increase in permeability may be that the microcapsules greatly increase the contact area between PTB and the skin, allowing greater PTB penetration. Crucially, the microcapsules can form a dense lipid nanomembrane, which has a locking effect on the skin surface and can increase the hydration between the active component and the skin cuticle, thus improving PTB penetration of the SC. In addition, the [Mat][Coc] IL can enhance transdermal, transcellular, and decellular transport by bypassing the SC barrier, allowing PTB to reach cells and achieve its therapeutic effects.

For safety, PTB should not enter the blood stream through the skin, even if its permeability is increased. Therefore, the PTB content in the test receiving tanks was determined. The amount of PTB passing through the epidermis was basically zero in each treatment group, suggesting that PTB would accumulate in the skin to form a drug reservoir. This suggests that PTB could maintain a high concentration in the skin for a long time, which is conducive to improving its bioavailability.

3.5 Antioxidant capacity of Free PTB and Mat-IL-MicCap@PTB

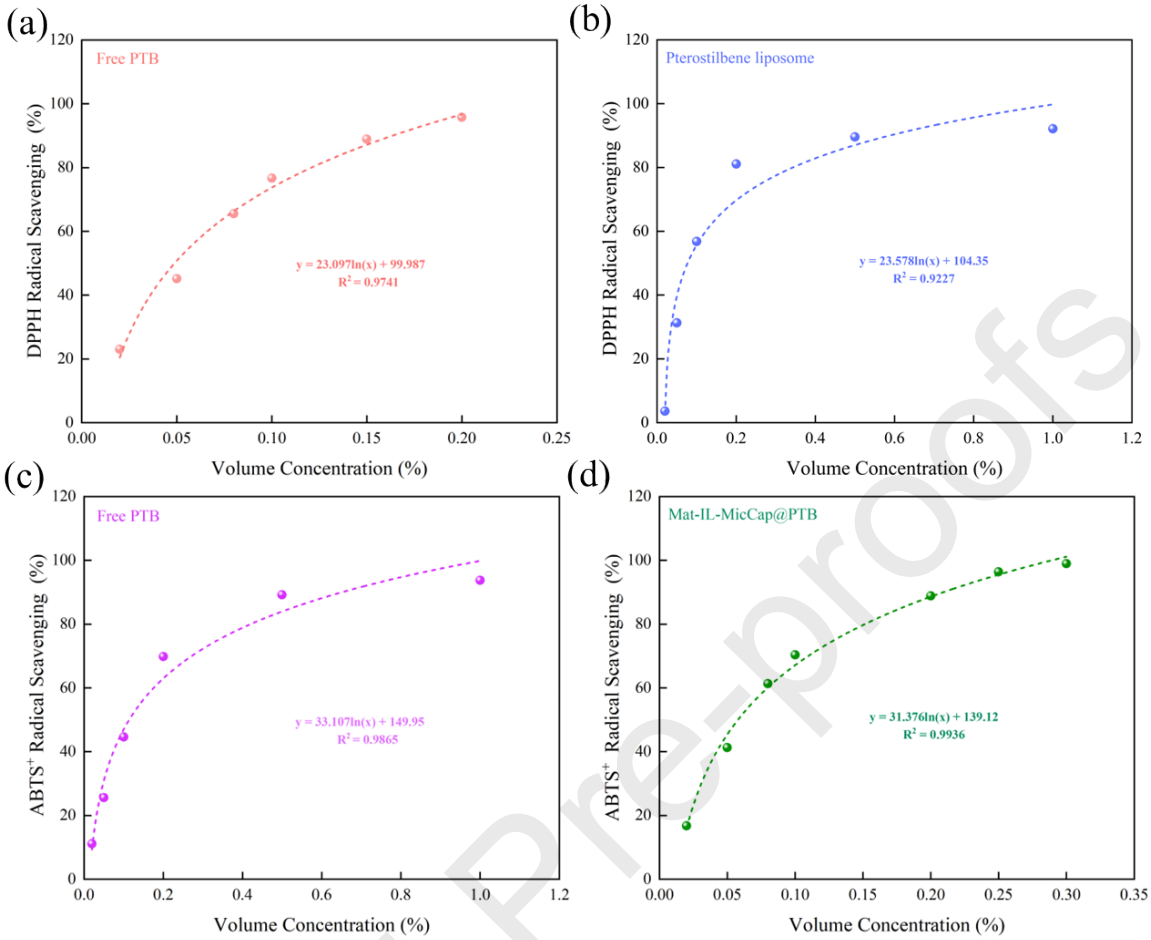

Figure 4. Antioxidant capacity of Free PTB and Mat-IL-MicCap@PTB. (a, b) Free PTB and Mat-IL-MicCap@PTB scavenging of free radicals (DPPH assay), respectively. (c, d) Free PTB and Mat-IL-MicCap@PTB scavenging experiments on free radical (ABTS assay), respectively.

