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Research Article

Skin Lightening Efficacy of Tranexamic Acid in Topical Delivery Systems

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Abstract

The off-label use of oral Tranexamic Acid (TA) has been proven to be an effective treatment for melasma, a commonly acquired skin hyperpigmentation disorder. Topical TA was introduced as a cosmetic skin lightening product in recent years. However, topical TA often does not achieve clinically significant lightening of melasma. It has been postulated that topical TA does not have good skin permeation properties, therefore, delivery systems have been deployed to improve the skin permeability of TA across the skin barrier. Using an *in vitro* 3D skin model (MelanoDerm, MatTek), the skin lightening effects of TA encapsulated in poly lactic co-glycolic acid (PLGA) polymer and TA in liposomes were compared against the lightening effect of free TA. The melanin content of MelanoDerm skin models subjected to the three TA variants was assessed, and images of the skin models were captured after 21 days of application. Liposomal-TA and PLGA-TA were found to be superior to free TA molecules in terms of greater reduction in melanin content. Liposomal-TA was deemed to be superior to PLGA-TA since it achieved a similar lightening effect with half the amount of TA in the liposomal delivery system.

Keywords: Melasma; Hyperpigmentation; Tranexamic acid; Whitening; Lightening; Topical delivery; Encapsulation; Liposome; Poly lactic co-glycolic acid polymer

Introduction

Melasma is a common acquired disorder of skin hyperpigmentation. It is characterized by symmetrical, brown or greyish-brown patches with serrated margins involving sun-exposed areas of the skin, most commonly affecting the face. Melasma is difficult to treat and is often a recurring condition especially if the sufferer does not practice sun avoidance. It can be triggered by UV light, which activates the plasminogen in the keratinocytes to produce plasmin, resulting in melanogenesis [1,2]. An increased number of mast cells has been observed in the skin at the site of melasma lesions [3]. Mast cells may produce vascular endothelial growth factor (VEGF), transforming growth factor-β, and basic fibroblast growth factor, all of which promote vascular growth and contribute to the development of melasma [4-6].

The off-label use of oral tranexamic acid (TA) 250 mg twice daily has been demonstrated in studies [7-9] and proven to be an effective treatment for melasma. Although the mechanism of action of TA is still unclear, the proposed mechanism involves the binding and prevention of plasminogen transfer from epithelial cells to keratinocytes, as well as the inhibition of VEGF to induce angiogenesis [10]. TA has anti-plasmin action [2] and has recently been shown to readily compete with tyrosinase activity, inhibiting hyperpigmentation by decreasing melanin synthesis [11]. Despite its efficacy, long-term use of oral TA, an anti-fibrinolytic agent,

may be needed because recurrence of melasma is observed after discontinuation [12,13]. However, long term use of oral TA raises safety concerns due to the risk of thromboembolic events in susceptible individuals [14].

Topical TA at concentration of 3% to 5% has been introduced as a cosmetic product for skin lightening effect. However, topical TA does not achieve the same lightening effect as oral TA and often does not achieve clinically significant lightening for melasma. It has been postulated that topical TA does not have good skin permeation properties due to its hydrophilic nature, with log P of -0.09 at pH 5 [15]. Therefore, it is unable to permeate sufficiently across the skin barrier to disrupt the signalling of keratinocytes, mast cells, and melanocytes involved in melanin production.

Materials and Methods

Three variants of TA were purchased and made into topical creams by Chaks Cosmetic Design & Services Pte Ltd. A 3% w/w free TA was used to create a pleasant lightweight cream. The same cream base was also used for the other variants of TA, namely (a) 5% w/w TA (10)- poly lactic co-glycolic acid (PLGA) cream containing 0.5% of TA, and (b) 10% w/w liposomal-TA (2.5) cream containing 0.25% TA. Free TA, USP powder, was procured from Astral Scientific Australia, while TA in PLGA polymer was specially encapsulated by Hosokawa Micron Corporation, Japan, for this work. Briefly, PLGA-TA nanospheres were fabricated with emulsion solvent diffusion method in an aqueous polyvinyl alcohol solution with inference from the published work on PLGA-Coumarin-6 nanospheres by Hosokawa Micron Corporation [16]. The actual methodology was not revealed in detail due to a non-disclosure agreement between the manufacturer and the formulator to protect the technology. The PLGA-TA presented as a lyophilized powder with 10% TA loaded in the particles.

