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"Preparation and Characterization of PDRN-Modified Ceramide Cationic Nanoemulsion (PDRN-CER-CNE) for Anti-Photoaging Cosmetics"

Huanfan He [1], Xiaojing Pei [1,*], Ze Zhang [1], Shujing Li [1], Yifan He [1], Hong Meng [1,*], Yinmao Dong [1], Li Chen [2], Li Lan [2]

[1] School of Light Industry Science and Engineering, Beijing Technology and Business University;
[2] PECHOIN Global Cutting-Edge Science Institute

1. Introduction

Skin photoaging is a complex physiological process primarily caused by cumulative environmental insults, particularly solar radiation. It is characterized by progressive disruption of the skin's multilayered structure, dysregulation of cellular signaling pathways, and molecular-level alterations, ultimately manifested as critical functional abnormalities including impaired barrier function, imbalanced pigment metabolism, and degradation of the dermal matrix [1, 2]. The solar spectrum comprises radiation across multiple wavelengths, with the full spectrum partitioned into ultraviolet (UV, 5%), visible light (VL, 45%), and infrared (IR, 50%). UV radiation (UVA: 315-400 nm; UVB: 280-315 nm) primarily mediates DNA damage, oxidative stress, and inflammatory responses, whereas VL (400-700 nm) and IR (>700 nm) predominantly contribute to oxidative damage and mitochondrial dysfunction, thereby amplifying reactive oxygen species (ROS) production [3]. Clinically, these effects present as diminished barrier function, hyperpigmentation, wrinkle formation, and even skin cancer [4]. Therefore, mitigating oxidative stress and enhancing antioxidant capacity represent primary strategies to counteract skin photoaging.

Ceramides, constituting 40–50% of stratum corneum lipids, play essential roles in maintaining epidermal barrier function and regulating keratinocyte differentiation [5]. By reinforcing the structural integrity of the stratum corneum, ceramides strengthen the skin's defense mechanisms against photodamage. However,

their extremely low aqueous solubility and poor transdermal permeability have hindered clinical translation, driving the development of nanodelivery systems to address these physicochemical limitations. Consequently, nanocarrier-based ceramide delivery systems have emerged as a focus of current research. Nanoencapsulation not only improves ceramide solubility and chemical stability but also facilitates their transdermal delivery efficiency.

Polydeoxyribonucleotides (PDRN), DNA polymers (50-1500 kDa) derived from salmonid testicular cells, exhibit a double-helical structure that activates adenosine A2A receptor signaling, thereby mediating diverse biological effects [6]. PDRN suppresses UV-induced matrix metalloproteinase-1 (MMP-1) expression via inhibition of nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. Moreover, it enhances collagen synthesis by activating the transforming growth factor-β (TGF-β)/Smad axis, thereby attenuating skin photoaging phenotypes [7]. However, their high molecular weight (>500 kDa) limits penetration efficiency via conventional transdermal routes, posing a major barrier to clinical translation.

To overcome these limitations, a PDRN-modified ceramide cationic nanoemulsion (PDRN-CER-CNE) was developed via electrostatic self-assembly. This system leverages electrostatic interactions between the cationic nanoemulsion surface and the anionic phosphate backbone of PDRN, enabling synergistic effects between ceramide (barrier restoration) and PDRN (anti-inflammatory activity). The proposed strategy addresses the transdermal limitations of ceramide (low solubility) and PDRN (high molecular weight), with co-delivery efficiency being markedly enhanced. Furthermore, the inherent physicochemical stability of nanoemulsions ensures compatibility with cosmetic formulations such as creams and lotions, providing a practical platform for developing multi-target anti-photoaging products.

2. Materials and Methods

Phytosphingosine was dissolved in a mixture of octyldodecanol and caprylic/capric triglyceride at high temperature with continuous stirring. After cooling, ceramides, linoleic acid, cholesterol, lecithin, and pentaerythritol tetrakis(bis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate)) were added to the oil phase. Separately, steareth-21 was dissolved in glycerol-containing water under magnetic stirring. The oil and aqueous phases were mixed and homogenized using a high-speed blender (8,000 rpm, 5 min), followed by homogenization with a high-pressure homogenizer. The pH of the nanoemulsion was adjusted to

5.5 ± 0.1 using citric acid solution to obtain ceramide cationic n-anoemulsions (CER-CNE).

