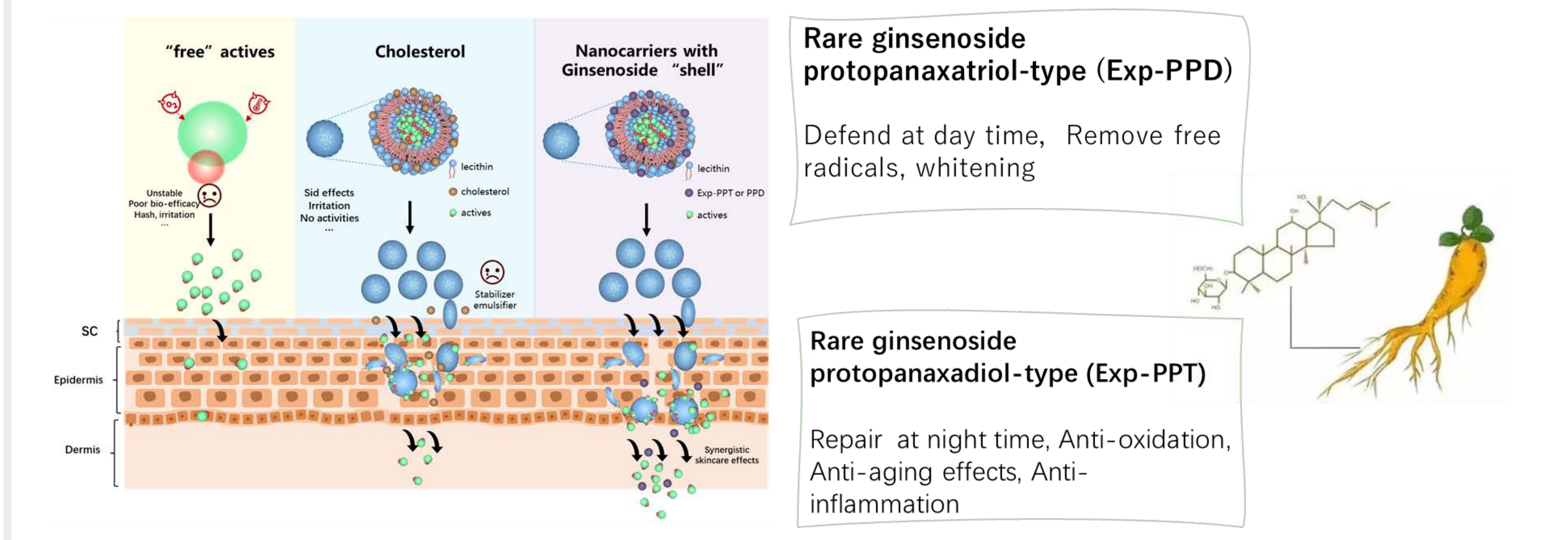


Novel nanocarriers with unique ginsenoside "shell" for regulating circadian rhythm skin care: Ginseng Loves Your Skin Day and Night

Li, Dongcui^{1*}; Ren, Shaohua¹; Wang, Yunsi¹; Liu, Xinyi¹; Zhang, Zhiqian²;
1 cBiomey (Guangzhou) Technology Co., Ltd., Guangzhou, China;
2 Beijing Tidetron Bioworks Company, Beijing, China

Introduction:

Panax ginseng C.A.Meyer, a traditional and precious Chinese medicine, has attracted attention for thousands of years. *Panax ginseng* extracts are widely used in medicine, health food, cosmetics and other fields. Ginsenosides are the key functional component of ginseng extract. Ginsenosides mainly belong to two categories, namely Protopanaxadiol-type (PPD) saponins and protopanaxatriol-type (PPT) saponins. Due to their structural differences, disparate pharmacological effects and cosmetic efficacy, they must be well separated and enriched for use. In this study, the PPD and PPT ginsenosides were enriched, separated and transformed efficiently to rare ginsenosides protopanaxadiol-type (Exp-PPD) and protopanaxatriol-type (Exp-PPT) by natural extraction process, resulting in improved bioactivity, clearer components, more unified standards and stable quality of the final product, as opposite to conventional ginseng extracts. We further explored and proved that exclusive applications of Exp-PPD and Exp-PPT can well meet the specific requirements of skin care during daytime and nighttime, respectively. Many skin care actives are hard to penetrate through the stratum corneum, epidermis to dermis layer. According to the characteristics of molecular polarity, we further explored the unique use of Exp-PPD and Exp-PPT as a replacement of traditional stabilizer such as cholesterol to encapsulate actives, forming various nanocarriers including micelles, liposomes, and nanoparticles etc. This innovative nanocarrier "shell" can largely improve the performance of both oil-soluble and water soluble active substances, achieving excellent skin care effects, soothing and less irritation, improving transdermal permeation and bioactivities.



