

# Development of Multifunctional Cosmetic Products with Anti-Photoaging Effect

Eun-Suk Kim<sup>1</sup>, Hye-Jin Kwon<sup>2</sup>

<sup>1</sup>Professor, Seokyeonh Univ, Dept. Beauty Art, Seoul Korea, <sup>2</sup>Professor, Soongsil Univ, Dept. Chemical Engineering, Seoul Korea

## Abstract

**Background/Objectives:** Ultraviolet rays induce erythema, allergy, and skin aging. We investigated the anti-photoaging effect of stem-cell culture media and Polygona Multiflora Radix extract (GMF).

**Methods/Statistical analysis:** An emulsion containing natural GMF and a mixed emulsion containing stem-cell culture medium and Polygona Multiflora Radix emulsion (DMF) were prepared. Photoaging was induced in hairless mice (SKH-1) by irradiation of UVB rays thrice per week for 6 weeks. Pathological changes were examined following 5 or 10 days of emulsion application.

**Findings:** These mice exhibited typical signs of photoaging including increased epithelial thickness, accumulation of abnormal elastic and collagen fibers, and dermal accumulation of inflammatory and mast cells. Photoaging was significantly inhibited in all experimental groups. The group treated with DMF for 10 days showed similar levels of improvement as those observed in the control group.

**Improvements/Applications:** Thus, both natural GMF and DMF can reduce wrinkles caused by photoaging. In addition, our findings verify the potential of GMF and stem-cell culture media as ingredients for anti-photoaging cosmetic products.

**Keywords:** Polygona Multiflora Radix, photoaging, multifunctional cosmetics, stem-cell, Korean herbal

## Introduction

Ultraviolet (UV) rays, a major cause of skin aging, cause wrinkle formation, pigmentation, and dermal deformation. Aging induced by UV rays differs from natural aging<sup>[1,2]</sup>. Photoaging causes deeper wrinkles than natural aging and accelerates aging by inducing the accumulation of abnormal collagen fibers that leads to reduced skin elasticity<sup>[3]</sup>. UV rays increase the synthesis of MMP-1, -3, -9 and -12 enzymes in fibroblasts and epithelial cells, thereby destroying matrix proteins including collagen and elastic fibers. Inflammatory cells gather and release cytokines to signal the production of enzymes required to eliminate UV-damaged cells. During this process, collagen and elastic cells are broken down, thereby promoting aging<sup>[4,5]</sup>. Researchers have

been proposing various scientific methods that combine oriental medicine, microbiology, and food science to identify novel functional molecules that can prevent and reduce skin damage due to photoaging. Stem-cell culture media, commonly used in the field of cosmetics, contain active compounds such as proteins, cytokines, minerals, and vitamins that promote stem-cell differentiation and activity. In addition, stem-cell culture media can regulate skin differentiation and immune functions, prevent apoptosis, and treat wounds<sup>[6,7]</sup>. Media containing only those substances that are approved to be safe can be used to make highly functional and biocompatible cosmetic products<sup>[8]</sup>. Interest in cosmetic products containing natural herbal medicine has increased in Korean as well as in Asian and North American markets. Oriental herbal medicines contain glucose and amino acids involved in the primary metabolism of plants, and secondary substances including phenol, terpenoids, and alkaloids. Oriental herbal medicines have high potential as an ingredient for functional skin-whitening anti-aging

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**Corresponding Author:**

**Hye-Jin Kwon**

Soongsil University,

e-mail : kwonhj0070@ssu.ac.kr

cosmetic products<sup>9,10</sup>. In the present study, we prepared multifunctional cosmetic products with stem-cell culture media and natural substances and tested their effect on photoaging in hairless mice.

## Method

Polygonia Multiflori Radix extract (GMF), listed in Korean Herbal Pharmacopoeia, was obtained by hot water extraction. A 10% o/w emulsion (GMF) was prepared and used in the experiment. Stem-cell culture media (DCCM4), used as a cosmetic ingredient, listed in the INCI were provided by Eseumedio Co., Ltd. The culture media and GMF were concentrated two-fold to produce a 10% o/w emulsion (DMF) to be used in the final experiment. All reagents used were special grade chemicals.