With increasing age, the human body loses its ability to eliminate reactive oxygen species (ROS), and the amount of ROS in skin cells increases, causing skin damage and signs of aging.[32] Anti-aging cosmetics work by scavenging free radicals, thus bringing the oxygen metabolism of skin cells back into balance.[33] PTB has strong free radical scavenging ability and can remove DPPH and ABTS radicals in a concentration-dependent form.[34] DPPH is a stable free radical commonly used to evaluate the free radical scavenging activity of antioxidants.[35] Therefore, the antioxidant capacity of Mat-IL-MicCap@PTB was analyzed. As shown in Figures 4a and 4b, the scavenging rate of DPPH free radicals gradually increased with the increase in dose. Further, the IC_{50} of Free PTB against DPPH free radicals was 0.11%, whereas that of Mat-IL-MicCap@PTB was only 0.093%, indicating that the PTB preparation had a significantly enhanced radical scavenging ability. The same conclusion was drawn from the $ABTS^+$ free radical scavenging experiment (Figures 4c and 4d). The IC_{50} of Free PTB against $ABTS^+$ free radicals were 0.051%, whereas that of Mat-IL-MicCap@PTB was 0.058%. Therefore, PTB retains considerable antioxidant capacity after being coated with microcapsules.

3.6 Biological activity of Free PTB and Mat-IL-MicCap@PTB

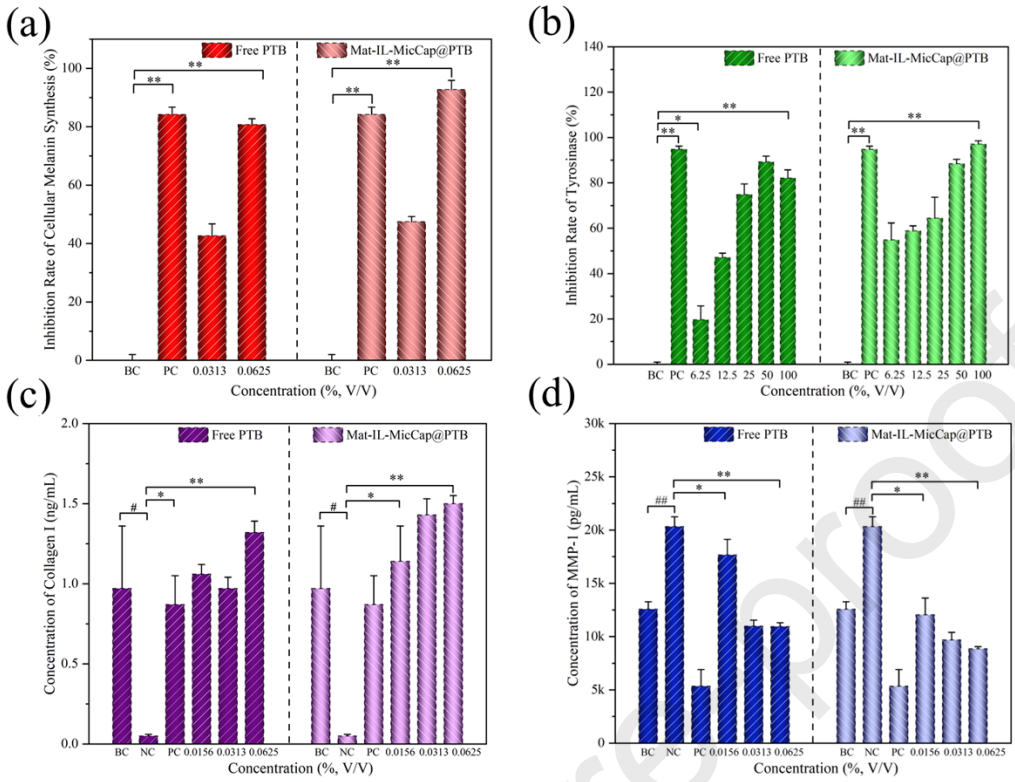

Figure 5. Biological activity of Free PTB and Mat-IL-MicCap@PTB. (a) Inhibition rates of Free PTB and Mat-IL-MicCap@PTB on melanin synthesis in G361 cells. (BC = blank control; PC group was treated with 0.3% concentrated kojic acid, $^{**}p < 0.01$). (b) Tyrosinase inhibition rate of Free PTB and Mat-IL-MicCap@PTB (BC = blank control; PC group was treated with 0.3% concentrated kojic acid, $^{*}p < 0.05$, $^{**}p < 0.01$). (c) Effect of Free PTB and Mat-IL-MicCap@PTB on type-I collagen production in HKF cells (BC = blank control; NC = negative control; PC group was treated with 100 μg mL^{-1} vitamin C (VC) and 7 μg mL^{-1} vitamin E (VE), $^{\#}$ or $^{*}p < 0.05$, $^{**}p < 0.01$). (d) Effect of Free PTB and Mat-IL-MicCap@PTB on MMP-1 production in HKF cells ((BC = blank control; NC = negative control; PC group was treated with 100 μg mL^{-1} VC and 7 μg mL^{-1} VE, $N = 3$, mean ± SD; $^{*}p < 0.05$, $^{\#\#}$ or $^{**}p < 0.01$).

