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# Excimer Laser Penetrates Deeper into Hair Follicles and Activates More Melanocyte Lineage Cells than Excimer Light

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**Background:** Both ultraviolet B (UVB) phototherapy with 308-nm excimer light and excimer laser devices are widely used to treat vitiligo. While the devices share the 308-nm wavelength, they have distinct characteristics. Notably, the excimer laser exhibits laser properties (monochromatic coherent light) and unique device specifications (a high frequency of 400 Hz and a remarkably high irradiance of 83 million mW/cm$^2$). This study compared excimer light and laser irradiation, focusing on the depth of penetration into hair follicles and the effects on activation of melanocyte lineage cells, including melanocyte stem cells (McSCs) and melanoblasts.

**Methods:** We irradiated the dorsal skin of mice with both devices at 1,000 mJ/cm$^2$. Samples taken at 15 min and 3, 24, and 72 h after irradiation were used for immunostaining analysis. We evaluated penetration depth by using the staining pattern of cyclobutane pyrimidine dimers (CPDs), induction of apoptosis by using a terminal deoxynucleotidyl transferase-mediated deoxy-uridine triphosphate nick end-labeling (TUNEL) assay, and activation of melanocyte lineage cells by using fluorescent double immunostaining for TRP2 and β-catenin.

**Results:** The excimer laser induced significantly more CPDs in the deeper regions of hair follicles while causing significantly faster removal of CPDs and less apoptosis in the epidermis. Moreover, the percentage of TRP2-positive cells with nuclear β-catenin in the follicles was significantly higher with the excimer laser.

**Conclusions:** As compared with excimer light, the excimer laser penetrated more deeply into hair follicles, resulted in fewer epidermal side effects, and activated significantly more melanocyte-lineage cells. (J Nippon Med Sch 2025; 92: 52–60)

**Key words:** excimer laser, irradiance, repigmentation effect, UVB phototherapy, vitiligo

## Introduction

Vitiligo is an acquired depigmentation disorder characterized by the loss of melanocytes. It is mainly caused by autoimmune mechanisms, but genetic and environmental factors may also be involved[1]. Currently, ultraviolet B (UVB) phototherapy, particularly with 308-nm excimer light and excimer laser devices, is widely used as a primary treatment method. These light sources have distinct technical characteristics: excimer light devices emit incoherent light at wavelengths of 308 nm ±2 nm in a continuous wave mode, while excimer laser devices emit coherent, monochromatic light at 308 nm in pulses. Note that coherent light has an aligned phase and direction, whereas incoherent light does not. In addition, monochromatic light in this case refers to light with a single wavelength of 308 nm.

Understanding the mechanisms of repigmentation in vitiligo is essential for comprehending the mode of action of these treatments. The perifollicular distribution pattern is the most common type of repigmentation, suggesting that hair follicles are the primary source of epidermal pigmentation[2]. Nishimura et al.[3] reported that melanocyte

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stem cells (McSCs) were present in the bulge area of hair follicles. Later, Yamada et al.[4] revealed that UVB irradiation activated these McSCs, causing them to differentiate into melanoblasts, which then migrated to the epidermis and matured into melanocytes, ultimately inducing epidermal pigmentation. In this process, the Wnt/β-catenin signaling pathway plays a crucial role in regulating activation and differentiation of McSCs[4–6].

Both excimer light and laser irradiation are effective for this pigment regeneration[7–11]. However, an increasing number of reports suggest that excimer laser is particularly effective[12–14]. Alhowaish et al.[12] reported that excimer laser treatments had a faster onset of repigmentation, required fewer treatment sessions, and achieved repigmentation with a lower cumulative dose than traditional phototherapy. Furthermore, Noborio et al.[14] found that excimer laser irradiation produced satisfactory repigmentation in vitiligo that was unresponsive to treatment with narrow band-UVB and excimer light. These findings led us to hypothesize that excimer laser devices may have a stronger repigmentation effect than devices using excimer light. Nevertheless, it is unclear how differences in the characteristics of these two devices affect activation of McSCs.

In this study, we compared excimer light and laser irradiation by using C57BL/6J mice to focus on penetration depth into hair follicles and the effects on activation of melanocyte lineage cells, including McSCs and melanoblasts. Specifically, we assessed penetration depth by using the staining pattern of cyclobutane pyrimidine dimers (CPDs) and evaluated activation of melanocyte lineage cells by using fluorescent double immunostaining for TRP2 and β-catenin, with TRP2 as a melanocyte-lineage maker[6,15]. In this comparative study, we hoped to gain a deeper understanding of the characteristics of both light sources and to establish improved treatments for vitiligo.

