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Study on the regulation of collagen and anti-aging by hydroxypropyl tetrahydropyrantriol nanocarriers

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Abstract

In this study, hydroxypropyl tetrahydropyrantriol was encapsulated using nanocarrier technology to obtain Hydroxypropyl Tetrahydropyrantriol Nanocarriers (HPRO Ncs). The safety of HPRO Ncs was assessed via the hen's egg test on the chorioallantoic membrane (HET-CAM test) and a patch test. Stability tests on HPRO Ncs focused on monitoring changes in pH levels, particle size, distribution, and hydroxypropyl tetrahydropyrantriol (HPRO) content within HPRO Ncs solutions across various temperatures. Transdermal permeation experiments were conducted to evaluate the permeation performance of HPRO Ncs. Additionally, the impact of HPRO Ncs on Collagen I and Collagen IV content in ex vivo skin tissues of Chinese individuals following UVA and UVB irradiation was examined. Human efficacy tests further confirmed the anti-aging properties of HPRO Ncs. Results indicated that HPRO Ncs demonstrated favorable safety and enhanced stability post-encapsulation, effectively preventing unwanted chemical reactions involving HPRO during formula application. Transdermal permeation test results revealed that nano-encapsulated HPRO Ncs exhibited higher permeation amounts compared to the control group. Specifically, cumulative permeation amounts at 5 hours were approximately 2.2 times higher than the control group, 2.53 times higher at 8 hours, and 2.6 times higher at 12 hours. In vitro tissue tests showed that 2% (v/v) HPRO Ncs increased COL-1 (type I collagen) by 80.65% and COL-4 (type IV collagen) by 109.38%. Meanwhile, 10% (v/v) HPRO Ncs led to a 283.87% increase in COL-1 and a 134.38% increase in COL-4. Human experiments demonstrated that 10% HPRO Ncs significantly enhanced skin elasticity (R2 value) and compactness (F4 value), showcasing remarkable anti-wrinkle and tightening effects. Overall, experiments highlighted that HPRO Ncs, prepared via flexible liposome encapsulation technology, offers superior stability and broader applicability in formulae. This technology addresses the risks of inactivation and discoloration associated with HPRO, while also improving its bioavailability and efficacy.

Keywords: Aging signs evaluation, Anti-aging, Biotics formulation, Ingredients combination, Regenerative beauty

1. Introduction

The aging of the skin is a complex biological process, the molecular mechanisms underlying both intrinsic and photoaging share similarities, primarily characterized by the loss of mature collagen and an increase in matrix metalloproteinase expression. However, various strategies targeting collagen turnover have shown limited efficacy in preventing or reversing skin aging. As studies on the mechanisms of skin aging progress, researchers have identified glycosaminoglycans (GAGs) and proteoglycans (PGs)—the most abundant structural components in the extracellular matrix aside from collagen fibers—as being closely related to skin aging and wrinkle formation [1]. Skin aging is accompanied by abnormalities in the

structure, types, and content of GAGs and PGs; for instance, there is a decrease in total sulfated GAG levels [2], shortening of GAG chains linked to core proteoglycans [3], and abnormal deposition sites for GAGs [4].

GAGs and PGs are abundantly present within the dermal extracellular matrix, as well as on the surfaces of fibroblasts and keratinocytes [5]. There are four main types of GAGs: chondroitin sulfate/dermatan sulfate, heparin/heparan sulfate, keratan sulfate, and hyaluronic acid. The negatively charged carboxyl groups and sulfate groups on GAG chains are crucial for maintaining tissue hydration. For example, HA can bind up to 1,000 times its own molecular weight in water molecules [1], thus effectively preserving tissue structural function [6]. PGs consist of a core protein covalently linked to one or more GAG chains. These core proteins can interact with key proteins such as collagens (types I-IV), growth factors like epidermal growth factor or platelet-derived growth factor, as well as transforming growth factor beta. These interactions influence collagen fiber formation and signaling pathways involving growth factors or cytokines [3,7]. Heparan sulfate proteoglycans, including basement membrane proteoglycan, type XVIII collagen, transmembrane multi-ligand proteoglycan, and phosphatidylinositol-anchored proteins, are vital components of the skin's basement membrane [1,8,9,10]. The basement membrane proteoglycan plays an essential role in keratinocyte survival and proliferation at the epidermal level.

