

“A Supramolecular Co-crystal Strategy for Enhancing the Protective Efficacy of Naringin Against Skin Photo-damage”

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1. Introduction

Photoaging is the main factor in extrinsic skin aging. Long-term exposure to ultraviolet radiation can cause the skin to produce excessive free radicals, trigger inflammatory responses, degrade extracellular matrix (such as collagen), and damage cellular DNA, thereby leading a series of issues including skin sagging, wrinkle deepening, pigmentation, and even skin cancer [1]. Consequently, the development and application of active pharmaceutical ingredients (APIs) with anti-photoaging effects have become a research hotspot in cosmetics and dermatology.

Naringin (Nar) is a natural dihydroflavone compound mainly derived from Rutaceae plants (e.g., grapefruit). It has many significant biological activities such as antioxidant, anti-aging, anti-inflammatory, and antibacterial properties [2,3]. Studies have shown that Nar can effectively scavenge free radicals and inhibit the expression of ultraviolet-induced matrix metalloproteinases, thereby protecting skin collagen and demonstrating promising anti-photoaging potential [4]. However, due to the polyphenolic hydroxyl groups in its molecular structure, Nar exhibits strong intra- and intermolecular forces, resulting in extremely low water solubility (1 mg/mL at 40 °C) [5]. This drawback severely limits its direct application in water-based cosmetic formulations, compromises its skin penetration and bioavailability, and consequently restricts the full expression of its skincare efficacy.

To improve the solubility and biological activity of poorly soluble API, supramolecular chemistry offers innovative solutions. Among these, supramolecular co-crystal technology involves combining an API with co-crystal formers (CCFs) within the same crystal lattice at a fixed stoichiometric ratio via non-covalent interactions (e.g., hydrogen bonding, π - π stacking), without altering the covalent structure of the API [6]. Selecting appropriate CCFs for the API is key to successful co-crystal design. The structure of a CCF typically contains aromatic rings, pyridine rings, or heteroatoms such as O and N, which are more prone to form intermolecular hydrogen bonds or π - π stacking interactions with flavonoids.

Based on this rationale, our study adopted a supramolecular co-crystal strategy, using Nar as the API and selecting ferulic acid (Fer), 4,4'-bipyridine (4B), and mandelic acid (Man) as CCFs.

First, molecular electrostatic potential surface (MEPS) was used for virtual screening of co-crystal formation to assess the likelihood of co-crystal production, followed by solvent evaporation to attempt the preparation of Nar co-crystals. Our article aimed to explore the principles of synthesizing Nar co-crystals and to thoroughly analyze their structural characteristics using a variety of characterization methods; to evaluate the improvement in Nar's water solubility upon co-crystal formation; and to further investigate, through in vitro cell experiments, the differences in the protective effects of Nar against skin photodamage before and after co-crystallization. Our study was expected to provide a theoretical and experimental basis for the development of novel cosmetic raw materials that enhance the bioavailability of Nar, and also serve as a reference for the supramolecular modification of other poorly soluble flavonoids.

2. Materials and Methods

2.1 Co-crystal design based on MEPS

The calculation of MEPS followed the method of Musumeci [7]. The maximum electrostatic potential point of the hydrogen bond donor and the minimum point corresponding to the hydrogen bond acceptor were used to calculate the α parameter and β parameter. The specific calculation formulas were as follows:

$$\alpha = 0.0000162MEP_{\max}^2 + 0.00962MEP_{\max}$$

$$\beta = 0.000146MEP_{\min}^2 - 0.00930MEP_{\min}$$

Where $MEP_{\min}$ and $MEP_{\max}$ were the local minima and maxima on the MEPS in $\text{kJ}\cdot\text{mol}^{-1}$. The electrostatic potential pairing energy was as follows:

$$E = -\sum_{ij} \alpha_i \beta_j$$

In the formula, α_i represented the hydrogen bond donor parameter, and β_j was the hydrogen bond acceptor parameter; E was the total interaction site pairing energy of these molecules. Therefore, the ability to form co-crystal could be estimated as:

$$\Delta E = E_{CC} - mE_1 - nE_2$$

In the formula, E_1 was the electron interaction site energy of compound 1; E_2 was the electron interaction site energy of compound 2; E_{CC} was the co-crystal interaction site pairing energy, formed by combining the stoichiometric ratio $m:n$. The lower the value of E_{CC} , the more likely a co-crystal was to form.