Materials & Methods:

[Materials]: 0.0015-0.003% Exp-PPD, 0.0015-0.003% Exp-PPT, Exp-PPD day serum and Exp-PPT night serum, 0.008% tocopherol acetate, 0.05% 4-Butylresorcinol, 50 μ M Coenzyme Q10, Benchmarking commercial ginsenoside

[Methods]
Extraction and condensation: Exp-PPD and Exp-PPT were extracted and separated from ginseng root, and then were converted to rare ginsenosides by hydrolyzing with acids and freeze-drying.

Circadian rhythm skin care by EXP-PPD and EXP-PPT Ginsenosides:

- In-vitro test:** 3T3 cell test ROS scavenging caused by UV damage or H₂O₂. HSF cell test SOD enzyme activity; COL I and COL III expression; intracellular ROS scavenging. B16 cell test tyrosinase inhibition and melanin content. HaCat cell test MMP-1; anti-inflammation; soothing effect; skin barrier by filaggrin expression. The melatonin related proteins AANAT and ASMT related with many circadian rhythm clock genes are measured
- In-vivo test:** EXP-PPD serum for day and EXP-PPT serum for night skin care are evaluated by an in vivo study (N=8) for 28 days. VISIA-CR and PRIMOS-CR are used to collect the wrinkle images of the subject; CL400, GL200 and elasticity test probes are used to detect the ITA° value, gloss and skin elasticity R2 and F4 values.

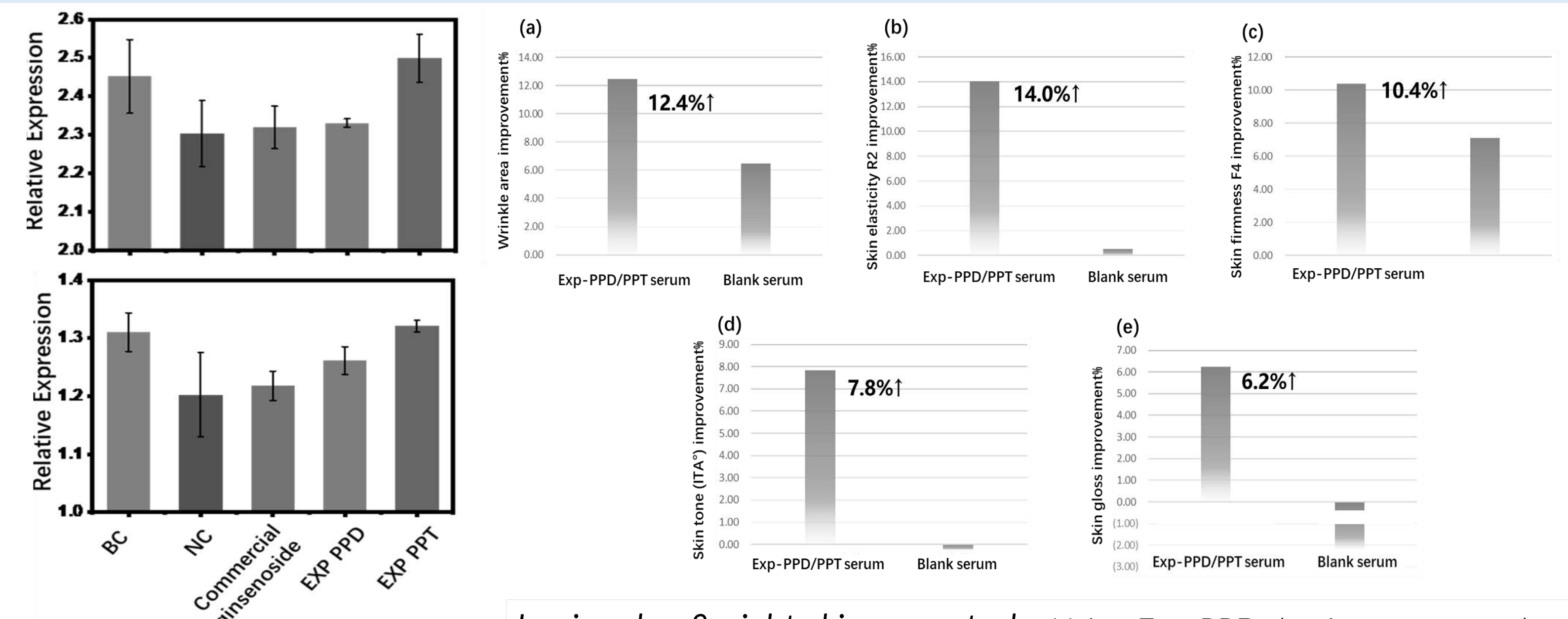
Nanocarrier with Ginsenoside "shell":