Liposomal-TA was a commercial ingredient sourced from Spain. Briefly, phosphatidylcholine was dissolved with cholesterol in ethanol, followed by the evaporation of ethanol to create a thin film. An aqueous solution of TA, mannitol, and glycerine was added to the thin film and subjected to heat and homogenization to create liposomal nano-vesicles. The liposomal system utilized in the experiment was composed of micron-sized spherical particles of mean diameter of 290 nm (ranging from 150 to 300 nm) suspended in a water medium with a pH of 5.6, with a polydispersity index of 0.4. The TA loaded in liposomal carrier is about 2.5%.

The skin lightening effect of three TA variants were investigated using an *in vitro* 3D skin model, MelanoDerm (MatTek, USA). MelanoDerm is a commercially available, highly differentiated 3D tissue culture model comprising normal human-derived melanocytes and keratinocytes from Asian origin (MEL-300-A). It is a useful screening tool to assess the pigment lightening effect of a product, which the product can be applied directly on the epidermis model over a period of 21 days.

MelanoDerm Skin Brightening Assay Protocol [17]. MelanoDerm experiments typically extend for 7 to 21 days. To maintain good skin morphology, MelanoDerm tissues must be fed with 5.0 mL of medium underneath each tissue and replaced with fresh medium every other day. The use of the hanging top plates (Figure 1) facilitates culture of MelanoDerm tissues for melanogenesis and skin lightening studies. To prepare for the MelanoDerm tissues, the following steps are performed:

a. Pre-warm media: Pre-warm the required volume of maintenance medium (provided) to 37 °C. Label the well plates indicating the treatments/ sample creams to be used. At this step, and for each feeding, warm only the required volume of medium.

b. Transfer MelanoDerm samples: Using sterile technique, pipette 5.0 mL of the pre-warmed maintenance medium into each well of the sterile well plates. Place the hanging top lid on the plate bottom containing the maintenance medium. Remove the MelanoDerm tissue from the agarose-containing package using sterile forceps and transfer the MelanoDerm inserts into the hanging top lid (Figure 1C). Cover the plate with the regular plate lid (Figure 1D). When removing the tissue samples from the agarose shipping plate, care should be taken to remove any adherent agarose sticking to the outside of the cell culture inserts containing the MelanoDerm samples.

c. Pre-equilibration: Incubate the well plates containing the MelanoDerm samples in a humidified 37 °C, 5% CO_2 incubator overnight (18-24 hours) prior to applying treatment.

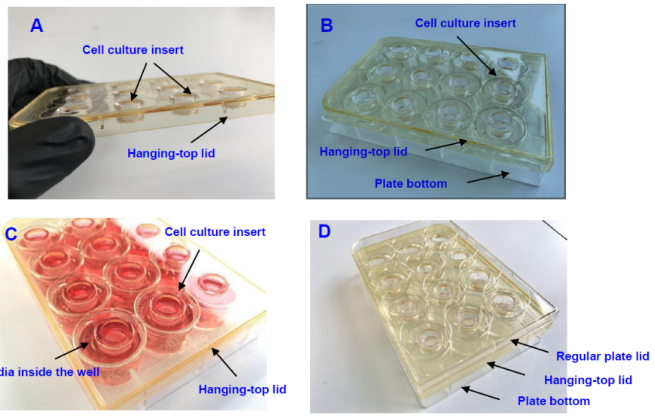

Figure 1 (A) Cell culture inserts (8.8 mm ID) in the hanging-top lid. (B) Hanging-top lid with inserts on top of the bottom plate without medium. (C) Hanging-top lid on top of the plate bottom containing medium. (D) Fully assembled hanging top well plate. Figure 1 was extracted from MelanoDerm Skin Brightening Assay Protocol [17].

Treatment Conditions: Sample application (10 µl) was carried out on Day 0, 1, 3, 6, 8, 10, 13, 15, 17 and 20. Prior to sample application, the surface of epidermis of skin model was washed with phosphate buffered saline (PBS) to remove residual sample product from previous application. *In vitro* skin models were also treated with 2% kojic acid (25 µl) as positive control and deionized (DI) water (25 µl) as negative control.

