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ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

Comparative Evaluation of the Biodegradability and Wrinkle Reduction Efficacy of Human-Derived Collagen Filler and Hyaluronic Acid Filler

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Abstract

Background The development of fillers for wrinkle prevention is growing to meet rising demands to reduce the aging of skin.

Objective In this experiment, we confirmed the effects of human collagen and hyaluronic acid filler biodegradation for wrinkle reduction using a photo-aging mouse model.

Materials and Methods A total of 10 hairless mice (SKH1-Hrhr) were randomly divided into two groups and injected with hyaluronic acid and human-derived collagen filler. At 0, 2, 4, 8, and 12 weeks, PRIMOSlite®, folliscope, and MRI were used to evaluate the biodegradability of the fillers after the injections. We also studied the photo-aging mouse model for skin roughness and histological evaluation and confirmed that the filler injection had excellent anti-wrinkle effects.

Results Human-derived collagen fillers had excellent biodegradability compared to that of hyaluronic acid fillers. The skin surface roughness in the photo-aging mouse models was significantly reduced after injections of human-derived collagen filler.

Conclusion Our results showed that the human-derived collagen filler had excellent biodegradability and effectively reduced wrinkle formation in a photo-aging mouse model.

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Keywords Collagen filler · Ultraviolet · Photo-aging · Wrinkle reduction

Introduction

Three major structure components of the dermis, collagen, elastin and GAGs, have been the focus of most anti-aging research and efforts for aesthetic-anti-aging strategies pertaining to the skin, including "anti-wrinkle creams" and various fillers [1]. The most commonly used products in anti-aging studies fall into four major categories: autologous fats, collagens, hyaluronic acid (HA), and biosynthetic polymers [2]. Among these products, human-derived collagen fillers include CosmoDerm™, Cymetra™, and Dermologen™. Human-derived collagen fillers do not require skin testing and have fewer side effects than other types of collagen fillers [3].

The characteristics of an ideal dermal filler include biocompatibility, safety, minimal immune system response, stability at the injection site, volume maintenance, lifting capacity, and a non-migratory nature [4, 5]. Collagen-based dermal fillers have been reported to reduce downtime, bruising, and pain during injection, and restore the lost structural components to aging skin [6].

However, dermal fillers are the highest-risk (Class III) devices that require premarket approval (PMA)

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applications and PMAs combined with preclinical data (e.g., animal studies), with clinical trials using clearly defined objectives to evaluate their effectiveness and safety [7]. The FDA (Food and Drug Administration) also requires animal or clinical studies for the registration and approval of injectable filler substances or surgically introduced artificial implants [8]. Therefore, it is necessary to study the safety and efficacy using preclinical testing of fillers.

To study photo-aging, we used hairless mice, a well-established model for studying the development of photo-aging [9]. Mouse models have been used extensively in photo-aging research and for other filler injections [10].

Therefore, we used artificial UVB irradiation to produce a model of photo-aging mice and confirmed the biodegradability and wrinkle reduction effects of human-derived collagen fillers and HA fillers in animal models.

Materials and Methods

Study Design

Human-derived collagen and HA biocompatible substance-based fillers were used. Human collagen filler was purchased from Bellacol (Hans Biomed, Seoul, Korea), and the HA filler was purchased from Juvéderm VOLUMA (Allergan, Pringy, France).

Two weeks after the induction of photo-aging, 100 µl of filler was injected into the dorsal skin of the posterior limbs of hairless mice (6-week-old females, a total of 10 mice per group).

Scanning Electron Microscopy

The fillers were evaluated for their morphologies using standardized scanning electron microscopy (SEM) methods. The human-derived collagen filler was diluted with water for injection (WFI) and filtered with a syringe through a 0.22-µm membrane filter mounted in a clean stainless-steel filter housing to remove the carrier matrix. The contents of each filter were rinsed 9 or 10 times with WFI to rinse all carrier materials through the filter mesh. The filter contents were placed in an oven until completely dry. The clean dry product was transferred to a clean dry sample tube and labeled. The filler sample was mounted on a stainless-steel SEM pedestal pre-labeled with a double-sided adhesive mounting disk. The samples were coated with AuPd imaging powder, prepared, and imaged using conventional SEM technology (LEO SUPRA 55, Carl Zeiss, Germany).