- Nanocarrier preparation:** The ginsenosides and benchmark cholesterol nanocarriers are prepared using flash nanoprecipitation method.
- Transdermal permeation efficacy:** The Franz diffusion cell is used for measuring transdermal permeation of model active encapsulated by ginsenosides and by cholesterol nanocarrier.
- Improved bioactivities:** Various in-vitro cell models have been established to elucidate the advantages of ginsenosides nanocarriers versus traditional cholesterol stabilized nanocarriers in terms of active efficacy.

References:

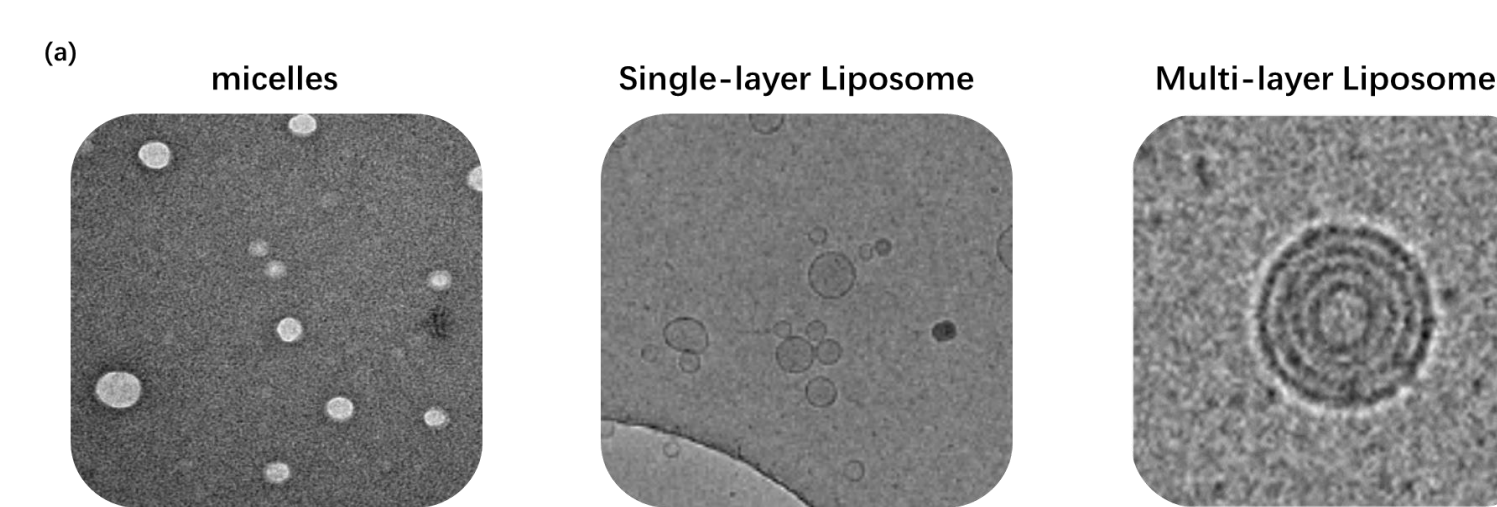
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Results & Discussion:

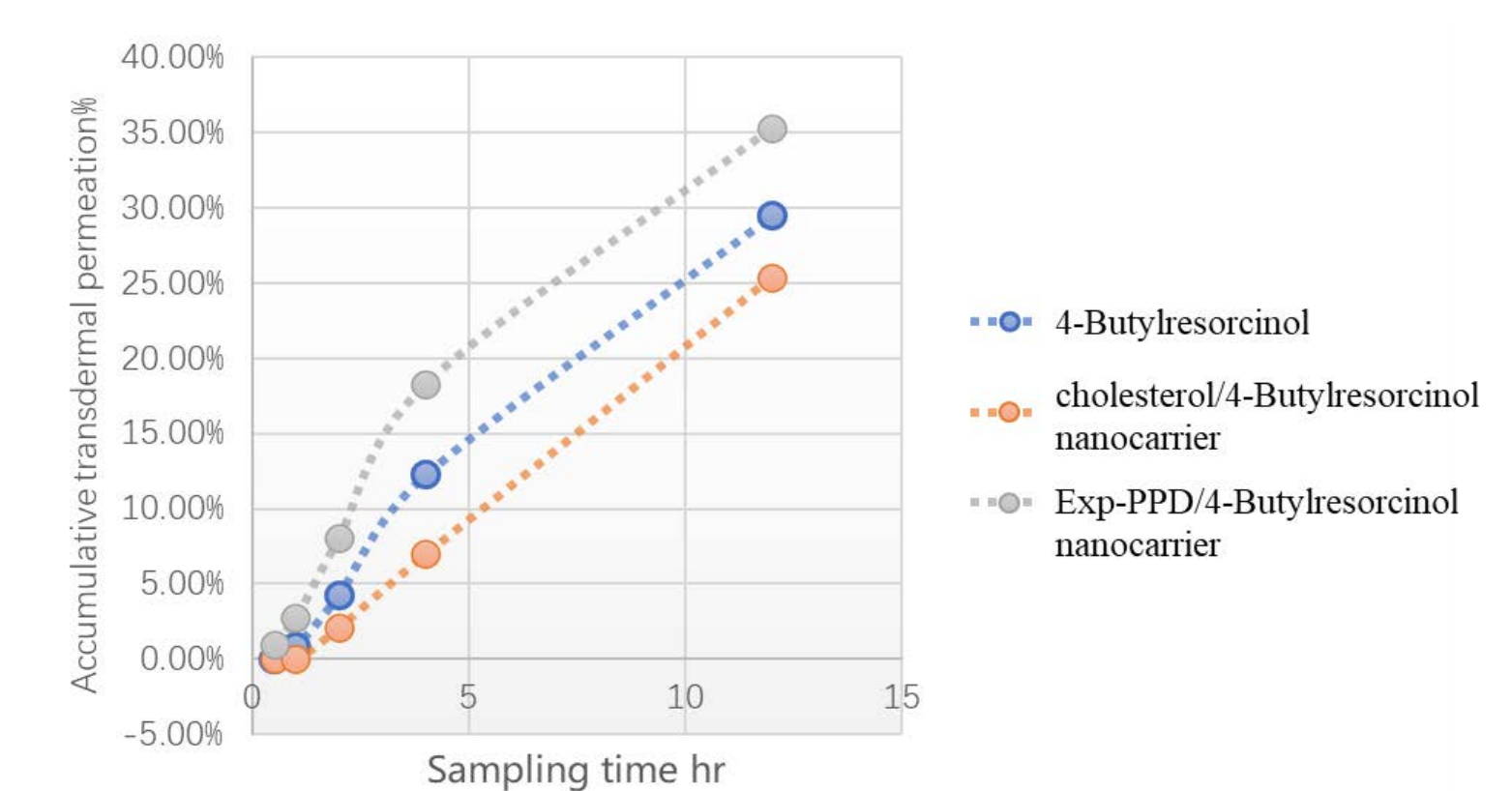


In vitro circadian rhythm: Exp-PPD and Exp-PPT ginsenosides, can restore the expression of ASMT/AANAT.