Melanin Content Quantification: On Day 21, melanin content was assessed by (i) macroscopic images were captured with iPhone 14 with the same light setting, (ii) microscopic images were captured with EVOS XL Core Imaging System using a10x LPlan Phase, and (iii) SOLVABLE™ melanin assay to evaluate melanin content in the *in vitro* models (MelanoDerm, MatTek). The *in vitro* skin models were placed in SOLVABLE™ solubilizer and heated at 95 °C overnight. The samples were cooled and centrifuged to pellet any insoluble materials. Supernatants were placed on 96-well plate and read at 490 nm using a microplate reader (HTX Synergy, Biotek) to determine the melanin content. Statistical analysis of the melanin contents in different treatment samples was performed using Student's t-test (GraphPad, Dotmatics).

Results

The objective of this study was to assess the lightening effect of TA in encapsulation delivery systems compared to its free molecules. The negative control was deionized (DI) water. Kojic acid, a commonly used positive control for *in vitro* lightening, exhibited observable skin lightening effect on the pigmented skin model. Macroscopic and microscopic images of the Wells are shown in Figure 2. The 3D skin model in the Wells subjected to DI water (blank) served as a negative control to demonstrate the amount of melanin produced without any intervention. Conversely, those subjected to kojic acid showed less melanin and, thus, brighter skin images. TA in PLGA and liposomal delivery systems exhibited a greater melanin content reduction than 3% w/w free TA. The melanin reduction effects of the PLGA and liposomal delivery systems of TA were similar to each other.

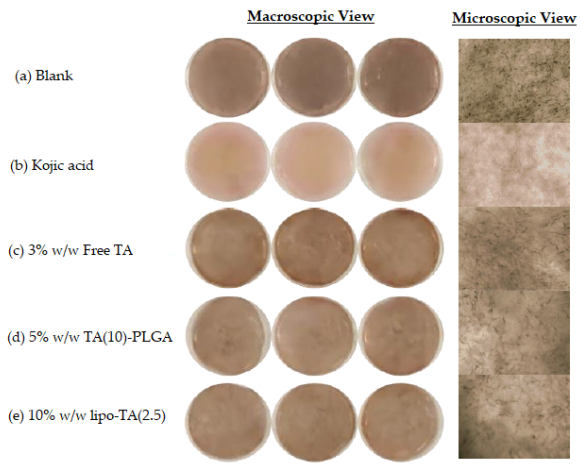

Figure 2: Macroscopic and microscopic view of *in vitro* pigmented model after 21 days in culture. (a) Blank (negative control); (b) Kojic acid (positive control); (c) 3% w/w Free TA; (d) 5% w/w TA(10)-PLGA; (e) 10% w/w liposomal-TA(2.5).

The application of kojic acid 2% solution resulted in a relative reduction of melanin content to approximately 45.56% ($p = 0.0003$) with reference to the Blank. Applications of 3% w/w free TA, 5% w/w TA (10)-PLGA, and 10% w/w liposomal TA creams resulted in relative reductions of melanin content to 83.9% ($p = 0.0559$), 75.71% ($p = 0.0118$), and 76.88% ($p = 0.0186$), with reference to the Blank respectively (Figure 3, Table 1).

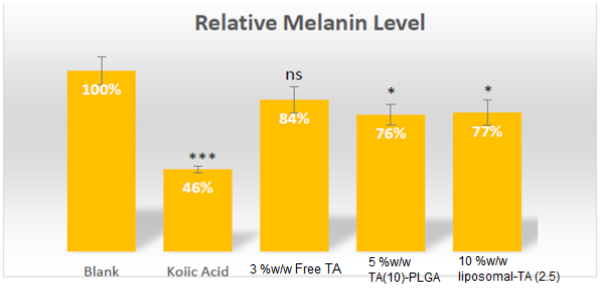

Figure 3: Melanin content quantification. Student T-test: ***P value≤0.001; ** P value⩽0.01; * P value ⩽0.05. (With reference to the Blank); ns: P value > 0.05.

Sample Treatment	Relative Melanin Content to Blank (-ve control) in %				
	Well 1	Well 2	Well 3	Average (%)	SD
Blank (-ve)	91.51%	106.44%	102.05%	100.00%	7.67%
Kojic acid (+ve)	44.98%	47.61%	44.10%	45.56%	1.83%
3% w/w Free TA	88.00%	88.00%	75.71%	83.90%	7.10%
5% w/w TA (10)-PLGA	81.85%	74.83%	70.44%	75.71%	5.76%
10% w/w liposomal-TA (2.5)	84.49%	70.44%	75.71%	76.88%	7.10%

Table 1: Relative melanin content in percentage with reference to the Blank (Negative Control).