Six-week-old male and female ICR/HR hairless mice (SKH-1) purchased from Orient. Co., Ltd. were bred for 18 months. The mice had *ad-libitum* access to food and water. The cage conditions were as follows: temperature,  $24 \pm 2^\circ\text{C}$ ; humidity,  $50\% \pm 10\%$ ; and 12:12 day/night cycle. Six experimental groups were formed containing six mice each. The mice were subjected to one of the following treatments: natural aging, photoaging, 5 days of GMF treatment after photoaging, 5 days of DMF treatment after photoaging, 10 days of GMF treatment after photoaging, and 10 days of DMF treatment after photoaging. The study was approved by the Institutional Review Board of Chonbuk National University.

A UVB lamp (Sankyo Denki, Tokyo, Japan) with an emission spectrum of 280–360 nm and a maximum peak at 360 nm was used for UVB irradiation. UVB rays were irradiated on the dorsal skin of the experimental animals thrice per week with a minimal erythral dose (MED) of 60 mJ/cm<sup>2</sup>. The irradiation was performed at 1, 2, and 4 MED at 1, 2, and 4 weeks, respectively, and thereafter for total 6 weeks. Irradiation was not performed for the last 3 days to eliminate the direct effect of UVB rays. An AHA peeling solution was prepared by dissolving 30 g of glycolic acid in 50 mL of distilled water, titrating it with NaOH to pH 3.2, and adjusting the total volume to 100 mL. The solution was stored in a fridge. AHA peeling was performed once for 10 min. After neutralization with PBS and complete drying of the skin, an emulsion corresponding to the designated treatment of each group was applied. The sample was applied twice a day for 10 days, and 200  $\mu\text{L}$  of the cosmetic product was applied to the entire surface of dorsal skin each time. Skin tissues

were extracted at 5 and 10 days after AHA peeling, fixed in 10% neutral formalin, and used in for histological analysis.

To study microstructures on the skin surface following irradiation, skin sections were made and examined at 40 $\times$ , 100 $\times$ , 400 $\times$ , 1000 $\times$ , and 1500 $\times$  magnification using a scanning electron microscope.

A sharp knife was used to obtain skin tissues from the mice. The tissues were fixed in 2.5% paraformaldehyde-glutaraldehyde, washed several times with a phosphate buffer solution ( $4^\circ\text{C}$ , 0.4 M phosphate buffer, pH 7.4), and fixed in 1% OsO<sub>4</sub>. The fixed samples were washed with the same buffer solution, dehydrated (60%–100% ethanol), and substituted with isoamyl acetate. The treated samples were completely dehydrated and placed on a sample holder. The samples were then coated with platinum until their thickness was 20 nm using an ioncoater (IB-5ioncoater, Eiko, Japan) and examined under a scanning electron microscope.

The skin tissues were fixed in a 10% neutral formalin solution at room temperature for 24 hours. They were washed, dehydrated, cleared, infiltrated, and embedded in paraffin. Thereafter, 4- $\mu\text{m}$  sections were prepared and examined under an optic microscope to study changes in the skin tissues. Paraffin blocks were stained using Hematoxylin & Eosin (H&E), Masson trichrome, and Toluidine blue Verhoeff-van Gieson stains. The stained tissue slides were histomorphometrically analyzed using an image program. Tissue thickness, number of inflammatory and mast cells, surface area of collagen and elastin, and distribution and surface area of lamella were quantified.

