의학석사 학위논문

# The Nude Mouse Model for Photoaging

# 누드마우스의 광노화 모델

**2013 년 6 월**

서울대학교 대학원

의학과 성형외과학 전공

**YINGFANG FAN**

**A thesis of the Degree of Master**

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**June, 2013**

**The Department of Plastic Surgery**

**Seoul National University**

**College of Medicine**

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이 논문을 의학석사 학위논문으로 제출함

2013 년 6 월

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2013 년 6 월

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# The Nude Mouse Model for Photoaging

**by**

**YINGFANG FAN**

**A thesis submitted to the Department of Plastic Surgery in partial fulfillment of the requirement of the Degree of Master in Plastic Surgery at Seoul National University College of Medicine**

**June, 2013**

**Approved by Thesis Committee:**

**Professor _____Chairman**

**Professor _____Vice chairman**

**Professor _____**

# ABSTRACT

**Introduction:** Autologous adipose-derived stem cells (ADSCs) and their secretory factors have great promise for applications in treating photodamaged skin. What is more significant is that ADSCs also have an antiwrinkle effect and have thus become a topic of primary interest. Nude mice have been used extensively in studies on ADSCs, human dermal fibroblasts, and other injections. However, a photoaging model of the nude mouse has not yet been developed. The purpose of this study was to develop a nude mouse model for photoaging.

**Materials and Methods:** Fourteen 5-week-old female BALB/c nude mice were irradiated with ultraviolet-B (UVB) rays 6 times a week for 6 weeks. The minimum erythema dose was determined before UV irradiation in order to minimize inflammation of the irradiated skin and define the initial irradiation dosage. The total wrinkle area and mean depth of the wrinkles were compared by replica analysis. At the sixth and 10th weeks, skin biopsies were performed.

**Results**: The mean depth of the wrinkles in the UVB-irradiated nude mice significantly increased, and the epidermal and dermal thickness of the upper and lower back skin was significantly thicker following continuous UVB irradiation up to the sixth week ($p < 0.05$). Furthermore, a marked decrease in

collagen bundles was observed in the UVB-irradiated group by Masson's trichrome staining.

**Conclusions**: This study successfully developed a nude mouse model for the experimental analysis of photoaging. The results of this study indicate that the nude mouse is a good model for photoaging studies.

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**Keywords:** UV-B, Wrinkle, UV-induced photoaging, BALB/c nude mouse

**Student number**: 2011-24148

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# INTRODUCTION

Skin aging is a complex process that is influenced by intrinsic and extrinsic factors. Intrinsic, or chronological skin aging, results from the passage of time and is influenced by genetic factors. Extrinsic skin aging is mainly determined by UV irradiation, which is also called photoaging. These 2 types of aging processes are superimposed onto sun-exposed skin, and have the common feature of causing dermal matrix alterations that primarily contribute to the formation of wrinkles, laxity, and fragility of aged skin.[1]

As individuals age, the skin undergoes changes such as irregular pigmentation, thinning, and loss of elasticity that are caused by both genetic and environmental factors.[2] Quantitative and qualitative changes in dermal collagen and elastin occur in response to chronic ultraviolet (UV) irradiation. These changes have been implicated in the onset of wrinkling observed in chronically irradiated, or photoaged skin.[3] The hairless mouse is proving to be a relevant model for the systematic study of photoaging.[4,5,6] However, this model presents a major disadvantage because it is not widely used to study adipose-derived stem cells (ADSCs). Autologous ADSCs and their secretory factors have great promise for applications in treating photodamaged skin.[7] Morever, ADSCs also have an antiwrinkle effect, and have thus become a major topic of concern. Mouse models have been extensively employed for investigating photoaging.[8] Nude mice have been used in numerous studies on

ADSCs, human dermal fibroblasts, and other injections .[1,8] Thus, the purpose of this study was to develop a nude mouse model for photoaging.

