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ARTICLE

Effects of Egg Shell Membrane Hydrolysates on Skin Whitening, Wound Healing, and UV-Protection

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Abstract

This study was conducted to examine the effects of egg shell membrane hydrolysates (ESMH) on skin whitening, wound healing, and UV-protection. ESMH was divided into three groups by molecular weight (Fraction I: above 10 kDa of ESMH, Fraction II: 3 kDa-10 kDa of ESMH, Fraction III: below 3 kDa of ESMH). As a result, all of ESMHs showed over 90% of protein contents. The wound healing experiment using HaCaT cells showed that the fraction I was slightly superior to other fractions depending on the concentration though it was not significantly different. In the experiments of inhibition of tyrosinase and L-3,4-dihydroxyphenylalanine (L-DOPA) oxidation to verify the L-DOPA whitening effect, the whole ESMH (before fractioning) showed a similar amount of inhibition effect with arbutin (control). In the inhibition of melanin formation in B16-F1 melanoma cells, the fraction I showed a high inhibitory effect. In the experiment for protecting the skin from ultraviolet rays using HaCaT cells, all the fractions showed a higher rate of cell viability than the control. In conclusion, this study confirmed that the cosmetic effects of ESMHs such as skin whitening, wound healing, and UV-protection, which were divided depending on the molecule weight. We could confirm that the possibility of ESMHs as a material for functional cosmetics.

Key words: egg shell membrane, skin whitening, wound healing, UV-protection

Introduction

Skin color is affected by melanin produced by melanin cells, hemoglobin present in blood vessels, the amount and spread of carotene in the skin, the thickness of the skin and the amount of blood present. The skin experiences an endless number of changes, with aging being the most prominent change. Skin aging is commonly divided into either internal or external aging. The internal aging is primarily affected by genetic factor and the external aging is affected by UV light, stress and pollution (Kang, 1997). In order to prevent the three main factors causing skin aging, such as UV light, oxidization and drying, many cosmetic products are being developed with a focus on preventing damage by UV light, promoting better blood circulation, maintaining moisture and preventing harmful oxidation of skin cell. Whitening, anti-wrinkle and UV light protection products are some of the example products made to prevent the process of aging.

A person's skin color is determined by the level of melanin, but other factors, such as UV light exposure, specific hormones and inflammatory agents resulting in overproduction of melanin which stays in the body without being properly removed through the skin can affect the color of the skin as well (Pawelek and Korner, 1982). Research is currently underway to suppress such melanin production and to study about arbutin, which inhibits tyrosinase in melanin synthesis, and vitamin C, which suppresses melanin production (Yada *et al.*, 1991). There are also a high number of studies being conducted on the functional cosmetic products including whitening products, which are able to prevent skin aging. It is known that skin aging is due to UV light, environmental factors and stress. On the other hand, skin moisture, UV-protection and skin elasticity are good factors to delay skin aging (Nakagawa *et al.*, 2011). Studies are being conducted on ways to increase moisturization (Held *et al.*, 1999), better protect the skin against UV light (Gonzaga, 2009), increase the recovery rate of the skin from UV light damage (Oikarinen *et al*., 1985) and increase blood circulation and skin cell proliferation (Matsumoto *et al*.,

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2006).

Retinol and (-) epi gallocathechin-3-gallate (EGCG) are known to prevent collagen from being broken down under the influence of denatured MMP proteins, which is a consequence of UV light exposure, and from the skin aging caused by the accumulation of denatured collagen. Such compounds have been used in increasing cell activity and collagen storage (Karna *et al.*, 2011). With the recent attention to UV light skin damage due to the increased destruction of the ozone layer, there has been an increase in the use of cosmetic products with the function of UV light protection (Gonzaga, 2009). Recently, the consumers demand for cosmetic products using natural ingredients, more research has been done on developing organic cosmetic products that have whitening or skin recovery properties (Park *et al*., 1993).