Based on this evidence, we believe that simultaneously targeting collagen turnover and modulating anti-aging active ingredients related to GAGs and PGs may yield promising results toward reversing or delaying signs of aging. HPRO has emerged as a noteworthy anti-aging active ingredient. Research findings indicate that it can markedly upregulate mRNA expression levels associated with collagen synthesis. It also regulates expressions of various forms, including free GAGs, and stimulates HSPGs at dermal-epidermal junction areas. For instance, it increases syndecan-1, syndecan-4, CD44, and basement membrane proteoglycan expressions. These actions enhance the assembly and deposition of critical DEJ layer constituents, thereby strengthening cohesion between the epidermis and DEJ layers [11,12]. In addition, it adjusts keratinocyte proliferation and differentiation, avoiding thinning caused by age-related changes while promoting wound healing capabilities [13-15]. Numerous clinical trials have demonstrated its potential, confirming improvements against signs associated with cutaneous senescence without irritation, making it a highly promising candidate for cosmetic formulations.

However, challenges arise when incorporating HPRO into cosmetic formulations. This is largely attributed to the inherent barrier properties of human skin, which lead to low transdermal absorption rates, particularly for hydrophilic compounds. Tong Ha et al. [17] conducted a layered quantitative analysis using integrated micro-infrared spectroscopy, chemometrics, and machine learning techniques. Their study revealed that after six hours of application, O/W emulsions containing 10.0% HPRO resulted in an average actual penetration concentration of 0.26%, while aqueous solutions only achieved 0.06%. Although O/W emulsion formulations demonstrated better permeation behavior, overall transdermal absorption remained below expectations. This limits the bioavailability of HPRO and hinders the effective realization of its intended anti-aging benefits. Consequently, higher concentrations are often required in practical applications to achieve the desired outcomes.

Flexible liposomes are biomimetic delivery systems characterized by their dual-layer phospholipid structures, which exhibit high deformability. This allows for improved penetration efficiency of hydrophilic drugs through the stratum corneum barriers. In this investigation, we aim to leverage the excellent deformation capacity and stable architecture of flexible liposomes as a vehicle to facilitate enhanced transdermal delivery efficacy. We are also exploring new carrier technology avenues to assess the impact of HPRO on combating visible signs of aged-skin conditions.

2. Experimental Section

2.1. Experimental Materials and Instruments

Hydroxypropyl tetrahydrofuran triol, Tianjin Taipu; Phosphatidylcholine, butanediol, polyquaternium-51, 1,2-hexanediol, glycerin and propylene glycol, Sinopharm; SIMULGEL FL (acrylic acid hydroxyethyl ester & sodium dimethyl taurine acrylate copolymer & isododecane & polysorbate 60), Seppic; Vitamin C (VC), Vitamin E (VE) and sodium dodecyl sulfate (SDS), Sigma; Paraformaldehyde, Biosharp; Physiological saline solution, Kelun; PBS, Solarbio; Ex vivo skin tissue culture medium and ex vivo skin tissue, Guangdong Boxi; Collagen I antibody, CST; Collagen IV antibody, Abcam. 9-day-old SPF-grade fertilized eggs of White Leghorn chickens (2040822Ahzz0215000570), Zhejiang Lihua Agricultural; Strat-M membrane, Merck Millipore.

150l CO₂ incubator, Thermo; SW-CJ-1F ultra-clean workbench, Sujing Antai; UVA irradiation device, Philips; UVB irradiation device, Philips; BX43 fluorescence microscope, Olympus; incubator, Grumbach; stereo microscope, Leica; 1100 high-performance liquid chromatography system, USA Agilent Technologies Inc.; Zetasizer/Nano-ZS90 laser particle size analyzer, UK Malvern; TK-12A transdermal diffusion system and improved Franz diffusion cell, Shanghai Kaikai; 2000 high-pressure homogenizer, UK APV.

2.2. Preparation of HPRO Ncs

Weigh 6.0 g of phosphatidylcholine and 10.0 g of butanediol, then heat and stir them in a water bath at 65 °C. Preheat 30.0 g of hydroxypropyl tetrahydrofuran triol to the same temperature and add it to the mixture. Stir for 10 minutes, then add 1.0 g of polyquaternium-51 and 1.5 g of 1,2-hexanediol to the preheated mixture and continue stirring for an additional 30 minutes at the same temperature. After this step, stir for another 10 minutes to obtain Mixture B, then cool it down to 35-45 °C. Inject preheated deionized water (51.5 g) into Mixture B at a rate of approximately 1 g per minute per 100 g of the total system weight, while maintaining continuous stirring at a speed of 600-800 rpm during the injection. After completing the injection, hydrate for an additional 20 minutes to obtain the initial mixture. Finally, homogenize the mixture using a microjet homogenizer set at a pressure of 14,000 psi with three cycles, controlling the discharge temperatures at 25 °C, 20 °C, and 15 °C respectively, to yield HPRO Ncs.