## Materials and Methods

### Animals

C57BL/6J mice obtained from CLEA Japan (Tokyo, Japan), and their inbred offspring, were maintained under controlled temperature and humidity in a 12-h light-dark cycle with a standard diet and water ad libitum in our University Animal Facility (Nippon Medical School). This study was approved by the Institutional Animal Care and Use Committee of Nippon Medical School (approval number: 2020-067) and was conducted in accordance with the Nippon Medical School Animal Experimentation Regulations.

### Irradiation and Sample Collection

Ten-to eleven-week-old mice of both sexes were used for irradiation by excimer light (ExSys308 model 50; GME German Medical Engineering GmbH, Erlangen, Germany) and excimer laser (XTRAC; Strata Skin Sciences Inc., Pennsylvania, USA). We irradiated the shaved dorsal skin of mice with the excimer light and excimer laser at doses of 500, 750 and 1,000 mJ/cm$^2$. All irradiation was performed on a light-shielding sheet with a 1 cm × 1 cm spot size (Ishizuka Corporation Inc., Tokyo, Japan).

The ExSys308 is a compact device featuring a handpiece with a 5 cm × 3.5 cm treatment area that can be customized to various sizes by selecting the included adapter. In our study, we used an adaptor with a diameter of 10 mm for mouse irradiation. The ExSys308 emits incoherent light at a wavelength of 308 nm ±2 nm in a continuous wave mode from a xenon chloride (XeCl) lamp, delivering irradiance of 50 mW/cm$^2$. In contrast, the XTRAC is based on a self-contained XeCl gas system and outputs coherent, monochromatic light at a wavelength of 308 nm. It features a 2 cm × 2 cm square treatment area on its handpiece and is activated by a hand switch. The XTRAC operates in a pulsed mode, delivering 10 mJ per pulse in short trains with a 30 ns pulse width. This results in an extremely high irradiance of 83 million mW/cm$^2$ (2.5 mJ/cm$^2$ divided by 30 ns). Additionally, with its rapid pulse repetition rate of 400 Hz, the XTRAC enables short exposure times for treatment.

Skin specimens were collected at 15 min and 3, 24, and 72 h after irradiation, fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4), and then embedded in paraffin. Sections (thickness 4 μm) were used in all histological analyses.

### Histology

To examine differences in the penetration depths of the excimer light and laser, we anatomically separated hair follicles into four compartments using skin sections stained with hematoxylin and eosin (**Fig. 1A**), as detailed in a report by Goldstein et al.[15]: the epidermis, infundibulum (between the epidermal ostium superiorly and sebaceous gland duct inferiorly), bulge (from the insertion of sebaceous gland duct above to the arrector pili muscle below), and lower portion (proximal to the bulge and below).

For immunostaining of CPDs, sections were deparaffinized and washed in PBS. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen per-