The eggs are consumed and the egg shell membranes are used for animal feed or discarded with the egg shells. However, the main compound of egg shell membrane (ESM), an amino acid, has excellent characteristics in maintaining the moisture and elasticity of the skin and shows a similar structure of amino acid that composes collagen and elastin in the skin. Utilization of the ESM may be a new valuable material of cosmetics for the whitening and wrinkle care with the emphasis on its function of controlling aging, wrinkles and skin damage from ultraviolet rays. Egg shell membrane, an organic substance, is known to increase cellular activity, increase collagen production, slow down skin aging and reduce the detrimental effects of damage from UV light and inflammation (Candilish *et al*., 1969).

According to the data by Korean Statistical Information Service (KOSIS, 2011), approximately 150 million of eggs were produced in 2011 in Korea. While the albumen and egg yolk are being used for food, the egg shells, which have the ability to block bacteria while allowing oxygen to pass through, are being discarded as waste. The egg is made of shell, albumen and yolk. The egg shell membrane exists between the shell and the albumen. The egg shell membrane is either present as two layers, outer and inner, or as a web without a specific pattern. The outer and inner layers of the egg are made up of fibrous protein keratin of 50 μm and 20 μm thickness, respectively, and the egg shell membrane is made up of 70% organic compounds, 10% inorganic compounds, and 20% water. Furthermore, among the organic compounds, 80% is made up of proteins, followed by 2.3% lipid and 3.4% carbohydrates (Arias *et al*., 1991; Okubo *et al*., 1997). Egg shell membranes are made of the insoluble protein keratin and are known to be quite resistant to physical, chemical and biological reactions, as in cases with feathers, hair, horns, scales and nails (Vignardet *et al*., 2001). Daniel *et al*. (2008) found that ESM are potential ingredients for the cosmetic effects such as moisturization, wound recovery, skin growth and reducing wrinkles. However, since the egg shell membrane does not readily break down by enzymes such as trypsin and pepsin, in order to make it become soluble, the compound has to be oxidized or reduced to decrease its insolubility, then the fragmented proteins must be divided using pepsin (Xiang *et al*., 1992).

In this study, egg shell membranes were fragmented as whole ESMH (before fractioning), fraction I (above 10 kDa of ESMH), fraction II (3-10 kDa of ESMH) and fraction III (below 3 kDa of ESMH), then checked for molecular mass and protein content using Urea-PAGE. The functional properties of an individual fragment for cosmetic use were assessed with the changes in the HaCaT cells, which is an epithelial cell, over time. Whitening effects, the inhibition of tyrosinase, the inhibition of the oxidization of L-DOPA and the inhibition of melanin production in the B16-F1 melanoma cells, which produce melanin, were then tested. The UV-protection effect of ESMHs was also conducted in this study.

Materials and Methods

Egg shell membrane hydrolysates

Egg shell membrane hydrolysates manufacture

ESMH manufacture was slightly modified by the method of Morimura *et al*. (2002). The egg shell membrane used in this study was provided by Sungkyun Biotech Co., Ltd. The 100 g of dried egg shell membranes were added to 1100 mL of distilled water, 2 N NaOH and 40% EtOH before being kept at 70°C for 2 h for the reactions. After the reactions, 99% acetic acid was added for neutralization (pH 7.0) and 40 g of the activated carbon was added for decolorization for 20 min. The extract ran through Whatman filter No.1 and desalinated with Micro acilyzer G3. It was then evaporated and freeze dried (FD 3, Heto, Gydevang, Denmark).

Membrane cutting

The ESMHs filtered by ultra-filtration membranes (Milipore-Amicon Series 8000 Stirred Cells, EMD Millipore Corp., USA) were divided into four fragments: whole ESMH, fraction I, II, and III. The molecular mass of the fragments were determined by using Urea poly-

acrylamide gel eletrophoresis (Urea-PAGE). mPAGE Minicell (AE-6530, ATTO. Inc., Japan) was used as the electrophoresis and blotting instrument and the ultra-low range molecular weight marker (M-3546, Sigma-Aldrich Co., USA) was used to mark the molecular weights. A sample buffer was added to each sample, and was heated for 5 min at 80°C using a circulator (Vision Scientific Co., Ltd., Korea), then ran through a gel for 10 h at 60 V. After the gel electrophoresis, the gel was stained for 1.5 h using Coomassie Brilliant Blue R-250, and the dyed gel was decolored using destaining solution (acetic acid 10%, iso-propanol 12.5%, distilled water 77.5%).