# MATERIALS AND METHODS

## Animal experiments

Fourteen 5-week-old female BALB/c nude mice were provided from ORENT Inc. (Seongnam, Korea). They were fed a standard diet and water. In addition, they were irradiated dorsally using the UVB-emitting system 6 times a week for 6weeks. Mice were allowed to rest for 1 week in the animal facility before the start of the experimental procedures. The irradiation source included 4 UVB lamps (TL20W/12RS; Philips, NY, USA) filtered with a Kodacel filter (TA401/407; Kodak, Rochester, NY, USA) to remove the UV-C radiation associated with these bulbs. The irradiance used was between 290 and 320 nm. The distance from the lamps to the backs of the animals was 20cm. During exposure, the animals could move around freely in their cages.

## Minimum erythema dose assessment for nude mice

The MED is defined as the minimum dose of radiation that produces perceptible skin erythema and is expressed as joules per square centimeter ($J/cm^2$). The MED must be determined before UV therapy in order to minimize the inflammation of irradiated skin and to define the initial irradiation dosage.[9]

The skin was divided into 6 sections, 24 $cm^2$ in size, which included the testing area and the standard product application site. The minimum

irradiation area was 0.5 $cm^2$. UV irradiation was conducted after isolation of the test area (the nude mice were kept in a comfortable position). During the irradiation, the nude mice were kept still due to anesthesia. Within 24h after UV exposure, the test sites of the nude mice, where the erythema reaction might appear, were selected. Erythema was identified by a number of trained evaluators under a sufficient light source. The minimum intensity of UVB irradiation that produced erythema on a given test area were regarded as the MED.

## Groups and UVB-irradiation protocol

The mice were divided into 2 groups: (1) the control group ($n$ = 7), and (2) the UVB-irradiated group ($n$ = 7). The irradiation dose was one minimum erythemal dose (MED; 150 $mJ/cm^2$) in the first 2 weeks, 1.2 MED (180 $mJ/cm^2$) in the third week, 1.4 MED in the fourth week (210 $mJ/cm^2$), 1.6 MED (240 $mJ/cm^2$) in the fifth week, and 1.8 MED (270$mJ/cm^2$) in the sixth week. The total UVB dose was approximately 48 MED (6.9 $J/cm^2$ ; Table 1).

## Skin image and replica analysis

At weeks 0, 2, 4, 6, 7, 8, 9, and 10, negative replicas of the upper and lower skin surfaces were taken using a silicon-base impression material (Courage + Khazaka electronic GmbH, Koln, Germany). The replicas were cut into circular pieces with a diameter of 1 cm and processed so that their backs became flat. Images of the negative replicas were observed using a wrinkle

analysis system Visioline® VL 650 (Courage + Khazaka electronic GmbH, Koln, Germany).

## Histological observations

At weeks 6 and 10, the upper and lower back (1 cm × 1cm) sections were fixed in a 10% formaldehyde solution for 24 h, embedded in paraffin, and sectioned at 6 mm. The sections were subjected to Hematoxylin & Eosin (H&E) and Masson's trichrome staining, and analyzed using an image analysis software (Leica Qwin V3 and Leica Microsystems CMS GmbH).

## Statistical analysis

All statistical analyses were performed using Wilcoxon signed rank test. SPSS Version 20.0 (SPSS, Inc., Chicago, IL, USA) was used for all calculations. A *p*-value of $< 0.05$ was considered statistically significant.

# RESULTS

## Skin changes caused by UV irradiation

The photoaged group developed erythematous papules on their backs after 2 weeks of UV irradiation and showed deep, rough wrinkles on the upper back skin after 6 weeks of UV irradiation (Fig. 1). The skin of the control group showed no changes (Fig. 2).

## Wrinkle analysis

The images of the negative replicas were observed using a wrinkle analysis system Visioline® VL 650 to quantify the degree of wrinkle formation. The mean depth and total number of wrinkles in the UVB-irradiated group were significantly greater than that in the control group (Fig. 3). Furthermore, the upper back skin was substantially than lower back skin. The mean depth and total number of wrinkles in the UVB-irradiated group decreased up to week 10 ($p < 0.05$).