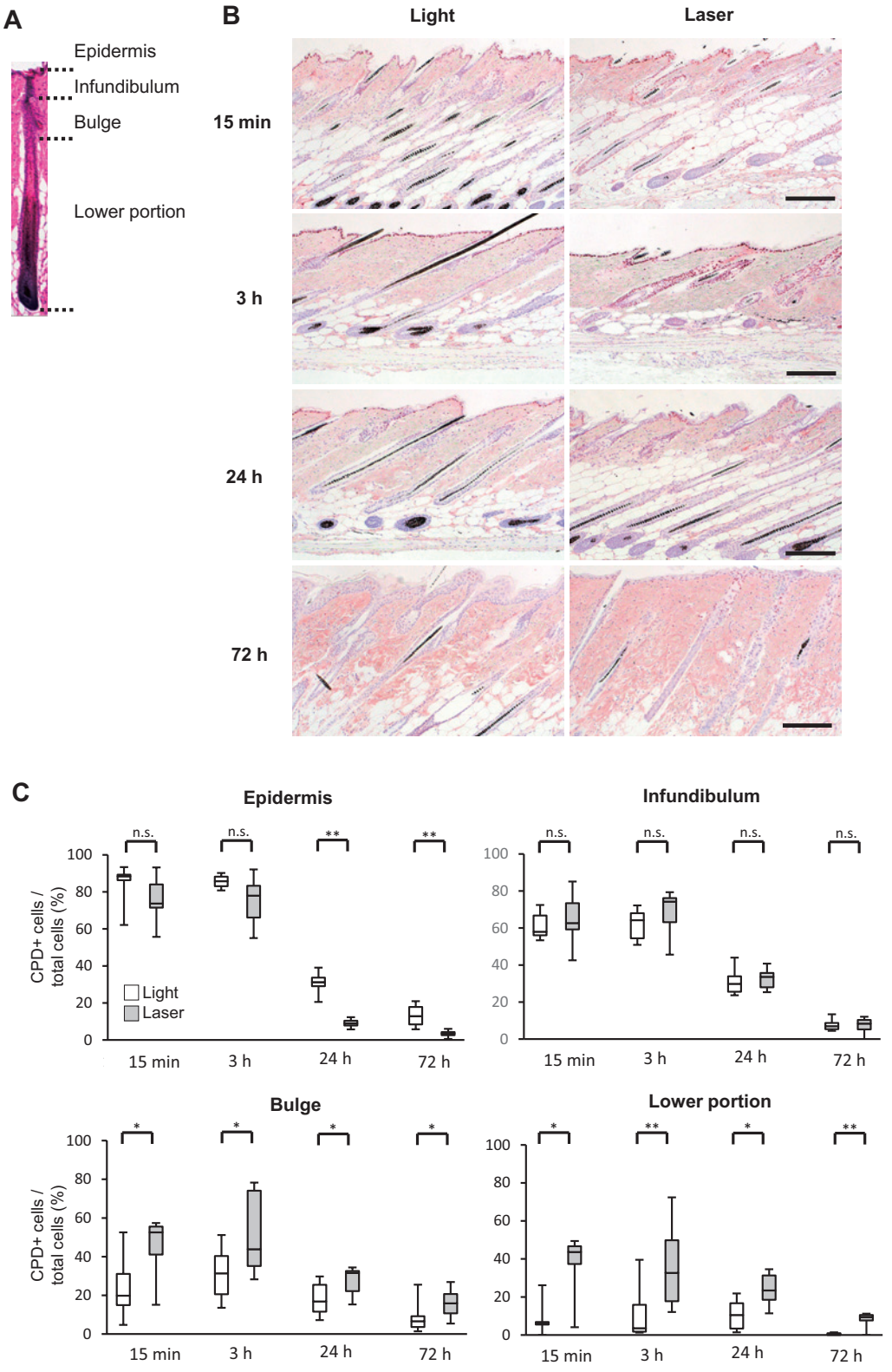


Fig. 1 CPD induction after excimer light and laser irradiation.

(**A**) Compartment of the hair follicle.

(**B**) Immunostaining of CPDs in skin sections at 15 min and 3, 24, and 72 h after excimer light or laser irradiation. Scale bars = 200 μm.

(**C**) Percentages of CPD-positive cells in the epidermis (upper left), infundibulum (upper right), bulge (lower left), and lower portion (lower right) of the hair follicle. N = 7 per group, except for the light at 15 min and 72 h (n = 6 each).

*p<0.05, **p<0.01 as determined using the Mann-Whitney U test.

oxide (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) in distilled water for 15 min, and then treated with 2M HCL (Fujifilm Wako) for 30 min for DNA denaturation. Blocking of nonspecific staining from anti-mouse IgG was done by using a mouse on mouse (M.O.M.) kit (Vector Labs Inc., Burlingame, CA, USA) according to the manufacturer's instructions. The sections were incubated at 4°C overnight with an antibody against CPDs (1:3,000 dilution) (Cosmo Bio Company Ltd., Tokyo, Japan). Staining was performed with an indirect immunoperoxidase technique. For visualization, NovaRED (Vector Labs) was used as a substrate, and slides were counterstained for nuclei with hematoxylin. Mouse IgG was used for the negative control.

For the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) assay, sections were deparaffinized, washed in PBS, incubated first with 400 μg/mL of proteinase K (Agilent Technologies, Santa Clara, CA, USA) for 15 min at room temperature, and then quenched in 3% hydrogen peroxide in distilled water for 15 min. The sections were labeled with a mixture of 1:9 terminal deoxynucleotidyl transferase enzyme to Labeling Safe Buffer (In Situ Apoptosis Detection Kit; Takara Co. Ltd., Osaka, Japan). The slides were incubated for 90 min at 37°C, treated with anti-fluorescein isothiocyanate horseradish peroxidase conjugate for 30 min at 37°C, and visualized using NovaRED as substrate and then counterstained for nuclei with hematoxylin. Labeling Safe Buffer without the transferase enzyme was used as the negative control.

Immunostaining of CPDs in skin samples taken at 15 min and 3, 24, and 72 h after irradiation and TUNEL assay at 24 h was performed as described above. The samples were observed with an optical microscope. The percentages of CPD-positive cells and TUNEL-positive cells were calculated per 400 cells in the epidermis and per 200 cells in the infundibulum, bulge, and lower portion.

