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"Polymeric nanomicelles with enhanced stability of retinoids through targeted peptide conjugation and efficient drug loading capabilities."

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1. Introduction

Skin aging is a multifactorial biological process influenced by intrinsic factors (chronological aging) and extrinsic factors such as ultraviolet (UV) radiation, pollution, and lifestyle habits. These cumulative influences lead to progressive physiological changes in the skin, including collagen degradation, reduced elastin fiber organization, decreased hyaluronic acid content, and the formation of wrinkles. Among these, the degradation of collagen and the structural disorganization of the extracellular matrix (ECM) are considered the most prominent hallmarks of aging skin, resulting in loss of firmness, elasticity, and overall skin integrity.

To counteract these effects, a wide array of active ingredients has been employed in topical and systemic dermatological therapies. Among them, retinoids—vitamin A derivatives such as tretinoin, retinal, and retinol—are extensively studied and clinically validated for their potent anti-aging properties. Retinoids exert their effects by stimulating fibroblast proliferation, enhancing collagen synthesis, and promoting epidermal turnover. However, despite their efficacy, conventional formulations of retinoids are limited by poor water solubility, chemical instability (especially in light and oxygen), and a narrow therapeutic window that often leads to skin irritation, dryness, and erythema when not properly delivered.

A critical challenge in the field of dermatological drug delivery lies in the non-specific nature of most conventional drug delivery systems (DDSs). These systems often disperse active ingredients indiscriminately across both target and non-target cells, resulting in reduced therapeutic efficacy and increased risk of side effects. This is particularly problematic in skincare applications where localized, cell-specific delivery is crucial for ensuring safety and maximizing benefit.

To overcome these limitations, recent advances in targeted drug delivery platforms have focused on the use of nanotechnology-based carriers such as polymeric micelles, liposomes, dendrimers, and solid lipid nanoparticles, which offer enhanced stability, controlled release, and improved skin penetration. Moreover, targeting strategies that exploit cell-specific receptors or surface markers have shown promise in improving delivery precision. In the context of skin aging, fibroblasts—which reside in the dermal layer—are of particular interest due to their central role in ECM remodeling and collagen production. Thus, selective targeting of fibroblasts represents a highly effective strategy for improving the therapeutic index of anti-aging agents like retinoids.

In this study, we present the development of a novel polymeric nanomicelle drug delivery system (DDS) conjugated with the collagen-derived peptide KTTKS (Lys–Thr–Thr–Lys–Ser). KTTKS is known to bind specifically to the protease-activated receptor-2 (PAR-2), which is highly expressed on the surface of dermal fibroblasts. By functionalizing the micelle surface with KTTKS and encapsulating retinoids within the micelle core, our system aims to achieve dual benefits: enhanced retinoid stability and fibroblast-specific targeting.

2. Materials and Methods

Materials

Two types of poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCL) copolymers were provided by Mizon (Korea): one with a short PEO block (Mn = 2,000 g•mol^{-1}) and the other with a long PEO block (Mn = 5,000 g•mol^{-1}), both with a fixed PCL block of 2,000 g•mol^{-1}. MEL was obtained from DK Bio (Korea). The peptide KTTKS was synthesized with a cysteamide group conjugated at the C-terminus and supplied by Peptron (purity >90%, Korea).

Anhydrous methylene chloride, pyridine, N,N-diisopropylethylamine (DIPEA), 4-nitrophenylchloroformate, N-(2-aminoethyl)maleimide trifluoroacetate salt, retinol, and retinal were purchased from Sigma-Aldrich (USA). Acetonitrile was obtained Daejungchem Co., Ltd. (Korea). Hydroxypinacolone retinoate (HPR) was obtained from Shanghai Sunway Pharmaceutical Technology Co., Ltd. (China). Tetrahydrofuran (THF, purity >98%) was pur-chased from TCI (Japan) and used as a removable solvent.