The average amount of melanin extracted from skin models after treatment with 3% w/w free TA, 5% w/w TA (10)-PLGA, and 10% w/w liposomal TA creams were 29.218 µg, 26.364 µg, and 26.772 µg, respectively (Table 2).

Sample Treatment	Amount of Melanin (µg)			
	Well 1	Well 2	Well 3	Average
Blank (-ve)	31.867	37.065	35.536	34.823
Kojic acid (+ve)	15.663	16.580	15.357	15.867
3% w/w Free TA	30.644	30.644	26.364	29.218
5% w/w TA (10)-PLGA	28.504	26.058	24.529	26.364
10% w/w liposomal-TA (2.5)	29.421	24.529	26.364	26.772

Table 2: Melanin content in micrograms in well plates corresponding to the negative control, positive control and TA with and without delivery systems.

Discussion

Tranexamic acid (TA) is also known as trans-4-aminomethylcyclohexanecarboxylic acid. It is a lysine analogue that exerts antifibrinolytic effects by reversibly blocking lysine binding sites on plasminogen [2]. While it is commonly used as a homeostatic agent, various dosage forms of TA have been explored in the treatment of melasma, including topical creams, localized microneedles, and oral tablets [18-22].

TA inhibits ultraviolet light-induced plasminogen activator-plasmin systems in keratinocytes, which leads to less free arachidonic acid and a subsequent decrease in the production of prostaglandins. This, in turn, leads to a reduction in melanocyte tyrosinase activity. It is also postulated to be effective by modulating the vascular component of melasma, indirectly reducing the production of basic fibroblast growth factor, which leads to decreased angiogenesis and neovascularization [23].

In a local retrospective review of 561 patients with melasma, 89.7% of the patients showed documented improvement with oral TA 250 mg twice daily, whilst 10.0% had no response and 0.4% worsened. The median duration of treatment was 4 months (range 0.03 to 22 months), whereas the median time to initial response was 2 months. Better response rates were observed in those with an older age of onset, a longer duration of disease, and no family history. There was a relapse rate of 27.2% with a median duration of 7 months upon cessation of oral TA in those who had improved [13].

Although oral TA serves as an effective option to reduce the overall disease outlook, it cannot be prescribed long-term due to the possible risk of thromboembolism events [14]. Topical TA seems to be an ideal solution, yet local clinical experiences with topical free TA creams or serums have been disappointing, showing poor improvement in melasma. In this study, we utilized free TA as 3% cream to represent the most commonly available concentration in skincare products in the local setting.

There is a Lipinski-like Rule of Five to predict the skin permeability or flux, and skin bioavailability of a molecule based on its physicochemical properties, although there are exceptions. In general, the inherent permeability of a molecule is inversely dependent on molecular weight, ideally less than 500 Daltons. It also increases with increasing lipophilicity to certain extent, measured as log P, ideally around 2 to 3, with measurable solubility both in oil and in water. The number of hydrogen bond donor should be less than 10,and the number of hydrogen bond acceptor should be less than 5 [24]. TA is a small hydrophilic molecule, with

a molecular weight of 157 Daltons, water solubility of 167 mg/ml, and a predicted log P of -0.09 at pH 5 [15]. Therefore, free TA in topical formulation is predicted to remain in aqueous phase of a formula with limited skin permeability across skin barrier and cell membrane if there was no chemical enhancer in the formulation. As such, delivery systems such as PLGA polymers and liposomes were investigated to assess their effect on the skin permeability of TA using MelanoDerm Skin Brightening Assay Protocol as a surrogate marker.

Poly lactic co-glycolic acid (PLGA) polymers Delivery System

PLGA polymer was selected because it is a safe, FDA-approved biodegradable polymer that can be used in cosmetic and medical applications as implants and drug carriers for penetration enhancement [25]. The PLGA-TA particles were incorporated into the study cream as composite particles, which were the agglomeration of PLGA-TA nanospheres bound together with water soluble excipients (Figure 4). The composite showed excellent capability to pass through skin pores and demonstrated good cutaneous penetrability to the deep epidermis layer, as shown by the green fluorescence dye tagged to Coumarin-6 molecules in the percutaneous absorption study of PLGA nanospheres via human skin biopsies by Tsujimoto et al (Figure 5) [16,26].