2.3. particle size and stability test

Prepare HPRO Ncs and store it at room temperature for 24 hours. Then, measure the average particle size and polydispersity index (PDI) of a 1.0% HPRO Ncs aqueous solution, and measure the pH of a 10.0% HPRO Ncs solution. HPRO Ncs were stored at -20 °C, 4 °C, 25 °C, and 45 °C for 30 days. The average particle size, PDI, and pH were measured on days 2 and 30. Stability evaluation of HPRO Ncs was based on changes in appearance, average particle size, PDI, and pH.

2.4. CAM test

The negative control group received physiological saline, while the positive control group was treated with a 1.0% SDS solution, each containing one chicken embryo. The test group used a 1.0% HPRO Ncs solution with six chicken embryos per sample.

Fertilized chicken eggs incubated for nine days were selected. Using dental serrated forceps, remove the shell at the air cell end. Inject physiological saline (0.90% NaCl) into the shell membrane, then carefully remove the membrane to expose the chorioallantoic membrane (CAM). Apply a volume of 0.3 mL of the test substance to cover at least 50% of the CAM surface. Observe and record the reaction of the CAM within five minutes to evaluate the onset of each type of toxic effect. Reaction Evaluation Method: Irritation Score (IS)

$$IS = \frac{(301 - \text{sec H}) \times 5}{300} + \frac{(301 - \text{sec L}) \times 7}{300} + \frac{(301 - \text{sec C}) \times 9}{300}$$

sec H represents the average time in seconds for congestion to first appear on CAM;

sec L denotes the average time in seconds for bleeding to first occur on CAM;

sec C indicates the average time in seconds for coagulation to begin on CAM.

Substances irritation: IS < 1.00, no irritation; IS between 1.00 and 5.00, mild irritation; IS

between 5.00 and 9.00, moderate irritation; IS ≥ 9.00 , severe irritation.

2.5. Human Patch Test

In this experiment, 30 subjects were asked to record their reactions to various concentrations of HPRO Ncs physiological saline solution (1.0%, 2.5%, 5.0%, 10.0%, and 20.0%). The solutions were applied using filter paper discs in a patch testing device, with a physiological saline solution patch serving as the blank control. Both the sample and control patches were affixed to each subject's inner forearm, and gentle pressure was applied to ensure even skin contact for 24 hours.

After removing the patches, skin reactions were observed after a waiting period of 30 minutes, allowing any indentations to disappear. Further evaluations of skin responses were then conducted at 24 hours and 48 hours post-removal of the patches.

2.6. Transdermal permeability

Essence: 0.8% SIMULGEL FL, 3.0% Propylene Glycol, 3.0% Glycerin, and water as the remainder. HPRO Ncs and HPRO were added separately to the essence to create control and treatment group samples, both with 2.5% HPRO. Strat-M was placed between the diffusion chamber and receiving chamber of a Franz diffusion cell, with the rough side facing the diffusion chamber. Then, 8 mL of receiving liquid (7.4 PBS) was added to ensure close contact with the Strat-M membrane. The Franz diffusion cell was fixed in a transdermal diffusion apparatus at 32 °C, and the magnetic stirrer rotor speed was maintained at 600 rpm. After achieving thermal equilibrium, 0.1 g of the sample was evenly applied on top of the Strat-M membrane surface. At time points of 2, 5, 8, and 12 hours, aliquots of 300 μ L were taken from the receiving liquid while replenishing it with fresh solution for quantitative analysis using HPLC-ELSD for HPRO content.

The cumulative permeation amount of HPRO in the receiving liquid was quantitatively calculated using the following formula:

$$Q = \frac{C_n * V + \sum_{n-1} C_{n-1} * V_0}{A}$$

Where: Q = Cumulative permeation amount per unit area; indicating sample permeation quantity per unit area over cumulative time; C_n = Concentration of sample at sampling point n; V = Volume of receiving liquid in receiver pool; V₀ = Volume sampled each time; A = Area of receiver pool.

2.7. In Vitro Skin Model Anti-Wrinkle and Firming Test

Fresh skin tissue was immersed in 75% ethanol for 30 s, followed by 3 washes with sterile PBS. The tissue was then cut into small pieces (24 \pm 2 mm²), placed epidermis-side up in a culture mold, and transferred to a 6-well plate with 3.7 mL of culture medium per well. The tissue was cultured in a humid environment (37 °C, 5.0% CO₂), and the medium was changed daily. After two days, the sample group and positive control group were exposed to UVA (30 J/cm²) and UVB (50 mJ/cm²) for four consecutive days. After each irradiation, fresh medium was added. The PC group received treatment via immersion with PC (100 μ g/mL VC + 7 μ g/mL VE), while the sample group received surface application of test samples (2% HPRO Ncs and 10.0% HPRO Ncs). After four days of irradiation, the ex vivo skin continued to be cultured for three more days without further irradiation but still received sample treatment during this period. The blank group had fresh medium replaced daily.

