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Preparation of Micrometer-Sized, Multifunctional Capsule Particles for Cosmetic by Microsuspension Polymerization Utilizing the Self-Assembling of Phase Separated Polymer Method

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ABSTRACT: Micrometer-sized polymer particles with encapsulated ultraviolet (UV) absorbent, fluorescent agent, and blue pigment were successfully prepared by microsuspension polymerization utilizing the Self-assembling of Phase Separated Polymer method. The particles were characterized by optical microscope and particle size distribution analysis, and they were evaluated on their usefulness for cosmetic using UV spectrometry, colorimetry, VISIA™ Evolution, and bioassay. The capsule particles had multifunctional properties, which are very attractive in the cosmetic field, especially in whitening, brightening, the improvement of face-texture, and less noticeable pores in face, as well as the protection from UV. Moreover, bioactivities of the particles under the UV irradiation, which were examined with the films prepared from capsule components, revealed not only makeup effect but also the activation of human epidermal keratinocytes. The results suggest the importance of blue light in the field of cosmetics. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: fluorescence; particle; microsuspension polymerization; microencapsulation; biological application of polymers

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INTRODUCTION

Consierable recent interests in cosmetic field have centered on whitening and brightening, because they are ladies' desires for many years. Particularly, looks such as transparent bare skin are required finally. Such a skin shows high reflectance of blue-green color with wavelength of 450–550 nm.[1] Based on this concept, Igarashi and Hasegawa[2] have developed hollow-pearl pigments. Typical conventional applications have been devised to give clarity to the skin using blue and/or green substrates. However, there is a problem to reduce the overall reflectivity, despite increasing reflection of 450–550 nm. Pearl pigments are also used for the same purpose, although it has defects of roughness and white float under the sunlight and flashlight. Hydroquinone, kojic acid, azelaic acid, arbutin, and their derivatives are known as bioactive ingredients for whitening, but it is necessary to use large amounts of the ingredients to demonstrate efficacy. Further, the application of the large amounts of antioxidant like hydroquinone causes damage to the skin.

One of authors has already reported the preparation of a novel type of capsule particles including valuable substrates[3,4] by microsuspension polymerization using the *S*elf-*a*ssembling of *P*hase *Se*parated *P*olymer (SaPSeP) method.[5,6] Recently, we extended this technique to the preparation of capsule particles including ultraviolet (UV) absorbent, fluorescent agent, and blue pigment for whitening agents.[7]

In this article, moreover, micrometer-sized, multifunctional capsule particles for cosmetic having not only makeup effect but also the activation of human epidermal keratinocytes under blue light irradiation will be prepared by microsuspension polymerization using the above SaPSeP technique. To our knowledge, there is no previous report on the latter topic.

EXPERIMENTAL

Materials

Ethyleneglycol dimethacrylate (EGDM; Light-ester EG: Kyoeisha Chemical, Osaka, Japan), methyl methacrylate (MMA) and

methacrylic acid (MAA; Wako Pure Chemical Industries, Osaka, Japan), styrene (S) (Tokyo Chemical Industries, Japan), poly(vinyl alcohol) (PVA; Gohsenol EG-05: degree of polymerization, 600; degree of saponification, 86.5–89%; The Nippon Synthetic Chemical Industry, Osaka, Japan), 2-ethylhexyl *p*-methoxycinnamate (octyl *p*-methoxycinnamate, OMC; Uvinal MC-80: BASF AG, Germany), 7-*N*,*N*-diethylamino-4-methylcoumarin (DEAMC) and 7-amino-4-methylcoumarin (DKSH AG, Ludwigshafen am Rhein, Germany), 7-*N*,*N*-diethylamino-4-methylcoumarin (DEAMC) and 7-amino-4-methylcoumarin (Synthon Chemicals GmbH & Co.KG, Wolfen, Germany), polystyrene (PS; degree of polymerization, 2000: Wako Pure Chemical Industries, Osaka, Japan), acrylic acid–alkyl methacrylate copolymer (ULTREZ-21: The Lubrizol, Tokyo, Japan), diglycerin (Diglycerin 801: Sakamoto Yakuhin Kogyo, Tokyo, Japan), dimethylpolysiloxane (KF96-20CS), polyglycerin-3-disiloxysimeticone (KF6100) (Shin-Etsu Chemical, Japan), and 3-(4-carboxy-2-oxo-1-pyrrolidinyl)propyl methyl siloxane, polymer with dimethylsiloxane, trimethylsilyl-terminated (cosmetic ingredient: PCA DIMETHICONE) (Monasil PCA: Croda Japan KK, Tokyo, Japan) were obtained commercially. 2,2′-Azobis(isobutyronitrile) (AIBN), 2,2′-azobis(2,4-dimethylvaleronitrile) (V-65), and dehydrated *N*,*N*-dimethyl formamide (DMF; water content <50 ppm; Wako Pure Chemical Industries, Osaka, Japan) were obtained commercially and used without further purification. All other reagents were of reagent grade and were used without further purification.

