

Effect of LED Irradiation on Proliferation of Human Epidermal Keratinocyte for Convergence

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LED조사가 인간 피부 각질세포의 증식에 미치는 융복합적인 영향

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Abstract The purpose of this study is to determine the effect of the light-emitting-diode (LED) to investigate proliferation of human epidermal keratinocyte and collagen, procollagen expression. In order to determine whether LED irradiation can safely be applied to human skin, the proliferative effects of LED irradiation were determined by MTS assay in Human Epidermal Keratinocytes. Wavelength of 470nm LED irradiation increased mRNA expression of collagen, procollagen without cytotoxicity. Our results suggest that 470nm LED irradiation may have a proliferative effects and collagen synthesis property. In order to determine whether LED irradiation can safely be applied to human skin, the cytotoxic effects of LED irradiation were determined by MTS assay in Human Dermal Fibroblasts (HDF). As far as we know, this is the first report demonstrating in vitro collagen synthesis activity of 470nm LED irradiation and being a scientific basis for the cosmetic.

Key Words : LED(Light emitting diode), Human Epidermal Keratinocytes, Collagen, Procollagen, Light therapy, Convergence

요 약 본 연구의 목적은 발광 다이오드 (LED)을 인간 피부 각질 세포에 조사 시 콜라겐, 프로 콜라겐의 증식 발현을 조사하기 위해 실시되었었다. LED 조사 시 안전하게 인간의 피부에 적용할 수 있는지 여부를 결정하기 위해, LED 조사의 증식 효과는 인간 표피 각질세포에서 MTS 분석으로 결정하였다. 470nm의 파장 조사는 세포 독성 없이 mRNA의 콜라겐의 발현, 프로 콜라겐을 증가시켰으며, 이 결과는 470nm LED 조사가 피부각질세포 증식 효과와 콜라겐 합성에 영향을 미칠 수 있음을 시사한다. 또한 LED 조사시 독성 효과는 인간 피부 섬유 아세포 (HDF)에서 MTS 분석으로 결정 한 결과 세포 증식에 독성을 나타내지 않았다. 470nm LED 조사시 시험 관내 콜라겐 합성 활동을 증가시킴으로 피부미용 및 융복합적인 부분에 활용할 수 있는 기초 자료로 활용이 가능하다고 사료된다.

주제어 : 엘이디, 인간피부각질세포, 콜라겐, 프로콜라겐, 광치료, 융복합

Received 29 September 2016, Revised 31 November 2016
Accepted 20 November 2016, Published 28 November 2016
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ISSN: 1738-1916

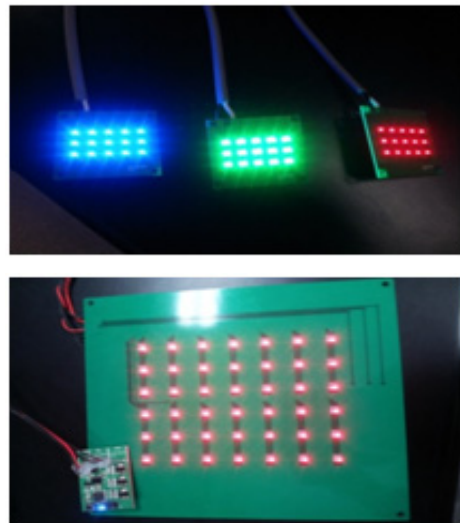
1. Introduction

In curing the various diseases, a laser and LED are now applied to many people of a worldwide. LED related research has been progressed and in the specific LED wavelength, it was reported that there was an effect including wound healing acceleration [1,2,3,4], anti-inflammation[5,6,7,8], pigmentation prevention [9,10], and etc. Besides, it was clarified that there is an effect of the cellulite removal [11,12], and depilation[13]. And in the recent research[14], it reported to could be applied to the treatment of the patient in which the visibleray(400-500nm)has the atopic eczema. Collagen is the most abundant protein in mammals, it is a major structural protein in the extracellular space as a major component of connective tissue of animals. Collagen is found primarily in the fibrous tissue of these tendons, ligaments, skin, etc. in the form of elongated fibrils[15]. It is abundant in many bone, cornea, cartilage, intestines, intervertebral discs and dentin of the tooth. In the muscle tissue, it is a major component of the endomysium function[16]. Collagen forms a strong muscle weight of 1-2% and configured to 6% of the muscle tissue. In addition, fibroblasts are the most common cells that produce collagen. Gelatin that is used in the food industry is the regional ratio of hydrolyzed collagen. Collagen has been used in many medical complications, treatment of bone and skin [17,18]. So we selected the wavelength in the visible rays which the side effect is not reported until now in this research and in order to replace the risk of the laser injuring the skin tissue and eye[19,20]. In this research, by using the LED light source of the visible ray wavelengths 470nm, 525nm and 630nm, we proved the collagen synthesis effect of LED at human epidermal keratinocyte(neonatal, HEKn). As far as we know, this is the first report demonstrating in vitro collagen synthesis activity of 470nm LED irradiation and being a scientific basis for its cosmetic.