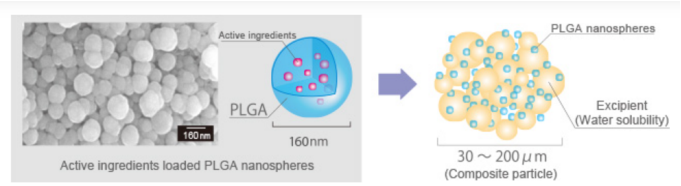

Figure 4: Image of PLGA nanospheres (NS) and the composite particle. Figure extracted from Hosokawa Micron Corporation [26].

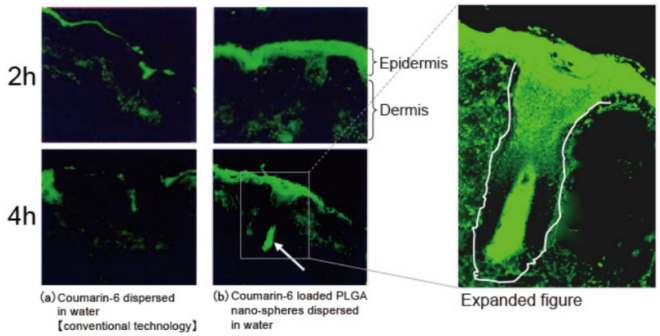

Figure 5: Photos of permeability of PLGA NS into human skin biopsies of under arm skin of 35-year-old woman (a) Coumarin 6 (conc. 10% w/w) dispersed in aqueous solution with surfactant (Pluronic F68) of 0.5% w/w. Spread: 0.3ml. (b) Aqueous dispersion of Coumarin-6 loaded PLGA NS (conc. 0.2% w/w). PLGA: Coumarin-6 = 2000: 1. Coumarin-6 conc. in aqueous dispersion is 0.0001% w/w. Spread: 0.3ml. Figure extracted from Hosokawa Micron Corporation [25,26].

PLGA polymers undergo hydrolysis of their ester linkages in the presence of water to produce the original monomers, glycolic acid and lactic acid, which are byproducts of various metabolic pathways in the body under normal physiological conditions [27]. These degradation products can help maintain the healthy acidic microenvironment of the skin. In addition to pH conditioning, glycolic acid has been found to augment skin desquamation by stimulating the activity of enzymes involved in the degradation of corneodesmosomes, the structures responsible for maintaining the integrity of the stratum corneum. This process promotes the removal of superficial damaged layers, unveiling a smoother and more vibrant skin texture [28]. Lactic acid is well known for its gentle exfoliative properties and compatibility with sensitive skin types [29]. Together, glycolic acid and lactic acid are proposed to induce upregulation of collagen production in the dermis, the deeper layer of the skin [30]. Moreover, both glycolic acid and lactic acid (300–500 μg/mL) have been reported to suppress melanin formation by directly inhibiting tyrosinase activity in human and mouse melanoma cells *in vitro* [31]. These additional benefits from degradation products of PLGA are postulated to be synergistic for overall skin lightening and glow.

Liposomal Delivery System

The liposomal system made up of phospholipids was selected as a comparison due to its similarity with skin lipids, good safety profile, skin tolerability, and wide application in cosmetic products [32]. Liposomes composed of phospholipids can easily penetrate the skin and pass across the skin's stratum corneum via intracellular or transcellular routes, aided by two elongated elastic layers on their surface, their inherent low weight, water and oil solubilities, and small molecular size [32,33]. Phospholipids are amphiphilic molecules; they form unilamellar or multilamellar nanovesicles in the presence of water. In this manner, water-soluble molecules like TA can be entrapped in these nanovesicles.

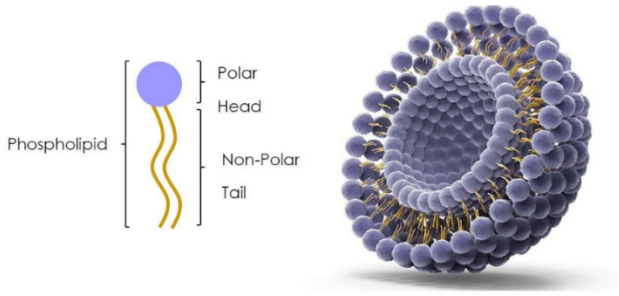

Figure 6: A pictorial illustration of a liposomal nano-vesicle composed of phospholipids [34].