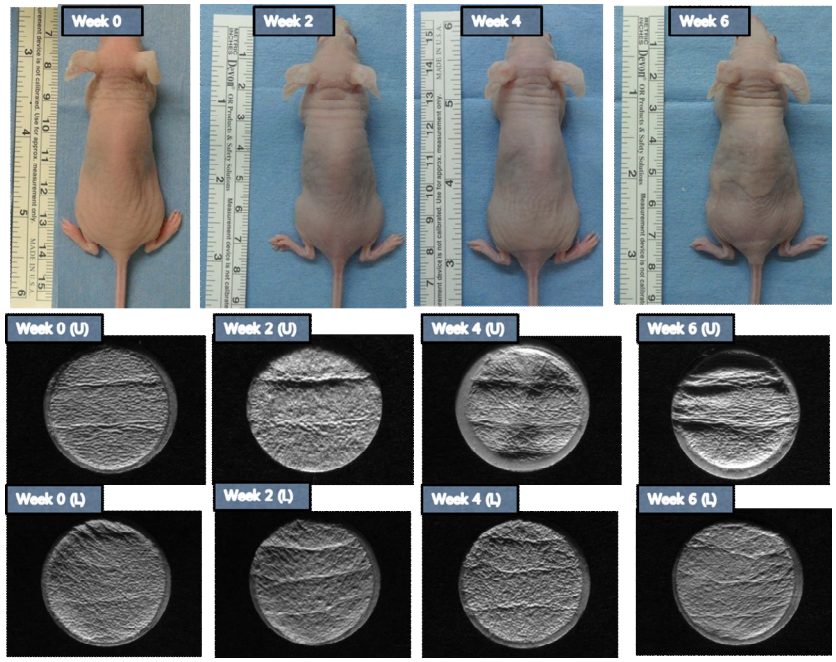
## Histological observations

From the histological observations, the thickness of the epidermis and dermis were significantly increased by UVB irradiation as shown by the H&E staining (Fig. 4). The epidermal and dermal thickness of the upper and lower back skin was significantly thicker following continuous UVB irradiation (Fig.

5; $p < 0.05$). Furthermore, epidermal and dermal thickness significantly reduced up to week 10. Figure 6A shows the Masson's trichrome staining results of collagen (blue area in the dermis) in the UVB-irradiated and control mice groups. The collagen fibers in the UVB-irradiated group were markedly less than those in the control group up to week 6 (Fig. 6B; $p < 0.05$).

| Period | Exposure duration<br>UV dosage : 1 MED = 150 mJ | | | | | | Total dosage: 48 MED |
|---|---|---|---|---|---|---|---|
| | Mon | Tue | Wed | Thu | Fri | Sat | Dosage/wk |
| 1st wk | 1.0 MED | 1.0 MED | 1.0 MED | 1.0 MED | 1.0 MED | 1.0 MED | 6.0 MED |
| 2nd wk | 1.0 MED | 1.0 MED | 1.0 MED | 1.0 MED | 1.0 MED | 1.0 MED | 6.0 MED |
| 3rd wk | 1.2 MED | 1.2 MED | 1.2 MED | 1.2 MED | 1.2 MED | 1.2 MED | 7.2 MED |
| 4th wk | 1.4 MED | 1.4 MED | 1.4 MED | 1.4 MED | 1.4 MED | 1.4 MED | 8.4 MED |
| 5th wk | 1.6 MED | 1.6 MED | 1.6 MED | 1.6 MED | 1.6 MED | 1.6 MED | 9.6 MED |
| 6th wk | 1.8 MED | 1.8 MED | 1.8 MED | 1.8 MED | 1.8 MED | 1.8 MED | 10.8 MED |

**Table 1. Schematic representation of the UVB-irradiation protocol. Nude mouse UVB-irradiation schedule for a 6 week periods.**


Week 0
Week 2
Week 4
Week 6
Week 0 (U)
Week 2 (U)
Week 4 (U)
Week 6 (U)
Week 0 (L)
Week 2 (L)
Week 4 (L)
Week 6 (L)