Protein assay

Lowry assay (Bollag and Edelstein, 1991) was used for measuring the protein levels of the fragments. After the preparation of Solution A (100 mL of distilled water with 0.5 g of $CuSO_4 \cdot 5H_2O$, 1 g of $Na_3C_6H_5O_7 \cdot 2\ H_2O$) and Solution B (1 L of distilled water with 20 g of Na_2CO_3, 4 g of NaOH), Solution C (1 mL of Solution A and 50 mL of Solution B) was prepared, followed by Solution D (10 mL of Folin-Ciocalteu phenol reagent and 10 mL of D.W). Each 500 mL sample of the whole ESMH, fraction I, II, and III was added with 2.5 mL of solution C and then was left at room temperature for 10 min. After this, 250 μL of Solution D was and left for 20 min at room temperature, followed by tests to measure absorbance at 750 nm.

Whitening effect

Inhibition of tyrosinase

The measurement of the inhibitory activity of the egg shell membrane on tyrosinase was conducted with the process developed by Ishihara *et al*. (1991). The mixture of 220 μL of 0.1 M potassium phosphate buffer (pH 6.5), 20 μL of appropriately diluted samples and 20 μL of mushroom tyrosinase (1,500-2,000 U/mL) were added to a 96 well plate. 40 μL of 1.5 M tyrosine was then added before being placed for 10 to 15 min at 37°C. Afterwards, an ELISA microplate reader was used to measure absorbance at 490 nm, and arbutin was used as a positive control.

$$\text{Inhibition of tyrosinase (\%)} = 100 - \frac{b - b'}{a - a'} \times 100$$

a: Control OD_{490}
b: Sample OD_{490}
a', b': Mushroom tyrosinase instead of buffer OD_{490}

Inhibition of oxidization of L-DOPA

The measurement of the suppression activity of the egg shell membrane on the oxidization was conducted with a process developed by Jeon *et al* (2005). The mixture of 850 μL of 0.1 M potassium phosphate buffer (pH 7.0), 50 μL of appropriately diluted samples and 50 μL of mushroom tyrosinase (1,500-2,000 U/mL) were added and kept at 37°C for 6 min. Following this, 50 μL of 0.06 M L-DOPA was added before being placed for 1 min at 37°C. Afterwards, the ELISA microplate reader was used to measure absorbance at 490 nm. In the control, 0.1 M potassium phosphate buffer (pH 7.0) was used in place of the sample fluid, and arbutin was used as control.

$$\text{Inhibition of oxidization of L-DOPA (\%)} = 100 - \frac{OD_{sample}}{OD_{control}} \times 100$$

Cell culture and MTT assay for melanogenesis inhibition activities

The B16-F1 cells from murine melanoma cell line used in the experiment were supplied from the Korean Cell Line Bank (KCLB). 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (17-602, BioWhittaker. Inc., USA) were added to DMEM (Dulbecco's Modified Eagle's Medium) (LM 001-05, WelGENE Inc., USA). 25 nM of á-melanocyte stimulating hormone (á-MSH) (M 4135, Sigma-Aldrich Co., USA) was used to promote melanin production. For the cultivation of B16-F1 cells, a DMEM medium with 10% FBS and 1% penicillin-streptomycin was used. When cells filled 90% of the culture dish, it was centrifuged at 2,000 rpm for 3 min, and then collected for dilution in a 1:5 ratio.