Skin tissues intended for analysis were fixed in 4.0% paraformaldehyde solution for 24 hours before undergoing immunofluorescence detection. Observations were made under a fluorescence microscope, and images were captured for subsequent analysis.

Enhancement rate was calculated using the formula: (Sample Group - Negative Control Group) / Negative Control Group \times 100%.

2.8. Verification of Human Efficacy

A total of 13 subjects aged between 28 and 55 years were selected for the study. Each

subject's left cheek was assigned to the control group, and the right cheek to the sample group. The subjects applied the products twice daily as part of their regular skincare routine for 28 days. Skin physiological parameters were assessed before the application, and on days 14 and 28 post-application. Prior to testing, all participants underwent a standardized facial cleansing procedure followed by a drying process to remove moisture from the skin. After sitting in a controlled environment at $21 \pm 1^\circ\text{C}$ and $50 \pm 10\%$ humidity for 30 minutes, skin parameter measurements were conducted on both sides of the face. Cutometer® for measuring skin elasticity and firmness; Tewameter® TM HEX for assessing transepidermal water loss; and Corneometer® CM825 for evaluating stratum corneum hydration levels.

Composition of essence: 1.0% Carbopol940, 0.5% Hydroxyacetophenone, 3.0% 1,2-Hexanediol, 95.5% Deionized Water. Control group: 10 mL of HPRO + 90 mL of essence. Sample group: 10 mL of HPRO NCs + 90 mL of essence.

3. Result

3.1. particle size and stability test

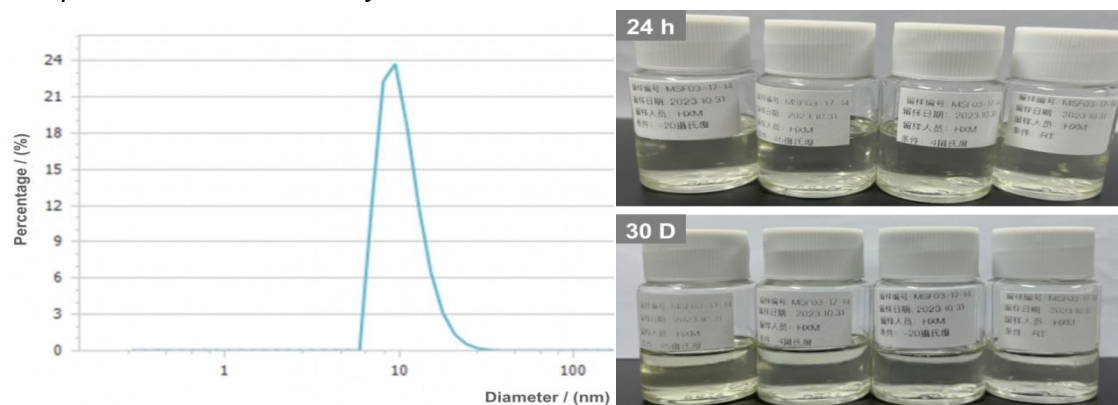


Figure 1. The particle size distribution of the freshly prepared HPRO NCs. **Figure 2.** Appearance of HPRO NCs after storage for 24 hours and 30 days at different temperatures.

The particle size distribution of the freshly prepared HPRO NCs are illustrated in Figure 1. The number-average particle size is measured to be 10.48 nm, with a peak standard deviation of 3.24 nm, and the majority of HPRO NCs vesicles exhibit a particle size around 10 nm. Additionally, the Zeta average particle size of HPRO NCs was determined to be (23.25 ± 0.80) nm, with a polydispersity index (PDI) of 0.299, which indicates a relatively narrow vesicle size distribution and uniform particle sizes throughout the sample.

Table 1. Changes in particle size, PDI, pH and HPRO content of HPRO NCs under different storage conditions.