Human dermal fibroblast (HDF) cells were obtained from the American Type Culture Collection (ATCC, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Welgene (Korea) and used as a reagent-grade culture medium. Fetal bovine serum (FBS), penicillin–streptomycin (P/S), and phosphate-buffered saline (PBS) were supplied by GIBCO-Thermo Fisher Scientific (USA). All experiments were conducted using double-distilled deionized (DI) water.

Fabrication of MEL-Linker

To synthesize the orthoformate intermediate, MEL (66.0 g, 0.15 mol), 4-nitrophenylchloroformate (60.7 g, 0.30 mol, 2.0 eq), and pyridine (30.4 mL, 0.38 mol, 2.5 eq) were dissolved in anhydrous dichloromethane (DCM, 3.0 L, 0.05 M) and stirred at room temperature for 1 h. The reaction was quenched with distilled water, followed by phase sep-aration. The organic layer was washed sequentially with saturated NH_4 Cl and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to obtain orthoformate intermediate.

Orthoformate intermediate (3.81 g, 0.06 mol) and N,N-diisopropylethylamine (DIPEA, 34.9 mL, 0.19 mol, 3.0 eq) were dissolved in acetonitrile (ACN, 1.3 L, 0.05 M). N-(2-aminoethyl)maleimide trifluoroacetate salt (32.1 g, 0.13 mol, 2.0 eq) was added, and the mixture was stirred at room temperature for 18 h. After completion, the solvent was evaporated under reduced pressure. The residue was partitioned between Dichloromethane (1.3 L) and distilled water (1.3 L), stirred for 30 min, and the organic phase was separated. The organic layer was washed with brine, dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography to obtain MEL-Linker.

Preparation of Retinoids-TNM-KTTKS Nanomicelles

To prepare Retinoids-TNM-KTTKS, PEO-b-PCL (1% w/w) and MEL-Linker (0.2 mg mL^{-1}) were dissolved in tetrahydrofuran (THF) and stirred at 40 °C for 10 min in a round-bottom flask. For wrinkle care ingredients, retinoids (0.01% w/w relative to the total mass) were also in-corporated into the solution.

Deionized (DI) water (9.9 mL) was then added dropwise using a syringe pump (Pump 11 Elite, Harvard Apparatus, USA) at a flow rate of 100 µL min^{-1} under continuous stirring to induce nanomicelle formation. After self-assembly, THF was removed by rotary evaporation at 40 °C for 20 min. The dispersion was filtered through a 0.2 µm syringe filter to remove unencapsu-lated retinoids.

Finally, KTTKS was added in a predetermined amount, and the mixture was gently stirred at room temperature for 12 h to achieve conjugation, resulting in the final Retinoids-TNM-KTTKS nanomicelles.

Characterization of Retinoids-TNM-KTTKS

The hydrodynamic diameters and zeta potentials of Retinoids-TNMs and Ret-in-oids-TNM-KTTKS were measured using dynamic light scattering (DLS) with an ELS-Z an-alyzer (Otsuka Electronics, Japan) at 25 °C. The morphological characteristics of the na-nomicelles were observed by energy-filtering transmission electron microscopy (EF-TEM; LIBRA 120, Carl Zeiss, Germany).

To evaluate the encapsulation efficiency (EE) of retinoids in the Retinoids-TNM-KTTKS, 1 mg of freeze-dried nanomicelles was dissolved in 1 mL of methanol. The concentration of en-capsulated retinoids was determined by UV–visible spectrophotometry (V-730, JASCO, JA-PAN) based on the standard calibration curves prepared using known concentrations of each retinoid (Retinol, Retinal, and Hydroxypinacolone Retinoate [HPR]) in methanol. Ab-sorbance was measured at 325 nm for Retinol and 380 nm for Retinal and for HPR 360nm. The en-capsulation efficiency (%) was calculated by comparing the amount of retinoids detected in the micelles to the initial amount added. The EE of the prepared nanomicelles was found to be in the range of 85–90%.

Cell culture and viability assay

Human dermal fibroblasts (HDF) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin–streptomycin (Gibco, USA). Cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

The cytotoxicity of Retinoids-TNM-KTTKS was evaluated using the EZ-Cytox Cell Viability Assay Kit (EZ-3000, Dogen, Korea). Retinoids were dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO in the culture medium was controlled to be below 1.0 ppm.

