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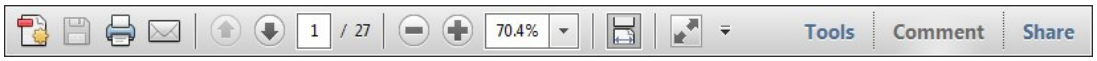

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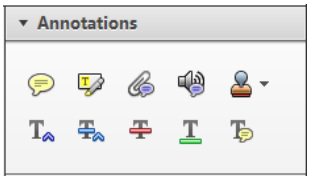

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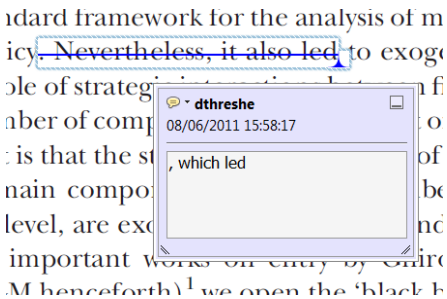

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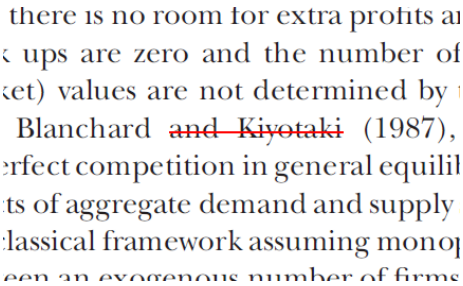

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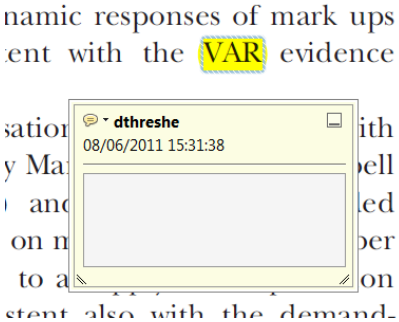

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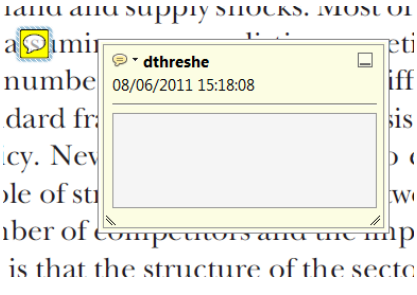

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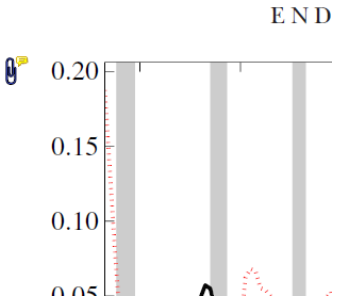

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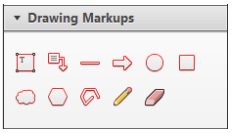

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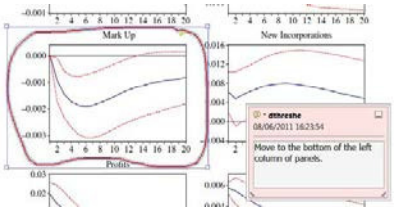

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Skin Research and Technology

High intensity focused ultrasound as a potential new modality for the treatment of pigmentary skin disorder

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Background/Purpose: The clinical skin tightening benefits of high intensity focused ultrasound (HIFU) have been established, but its mechanism of action in pigmented skin disorders remains unknown. We macroscopically and histopathologically investigated dermatological changes after HIFU at different exposure doses in a UVB-induced guinea pig model of hyperpigmentation.
Methods: We applied HIFU irradiation at 0.1 and 0.2 J/cm^2 to UVB-induced spotty hyperpigmentation in guinea pig skin. The therapeutic effects of HIFU were judged based on gross appearance using photography, dermoscopy, and chromametry during a period of 3 weeks after HIFU irradiation. Histological assessments were performed using Fontana-Masson staining 1 day before and 3 weeks after HIFU irradiation.
Results: Macroscopically, UVB-induced hyperpigmentation was significantly reduced 2 weeks after HIFU with 0.2 J/cm^2, and 3 weeks after HIFU with 0.1 J/cm^2. Histopathologically, the heavy deposition of melanin in the epidermis induced by UVB exposure was reduced 3 weeks after HIFU irradiation.
Conclusion: We confirmed that HIFU has a positive effect on UVB-induced hyperpigmentation as well as mechanical destructive activity. We suggest that HIFU may be useful as an alternative modality for human patients suffering from skin pigmentary conditions.