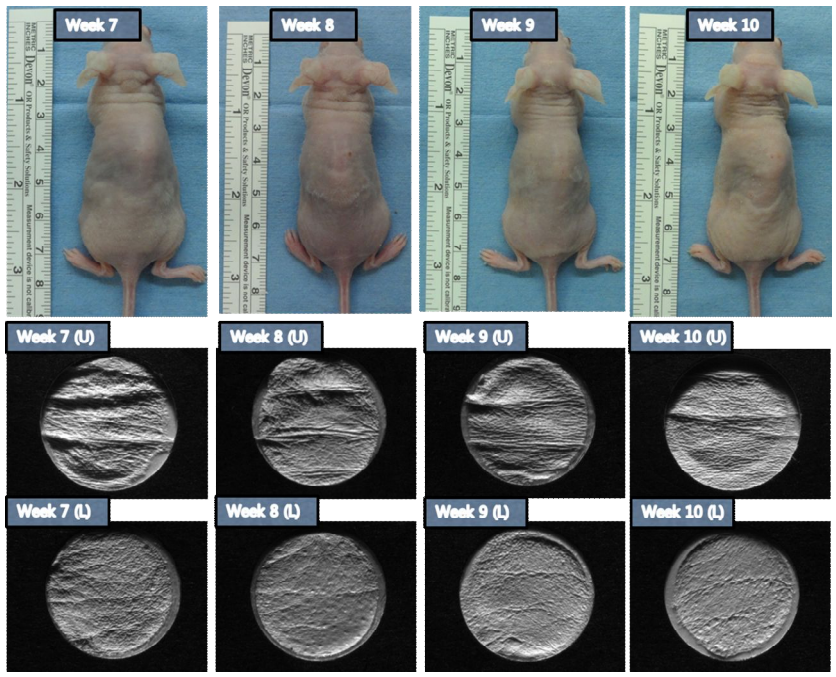

Week 7
Week 8
Week 9
Week 10
Week 7 (U)
Week 8 (U)
Week 9 (U)
Week 10 (U)
Week 7 (L)
Week 8 (L)
Week 9 (L)
Week 10 (L)

**Figure 1. Photographs of the skin surface and a replica report. Observations for the UV-irradiated group at 0, 2, 4, and 6 weeks (irradiated), and 7, 8, 9, and 10 weeks. The UV-irradiated group developed erythematous papules on their backs after 2 weeks of UV irradiation and showed deep, rough wrinkles on the upper back skin after 6 weeks of UV irradiation, furthermore the wrinkles improved once the exposure ceased up to week 10. U: upper back; L: lower back.**

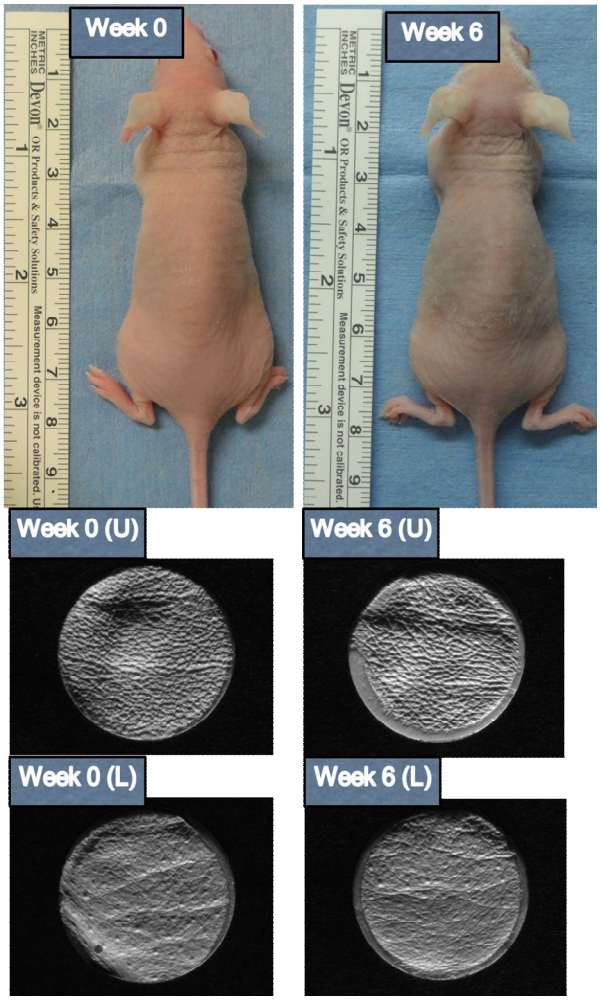



**Figure 2. Photographs of the skin surface and a replica report. Control group at 0 and 6 weeks (non-irradiated). The skin of the control group showed no changes. U: upper back; L: lower back.**