Instruments

Infrared (IR), UV, and ^{1}H-NMR spectra were obtained using JASCO FT/IR-4200, Shimadzu UV-2450 UV–VIS. spectrometer, and JEOL JNM-ECP500 Spectrometer, respectively. Particles were observed with a Kyence digital microscope VHX-900, and the particle size distribution was estimated with a Horiba laser scattering particle size distribution analyzer LA-920. T. K. Robomics (Primix, Osaka, Japan) was used as a homogenizer. Makeup effects were evaluated using a colorimeter (CR-221, Konica Minolta Holdings, Tokyo, Japan) and a VISIA^{TM} Evolution (VISIA, Canfield Scientific, Fairfield, NJ, USA).

Synthesis of 7-*N*-(*p*-Vinylbenzyl)amino-4-methylcoumarin

7-Amino-4-methylcoumarin (1576.6 mg, 9.0 mmol), *p*-vinylbenzyl chloride (1376.1 mg, 9.0 mmol), sodium carbonate (953.9 mg, 9.0 mmol), dehydrated DMF (20 mL), and a Teflon-covered magnetic stirring bar were placed in a round-bottomed flask (50 mL) equipped with a reflux condenser.[8] The mixture was stirred at 85°C for 80 h. Water (50 mL) was added to the stirred solution. To precipitates collected by centrifugation, chloroform (200 mL) and water (100 mL) were added. The separated chloroform layer was dried with anhydrous $MgSO_4$ and filtered to remove it. The filtrate was evaporated *in vacuo*. The residue was subjected to column chromatography on silica gel with chloroform/methanol (50/1, v/v) as eluant to afford 2040 mg (82.3 %) of a white solid, mp 170°C. Thin layer chromatography (rate of flow = 0.9, chloroform/methanol = 20/1, v/v).

^{1}H-NMR ($CDCl_3$: ppm): δ = 2.35 (3H, s, Me), 4.38 (2H, d, J = 5.5 Hz, CH_2), 4.65 (1H, m, NH), 5.25 (1H, d, J = 11.0 Hz, CH=), 5.74 (1H, d, J = 17.7 Hz, CH=), 6.00 (1H, s, =CHCO), 6.47 (1H, d, J = 1.7 Hz, CH), 6.53 (1H, d of d, J = 1.7/8.6 Hz, CH), 6.72 (1H, d of d, J = 11.0/17.7 Hz, =CH-Ar), 7.32 (2H, d, J = 7.9 Hz, Ar), 7.35 (1H, d, J = 8.6 Hz, CH), and 7.40 (2H, d, J = 7.9 Hz, Ar).

IR (KBr: cm^{-1}): 3359 (NH), 3068, 2989, 2919 (CH_2, CH_3), 1704 (CO), 1625 (C=C), and 1569 (C=C).

Elemental analysis: Calcd for C19H17NO2: C, 78.33; H, 5.88; N, 4.81%. Found: C, 78.17; H, 5.80; N, 4.79%.