Key words: high intense focused ultrasound – hyperpigmentation – pigmentation – UVB

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SKIN COLOR is related to the amount and distribution of melanin. Abnormal melanin accumulations on the unevenness of skin tone and the effects of surface imperfections have been discussed (1). Melanin accumulation can be due to many different causes such as hormonal imbalance or sun exposure, and may be either transient or permanent, as observed clinically in conditions such as melasma, chloasma, or lentigo. Unwanted pigmentation can cause patients to be uncomfortable, self-conscious, and reduce feelings of self-worth (2). Several treatments are used to reduce hyperpigmentation, including disruption of the distribution of melanosomes and inhibition of the tyrosinase enzyme.

High intensity focused ultrasound (HIFU) has been investigated as a tool for the treatment of solid benign and malignant tumors for many decades (3). Recently, HIFU was explored as a new treatment modality for skin tightening and rejuvenation (4). High intensity focused ultrasound can produce small, micro-thermal lesions at precise depths in the dermis up to the fibromuscular layer, causing thermally induced contraction of collagen and tissue coagulation with subsequent collagenesis, while sparing the epidermis (5–7). To date, no experimental or clinical studies have evaluated the efficacy of HIFU for the treatment of pigmented skin lesions. In this study, we evaluated the effects of HIFU on hyperpigmentation using an animal model.

Guinea pigs are commonly used for studies of skin reactions to UV irradiation and of the protective effects of sunscreen on sunburn and tanning reactions in the skin. The effects of depigmenting agents on spotty pigmentation have also been evaluated using guinea pig models (8, 9). This study was undertaken to macroscopically and histopathologically investigate dermatological changes in UVB-induced guinea pig skin pigmentation after HIFU at different exposure doses.

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Materials and Methods

Guinea pig model

One 6-week-old female brownish guinea pig (Tokyo Laboratory Animals Science Co., Tokyo, Japan) was used in this study. The guinea pig was bred and housed under conventional conditions (temperature: 23 ± 3°C, relative humidity: 55 ± 15%) at the R&D Center of the College of Medicine at Chung-Ang University, Korea. All procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Chung-Ang University (IRB number: 13-0020). After acclimatization for 7 days, the dorsal skin of the guinea pig was separated into four areas (2 × 2 cm) as follows: area 1: no UVB-induced tanning (control), area 2: UVB-induced tanning (control), area 3: UVB-induced tanning with HIFU at 0.1J/cm^2, and area 4: UVB-induced tanning with HIFU at 0.2J/cm^2.

UVB irradiation regimen

The guinea pig was anesthetized with Zoletil 50 (Virbac S.A, France) (40 mg/kg) and Rompun (Bayer, Korea) (5 mg/kg) in saline (Huons, Korea). To develop pigmentation, the back of the guinea pig was cleanly shaved with electric clippers. The guinea pig was exposed to weekly sessions of narrow band UVB (NB-UVB) irradiation for 4 weeks at a dose of 490 mJ/cm^2 per session using a NB-UVB lamp (Dermalight®80, National Biological Corp., OH, USA).

HIFU ultrasound device protocol

A HIFU device (Ultraformer2®, Classys Inc., Seoul, Korea) was used in this study. An ultrasound probe was connected to a generator system operating in the MHz frequency regime. The ultrasound energy was coupled from the transducer (operating at 7 MHz) to skin by ultrasound coupling gel applied to the skin surface. The nominal focal depth for this study was 1.5 mm below the skin surface. Each probe delivered a set of pulses in a linear array, pulses spaced 1.0–2.0 mm apart, and an entire linear array was up to 25 mm long. The spacing of pulses within each linear array was set at 1 mm, resulting in 25 thermal coagulative zones created with each probe discharge. Linear arrays were spaced in parallel at 1-mm intervals. Ultrasound transmission gel (Supersonic®, Sungheung Co., Korea) was applied to the skin, and handpiece 2 was pressed perpendicularly, uniformly and firmly to the skin surface. The guinea pig was treated only once with a 7-MHz, 1.5-mm handpiece, at 0.1J/cm^2 and 0.2J/cm^2. After treatment, the ultrasound transmission gel was wiped off of the guinea pig's skin. The treated skin showed mild redness and swelling that persisted for several days.