Magnetic Resonance Imaging

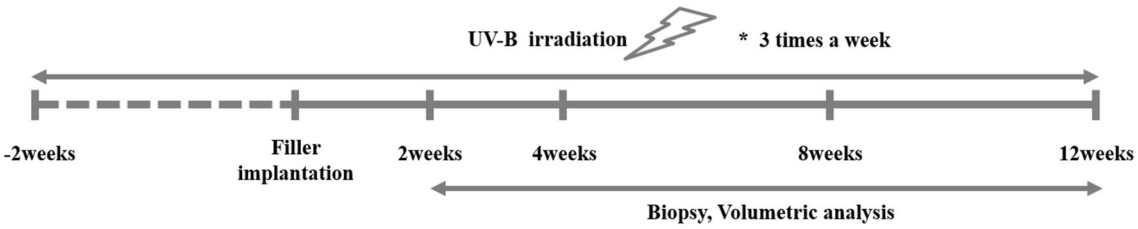
The biodegradability of injected filler was confirmed by three-dimensional magnetic resonance imaging (MRI). A magnetic resonance (MR) image was acquired using a T2WI pulse sequence using a 3.0 T MRI system (Achieva, Philips Healthcare, Best, the Netherlands) with a T2-weighted imaging wrist coil (SENSE wrist 4 channel, Philips Healthcare). The sequence parameters were T2WI (Turbo Spin Echo), TR, 2863.3 ms; TE, 100.0 ms; flip angle, 90°; FOV, 80.80 mm; slice thickness, 2 mm; matrix size 248 × 208; resolution, 0.32. The images were evaluated by a trained radiologist to analyze the injected human-derived collagen fillers and the HA fillers separately from surrounding tissues.

Hairless Mouse Skin Photo-Aging Model

We obtained 6-week-old female SKH1 hairless mice from Orient Bio (Orient Bio Inc., Seongnam, Korea), and they were fed a standard diet. After resting for 1 week, the mice were divided into four groups: UVB-non-irradiated ($n = 5$), PBS + UVB-irradiated ($n = 5$), HA filler + UVB-irradiated ($n = 5$), and human-derived collagen filler + UVB-irradiated ($n = 5$) groups. UVB was irradiated three times for 12 weeks using a BIO-SPECTRA (Vilber Lourmat, Marne-la-vallée France). The wavelength used was between 290 and 320 nm. The distance from the lamp to the backrest was 20 cm. During the irradiation, the mice moved freely around their cages. UVB was irradiated at 1 MED (50 mJ/cm^2) at 1 week, 1.2 MED (75 mJ/cm^2) at 2–3 weeks, 1.4 MED (100 mJ/cm^2) at 4–5 weeks, 1.8 MED (125 mJ/cm^2) at 6–8 weeks, 1 MED (50 mJ/cm^2) and 1.8 MED (150 mJ/cm^2) at 9–11 weeks, and 1.4 MED (125 mJ/cm^2) at 12 weeks. The total dose was 4050 mJ/cm^2. Two weeks after induction of photo-aging, 100 µl of phosphate-buffered saline (PBS) and fillers were injected into the dorsal skin of the posterior limbs of hairless mice (6-week-old female mice, total of 15 mice per group) (Scheme 1). All procedures involving animals were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Chung-Ang University, Korea (IACUC No. 15-00011).

Volumetric and Skin Roughness Measurement

After the filler injection, the increased volume was measured and quantified using a three-dimensional (3-D) micro-topography imaging system PRIMOSlite® (GFMesstechnik GmbH, Berlin, Germany) measuring device. The change in volume can be quantitatively determined through a three-dimensional measurement of the skin area at different times before and after the



Scheme 1 UVB irradiation schedule for induction of photo-aging in mouse

treatment, as well as a computer-assisted comparison of the measured profile. To determine the effect of the filler on skin roughness, the roughness of the same area was measured as time dependent using PRIMOSlite®. Quantification of the roughness was analyzed using the roughness parameters (Rmax, Rz, Rt) calculated according to DIN 4288.