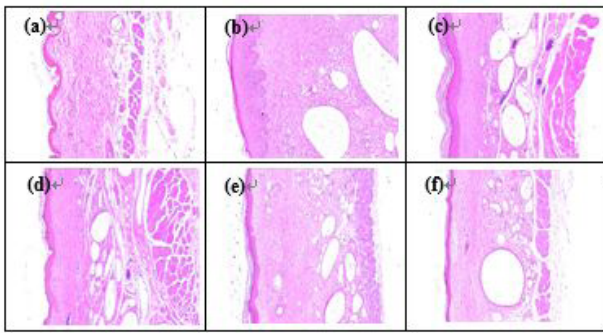
PASW Statistics 18.0 for Windows (SPSS Inc., USA) was used for all statistical analyses. A one-way ANOVA was performed to compare differences between the experimental groups. For measurements repeated during a certain period, repeated measures ANOVA was used to examine interaction between time and the experimental groups. A post-hoc analysis was performed to confirm statistical significance. Statistical significance was set at  $p < 0.05$ .

## Result and Discussion

### 1. Changes in epithelial thickness

The skin tissues of SKH-1 hairless mice were stained with H&E, and proliferation of epithelial cells

and epithelial thickness were examined as skin markers. The control group, not subjected to UVB irradiation, had an epithelial thickness of 22.46  $\mu\text{m}$  for the dorsal skin, whereas the photoaging group subjected to 4 weeks of UVB irradiation had an epithelial thickness of 203.38  $\pm$  23.07  $\mu\text{m}$ , indicating epithelial cell proliferation. After UVB irradiation, GMF and DMF were applied for 5 and 10 days, respectively. Epithelial thickness was 86.19  $\pm$  12.06, 53.69  $\pm$  12.60, 69.76  $\pm$  13.31, and 32.52  $\pm$  11.02  $\mu\text{m}$  in the GMF-5, DMF-5, GMF-10, and DMF-10 groups, respectively. These treatments reduced epithelial thickness by -40.84%, -67.16%, -62.99%, and -75.58%, respectively. The DMF-10 group showed the highest reduction in epithelial thickness and similar epithelial thickness as that of the control group [Figure 1].



**Figure 1. Photographs of histological sections of hairless mice (SKH-1) dorsal back skin by Hematoxylin&Eosin staining (Original magnification: x40, scale bar : 100  $\mu\text{m}$ , a: Intact, b: Aging, c: GMF-5, d: DMF-5, e: GMF-10, f : DMF-10)**

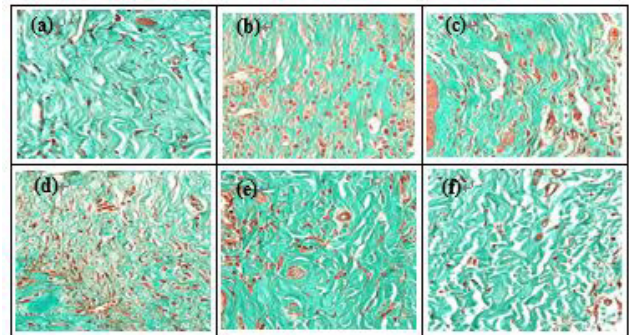
The epithelium repeatedly undergoes differentiation and proliferation to act as a barrier. Its permeable membrane minimizes the loss of skin moisture. Abnormal epithelial proliferation, which is a characteristic of photoaging, compromises the skin and causes allergy. When the skin loses its protective function, skin aging and secondary diseases can be induced, which in turn leads to inflammation. Frequent inflammation promotes aging in the surrounding cells<sup>[11]</sup>. The reduction in abnormal epithelial proliferation observed in the DMF-10 group indicates that the mixed extract has anti-aging effect.

## 2. Change in dermal collagen and elastin

The dermis consists of elastic and collagen fibers. Collagen fibers are the major components of the dermis responsible for the elasticity and structural integrity of the skin<sup>[12]</sup>. Elastic fibers, which are less abundant than

collagen fibers, contribute to skin elasticity. Skin aging is histologically defined as a decrease in the number of fibroblasts as well as elastin and collagen fibers, resulting in shrinkage of the extracellular matrix<sup>[13,14]</sup>. In the present study, we used wrinkle patterns as a marker of photoaging and observed the accumulation of abnormal elastic and collagen fibers.