Evaluation of tanning reduction

We evaluated tanning reduction 1, 2, and 3 weeks after HIFU treatment using photography, dermoscopy, and chromametry. Clinical changes were measured using digital photographs (Canon 3000D, Canon Inc., Tokyo, Japan). We used a dermoscope to produce images with enhanced magnification (DermLite Pro, CA, USA). The lightening effect was determined by measuring the L* value with a CR-10 reflectance spectrophotometer (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan) as a chromameter. The L* value (luminance) defines the relative lightness ranging from total black (L* = 0) to total white (L* = 100). The blanching effect was quantified by the increase in L* value: ΔL* = L* (on the measuring day) – L* (on the first day of the test, before HIFU treatment).

Histological analysis

Three weeks after HIFU treatment, the guinea pig was sacrificed and skin samples were removed from each quadrant of the test site. Samples were fixed in 10% formaldehyde, embedded in paraffin, and stained with standard hematoxylin and eosin (H&E). Changes in melanin deposition were measured by Fontana-Masson (FM) staining. All staining was examined under a phase-contrast microscope (Eclipse TS100®, Nikon Instruments Inc., Melville, NY, USA).

Statistical analysis

Data are presented as mean ± standard deviation. Statistical comparisons between the treated and untreated areas were performed using one-way ANOVA followed by Tukey's post hoc test for direct comparisons between groups.

P values < 0.05 and <0.01 were considered statistically significant.

Results

Clinical and dermoscopic changes

In both digital photographs and dermoscopic pictures, UVB-induced hyperpigmentation started to decrease 1 week after HIFU treatment in areas 3 and 4, while no reduction occurred in area 2. At 3 weeks after HIFU treatment, the tanning induced by UVB radiation was markedly reduced in areas 3 and 4. Compared with area 3, tanning in area 4 decreased more quickly (Figs 1 and 2).

Changes of brightness index

Compared with ΔL* in area 2, the L* values of areas 3 and 4 were significantly decreased from baseline (before HIFU treatment), at 3 weeks and 2 weeks after HIFU treatment, respectively ($P < 0.01$) (Fig. 3). The details of the L* values are shown in Table 1.

Histological changes

Microscopic examinations of H&E-stained sections confirmed that there were no signs of inflammatory or necrotic reactions in any of the four tested areas (Fig. 4). In FM-stained sections, marked increases of melanin in the basal layer of the epidermis were detected in area 2. However, in areas 3 and 4, heavy deposition of melanin in the epidermis induced by UVB exposure was reduced compared with area 2 (Fig. 5).

Discussion

Melanin pigment is a heterogeneous biopolymer synthesized from intermediate products derived from dopaquinone in the epidermis. The perceived color of skin is determined by the ratio of eumelanins to phaeomelanins, and in part by blood within the dermis. Exposure of skin to UVB irradiation upregulates the synthesis of melanocyte tyrosinase, regulating in increased melanogenesis and, thus tanning (10, 11).

In the present study, depigmentation with HIFU was investigated macroscopically and his-