Preparation of S–MAA Copolymer

S (12.5 g, 0.12 mol), MAA (344.2 mg, 4 mmol), AIBN (100 mg, 0.6 mmol), and ethanol (50 mL) were placed in a round-bottomed flask (100 mL) equipped with a reflux condenser. Solution polymerization was carried out with stirring at 75°C for 12 h under argon atmosphere. Prepared S–MAA copolymer (P(S-MAA)) was precipitated in diethyl ether (100 mL) and purified by reprecipitation from chloroform solution with diethyl ether.

IR (Film: cm^{-1}): 3026, 2925 (CH_2, CH_3), 1698 (CO_2H), 1601, 1493, and 1452 (Ph).

Microsuspension Polymerization

Typical examples of the procedure are described in the following subsections.

Preparation of Capsule Particles Including Fluorescent Agent. A homogeneous solution of EGDM (49 g), MMA (2 g), PS (5.1 g), OMC (51 g), indigo (2 mg), V-65 (0.6 g), and DEAMC (70 mg), which is a fluorescent agent, was dispersed as droplets in 1.01 wt % PVA aqueous solution (192.3 g) under vigorously stirring with a T. K. ROBOMICS homogenizer at 5000 rpm for 3 min, 7000 rpm for 5 min, 9000 rpm for 7 min, and 13,000 rpm for 10 min. The dispersion was placed in a round-bottomed separable flask equipped with a mechanical stirrer and polymerized at 60°C for 8 h and further at 80°C for 2 h under stirring. The amount of the capsule particles was measured by gravimetry after centrifugal washing and drying.

Preparation of Capsule Particles Having Fluorescent Moiety. A homogeneous solution of EGDM (49 g), MMA (2 g), PS (5.1 g), OMC (51 g), indigo (2 mg), V-65 (0.6 g), and 7-*N*-(*p*-vinylbenzyl)amino-4-methylcoumarin (VBAMC; 88.3 mg), which gives fluorescent moiety in formed capsule shell, was dispersed as droplets in 1.01 wt % PVA aqueous solution (192.3 g) under vigorously stirring by the T. K. ROBOMICS homogenizer as described earlier. Capsule particles were obtained by a microsuspension polymerization for the dispersion and followed by separation and dry process.

Determination of Fluorescent Agent in Capsule Particles

To each dispersion (250 μL), ethanol (750 μL) and ethyl acetate (750 μL) were added. The mixture was centrifuged at 15,000 rpm for 15 min. The supernatant of 500 μL was adjusted to 10 mL by addition of ethanol in a volumetric flask and subjected to UV measurement. The amount of the fluorescent agent was determined by comparing the absorption of the above solution with that of an authentic sample at 377 nm. When the corresponding amounts of fluorescent agent were added to water

Table I. Recipes for Films

Film	EGDM (g)	MMA (g)	PS (g)	OMC (g)	Indigo (mg)	DEAMC (mg)	V-65 (mg)
A	2.45	0.1	0.26	-	-	-	30
B	2.45	0.1	0.26	2.55	0.1	-	30
C	2.45	0.1	0.26	2.55	0.1	1	30
D	2.45	0.1	0.26	2.55	0.1	50	30

(250 μL), ethanol (750 μL), and ethyl acetate (750 μL), the calibration curve could be easily obtained.

Simple Formulation for Cosmetic Products

Water (29.35 g) was dropped slowly to a solution of polyglycerin-3-disiloxysimeticone (1.7 g), diglycerin (10 g), dimethylpolysiloxane (10 g), and PCA dimeticone (0.8 g) under stirring with a homogenizer. Acrylic acid–alkyl methacrylate copolymer powder (0.05 g) swollen with water (5 g), ethylenediaminetetraacetic acid disodium salts (0.05 g), 1,2-pentandiol (2 g), and triethanolamine (0.05 g) were added to the dispersion under stirring with a homogenizer. To the mixture (55 g), aqueous dispersion (45 g) containing capsule particles (ca. 15 g) was added and stirred well.

Evaluation of UV Protection

Coating films (8-μm thickness) of the above formula were formed on a crystal glass plate using a film applicator (Nippon Seeders Service, Osaka, Japan). The UV protection of the formula, that is, Sun protection factor (SPF) value against UV-B (280–315 nm) and Protection Grade of UV-A (PA) value against UV-A (315–400 nm) was evaluated by comparison with that of commercial sunscreen cosmetic (SPF24, PA2+).