HDF cells were seeded in 96-well plates at a density of 4×10^3 cells/well and incubated overnight. The culture medium was then removed, and the cells were treated with various concentrations of Retinoids-TNM-KTTKS diluted in DMEM for 24 h. After treatment, cells were

washed with phosphate-buffered saline (PBS), and 100 µL of EZ-Cytox solution was added to each well. The plate was incubated for 2 h at 37 °C, and absorbance was measured at 450 nm using a microplate reader (Spark, Tecan, Switzerland).

Measurement of Collagen Production

Human dermal fibroblasts (HDF) were seeded in 96-well plates at a density of 4×10^3 cells/well and incubated for 24 h under standard conditions (37 °C, 5% CO_2 , humidified at-mosphere). After incubation, the cells were treated with Retinoids-TNM-KTTKS diluted in DMEM to achieve final retinoid concentrations ranging from 0.0001 to 1 ppm.

Following treatment, the cells were washed with phosphate-buffered saline (PBS) and fixed in situ with ice-cold 70% ethanol. The ethanol was removed, and each well was rinsed with de-ionized (DI) water. A Sirius red–picric acid staining solution was then added, and the plate was incubated overnight at 4 °C with gentle agitation.

After staining, unbound dye was removed by repeatedly rinsing the wells with DI water until no residual dye was observed. To extract the collagen–dye complexes, 1 N NaOH was added to each well and incubated at room temperature for 10 min with gentle agitation. The absorbance of the solubilized dye was measured at 540 nm using a microplate reader (Spark, Tecan, Switzerland).

Stability Evaluation of Retinoids in Retinoids-TNM-KTTKS

To evaluate the stability of retinoids encapsulated within Retinoids-TNM-KTTKS, both free retinoid solutions and Retinoids-TNM-KTTKS were prepared at the same retinoid con-centrations. The samples were stored under three different temperature conditions—high temperature (45 °C), room temperature (25 °C), and refrigeration (4 °C)—for a period of three months.

To analyze the encapsulated retinoids, micelle structures were disrupted by treating Ret-in-oids-TNM-KTTKS samples with methanol, followed by filtration using a 0.2 µm syringe filter to remove micelle residues and obtain the released retinoid content.

The concentrations of three retinoid types—retinol, retinal, and hydroxypinacolone retinoate (HPR)—were quantified using UV–visible spectroscopy (V-730, JASCO, Japan). Absorbance measurements were performed at 325 nm for retinol, 380 nm for retinal, and 360 nm for HPR. Quantification was based on standard calibration curves generated using known concentra-tions of each retinoid.

The stability of both free retinoid and Retinoids-TNM-KTTKS was determined by calculating the relative retention of absorbance values over time, based on measurements at Day 1. The stability (%) was calculated using the following equations:

For free retinoid:

$$\text{Stability (\%)} = (\text{Absorbance on each day} / \text{Absorbance on Day 1}) \times 100$$

For Retinoids-TNM-KTTKS:

$$\text{Stability (\%)} = (\text{Absorbance on each day} / \text{Absorbance of Retinoids} - \text{TNM} - \text{KTTKS on Day 1}) \times 100$$

3. Results

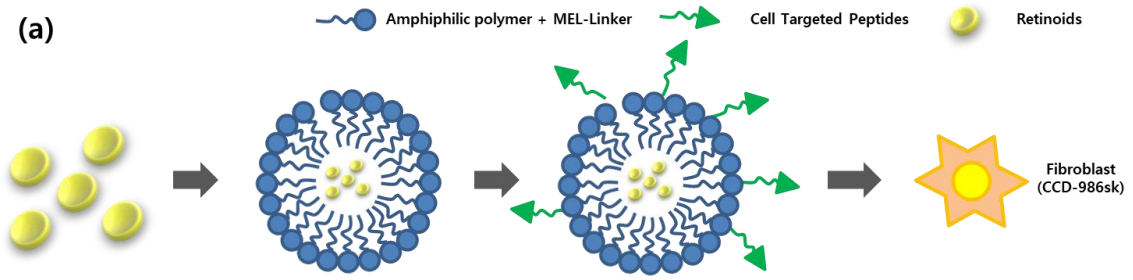
Design of a fibroblast-targeting Retinoids-TNM-KTTKS system.