Time	T / °C	pH	D / nm	PDI	HPRO concentration / %
24h	45	5.69	28.42±0.62	0.333	30.012
24h	4	5.70	18.39±0.84	0.071	29.989
24h	-20	5.73	28.32±0.61	0.309	30.101
24h	25	5.68	32.36±0.40	0.331	30.007
30D	45	5.57	25.78±0.56	0.251	29.874
30D	4	5.69	30.59±0.38	0.341	30.091
30D	-20	5.70	24.99±0.63	0.328	30.001
30D	25	5.64	27.22±0.59	0.339	30.034

Table 1 presents the Zeta particle size, PDI, pH, and HPRO content of HPRO NCs stored at different temperatures for 30 days. As shown in Table 1, both the pH and HPRO content exhibited minimal changes. After storage at 45°C for 30 days, the average particle size and PDI showed only slight variations. The appearance of HPRO NCs after being stored at different temperatures for 30 days is illustrated in Figure 2; negligible changes were observed in the appearance post-storage. These findings indicate that HPRO NCs possess commendable stability.

3.2. Stimulation of the Chicken Embryo Allantoic Membrane

In this test, the IS value for the negative control group using physiological saline was 0, indicating that the IS value for the negative control group was less than 1. The positive control group treated with 1% SDS exhibited an IS value of 17.59, which is greater than 10. These results demonstrate that the testing conditions meet established standards. The average IS value is (0.46 ± 0.17) , indicating that HPRO Ncs exhibits no irritant properties.

3.3. Skin irritation

Human patch tests with HPRO Ncs at concentrations of 1.0%, 2.5%, 5.0%, 10.0%, and 20.0% over different time intervals. The study found that none of the 30 subjects exhibited any signs of erythema, edema, significant swelling, infiltration, papules, or associated vesicles or pustules. This indicates that HPRO Ncs demonstrated no irritant effects on human skin during this trial.

3.4. Permeability

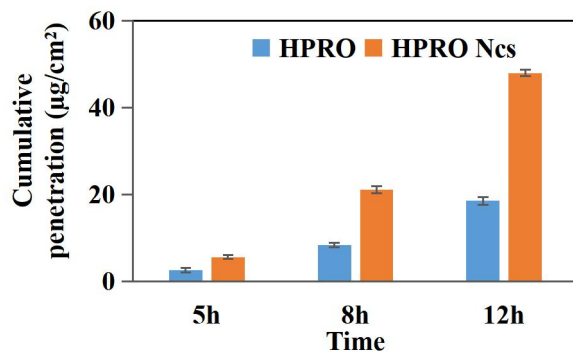


Figure 3. Cumulative Permeation at Various Times

The results of the transdermal permeation tests showed that the permeation amount of HPRO Ncs, obtained by nano-encapsulation, was higher than that of the control group. At 5 hours, the cumulative permeation amount was approximately 2.2 times higher than the control group. By 8 hours, this amount increased to 2.53 times that of the control group, and by 12 hours, it was 2.6 times higher than the control group.