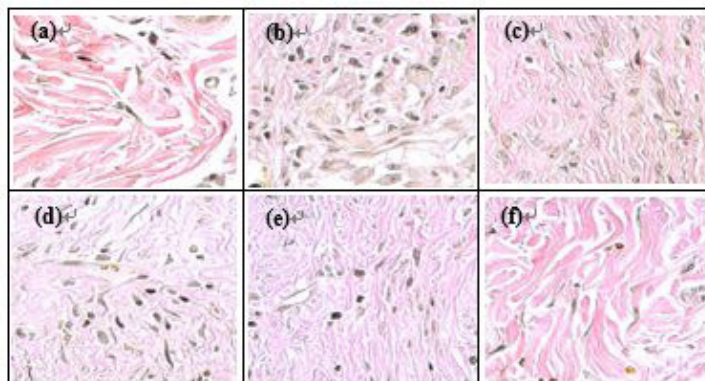
Collagen networks are destroyed and become dysfunctional due to UV rays. The accumulation of damaged collagen networks induces aging. We performed Masson's trichrome staining to evaluate the number and density of collagen fibers in the dermis [Figure 2]. The control group showed dense and regular patterns of collagen fibers, whereas the photoaging groups showed an irregular and less dense arrangement of collagen fibers due to destroyed collagen fibers. The positive control group and treatment groups showed more dense and regular patterns of collagen fibers compared with those in the photoaging groups. The density of collagen fibers increased as the concentration of emulsion increased. Elastic fibers are degraded in the dermis upon exposure to UV rays. UV rays activate elastase that promote elastic fiber degeneration, destroys the elastic fiber network, and induces neutrophil-mediated inflammation, thereby causing elastosis. We performed Verhoeff's staining to evaluate the number and density of elastic fibers in the dermis [Figure 3].



**Figure 2. Photographs of histological sections of hairless mice (SKH-1) dorsal back skin by Masson trichrome staining (Original magnification: 200x, Arrow : collagen fiber, scale bar : 100  $\mu\text{m}$ , a: Intact, b: Aging, c: GMF-5, d: DMF-5, e: GMF-10, f : DMF-10)**

The control group showed dense and regular patterns of elastic fibers, whereas the photoaging group showed clumped and distorted elastic fibers, resulting in elastosis. The positive control group and treatment groups had reduced size and frequency of defective elastic fibers compared with those in the photoaging groups. The size and frequency of defective elastic fibers

evidently decreased as the concentration of emulsion increased. Table 1 shows changes in the measurements of the markers in the dermis. The level of dermal elastic fibers decreased by -30.48%, -52.96%, -38.58%, and -62.7% following 5 and 10 days of GMF and DMF treatments, respectively, compared with the non-photoaging control group.



**Figure 3.** Photographs of histological sections of hairless mice( SKH-1) dorsal back skin by Verboeff-van Gieson staining(Original magnification: 400x, Arrow : elastin fiber, scale bar : 100 μm, a: Intact, b: Aging, c: GMF-5, d: DMF-5, e: GMF-10, f : DMF-10)

**Table 1. Histomorphometrical Values Detected on the Intact or UVB Irradiated Hairless Mice Dosal Back Skins-Dermis connective tissues**

	Controls		Test materials			
H	Intact	Ag-ing	GMF-5	DMF-5,	GMF-10,	DMF-10
C	52.88±5.96	78.72±4.07**	68.27±4.19*	57.40±6.06**	66.91±3.20*	52.43±4.9**
E	6.45±2.76	17.43±2.56**	12.12±2.24*	8.20±1.87**	10.70±2.27*	6.50±1.32**

(p\* < 0.05, p\*\* < 0.01).