To enable fibroblast-specific delivery of retinoids, we developed a smart polymeric nanovehicle (TNM) platform functionalized with the collagen-derived peptide KTTKS. KTTKS is a type I collagen subfragment comprising the minimal sequence required for binding to the protease-activated receptor-2 (PAR-2), which is selectively expressed on the membrane of dermal fibroblasts. Leveraging this specific ligand–receptor interaction, we aimed to enhance the cellular uptake and therapeutic efficacy of retinoids for anti-winkle applications.

To achieve targeted functionality, KTTKS was conjugated to the surface of micelles through a lipid linker system. We synthesized a maleimide-functionalized lipid linker (MEL), starting from MEL molecules modified at the hydrophilic head with a maleimide group. During micellization, the MEL linker successfully co-assembled with poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCL) due to hydrophobic interactions between the dialkyl chains and the PCL block. This self-assembly process enabled the maleimide moieties to be exposed on the micelle surface.

Subsequently, the KTTKS peptide—engineered to include a cysteamine group at the C-terminus—was covalently conjugated to the micelle surface via a thiol–maleimide click reaction, resulting in the formation of KTTKS-functionalized TNMs (Retinoids-TNM-KTTKS). Retinoids, a known promoter of fibroblast proliferation, was initially used as a model compound to validate the drug-loading and delivery capabilities of the nanocarrier. Finally, retinoids were encapsulated into the hydrophobic micelle core, yielding Retinoids-TNM-KTTKS as the final delivery system (Fig. 1a).

Retinoids-TNM-KTTKS enhances cellular uptake via PAR-2 receptor-mediated endocytosis, followed by lysosomal escape, thereby improving intracellular delivery and bioactivity of the loaded retinoids. This targeted delivery approach is expected to significantly enhance anti-wrinkle efficacy compared to non-targeted (Fig. 1b).



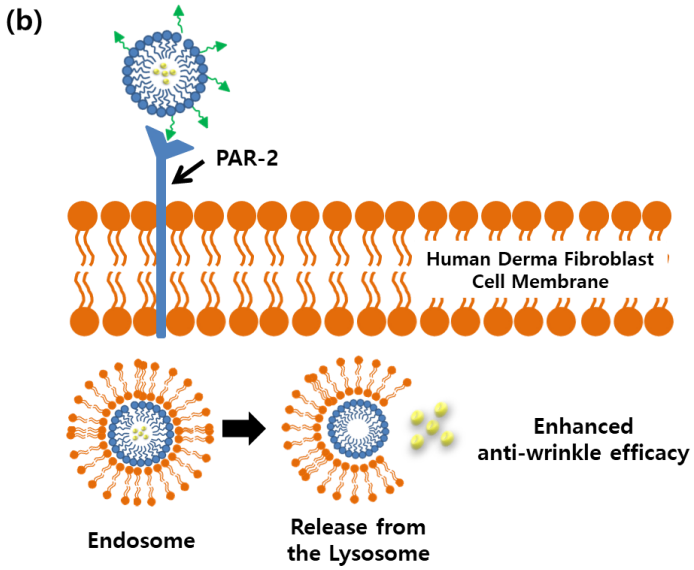


Figure 1. *(a) Schematic representation of the fabrication process of Retinoids-loaded targeted nanomicelles (Retinoids-TNM-KTTKS), constructed via co-assembly of PEO-b-PCL and MEL-KTTKS linker followed by retinoid encapsulation.(b) Proposed mechanism of anti-wrinkle activity: Retinoids-TNM-KTTKS targets fibroblast cells through PAR-2 receptor-mediated endocytosis, enhancing intracellular delivery of retinoids and promoting collagen synthesis for improved anti-aging efficacy.*

Characterization of Retinoids-TNM-KTTKS.