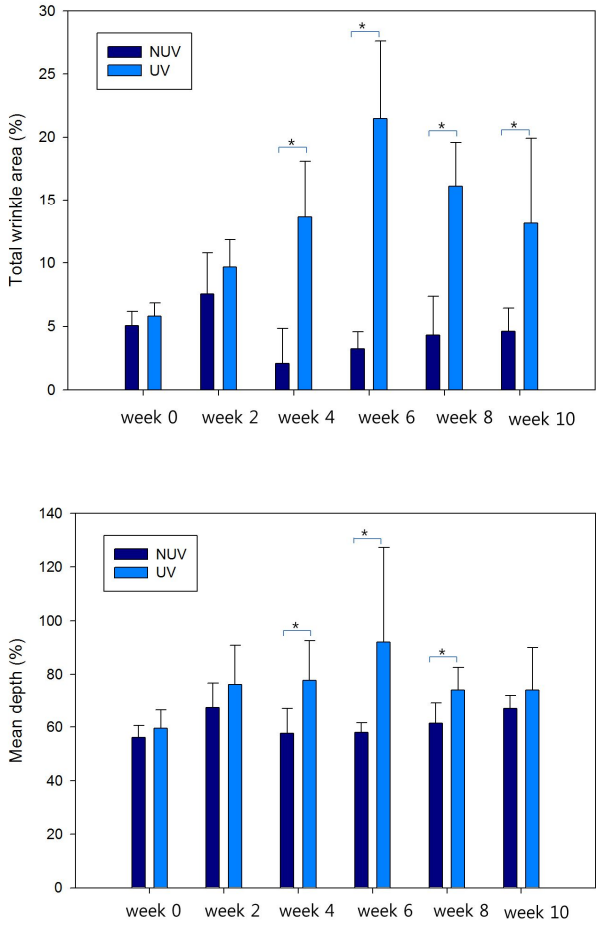



**Figure 3. Analysis of the replicas for the UV-irradiated(UV) and non-irradiated(NUV) control groups for UVB-induced mean depth and total area of the skin's wrinkles. Each measurement is shown as mean± standard deviation. The mean depth and total number of wrinkles in the UVB-irradiated group were significantly greater than that in the control group. Although the wrinkles improved once the exposure ceased, the data are still meaningful. (*$p < 0.05$; Wilcoxon signed rank test)**

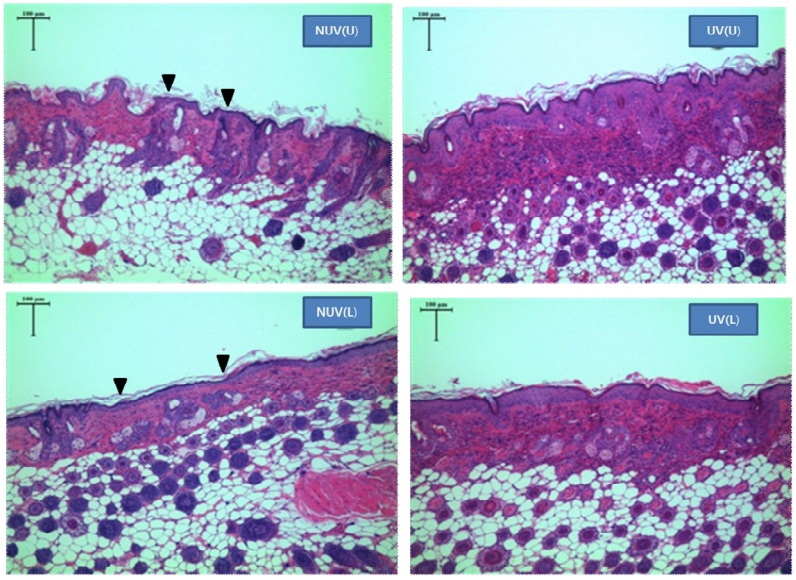
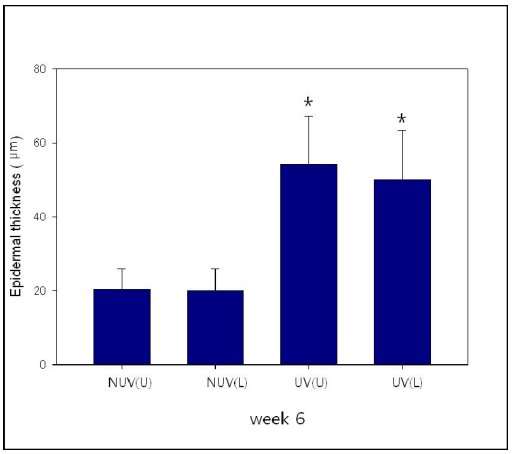



