Inhibitory effect of the nano-capsule configured with hydrolyzed egg shell membrane and VCP-IS-2Na against melanogenesis

NT_612

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INTRODUCTION

Melanin production in melanocytes involves various proteins such as tyrosinase (TYR) and microphthalmia-associated transcription factor (MITF). Furthermore, alpha-melanocyte-stimulating hormone (α-MSH), which is generated from cleavage of proopiomelanocortin (POMC) peptide, is secreted from keratinocytes and binds to melanocortin-1 receptor (MC1R) on plasma membrane of melanocytes to promote melanogenesis.

Previously, we have developed the novel nano-capsule (NC-ESMVC) configured with egg shell membrane hydrolyzed extract (ESM) and sodium isostearyl 2-O-L-ascorbyl phosphate (VCP-IS-2Na), an amphiphilic ascorbic derivative. VCP-IS-2Na was reported to have the inhibitory effects on melanogenesis. However, the inhibitory effect of the combination of ESM and VCP-IS-2Na on melanogenesis and the promotion of NC-ESMVC on that effect with a nano-encapsulation technology have not been evaluated.

In this study, we investigated whether the combination of ESM and VCP-IS-2Na effectively suppressed melanogenesis and inhibited the melanogenesis-related gene expression level. Furthermore, NC-ESMVC was investigated in comparison with non-capsule.

MATERIALS AND METHODS

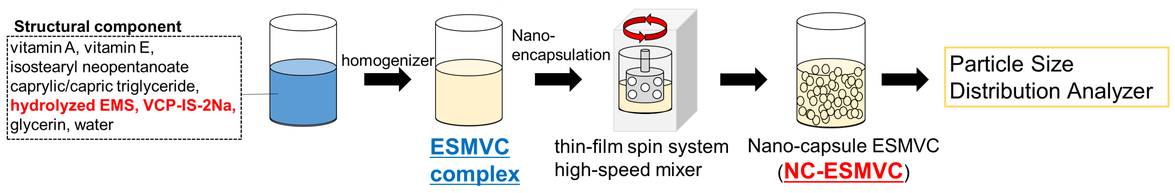
Measurement of melanin content

HMV-II cells(RIKEN BRC, JAPAN) were treated with ESM and/or VCP-IS-2Na for 48h. Cells were trypsinized and centrifuged. The cell pellet was dissolved in 2 N NaOH at 98℃ for 2 min and the melanin content was measured at absorbance of 405nm.

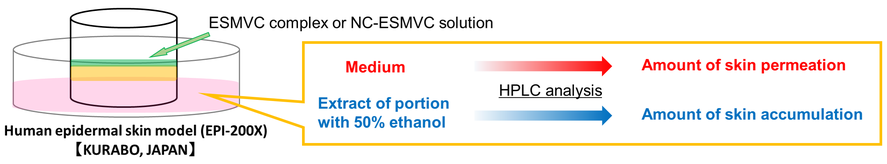
Effects of ESM and VCP-IS-2Na on gene expression.

HMV-II cells were treated ESM and/or VCP-IS-2Na for 24 h, followed by total RNAs were extracted using NucleoSpin® RNA Plus (TaKaRa Bio, Japan). Normal human epidermal keratinocytes(NHEKs) were treated with 500 μM H_2O_2 for 30 minutes before ESM and/or VCP-IS-2Na treatment. Total RNAs were converted to cDNAs using a PrimeScript™ RT Master Mix (Takara Bio, Japan). Real-time PCR was performed and relative expression of mRNA was calculated by $2^{-\Delta\Delta CT}$ method and normalized to the expression of GAPDH.

Preparation and morphological analysis of NC-ESMVC



Skin permeation and accumulation assay of NC-ESMVC



Inhibitory effect on melanogenesis using cultured human skin models

NC-ESMVC or ESMVC complex solution was applied to the human skin model (MEL300; Kurabo, JAPAN). After 2weeks incubation, macro- and microscopic observations were conducted.