H : Histomorphometry

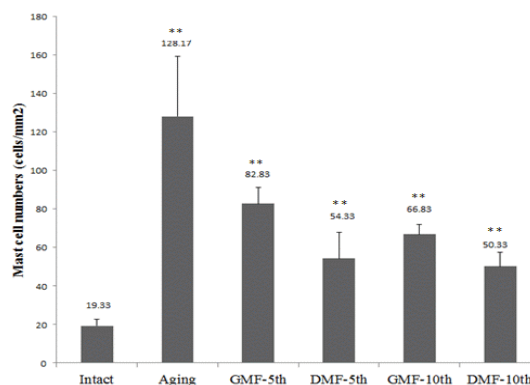
C: Collagen fiber (%/mm<sup>2</sup>)

E : Elastic fiber (%/mm<sup>2</sup>)

### 3. Change in the number of mast cells in the skin

Examined changes in the number of mast cells by staining the skin tissues obtained from SKH-1 hairless mice with Toluidine blue. A total of 19.33 ± 3.27 mast cells were found in the dorsal dermis of mice that were not exposed to UVB rays. On the other hand, the number of mast cells in the dermis significantly increased to 128.17 ± 31.29 in the toluidine photoaging group subjected to 4 weeks of UVB irradiation compared with the control group (p < 0.01). The number of mast cells decreased by -35.37%, -57.61%, -47.85%, and -60.73% following 5 and 10 days of GMF and DMF treatments, respectively. Mast cell overproduction was inhibited in the DMF and GMF

groups. Moreover, UVB-induced mast cell overproduction was significantly inhibited in the GMF group (p < 0.01) [Figure 4].



**Figure 4.** Photographs of histological sections of hairless mice( SKH-1) dorsal back skin by Verboeff-van Gieson staining(Original magnification: 400x, Arrow : elastin fiber, scale bar : 100 μm, a: Intact, b: Aging, c: GMF-5, d: DMF-5, e: GMF-10, f : DMF-10)

**4. General characteristics of the subjects**

Table 2 shows inflammatory counts. The control group showed almost no inflammatory cells, whereas the number of inflammatory cells significantly increased in the aging group. The number of inflammatory cells decreased following 5 and 10 days of GMF and DMF treatments. Similar results were reported by studies on anti-aging effect in animal skin models of photoaging. Typical characteristics of photoaging including increased epithelial thickness, accumulation of abnormal collagen and elastic fibers, and dermal accumulation of inflammatory and mast cells could be observed

following the induction of photoaging using UVB rays. The normalization of the epithelial thickness may indicate that the GMF and DMF treatments can protect and recover the skin barrier as reported in previous studies<sup>[15]</sup>. Normalization of the skin barrier helps alleviate inflammation in the epithelium and dermis. In the present study, 5 and 10 days of DMF treatments significantly inhibited UVB-induced photoaging ( $p < 0.01$ ,  $p < 0.05$ ). Photoaging was more significantly inhibited in the DMF group than that in the GMF group ( $p < 0.01$ ). Thus, GMF and DMF may be useful as ingredients of functional cosmetic products.

**Table 2. Histomorphometrical Values Detected on the Intact or UVB Irradiated Hairless Mice Dosal Back Skins-Dermis connective tissues**

	Controls		Test materials			
H	Intact	Ag-ing	GMF-5	DMF-5,	GMF-10,	DMF-10
I	42.50 ± 18.88	651 .67 ± 140.28 **	385 .50 ± 114.31 *	214 .00 ± 42.83 **	241 .17 ± 21.66 *	15 9.17 ± 49.18 **

( $p^* < 0.05$ ,  $p^{**} < 0.01$ )

H : Histomorphometry

I : Inflammatory cell numbers (cells/mm<sup>2</sup>)

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**Conflict of Interest:** Nil

**Conclusion**

We induced wrinkle formation in hairless mice using UVB rays and found that an emulsion containing natural GMF and DMF can improve skin damage due to photoaging. The emulsions inhibited and mitigated the characteristics of photoaging including increased epithelial thickness, accumulation of abnormal elastic and collagen fibers, and dermal accumulation of inflammatory and mast cells. DMF has a significantly higher efficacy than GMF. These results demonstrate that an emulsion containing natural GMF and DMF inhibits the activity of skin aging factors induced by UVB. Thus, GMF and DMF may be used as functional, bioactive materials for anti-photoaging cosmetic products.