Histological Analysis

After injection of the filler, the mice were killed at 24 weeks and the injection site of the filler was biopsied, and fixed with 10% formaldehyde. The specimens were embedded in paraffin and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT). The stained tissues were observed under a microscope (Leica Mikroskopie, Wetzlar, Germany), and the images were captured. The H&E and Masson's trichrome (MT)-stained sections were assessed to evaluate the histopathological changes.

Statistical Analysis

Statistical analysis was performed with the SPSS 18 statistical program. Shapiro–Wilk, Kruskal–Wallis, and a one-way ANOVA testing were utilized. $p < 0.05$ was considered significant.

Results

Determination of Filler Biodegradability in a Mouse Model

The particle morphology of human-derived collagen filler and HA filler was confirmed by SEM (FE-SEM-LEO SUPRA 55, Carl Zeiss, Germany). Human-derived collagen filler was interconnected and highly porous (Fig. 1a), and the HA filler was shaped as irregular compact polygonal particles (Fig. 1b).

To observe the biodegradability of the injected fillers, human collagen and HA fillers were injected into the subcutaneous layer of the mice, and their appearance distribution was monitored by measuring the PRIMOS image (Fig. 2a). Immediately after the injections, there was no observable leakage of the injected fillers. Further, the altered volume of the filler injected into the subcutaneous layer in the mice was quantitatively analyzed compared to the initial volume (Fig. 2b). The human-derived collagen fillers showed a significant time-dependent decrease in the volume of the injected fillers; in contrast, the HA filler showed an increase of 189.3 ± 16.1 (%) when compared to the initial volume, at 12 weeks after the filler injection. As a result, comparing the quantitated values of apparent in vivo injected filler volume changes showed that the biodegradability of human-derived collagen fillers was better than that of HA fillers. However, additional measurements of the volume changes of filler degradation in vivo are necessary.

Identification of the Filler Biodegradability by MRI

The three-dimensional volume changes were monitored using MRI to measure the biodegradability of the injected fillers. The human-derived collagen fillers showed a time-dependent decrease in volume of the injected filler, but the HA filler was observed to maintain its volume for 12 weeks (Fig. 3a). The HA filler in the sagittal T2-weighted image had a high signal, and the human-derived collagen filler was detected at a relatively low signal. However, the signal observed for the fillers was clearly distinguishable from other tissues (Fig. 3b). The HA filler remained without degradation for 12 weeks, whereas the biodegradation of the human-derived collagen filler was observed to be 79.6 ± 6.9% after 12 weeks.

Evaluation of Anti-wrinkle Effect of Filler Injection in Photo-Aging Mouse Model

The change in the pattern of wrinkles due to the injected filler in a photo-aging mouse model using UVB irradiation was confirmed by imaging and roughness parameters using PRIMOSlite®, which is an optical three-dimensional skin measurement system. Consequently, it was confirmed that the group with PBS injections had numerous wrinkles and