2. Materials and Methods

2.1 LED Irradiation

A light source was irradiated from 10cm distance for one hour on the cell surface, and 470 to 525 nm to 630 nm, the current of 50mA was investigated[Fig. 1], continuous wavelength LED emitting at the wavelength of each LED (U-JIN LED Goyang city, Korea), 37 °C, 5% was performed in CO₂incubator.



[Fig. 1] LED device 470 to 525 nm and emitted 630 nm

2.2 Cell culture

Human epidermal keratinocytes (neonatal) (HEKn), animal product free EpiLife® basal medium and growth supplements (HKGS), recombinant trypsin/EDTA, defrinded trypsin inhibitor, and streptomycin had bought from Gibco (NY, USA). Human skin fibroblasts (HDF) was taken at Chungnam National University, Korea. HDF is then incubated with Dulbecco's modified Eagle's medium, penicillin 100 U / ml, supplement (DMEM GIBCO Inc., NY, USA) streptomycin and 10% fetal bovine serum (FBS in / ml of 100 µg; GIBCO Inc., NY, USA). Culturing the cells under an atmosphere of 5% CO₂ at 37 °C, which was subcultured every 3 days.

2.3 Cell viability

Cell viability effects of 470nm, 525nm, 630nm was measured using a solution for using the CellTiter 96@ colorimetric by counting the number of viable cells proliferation. This analysis was performed to determine the number of viable cells remaining after the completion of the culture process. Human epidermal keratinocytes play in a 96-well flat bottom plates at a density of 1×10^4 cells of the well, 470nm to 525nm and 630nm light irradiation after a first cell culture 24hours after the time of the manufacturer of the number of viable cells. It was calculated according to the instructions.

2.4 Western immunoblotting

HEKn cell or tissue was dissolved in a protein extraction reagent (Pierce Biotechnology Inc., IL, USA). Cell or tissue lysate was centrifuged at $15,000 \times g$ 15 minutes at 4 °C, and the supernatant was collected for Western blotting. It was checked the protein value concentration BCA assay kit (Pierce, Rockford) using (10 µg) the same amount of protein was separated by 10% SDS-PAGE and blotted on a polyvinylidene difluoride membrane. The membrane is the primary antibodies all night 3% BSA (Phosphate solution, 0.1% Tween 20 buffer for the pH 7.4). After the secondary antibody conjugated for 1 hour at room temperature after the incubation by joining and horseradish PBS with peroxidase. It was blocked by PBS-T. The protein-enhanced chemiluminescence (Pierce) and image quant method was visualized by LAS 4000 (GE Healthcare, USA).

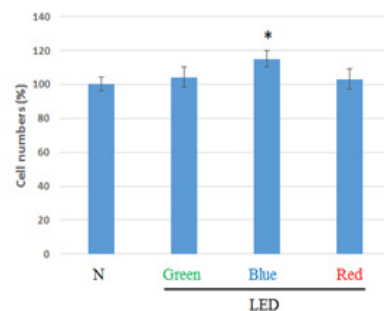
2.5 Statistical analysis

The result is \pm S.D. Of triplicate experiments are represented as means. Statistically compared using the Student's t-test a significant value, P- value of 0.05 was considered as being statistically significant.

3. Results

3.1 LED irradiation on the cell viability of Human epidermal keratinocytes

In order to determine whether LED irradiation can safely be applied to human skin, the proliferative effects of LED irradiation were determined by MTS assay in Human Epidermal Keratinocytes. In our study, 470nm LED irradiation significantly increased proliferation of Human epidermal keratinocytes more than 525nm and 630nm LED irradiation without cytotoxicity in Human epidermal keratinocytes [Fig. 2]. And 470nm LED irradiation in increase no cytotoxicity in human epidermal keratinocytes. It is considered to be harmless to human body at 470 nm.