MTT assay is based on the process of mitochondria dehydrogenase inside living cells reacting with MTT to form dark blue MTT formazan crystals. The process developed by Carmichael *et al*. (1988) was used. The B16-F1 cells were placed in a 96 well plate at a concentration of 5×10^4 cells/mL, and the plate was incubated in a 5% CO_2 incubator for 37°C for 24 h in order to have the cells attach to the bottom of each well. After the incubation, the medium was removed to be replaced with a new medium, and they were incubated for 24 h. Afterwards, the medium without any samples was removed, and after adding 100 μL of 0.5 mg/mL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution to each well, the plate was placed in a 5% CO_2 incubator at 37°C, without light for 4 h. After the formation of MTT formazan, DMSO was added, and then the plate was placed at room temperature for 5 min before measuring

absorbance at 540 nm using the ELISA microplate reader. The cell survival was then calculated in percentage.

The B16-F1 cells were added to a 6 well plate at a concentration of 1×10^5 cells/mL and then left in a 5% CO_2 incubator at 37ºC for 24 h. After the incubation, a DMEM medium and egg shell membrane fragments diluted to appropriate concentrations were incubated for 72 h. The pellets obtained via trypsinization were left at 60ºC for an hour to dry, and then immersed with 10% DMSO and 1 N NaOH to be read through the ELISA microplate reader to measure the light absorbance at 490 nm. Arbutin was used as a positive control.

Wound healing effect

Cell culture and MTT assay for wound healing effect

10% heat-inactivated FBS and 1% penicillin-streptomycin (17-602, BioWhittaker, Inc., USA.) were added to the DMEM medium. HaCaT cells from the human keratinocyte cell line were supplied by the dermatological sciences research center of Chung-Ang University, Korea.

For the HaCaT cells, a DMEM medium with a 10% FBS and 1% penicillin-streptomycin was used. When cells filled 90% of the culture dish, it was centrifuged at 2,000 rpm for 3 min, and then collected for dilution in a 1:5 ratio. The MTT assay method used is as explained above.

Blocking of ultraviolet ray

Cell protection against UVB

HaCaT cells were added to a 96 well plate at a concentration of 1×10^5 cells/mL and then left in a 5% CO_2 incubator at 37ºC. After 24 h, the medium was drained and cells were washed twice in PBS and then covered with a thin film of the same buffer. Then cells were grown in culture dishes with the addition of different concentrations of ESMH (whole ESMH, fraction I, fraction II, fraction III). Cells were irradiated with a UV-B lamp with 2 J/cm^2 for 60 s. After the assessment, MTT assay was conducted and the survival rates of treated and untreated samples were compared.

Statistical analysis

All the data were treated for significance by using one-way analysis of variance (ANOVA) at $p<0.05$ with the IBM SPSS Statistics 19.0 (IBM Corporation, Somers, NY, USA). Data were analyzed using ANOVA followed by Tukey's and Duncan's test. Results were considered significant when $p<0.05$, unless otherwise stated.

Results and Discussion

Confirmation of egg shell membrane hydrolysates

Confirmation of molecular mass using Urea-PAGE

The molecular fragments of the egg shell membrane which has been fragmented according to molecular mass via Urea-Page are shown on Fig. 1. Using an ultrafiltration membrane, the fragments were divided into above 10 kDa of ESMH (fraction I), between 3 kDa-10 kDa of ESMH (fraction II) and below 3 kDa of ESMH (fraction III).

Protein content of egg shell membrane hydrolysates

Table 1 shows the results of the assessment via protein to find the resolvent protein content of individual egg shell membranes of the whole ESMH, fraction I, II, and III. Protein contents were 92.2%, 94.1%, 93.3% and 92.7%, respectively. Leach *et al*. (1981) also reported similar results showing that the organic components of egg shell membrane were made primarily of proteins.

