L-carnitin-based supramolecular solvent loading macromolecule collagen as enhanced transdermal delivery system

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Abstract

Background: Macromolecular collagen, a functional protein with molar weight around 300000, is the main composite of consumption medical and plays a significant role in traditional medical health market. This study is designed to solve the drawbacks of macromolecular collagen, such as low bioavailability, difficulty in permeating epidermis and being absorbed by dermis, and poor stability during transportation.
Methods: In this study, a series of L-carnitin-matrix ionic liquids, as supramolecular solvents, were developed based on the density functional theory (DFT) calculation results.
Results: The L-carnitin-based supramolecular solvent loading macromolecule collagen has a particle size of less than 70nm accompanying good dispersity and biocompability. The solubility and the skin permeability of macromolecule collagen are increased by 3.4 times. The content of collagen in cuticle, epidermis and dermis are increased by 1.2 times, 2.7 times and 4.3 times, respectively, and the bioavailability is increased by nearly 5 times. This system can also effectively relieve wrinkles and pigmentation caused by aging. This study also explores the mechanism of IL solubilization in promoting infiltration. With hydrophilic and lipophilic groups, the resulting IL carries out non-biding lipids to the collagen to enhance the encapsulation rate. In addition, it opens the tight junction to promote collagen bypass, and improves the permeability of keratinocytes when promoting across cell transport of collagen.
Conclusion: This study breaks the combined limitations of biological ILS, provides a new solution for solving, stabilizing and permeating generic proteins and macromolecular drugs in dressing, manual organ regeneration medicine, tissue engineering, biological skin care, etc.

Keywords: Macromolecular collagen, ionic liquids, bioavailability, stability, permeability.

Introduction

As the outermost physical barrier of the body, skin aging is the most intuitive manifestation of organismal aging. Skin aging is a complex process regulated on multiple factors and scales, and is briefly influenced by a combination of endogenous and exogenous elements. Endogenous factors are slowly evolving and uncontrollable over time. Clinical manifestations include fragile, inelastic,

dry, sagging skin. Exogenous factors are due to the lifelong exposure of the skin to various environments, in which UV light, air pollution, tobacco, oil smoke, and mechanical forces all accelerate skin aging to varying degrees. Exogenous skin aging is manifested by the accumulation of amorphous elastic fibers, collagen disorders, capillary dilation, weakened barrier function, and reduced number of fibroblasts. Clinically, it manifests as skin roughness, laxity, deep wrinkles, dullness, dryness, and spot discoloration.

Skin aging is a process that cannot be reversed, but is malleable. Currently anti-aging focuses on repair and protection, which regulate the speed of aging from anti-aging pathways to prevent and delay aging. However, it is still impossible to avoid the aging of the skin, so repair is essential. Repair generally targets the surface of the skin, care for collagen synthesis and enhancing the skin's repair capacity. The common anti-aging approaches include transdermal application, injections, oral, radiofrequency and electrodermabrasion, but injections and electrodermabrasion are often associated with irreparable side effects. Among them, transdermal delivery of anti-aging drugs is a very valuable way. Most of the current methods Pay attention to single pathway, and there is a lack of multi-pathway and multi-targeting research. With the rising demand for anti-aging in modern society, it is urgent to investigate and explore transdermal delivery ways for skin aging.

Transdermal delivery is a delivery system that is superior to other modes in terms of safety, practicality and convenience. It not only avoids the first-pass elimination of the drug, but also ensures that the entire process is painless. Current transdermal delivery systems include chemical promoters, liposomes, micelles, microemulsions, microneedles, ion introduction, and ionic liquid delivery. Of these, ionic liquids (ILS) have shown excellent potential for drug delivery. They are compounds composed entirely of anions and cations that are liquid at room temperature.[1] It has many unique properties including non-volatility, non-flammability, low vapor pressure, broad liquid range and excellent solubility. The most attractive of these properties is the availability of tunable physical, chemical and biological properties, which are rarely achieved in other molecular compounds.[2] Therefore ILS are frequently used for the synthesis active pharmaceutical ingredients (APIS) and deliver drugs. Zhang et al. prepared an ILs -based salicylic acid microneedle patch (SA-PIL-MN) by photocrosslinking imidazole IL monomers in a mold, which can effectively suppress the growth behavior of Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), and successfully restrained the activity of P. acnes in a mouse acne model, eliminating the symptoms of acne in mice.[2-3,6] Moniruzzaman et al. used dimethylimidazolium dimethyl phosphate ILs as the water phase, Tween-80 and Span-20 as nonionic surfactants, and IPM as the oil phase to prepare an oil-in-ionic liquid microemulsion system, which can dissolve water-insoluble drugs as well as most pharmaceutical grade organic liquids. Banerjee et al. employed ILs (CAGE) obtained from choline and geranylic acid to improve topical delivery of proteins. CAGE significantly enhanced the penetration of BSA, OVA and insulin in isolated porcine skin versus the control group. In a hyperglycemic rat body model, insulin contained in CAGE dramatically reduced blood glucose levels for 12 hours. All of the above researches show the tremendous potential of ionic liquids for drug delivery. However, there are fewer studies for Bio-ILs systems and no use in the direction of bioactive peptides.[4-5]

Collagen is a family of proteins that can be divided into interstitial collagen, basement membrane collagen and pericellular collagen according to their distribution and functional characteristics in the body. The interstitial collagen molecule accounts for the vast majority of the collagen in the

whole body, and the relative molecular mass is large. There are 3 swimming bands in the electrophoresis picture. The 2 swimming bands that appear near 100kD are the α1 chain and α2 chain of the collagen molecule, respectively. At 200kD The one band that appears nearby is the beta chain of the collagen molecule. That is, the relative molecular mass of each polypeptide chain of collagen can reach 100kD, and the relative molecular mass of one collagen molecule is 300kD.
Bio-ILs can highly encapsulate and stabilize collagen, and greatly enhance the skin permeability of collagen. The prepared collagen/ILs exhibits a variety of excellent antioxidant and anti-inflammatory effects in cellular level, animal level and population efficacy tests. Therefore, the ILs have favorable stability, biocompatibility, and are a natural, environmentally friendly and effective transdermal drug delivery vehicle. The combination of collagen and ILs solves the difficulty in usage of collagen, high cost and poor effect. problems, greatly promoting the application of collagen in the clinical field.

Materials and Methods

Characterization of IL： The chemical structures of L-carnin-based ILs dispersed in deuterium oxide (D2O) were analyzed by proton nuclear magnetic resonance (1H NMR) and carbon nuclear magnetic resonance (13C NMR) (Bruker Avance III 400 MHz) spectroscopy. Fourier transform infrared (FTIR) spectroscopy (Thermo Scientific Nicolet iS 50) measurements of the ILs were carried out in the attenuated total reflectance mode.

Preparation of the L-carnin-based IL: A certain amount of malic acid was dissolved in water at room temperature, following which the aqueous solutions of L-(−)-carnitine were added dropwise to the Tau-containing solution. The reaction was carried out for 8 h at 25℃. The malic acid：L-(−)-carnitine molar ratios were 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, and 4:1. After the reaction, the aqueous solution was removed by vacuum distillation at 60℃, and the obtained malic acid-based ionic liquids.

ILs encapsulated with collagen: As a drug delivery vehicle, its ability to encapsulate drugs is very important. The MAC-ILs was used as a carrier to encapsulate the collagen. High-performance liquid chromatography (HPLC) testing shows that the encapsulation rate of the MAC-ILs on the collagen, which indicates that MAC-ILs have a high loading capacity for collagen.

Cell Culture Assays: NHF cells and L929 cells were obtained from the Institute of Cells, Chinese Academy of Sciences (Shanghai, China). The cells were cultured in a DMEM complete medium containing 10% FBS followed by incubation at 37 °C in a 5% CO2 incubator. The cells were then subjected to logarithmic growth for the subsequent experiments.

Results and discussion

1、Design, Synthesis, Optimization, and Characterization of L-carnitin-based IL.

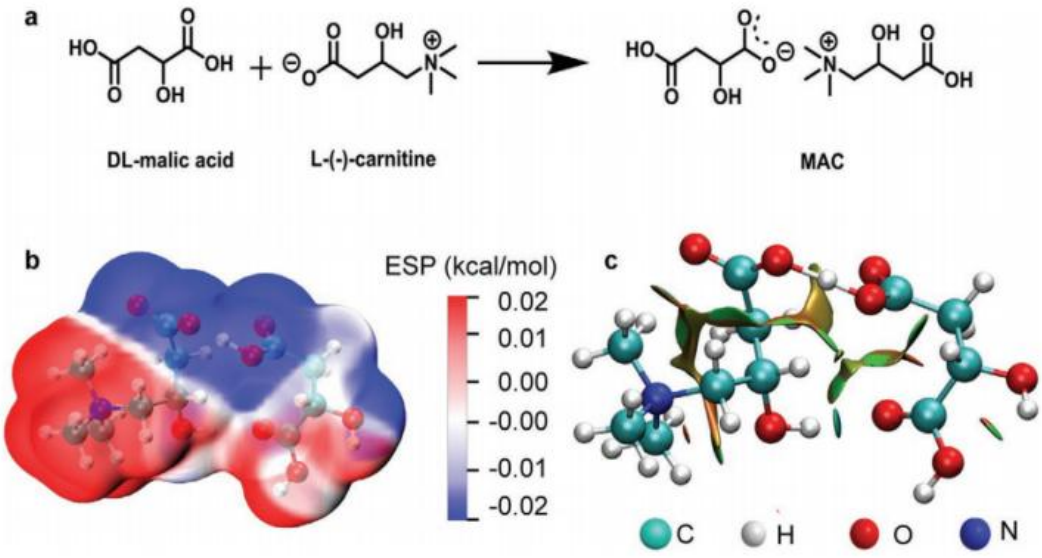


Fig.1 Malic acid and L-(-)-carnitine

MAC was prepared using L-(-)-carnitine and MA. The ESP analysis indicates that L-(-)-carnitine-based ILs have good stability. RDG was also used to analyze the noncovalent interactions in real space based on the electron density and derivatives of MAC. The surface is colored using a blue-green-red scale. Two large flakes of color are shown between L-(-)-carnitine and MA located in the transition area, revealing the presence of van der Waals interactions in the monomer structure of MAC. These analyses indicate that van der Waals interactions exist in the monomer structure of L-(-)-carnitine-based ILs, contributing to the formation of MAC.

2、Stability and safety of L-carnitin-based supramolecular solvent loading macromolecule collagen.

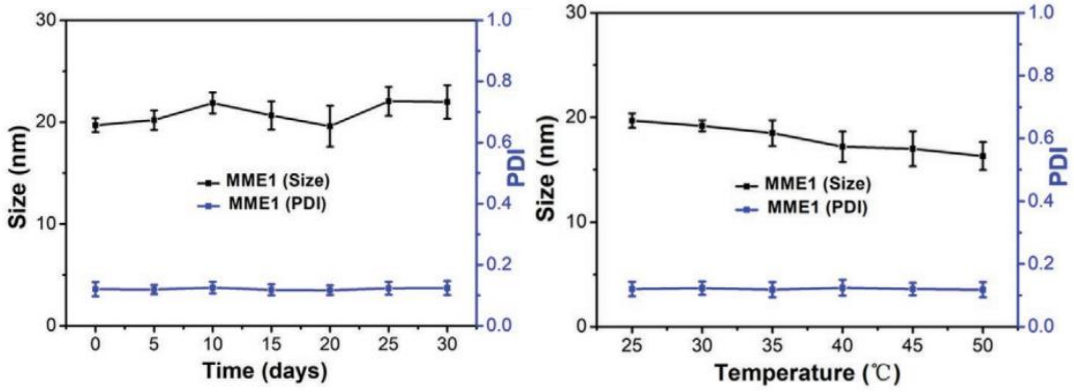


Fig.2 Stability of supramolecular solvent loading collagen after storage for 1 month and at different temperatures (n = 3).

The size and PDI of supramolecular solvent loading collagen do not significantly change over 1 month. Supramolecular solvent loading collagen have good stability and biocompatibility, showing promising application prospects in drug delivery.

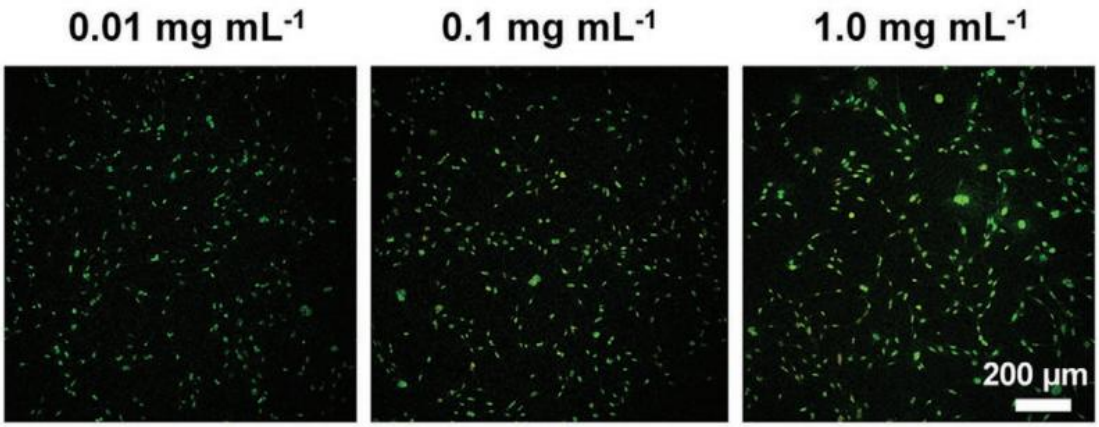


Fig.3 CLSM images of supramolecular solvent loading collagen treated cells stained with calcein-AM (live cells, green fluorescence) and propidium iodide (dead cells, red fluorescence).

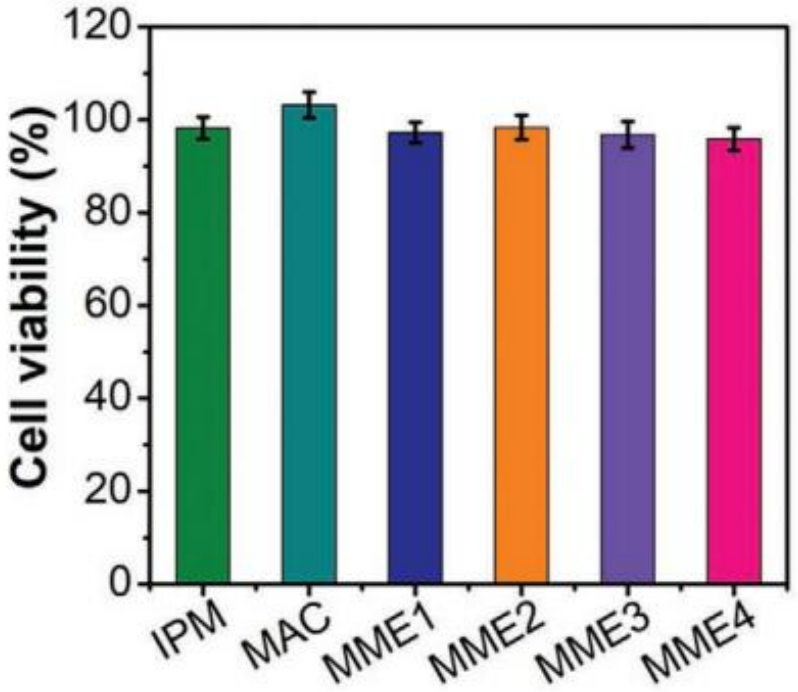


Fig.4 Cell viability of cells treated with different systems (n = 6).

At a concentration of 1.0 mg mL^{-1}, supramolecular solvent loading collagen can lead to less than 5% of cell death. In vitro cytotoxicity using CCK-8 to evaluate the safety of MAC, CAC, MME, and CME shows that, in comparison with the control IPM, their cell viability is about 97%. These results indicate that L-carnitin-based supramolecular solvent as drug carriers have low cytotoxicity.

3、Skin Penetration and drug delivery efficiency of L-carnitin-based supramolecular solvent loading macromolecule collagen.

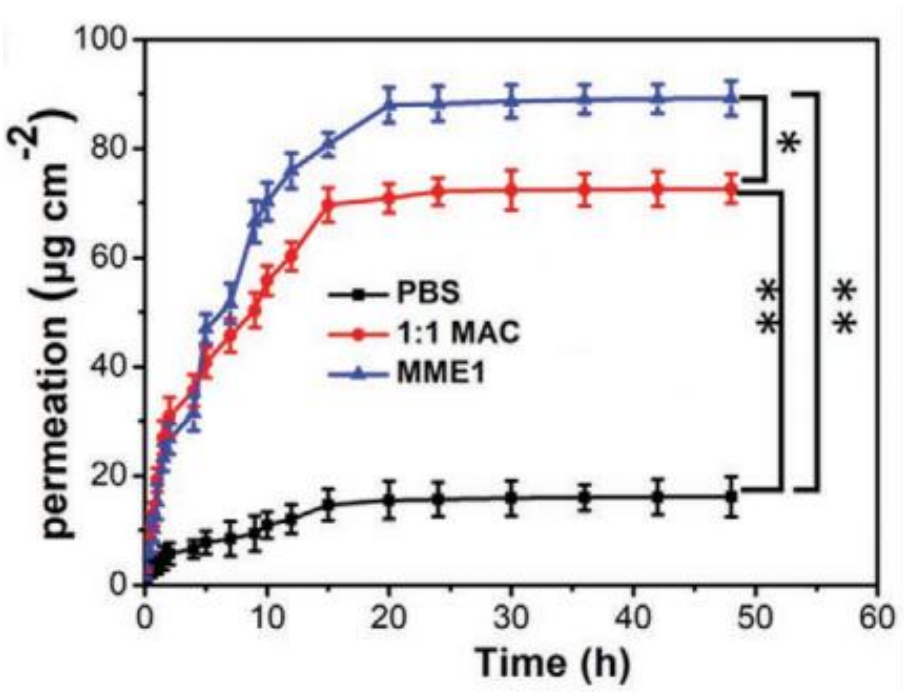


Fig.5 Permeation profiles of collagen across the skin delivered by PBS, 1:1 MAC, and MME1 ($n = 3$, $^{*}p < 0.05$, and $^{**}p < 0.01$).

The cumulative transdermal penetration of MME1 is higher than that of 1:1 MAC.

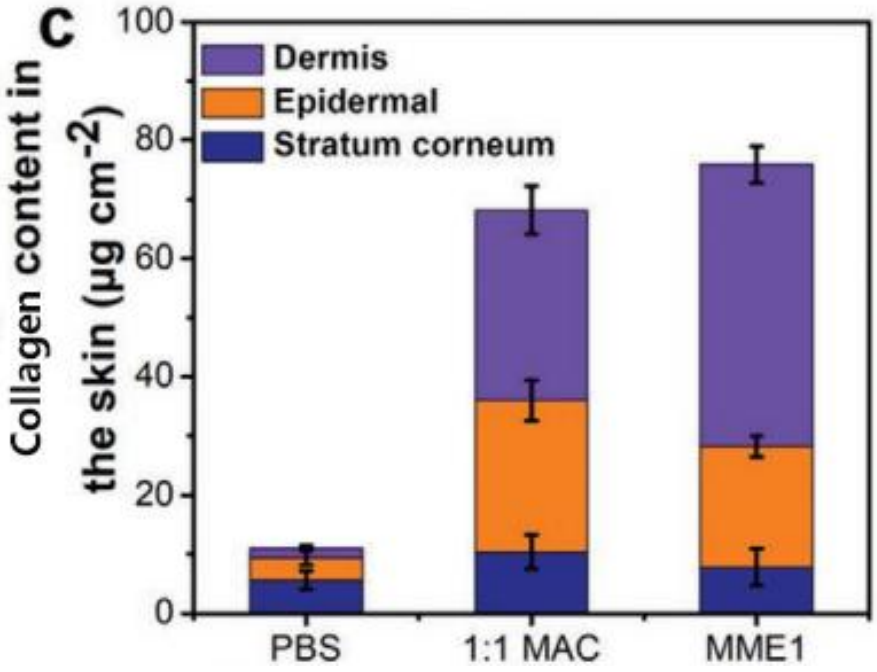


Fig.6 Total penetration of collagen into skin by PBS, 1:1 MAC, and MME1 with blue, yellow, and purple bars representing stratum corneum, epidermis, and dermis, respectively.

The results show that most of collagen delivered by MME1 is distributed in the dermis, while the drug in PBS gives low penetration efficiency.

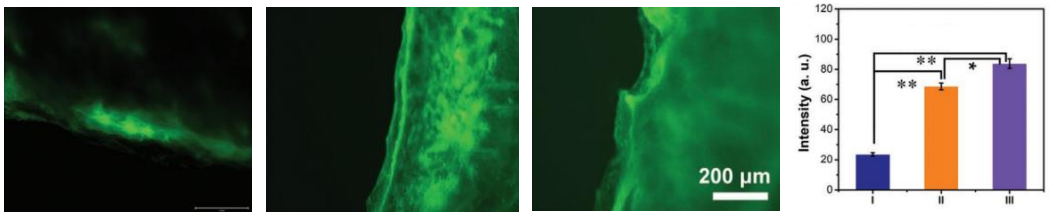


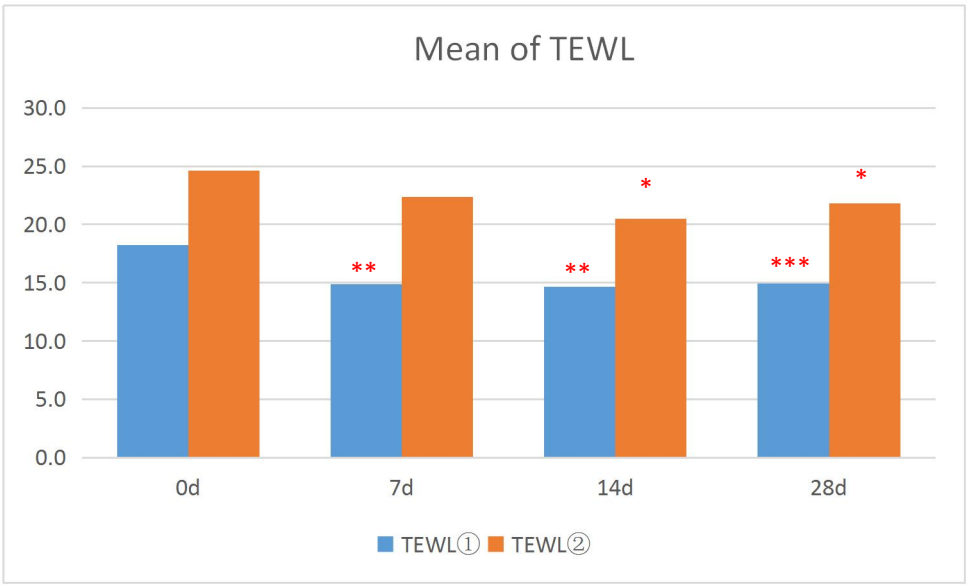
Fig.7 CLSM images of collagen on mouse skin solvated in PBS, 1:1 MAC, and MME1.

Quantitative fluorescence data of collagen on mouse skin delivered by PBS, 1:1 MAC, and MME1 (n = 3, *p < 0.05, and **p < 0.01).

The drug delivered by 1:1 MAC and MME1 exhibits high staining expression in stratum corneum, epidermis, and dermis. The fluorescence intensity is 3 fold higher than that of the PBS group, respectively, indicating that L-carnitin-based supramolecular solvent loading macromolecule collagen can effectively deliver insulin through the stratum corneum.

4、Human safety and efficacy

Seventeen healthy women aged 20-60 were selected for a 28-day evaluation



Marking method of significance : "n.s." means no significant difference, "*" means significant difference, 0.01≤P<0.05; "**", 0.001≤P<0.01; "***", P<0.001.

Fig.8 Transepidermal water loss (TEWL) test results show.

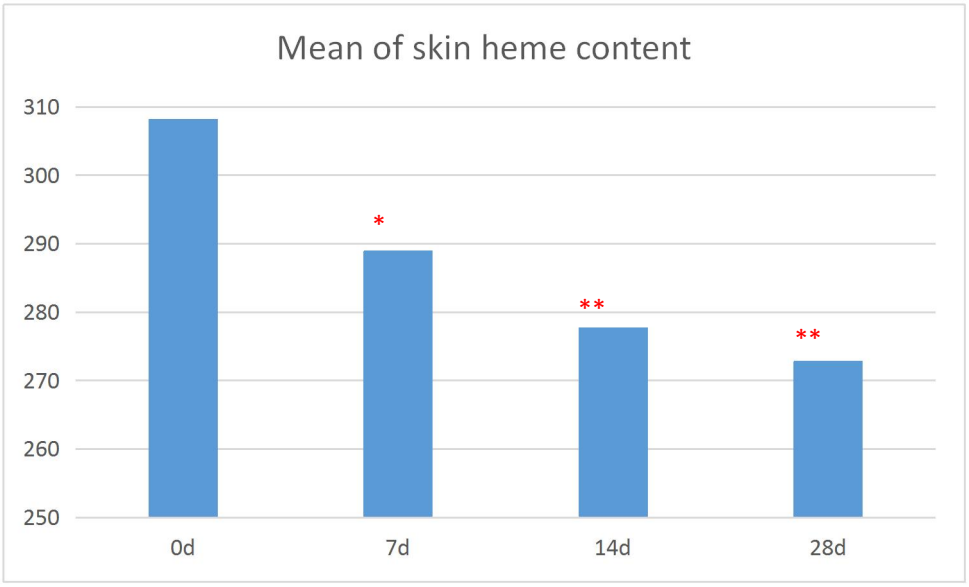


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Fig.9 Skin red pigment test results show

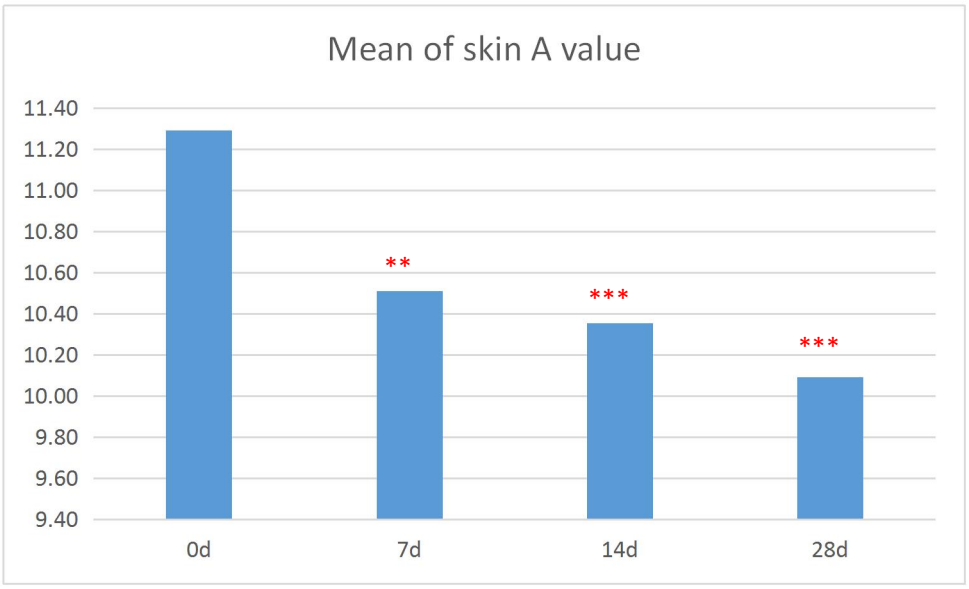

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Fig.10 Skin A value test results show

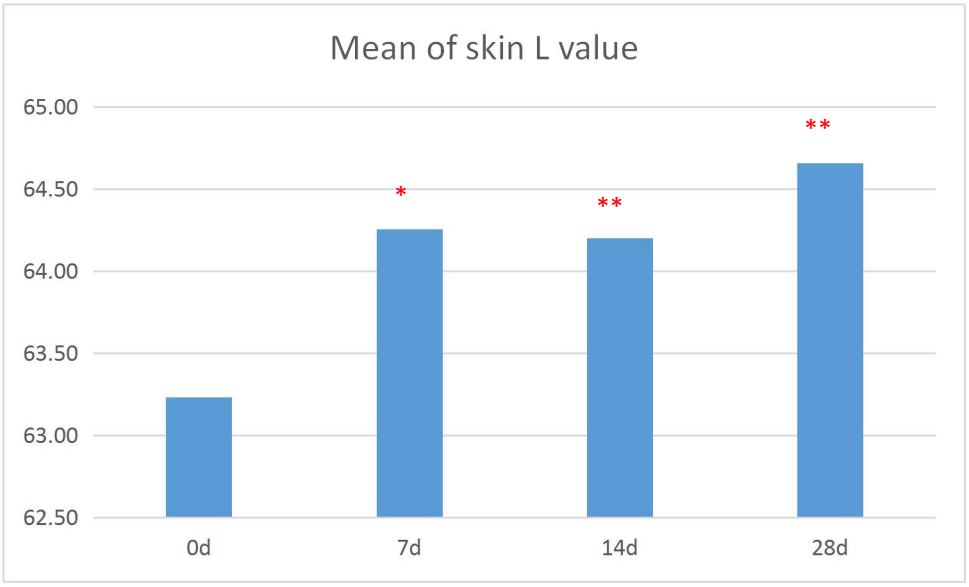

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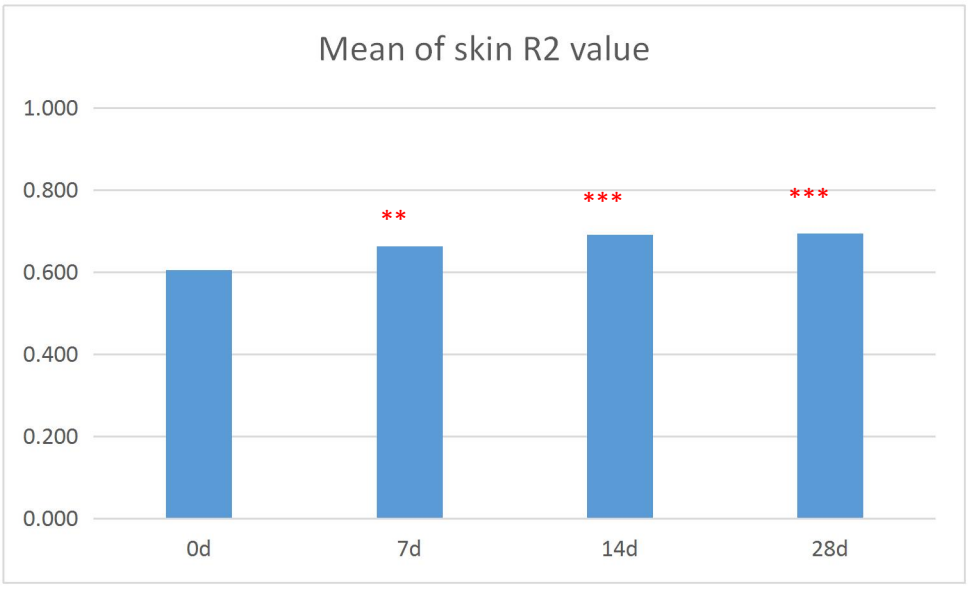
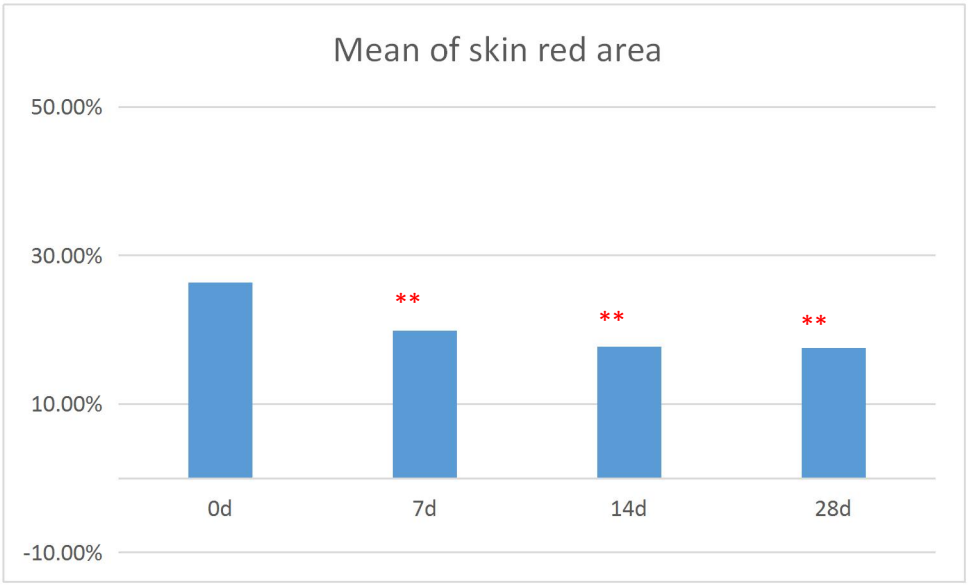
Fig.11 Skin L value test results show



Marking method of significance : "n.s." means no significant difference, "*" means significant difference, 0.01≤P<0.05; "**", 0.001≤P<0.01; "***", P<0.001.

Fig.12 Skin R2 value test results show



Marking method of significance : "n.s." means no significant difference, "*" means significant difference, 0.01≤P<0.05; "**", 0.001≤P<0.01; "***", P<0.001.

Fig.13 Skin red area ratio test results show

Based on the above data, 17 subjects can effectively reduce the transepidermal water loss rate, reduce skin redness, reduce skin heme value, improve skin dullness, and make skin whiter and more elastic after 28 days of efficacy test.

Conclusions

In summary， we successfully prepared malic acid and L-carnitine into L-carnitin-matrix ionic liquids by using supramolecular modification technology, and used it to load collagen for transdermal delivery. It can simultaneously improve the stability of collagen and enhance the ability of collagen to penetrate the skin. L-carnitin-matrix ionic liquids can significantly enhance the penetration of collagen and has no obvious stimulating effect on mouse skin. L-carnitin-matrix ionic liquids has been demonstrated to show good safety, capable of serving as drug delivery systems for penetrationenhanced delivery.

The conclusions can be summarized as following:

1、 Malic acid and L-carnitine successfully prepared highly safe supramolecular solvent delivery carrier.

2、 The supramolecular carrier has good encapsulation and drug loading efficiency for collagen.

3、 Supramolecules promote the transdermal delivery of collagen, which is increased by 2 times.

4、 Supramolecular collagen can effectively improve the aging phenomenon such as wrinkles, fine lines, dull spots, dryness and roughness caused by the loss of collagen. Collagen has excellent long-lasting moisturizing and skin barrier repair ability. Make skin fairer, smoother and younger.

Acknowledgments

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Conflict of Interest Statement

The authors declare no competing financial interest.

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