For immunohistochemical detection of TRP2 and β-catenin, the sections were incubated overnight at 4°C with the primary antibodies: rabbit anti-TRP2 (1:2,000) (Abcam, Cambridge, UK) and mouse anti-β-catenin (1: 200) (BD Transduction Laboratories, Franklin Lakes, NJ, USA). After washing with PBS, the sections were incubated with biotinylated anti-mouse IgG (1:250) (Vector Labs) for 10 min at room temperature and with Alexa Fluor 594 goat anti-rabbit IgG (1:500) (Thermo Fisher Scientific, Waltham, MA, USA) for 30 min at room temperature, followed by incubation with Fluorescein Avidin DCS (1:62.5) (Vector Labs) for 5 min at room temperature. The biotinylated anti-mouse IgG and Fluorescein Avidin DCS were used to detect β-catenin.

The sections were then mounted with VECTASHIELD HardSet Mounting Medium with 4',6-diamidino-2-phenylindole (DAPI) (Vector Labs) and observed with a BX53 fluorescence microscope (Olympus, Tokyo, Japan). Images were obtained with a DP74 camera (Olympus).

Fluorescent double immunostaining for TRP2 and β-catenin of skin sections at 24 h was performed as described above, and the percentages of TRP2-positive cells and double-positive cells (defined as TRP2-positive cells with nuclear β-catenin) were calculated per 200 cells in the bulge and lower portion.

**Statistical Analysis**

Sample sizes were determined on the basis of our previous study[16]. Data are represented as box-whisker plots (box, median and quartiles; whiskers, range). The statistical differences between the effects of the excimer light and laser were analyzed using the Mann-Whitney U test in Microsoft Excel 2019. P values of $<0.05$ were considered statistically significant.

## Results

**The Excimer Laser Penetrates Deeper into Hair Follicles than Excimer Light**

The results of immunostaining of CPDs suggested that the excimer laser induced more CPDs in deeper hair follicles at all irradiation doses; staining intensity was the strongest at a dose of 1,000 mJ/cm$^2$. Therefore, we decided to use an irradiation dose of 1,000 mJ/cm$^2$, and the backs of seven mice were irradiated on one side with the excimer light and on the other side with the excimer laser. These sides were selected randomly.

In the epidermis, we found many CPDs after irradiation by excimer light and laser, and there was no significant difference between them at 15 min and 3 h after irradiation. However, at 24 h and 72 h, significantly more CPDs remained in skin irradiated with excimer light than in skin irradiated with the excimer laser (**Fig. 1B, C**). In the infundibulum, induction of CPDs was similar for the excimer light and excimer laser throughout the time course. In the bulge and lower portion, more CPD-positive cells were observed after excimer laser irradiation than after excimer light irradiation at all time points. Note that both the excimer light and laser seemed to induce more CPDs in the shallower area than in the deeper area of the skin at 15 min and 3 h. These results indicate that the excimer laser penetrated more deeply into hair follicles than excimer light.