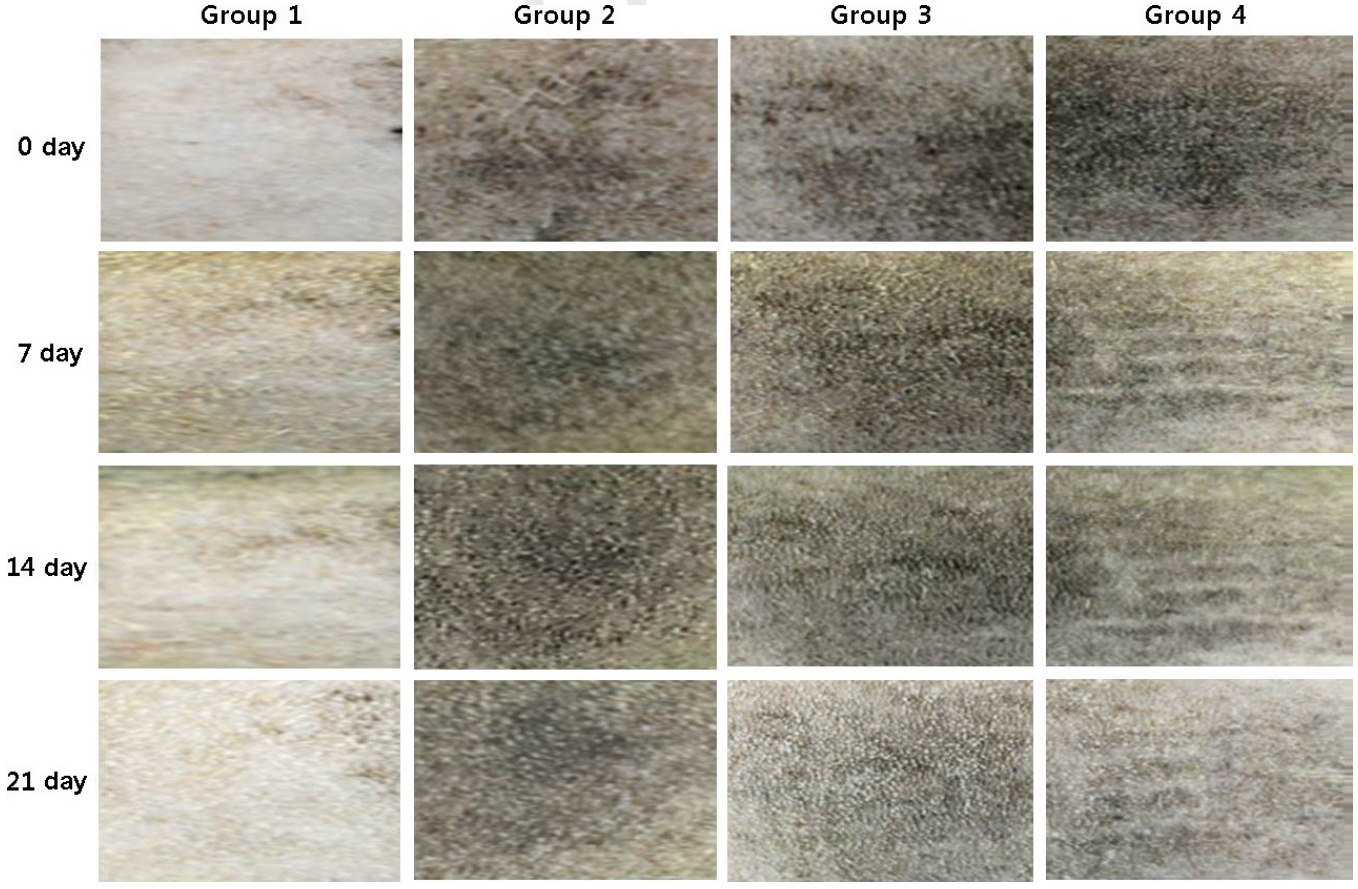

Fig. 1. Digital photographs show that tanning induced by UVB exposure was markedly reduced in areas 3 and 4 at 3 weeks after HIFU treatment.