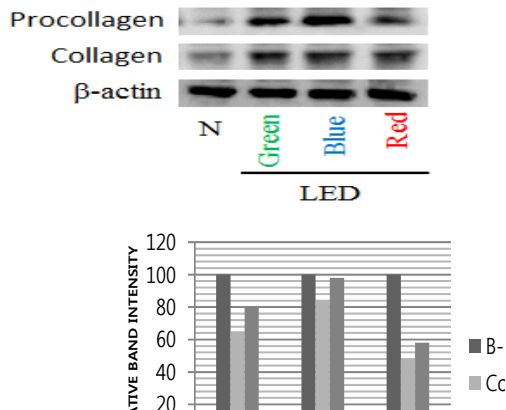


[Fig. 2] LED irradiation on the cell viability of Human epidermal keratinocytes.

Human epidermal keratinocytes were disposed of 470nm(Blue), 525nm(Green), 630nm(Red) LED irradiation for 1 hr. Cell viability was measured by MTS assay. The results of experiment three times are indicated by three independent experiments (SD = bars) percent of the average cell viability compared to the average, and the untreated control cell and survival of the asterisk denotes a significant difference

3.2 LED irradiation on the expression of collagen, procollagen in HEKn cells

The increase of collagen and procollagen expression may be one of the mechanisms responsible for the collagen synthesis action of 470nm LED irradiation [Fig. 3]. It is considered to be harmless to human body at 470 nm.

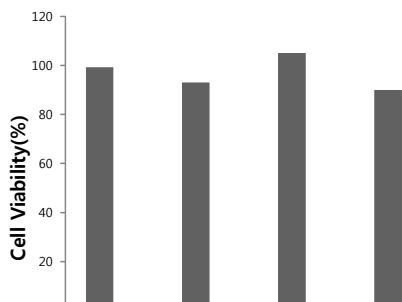


[Fig. 3] LED irradiation on the expression of collagen, procollagen in HEK293T cells.

Like those described in the Materials and methods, the expression of collagen was determined by Western blot was the procollagen. β -actin level was used as an housing marker for the load change. Experiment result was the average of the three independent experiments

3.3 LED irradiation on the cell viability of Human Dermal Fibroblasts

In our study, 470nm LED irradiation significantly increased proliferation of Human Dermal Fibroblasts more than 525nm and 630nm LED irradiation without cytotoxicity in Human Dermal Fibroblasts [Fig. 4]. And 470nm LED irradiation in increase no cytotoxicity in human Dermal Fibroblasts. It is believed that the skin is not toxic.



[Fig. 4] LED irradiation on the cell viability of Human Dermal Fibroblasts

Human Dermal Fibroblasts was treated with 470nm(Blue), 525nm(Green), 630nm(Red) LED irradiation for 1 hr. The results of experiment three times are indicated by three independent experiments percent of the average cell viability compared to the average, and the untreated control cell and survival of the asterisk denotes a significant difference

4. Conclusion

In recent years, many reports have specific wavelengths in the optical field of clinical research has suggested could be an alternative healing[21,22]. Different wavelengths may have a different effect on the tissue[23] have different chromophores. Variety of cell and tissue types of the body can absorb light of a particular wavelength according to the irrespective unique light absorption characteristics[24]. LED irradiation securely to determine whether or not to apply to the human skin, proliferation of LED irradiation was measured by MTS analysis in human epidermal keratinocytes. This study is the 470nm ~ 630nm the cells in the human epidermal keratinocytes toxicity [Fig. 2] examine the LED, more research is greatly increased proliferation of human epidermal keratinocytes 525nm LED no. There is an increase in collagen and procollagen expression can be one of the mechanisms responsible for the synthesis of collagen acts of 470nm [Fig. 3] were investigating. And the survey is the increase [Fig. 4] 470nm LED no cytotoxicity in human skin fibroblasts. Thus, LED is 470nm radiation can be used as therapeutic agents for improving skin conditions. As far as we know, is the first LED to provide a scientific basis for investigation and report on bio-cosmetics show in collagen synthesis activity of 470nm[25,26].

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