Evaluation of Brightening

Prepared cosmetic products were applied to BioSkinPlate (FR40: Beaulac, Saitama, Japan) at 2 μL cm^{-2}. Color difference was evaluated using a colorimeter by comparison with a blank test, which was measured on only BioSkinPlate without the cosmetic. Each measurement was carried out three times, and the average value of $n = 3$ ($M \pm$ SD) was calculated.

Evaluation of Makeup Effects by VISIA^{TM} Evolution

An appropriate amount (ca. 3 μL cm^{-2}) of prepared cosmetic products was applied to cheek or forehead of man (age: 58). Makeup effects like improvement of face-texture and less noticeable pores using cosmetic products were evaluated with a VISIA^{TM} Evolution.

Evaluation of the Activation of Human Epidermal Keratinocytes by UV Radiation through Films

Films (A, B, C, D) Obtained by Bulk Copolymerization of EGDM and MMA under the Conditions Listed in Table I. Mixtures (2.5 g) of compounds listed in Table I were separately spread on a PS plate (5 × 7.5 cm^2), and EGDM and MMA therein were copolymerized in oven at 70°C for 4 h. Films (B–D) contained components used for the preparation of capsule particles (1–3).

Bioassay by UV Radiation through Films. Human epidermal keratinocyte cell line (HaCaT) was seeded in the medium of Dulbecco's modified Eagle's medium (GIBCO) containing 10% of fetal calf serum at a density of 20,000 cells per well in a Cellbind-96well plate (Corning). After 24 h, HaCaT was irradiated through prepared film with UV-A (315–400 nm, 8160 J m^{-2}) and UV-B (280–315 nm, 6240 J m^{-2}) using Mini-Transilluminator NTM-10 (Funakoshi, Tokyo, Japan). After further 24 h, cell survival rates were calculated using Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the procedure reported by Yoshimura et al.[9] in comparison with that without UV irradiation.

RESULTS AND DISCUSSION

Microsuspension Polymerization

Four kinds of capsule particles were prepared using the SaPSeP method.[3–6] The mechanism can be simply explained as follows. The shell wall is formed by diffusion and adsorption of phase-separating P(EGDM-MMA) to oil–water interphase along with polymerization, and further the polymerization in the inside of shell wall also proceeds with crosslinking reaction. As the results, the components without monomers and PS in the organic phase remain in the core part of capsule particles.

Figure 1 shows optical micrographs of the four kinds of capsule particles. Aqueous dispersion of all capsule particles (1–4) had a pale color and emitted blue fluorescence under the irradiation of UV light (365 nm). The size distributions (number-average diameter: d_n) of the capsule particles (1), (2), (3), and (4) were, respectively, in the ranges of 0.5–4.5 μm (1.53 μm), 0.3–5.2 μm (1.25 μm), 0.5–8.9 μm (1.99 μm), and 0.5–5.9 μm (1.54 μm). The contents of the capsule particles (1), (2), (3), and (4) in the dispersions were, respectively, 35, 32, 34, and 31%. The supernatants obtained from the dispersions by centrifugation showed neither fluorescence nor UV absorption.

In the case of the capsule particles (1), encapsulation of DEAMC (fluorescent agent), indigo (pigment), and OMC (UV-B absorbent + solvent) was carried out. An aqueous dispersion of monomer droplets dissolving them was prepared using a T. K. ROBOMICS homogenizer, and it was stable throughout the microsuspension polymerization. Capsule shell was formed by adsorption of phase separated P(EGDM-MMA) promoted by PS. The amount of the fluorescent agent extracted with ethanol/ethyl acetate (1/1) mixture from the capsule particles (1) was almost in accord with that of charge-in quantity. According to the mechanism of the SaPSeP method,[3–6] the above results suggest that DEAMC (fluorescent agent) and indigo dissolved in OMC were completely included by the capsule shell composed of P(EGDM-MMA) containing PS. In this experiment, generally accepted concentration of the fluorescent agent was added as one of effective compounds for whitening, brightening, the improvements of face-texture, and less noticeable pores in face.