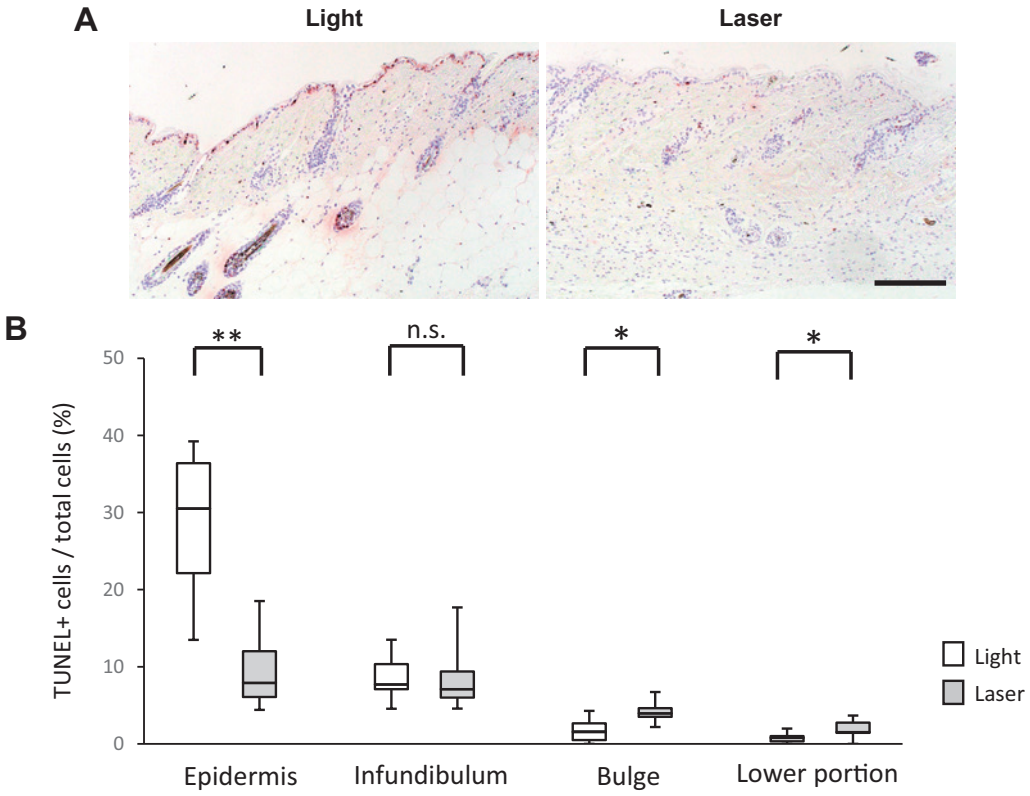


Fig. 2 Apoptosis induction after excimer light and laser irradiation.
(**A**) TUNEL assay in skin sections at 24 h after excimer light or laser irradiation. Scale bar = 200 μm.
(**B**) Percentages of TUNEL-positive cells in each compartment of the hair follicle. N = 7 per group.
*p<0.05, **p<0.01 as determined using the Mann-Whitney U test.

**Excimer Laser Induces Less Apoptosis in the Epidermis than Excimer Light**

Next, we examined induction of apoptosis by excimer light and laser irradiation. In our previous study[16], apoptosis was most frequent at 24 h after UVB irradiation. Therefore, skin samples taken from mice at 24 h after excimer light and laser irradiation were used for the TUNEL assay (**Fig. 2A**). Differences in apoptosis induction were assessed by comparing the percentage of TUNEL-positive cells in each compartment of the hair follicle and in the epidermis (**Fig. 2B**).

In the epidermis, significantly fewer TUNEL-positive cells were detected in the skin irradiated with excimer laser than in skin irradiated with excimer light. There was no significant difference in apoptosis induction between excimer light and laser irradiation in the infundibulum, and the positive percentage was low for both. In the bulge and lower portion, although only a few positive cells were observed, excimer laser-irradiated skin had a significantly higher percentage of TUNEL-positive cells than did skin irradiated with excimer light, perhaps because penetration of hair follicles was deeper with excimer laser than with excimer light. These results indicate that the excimer laser induced less apoptosis in the epidermis than did excimer light.

**Excimer Laser Activated More TRP2-positive Cells than Excimer Light**

Finally, we examined the effects of excimer light and laser irradiation on activation of melanocyte lineage cells. It has been reported that UVB irradiation induced epidermal pigmentation through differentiation of McSCs via the Wnt/β-catenin signaling pathway[4-6]. In a study by Yamada et al.[4], Wnt7a mRNA expression was markedly elevated at 24 h after UVB irradiation and returned to the basal level at 72 h. Therefore, skin samples collected from mice at 24 h after excimer light and laser irradiation were subjected to fluorescent double immunostaining for TRP2 and β-catenin (**Fig. 3A, B**). We compared the percentages of double-positive cells, defined as TRP2-positive cells with nuclear β-catenin.

In the lower portion, the percentage of double-positive cells per total number of cells was significantly higher with excimer laser irradiation than with excimer light irradiation (**Fig. 3C**). In both the bulge and lower portion, the proportion of double-positive cells among TRP2-positive cells was also significantly higher with excimer