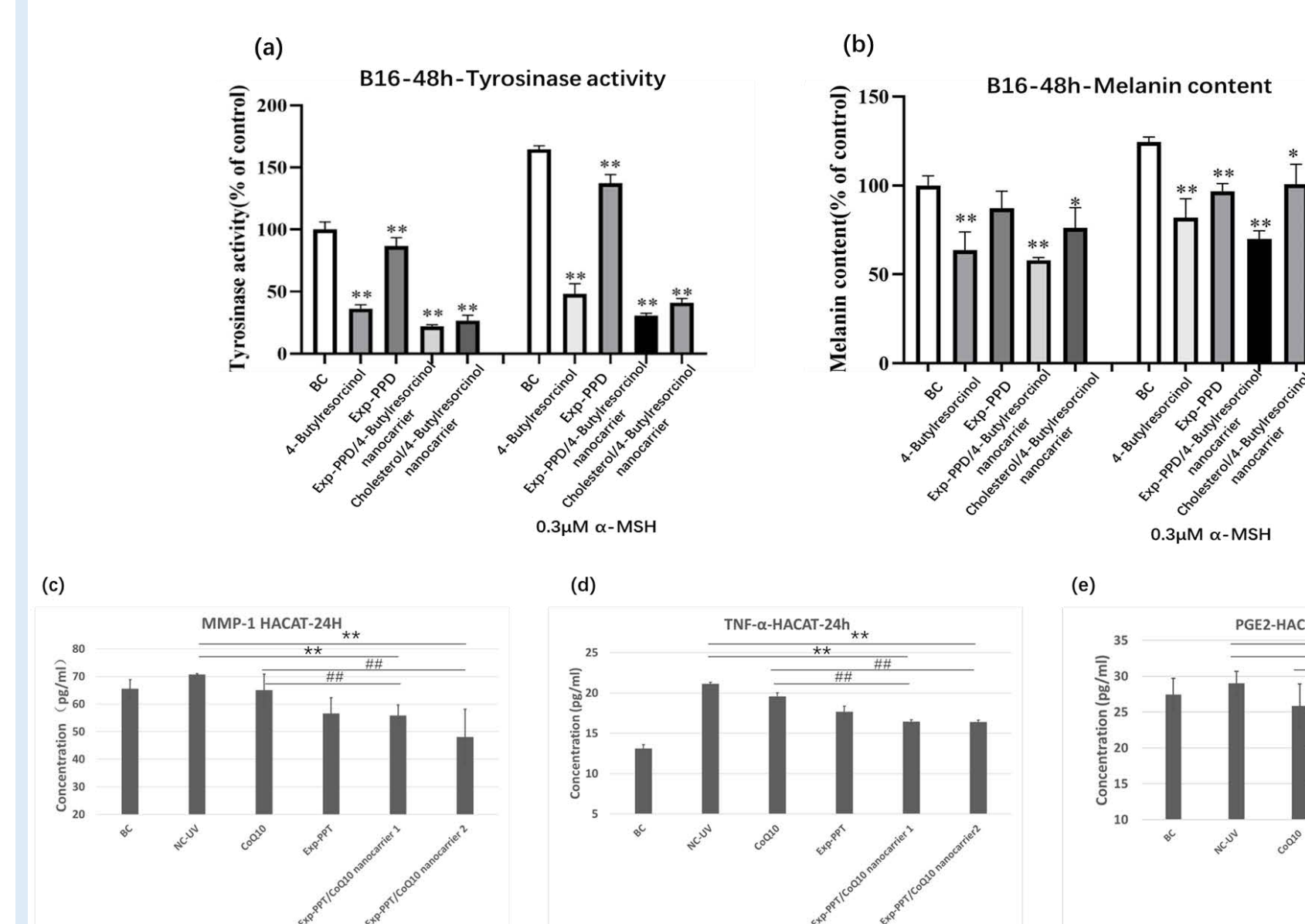
In vivo day & night skin-care study: Using Exp-PPD daytime serum and Exp-PPT night serum for 28 days, compared to the blank serum, the subject's eye wrinkle area was improved by 12.4%, skin elasticity by 14%, the skin firmness was improved by 10.4%, skin color (ITA° value) was improved by 7.8% and skin radiance improved by 6.2%.