The physicochemical properties of the fabricated nanomicelles were evaluated to confirm the successful formation of Retinoids-TNM-KTTKS.

According to dynamic light scattering (DLS) analysis, the hydrodynamic diameter of Retinoids-TNM-KTTKS were approximately 45.43 ± 1.59 nm, and the zeta potential was measured to be -0.85 ± 0.95 mV. (Fig. 2a~d).

The morphology of the nanocarriers was further investigated using energy-filtering transmission electron microscopy (EF-TEM). (Fig. 2a~c) The micelles exhibited a spherical shape with uniform size distribution and a well-defined core–shell structure, consistent with the DLS results.

To evaluate the encapsulation efficiency (EE) of the retinoids within the micelles, UV–visible spectrophotometry was performed after dissolving freeze-dried samples in methanol. Standard calibration curves were established for each retinoid—retinol (λ = 325 nm), retinal (λ = 380 nm), and hydroxypinacolone retinoate [HPR] (λ = 360 nm). The EE of the Retinoids-TNM-KTTKS ranged from 85% to 90%, confirming the efficient entrapment of hydrophobic active ingredients in the micelle core. (Fig. 2d).

These results collectively indicate that the Retinoids-TNM-KTTKS nanocarriers were successfully fabricated with appropriate size, stability, morphology, and drug-loading capacity suitable for fibroblast-targeted delivery.

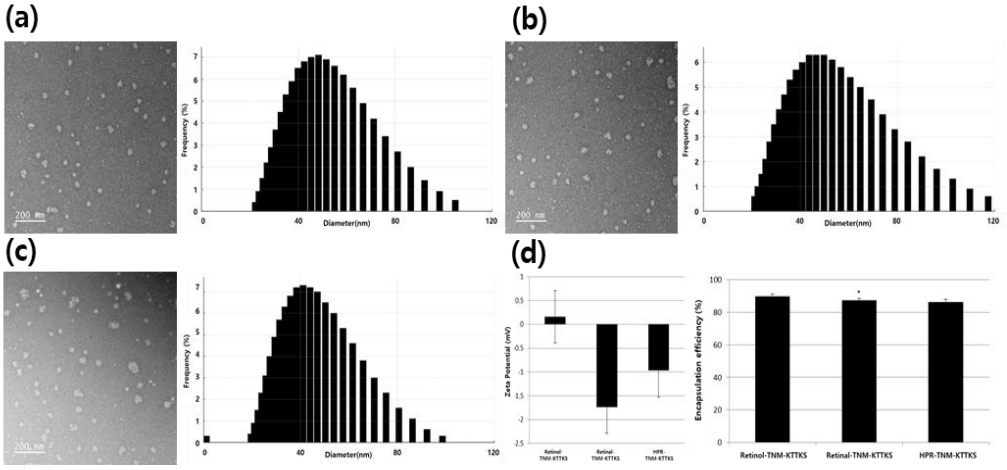


Figure 2.

Transmission electron microscopy (TEM) image and particle size distribution of (a) Retinol-TNM-KTTKS, (b) Retinal-TNM-KTTKS, and (c) HPR-TNM-KTTKS nanomicelles.

(d) Zeta potential and encapsulation efficiency of the retinoid-loaded nanomicelles.

Error bars represent the standard deviation of the mean (n = 3). Statistical significance was determined using one-way analysis of variance (ANOVA), with $p < 0.05$ considered significant.

Effect of Retinoids-TNM-KTTKS on Cell Viability and Collagen Production.

Retinoids are well-established active ingredients for anti-wrinkle applications. In this study, we engineered Retinoids-TNM-KTTKS and evaluated its ability to activate human dermal fibroblast (HDF) cells.

Since retinoid-based compounds are known to induce skin irritation and cytotoxicity at high concentrations, it is crucial to develop delivery systems that can maximize efficacy at low concentrations while minimizing safety concerns. This was examined through cytotoxicity testing.