LOW RESOLUTION COLOR FIG

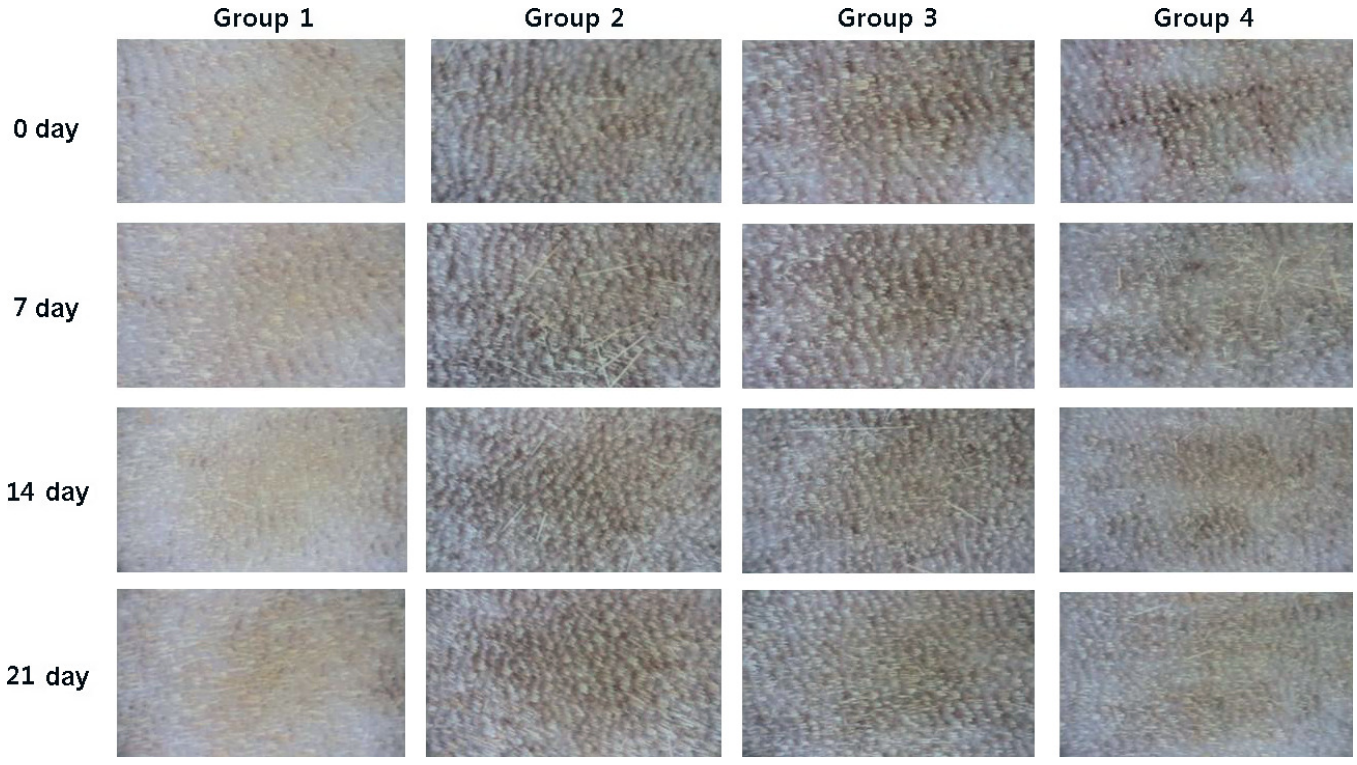

Fig. 2. Dermoscopic pictures show that tanning induced by UVB exposure was markedly reduced in areas 3 and 4 at 3 weeks after HIFU treatment.

COLOR

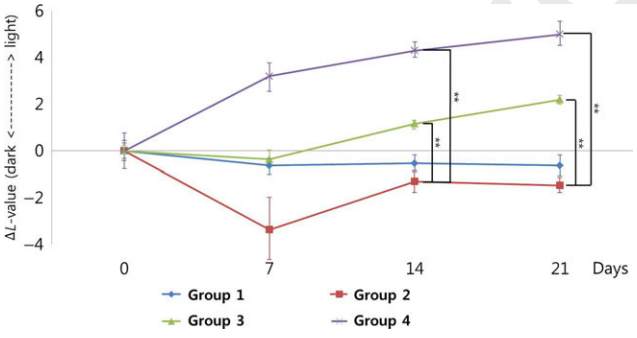

Fig. 3. The L values of areas three and four were significantly decreased from baseline at 3 weeks and 2 weeks after HIFU treatment, respectively, compared with area 2 (P < 0.01**).*

TABLE 1. The details of the L values*

Days		0	7	14	21
Group 1	L*1	66.7	65.4	66.3	66.2
	L*2	66.4	65.5	65.4	65
	L*3	65.6	65.9	65.4	65.6
	L* Mean	66.23	65.6	65.7	65.6
	L* SD	0.57	0.26	0.52	0.6
Group 2	L*1	56	51.3	55.9	54.9
	L*2	57.5	54.4	55.3	55.6
	L*3	56.7	54.4	55.1	55.3
	L* Mean	56.73	53.36	55.43	55.27
	L* SD	0.75	1.79	0.42	0.35
Group 3	L*1	55	55	55.7	57.2
	L*2	54.7	54.8	56.4	56.8
	L*3	55.1	53.9	56.2	57.4
	L* Mean	54.93	54.56	56.1	57.13
	L* SD	0.21	0.56	0.36	0.31
Group 4	L*1	52.3	55.7	58	59
	L*2	54.6	58	57.2	57.7
	L*3	53.6	56.4	58.2	58.8
	L* Mean	53.5	56.7	57.8	58.5
	L* SD	1.15	1.18	0.53	0.7

SD, Standard deviation

topathologically, using a UVB-induced hyperpigmentation model in the skin of a guinea pig. We macroscopically confirmed that UVB-induced hyperpigmentation significantly decreased after HIFU treatment with 0.1 J/cm^2 and 0.2 J/cm^2. The effects of HIFU on UVB-induced hyperpigmentation were enhanced when applied with 0.2 J/cm^2 energy compared with 0.1 J/cm^2 energy. Histologically, we also confirmed that the melanin deposition in the epidermis induced by UVB exposure was markedly reduced after HIFU treatment with 0.1 J/cm^2 and 0.2 J/cm^2. Therefore, we suggest that HIFU has skin lightening effects on areas with UVB-induced hyperpigmentation.