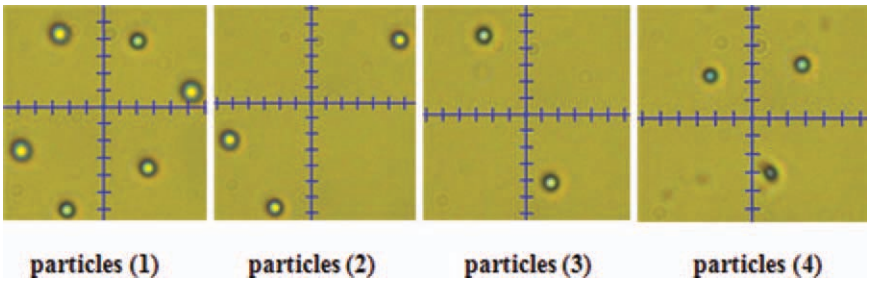

Figure 1. Optical micrographs of four kinds of capsule paricles (1 scale: 1 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

To keep effectiveness of encapsulation, which prevents direct contact of the fluorescent agent to skin, it is needed to make it remain inside capsule particles in cosmetic formulations. The extraction result of the fluorescent agent from the capsule particles (1) with ethanol/ethyl acetate (1/1) mixture suggests that encapsulated fluorescent agent may be released by organic solvents often used in cosmetic formulations.

In the case of the capsule particles (2), it was tried to bind the fluorescent agent strongly to the capsule shell by ionic interaction between the fluorescent agent and MAA unit. IR spectrum of MAA reveals an absorption band due to —COOH group at 1697 cm^{-1}. By the addition of DEAMC to MAA, a new absorption band due to —COO^- group appeared at 1604 cm^{-1} instead of 1697 cm^{-1}, supporting the ionic interaction between diethylamino group of DEAMC and carboxylic group of MAA (see Figure 2). MAA (261 mg in the scale of experimental part) was added to a homogeneous solution of EGDM, MMA, PS, OMC, DEAMC, indigo, and V-65. Subsequent treatment was carried out as described in the experimental part of the capsule particles (1). MAA dissolves much better in the oil phase composed of EGDM and MMA than the PVA aqueous phase, and capsule shell composed of MMA, EGDM, and MAA binding the fluorescent agent was formed by the SaPSeP method.[3–6] However, this attempt could mot make fluorescent agent remained inside the capsule particles (2) by the addition of the ethanol/ethyl acetate (1/1) mixture, too. This indicates that the ionic interaction between the fluorescent agent and MAA was not enough to suppress of the release by the organic solvents as well as the capsule particles (1).

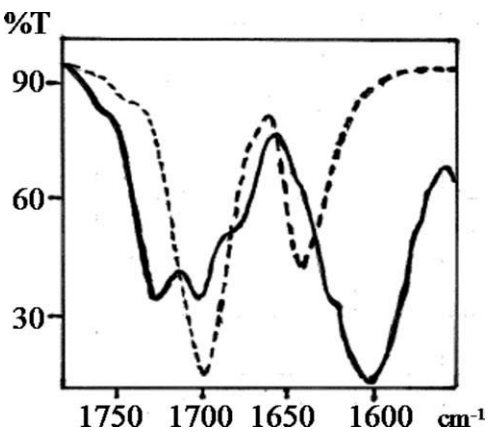

Figure 2. IR spectra of MMA before (dotted line)/after (solid line) the addition of the equivalent of DEAMC.

In the case of the capsule particles (3), it was tried to prevent release of the fluorescent agent therefrom by the organic solvents using the ionic interaction between the fluorescent agent and MAA units in a P(S-MAA) as promoting agent for phase-separation, which was prepared by solution polymerization of styrene and a small amount of MAA in ethanol at 75°C for 12 h with AIBN as initiator (see "Experimental" section). Capsule particles (3), in which P(S-MAA) seems to form an inner wall of the capsule particles,[6] were obtained by the similar method as described in the case of the capsule particles (1). However, this attempt also could not stop the release of fluorescent agent from the capsule particles (3) by the addition of the ethanol/ethyl acetate (1/1) mixture, which indicates that the ionic interaction between the fluorescent agent and MAA units in the P(S-MAA) inner wall was not enough for the fixation of the fluorescent agent as well as the capsule particles (1 and 2).