As a result, both free retinoids and the Retinoids-TNM-KTTKS exhibited no detectable cytotoxicity under the tested conditions, confirming their biocompatibility in HDF cells.(Fig. 3a).

To further quantify the biological efficacy of Retinoids-TNM-KTTKS, we evaluated its ability to stimulate collagen synthesis in human dermal fibroblasts (HDFs).

Experimental results revealed that treatment with Retinoids-TNM-KTTKS led to a statistically significant increase in collagen production, ranging from 11.09% to 31.23% higher compared to treatment with the same concentration of free retinoids. (Fig. 3b~d).

These findings suggest that the enhanced biological activity of Retinoids-TNM-KTTKS is not solely attributed to the delivery of active ingredients, but also to the selective targeting of fibroblasts and efficient intracellular uptake facilitated by the nanocarrier system.

Therefore, Retinoids-TNM-KTTKS demonstrates strong potential as a high-performance delivery platform capable of improving collagen biosynthesis relative to conventional retinoids.

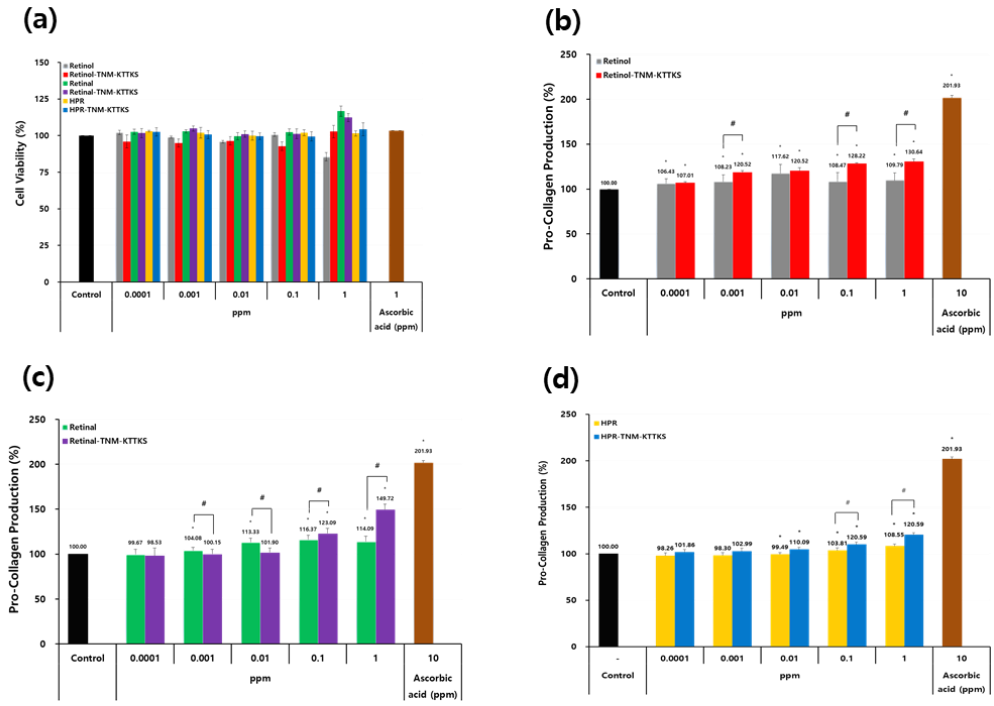


Figure 3.

(a) Cytotoxicity evaluation of free retinoids and Retinoids-TNM-KTTKS in human dermal fibroblasts (HDFs). Evaluation of collagen synthesis in human dermal fibroblasts (HDFs) stimulated by (b) Retinol and Retinol-TNM-KTTKS, (c) Retinal and Retinal-TNM-KTTKS, and (d) HPR and HPR-TNM-KTTKS nanomicelles.

Error bars represent the standard deviation of the mean (n = 9). Statistical significance was determined using one-way analysis of variance (ANOVA), with $p < 0.05$ considered significant.

Comparative Stability Analysis of Free and Encapsulated Retinoids.