**Figure 4. Mouse skin samples stained with hematoxylin and eosin (H&E) at week 6. NUV(U): Upper back site of the non-irradiated control group. The very thin epidermis(head of arrow). NUV(L): Lower back site of the non-irradiated control group. UV(U): Upper back site of the irradiated group. UV(L): Lower back site of the irradiated group. H&E staining shows that the epidermis and dermis in UV(U) and UV(L) were thicker than that in NUV(U) and NUV(L) at week 6. Scale bars are 100 μm.**

A




B

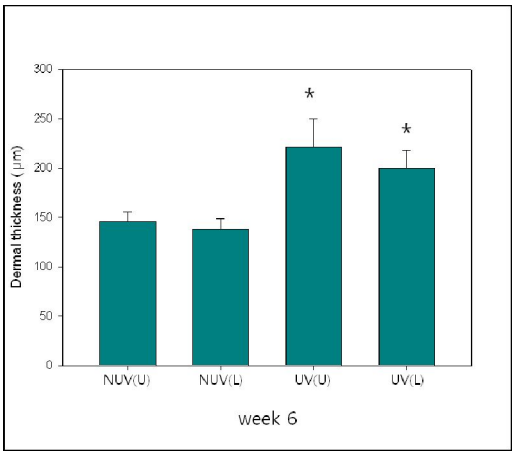
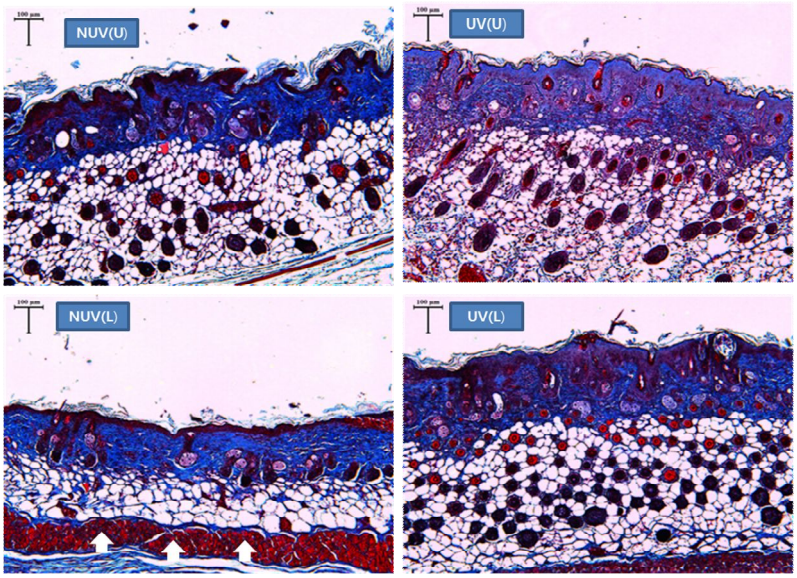



**Figure 5. Epidermal and dermal thickness as determined by H&E staining. (A) Epidermal thickness was measured as the mean value from 5 different images on the same slide. (B) Dermal thickness was measured as the distance from the epidermal-dermal junction to the dermo-subcutaneous fat layer. Each measurement is shown as mean± standard deviation. The epidermal and dermal thickness of the upper (U) and lower (L) back skin was significantly thicker following continuous UVB irradiation. (*$p < 0.05$; Wilcoxon signed rank test)**

