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# Oral administration of a novel hydrolyzed chicken sternal cartilage extract (BioCell Collagen®) reduces UVB-induced photoaging in mice

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ABSTRACT

Excessive exposure to ultraviolet B (UVB) radiation causes skin aging (a process known as photoaging). This study examined whether subchronic (14-week) oral administration of a novel hydrolyzed chicken sternal cartilage extract—comprising naturally-occurring primarily hydrolyzed type II collagen peptides, chondroitin sulfate and hyaluronic acid, extracted from a single source—could reduce signs associated with photoaging in hairless mice. Administration of the extract to UVB-irradiated mice led to significant increases in skin elasticity and hyaluronic acid content, compared with UVB-irradiated controls. Matrix metalloproteinase expression, transepidermal water loss, dermal inflammatory cells and wrinkles were all significantly reduced by administration of hydrolyzed chicken sternal cartilage extract. This study is the first to demonstrate that oral administration of this novel extract, containing a unique single-source complex of primarily hydrolyzed type II collagen (rather than the more widely studied type I form), chondroitin sulfate and hyaluronic acid, reduces UVB-induced photoaging in hairless mice.

## 1. Introduction

The skin is a vital organ for human survival, acting as the primary barrier to external insults such as infectious agents and physical injury, as well as fulfilling several important sensory roles, including regulation of temperature and controlling under/over-hydration (Vollmer, West, & Lephart, 2018). The dermis makes up the bulk of the skin and is responsible for its tensile strength, elasticity, and pliability (Brown & Krishnamurthy, 2018). Although primarily composed of collagen (comprising approximately 70% of the total dry mass of skin), many other extracellular components, including glycosaminoglycans (such as hyaluronic acid and dermatan sulfate), blood vessels, nerve fibers, hair follicles, and numerous different cell types [including regenerative cells (fibroblasts, adipocytes, and Schwann cells) and immune cells (macrophages and mast cells)] also perform vital roles in the extracellular matrix (ECM) of the dermis (Brown & Krishnamurthy, 2018; Gniadecka et al., 1998; Lodish et al., 2000; Rivera-Gonzalez, Shook, & Horsley, 2014; Schwartz & Park, 2012). Several cell types within the dermis express matrix metalloproteinases (MMPs), a family of zinc-containing endopeptidases that are responsible for degradation of the ECM in response to stimuli such as physical injury and ultraviolet (UV) radiation (Pittayapruek, Meephansan, Prapapan, Komine, & Ohtsuki, 2016). Impairment of the epidermis can also be caused by excessive exposure to UV radiation and this results in loss of moisture from the skin, referred to as transepidermal water loss (TEWL) (Ito, Seki, & Ueda, 2018).

As with all organs, human skin undergoes chronological (intrinsic) aging and environmental (extrinsic) aging (WHO, 2011 in Durai, Thappa, Kumari, & Malathi, 2012); extrinsic skin aging is primarily caused by chronic UV exposure, thus often being referred to as “photoaging” (Shin et al., 2019). Although these two forms of skin aging have different characteristics (intrinsic aging is associated with fine wrinkles and reduced epidermal thickness, whereas photoaging is associated with deep wrinkles, hyperpigmentation, and laxity) and etiologies, they share many similarities in the physiological

*Abbreviations:* ARRIVE, Animal Research: Reporting of *In Vivo* Experiments; ANOVA, analysis of variance; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; HAS, hyaluronic acid synthase; HYAL, hyaluronidases; MED, minimal erythema dose; MMP, matrix metalloproteinase; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; Smad, small mother against decapentaplegic; TGF-β, transforming growth factor-β; TEWL, transepidermal water loss; UV, ultraviolet; UVB, ultraviolet B