The stability of both free retinoids and Retinoids-TNM-KTTKS was monitored over a period of three months under various storage conditions (4 °C, 25 °C, and 45 °C).

The nanomicelle-encapsulated retinoids exhibited significantly improved stability across all temperature conditions compared to their free counterparts.

In particular, under high-temperature conditions (45 °C), the residual content of free retinol, retinal, and HPR sharply decreased to approximately 35%, 10%, and below 60%, respectively, by Day 90.

In contrast, the corresponding Retinoids-TNM-KTTKS retained more than 80%, 45%, and 80% of their initial content under the same conditions.

Even at room temperature (25 °C) and refrigeration (4 °C), the encapsulated forms consistently demonstrated superior retention compared to the free retinoids, confirming an overall enhancement in stability.

These findings indicate that the TNM-KTTKS-based nanomicellar system effectively protects retinoids from thermal, oxidative, and photodegradation, thereby extending their shelf life and preserving biological activity.

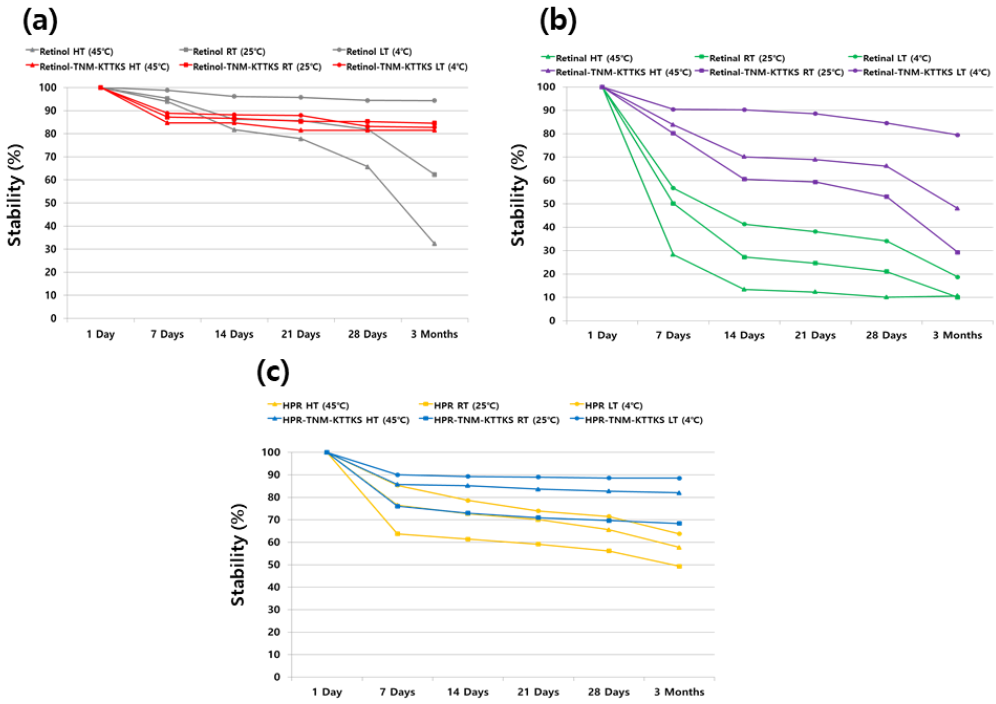


Figure 4.

Stability evaluation of (a) Retinol and Retinol-TNM-KTTKS, (b) Retinal and Retinal-TNM-KTTKS, and (c) HPR and HPR-TNM-KTTKS nanomicelles under various storage conditions (4 °C, 25 °C, and 45 °C) over a period of 90 days.

Residual retinoid content was quantified by UV–visible spectroscopy to assess the protective effect of nanomicellar encapsulation.

4. Discussion

In this study, we developed a nanomicellar delivery system (Retinoids-TNM-KTTKS) to enhance the bioavailability, safety, and efficacy of retinoid-based compounds for anti-aging applications. The results demonstrated that the nanocarrier system offers several advantages over free retinoids in terms of biocompatibility, functional activity, and physicochemical stability.