**A**




**B**

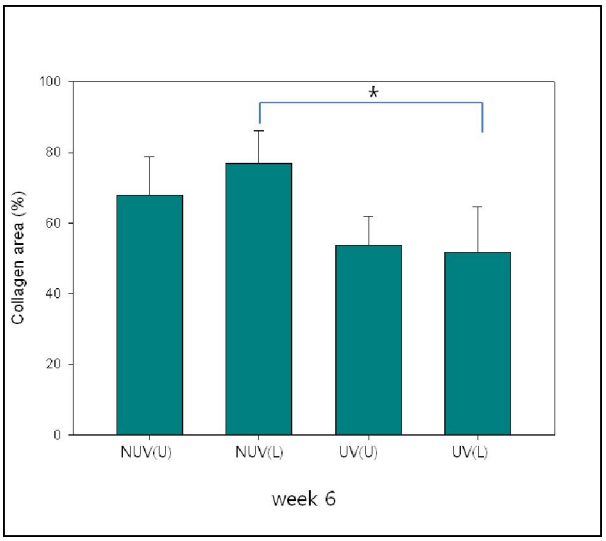



**Figure 6. (A) Collagen distribution determined by Masson's trichrome staining. Using Masson's trichrome staining, collagen fibers were stained blue, Blank arrow is the panniculus carnosus. Scale bars are 100 μm. (B)**

**Collagen (blue area in the dermis) was measured as the mean value of 5 different images on the same slide. Each measurement is shown as mean± standard deviation. The significantly lesser collagen contents were found in UV(U) and UV(L) than in NUV(U) and NUV(L) at week 6. NUV(U): Upper back of the non-irradiated control group. NUV(L): Lower back of the non-irradiated group. UV(U): Upper back of the irradiated group. UV(L): Lower back of the irradiated group. (*$p < 0.05$; Wilcoxon signed rank test)**

# DISCUSSION

The skin is composed of the epidermis, dermis, and 3 subcutaneous tissue layers. The epidermis is the most superficial layer and can protect the body from harmful environmental stimuli and fluid loss. The dermis is located under the epidermis and is attached to it by the dermo-epidermal junction.[10,11] UVB radiation (290~320 nm) can penetrate the epidermis and reach the dermis.[12] Extrinsically aged skin shows hyperplasia, with an increase in the thickness of the epidermis and dermis. There is a complete perturbation of the structural content (e.g., reduced interstitial collagen and increased elastic fibers) associated with damaged fibers, thus leading to severe disorganization of the connective tissue structure.[13] Dermal connective tissue forms the dermis and contains extracellular matrix proteins such as collagen, elastic fibers, fibronectin, glycosaminoglycans, and proteoglycans, which are produced and secreted into the extracellular space by fibroblasts (i.e., the main cell type of the dermis). Collagen is the most abundant protein in dermal connective tissue.[13]

A common mechanism of type I collagen includes alterations due to intrinsic aging and photoaging in dermal connective tissue.[1, 11, 13] Intrinsic aging induces the down-regulation of transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) in the TGF-β/ Smad/ CTGF/ procollagen axis and leads to decreased synthesis of type I collagen.[1, 11] In

photoaging, UV irradiation from the sun generates reactive oxygen species (ROS), which activate fibroblast growth factor and cytokine receptors in the skin. Activated receptors stimulate p38 and JNK, members of the mitogen-activated protein kinase (MAPK) signaling cascade, and c-Fos and c-Jun, which subsequently combine to form the activator protein 1 (AP-1) complex and stimulate matrix metalloproteinase (MMP) transcription.[1] Increased MMP transcription accelerates the degradation of collagen which induces dermal matrix alterations. ROS generation and AP-1 formation induced by photoaging can also lead to decreased collagen synthesis by blocking TGF-β type II receptor/Smad signaling. ROS can also be generated from oxidative metabolism and accumulate during the intrinsic aging process.[1, 11]