Skin whitening effect

Inhibition of tyrosinase

Tyrosinase is an important enzyme responsible for com-

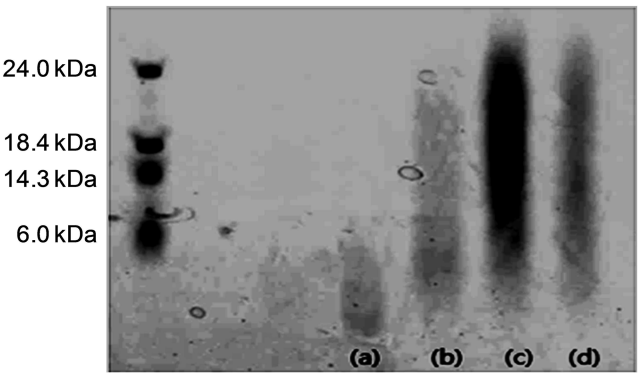

Fig. 1. Urea-PAGE of fractionated egg shell membranes. (a) Fraction III (Below 3 kDa of ESMH), (b) Fraction II (3 kDa-10 kDa of ESMH), (c) Fraction I (Above 10 kDa of ESMH), (d) Whole ESMH (ESMH before fractioning). *ESMH, Egg Shell Membrane Hydrolysate.

Table 1. Protein content of the egg shell membrane hydrolysates fractionated by molecular weight

Extracts	Protein content (%)
Whole ESMH	92.2±0.3
Fraction Ⅰ(above 10 kDa of ESMH)	94.1±0.6
Fraction Ⅱ(3 kDa-10 kDa of ESMH)	93.3±0.5
Fraction Ⅲ(below 3 kDa of ESMH)	92.7±0.3

Data are presented as mean±standard deviation of three independent experiments.

bining with Cu in the production of melanin. It is known that tyrosine, commonly found in the skin of plants and animals, undergoes an enzymatic reaction by tyrosinase to convert to 3, 4-dihudroxyphenylalanine (dopa) and L-dopaquinone, which is then oxidazed to convert to dopachrome and then converted to melanin after undergoing decarboxylation and polymerization (Borski *et al*., 2000). Compounds which have been found to date have whitening properties include vitamin A, mannitol and oxyresveratol. Chemical tyrosinase inhibitors including ascorbic acid and hydroquinone are also known to have such whitening properties. However, these compounds are found to be linked to problems such as toxicity of melanin cells and permanent discoloration of the skin thus their usage to less than 5% (Meyskens and Fuller, 1980). The results of the treatment of the whole ESMH, fraction I, II, and III with concentrations of 0.01 mg/mL, 0.1 mg/mL and 1 mg/mL are shown in Fig. 2. In the case of the whole ESMH, the results were similar to that with arbutin as a positive control, and in all the cases of fraction I, II, and III, inhibition was evident but not as significantly as that of the whole ESMH.

Inhibition of oxidization of L-DOPA

Fig. 3 shows the result of the inhibitory effect of tyrosinase after assessing the optical density of L-dopaquinone produced by the reaction between L-3,4-dihydroxyl-L-phenylalanine (L-DOPA) and mushroom tyrosinase, which was used as the enzyme in the study and played a role in deciding the initial speed of the process of biosynthesis of melanin. In other words, when the whole ESMH, fraction I, II, and III were treated at concentrations of 0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL, the results were similar to the inhibitory effect displayed by Arbutin, which was used as a positive control. There were no significant differences among the fragments. However, at 0.01 mg/mL, the inhibition of fraction II did not show as much effect as the others, with all showing similar inhibitory effects at 1 mg/mL, with the exception of fraction I. Similar to the case in the inhibitory result of tyrosinase, the whole ESMH showed a high inhibitory effect. In this case, melanin is the end product, so in terms of biosynthesis, that causes whitening seems to have come from the conversion of dopamine in the process of melanin being converted to tyrosine in order to prevent melanin production (You *et al*., 2009).