HIFU has recently been used for skin tightening and rejuvenation. Usually, 3- and 4.5-mm transducers are applied to deliver energy to the deep

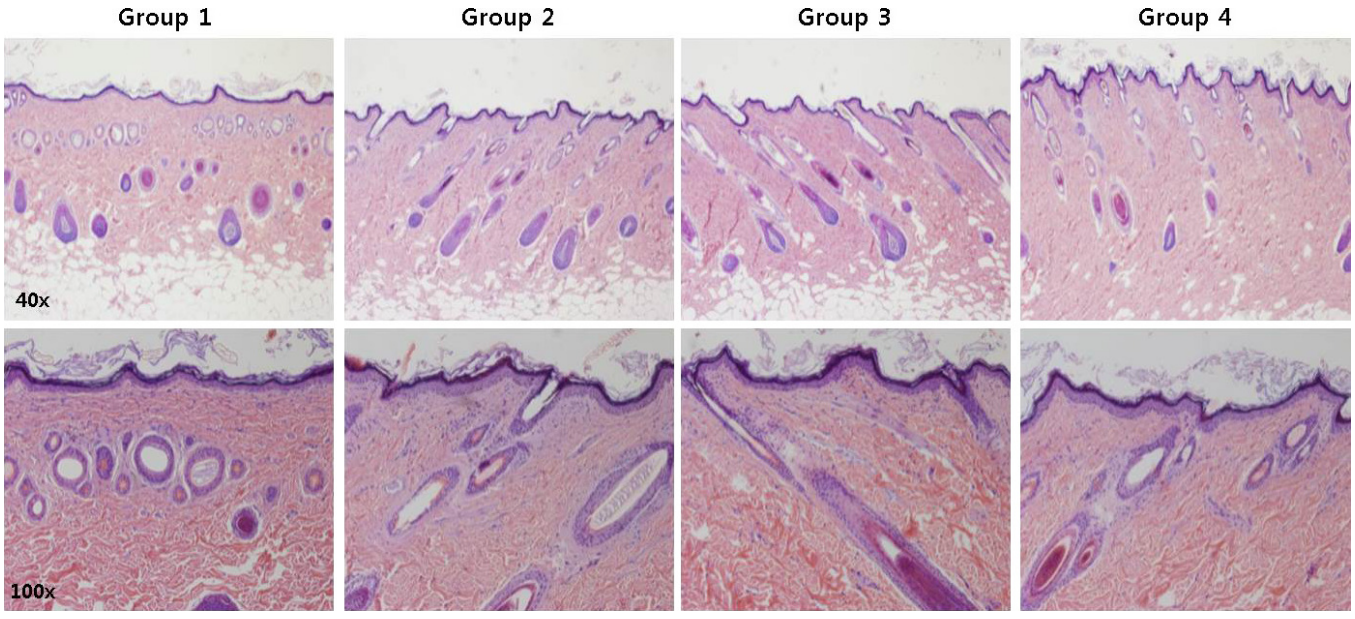

Fig. 4. There were no signs of inflammatory or necrotic reactions of skin tissue (hematoxylin & eosin stain). 6