First, the cytotoxicity assay using human dermal fibroblasts (HDFs) confirmed that both free retinoids and Retinoids-TNM-KTTKS formulations did not induce any detectable toxicity under the tested concentrations, suggesting good biocompatibility of the system. This is particularly important, as retinoids are known to cause irritation and cytotoxicity at high doses. The ability of the TNM-KTTKS platform to deliver retinoids effectively at low concentrations while minimizing cellular stress highlights its potential as a safe topical.

More importantly, collagen synthesis assays revealed that Retinoids-TNM-KTTKS significantly enhanced collagen production compared to free retinoids. Quantitative analysis showed an increase of 11.09% to 31.23% in collagen levels in HDFs treated with the nanomicellar formulations. This improvement is likely due to the fibroblast-targeting capability of the KTTKS peptide and the enhanced cellular uptake facilitated by the nanocarrier structure, enabling more efficient intracellular delivery of the active compounds.

Furthermore, the encapsulated retinoids showed superior stability under various storage conditions. While free retinoids exhibited rapid degradation—particularly at elevated temperature (45 °C), where the residual content dropped below 35% for retinol and even 10% for retinal—the Retinoids-TNM-KTTKS formulations retained over 45% to 80% of their original content. This indicates that the nanomicellar system provides substantial protection against thermal and oxidative degradation. Enhanced stability was also observed at room temperature and refrigeration conditions, supporting the role of the nanocarrier in extending shelf-life and maintaining biological function.

Collectively, these findings suggest that TNM-KTTKS-based nanomicelles not only mitigate the inherent instability and irritation issues associated with retinoids but also improve their biological performance. This multifunctional delivery platform holds strong potential for future application in cosmetic and dermatological aimed at skin regeneration and anti-aging.

5. Conclusion

In conclusion, we successfully developed a retinoid-loaded nanomicellar delivery system, Retinoids-TNM-KTTKS, designed to enhance the stability, biocompatibility, and efficacy of retinoid compounds for anti-aging applications.

This system exhibited excellent biocompatibility in human dermal fibroblasts (HDFs), with no detectable cytotoxicity observed under the tested conditions. Furthermore, Retinoids-TNM-KTTKS significantly enhanced collagen synthesis compared to free retinoids, highlighting its improved biological activity through fibroblast-targeted delivery and efficient cellular uptake.

Importantly, nanomicellar encapsulation markedly improved the physicochemical stability of retinoids, particularly under elevated temperature conditions where free compounds typically undergo rapid degradation.

Collectively, these findings demonstrate the strong potential of TNM-KTTKS nanocarriers as a safe and effective platform for retinoid delivery, offering considerable advantages for the development of next-generation cosmetic and dermatological formulations aimed at skin regeneration and anti-aging.

Reference

[1]. Cheeun Song, Seung Ju Jang, Woo Hyeok Jeon, et al (2022) Nanomedicines for Therapy of Bladder Cancer. Korean J Urol Oncol 20:235-247.
[2]. Yousong Lee,a Seulgi Kim,a Jihye Seo, et al (2023) Fibroblast-targeting polymeric nanovehicles to enhance topical wound healing through promotion of PAR-2 receptor-mediated endocytosis. Biomater. Sci. 11:450.
[3]. Emine Kahraman a, Neşet Neşetoğlu b, Sevgi Güngör. et al (2018) The combination of nanomicelles with terpenes for enhancement of skin drug delivery. International Journal of Pharmaceutics. 551:133-140.
[4]. Uitto J, Olsen DR, Fazio MJ. (1989) Extracellular matrix of the skin: 50 years process. J.Invest Dermatol 92:61s-77s.
[5]. Nimni ME, Harkness RD. (1993) Molecular structure and functions of collagen. In Collagen 1-3.
[6]. Kim NM, Koo BS, Lee SK, et al (2007) Effect of Korean red ginseng on collagen biosynthesis and MMP-1 activity in human dermal fibroblast. J. Ginseng Res 31:86-92.