In case of the capsule particles (4), the terpolymerization of EGDM, MMA, and VBAMC having the fluorescent moiety was carried out for the formation of capsule shell covalently bonding fluorescent agent. The extract with the ethanol/ethyl acetate (1/1) mixture from the dispersion also showed no fluorescence. This indicates that all fluorescent moiety attached to the shell layer by chemical bond cannot be extracted by the organic solvents as expected.

As described earlier, four types of micrometer-sized particles with encapsulated UV-B absorbent, the fluorescent agent, and the blue pigment were prepared according to the SaPSeP method.[3–6] Essentially, the same results were obtained in the capsule particles (1–3), and the fluorescent agent was extracted with the ethanol/ethyl acetate (1/1) mixture from capsule particles because of insufficient ionic interaction between the diethylamino group of fluorescent agent and the carboxyl group of MAA. The terpolymerization with the fluorescent monomer (VBAMC) completely suppressed the release of fluorescent moiety from capsule particles.

Evaluation of UV-Protection

UV protections of cosmetic formula containing 15% of the capsule particles (1–4) were evaluated by comparison with that of a commercial sunscreen cosmetic (SPF 24; PA2+). Figure 3

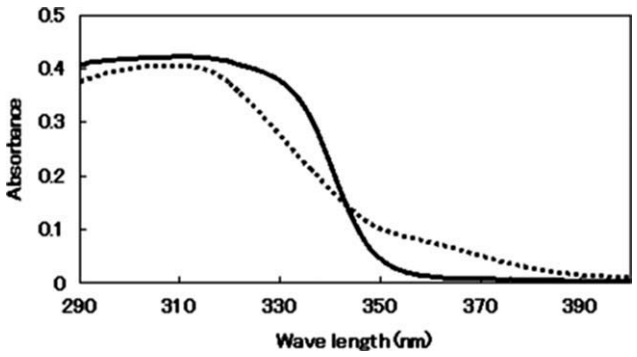

Figure 3. Comparison of UV protection: (solid line) formula containing 15% of capsule particles (1) and (dotted line) commercial sunscreen cosmetic (SPE24, PA2+).

shows the result of the capsule particles (1). Similar results were obtained in the other cases of the capsule particles (2–4). The SPF values of the formula (1–4) corresponded to about SPF24. On the other hand, because the additive amount of fluorescent agent, which absorbed UV-A, was very slight, PA value was negligible.

Evaluation of Makeup Effect

The particles with encapsulated fluorescent agent, indigo, and OMC can be expected excellent makeup effects owing to not only optical properties of plastic particles like reflection, refraction, and light scattering but also encapsulation of the fluorescent agent and blue pigment. The makeup effects of cosmetic formula containing 15% of the capsule particles (1–4) were evaluated using a colorimeter[10] and a VISIA™ Evolution.[11]

By Colorimeter. Prepared cosmetic products were put on a BioSkinPlate (FR40: Beaulac, Saitama, Japan) at 2 μL cm^{-2}, and the behavior of parameters in colorimeter was investigated. The results are summarized in Table II. ΔL, which is a parameter of brightness showing white in the increasing direction and black in the decreasing direction, was increased by the use of the capsule particles (1–4). Δa, which is a parameter showing red in the positive direction and green in the negative direction, was decreased by the use of the capsule particles (1–4), resulting in color change toward a tinge of green. Δb, which is a parameter showing yellow in the positive direction and blue in the negative direction, was greatly decreased by the use of the capsule particles (1–4), resulting in color change toward a tinge of blue. ΔE is represented by $(\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ to show a significant color difference. Four kinds of the capsule particles showed the similar performance. These results indicate that cosmetics containing the capsule particles (1–4) were very useful for increases in the brightness and whiteness for having the color toward greenish blue, which give looks like transparent bare skin.[1]

By VISIA™ Evolution. Prepared cosmetic products were applied to the cheek or forehead of man, and the makeup effect was evaluated by comparison with and without use of the cosmetic. The results are summarized in Table III. In all cosmetic products containing the capsule particles (1–4), both numbers of detected features for the face-texture and pores were decreased, indicating the improvement of face-texture and less noticeable pores.