CER-CNE was slowly added dropwise into PDRN solution under magnetic stirring. The PDRN concentration was optimi-zed based on particle potential measurements. The resulting PDRN-CER-CNE was collected after stabilization.

PDRN-CER-CNE was incorporated into a cream base at l-ow temperature to prepare the final product.

3. Results

3.1 The impact of PDRN on the zeta potential of CER-CNE

It was observed that the zeta potential of the CER-CNE system stabilized at a PDRN concentration of 6 mg / mL, which was selected as optimal.

Table 1. Impact of PDRN on the zeta potential of CER-CNE.

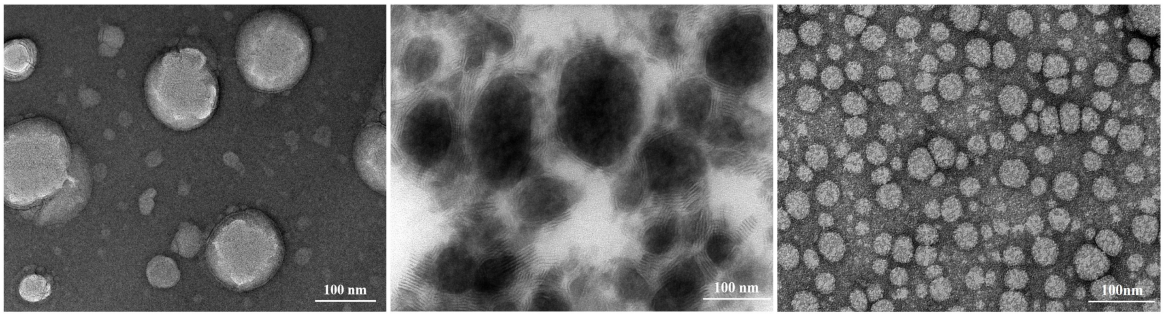
PDRN (mg/mL)	0	1	2	4	6	10	15	20
Zeta potential(mV)	+35.34	+29.21	-5.998	-28.14	-34.08	-37.47	-34.39	-36.09

3.2 Characterization of nanoemlusion

PDRN-CER-CNE, prepared via electrostatic adsorption, exhibited an increased particle size of 124.52 ± 2.89 nm and a reversed zeta potential from 28.56 ± 2.67 mV to -34.08 ± 2.12 mV. Transmission electron microscopy (TEM) imaging further revealed a spherical morphology with lamellar PDRN adsorption, confirming successful surface modification.

Table 2. Characterization of nanoemulsions.

	Particle size （nm）	**PDI**	**Zeta potential (mV)**
CER-CNE	112.61±2.42	0.09±0.013	28.56±2.67
PDRN-CER-CNE	124.52±2.89	0.15±0.019	-34.08±2.12



a **b** **c**

Figure 1. TEM images of (a) CER-CNE; (b) PDRN-CER-CNE and (c) PDRN-CER-CNE in the cream

3.3 Cellular uptake of PDRN-CER-CNE

PDRN-CER-CNE enhanced HaCaT cellular uptake via adenosine A2A receptor-mediated targeting and the nanoemulsion structure. This enhancement was confirmed by confocal laser scanning microscopy (CLSM), with the PDRN-CER-CNE group exhibiting significantly higher fluorescence intensity than the control group (Figure 2).