Microencapsulation could retain the biological activity of active ingredients in cosmetic formations. In particular, PTB has been used for brightening and photoprotection, mainly because it can inhibit the growth of melanocytes.[36] Therefore, the rate of inhibition of melanin synthesis in G361 cells was studied (Figure 5a). After treatment with 0.0313% and 0.0625% (v/v) of free PTB, the inhibition rates were 42.7% and 80.8%, respectively. Compared with free PTB group, the inhibition rate of Mat-IL-MicCap@PTB group was increased by 4.8% and 11.9%, respectively, and the inhibition rates were significantly higher than those of the blank control (BC). A reduction in melanin synthesis has a brightening effect. Thus, on the basis of these results, the microcapsules have a better brightening effect than Free PTB. In addition, the inhibitory effect of Mat-IL-MicCap@PTB on tyrosinase was higher than that of free PTB under different experimental concentrations (Figure 5b), further verifying the brightening effect.

Collagen is a major component of the human dermal extracellular matrix (ECM), of which type-I collagen accounts for 80%. A reduction in the amount and changes to the structure of collagen fibers is a key factor contributing to the appearance of aging skin.[37] Therefore, the effects of Free PTB and Mat-IL-MicCap@PTB on type-I collagen production were tested. Compared to the negative control (NC) group, the use of vitamins C and E as a positive control (PC) group significantly increased the content of type-1 collagen, which confirmed the validity of the experiment (Figure 5c). In addition, the use of Mat-IL-MicCap@PTB resulted in a significant increase in the UVA-induced type-I collagen content at concentrations of 0.0156%, 0.0313%, and 0.0625% (v/v). Notably, Mat-IL-MicCap@PTB increased the type-I collagen content in fibroblasts more significantly than Free PTB, increasing by 0.08, 0.43 and 0.18, respectively, indicating that the former has better anti-wrinkle and skin-tightening effects.

MMPs are a family of ubiquitous endopeptidases that degrade ECM proteins. MMP-1 is the main protease that triggers the degradation of collagen fibrils. After cleavage by MMP-1, collagen is further degraded by MMP-3 and MMP-9.[38, 39] Therefore, the effects of CTX/Mat-IL on MMP-1was were examined. As shown in Figures 5d, compared with the NC group, Free PTB and Mat-IL-MicCap@PTB showed significant inhibitory effects on the MMP-1 content of fibroblasts induced by UVA radiation at concentrations of 0.0156%, 0.0313%, and 0.0625%. Compared with free PTB group, the concentration of MMP-1 in Mat-IL-MicCap@PTB group decreased by 31.6%, 11.7% and 20.1%, respectively. These results further suggest that Mat-IL-MicCap@PTB exhibits anti-wrinkle properties.