The above desirable properties as whitening, brightening, the improvements of face-texture, and less noticeable pores seem to be derived from blue fluorescence, blue pigments, and optical properties of the capsule particles.

Evaluation of the Activation of Human Epidermal Keratinocytes by UV-Radiation through Films

Not only makeup effect but also activation of the skin cells are essentially important for making transparent bare skin, but there have been no attempts to satisfy both issues. We evaluated bioactivities using films (A–D) prepared by bulk copolymerization of EGDM and MMA under the conditions listed in Table I, because the evaluation using capsule particles was difficult. Films (B–D) contained components used for the preparation of capsule particles (1–3). Human epidermal keratinocyte cells were irradiated through the films with UV-A (8160 J m^{-2}) and UV-B (6240 J m^{-2}).

Figure 4 shows the results. In the film (A) containing no UV absorbents, the number of epidermal keratinocyte cells was remarkably decreased by damage due to UV. In the films (B–C) containing UV absorbents, the UV damage was obviously suppressed by UV-B absorption of OMC. However, in the film (C) the damages due to UV could not be completely suppressed because of a slight amount of the fluorescent agent having UV-A absorption ability. In the film (D), surprisingly, the number of epidermal keratinocyte cell was larger than that under no UV radiation. It was obvious that human epidermal keratinocyte cells were activated by the fluorescence, that is, blue light. The

Table II. Evaluation of Makeup Effect by a Colorimeter

Capsule particles	ΔL	Δa	Δb	ΔE
1	4.19 ± 0.50	−1.50 ± 0.21	−8.16 ± 1.05	9.29 ± 1.17
2	2.40 ± 0.67	−1.67 ± 0.13	−8.06 ± 0.99	8.42 ± 1.32
3	3.62 ± 1.30	−1.18 ± 0.31	−8.03 ± 0.73	8.92 ± 1.16
4	3.38 ± 0.60	−1.28 ± 0.20	−7.78 ± 1.10	8.60 ± 0.88

Each number is expressed in $M \pm SD$ (the average of $n = 3$).

Table III. Makeup Effects Evaluation Using a VISIA™ Evolution

Capsule particles	Evaluation site	Evaluation item	Number of detected feature		
			Without cosmetic	With cosmetic	Ratio (with)/(without)
1	Cheek	Face-texture	133	107	0.80
		Pores	140	120	0.86
	Forehead	Face-texture	1086	966	0.89
		Pores	487	413	0.85
2	Cheek	Face-texture	153	105	0.69
		Pores	122	105	0.86
	Forehead	Face-texture	928	669	0.72
		Pores	369	321	0.87
3	Cheek	Face-texture	110	92	0.84
		Pores	117	108	0.92
	Forehead	Face-texture	882	635	0.72
		Pores	385	318	0.83
4	Cheek	Face-texture	157	121	0.77
		Pores	168	145	0.86
	Forehead	Face-texture	947	722	0.76
		Pores	451	408	0.90