**Ethical Clearance:** Not required

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**References**

1. Farage Ma, Miller Kw, Elsner P, Maibach Hi. Intrinsic And Extrinsic Factors In Skin Ageing. Int. J. Cosmet. Sci. 2008 Apr;30(2):87-95. DOI: 10.1111/j.1468-2494.2007.00415.x
2. Larroque CP, Camaré C, Nadal WF, Grazide MH, Pucelle M, Garoby SS, et al. Elastin Modification by 4-Hydroxynonenal in Hairless Mice Exposed to UV-A. Role in photoaging and actinic elastosis. Journal of Investigative Dermatology. 2015 Jul;35(7):1873-81. DOI:10.1038/jid.2015.84..
3. Walter AR, Brandon RG, Lynne TS. rectomy, Oophorectomy, estrogen treatment, and cognitive aging: Clinical evidence for a window of opportunity. Brain research. 2011 Oct;1379:188-286.

4. Sakura M, Chiba Y, Kamiya E, Furukawa A, Kawamura N, Niwa M, et al. Differences in the Histopathology and Cytokine Expression Pattern between Chronological Aging and Photoaging of Hairless Mice Skin. *Modern Research in Inflammation*. 2014 Jun;3(3):82-9. DOI: 10.4236/mri.2014.33010.
5. Zhang Q, Fong CC, Yu WK, Chen Y, Wei F, Koon CM, et al. Herbal formula Astragali Radix and Rehmanniae Radix exerted wound healing effect on human skin fibroblast cell line Hs27 via the activation of transformation growth factor (TGF- $\beta$ ) pathway and promoting extracellular matrix (ECM) deposition. *Phytomedicine*. 2012 Jan;20(1):9-16. DOI:10.1016/j.phymed.2012.09.006.
6. Yang, Y, Li S. Dandelion Extracts Protect Human Skin Fibroblasts from UVB Damage and Cellular Senescence. *Oxid Med Cell Longev*. 2015 Dec;2015:1-10. DOI:10.1155/2015/619560.
7. Esser PR, Wöolfle U, Dürr C, Loewenich FD, Schempp CM, Freudenberg MA, et al. Contact sensitizers induce skin inflammation via ROS production and hyaluronic acid degradation. *PLOS ONE*. 2012 Oct;7(7):41340.
8. Ichihashi M, Ueda M, Budiyo A, Bito T, Oka M, Fukunaga M, et al. UV-induced skin damage. *Toxicology*. 2003 Jul;189(1-2):21-39. DOI: 10.1016/s0300-483x(03)00150-1.
9. Labat RJ, Fourtanier A, Boyer LB, Robert L. Age dependent increase of elastase type protease activity in mouse skin: Effect of UV-irradiation. *Journal of Photochemistry and Photobiology B: Biology*. 2000 Nov;57(2-3):113-121.
10. Fisher GJ, Talwar HS, Lin J, Voorhees JJ. Molecular mechanism of photogaging in human skin in vivo and their prevention by all-trans retinoic acid. *Photochem Photobiol*. 1999 Apr;69(2):154-157.
11. Taihao Q, Tianyuan H, Sewon K, John JV, Gary JF. Solar ultraviolet irradiation reduces collagen in photoaged human skin by blocking transforming growth. *Am J Pathol*. 2004 Jun;165(3):741-751.
12. Oikarinen A. Aging of the skin connective tissue: how to measure the biochemical and mechanical properties of aging dermis. *Photodermatology, Photoimmunology and Photomedicine*. 1994 Mar;10(2):47-52.
13. Braverman, IM, Fonferko E. Studies in cutaneous aging. I. The elastic fiber network. *J Invest Dermatol* 1982 Oct;78(5): 434-77. DOI :10.1111/1523-1747.ep12507866.
14. Uitto J. Connective tissue biochemistry of the aging dermis. Age-related alterations in collagen and elastin. *Dermatologic Clin* 1986 Sep;4(3):433-446.
15. Moloney SJ, Edmonds SH, Giddens LD, Learn DB. The hairless mouse model of photoaging: evaluation of the relationship between dermal elastin collagen, skin thickness and wrinkles. *Photochemistry and photobiology*. 1992 Aug; 56(4):505-511. DOI:10.1111/j.1751-1097.1992.tb02194.x.