Various phenomena and mechanisms of skin aging have been verified by *in vivo* experimental data from mouse models. Mice are useful models for studying cutaneous aging as they are genetically similar to humans, affordable for experiments, and easy to manage.[1] The hairless mouse is proving to be a relevant model for the systematic study of photoaging.[4, 5, 6] At present, the most commonly used hairless mouse for studying photoaging is the albino hairless-Skh-1.[14] The present study is the first to use a nude mouse to examine photoaging. The UVB-treated group of nude mice was irradiated over a 6-week period, 6 times a week. At the end of the irradiation period, replica analyses showed deep, rough wrinkles on the upper back, but no changes were observed in the non-irradiated control group (Fig. 2). H&E staining showed that the hyperplastic epidermis/ dermis and skin thickened (Fig. 4). In the

study group both epidermal and dermal thickness were significantly increased by UVB-irradiation, especially when the upper back skin of mice in the UVB-treated group was compared to the control group (Fig. 5; $p < 0.05$). To visualize changes in the collagen fiber, which were noted in the dermal areas, Masson's trichrome staining was performed; the results showed that collagen fibers were stained blue (Fig. 6A), and the number of collagen fibers in the study group were significantly less than those in the control group (Fig. 6B; $p < 0.05$). It is reported that, following UVB irradiation, the collagen fibers were partially damaged.

It has been recognized that photoaged nude mouse skin undergoes partial repair once the exposure has stopped (Figs. 1 and 3). This occurrence has also been shown to take place in hairless mice. This striking deposition, 15 weeks after UV exposure, of an observed l00-µm-deep subepidermal band of new collagen led to the development of an assay to test topical substances that might enhance the repair process.[14]

In this study, we observed that the thickness of the epidermal and dermal layers in UVB-irradiated nude mice was significantly great than that of the non-irradiated control mice. Although the wrinkles improved once the exposure ceased, the data are still meaningful as they demonstrate the use of a nude mouse model to study photoaging.

# CONCLUSION

On the basis of the results obtained in this study, we conclude that this model can be used to better understand the process of wrinkle formation. These results indicate that the nude mouse is a good model for investigating the development of photoaging. This study led to the successful development of a nude mouse model for photoaging analysis.

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# 국문 초록

**서론**: 광노화의 모델은 hairless mouse 를 많이 사용하고 있고, nude mouse 를 이용한 실험은 거의 없었다. 최근에는 성체줄기세포인 지방조직 유래줄기세포(ADSCs)의 광노화 효과가 새롭게 증명되고 있으며, 새로운 노화 방지의 해결책으로 떠오르고 있다. 지방조직 유래줄기세포(ADSCs)를 연구하는 동물 모델은 주로 nude mouse 를 이용하였다. 그로 인해 nude mouse 를 이용한 광노화 모델의 필요성이 증가하고 있다. 본 연구에서는 nude mouse 를 이용하여 광노화 모델을 만들고자 한다.

**대상 및 방법**: 5 주령의 누드마우스(BALB/c nude mouse) 14 마리를 사용하였고, 자외선 B 를 조사하는 실험군 7 마리, 자외선을 조사하지 않은 대조군 7 마리로 나누어 실험을 진행하였다. 주름 변화의 평가 방법은 실리콘을 이용하여 피부에 negative replica 를 제작하였고, 각 군의 주름의 정도를 비교하였다. 6 주에 조직학 검사를 위해 hematoxylin and eosin staining 과 Masson′s trichrome staining 을 하였다.

**결과**: 자외선을 조사한 실험군에서 주름이 대조군에 비해 유의하게 잘 생겼다. 표피 와 진피의 두께가 대조군에 비해 유의하게

증가하였다. 조직학 소견으로는 대조군에 비해 실험군에서 collagen 의 양이 유의하게 감소하였음을 알 수 있었다($p < 0.05$).

**결론**: 연구자는 nude mouse (BALB/c nude mouse)에 자외선 B 를 조사하여 유의한 주름을 만들었다. 본 연구를 통해 nude mouse (BALB/c nude mouse)의 광노화 모델을 정립할 수 있었다.

**주요 단어**: 자외선 B, 누드마우스, 주름모델, 광노화

**학번**: 2011-24148