3.7 Clinical safety and efficacy

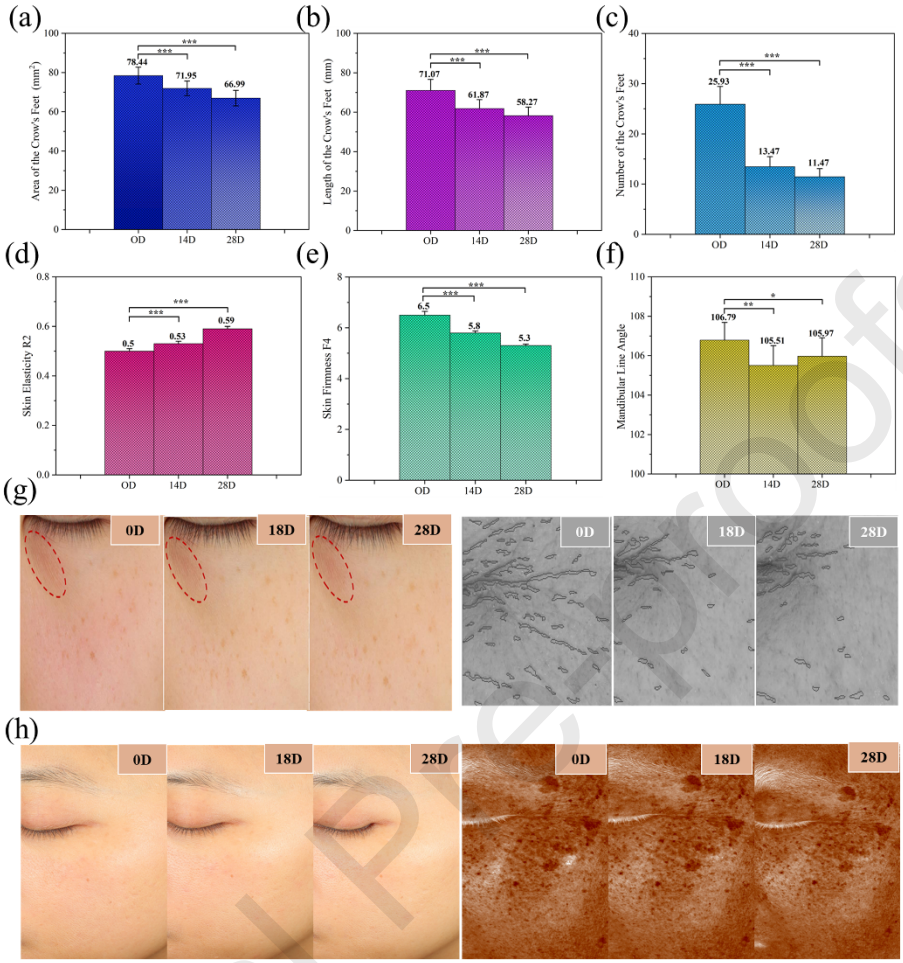

Figure 6. Evaluation of the efficacy of the Mat-IL-MicCap@PTB emulsion. (a, b, c) Improvement in the area, quantity, and length of wrinkles in the corner of the eye after using the product for 14 and 28 days, respectively. (d) Improvement in the mandibular line angle (skin tightness) after using the product for 14 and 28 days (e, f) Skin elasticity (R2) and skin tightness (F4) after using the product for 14 and 28 days, respectively. VISIA skin analysis of the anti-wrinkle effects on the corners of the eyes (g) and changes in facial skin tone and appearance of brown spots (h) in the control group and Mat-IL-MicCap@PTB group after 28 days of treatment.

To identify any adverse reactions, skin-sealing patch experiments were performed with diluted PTB preparations. No adverse reactions were found in any of the 31 subjects (Table S1). Based on these results, a clinical evaluation was conducted.[40, 41] All tests were performed in accordance with ethical regulations. Thirty healthy Chinese male and female participants of different ages with loose facial skin, fine lines, or wrinkles, as well as canthus wrinkles, were selected. We used the professional VISIA skin analysis tool to track changes in skin appearance after treatment with Mat-IL-MicCap@PTB. Specifically, the facial wrinkles, skin elasticity (R2), and firmness (F4) were assessed. After 14 days of continuous product use, the area, length, and number of eye wrinkles, as analyzed by PRIMOS®, decreased

significantly compared with the baseline value by 8.27%, 12.95%, and 48.07% ($p < 0.001$), respectively. After 28 days of use, the rates of decline increased to 14.60%, 18.01%, and 55.78%, respectively (Figures 6a, 6b, and 6c). Further, using the VISIA-CR instrument, we found that the proportion of wrinkles at the eye corners, under the eyes, and on the cheeks decreased by more than 20% (Figures S3–S5). Skin smoothness was also greatly improved (Figure S6), and skin elasticity (R2) increased by 18.97% (Figure 6d), whereas skin firmness (F4) decreased by 18.53% (Figure 6e). In addition, the mandibular line angle of the participants significantly decreased from the baseline value (Figure 6f). Figure 6g shows the changes in the length and number of fine lines in the corners of the eyes after using the emulsion for 28 days.