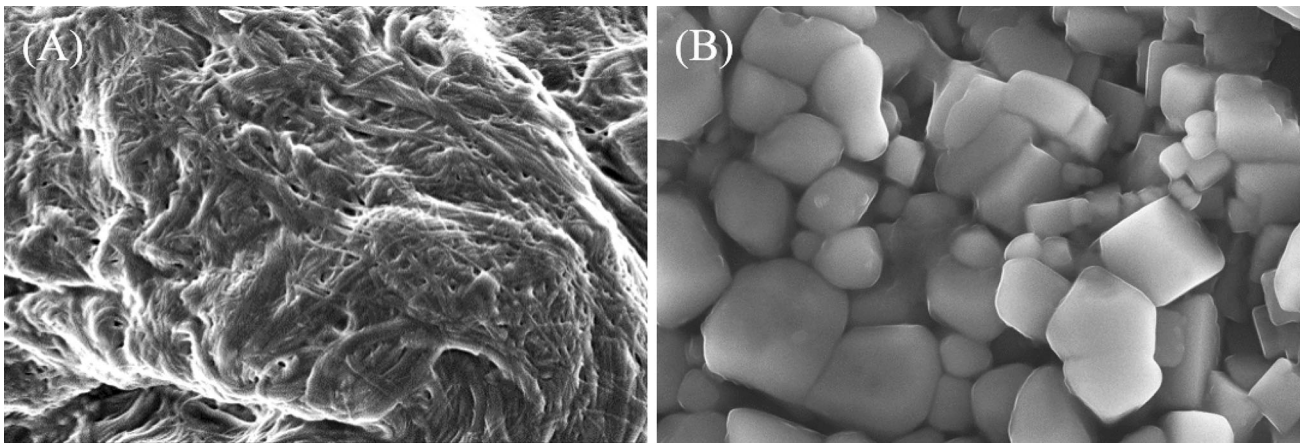


Fig. 1 SEM image of particle morphology. **a** Hyaluronic acid filler (Juvéderm VOLUMA), **b** human-derived collagen filler (Bellacol A10)

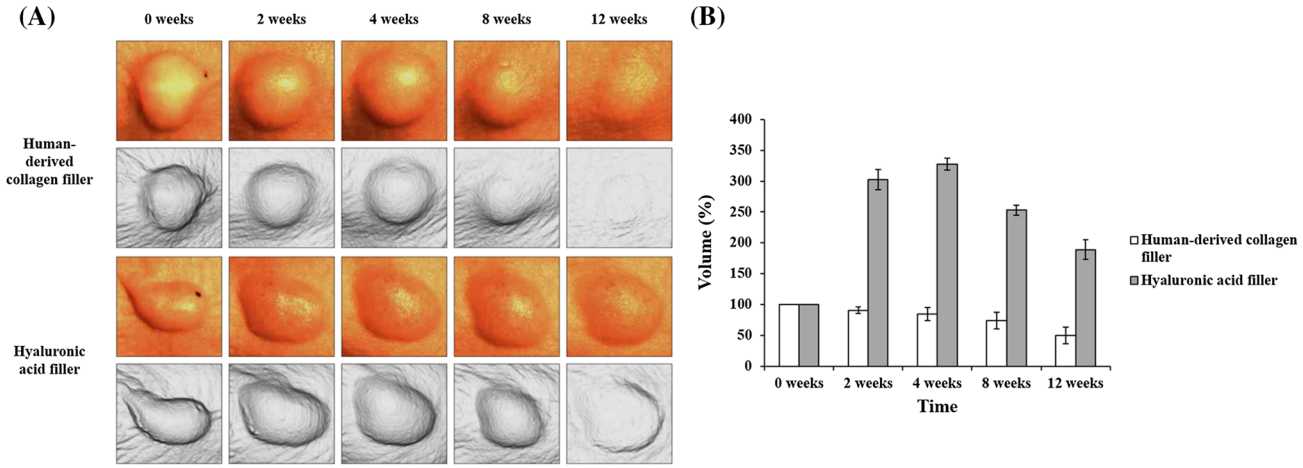


Fig. 2 Evaluation of degradation after mouse dorsal skin injection of human-derived collagen filler (Bellacol A10) and hyaluronic acid filler (Juvéderm VOLUMA) by PRIMOSlite®. After injection of filler, we analyzed degradation of injected filler by volume change using the stereoscope and 3D simulation image. **a** Stereoscopic and 3D simulated images were observed at 0, 2, 4, 8, and 12 weeks after filler injection. **b** Average percentage of volume change due to degradation of the injected filler