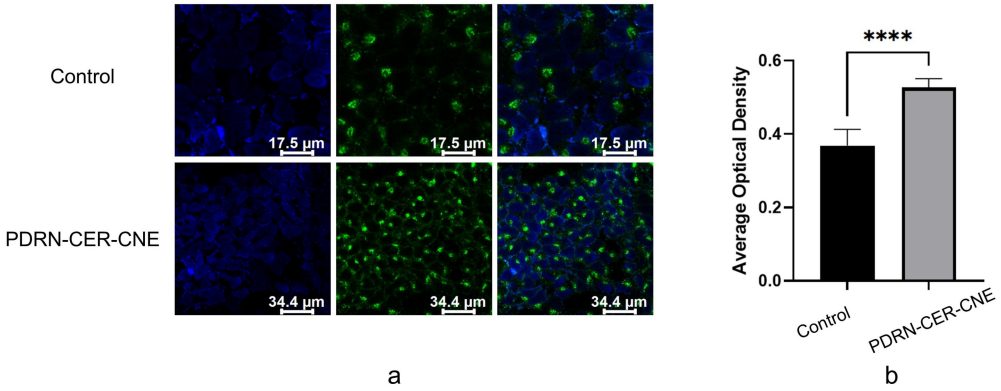


Figure 2. (a) Confocal images of PDRN-CER-CNE bound to HaCaT cells; (b) Difference in average optical density between PDRN-CER-CNE and Control

4. Discussion

This study systematically evaluated the effect of PDRN concentration on the surface charge properties of CER-CNE nanoemulsions through dropwise addition of varying PDRN concentrations. The zeta potential of the CER-CNE nanoemulsions decreased progressively with increasing PDRN concentration. At 6 mg/mL PDRN, the zeta potential reduction plateaued, with no further observable changes. Thus, 6 mg/mL PDRN was selected as the optimal concentration for the CER-CNE system.

PDRN-CER-CNE was successfully prepared via electrostatic adsorption. Key physicochemical properties—including particle size, zeta potential, and morphology—were compared before and after modification. As summarized in Table 2, the particle size of PDRN-CER-CNE (124.52 ± 2.89 nm) increased by approximately 10 nm compared to unmodified CER-CNE, with a polydispersity index (PDI) of 0.15 ± 0.019. The zeta potential reversed from 28.56 ± 2.67 mV (CER-CNE) to -34.08 ± 2.12 mV (PDRN-CER-CNE), confirming successful surface charge inversion due to PDRN modification. TEM revealed spherical structures for both CER-CNE and PDRN-CER-CNE (Figure 1a-b). Laminar PDRN adsorption was evident in PDRN-CER-CNE

(Figure 1b). Furthermore, Figure 1c demonstrates that PDRN-CER-CNE maintains structural integrity within the cream matrix, with no disruption to its nanodroplet architecture.

The cellular uptake efficiency of PDRN-CER-CNE was evaluated in HaCaT cells using CLSM. Cells were incubated with the formulations for 30 min prior to imaging. As shown in Figure 2, PDRN-CER-CNE incorporated into the cream exhibited significantly enhanced cellular uptake. In the control group (free PDRN in cream), minimal fluorescent signals were observed around the cell periphery (Figure 2a), whereas PDRN-CER-CNE treatment resulted in markedly higher intracellular fluorescence intensity (Figure 2b). This demonstrates that the nanoemulsion structure promotes PDRN delivery. Furthermore, the enhanced accumulation of PDRN-CER-CNE in keratinocytes is attributed to the specific binding of PDRN to adenosine A2A receptors expressed on these cells.

5. Conclusion

In this study, a PDRN-modified ceramide cationic nanoemulsion (PDRN-CER-CNE) was successfully constructed through electrostatic self-assembly. This system addresses the dual challenges of low ceramide transdermal efficiency and poor PDRN macromolecule delivery. Dynamic light scattering and TEM analyses confirmed stable PDRN adsorption on the cationic nanoemulsion surface via electrostatic interactions. The composite exhibited a uniform particle size of 124.52 ± 2.89 nm with a surface charge reversal from +28.56 ± 2.67 mV to -34.08 ± 2.12 mV. The system synergizes ceramide-mediated skin barrier restoration with PDRN's A2A receptor targeting in keratinocytes. CLSM demonstrated substantially enhanced cellular uptake compared to the control group.

By integrating antioxidant, anti-inflammatory, and barrier-repair functions, PDRN-CER-CNE shows potential for developing multifunctional anti-photoaging cosmetics including sunscreens and repair serums to counteract UV-induced skin damage.

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