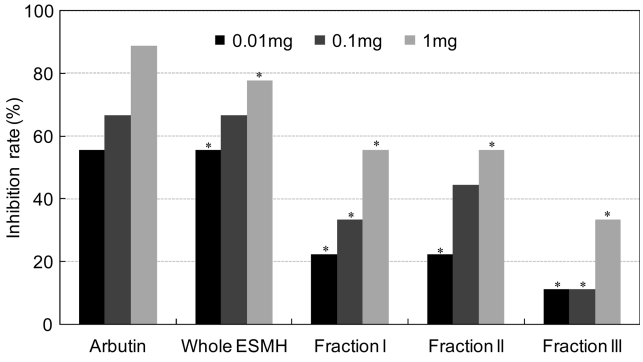

Fig. 2. Effect of the egg shell membrane hydrolysates fractionated by molecular weight on inhibition of tyrosinase activities. Data are presented as mean±SD of three independent experiments (* $p<0.05$ vs. control; Arbutin).

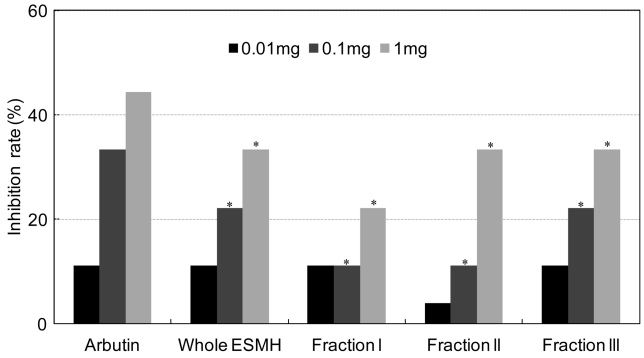

Fig. 3. Effect of the egg shell membrane hydrolysates fractionated by molecular weight on inhibition of L-DOPA activities. Data are presented as mean±standard deviation of three independent experiments. Full is defined as egg shell membrane hydrolysates before fractionation (*$p<$ 0.05 vs. control; Arbutin).

Inhibition of melanogenesis

Methods of suppressing the production of melanin include direct inhibition of tyrosinase, an important enzyme in the production of melanin and suppression of melanin within skin cells (Mishima *et al.*, 1988). The whole ESMH, fraction I, II, and III with concentrations of 0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL were tested to investigate their effects on the cell proliferation of B16-F1 cells. The fraction I with 1.0 mg/mL concentration showed the lowest level of cell proliferation at 91%, and other fractions with each concentration showed a survival rate of over 90%. As a result, all the egg shell membrane fragments are predicted to have no effect on the cell proliferation of B16-F1 cells (data not shown).

Among these, the results of the treatment of the whole ESMH, fraction I, II, and III are based on the result of B16-F1 cells not having an effect on cell proliferation in terms of melanin suppression are summarized in Fig. 4. In other words, when treated at concentrations of 0.01 mg/mL, 0.1 mg/mL and 1 mg/mL, the whole ESMH showed melanin suppressions of 1.5±3%, 5.6±5% and 6.3±3%,

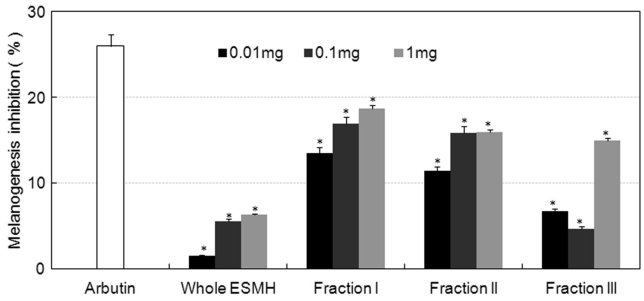

Fig. 4. Melanogenesis inhibition activities of the egg shell membrane hydrolysates fractionated by Molecular Weight. Data are presented as mean±standard deviation of three independent experiments (*p<0.05 vs. control; Arbutin).