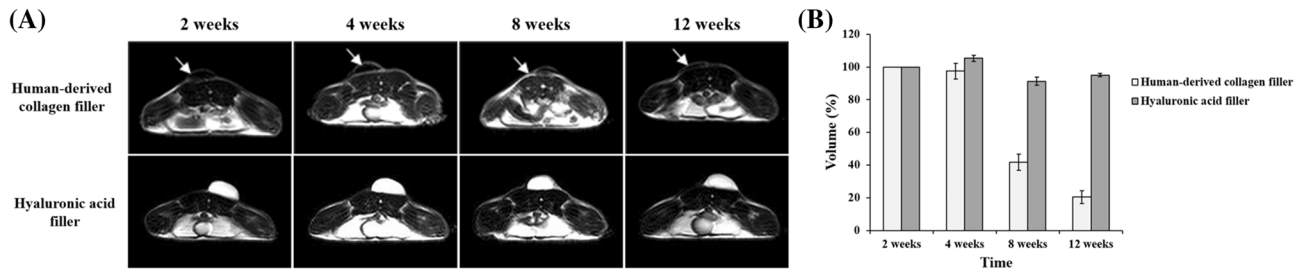


Fig. 3 Biodegradation monitoring by T2-weighted MRI (magnetic resonance imaging) analysis after injection of human collagen filler and hyaluronic acid filler. The MRI was made by Intera Achieva 3.0T (Philips, USA). White arrow: human-derived collagen dermal filler

a deep wrinkle. However, the wrinkles of the group injected with the human-derived collagen filler and HA filler were found to be similar to those of the group without UVB irradiation (Fig. 4a). These results show that wrinkles were induced by UVB, and that the wrinkles in the filler injection groups were reduced. It was also confirmed that the depth of wrinkles was quantitatively improved by the filler injection when comparing Rmax, Rt, and Rz (Fig. 4b–d).