In a published study, 5% topical liposomal TA was compared with topical hydroquinone 4% cream in an Iranian split-face study involving 30 women participants over 12 weeks [35]. Both treatment arms were shown to significantly reduce the Melasma Area and Severity Index (MASI) score ($p < 0.001$) after 12 weeks. This study suggested that liposomal TA could be an over-the-counter cosmetic alternative to the conventional melasma treatment using hydroquinone, which is only available as a prescription cream.

One limitation identified in our work was that TA loaded in the delivery systems were not set at the same concentration. Nonetheless, this was an exploratory work to compare the delivery systems, hence the inconsistent in TA loading concentration of the PLGA and liposomal systems were discussed and accepted.

MelanoDerm Skin Brightening Assay

The MelanoDerm Skin Brightening Assay is a commercially available, cost-effective screening tool to evaluate the efficacy of skincare formulations used to address skin pigmentation disorders. It is cheaper and more reproducible than using *ex vivo* human skin, which is more expensive, has high intrinsic variation, and is not readily available. It also provides a good alternative to animal testing, which was previously done in the cosmetics and pharmaceutical industries but has become ostracized due to greater consumer support for cruelty-free products [36].

In our work, the macroscopic and microscopic images served as the visual observation of the treatment options. Notably, the macroscopic and microscopic images from the Wells treated with 3% free TA cream did not seem to be significant different from the blank (negative control), while the images of the Wells treated with PLGA and liposomal delivery systems were visibly lighter than blank. These images provided visual cues suggestive of a better lightening effect with delivery systems compared to the free TA molecules.

SOLVABLE™ melanin assay of the 3D skin models provided a quantitative measure and was used as a surrogate marker to assess the permeability of TA in the three different forms. The 3% w/w free TA cream had a relative reduction of melanin content to 83.9% ($p = 0.0559$, not statistically significant) with reference to the blank, while 5% w/w TA (10)-PLGA and 10% w/w liposomal TA creams had relative reductions of melanin content to 75.71% ($p = 0.0118$, statistically significant) and 76.88% ($p = 0.0186$, statistically significant), respectively, with reference to blank (Figure 3, Table 1).

The hypothesis was that greater melanin reduction was proportionally related to better TA penetration into the skin. Free TA did not demonstrate a statistically significant reduction in melanin content, while TA encapsulated with either PLGA or liposomes did. The melanin assay result of free TA cream replicated the actual clinical experience with commercial topical TA products, where there was no significant improvement of melasma despite good clinical outcome from oral TA. The greater melanin reductions observed from TA encapsulated in delivery systems offer a promising way to improve the permeation of TA into deeper skin layers to elicit its anti-pigment effects on the responsible cell types.

Among the delivery systems, the liposomal delivery system was observed to be superior to PLGA encapsulation because the achieved results were similar (Table 1), despite the liposomal system only had half the amount of TA compared to the PLGA encapsulation system: 0.25% versus 0.5%. This could be due to the similarity of the phospholipid components in liposomes and the skin membrane, which allowed for easier penetration across the skin stratum corneum via intracellular or transcellular routes [32]. Notably, liposomal nanovesicles might also act as:

- Carriers to deliver entrapped molecules into or across the skin
- Penetration enhancers to modify the intercellular lipid lamellae
- Depot for sustained release of active compounds
- Site-limiting membrane barrier for a controlled transepidermal or transdermal delivery system [33]

Conclusions

Both PLGA and liposomal delivery systems are safe drug carriers and have been utilized in various clinical applications. They were compared head-to-head for encapsulating and delivering tranexamic acid, a hydrophilic molecule, in this study. It was interesting to observe the similarity in melanin reduction between the two delivery systems, despite different concentrations of drug loading. The liposomal delivery system was deemed to be more effective by achieving similar lightening results with half the amount of the TA. The liposomal delivery system will be chosen for future work to investigate the optimum concentration for skin lightening effect in 3D skin model, and possibly in subsequent clinical trial with melasma patients.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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