3.5. Effects of Anti-Aging Based on Ex Vivo Skin Models

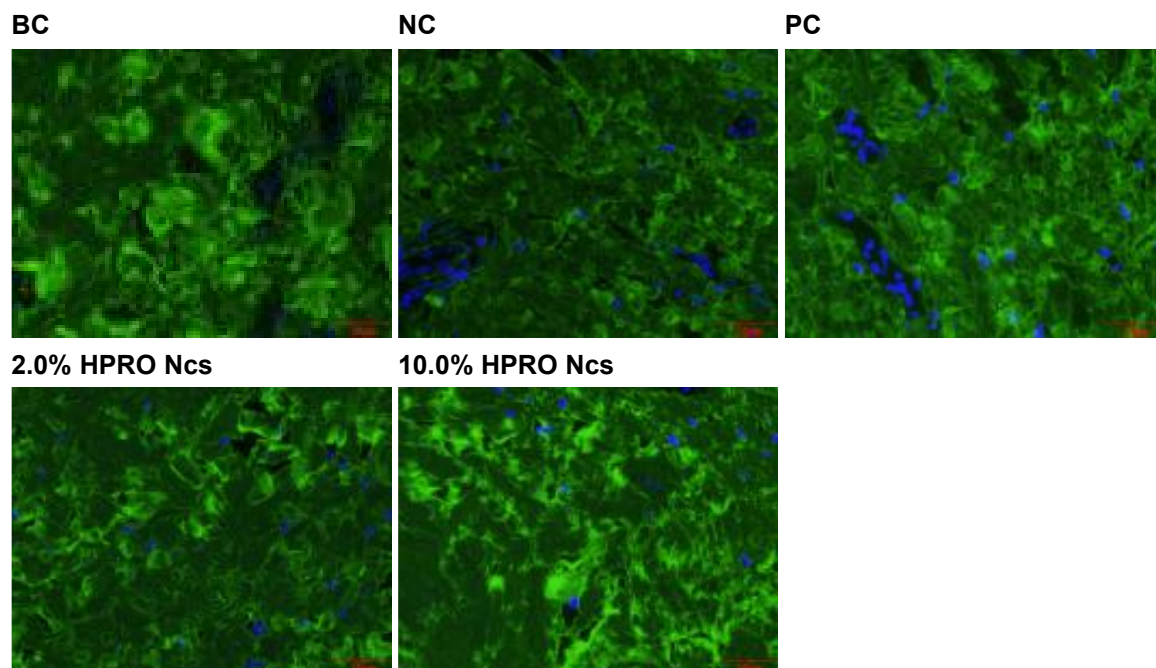


Figure 4. immunofluorescence for collagen type I in ex vivo skin (Note: The image was captured using a fluorescence microscope (Olympus, BX43) at a magnification of 20×. The blue color represents the

cell nuclei, while the green fluorescence indicates Collagen I. A stronger intensity of green fluorescence correlates with a higher content of Collagen I).

Figure 4 presents the immunofluorescence images of collagen type I in ex vivo skin samples. Compared with the NC group, both the 2.0% and 10.0% HPRO Ncs groups showed stronger green fluorescence intensity in the ex vivo skin samples. Notably, the green fluorescence intensity in the 10.0% HPRO Ncs group was greater than that in the BC group, indicating an increased presence of type I collagen after treatment with 2.0% and 10.0% HPRO Ncs. Furthermore, the content of type I collagen in the ex vivo skin from the 10.0% HPRO Ncs group surpassed that of the blank control group. These findings indicate that HPRO Ncs can enhance the synthesis of type I collagen in the skin. Moreover, its efficacy is concentration-dependent; as the concentration increases, so does its capacity to promote type I collagen production.

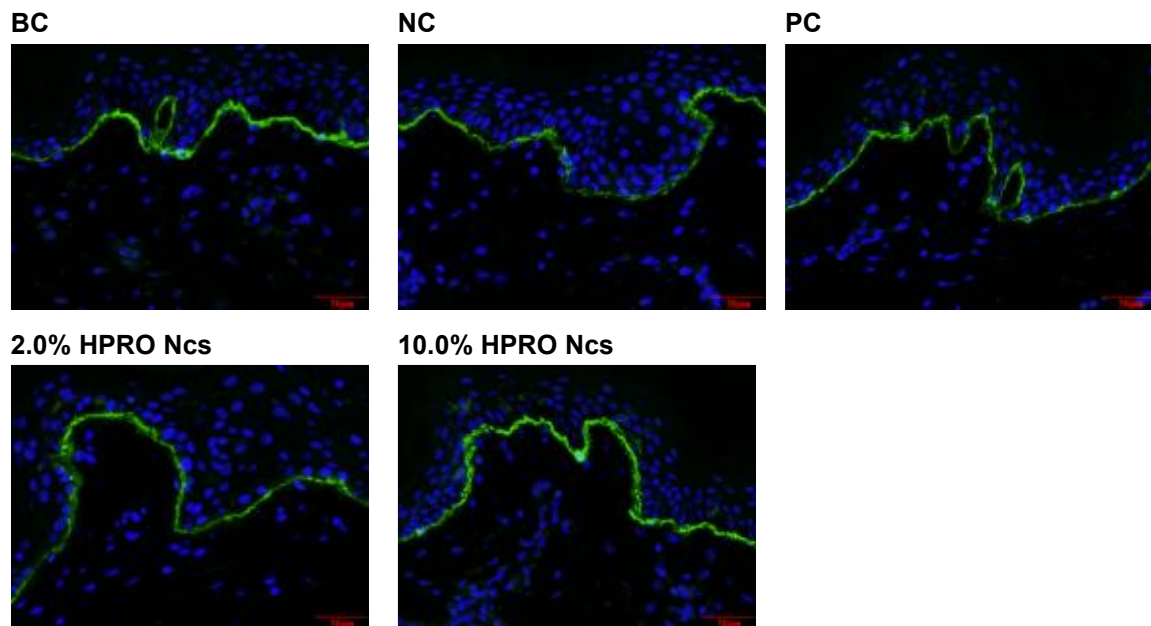


Figure 5. immunofluorescence for collagen type IV in ex vivo skin (Note: The image was captured using a fluorescence microscope (Olympus, BX43) at a magnification of 20 \times . The blue color represents the cell nuclei, while the green fluorescence indicates Collagen IV). A stronger intensity of green fluorescence correlates with a higher content of Collagen IV).