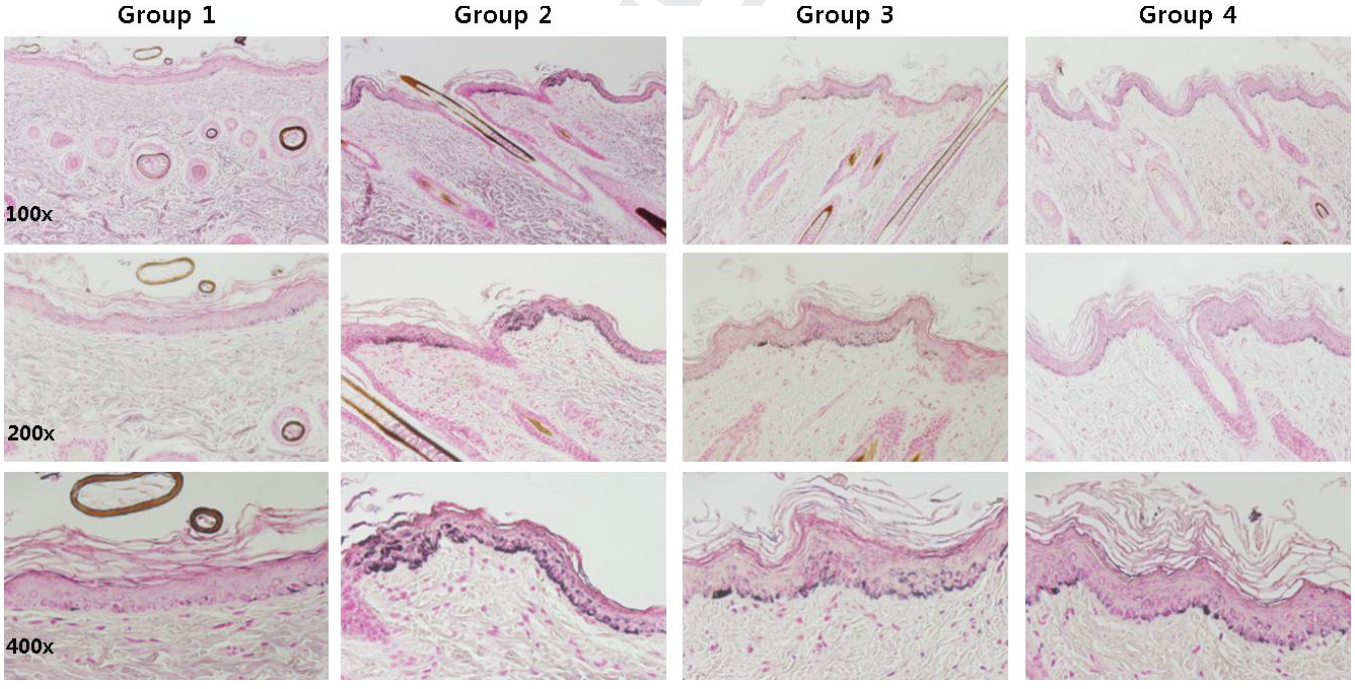

Fig. 5. Heavy deposition of melanin in the epidermis induced by UVB exposure was reduced in areas 3 and 4 (Fontana-Masson stain). 7

dermis, subcutis, and fibromuscular layer. Epidermal injury is minimized and ultrasound energy is directed into the deep skin tissue, resulting in well-defined thermal injury zones (5–7).

The mechanism underlying the lightening effects of HIFU is not understood. We hypothesize that when using a HIFU 1.5-mm transducer, the ultrasound energy is delivered beneath the dermoepidermal junction and upper dermis. The ultrasound waves induce vibrations in the composite molecules within skin tissue during propagation, and the friction that develops between intrinsic molecules is the source of the generated heat (12). We then propose that the mechanical destructive effects induced by vibration and friction are what eliminate melanin and pigmented debris from the epidermis and upper dermis.

Similarly, melasma has been successfully treated with fractional resurfacing lasers. Fractional

photothermolysis may induce ultrastructural changes, resulting in decreases in the numbers of melanocytes and melanin granules within keratinocytes (13, 14). HIFU is similar to fractional laser resurfacing in that thermal lesions are created, but is unique in that the thermal lesions are created below the surface and can be of variable geometry (10). As fractional resurfacing lasers have been used for the treatment of pigmented lesions including melasma, HIFU may be effective due to similar mechanisms for the elimination of melanocytes and melanin, and may be helpful to treat skin pigmentary conditions.

Our results demonstrate that a single session of HIFU treatment using a 1.5-mm-depth transducer is effective for improving UVB-induced hyperpigmentation in an animal model. The major limitation of this study is the use of tanning loss to assess depigmentation capacity. This method is widely used for testing pigmentary skin problems, but does not measure the ability to reduce long-lasting pigmentation such as freckles or melasma. Based on this animal study, we suggest that HIFU may be useful as an alternative modality for the treatment of skin pigmentary conditions in human patients. Further clinical studies are necessary to evaluate the effects of HIFU on pigmentary skin disorders.

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Conflicts of interest: None

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