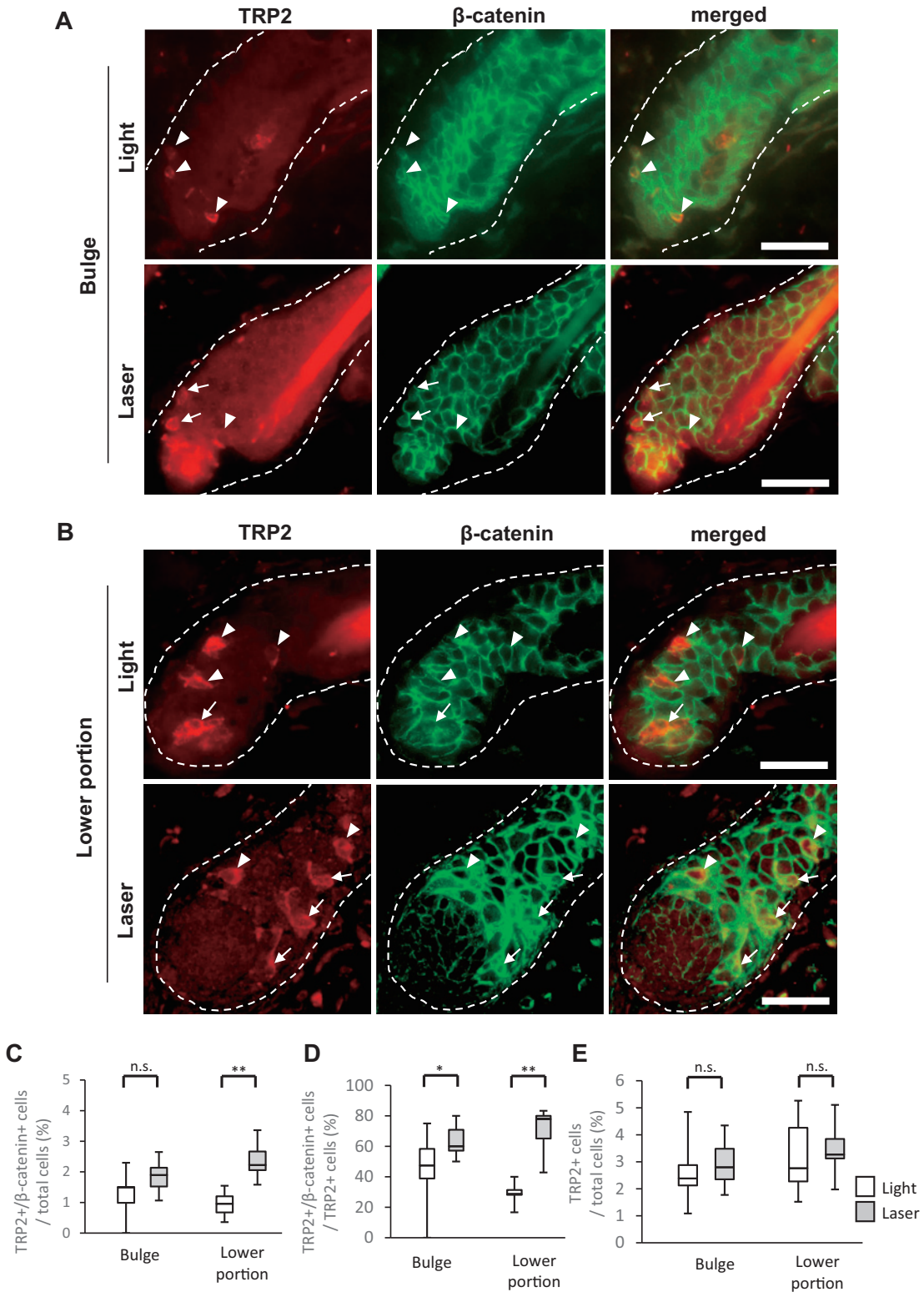


Fig. 3 TRP2-positive cells with nuclear β-catenin after excimer light and laser irradiation.
(**A**, **B**) Fluorescent double immunostaining for TRP2 and β-catenin in skin sections at 24 h after excimer light or laser irradiation of hair follicles in the bulge (**A**) and lower portion (**B**). Arrows show TRP2-positive cells with nuclear β-catenin. Arrowheads show TRP2-positive cells without nuclear β-catenin. Scale bars = 30 μm. (**C**) Percentages of double-positive cells per total cells. (**D**) Percentages of double-positive cells among TRP2-positive cells. (**E**) Percentages of TRP2-positive cells per total cells. Double-positive cells are noted as TRP2+/β-catenin+ cells. N = 7 per group.
*p<0.05, **p<0.01 as determined using the Mann-Whitney U test.

laser irradiation (**Fig. 3D**). However, in the bulge and lower portion there was no difference in the percentage of TRP2-positive cells per total number of cells (**Fig. 3E**). These results suggest that the excimer laser activated more TRP2-positive cells than did excimer light in the deeper regions of hair follicles.

## Discussion

UV irradiation induces epidermal pigmentation, and excimer light and laser devices are currently used in phototherapy for vitiligo. An increasing number of studies have investigated the efficacy of the 308-nm excimer laser[12–14]. On the basis of these reports, we hypothesized that excimer laser irradiation would be more effective than excimer light irradiation for pigment regeneration. To examine this hypothesis, we conducted experiments using mice to focus specifically on differences in the depth of penetration of hair follicles and effects on the activation of melanocyte lineage cells.