Figure 5 presents the immunofluorescence images of collagen type IV in ex vivo skin samples. Compared with the NC group, the 2.0% and 10.0% HPRO Ncs groups showed significantly stronger green fluorescence intensity in the ex vivo skin samples, indicating a higher abundance of collagen type IV after treatment with these concentrations of HPRO Ncs. These results suggest that HPRO Ncs can enhance the production of collagen type IV in the skin, and this effect is concentration-dependent; as the concentration increases, the ability to promote collagen type IV synthesis is more pronounced.

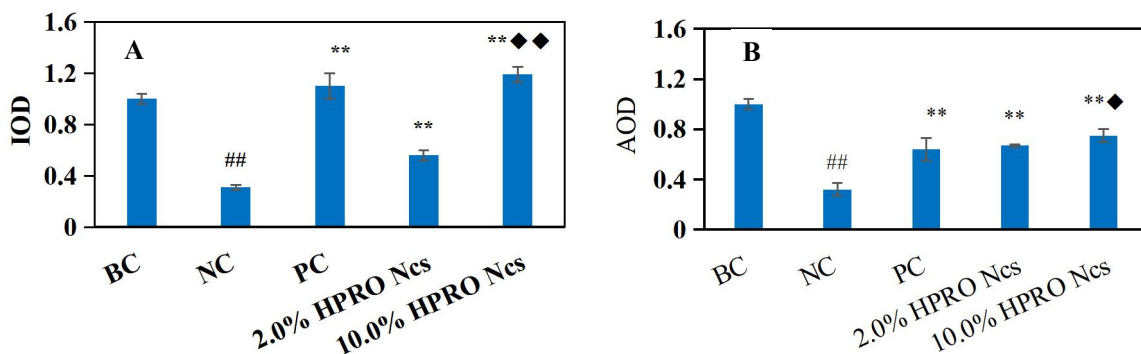


Figure 6. A) Mean IOD of immunofluorescence for collagen type I in ex vivo skin; B) Mean AOD of

immunofluorescence for collagen type IV in ex vivo skin. (Note: In statistical analysis using the t-test, significance is indicated as follows: compared to the BC group, # denotes P-value < 0.05 and ## denotes P-value < 0.01; compared to the NC group, * indicates P-value < 0.05 and ** indicates P-value < 0.01; compared to the 2.0% HPRO Ncs group, ◆ represents P-value < 0.05 and ◆◆ represents P-value < 0.01.)

The results presented in Figure 6A illustrate the cumulative optical density values (IOD) of collagen type I immunofluorescence in ex vivo skin samples. Figure 6B illustrates the average optical density values (AOD) of collagen type IV immunofluorescence in the same samples. As shown in Figure 6A, compared with the NC group, the IOD values for collagen type I immunofluorescence significantly increased in both the 2.0% and 10.0% HPRO Ncs groups ($P < 0.01$), with improvement rates of 80.65% and 283.87%, respectively. Furthermore, when compared with the BC group, the IOD value for collagen type I immunofluorescence was even greater in the 10.0% HPRO Ncs group, exhibiting an improvement rate of 19%.

In Figure 6B, it is evident that compared with the NC group, there was a significant increase in AOD values for collagen type IV immunofluorescence within both the 2.0% and 10.0% HPRO Ncs groups ($P < 0.01$), with corresponding improvement rates of 109.38% and 134.38%, respectively.

3.6. Efficacy of Human Skin in Wrinkle Resistance and Firmness

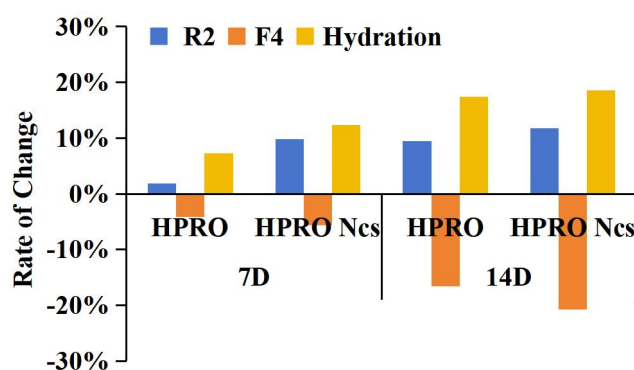


Figure 7. Efficacy testing data for HPRO Ncs and HPRO in human.