In addition, the subjects continuously used the Mat-IL-MicCap@PTB emulsion for 28 days to assess changes in melanin content, skin tone (ITA°), and skin brightness (Figures S7–S9) after 14 and 28 days, respectively. Compared with the baseline value, the statistical results showed that, the change rate of melanin was 0.47% ($p > 0.05$, 1.27%) ($p < 0.001$), and the increases in ITA° were 2.31% ($p > 0.05$) and 6.14% ($p < 0.001$), respectively. Further, the increases in skin brightness were 0.24% ($p > 0.05$) and 0.97% ($p < 0.05$), respectively. Overall, the brightening effect of the Mat-IL-MicCap@PTB emulsion is shown in Figures 6h, S10, and S11. After 28 d, the melanin content decreased and ITA° and skin brightness increased significantly, further demonstrating that Mat-IL-MicCap@PTB can inhibit melanin production and promote skin brightening.

Crucially, these results were reproducible in multiple experimental groups, indicating that the synthesized Mat-IL-MicCap@PTB increased skin firmness and elasticity and inhibited wrinkle formation without adverse effects, such as irritation or toxicity to the skin. In summary, it was concluded that Mat-IL-MicCap@PTB has high clinical safety and outstanding clinical efficacy in terms of antioxidant, anti-wrinkle, moisturizing, brightening, and repair properties.

4. Conclusions

In this study, we developed and optimized a novel ionic liquid microcapsule, Mat-IL-MicCap@PTB, by applying IL technology to enhance the biological activity and resolve issues limiting the application of PTB in drugs and cosmetics. The system showed good biocompatibility and stability, achieved the efficient transdermal delivery of PTB, and stabilized the active ingredients. Concerning its cosmetic effects, the system reduced melanin synthesis and tyrosinase activity, resulting in skin brightening. The system also stimulated type-I collagen production in fibroblasts, and a clinical trial further confirmed its remarkable ability to decrease wrinkles and increase elasticity. Overall, we have demonstrated the promise of microcapsule carriers for the local delivery of active ingredients from multiple perspectives and provided a simple, convenient, and effective delivery system for the clinical use of pterostilbene. The findings suggest the promise of this system for combination with similar active components, as well as its applications in the fields of biology, medicine, and materials science.

Data Availability: All the relevant data are available from the corresponding authors upon reasonable request.

Conflicts of Interest/Declaration of Competing Interests: The authors declare no conflict of interest.

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CRediT Author Contributions: De Bai: Investigation, methodology, writing original draft, conceptualization, data curation, formal analysis and project administration. Haowei Chen: Investigation, methodology, conceptualization, data curation and formal analysis. Lijuan Liu: Investigation, methodology, data curation and formal analysis. Nanxi Xiang: Supervision, data curation and formal analysis. Jichuan Zhang: Writing—review & editing. Chengyu Wu: Project administration, conceptualization, supervision and writing—review & editing. Jiaheng Zhang: Project administration, conceptualization, supervision and writing—review & editing. Fang Wang: Funding acquisition, project administration, conceptualization, supervision and writing—review & editing.

Ethical Declaration: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Shanghai Micro-spectial Chemical Analysis and Test Technology Co.,Ltd. (protocol code: 202302JC01).