the fraction I showed 13.5±3%, 16.9±3% and 18.7±2%, the fraction II showed 11.4±5%, 15.8±5%, 15.9±5%, and fraction III showed 6.7±4%, 4.7±5% and 15±5%, respectively. Arbutin, which was used as a positive control, showed a result of 26±5%, and it showed an effect in suppressing biosynthesis of melanin as it is able to reduce tyrosinase activity and melanin contents. Among these, the fraction I showed a high level of inhibitory effect at 18.7±2%. Furthermore, looking at the tyrosinase inhibition test result of the whole ESMH, the inhibitory activity of tyrosinase was greater in relation to the level of melanin inhibited. This increases during the initial stage of the process in the formation of tyrosinase activity but the level is expected to decrease depending on the stage and pathways of the process. In summary, egg shell membrane was found to suppress tyrosinase activity in melanoma cells, and it is predicted that further studies on the reaction of melanin production to the egg shell membrane will allow practical applications in whitening cosmetic products.

Wound healing effect

The skin plays an important role as the protective barrier between the body and the outer environment in controlling temperature and releasing sweat. A break in the skin as a result of physical impact and damage is referred to as a wound, and the recovery of this skin barrier is referred to as wound healing (Gurtner *et al*., 2008). A typical wound recovery process includes inflammation, granulation, epithelialization, fibroplasia and contraction. The process of epithelialization is characterized by a thickening of the epidermis and a migration of keratinocytes to the edges of the wound (Hosgood, 2003).

Each fraction with three different concentrations was tested to investigate their effects on the cell proliferation of HaCaT cells. The fraction III treated at a concentration of 1 mg/mL showed the lowest cell proliferation at 95%, but since all other fragments at all concentrations showed cell survival rates of over 90%. There is no significant effect on the cell proliferation of HaCaT cells (data not shown). According to this process, the effects of egg shell membranes on artificially wounded HaCaT cells were tested and the concentrations which did not have an effect on HaCaT cells were taken into consideration. Fig. 5 shows the results of the treatment of the whole ESMH, fraction I, II, and III with concentrations of 0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL. Comparing the treated samples to the untreated samples, overall, the cells were shown to gather 12 h later. Although there were minor differences in the relation to concentrations, when the empty space left by the artificial wound is considered to be 100% healed, the control showed 62% empty space after 12 h and 50% after 24 h at the highest concentration (1 mg/mL) but in the case of the whole ESMH showed 17% in 12 h, 8% for the fraction I, 23% for the fraction II and 20% for the fraction III, followed by 0% for all cells after 24 h. Among these, the fraction I showed the highest speed in terms of cell gathering and this is believed to be due to its molecular mass. During fibroplasia, tissues form in the place of the wound, and peptides in the membrane have properties which accelerate wound healing by stimulating cell proliferation to help cells mature through spreading of particles which aid in cell proliferation.

Cell protection against UVB

It is known that UV light causes a weakening of the skin, an overproduction of melanin, fiber damage and DNA damage, which can lead to skin cancer (Marrot and Meunier, 2008). This study was conducted to assess the protective activity of tissues in relation to UV light damage, and to find the time period in which UVB light has no effect on the cell proliferation of HaCaT cells. The cells were treated for up to 3 min, with 20 s increments. With 60 s being the standard time frame to have an effect on cell proliferation in terms of UVB light, the treated and untreated cells were compared. The 60 s UVB light treatment of the whole ESMH, fraction I, II, and III with concentrations of 0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL was done to test their effects on the survival of HaCaT cells. The control showed a survival rate of 77±1%, while the whole ESMH at the lowest concentration (0.01 mg/mL) showed 108±2%, followed by 111±1% for the fraction I, 99±1% for the fraction II and 106±2% for the fraction III, and showed protective effects of 31%, 34%, 22% and