content of DEAMC in the capsule particles (1) corresponded to 3.5 mg of that in Table I. For the clear activation of human epidermal keratinocyte cells, an increase of the fluorescent agent in the capsule particles may be needed. When a large amount of the fluorescent agent is used, the fixation of the fluorescent agent to capsule shell by chemical bond is very useful, because the release of fluorescent agent from capsule particles by organic solvents often used in cosmetic formulations does not occur completely. Our prepared capsule particles were very useful for cosmetic materials in terms of both makeup effect and the activation of human epidermal keratinocyte cells. Recently, much interest has centered about phototherapy. Karu et al.[12] reported the activation of mitochondrial pathway by visible-to-near infrared radiation and an increase of adenosine triphosphate level by irradiation with He–Ne laser (632.8 nm),[13] and Passarella et al.[14] also obtained a similar result. Moriwaki et al.[15] reported the suppression of melanin production under the irradiation of red light emitting diode (LED) (620–630 nm) and blue LED (465–470 nm). Gold[16] has recommended phototherapy with blue light to improve skin conditions in cases of acne and blemishes. Shnitkind et al.[17] have described that a narrow-band blue light (420 nm) had an anti-inflammatory effect on keratinocytes by decreasing the cytokine-induced production of IL-1α and ICAM-1. Harada and Okajima[18] have reported that insulin-like growth factor-1 (IGF-1) had many physiological activities as hypotensive, protein anabolism, improvement of cardiac function, skin effect, hair growth effect, and activation of immune system, and so on. Okajima has commented on the promotion of IGF-1 by blue light. Considering these reports, blue light has possibilities of skin effects as whitening, anti-inflammatory effects, and skin effect by the promotion of IGF-1, although we do not have direct experimental data supporting this idea.

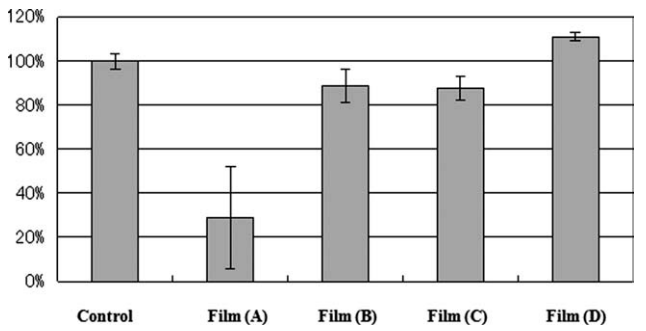

Figure 4. Cell survival rates after UV exposure relative to control (no UV radiation).

The polymer capsule particles have optical properties like reflection, refraction, and light scattering, suppressing UV damages. The encapsulations of UV absorbents, fluorescent agents, and pigments are useful because of the prevention of direct contact to skin. The formation of capsule shell bound the fluorescent agent by chemical bond is worth in the use of the large amount of fluorescent agent in cosmetic formulations, because there is no fear of releasing it to outside.

To our knowledge, there is no previous report on the activation of human epidermal keratinocyte cells under the blue light irradiation. Our result may expand the properties of blue light in skin care and cosmetic.

CONCLUSIONS

We have prepared four types of the capsule particles including UV absorbent, fluorescent moiety, and blue pigment by

microsuspension polymerization of EGDM and MMA using the SaPSeP method.[3–6] In all capsule particles, of which particle size distributions were almost the same and d_n values were 1.2–2.0 μm, UV absorbent, fluorescent moiety, and blue pigment were completely included therein. The prepared capsule particles (1–4) showed the increase of brightness and whiteness to give looks such as transparent bare skin, the improvement of face-texture, and less noticeable pores. Moreover, interestingly, they suggested the possibility of the activation of human epidermal keratinocyte cells under the blue light irradiation. This is very attractive, because they could turn bad UV-A for skin to useful blue light. To our knowledge, there has been no previous report on the activation of human epidermal keratinocyte cells under the blue light irradiation.

An usable amount of fluorescent agent was used in cosmetic formulations with the four capsule particles, but the perfect encapsulation of the fluorescent agent, which prevents direct contact to skin, even in cosmetic formulations containing organic solvents, further increases the safety of cosmetics. In the capsule particles (1–3), where there was no strong interaction between the fluorescent agent and capsule shell or ionic interaction operated between them, the fluorescent agent was released in the presence of organic solvents. However, the problem was improved by copolymerization of a monomer having fluorescent moiety, which was originally synthesized by the authors, in the shell formation (capsule particle 4).

From these results, it is concluded that the capsule particles including UV absorbent, fluorescent moiety, and blue pigment by microsuspension terpolymerization of EGDM, MMA, and monomer having fluorescent moiety using the SaPSeP method should be very useful for cosmetic materials in terms of both makeup effect and the activation of human epidermal keratinocyte cells.

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