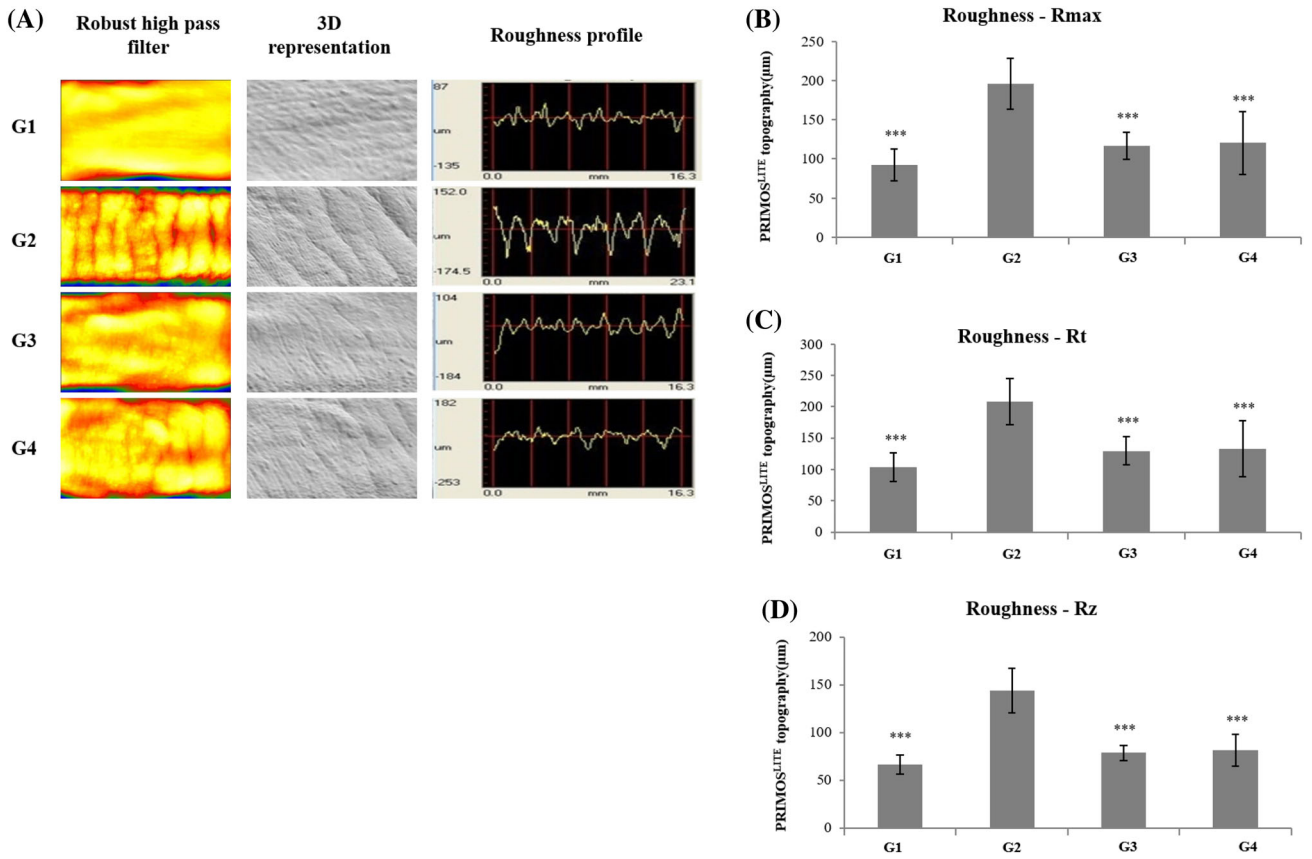


Fig. 4 Optical 3D skin image and quantitative analysis of skin roughness for evaluating wrinkle improvement of injected filler in photo-aging mouse model. **a** Using the optical 3D skin measurement system PRIMOSlite®, skin roughness measurements were taken at each filler injection site after 12 weeks of UVB irradiation. The robust high-pass filter observes changes in the depth of wrinkles in color-coded and shown as a 3D representation, and roughness profile shows a 2D section line with depth measurement. **b–d** Quantitatively shows the depth of the wrinkles reduced by filler injection. **b** Rmax: maximum roughness depth, **c** Rz: average maximum height of the profile, and **d** Rt: maximum height of the profile. (*p = 0.05, **p = 0.005, ***p = 0.0005)

Histological Observation

The effect of wrinkle reduction by the filler was observed histologically after 12 weeks of filler injection. The biopsied skin samples from each mouse group were stained with H&E to observe changes in the number and depth of wrinkles. As a result, we observed that the epidermal layer of the UVB-irradiated group was thickened (Fig. 5). In addition, compared to the PBS-injected group, the filler-injected group had a reduction in the number and depth of wrinkles. However, it was difficult to quantitatively show changes in the number and depth of wrinkles. Thus, our results indicated that the injection of human-derived collagen fillers helped to decrease wrinkle formation by increasing the collagen in the epidermis/dermis junction and the dermis.

Discussion

UVB is the most important section of the ultraviolet light spectrum that causes skin damage and aging [11, 12]. Studies on the prevention and treatment of light-induced skin aging and damage are important.

Human-derived collagen fillers do not require skin testing, and their side effects include bruising, erythema, and edema [13]. In addition, they cause platelet aggregation, which can also reduce the risk of bruising [14]. It has a short duration compared to that of HA fillers [15].

In this study, we visualized the time-dependent degradation patterns of the filler after injecting two fillers in dorsal skin. There have been many studies on the persistence of fillers [16–19]. Therefore, biodegradation of injectable fillers is of increasing importance.

In particular, the biodegradation of human-derived collagen filler causes a volume reduction in the filler (Fig. 2). The MRI showed that the degradation of the filler actually occurred in dorsal skin (Fig. 3). MRI is based on the