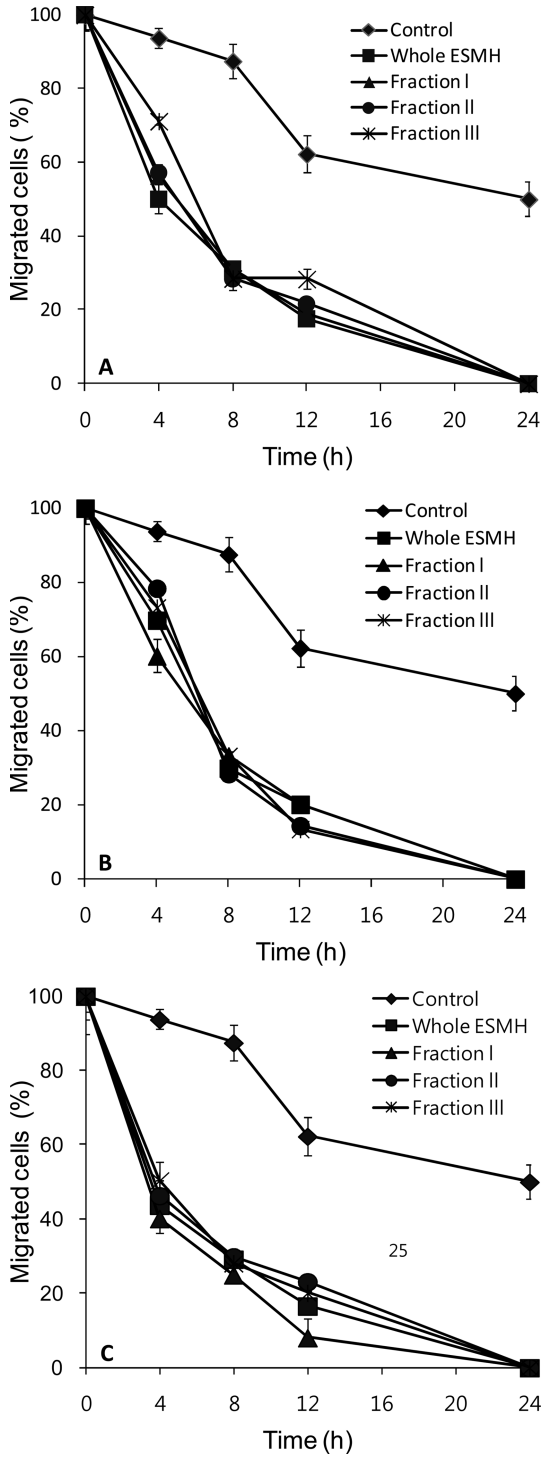

Fig. 5. Enhanced HaCaT cell migration in response to the egg shell membrane hydrolysates fractionated by molecular weight. A, 0.01 mg/mL; B, 0.1 mg/mL; C, 1 mg/mL. Data are presented as mean±standard deviation of three independent experiments.

29%, respectively, in comparison to the control (Fig. 6). Therefore, according to the effect of egg shell membrane on skin damage caused by UV light, egg shell membrane may be an effective material to use as a UV protector.

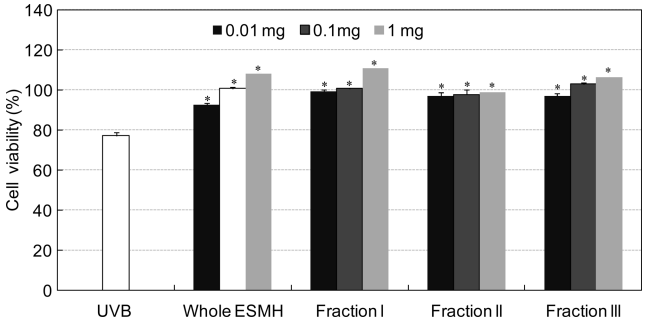

Fig. 6. HaCaT cell protection by UVB irradiation during 60 s. Data are presented as mean±standard deviation of three independent experiments. Full is defined as egg shell membrane hydrolysates before fractionation (*p<0.05 vs. control; only UVB treatment without ESMH).

In conclusion, the whole ESMH showed the skin whitening effect by inhibiting tyrosinase and oxidation of L-DOPA. Wound healing effect of ESMH by using HaCaT cell was also confirmed. Therefore, ESMH could be used as a good material for developing functional cosmetics.

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