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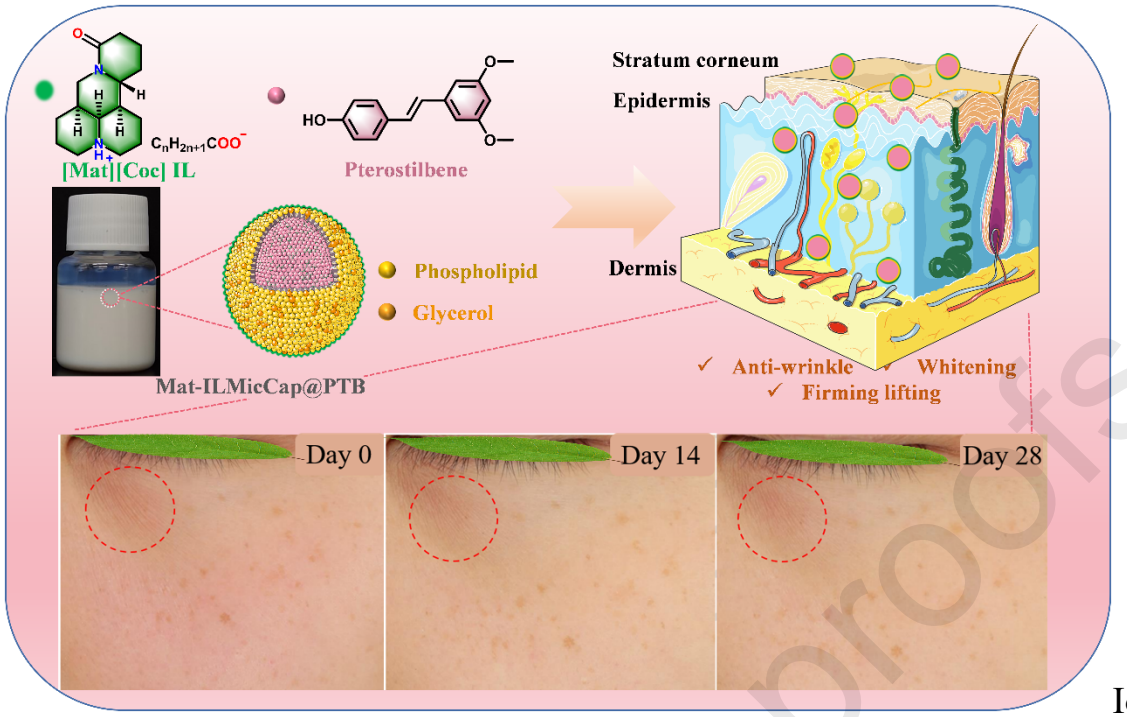

Ionic liquid microcapsules for the topical delivery of pterostilbene: Enhanced transdermal delivery and anti-wrinkle and skin brightening effects

In this study, we used a supramolecular solvent for microencapsulation, and successfully prepared a PTB-loaded ionic liquid microcapsule (denoted Mat-IL-MicCap@PTB), which effectively resolved the poor stability and low bioavailability of PTB. Further, transdermal experiments showed that the microcapsules improved the permeation and retention of the active component. In vitro experiments confirmed that the microcapsules reduced the irritation and side effects of PTB, and they not only achieved a lasting antioxidant effect but also inhibited the production of melanin, attenuated fine lines, and enhanced skin elasticity and health. Therefore, Mat-IL-MicCap@PTB is a feasible carrier for transdermal delivery and synergistic skin care, providing an effective strategy for improving the transdermal delivery of various dermatological drugs.

- The particle size and polydispersity index (PDI) of Mat-IL-MicCap@PTB, as measured by DLS, were 154.5 ± 3.4 nm and 0.178, respectively, indicating that the obtained microcapsule had a uniform particle size distribution without aggregation into large particles. The encapsulation efficiency was found to be $99.7 \pm 0.3\%$.

- Cell viability suggest that Mat-IL-MicCap@PTB has almost no harmful effect on healthy cells. Notably, the inhibitory effect of Mat-IL-MicCap@PTB on G361 cells was significantly greater than that of Free PTB, which is consistent with reported results for lipid nanoparticles and nanostructured lipid carriers.

- The results of transdermal experiment showed that skin retention of Mat-IL-MicCap@PTB was higher than that of Free PTB at different time points. After 8 h of reaction, the skin retention of Mat-IL-MicCap@PTB was 7.7-times higher than that of Free PTB.

- In addition, the use of Mat-IL-MicCap@PTB resulted in a significant increase in the UVA-induced type-I collagen content at concentrations of 0.0156%, 0.0313%, and 0.0625% (v/v). Notably, Mat-IL-MicCap@PTB increased the type-I collagen content in fibroblasts more significantly than Free PTB, indicating that the former has better anti-wrinkle and skin-tightening effects.

- Clinical trials indicated that the synthesized Mat-IL-MicCap@PTB increased skin firmness and elasticity and inhibited wrinkle formation without adverse effects, such as irritation or toxicity to the skin.

In summary, it was concluded that Mat-IL-MicCap@PTB has high clinical safety and outstanding clinical efficacy in terms of antioxidant, anti-wrinkle, moisturizing, brightening, and repair properties.

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Nanxi Xiang: Supervision, data curation and formal analysis.

Jichuan Zhang: Writing—review & editing.

Chengyu Wu: Project administration, conceptualization, supervision and writing—review & editing.

Jiaheng Zhang: Project administration, conceptualization, supervision and writing—review & editing.

Fang Wang: Funding acquisition, project administration, conceptualization, supervision and writing—review & editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: