Nano-capsules of naturally occurring phenylpropanoids and an amphiphilic vitamin C derivative provide synergistic protection against UVA irradiation-induced skin damage and collagen/ elastin fibre reconstruction

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Abstract

Background: The mix extract (CS-mix), *Coffea Robusta* seed extract and *Caffea Arabica* (Coffee) seed extract, containing many amounts of phenylpropanoids, and amphiphilic vitamin C derivative, disodium isostearyl ascorbyl phosphate (VCP-IS-2Na) were reported to prevent collagen and elastin fiber disintegration. We focused on the potential for synergistic reconstructive effect of CS-mix and VCP-IS-2Na against collagen and elastin fiber disintegration.

Methods: We evaluated the amount of collagen synthesis and pro-MMP-1 production on normal human dermal fibroblasts (NHDFs) using ELISA assay system. We also evaluated the potential of the downregulation of mRNA expression of four microfibril-relating genes using Real Time PCR System.

Results: The synergistic effects of CS-mix and VCP-IS-2Na on collagen synthesis and pro-MMP-1 production showed 111% and 198% compared with summation effect on each treatment. In the case of the combination of CS-mix and VCP-IS-2Na, the expression of Fibrillin-1 and EMILIN-1 was synergistically suppressed by 198% and 192% compared to summation effect. NC-CSVC suppressed the collagen and elastin fiber disintegration stronger than NC-CS, which is without VCP-IS-2Na.

Conclusion: The synergistic effects of CS-mix and VCP-IS-2Na were clarified on suppression against collagen and elastin fiber disintegration under UVA irradiation. Furthermore, it was clarified that approach with VCP-IS-2Na to NHDFs first and then with CS-mix is more effective. Therefore, NC-CSVC realized the component treatment to NHDFs in an appropriate order. These results suggest that combination of CS-mix and VCP-IS-2Na achieves a higher synergistic effect, and NC-CSVC is effective as a method for maximizing this synergistic effect for skin care cosmetics.

Keywords: Amphiphilic vitamin C derivative, Disodium isostearyl ascorbyl phosphate, Coffee seed extract, nano-capsulation, Collagen, Elastin

Introduction.

Ultraviolet (UV) irradiation causes not only direct cytotoxicity due to DNA damage but also oxidative stress by induction of reactive oxygen species (ROS) [1]. Skin aging is divided into two types, intrinsic aging and photoaging. Intrinsic ageing is an irreversible decline that happens to all living things. Photoaging is caused by chronic exposure to UV irradiation in sunlight and is characterized by alterations in facial skin appearance such as deep wrinkles and sagging [2]. The formation of facial wrinkles and sagging is closely related to loss of the elastic properties of the skin and these are linked to the dermal extracellular matrix composed of collagen and elastic fibers.

Normal human dermis consists primarily of an extracellular matrix (ECM) of connective tissue. Three major extracellular components have been recognized that contribute to the physiological properties of the skin. Specifically, fibers consisting of collagen, an abundant ECM protein that accounts for about 80% of the dry weight of the skin, provide tensile properties to the dermis, so as to allow the skin to serve as a protective organ against external trauma [3]. The elastic fibers, which account for 2-4% of the ECM in sun-protected skin, form an interconnecting network that provides elasticity and resilience to normal skin [4]. The ECM serves not only as a scaffolding to stabilize tissue structure, but also has been

observed to influence the development, migration, proliferation, shape, and metabolic function of cells with which it comes into contact. Thus processes that alter the relative proportions of ECM can result in clinical manifestations that are recognized as part of the cutaneous aging process.

Elastic fibers play a critical role in maintaining skin elasticity and firmness. There are three fiber types, oxytalan, elaunin, and elastin, and elasticity depends on the quantities of tropoelastin adsorbed on microfibrils. Chronic UV exposure results in qualitative and quantitative deterioration of elastin and induces the loss of elasticity. Causes of photoaged skin include epidermal–dermal junction flattening and the disappearance of oxytalan fibers, which are pure microfibrils [5]. Oxytalan fibers exhibit a candlestick-like structure in the papillary dermis anchored to the basement membrane and have an important role connecting the basement membrane to the dermis [6]. Oxytalan fibers are basically formed by microfibrils composed of fibrillin-1 (FBN-1). In order to form the framework of microfibrils, FBN-1 is firstly aligned around the cell and is cross-linked and stabilized by a γ-glutamyl-ε-lysine isopeptide bond of transglutaminase [7]. FBN-1 is organized by microfibrillar-associated protein 4 (MFAP-4), microfibrils are formed [8], then latent TGF-β-binding proteins (LTBPs) are deposited onto microfibrils, which become mature. These structural constituent proteins of elastin fibers are produced by human dermal fibroblasts. There is also a report suggesting the importance of elastin microfibril interface located protein-1 (EMILIN-1) for production of oxytalan fibers [9]. However, due to the involvement of many proteins in the microfibrils of oxytalan fibers, the reconstruction mechanism of oxytalan fibers has not yet been clarified.

We have been reported that the mix extract (CS-mix), *Coffea Robusta* seed extract (CRS) and *Caffea Arabica* (Coffee) seed extract (CAS), which contains many amounts of caffeic acid and chlorogenic acid, prevented UVA irradiation-induced decreased levels of collagen synthesis and increased level of pro-MMP-1 production [10]. Furthermore, CRS and CAS suppressed the decreases of the mRNA expression of microfibril-related genes exposed to UVA irradiation. Therefore, we developed a nano-capsule (NC-CS) containing CRS and CAS by polyglyceryl fatty derivative. NC-CS had greater suppressive potencies against the decrease in collagen, the MMP-1 production, and the downregulation of the mRNA expression of microfibril-related genes induced by UVA irradiation on NHDFs.

We have recently synthesized an amphiphilic ascorbic derivative, disodium isostearyl ascorbyl phosphate (VCP-IS-2Na), which exhibited high stability in various aqueous solutions at a wide range of pH values and satisfactory thermal stability [11]. VCP-IS-2Na has skin permeability superior to that of VC and exhibits VC activity in vitro and in vivo after enzymatic hydrolysis to free VC by phosphatase and/or esterase. So far, we have shown that VCP-IS-2Na has anti-melanogenesis effects [12] and stimulation on collagen synthesis [13].

Nanocapsule technology, which is defined as the process of encapsulating substances with various coating materials at the nanoscale range, is utilized as effective delivery system for active agents. This technique has advantages of controlling to release entrapped materials gradually and protecting susceptible materials such as vitamins from surrounding environments. However, in this study, nanocapsule technology was used not only as a means to improve the stable permeability of the inclusion component, but also to control the order of approach of the inclusion component and the shell component to the cell.

In this study, we investigated whether the combination of CS-mix and VCP-IS-2Na exerted a synergistic suppressive effect on on collagen synthesis, MMP-1 activity, mRNA expression of microfibril-relating genes in normal human dermal fibroblasts (NHDFs) under UVA irradiation. Furthermore, we also examined whether nanoencapsulation of CS-mix with VCP-IS-2Na enhanced its effect in human skin models.

Materials and Methods.

Materials

Coffea Robusta seed extract (CRS) and *Caffea Arabica* (Coffee) seed extract (CAS) were used for general cosmetics ingredients. CRS and CAS were mixed and CS-mix was dispensed. VCP-IS-2Na (disodium isostearyl ascorbyl phosphate) was synthesized by the methods described in our previous report [11].

Cells culture

Normal human dermal fibroblasts (NHDFs; Kurabo Co., Osaka, Japan) were maintained in FibroLife® basal medium (BM; Lifeline® Cell Technology, Maryland, USA) supplemented with 7.5 mM L-glutamine, hFGF-β (5 ng/mL), insulin (5 μg/mL), ascorbic acid (50 μg/mL), hydrocortisone (1μg/mL), and 2% fetal bovine serum (FBS) at 37 °C in 5% CO_2.

Suppression of collagen decrease and MMP-1 production stimulated by UVA irradiation on NHDFs

The suppressive effects on pro-MMP-1 production and amount of procollagen type I pN-peptide stimulated by UVA irradiation were evaluated of NHDFs using ELISA system. NHDFs were inoculated into 6-well plates at a density of 1.0×10^6 cells/well for 24 hr. The cells were changed to fresh medium containing various concentrations of samples and then cultured for 24 h. Control cells were cultivated without samples. They were exposed to 30 J/cm^2 UVA irradiation. The supernatants of each well were collected after 24 h cultivation, and the amount of procollagen type I pN-peptide and interstitial MMP-1 was measured by using the type I pN-peptide assay kit (TaKaRa, Japan) and human MMP-1 ELISA Kit (Amersham, Sweden).

Suppression of the mRNA expression of microfibril-related genes induced by UV irradiation in NDHFs

NHDFs were inoculated into 6-well plates at a density of 1.0×10^6 cells/well for 1 day. The cells were changed to fresh medium containing various concentrations of samples and then cultured for 24 h. They were exposed to 30 J/cm^2 UVA irradiation, the medium was changed to fresh medium, and the cells were cultured for a further 6 h before total RNAs were extracted. Total RNAs of NHDFs were extracted using NucleoSpin® RNA Plus (Machery-Nagel GmbH & Co. KG, Germany) and reverse-transcribed to cDNA with Oligo dT Primers and Random 6 mers using a PrimeScript® RT reagent Kit (TaKaRa Bio Inc., Japan). Real-time PCR was performed with SYBR® Premix Ex TaqTM II (TaKaRa Bio Inc.) and the products were analyzed using a Thermal Cycler Dice® Real Time System TP800 (TaKaRa Bio Inc.). Primers used for quantitative PCR are provided in Table 1.

Table 1. Primers used for quantitative PCR

	Sense	Anti-sense
FBN-1	5'-CTTCCACCTAACAGGCCATTAACA-3'	5'-CTATCACATGGTTCCATAGGTGCAG-3'
MFAP-4	5'-CCTATGCCAAGTACGCTGACTTCT-3'	5'-AACTTCTGGCCACTGTGGTAGGAC-3'
EMILIN-1	5'-CCTTCLACAGAGTCCTGCTCAA-3'	5'-CGCTCAGCAAGTAGCGTCCA-3'
LTBP-4	5'-TTCACLACTGTCAGCTGTGCTC-3'	5'-TTTCAAAGCCGGTTGGACAAG-3'

Evaluation of impact in the order of CS-mix and VCP-IS-2Na in the synergistic suppressive effects

To evaluate of impact in the order in the suppressive effects of combination of CS-mix and VCP-IS-2Na on pro-MMP-1 production, amount of procollagen type I pN-peptide, and the mRNA expression of microfibril-related genes stimulated by UVA irradiation were evaluated of NHDFs, which is treated with one sample for12 hr, then washed with PBS, then treated with other sample for 12 hr using the above methods. When treating with one type of sample, treating with the control for 12 hours after treating with the sample for 12 hours.

Preparation of nano-capsules of CS-mix with VCP-IS-2Na

CS-mix were mixed with isostearyl neopentanoate, caprylic/capric triglyceride, behenyl alcohol, squanane, dipropylene glycol, butylene glycol, pentylene glycol, sodium bicarbonate, xanthan gum crosspolymer, hydroxyethylcellulose, phenoxyethanol, and ethylhexylglycerin, and then VCP-IS-2Na, glycerin, and water were added to the solution. The solution was homogenated and made to CSVC complex (CP-CSVC). CP-CSVC was used with thin-film spin system high-speed mixer (FILMIX® Model 80, PRIMIX, Japan) and so made to sharp distribution of nanometer-sized particles, Nano-capsule CRVC (NC-CRVC). CS-mix were mixed with butylene glycol, phenoxyethanol, behenyl alcohol and squalane, and then polyglyceryl-10 diisostearate, polyglyceryl-10 myristrate, glycerin, and water were added to the solution. The solution was homogenated and made to CS complex (CP-CS). CP-CS was used with thin-film spin system high-speed mixer and so made to sharp distribution of nanometer-sized particles, Nano-capsule CS (NC-CS).

Skin permeation and accumulation assay with reconstructed human skin model

The skin penetration assay of CP-CSVC, NC-CSVC, CP-CS and NC-CS was performed by using epidermal human skin model, EPI-200X. 0.05% Sample solution (50 μl) was added to the interior of EPI-200X with 1.0 ml medium. After a fixed period of time, the collected mediums and extract, which is homogenated portion and 1.0 ml 50% ethanol, were subjected to HPLC analysis.

Suppression of NC-CSVC against skin damage stimulated by UVA irradiation on human reconstructed skin model

The suppressive effects of CP-CSVC, NC-CSVC, CP-CS and NC-CS on pro-MMP-1 production, amount of procollagen type I pN-peptide, and the mRNA expression of microfibril-related genes stimulated by UVA irradiation were evaluated of NHDFs, which is treated with UVA irradiated EPI-200X medium, using the above methods.

Results.

Suppressive effect of CS-mix and VCP-IS-2Na on collagen decrease and MMP-1 production stimulated by UVA irradiation in NHDFs

To investigate the synergistic suppression of CS-mix and VCP-IS-2Na on collagen decrease and MMP-1 production irradiated with UVA in biological tests, its effects against UVA irradiation was evaluated using NHDFs in culture. Pretreatment of NHDFs with each CS-mix and VCP-IS-2Na at 2 ppm showed the significant suppression of the decrease in collagen irradiated with UVA by 15% and 30%, respectively (Fig.-1[A]). The suppression rate of decrease in collagen was the highest when combining CS-mix and VCP-IS-2Na at 2 ppm, reaching up to 94%, which is twice as effective as the total effect of each treatment. Each CS-mix and VCP-IS-2Na at 2 ppm showed a significant 28% and 56% suppression of the excess production of MMP-1 induced by UVA irradiation (Fig.-1[B]). The suppression rate of excess production of MMP-1 was the highest when combining CS-mix and VCP-IS-2Na at 2 ppm, reaching up to 11%.

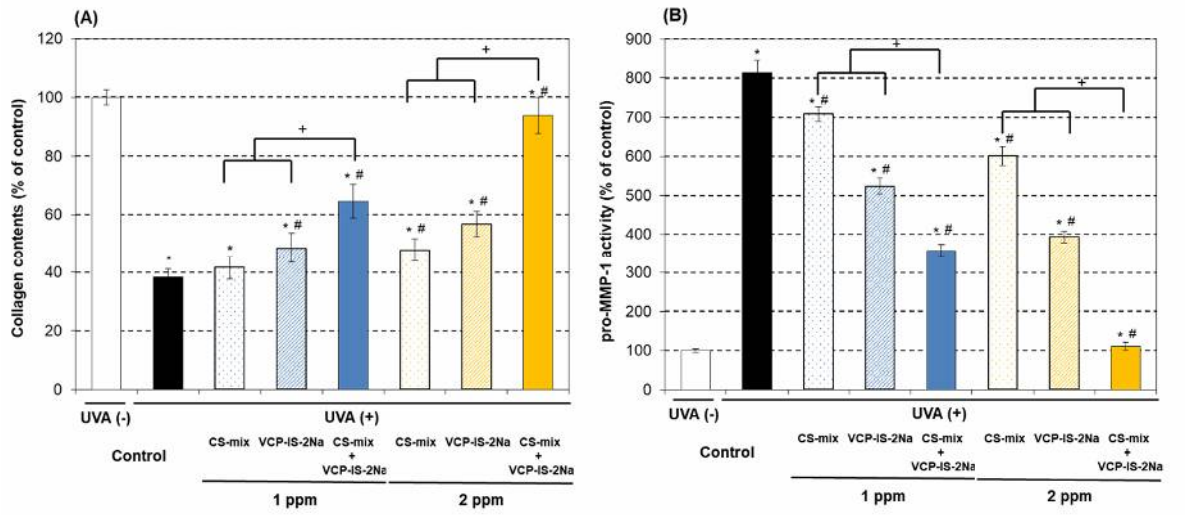

Fig.-1. Suppressive effect of CS-mix and VCP-IS-2Na on collagen decrease (**A**) and MMP-1 production (**B**) induced by UVA irradiation.

Each value represents the mean ± S.E. of three experiments. Values were significantly different from the non-irradiated group, UVA(-), at $p < 0.05$ (*). Values were significantly different from the irradiated group, UVA(+), at $p < 0.05$ (#). Values were significantly different in two groups at $p < 0.05$ (+).

Suppressive effect of the mRNA expression of microfibril-related genes induced by UV irradiation in NDHFs

The mRNA expression of the microfibril-related genes, FBN-1, MFAP-4, EMILIN-1, and LTBP-4, was decreased by 56%, 60%, 53%, and 67% after 30 J/cm^2 UVA irradiation compared in sham-irradiated cells (Fig.-2). The results indicated that ROS interferes with the reconstruction of oxytalan fibers in fibroblasts by decreasing expression of microfibril-related genes and that ROS scavengers may be effective as reconstructive agents.

To protect the reconstruction of oxytalan fibers against UVA, we evaluated the potential of CS-mix and VCP-IS-2Na for suppressing the downregulation of the mRNA expression of microfibril-related genes induced by UVA irradiation in NHDFs. We examined the effect of NC-CS on the downregulation of mRNA expression of microfibril-related genes induced with 30 J/cm2 UVA irradiation. NC-CS suppressed the downregulation of mRNA expression in a dose-dependent manner. CS-mix, at 2 ppm, suppressed the downregulation of FBN-1, MFAP-4, EMILIN-1, and LTBP-4 at 30 J/cm^2 UVA irradiation by 10%, 24%, 11%, and 20%,

respectively. VCP-IS-2Na, at 2 ppm, suppressed the downregulation of FBN-1, MFAP-4, EMILIN-1, and LTBP-4 at 30 J/cm^2 UVA irradiation by 31%, 39%, 41%, and 39%, respectively. The suppressive effects were showed by 80%, 64%, 99%, and 61% when combining CS-mix and VCP-IS-2Na at 2 ppm. These results indicated that the synergistic suppression of CS-mix and VCP-IS-2Na on the downregulation of FBN-1 (Fig.-2[A]) and EMILIN-1 (Fig.-2[B]) showed reaching up to 98% and 92%. On the other hands, the synergistic suppression of CS-mix and VCP-IS-2Na on the downregulation of MFAP-4 (Fig.-2[C]) and LTBP-4 (Fig.-2[D]) hardly showed.

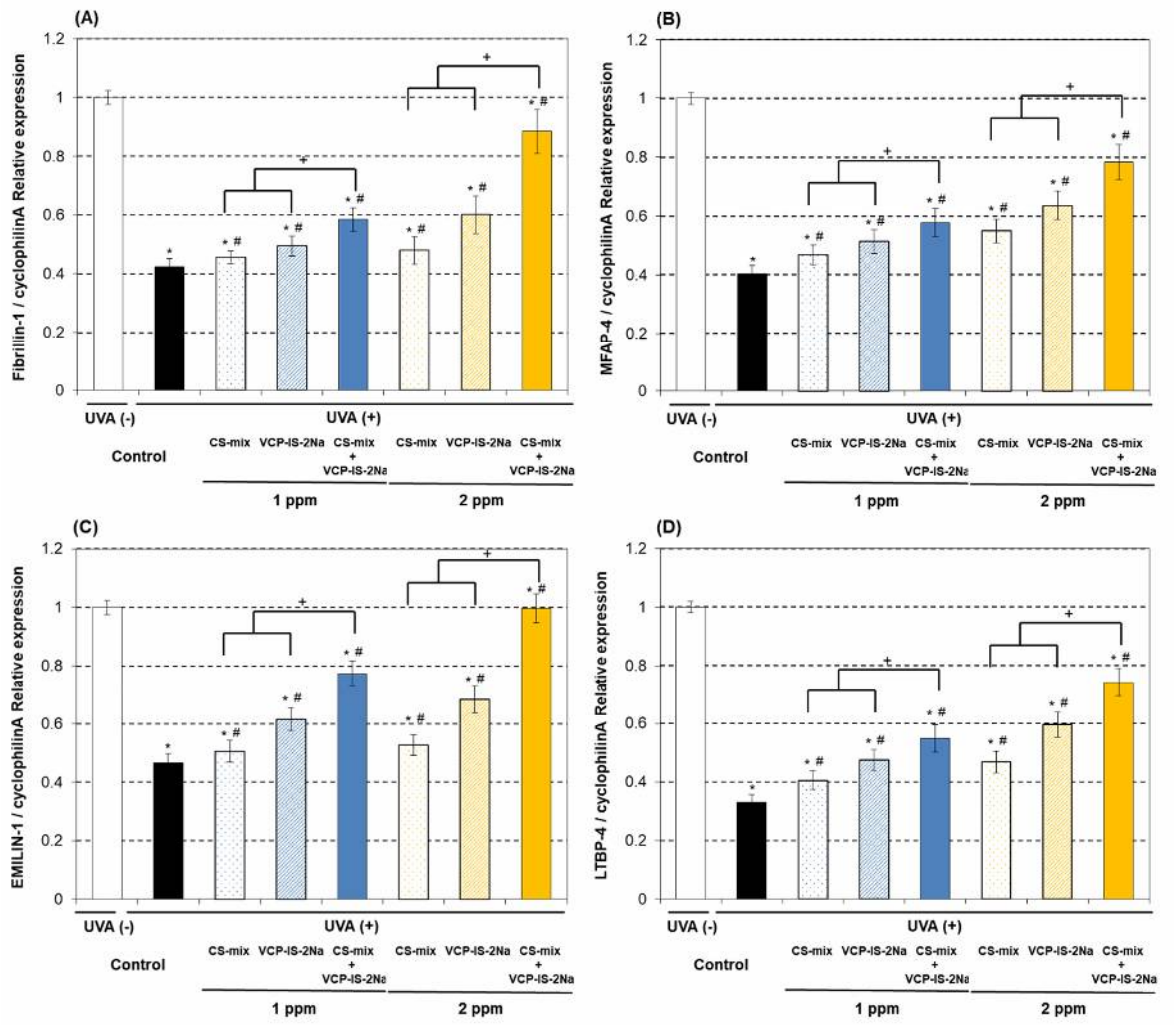

Fig.-2. Suppressive effect of CS-mix and VCP-IS-2Na on the downregulation of the mRNA expression of FBN-1, MFAP-4, EMILIN-1 and LTBP-4 in NHDFs exposed to UVA irradiation.

Each value reported represent means ± SD. Values were significantly different from the non-irradiated group, UVA(-), at $p < 0.05$ (*). Values were significantly different from the irradiated group, UVA(+), at $p < 0.05$ (#). Values were significantly different in two groups at $p < 0.05$ (+).

Impact in the order of CS-mix and VCP-IS-2Na in the synergistic suppressive effects

To investigate the impact in the order of CS-mix and VCP-IS-2Na in the synergistic suppressive effects on collagen decrease, MMP-1 production, and the downregulation of the mRNA expression of microfibril-related genes induced by UVA irradiation in biological tests, its effects against UVA irradiation was evaluated by changing the treatment order of CS-mix and VCP-IS-2Na in culture. Treatment with VCP-IS-2Na to NHDFs first and then with CS-mix suppressed the decrease in type I collagen production and the downregulation of FBN-1 and EMILIN-1 twice stronger than that with CS-mix to NHDFs first and then with VCP-IS-2Na (Fig.-3 and Fig.-4). Although, treatment with VCP-IS-2Na to NHDFs first and then with CS-mix suppressed the excess production of MMP-1 and the downregulation of MFAP-4 and LTBP-4 twice as strong as that with CS-mix to NHDFs first and then with VCP-IS-2Na.

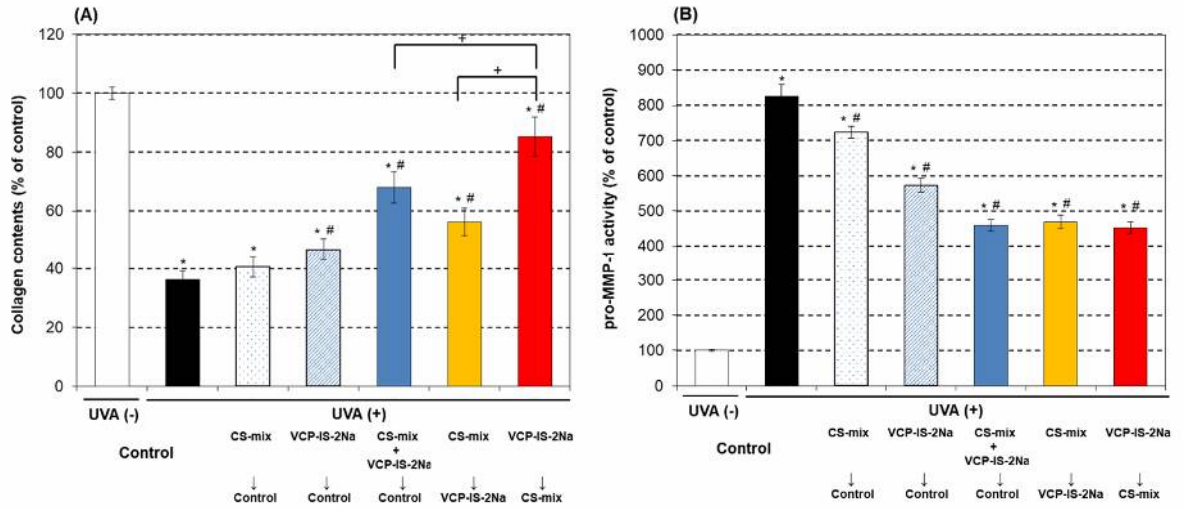

Fig.-3. Suppressive effect of CS-mix and VCP-IS-2Na on collagen decrease (**A**) and MMP-1 production (**B**) induced by UVA irradiation with changing the processing order of the samples.

Each value represents the mean ± S.E. of three experiments. Values were significantly different from the non-irradiated group, UVA(-), at $p < 0.05$ (*). Values were significantly

different from the irradiated group, UVA(+), at $p < 0.05$ (#). Values were significantly different in two groups at $p < 0.05$ (+).

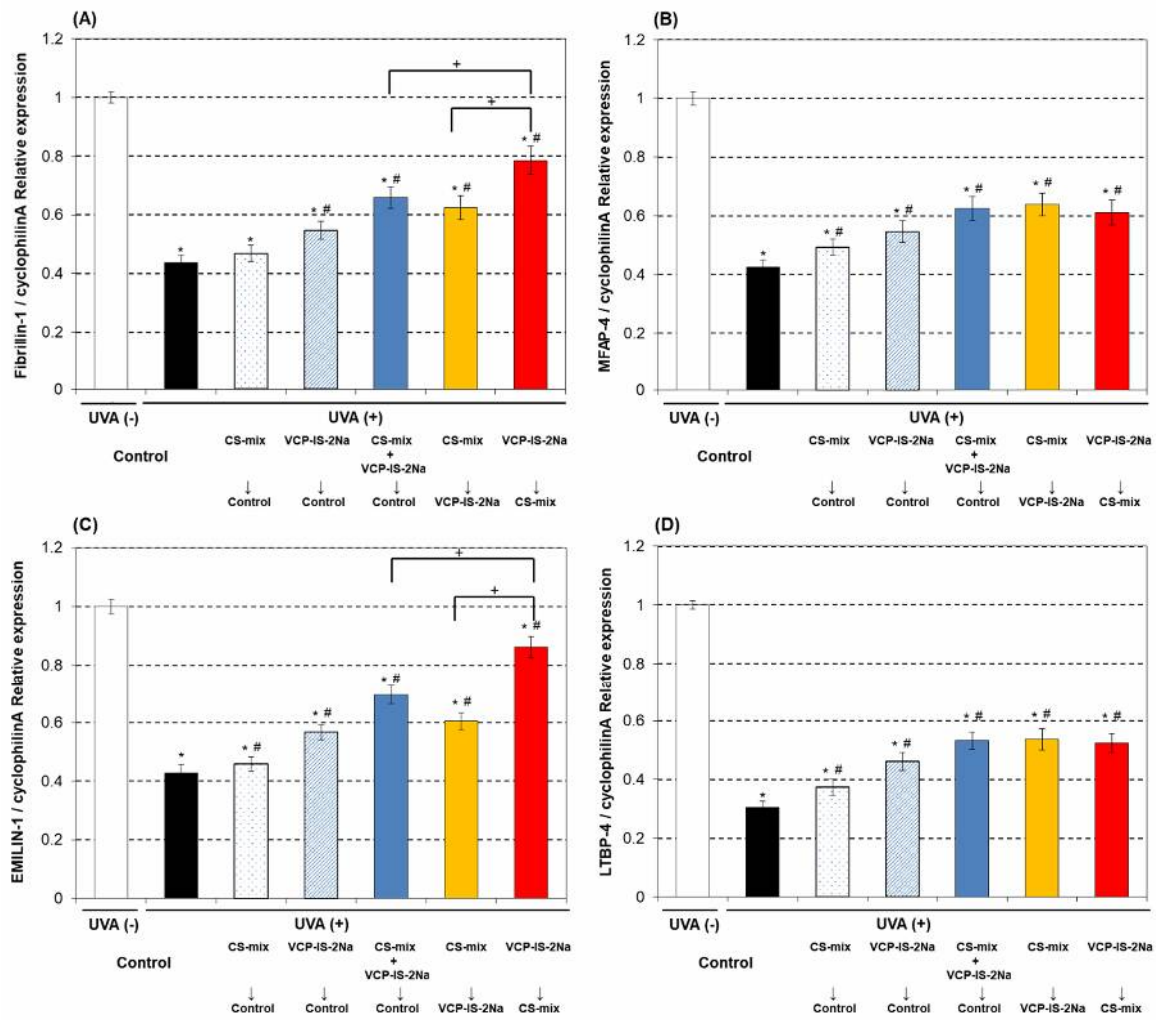

Fig.-4. Suppressive effect of CS-mix and VCP-IS-2Na on the downregulation of the mRNA expression of FBN-1 (**A**), MFAP-4 (**B**), EMILIN-1 (**C**) and LTBP-4 (**D**) in NHDFs exposed to UVA irradiation with changing the processing order of the samples.

Each value reported represent means ± SD. Values were significantly different from the non-irradiated group, UVA(-), at $p < 0.05$ (*). Values were significantly different from the irradiated group, UVA(+), at $p < 0.05$ (#). Values were significantly different in two groups at $p < 0.05$ (+).

Skin permeation and accumulation assay with reconstructed human skin model

The maximum penetration dose (ratio) of caffeic acid was ~22.6 nmol after 24 h treatment with CP-CS (Fig.-5[A]). Although, the maximum permeation dose (ratio) of caffeic acid was ~132.5 nmol after 24 h treatment with NC-CS. The maximum permeation dose (ratio) of caffeic acid was ~60.4 nmol after 24 h treatment with CP-CSVC. Although, the maximum permeation dose (ratio) of caffeic acid was ~258.4 nmol after 24 h treatment with NC-CSVC. The permeation of NC-CSVC is higher than that of CP-CSVC by 4.2 times. Furthermore, the permeation of NC-CSVC is higher than that of NC-CS by twice. The accumulation result is similar as the permeation results (Fig.-5[B]).

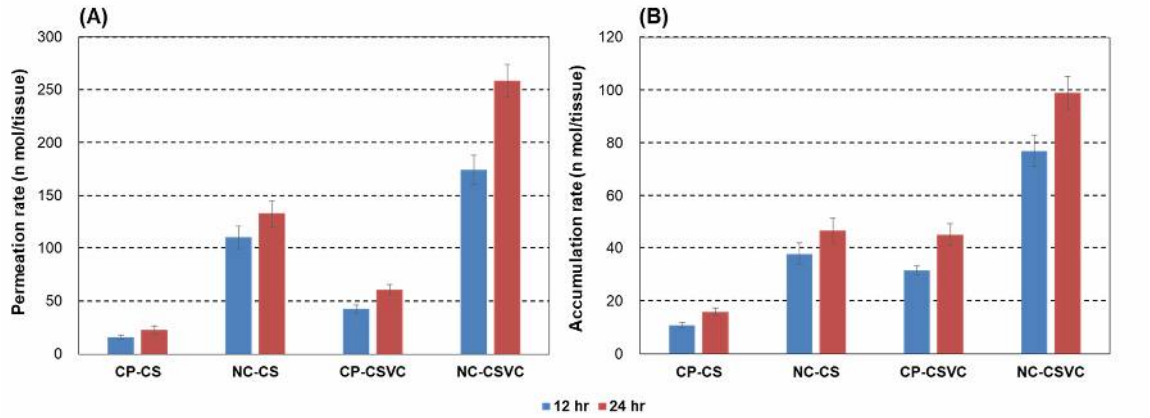

Fig.-5 Skin permeation (**A**) and accumulation (**B**) on EPI-200X.

Each value represents the mean±SD of three experiments, and values containing asterisks were significantly different (*P<0.05) in two groups.

Suppression of NC-CSVC against skin damage stimulated by UVA irradiation on human reconstructed skin model

Treatment with NC-CSVC to NHDFs suppressed the decrease in type I collagen production, the excess production of MMP-1 and the downregulation of FBN-1 and EMILIN-1 10 times over stronger than that with CP-CSVC (Fig.-6 and Fig.-7). Although, treatment with NC-CSVC to NHDFs suppressed the excess production of MMP-1 and the downregulation of MFAP-4 and LTBP-4 twice as strong as that with CP-CSVC to NHDFs. Treatment with NC-CSVC to NHDFs suppressed the decrease in type I collagen production, the excess production of MMP-1 and the downregulation of the microfibril-related genes stronger than that with NC-CS.

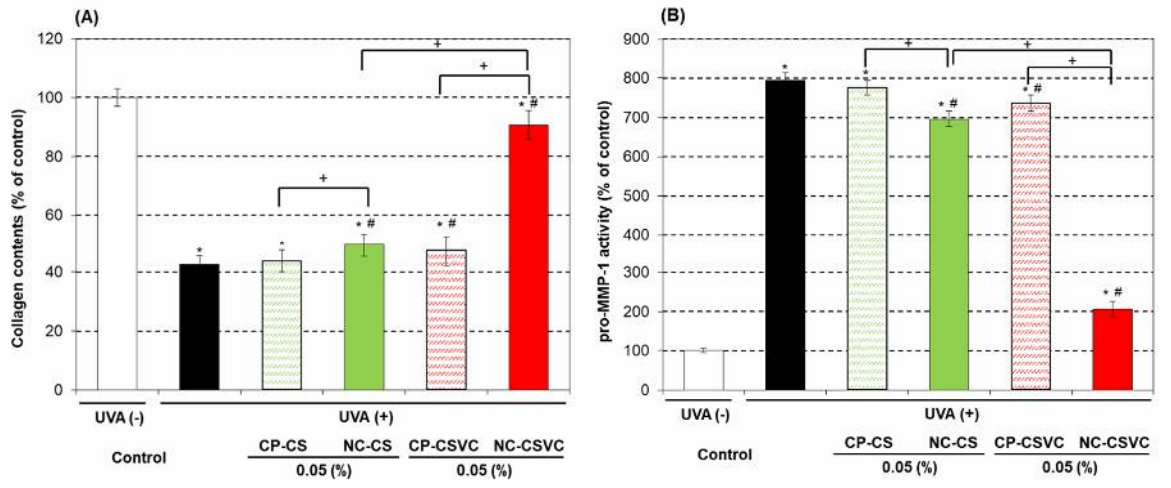

Fig.-6. Suppressive effect of NC-CSVC on collagen decrease (**A**) and MMP-1 production (**B**) induced by UVA irradiation.

Each value represents the mean ± S.E. of three experiments. Values were significantly different from the non-irradiated group, UVA(-), at $p < 0.05$ (*). Values were significantly different from the irradiated group, UVA(+), at $p < 0.05$ (#). Values were significantly different in two groups at $p < 0.05$ (+).

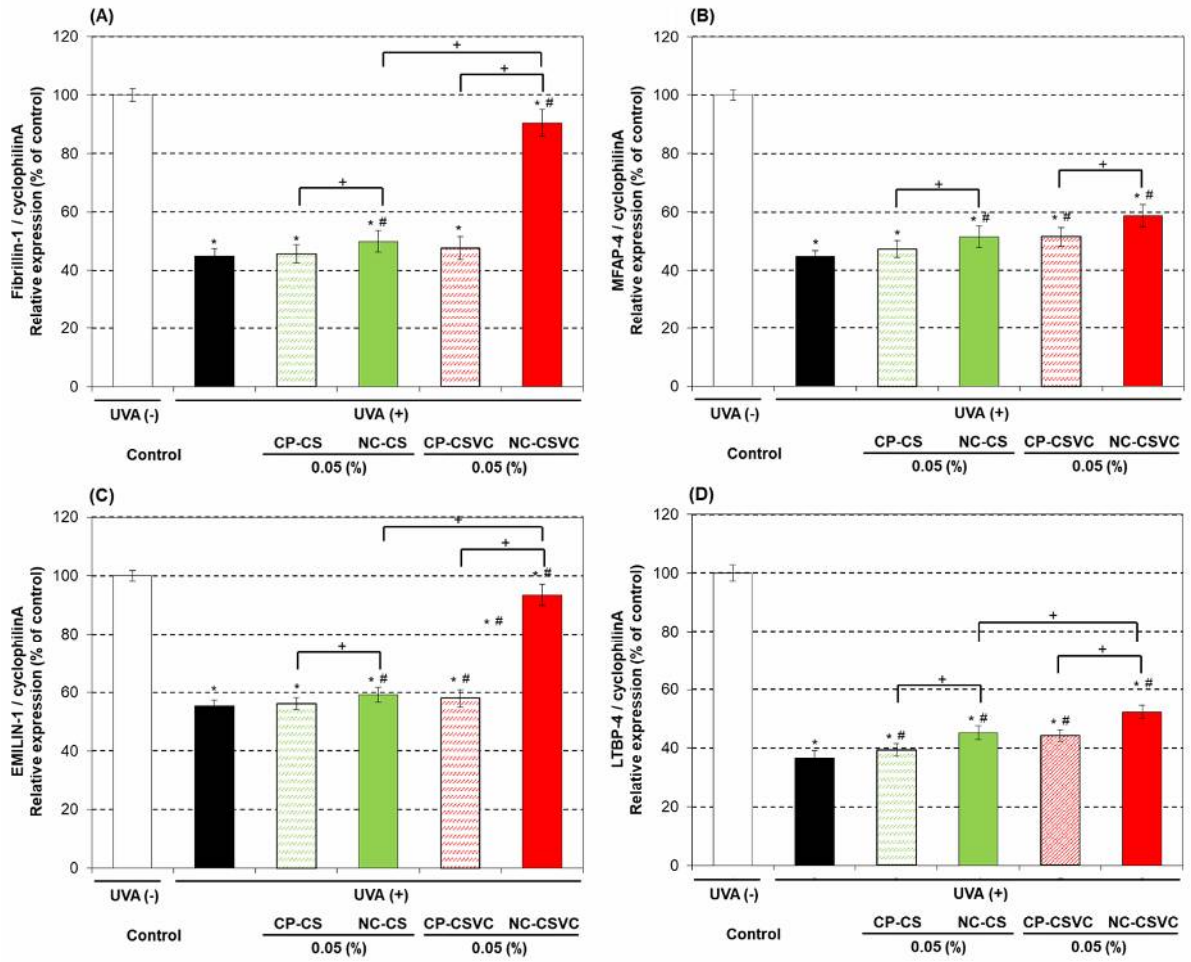

Fig.-7. Suppressive effect of NC-CSVC on the downregulation of the mRNA expression of FBN-1, MFAP-4, EMILIN-1 and LTBP-4 in NHDFs exposed to UVA irradiation.
Each value reported represent means ± SD. Values were significantly different from the non-irradiated group, UVA(-), at $p < 0.05$ (*). Values were significantly different from the irradiated group, UVA(+), at $p < 0.05$ (#). Values were significantly different in two groups at $p < 0.05$ (+).

Discussion.

The present study demonstrated that the combination of CS-mix and VCP-IS-2Na showed a synergistic protective effect against the decrease in type I collagen production, the excess production of MMP-1 and the downregulation of FBN-1 and EMILIN-1. Furthermore, we

clarified that these synergistic protective effect changed in the order of CS-mix and VCP-IS-2Na for approach to NHDFs. Treatment with VCP-IS-2Na to NHDFs first and then with CS-mix was more effective for these synergistic protective effect than that with CS-mix to NHDFs first and then with VCP-IS-2Na. We previously reported that nano-capsule of vitamins with VCP-IS-2Na was collapsed by enzymatic reaction, and then supplied intentional ingredients in the skin [14]. Therefore, we developed a novel nano-capsule NC-CSVC contains CS-mix as intentional ingredients with VCP-IS-2Na as shell constituents for not only high permeation and delivery of intentional ingredients but also achieve an effective ordered approach to NHDFs. Fig.-5 showed nanoencupsilation enhanced permeability and accumulation of CS-mix and VCP-IS-2Na by 4 times in human skin model. On the other hands, NC-CSVC protected UVA irradiation-induced the decrease in type I collagen production, the excess production of MMP-1 and the downregulation of FBN-1 and EMILIN-1 stronger than CP-CSVC. Furthermore, NC-CS suppressed the decreases of the mRNA expression of microfibril-related genes exposed to UVA irradiation stronger than non-capsule complex (CP-CPVC) by 10 times over. Therefore, it is considered that the increase of these synergistic protective effect on CS-mix and VCP-IS-2Na is brought by not only the improvement of the permeability and retention of the active reagents and the protection from the environments in which the activity of these materials are easily lost, but also the achieve of an effective ordered approach of CS-mix and VCP-IS-2Na to NHDFs by nanoencapsulation.

It is acknowledged that alteration in the quantity and character of collagen and elastin is a critical factor in the process of sagging and wrinkle formation. UV (UVB and UVA) irradiation of the skin causes changes in the metabolism of collagen and elastin matrix. UVA irradiated-fibroblasts have been shown to have decreased collagen production and increased MMP-1 production [15]. We previously reported that the mRNA expression of the microfibril-related genes was decreased in NHDFs induced with UVA irradiation [16]. Repetition of these reactions causes a significant decrease in dermal collagen and elastin, leading to the formation of very fragile dermis, sagging and wrinkles.

NC-CSVC is significantly suppressed the excess decrease in type I collagen production and excess production of MMP-1 following UVA irradiation in NHDFs (Fig.-6). It has been established that singlet oxygen produced in cells by UVA irradiation up-regulates MMP-1

gene transcription [17]. The results suggested that NC-CSVC suppressed MMP-1 production by quenching singlet oxygen, and therefore suppressed the decrease in type I collagen caused by inhibition of MMP-1 production in NHDFs. NC-CSVC is suppressed the decreases of the mRNA expression of FBN-1 and EMILIN-1 in NHDFs exposed to UVA irradiation (Fig.-7). Therefore, NC-CSVC is effective to reconstruct of forming oxytalan fibers with approach of basically microfibrils composed of FBN-1and EMILIN-1, which works as microfibril interface for production of oxytalan fibers. We also reported decreases in the mRNA expression of microfibril-related genes in fibroblasts exposed to H_2O_2 [16]. The anti-oxidant action of NC-CSVC promoted the recovery of the oxytalan fiber-related gene expression in NHDFs with UVA irradiation due to reduced intracellular ROS. Since the difference in action points and timing for NHDFs between CS-mix and VCP-IS-2Na may have led to synergistic effect, I would like to investigate that as the subject of our research in the future.

Conclusion.

We clarified the synergistic suppressive effect of CS-mix and VCP-IS-2Na on collagen synthesis, MMP-1 activity, mRNA expression of microfibril-relating genes in NHDFs under UVA irradiation. The combination of CS-mix and VCP-IS-2Na efficiently synergistically protected against the decrease in type I collagen production, the excess production of MMP-1 and the downregulation of FBN-1 and EMILIN-1. Furthermore, it was clarified that approach with VCP-IS-2Na to NHDFs first and then with CS-mix is more effective. Therefore, we developed a novel nano-capsule NC-CSVC, and NC-CSVC realized the component treatment to NHDFs in an appropriate order by transdermal enzymatic delivery system. These results suggest that combination of CS-mix and VCP-IS-2Na achieves a higher synergistic effect, and NC-CSVC is effective as a method for maximizing this synergistic effect. Combination of CS-mix and VCP-IS-2Na and NC-CSVC may be a novel effective anti-aging agent to progress the reconstruction of collagen and elastic fibers for the new approach of protective effect against collagen and elastin fiber disintegration for skin care cosmetics.

Conflict of Interest Statement.

NONE

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