If we assumed that the pure and co-crystal states were in equilibrium, then the probability of populating the co-crystal state would be described by a Boltzmann distribution:

$$P = \frac{e^{-\Delta G/RT}}{1 + e^{-\Delta G/RT}}$$

where ΔG was the free energy difference between the pure and the co-crystal states.

$$\Delta G = \Delta E + 11\text{kJ}\cdot\text{mol}^{-1}$$

2.2 Preparation of Naringin-ferulic acid (Nar-Fer) Co-crystal

Nar and the CCF were dissolved in 15 mL of 50% ethanol at a molar ratio of 1:1, and the mixture was magnetically stirred at 350 r/min for 24 hours. After mixing, the mouth of the bottle was completely covered with a sealing film, and 7–10 holes were pierced in the film to allow solvent evaporation. The solution was allowed to evaporate at room temperature in a fume hood for 2–3 days. When a large amount of solid precipitated from the solution and adhered to the walls and bottom of the beaker, the solid was collected, transferred to an oven, and dried overnight at 40°C . Finally, the solid was collected, sealed, and stored in a refrigerator at 4°C .

2.3 Structural characterization

Powder X-ray diffraction (PXRD) measurements were performed using an X'pert multifunctional powder X-ray diffractometer (PANalytical, Holland). Differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) were conducted on a DSC/DTA-TG STA 449 F5 Jupiter® instrument (NETZSCH, Germany). Fourier transform infrared spectroscopy (FTIR) studies were carried out using a Fourier-transform infrared spectrometer (PerkinElmer, USA). Morphological evaluation was performed using an XL-30 scanning electron microscope (SEM, Philips, Holland).

2.4 Ultraviolet (UV) absorption spectrum and solubility test

1 mg of sample was dissolved in 10 mL of distilled water, sonicated for 30 minutes, and allowed to stand for one hour. The absorbance was then measured using a UV spectrophotometer.

10 mg of Nar standard was weighed and diluted with absolute ethanol to a final volume of 50 mL in a volumetric flask, yielding a 0.2 mg/mL Nar standard solution. This solution was further diluted to five concentration gradients: 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL. The absorbance values of these dilutions were measured at 292 nm to construct the Nar standard curve.

10 mg of the sample was weighed, mixed with 5 mL of deionized water, and incubated at 37 °C with shaking at 200 rpm for 24 hours. The supernatant was then collected, centrifuged at 5000 rpm for 5 minutes, and filtered through a 0.22 µm water-based filter membrane. The filtrate was subsequently subjected to UV absorbance measurement at 292 nm.

2.5 Cell viability test

Human skin fibroblasts (HSFs, Boxi, China) were seeded into a 96-well plate. The experiment included a blank group and a sample group. After 24 h of culture (adherent and logarithmic phase), the sample was added and incubated for another 24 h. Then, 10 µL/well of 5 mg/mL MTT-PBS solution was added for 4–6 h. The supernatant was discarded, 100 µL/well DMSO was added, and the plate was shaken for 15 min. Absorbance (OD, A) was measured at 490 nm. Cell viability was calculated as follows:

$$\text{Cell viability}(\%) = \frac{A_{\text{sample}}}{A_{\text{blank}}} \times 100\%$$

2.6 Establishment of UVA-induced HSF model

HSFs were seeded into a 96-well plate. After 24 hours of culture, the cells adhered to the well bottom and entered the logarithmic growth phase. The experiment was divided into a blank group (non-irradiated) and a model group (UVA-irradiated). The cells in the model group were placed under a UVA lamp at a fixed distance from the lamp. The model group was exposed to various irradiation doses: 2, 5, 6.5, 8, 10, and 14 J/cm². Immediately after irradiation, the PBS was discarded and replaced with complete culture medium, and the cells were further cultured for an additional 24 hours. Cell viability was then measured.

2.7 Reactive oxygen species (ROS) test

HSFs were seeded in a 24-well plate. After 24 hours of culture, the cells adhered to the wall and entered the logarithmic growth phase. The sample was added to the sample wells, and the cells were incubated for 24 hours, followed by UVA irradiation (6.5 J/cm²). Then, DCFH-DA working solution was added to a final concentration of 10 µM, and the cells were incubated for 20 minutes. After washing twice with PBS, the cells were observed and photographed under a fluorescence microscope.

2.8 Kit test

HSFs were seeded in a 24-well plate. After 24 hours of culture, the cells adhered to the wall and entered the logarithmic growth phase. The sample was added to the sample wells and cultured for 4-6 hours, followed by UVA irradiation (6.5 J/cm²). After continuing culture for

another 24 hours, the cell culture supernatant was collected. The levels of human matrix metalloproteinase 1 (MMP-1), lipid oxidation (MDA), human type I collagen $\alpha 1$ (COL1 $\alpha 1$), human type III collagen (COL3), human elastin (ELN), and human fibronectin (FN) were measured according to the kit instructions. The cells were also collected and tested for human matrix metalloproteinase 9 (MMP-9) content and adenosine triphosphate (ATP) concentration following the kit instructions.

2.9 Statistical analysis

Statistical analysis was performed using SPSS version 22 (IBM, USA) and GraphPad Prism 9.0.0 (GraphPad Software, USA). For measurement data, normality was assessed using the Shapiro–Wilk test. Data that followed a normal distribution were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). Comparisons between groups were conducted using one-way ANOVA. When the data followed a normal distribution and satisfied the assumption of homogeneity of variance, post hoc comparisons were performed using Tukey's test. If the data followed a normal distribution but violated the assumption of homogeneity of variance, Dunnett's T3 test was used. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Analysis of MEPS of Nar and CCFs

Selecting an appropriate CCF for the API is key to successful co-crystal design. Experimental screening requires numerous attempts and is both expensive and time-consuming. Therefore, virtual prediction of the likelihood of co-crystal formation between an API and a CCF can provide guidance for subsequent experiments while reducing the number of tests and associated costs. In this study, the MEPS method was used to quantify and predict the potential for co-crystal formation between two substances through calculation and analysis of extreme point data.

As can be seen intuitively from Figure 1(A), the electrostatic potential near the hydroxyl group is positive, and the hydrogen atom on the hydroxyl group provides the maximum positive charge at the maximum point, with a value of $57.09 \text{ kcal}\cdot\text{mol}^{-1}$. This hydrogen can form $\text{O}-\text{H}\cdots\text{O}/\text{N}$ interactions with molecules that have a larger negative potential. Table 1 lists the electrostatic potential pairing energies and estimated probabilities of co-crystal formation for Nar and the selected CCFs. The smaller the ΔE value, the more readily a eutectic forms. Thus, from the electrostatic potential pairing energy, it can be seen that the different CCFs show slightly different tendencies for co-crystal formation, with the order $\text{Fer} > \text{Man} > 4\text{B}$. Fer and Nar exhibited the highest probability of co-crystal formation (49.41%). Consequently, Fer was subsequently selected as the CCF.

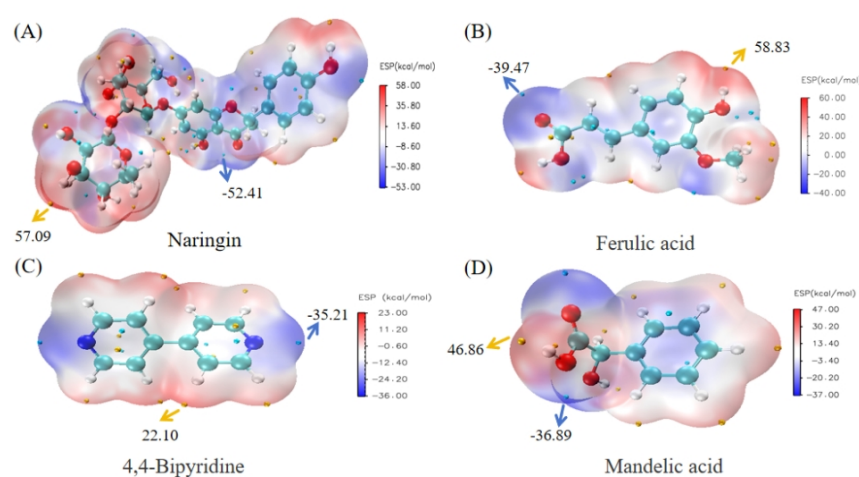


Figure 1. MEPS diagrams of Nar and the CCFs. Panels (A)-(D) show the MEPS of Nar, Fer, 4B, and Man, respectively. In the figures, ● represents C atom; ● represents O atom; ● represents N atom; ● represents H atom. Blue regions indicate negative electrostatic potential; the darker the blue, the more negative the potential. Red regions indicate positive electrostatic potential; the darker the red, the more positive the potential. Lighter colors indicate electrostatic potentials near zero. Small colored spheres mark the extreme points of the electrostatic potential: yellow spheres represent local maxima (most positive), and cyan spheres represent local minima (most negative). The values indicated in the figures are the specific electrostatic potential values at these extreme points, given in kcal·mol⁻¹.

Table 1. Calculation of MEPS of Nar and the CCFs¹

	MEP _{max} (kJ·mol ⁻¹)	MEP _{min} (kJ·mol ⁻¹)	α	β	E (kJ·mol ⁻¹)	E _{cc} (kJ·mol ⁻¹)	ΔE (kJ·mol ⁻¹)	ΔG (kJ·mol ⁻¹)	P(%)
Nar	238.86	-219.28	3.22	72.24	-232.61	/	/	/	/
Fer	246.14	-165.14	3.35	41.35	-138.52	/	/	/	/
4B	92.47	-147.32	1.03	33.06	-34.05	/	/	/	/
Man	196.06	-154.34	2.51	36.22	-90.91	/	/	/	/
Nar-Fer (1: 1)	/	/	/	/	/	-375.15	-4.02	6.98	49.41
Nar-4B (1: 1)	/	/	/	/	/	-180.86	85.80	96.80	41.95
Nar-Man (1: 1)	/	/	/	/	/	-297.95	25.57	36.57	46.94

¹Among these, MEP_{max} and MEP_{min} are the maximum and minimum points of the electrostatic potential, respectively, and the unit for both is kJ·mol⁻¹. α and β are the hydrogen bond donor parameter and hydrogen bond acceptor parameter, respectively. E refers to the interaction site energy of a single molecule. E_{cc} is the pairing energy of the interaction sites for a substance at a stoichiometric ratio of m:n; in this study, m = n = 1. ΔE refers to the pairing energy of a co-crystal formed between different molecules. ΔG is the free energy difference between the single-molecule state and the co-crystal state, and P is the probability of co-crystal formation.

3.2 Structural characterization analysis of Nar-Fer co-crystal

In this study, the co-crystal was prepared via the solvent evaporation method using 50% ethanol as the solvent. The final product was characterized by PXRD to confirm co-crystal formation. DSC, TG, FTIR, and SEM were further used to determine the melting point, structure, and other basic physicochemical properties of the co-crystal.

The PXRD patterns of the raw monomers and the co-crystal powder were shown in Figure 2(A). The characteristic diffraction peaks of Nar appeared at 4.3°, 9.6°, 11.4°, 14.4°, 16.4°, 18.6°, and 26.0°, while those of Fer appeared at 9.0°, 10.5°, 12.7°, 15.6°, 17.3°, 24.4°, 26.4°, and 29.3°. The Nar-Fer co-crystal exhibited distinct new diffraction peaks at 7.8°, 8.6°, 19.4°, 24.2°, and 34.6°, which differed from the peaks of Nar and Fer, confirming successful co-crystal formation.

The DSC and TG curves of the raw monomers and the co-crystal powder were shown in Figure 2(B-C). Nar exhibited an endothermic peak at 96 °C, which corresponded to a weight loss of 5.08% in the TG curve. This was likely due to the removal of water molecules from Nar during heating. The endothermic peak at 174 °C corresponding to the melting point of Nar. Fer showed a sharp endothermic peak at 175 °C, corresponding to its melting point. The Nar-Fer co-crystal displayed an endothermic peak at 93 °C, which was associated with a weight loss of 4.55% in the TG curve, indicating the loss of water molecules and confirming its hydrated nature. The melting point of the Nar-Fer co-crystal was 163 °C, which differed from those of

the two raw materials. This characteristic transition temperature confirmed the formation of a new crystalline phase, consistent with the PXRD results.

The FTIR spectra of the raw monomers and the co-crystal were shown in Figure 2(D). The characteristic peaks of Nar at 3430cm^{-1} and 2935cm^{-1} corresponded to O-H stretching vibrations, and the peak at 1645cm^{-1} corresponded to the C=O stretching vibration. In the infrared spectrum of Fer, the characteristic peaks at 2970cm^{-1} and 2840cm^{-1} were attributed to C-H vibrations of the methyl group, the peak at 3437cm^{-1} was assigned to the O-H stretching vibration, and the peak at 1674cm^{-1} corresponded to the C=O stretching vibration of the carboxylic acid group. After co-crystal formation, the O-H stretching vibrations of Nar shifted from 3430cm^{-1} and 2935cm^{-1} to 3435cm^{-1} and 2930cm^{-1} , and the C=O stretching vibrations of Nar and Fer shifted from 1645cm^{-1} and 1674cm^{-1} , respectively, to a common peak at 1650cm^{-1} . These shifts were attributed to hydrogen bonding in the Nar-Fer co-crystal, which caused the vibrational frequencies of certain functional groups to differ from those in the individual raw materials.

The SEM images of the raw monomers and the co-crystal were shown in Figure 2(E). Nar appeared as elongated rod-shaped crystals, Fer appeared as rod-shaped crystals, and the Nar-Fer co-crystal exhibited irregular block-shaped crystals, which were distinctly different from the morphology of the raw monomers.

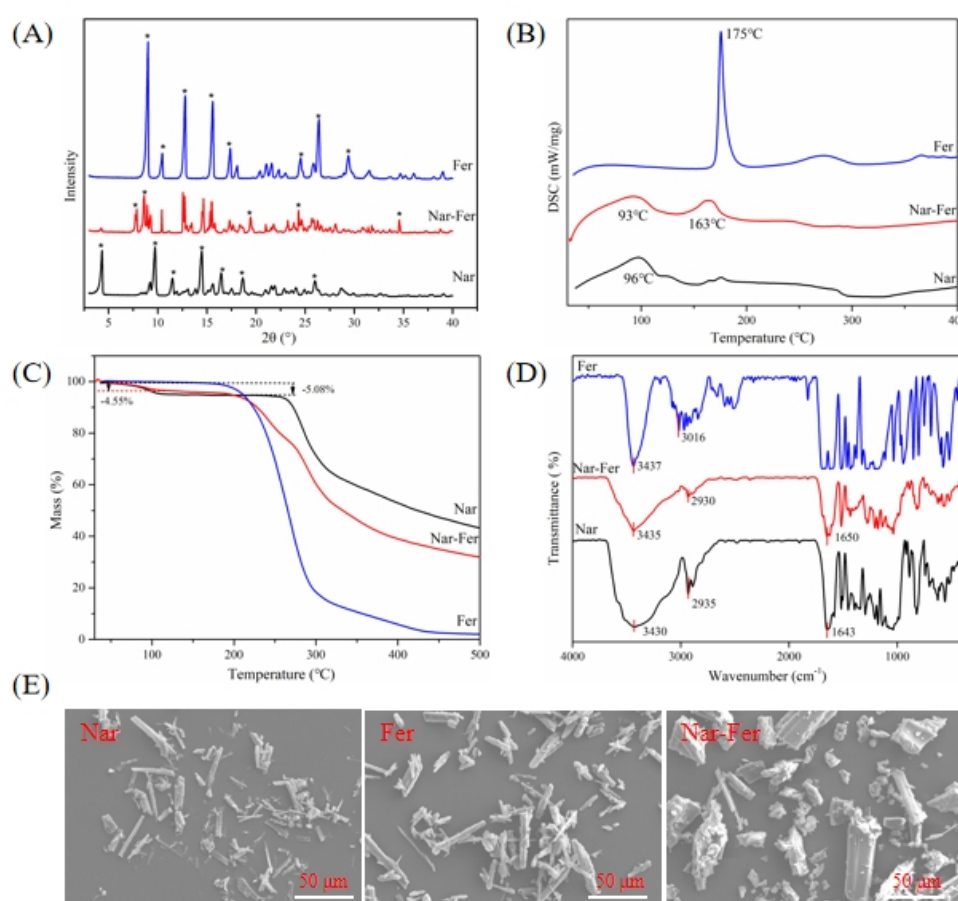


Figure 2. Structural characterization of Nar, Fer and Nar-Fer co-crystal. (A) PXRD, Hexagram symbol indicates diffraction peaks, (B) DSC, (C) TG, (D) FTIR, (E) SEM.

3.3 UV absorption spectrum and solubility analysis of Nar-Fer co-crystal

Most APIs have poor water solubility, which limits their bioavailability. Supramolecular co-crystals generally exhibit better solubility than the corresponding raw monomers. The UV

absorption spectra of Nar and the Nar-Fer co-crystal in water were shown in Figure 3(A). The main absorption peak of Nar in distilled water was approximately 285 nm. At this wavelength, the absorbance of Nar was about 1.2, whereas that of the Nar-Fer co-crystal was about 1.5. Thus, compared with the parent compound Nar, the co-crystal showed improved solubility. The water solubility of Nar and the Nar-Fer co-crystal was presented in Figure 3(B). The solubility of the Nar-Fer co-crystal was 2.21 times that of Nar.

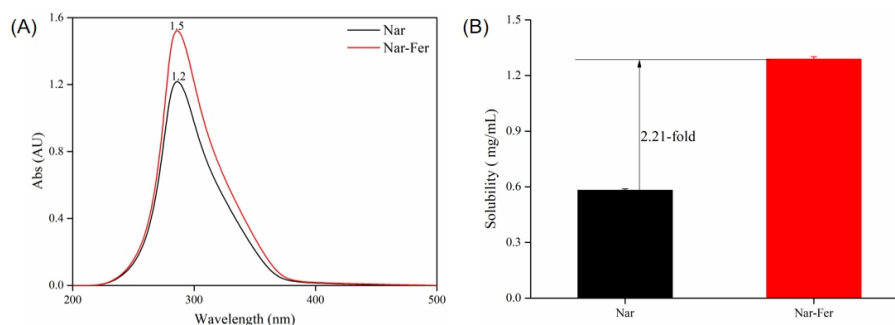


Figure 3. UV spectrum (A) and solubility (B) of Nar and Nar-Fer co-crystal.

3.4 Analysis of the protective effect of Nar-Fer co-crystal on skin photodamage

To further determine whether the improved physicochemical properties of the Nar-Fer co-crystal could enhance its protective effect against skin photodamage, we first screened the optimal working concentration of the Nar-Fer co-crystal on HSFs, then established a UVA-induced HSF model, and finally evaluated the efficacy of the Nar-Fer co-crystal in terms of antioxidant activity, extracellular matrix promotion, and DNA repair. The MTT assay was used to assess the effects of Nar, Fer, and the Nar-Fer co-crystal on the proliferation rate of HSFs (Figure 4(A-C)). The results showed that Nar at 0.01-100 $\mu\text{g/mL}$ had no significant toxicity to HSFs. Fer at 0.01-10 $\mu\text{g/mL}$ also had no significant toxicity to HSFs. The Nar-Fer co-crystal at 0.1-10 $\mu\text{g/mL}$ showed no significant toxicity to HSFs. Based on these findings, 1 $\mu\text{g/mL}$ was selected as the concentration for subsequent experiments.

During photoaging, HSFs in the dermal layer of the skin are the primary cells affected by ultraviolet radiation and are also the main effector cells responsible for the clinical signs of aging. Therefore, we used an in vitro model of UVA-irradiated HSFs to simulate the cellular changes that occur during skin photoaging under natural conditions. Twenty-four hours after irradiation with various doses of UVA, cell proliferation activity was assessed using the MTT method. The results showed that as the UVA dose increased, cell proliferation activity was gradually inhibited, and a significant difference was observed compared with the control group (without UVA irradiation) ($p < 0.05$), indicating that the in vitro HSF photoaging model was successfully established. When the UVA dose reached 6.5 J/cm^2 , cell viability decreased to approximately 50% of that of the control group (Figure 4(D)). Based on this result, we selected 6.5 J/cm^2 UVA for subsequent experiments.

The effects of the Nar-Fer co-crystal on oxidative stress in UVA-damaged HSFs were shown in Figure 4(E-I). Compared with the model group, the fluorescence intensity (indicative of ROS), MMP-1, MMP-9, and MDA levels in the Nar, Nar-Fer co-crystal, Fer, and vitamin E (VE) groups were significantly reduced ($p < 0.05$). Compared with Nar alone, the Nar-Fer co-crystal group showed no significant difference in ROS inhibition rate or MMP-9 level, but its MMP-1 and MDA levels were significantly lower ($p < 0.05$). These results demonstrated that Nar, the Nar-Fer co-crystal, Fer, and VE all exert photoprotective effects on cells by inhibiting oxidative stress. Moreover, compared with the parent compound Nar, the antioxidant effect of the Nar-Fer co-crystal was improved to a certain extent.

The effects of the Nar-Fer cocrystal on UVA-induced degradation of extracellular matrix proteins in HSFs were shown in Figure 4(J-M). Compared with the model group, the levels of COL1 α 1, COL3, ELN, and FN in the Nar, Nar-Fer co-crystal, and Fer groups were all significantly increased ($p < 0.05$). Compared with Nar alone, the Nar-Fer co-crystal group showed no significant difference in COL1 α 1 and COL3 levels, but its ELN and FN levels were significantly higher ($p < 0.05$). These results demonstrated that Nar, the Nar-Fer co-crystal, and Fer all exert photoprotective effects on cells by promoting extracellular matrix regeneration. Moreover, compared with the parent compound Nar, the Nar-Fer co-crystal showed improved efficacy in promoting the regeneration of ELN and FN.

Mitochondria are the primary sites of ROS generation. Excessive accumulation of ROS can lead to mutations in mitochondrial DNA (mtDNA), which ultimately reduces ATP production, resulting in insufficient cellular energy supply and contributing to skin aging. The effect of the Nar-Fer co-crystal on UVA-damaged HSF DNA was shown in Figure 4(N). Compared with the blank group, the ATP content in the model group was significantly reduced ($p < 0.05$), indicating that the photoaging model was successfully established. Compared with the model group, the Nar, Nar-Fer co-crystal, Fer, and VE groups all significantly promoted ATP production, with no significant difference observed between the Nar and Nar-Fer co-crystal groups. These results indicated that Nar, the Nar-Fer co-crystal, Fer, and VE exert photoprotective effects on cells by repairing DNA damage.

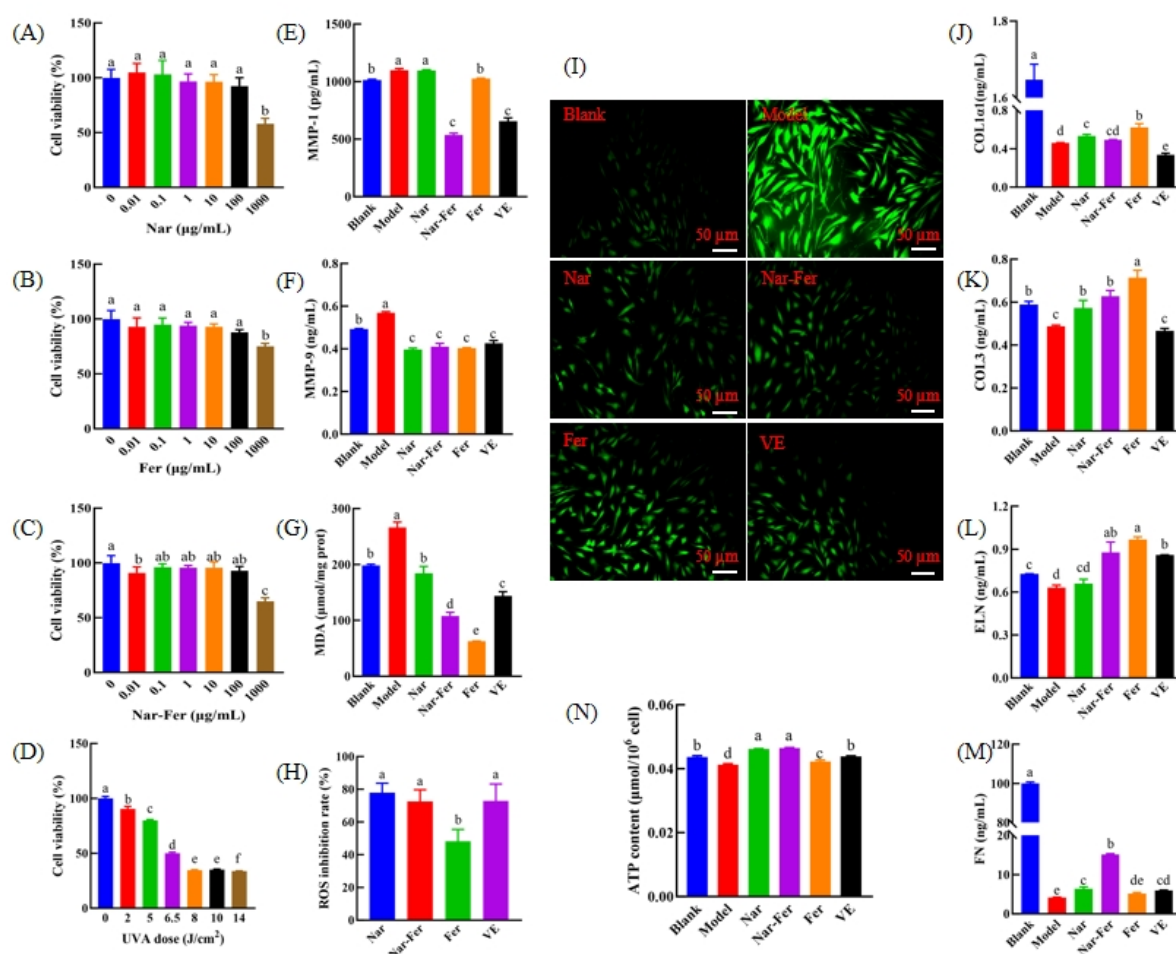


Figure 4. Protective effect of the Nar-Fer co-crystal against UVA-induced photodamage in HSFs. (A-C) Histograms showing the cell viability of HSFs treated with different concentrations of Nar, Fer, and the Nar-Fer co-crystal. (D) Effect of various UVA irradiation doses on the proliferation activity of HSFs. (E-I)

Effects of 1 $\mu\text{g/mL}$ Nar, Fer, and the Nar-Fer co-crystal on oxidative stress in UVA-damaged HSFs: (E) MMP-1 level, (F) MMP-9 level, (G) MDA level, (H) ROS inhibition rate, and (I) ROS fluorescence intensity. (J–M) Effects of 1 $\mu\text{g/mL}$ Nar, Fer, and the Nar-Fer co-crystal on UVA-induced degradation of extracellular matrix proteins in HSFs: (J) COL1 α 1 level, (K) COL3 level, (L) ELN level, and (M) FN level. (N) Effect of 1 $\mu\text{g/mL}$ Nar, Fer, and the Nar-Fer co-crystal on UVA-induced DNA damage in HSFs. The positive control group received 1 $\mu\text{g/mL}$ VE; the blank group was not exposed to UVA irradiation; the model group consisted of HSFs irradiated with a UVA dose of 6.5 J/cm². Identical letters indicate no significant difference between groups, whereas different letters indicate a significant difference ($p < 0.05$).

4. Discussion

This study first conducted a virtual screening of three CCFs using MEPS analysis. The results showed that Fer and Nar exhibited the lowest electrostatic potential pairing energy and the highest probability of co-crystal formation (49.41%). Subsequently, a Nar-Fer co-crystal was prepared via the solvent evaporation method, which verified the accuracy of the virtual prediction. Structural characterization confirmed that Nar and Fer formed a new crystalline phase through non-covalent interactions, including hydrogen bonding, rather than simple physical mixing, thus