Presentation of the data and statistical analysis

Statistical significance in the data was assessed running Student's t-test. Statistical significant differences compared with control are indicated by asterisks as follows: *P< 0.05, **P < 0.01 and ***P < 0.001.

RESULTS

The effects of ESM and VCP-IS-2Na

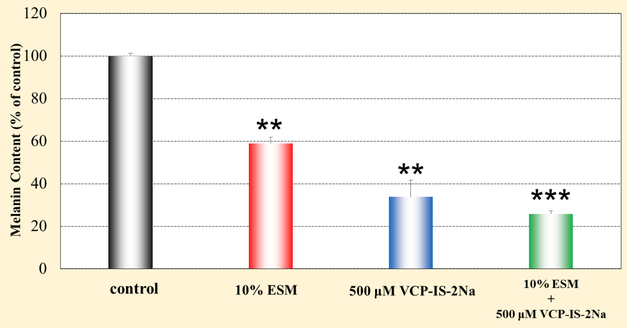


Fig.1 Effect of ESM and VCP-IS-2Na on melanin production in HMV-II cells

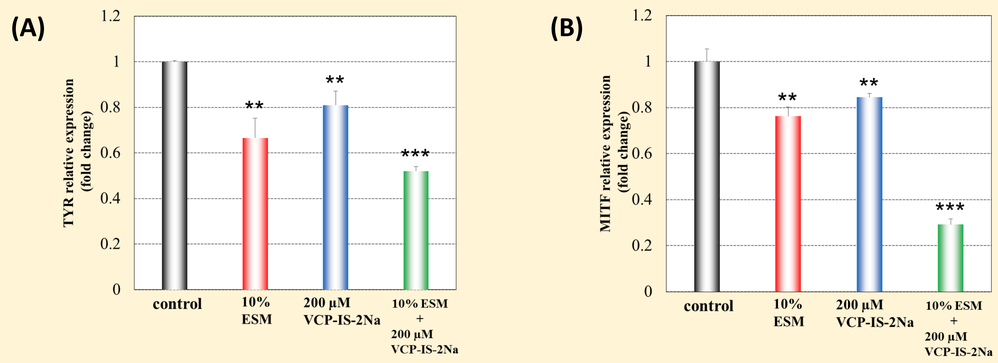


Fig.2 Effect of ESM and VCP-IS-2Na on gene expression involved in melanogenesis.

(A) Tyrosinase (TYR) gene expression HMV-II cells.

(B) Microphthalmia-associated transcription factor (MITF) expression in HMV-II cells.

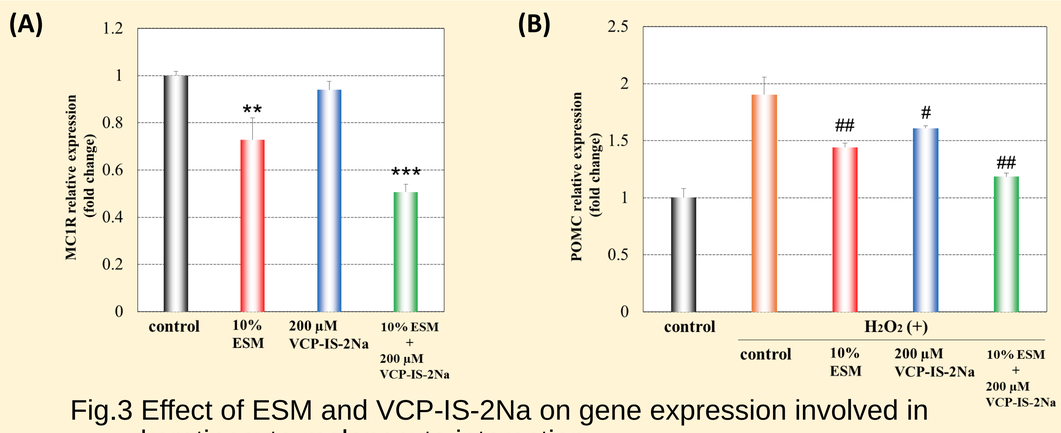


Fig.3 Effect of ESM and VCP-IS-2Na on gene expression involved in keratinocyte-melanocyte interaction.