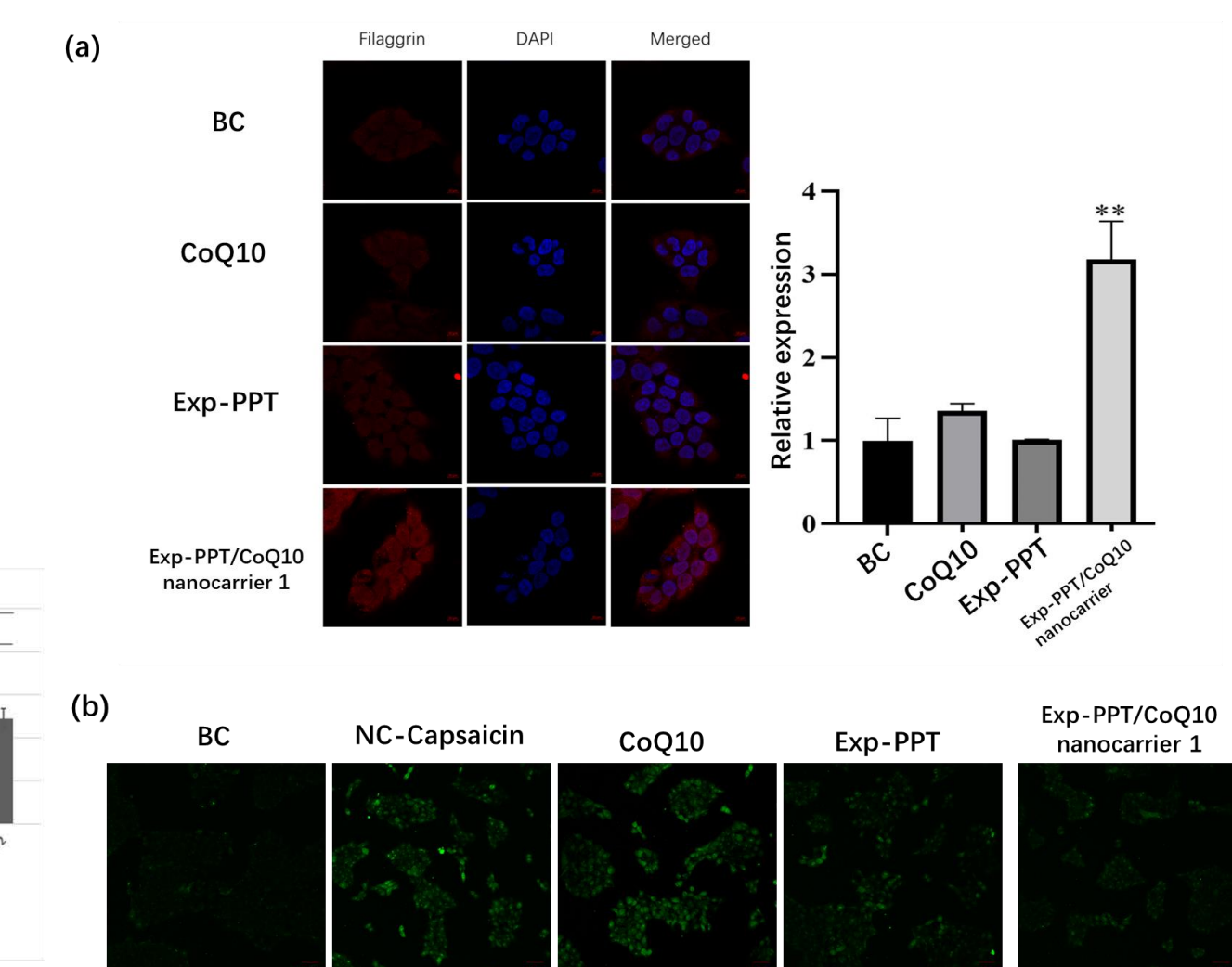
Encapsulation robustness and stability: Model Exp-PPD/4-Butylresorcinol nanocarriers, Exp-PPT/CoQ10 nanocarriers, and cholesterol nanocarriers have good stabilities for both water-insoluble and water-soluble actives.



Transdermal permeation efficiency by Franz cell: The ginsenosides nanocarriers show significant advantages over cholesterol nanocarriers for transdermal permeation efficiency of model active.



In-vitro bioactivity enhancement: the Exp-PPD or Exp-PPT nanocarriers' improvements on active bioactivities than "free" actives and cholesterol nanocarriers: (a) Tyrosinase activity inhibition and (b) Melanin content reduction (c) MMP-1 inhibition (d) (e) anti-inflammation of TNF- α , PGE2 reduction



Soothing & skin-barrier improvement: In-vitro models to demonstrate the Exp-PPT/CoQ10 nanocarriers' improvements on (a) skin barrier and (b) soothing of Capsaicin

Conclusions:

In summary, we report a novel nanocarriers comprised of a unique ginsenoside "shell" for regulating circadian rhythm skin care: Exp-PPD ginsenoside nanocarriers for daytime skin care and Exp-PPT ginsenoside nanocarriers for night skin care, exclusively. These nanocarriers show excellent properties and performance for skin care applications. This study provides an interesting pathway of better utilizing ginsenosides for advanced skin care applications that Ginseng Loves Your Skin Day and Night, and ginsenosides "shell" protect and improve the functional actives.

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