confirming the successful synthesis of the co-crystal. This may be attributed to the fact that the flavonoid skeleton of Nar is rich in hydrogen bond donor and acceptor sites (e.g., hydroxyl and carbonyl groups), while Fer possesses both carboxyl and phenolic hydroxyl groups. The significant hydrogen bond complementarity between the two provides a thermodynamic driving force for co-crystal formation [8].

In recent years, co-crystal technology has been used to improve the solubility and bioavailability of various flavonoids, including daidzein [9] and phloretin [10]. In this study, we also found that the water solubility of the Nar-Fer co-crystal was higher than that of the raw Nar monomer. These results indicate that supramolecular co-crystal technology can effectively control the solid-state stacking mode of Nar, thereby enhancing its hydrophilicity and laying a foundation for its application in aqueous cosmetic systems.

In the UVA-induced photoaging model of HSFs established in this study, cell viability decreased by approximately 50% when the UVA irradiation dose reached 6.5 J/cm², successfully simulating the photodamaging effect of ultraviolet radiation on HSFs. Using this model, the biological activities of the Nar-Fer co-crystal—including antioxidant activity, promotion of extracellular matrix regeneration, and DNA repair—remained largely unchanged or were even enhanced due to synergistic effects. This indicates that the introduction of bioactive Fer as a CCF not only improves the physicochemical properties of Nar but may also exert a positive synergistic effect. This study introduces the supramolecular cocrystal strategy into the field of skin photodamage protection, demonstrates the feasibility of the Nar-Fer co-crystal in improving Nar solubility and enhancing its skin protective effects, and provides new formulation concepts for the application of Nar in functional cosmetics. Given that Fer itself possesses good UV absorption properties, the Nar-Fer co-crystal holds potential for application in sunscreen or daily skincare formulations.

5. Conclusion

Our study demonstrated that supramolecular co-crystal technology was an effective means of improving the solubility of poorly soluble flavonoids such as Nar, and that the resulting co-crystal could enhance the anti-photoaging effect of the API. Our study provided a theoretical and experimental basis for the application and development of Nar in functional

cosmetics and skin protection preparations, and also served as a reference for the supramolecular modification of other poorly soluble flavonoids.

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