UV irradiation causes DNA damage. UVB is directly absorbed by DNA, generating dimers between adjacent pyrimidine bases[17,18]. These dimers include CPDs, pyrimidine-pyrimidone (6-4) photoproducts, and Dewar isomers, among which CPDs are generated in the highest quantities[18]. In other words, CPDs account for most UVB-induced DNA damage and thus can be considered a useful indicator of the penetration depth of UVB irradiation into hair follicles. In this study, to investigate differences in penetration depth between the excimer light and laser, we irradiated the dorsal skin of C57BL6/J mice with these light sources and analyzed temporal changes in the number and localization of CPD-positive cells. Particularly, CPD immunostaining at 15 min showed the depth and percentage directly reached by the excimer light and laser irradiation. In the bulge and lower portion of hair follicles, excimer laser-irradiated skin had significantly more CPD-positive cells than did skin exposed to excimer light (**Fig. 1C**). This indicated that the excimer laser penetrated more deeply than excimer light into hair follicles. The superior penetration of the excimer laser is attributable to its optical characteristics. It has a precise monochromatic wavelength of 308 nm and emits coherent light exhibiting phase alignment and directional consistency. These laser properties likely result in less scattering and absorption in the skin than with excimer light, allowing for deeper skin penetration. Furthermore, as discussed below, the excimer laser reached a depth sufficient to activate McSCs, as confirmed by activation of melanocyte lineage cells. Therefore, the penetration depth of the excimer laser is satisfactory.

Interestingly, significant differences between the excimer light and laser were also observed in the epidermis at 24 and 72 h after irradiation. Specifically, excimer light-irradiated skin persistently showed significantly more CPD-positive cells (**Fig. 1C**). While DNA damage, such as CPDs, is primarily repaired enzymatically through nucleotide excision repair (NER)[19], apoptosis is induced when DNA damage is severe. Because apoptosis may be involved in the removal of CPDs, we performed TUNEL assays on skin sections at 24 h after irradiation. The results revealed that excimer light-irradiated skin had significantly more TUNEL-positive cells in the epidermis, indicating greater induction of apoptosis (**Fig. 2 B**). Despite the significantly higher frequency of apoptosis, there was also greater persistence of CPDs in skin irradiated with excimer light. These results suggest that excimer light irradiation impairs mechanisms for removal of CPDs other than apoptosis, particularly the NER pathway. Greinert et al.[19] reported that the repair capacity of CPDs after UVB irradiation depends on the dose. However, in our study, although the same dose of 1,000 mJ/cm$^2$ was used, significant differences in the persistence of CPDs were observed between the excimer light and laser, suggesting that the irradiation dose alone could not explain this phenomenon. We focused on the difference in irradiation time as a potential factor in this discrepancy. The excimer laser, which has a pulse width of 30 ns and 10 mJ (2.5 mJ/cm$^2$) per pulse, emitted at a high frequency of 400 Hz, requires only 1 second to deliver 1,000 mJ/cm$^2$. In contrast, excimer light, with continuous emission at 50 mW/cm$^2$ irradiance, requires 20 seconds for the same dose. Kelfkens et al.[20] reported that the interval until tumor development was significantly shorter for a long-exposure group than for a short-exposure group, even at the same UVB irradiation doses, possibly because of DNA repair impairment due to decreased RNA and protein synthesis associated with increased exposure time at low irradiance. The delay in the removal of CPDs with excimer light in our study might have been due to the longer irradiation time, which might have caused some impairment in NER. In contrast, the excimer laser induced significantly faster removal of CPDs and less apoptosis in the epidermis, resulting in fewer epidermal side effects. This may be attributable to the short irradiation time resulting from its high frequency of 400 Hz and markedly high irradiance of 83 million mW/cm$^2$.

To compare the effects of excimer light and laser irradiation on the activation of melanocyte lineage cells, we

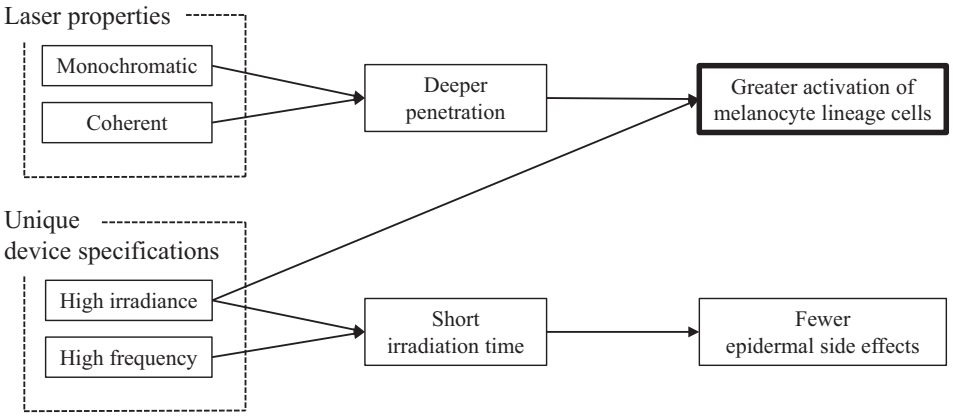


Fig. 4 Beneficial effects of the excimer laser.
Multiple components of the excimer laser work synergistically to generate its beneficial effects.