The data presented in Figure 7 illustrate the skin metrics following continuous application of 10.0% HPRO Ncs serum and 10.0% HPRO serum over a 14-day period. It is evident that after both 7 and 14 days of use, treatment with HPRO Ncs serum resulted in superior improvements in skin elasticity (R2), firmness (F4), and hydration levels compared to those treated with HPRO serum. Notably, the change rate for F4 exhibited a negative value; thus, a larger absolute value indicates a more significant improvement in firmness. After just 7 days of application, the enhancement effect on skin elasticity from the HPRO Ncs serum was markedly better than that observed with the standard HPRO serum treatment. This finding suggests that HPRO Ncs may facilitate faster improvements in skin elasticity.

4. Discussion

HPRO is recognized as one of the "three major anti-aging giants" in cosmetic ingredients. Its anti-aging effects are milder than retinoids, but it is non-irritating and more stable than peptide-based components that may lose effectiveness in formulations. This makes HPRO a promising raw material for all skin types. However, HPRO's penetration into the skin is limited because of its water-soluble nature. To address this issue, flexible liposomes were employed to encapsulate HPRO, leading to the formulation known as HPRO Ncs. The freshly prepared HPRO Ncs demonstrated a Zeta average particle size of (23.25 ± 0.80) nm and a PDI value of 0.299, indicating a narrow vesicle size distribution, uniform particles, and good stability as a pale yellow transparent liquid.

The patch test and chick embryo chorioallantoic membrane irritation assessment showed that HPRO Ncs is mild and non-irritating to the skin. Transdermal penetration tests indicated that after applying a 10% concentration of HPRO Ncs serum for 5h, 8h, and 12h, the cumulative

penetration of HPRO was 2.2 times, 2.53 times, and 2.6 times that of the control group, respectively. This demonstrates that flexible liposome technology enhances the permeability of HPRO.

Efficacy studies demonstrated significant increases in type I and IV collagen levels in ex vivo skin treated with either 2.0% or 10.0% concentrations of HPRO Ncs over seven days compared to NC groups subjected to UVA/UVB irradiation. Improvements for type I collagen were recorded at rates of 80.65% for the lower concentration group versus an impressive 283.87% for the group receiving treatment with 10.0% HPRO Ncs. This represents a 19.0% increase over controls compared to the blank group. Additionally, improvement rates for type IV collagen were noted as 109.38% and 134.38%, respectively.

Further human trials over fourteen days indicated marked enhancements in three dimensions: skin elasticity, firmness, and moisture content. All these parameters showed notable improvements beyond baseline measurements associated solely with standard use of HPRO. Interestingly, even after seven days of use, the benefits conferred by HPRO Ncs on skin elasticity, firmness, and moisture content were significant. This suggests that the flexible liposome encapsulation method allows for rapid enhancement of overall skin condition while accelerating the anti-aging effects associated with HPRO.

The findings from this research suggest that HPRO Ncs can be effectively utilized across various dosage forms within anti-aging cosmetics, particularly aqueous formulations. These formulations achieve favorable outcomes characterized by high stability, mildness without irritation, smaller particle size, and excellent deformability inherent to flexible liposomes. Furthermore, the affinity between lipid bilayers and human skin enhances both retention rates within the skin and bioavailability. This results in improved anti-aging efficacy while simultaneously mitigating risks related to hydroxylpropyl tetrahydropyran triol deactivation and discoloration.

5. Conclusion

This study successfully developed HPRO Ncs using flexible liposome encapsulation technology. The focus was on transdermal permeation efficiency, bioavailability, and anti-aging efficacy. The results showed that the flexible liposome particles in HPRO Ncs had a small particle size and narrow distribution, indicating good stability. Safety assessments confirmed that HPRO Ncs were non-irritating and that no adverse reactions occurred in a patch test with 30 human subjects. HPRO Ncs significantly enhanced penetration and retention of HPRO in the skin. 2.0% and 10.0% of HPRO Ncs increased type I collagen expression by 80.65% and 283.87%, respectively, while type IV collagen expression rose by 109.38% and 134.38%. In human efficacy tests, continuous use of 10% HPRO Ncs for fourteen days improved skin elasticity, firmness, and moisture content by approximately 11.76%, 20.81%, and 18.60%, respectively. Significant improvements were observed as early as seven days into treatment, confirming rapid anti-aging effects.

In summary, through flexible nanocarrier technology, HPRO Ncs effectively addressed issues related to low permeability and deactivation commonly associated with traditional formulations. It also exhibited high stability, enhanced permeability, gentleness, and efficacy, thus providing an innovative solution for developing efficient aqueous-based anti-aging cosmetics across various product forms. In this study, we primarily focused on the effects of HPRO Ncs in promoting the expression of type I and type IV collagen. Future research will further investigate the impact of HPRO Ncs on the generation of various GAGs and PGs, aiming to provide additional scientific evidence to substantiate the anti-aging effects of HPRO Ncs.

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