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mechanisms associated with their development (Shin et al., 2019; Varani, Quan, & Fisher, 2009). For example, in both conditions, reactive oxygen species (ROS) from oxidative metabolism induce upregulation of the c-Jun transcription factor, resulting in increased expression of MMPs, breakdown of collagen fibers, and prevention of collagen synthesis via inhibition of procollagen-1 (Freitas-Rodríguez, Folgueras, & López-Otín, 2017; Papakonstantinou, Roth, & Karakiulakis, 2012; Rivera-Gonzalez et al., 2014). Loss of skin moisture is also a prominent feature of skin aging, which is partially caused by a reduction in dermal hyaluronic acid (also known as hyaluronan). Hyaluronic acid retains skin moisture owing to its remarkable ability to absorb up to 1000 times its weight in water (Papakonstantinou et al., 2012; Rieger, 1998). As the only visible organ, physical changes to the skin have a notable physiological impact that can affect interpersonal relations and psychological wellbeing (Fore, 2006). While there a vast array of topical cosmetics intended to alleviate the effects of photoaging, dietary supplements intended for this purpose are becoming increasingly popular (Szyszkowska, Lepecka-Klusek, Kozlowicz, Jazienicka, & Krasowska, 2014).

This research article describes the evaluation of the efficacy of a novel hydrolyzed chicken sternal cartilage extract (BioCell Collagen®) in reducing parameters indicative of photoaging (such as increased wrinkles, TEWL and MMP expression, and reductions in skin moisture) when orally administered to hairless mice for 14 weeks. In terms of the individual components of the hydrolyzed chicken sternal cartilage extract, several studies have reported that ingestion of collagen hydrolysates reduces parameters associated with photoaging in humans (Inoue, Sugihara, & Wang, 2016; Kim, Chung, Choi, Sakai, & Lee, 2018; Proksch et al., 2014; Pyun et al., 2012) and animals (Oba et al., 2013; Zhuang, Hou, Zhao, Zhang, & Li, 2009). In human clinical studies, hyaluronic acid has been demonstrated to significantly improve skin moisture (Göllner, Voss, von Hehn, & Kammerer, 2017; Kawada et al., 2014, 2015) and reduce wrinkles (Oe et al., 2017). However, less emphasis should be placed on individual components, as the unique complex of these components in hydrolyzed chicken sternal cartilage extract may have a synergistic effect. Consumption of other dietary supplements containing a complex of collagen hydrolysate, chondroitin sulfate, and hyaluronic acid have been demonstrated to reduce numerous signs of skin aging in humans (Di Cerbo et al., 2015). Di Cerbo et al. (2015) used a formulation containing ingredients from external sources, *i.e.* the components were not naturally occurring or extracted from a single source, as is the case for hydrolyzed chicken sternal cartilage extract.

Two earlier studies by Schwartz and Park (2012) and Schwartz et al. (2019) were conducted using the same hydrolyzed chicken sternal cartilage extract as used in this study. Schwartz and Park (2012) conducted a preliminary study with 26 healthy females and reported that daily consumption of 1 g hydrolyzed chicken sternal cartilage extract for 12 weeks led to significant reductions in skin dryness/scaling and global lines/wrinkles (Schwartz & Park, 2012). In a larger-scale follow-up study including 128 females at the same daily dosage and duration as the previous study, Schwartz et al. (2019) reported that consumption of the extract led to significantly reduced facial lines and wrinkles, and increased skin elasticity and cutaneous collagen content (Schwartz et al., 2019). Despite these promising results, there were a number of unaffected parameters, which the authors believe may have been due to limitations (e.g., environmental UV exposure possibly varied between different participants and skin collagen content was only measured indirectly); these are limitations that can be addressed using a hairless mouse model with in-depth histomorphometric analyses. Most research to date regarding the effects of collagen on photoaging has focused on type I collagen, as it is the most abundant component of human skin (Henriksen & Karsdal, 2019; Waller & Maibach, 2006). However, research into whether other collagen types also have anti-photoaging properties is limited. To our knowledge, this is the first dermal efficacy study to examine whether subchronic (14-week) oral administration of a unique single-source complex of naturally-occurring primarily hydrolyzed type II collagen, chondroitin sulfate and hyaluronic acid, reduces UVB-induced photoaging in hairless mice.