performed fluorescent double immunostaining for TRP2 and β-catenin. TRP2, also known as dopachrome tautomerase (Dct)[21], is a melanocyte lineage marker expressed in McSCs, melanoblasts, and melanocytes[6,15]. In our study, TRP2-positive cells were not observed in the epidermis or infundibulum. This observation is consistent with the known characteristics of C57BL/6J mice, which possess almost no epidermal melanocytes on their dorsal skin. Therefore, our investigation primarily focused on the bulge and lower portion of hair follicles. Although multiple signaling pathways are involved in melanin synthesis, the Wnt/β-catenin pathway plays a central role in melanogenesis[4–6] because the target genes of this pathway include essential factors for melanin formation, such as microphthalmia-associated transcription factor (MITF), Dct, and tyrosinase[5]. Wnt ligands are secreted proteins that act via autocrine or paracrine signaling[6]. When Wnt ligands bind to their receptor Frizzled and co-receptor LRP5/6, degradation of cytoplasmic β-catenin is inhibited[4–6]. The stabilized β-catenin translocates to the nucleus and functions as a transcription factor, thus inducing cell proliferation, differentiation, migration, and adhesion[4]. Specifically, Wnt7a promotes differentiation of McSCs[4,6], and Wnt3a and Wnt10b promote differentiation of melanocyte precursors[6]. On the basis of these findings, we defined TRP2-positive cells with nuclear β-catenin, referred to as double-positive cells, as activated melanocyte lineage cells. Our experiments showed that the percentage of double-positive cells per total cells was significantly higher in the lower portion with excimer laser irradiation, while in the bulge there was a nonsignificant trend towards higher values with the excimer laser (**Fig. 3C**). Moreover, the proportion of double-positive cells among TRP2-positive cells was significantly higher with the excimer laser in both the bulge and lower portion (**Fig. 3D**). These results suggest that the excimer laser was more effective than excimer light in activating melanocyte lineage cells in hair follicles.

Two factors contribute to the more effective activation of melanocyte lineage cells by excimer laser irradiation. The first factor is the difference in the depth of penetration of hair follicles. UVB irradiation induces Wnt secretion in epithelial lineage cells such as hair follicle stem cells, hair follicle keratinocytes in the outer root sheath, and epidermal keratinocytes[4]. The results of CPD staining, described above, suggest that it is highly likely that the excimer laser penetrates deeper into hair follicles, thus stimulating a larger population of follicular keratinocytes, promoting Wnt secretion, and consequently contributing to activation of melanocyte lineage cells. In particular, the excimer laser, by penetrating deeper into hair follicles, may activate more McSCs in the bulge region, thereby promoting proliferation and differentiation of McSCs. The second factor is the difference in irradiance of the light sources. Lan et al.[22] found that reducing the irradiance of excimer light decreases expression of tyrosinase in melanoblasts, even with equivalent irradiation doses. They reported that irradiance was more important than irradiation dose in the development of immature pigment cells induced by UVB irradiation[22]. The irradiance of the excimer light used in our study was 50 mW/cm$^2$, whereas the excimer laser had a significantly higher irradiance of 83 million mW/cm$^2$. This marked difference in irradiance may have influenced the distinct levels of melanoblasts activation.

In conclusion, we have demonstrated that excimer light and laser irradiation bring about different photobiological effects in living tissues, although both primarily

emit 308-nm UVB. Specifically, the excimer laser penetrates deeper into hair follicles, results in fewer epidermal side effects, and activates more melanocyte lineage cells than excimer light. The various aspects of the excimer laser likely work in concert to produce its beneficial effects (**Fig. 4**). The laser properties (monochromatic coherent light) may account for the deeper penetration, while the short irradiation time may result in fewer epidermal side effects, and the combination of deeper penetration and remarkably high irradiance may contribute to greater activation of melanocyte lineage cells.

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**Conflict of Interest:** The authors declare no conflict of interest for this article.

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