(A) Melanocortin 1 receptor(MC1R) gene expression in HMV-II cells.

(B) Pro-opiomelanocortin(POMC) expression in NHEK cells. # p<0.05 ##p<0.01 compared with H2O2-treated control.

The functions of NC-ESMVC

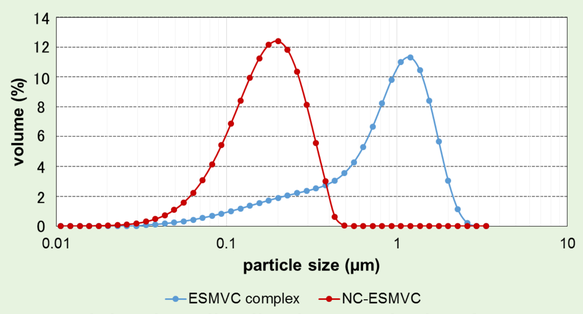


Fig.4 Particle size distribution analysis of NC-ESMVC

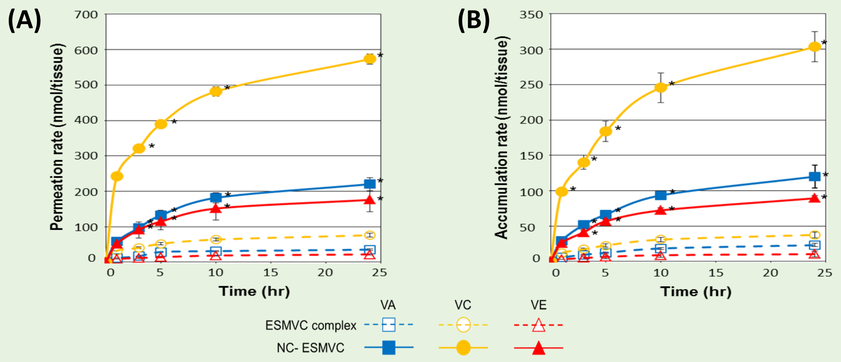


Fig.5 Skin permeation and accumulation assay of NC-ESMVC

(A) Permeation components through EPI-200X

(B) Accumulation components in EPI-200X

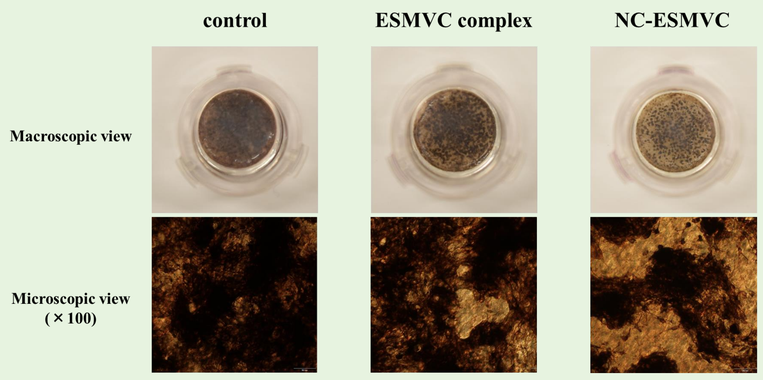


Fig.6 Effect of NC-ESMVC on pigmentation in human skin model MEL-300

CONCLUSION

1. The combination of ESM and VCP-IS-2Na efficiently inhibited melanin production due to suppressing effect of melanogenesis-related gene expression in HMV-II cells.
2. The combination of ESM and VCP-IS-2Na also suppressed the gene expression of POMC in NHEKs and MC1R in HMV-II cells.
3. Synergistic inhibition of MITF expression may have led to suppression of TYR, resulting in decrease of melanin production.
4. NC-ESMVC exerted an inhibitory effect on melanogenesis more than non-nano capsuled mixture in human skin model.
5. These results suggest that NC-ESMVC may be a novel effective brightness agents for the new approach of inhibitory effect on melanogenesis-related gene expression.

A proposed scheme of anti-melanogenesis effect of NC-ESMVC