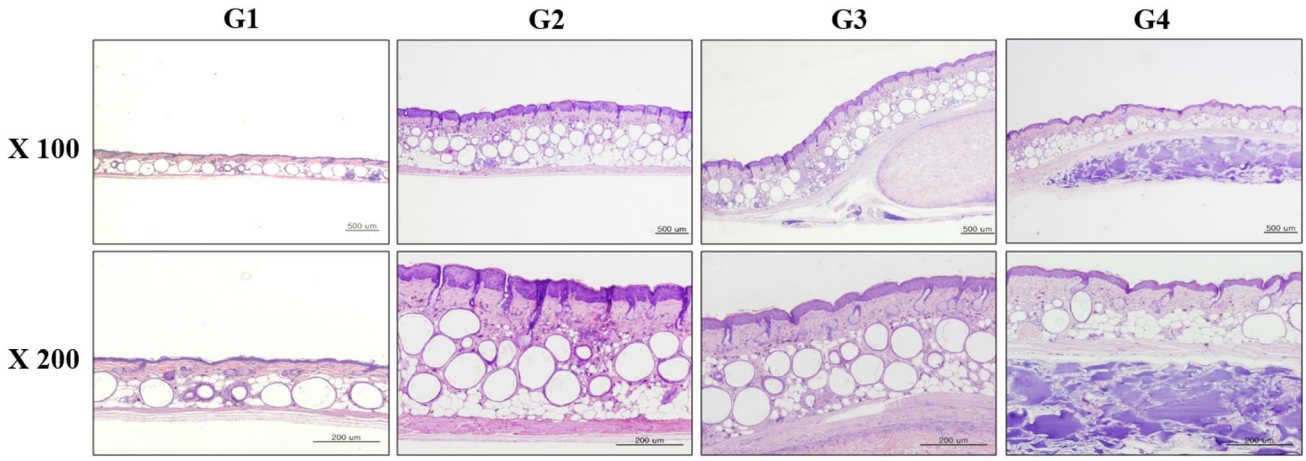


Fig. 5 Histological analysis in photo-aging mouse model after 12 weeks of filler injection. Observation of wrinkles in each group at H&E staining at 100 and 200 magnification using an optical microscope

principle of detecting hydrogen atoms [20]. The MRI images indicated the presence of relatively fewer hydrogen atoms in human collagen fillers than those in HA fillers. However, it was confirmed that the biodegradation of human-derived collagen fillers proceeds in a time-dependent manner via MRI image analysis. The difference in biodegradation of these fillers is thought to be due to the difference in the particle shape of HA filler and human collagen filler (Fig. 1). Most dermal filler particle morphologies are aimed at enhancing the stability of the material by the shape of a regular particle [21]. However, the particle shape of the human-derived collagen filler was irregular and not compact. Therefore, the human collagen filler was exposed to numerous sites capable of reacting with the collagenase-degrading enzyme in the skin, resulting in rapid biodegradation. The formation of wrinkles in the mouse model due to UVB irradiation has already been studied [22]. Mice are genetically similar to humans, easy to handle, and useful for studying skin aging. However, when UVB exposure ceased, the skin of the photo-aged mouse was partially repaired. Despite this, when we evaluated the effectiveness of the anti-wrinkle filler injections, there was partial repair in both the control and study groups, and this did not influence the results of the assessment. The number and depth of the wrinkles formed on the dorsal skin of the mouse after UVB irradiation were confirmed to have increased when compared with those of the non-UVB irradiation group (Fig. 3). We evaluated the degree of wrinkle reduction by filler injection as a parameter of skin roughness using a PRIMOSlite®. Using the evaluation results of Rmax, Rz, and Rt, we quantitatively showed the effects of wrinkle improvement in the mouse animal model by filler injection.

We also observed the wrinkle reduction effect of the injected filler in the tissue. In addition, the biodegradability of human-derived collagen fillers was confirmed in the mouse model, and the wrinkle reduction effect was demonstrated in a photo-aging mouse model. This is a novel method for quantitatively evaluating the wrinkle reduction effect of fillers in a photo-aging mouse model in which wrinkles are induced by UVB irradiation. Based on these results, the biodegradability of the biomaterial-based filler was evaluated in animal models, and the wrinkle reduction effect of the injected filler in the photo-aging mouse model was quantitatively evaluated.

The limitations of this study have been evaluated, and because a mouse model was used, the results can only be applied to marketing and preclinical identification data. Quantitative evaluation of the biodegradability of the injected filler and additional confirmation in non-rodents should be performed, and the related components such as collagen and elastin should be compared using immunohistochemistry (IHC) and mRNA and protein expression level analysis of the tissues. This will require a longer tracking period.

In conclusion, our results demonstrated that human-derived collagen fillers have excellent biodegradability. The human-derived collagen fillers also have anti-wrinkle effects and reduced skin roughness caused by UVB radiation exposure.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

Human and Animal Rights All applicable institutional and/or national guidelines for the care and use of animals were followed.

Informed Consent For this type of study, informed consent is not required.

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