## 2. Materials and methods

### 2.1. Animals and environmental conditions

Ethics approval for the study was received from the Animal Ethics Committee by Chemon's guideline (IACUC No. 2018-12-001). This article follows the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) Guidelines for reporting animal research (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010). A total of 44 female Hos®: HR-1 hairless specific pathogen-free mice (Joong Ah Bio, Suwon, Gyeonggi, South Korea) were received at 5 weeks of age and acclimated for 7 days before dose administration. The study was conducted using 40 of the animals. Body weights ranged from 16.83 to 21.26 g on arrival and from 20.48 to 23.78 g at the start of the administration period. All animals were uniquely identified by marker pen and housed individually in polycarbonate cages (170 × 235 × 125 mm) (Joong Ah Bio), which were labelled with color-coded identification cards. Room temperature and humidity were maintained at 20–26 °C and 40–70%, respectively, with values for both recorded hourly during the study. Ventilation (10–20 times per hour) and lighting (12-hour light/dark cycle) were also automatically controlled. Teklad certified irradiated global 18% protein rodent diet (DooYeol Biotech, Seoul, South Korea) was freely available to the animals (see Table S1 for diet composition).

All animals were observed daily for clinical signs and changes in behavior throughout the study. Body weights were recorded on arrival, once before dosing, once on the first day of dosing, and once weekly thereafter. Food consumption was recorded once weekly.

### 2.2. Test article administration and UVB irradiation schedule

The hydrolyzed chicken sternal cartilage extract was supplied by BioCell Technology LLC as an off-white to beige fine powder and comprises a complex matrix of primarily hydrolyzed collagen type II peptides, chondroitin sulfate, hyaluronic acid, extracted from a single natural source – chicken sternum cartilage (see Table 1 for test article composition). This unique biomolecular complex of components is

**Table 1**
Composition of hydrolyzed chicken sternal cartilage extract.

| Parameter | Value |
|---|---|
| Hydrolyzed collagen type II peptides (calculated as total protein) | ≥60% |
| Chondroitin sulfate | ≥20% |
| Hyaluronic acid | ≥10% |
| *Typical a mino acid profile* | |
| Aspartic acid | 5.29 g/100 g |
| Glutamic acid | 8.75 g/100 g |
| Alanine | 4.51 g/100 g |
| Glycine | 8.93 g/100 g |
| Threonine | 2.60 g/100 g |
| Proline | 5.25 g/100 g |
| Serine | 2.45 g/100 g |
| Leucine | 4.20 g/100 g |
| Isoleucine | 1.90 g/100 g |
| Valine | 2.43 g/100 g |
| Phenylalanine | 2.14 g/100 g |
| Tyrosine | 1.16 g/100 g |
| Methionine | 1.38 g/100 g |
| Cystine | 0.46 g/100 g |
| Lysine | 3.54 g/100 g |
| Histidine | 2.05 g/100 g |
| Arginine | 4.42 g/100 g |
| Tryptophan | 0.37 g/100 g |
| Hydroxyproline | 3.90 g/100 g |

naturally occurring (*i.e.* the components are not added from an external source), which differentiates this extract from many of the other compounds used as potential anti-photoaging ingredients in other studies. Another novel factor of the extract is its low molecular weight profile, with an average molecular weight of approximately 1500–2500 Daltons (Mn). Sterilized injectable saline (Daihan Pharmaceutical, South Korea) was the vehicle provided to control animals and used for formulation of the test article.

A total of four groups (10 animals per group) were included in the study and received either the vehicle alone with no UVB exposure (Group 1), the vehicle with UVB exposure (Group 2), or the test article at 200 or 600 mg/kg body weight/day with UVB exposure (Groups 3 and 4, respectively). These doses were calculated based on doses used in previous clinical studies with this compound (Lopez, Ziegenfuss, & Park, 2015; Schwartz & Park, 2012; Schwartz et al., 2019), which ranged from 1 to 3 g/day. The doses used in the current mouse study were calculated using the following equation: [human dose (mg/day) × conversion factor of 12.33 (Reagan-Shaw, Nihal, & Ahmad, 2008)] divided by 60 kg (default body weight for a human adult)], with the values then rounded to the nearest 100 mg for the final doses. Animals were administered the test article or vehicle by oral gavage using a 1 mL syringe (Korea Vaccine Co., Ltd., Ansan, Gyeonggi, South Korea) at a constant dose volume of 10 mL/kg body weight, once daily for 14 consecutive weeks. For UV exposure, each animal was exposed to UVB radiation (290–320 nm) three times per week. UVB exposure was gradually increased during the study [60 mJ/cm$^2$, equivalent to one minimal erythema dose (MED), in Weeks 1 and 2; 120 mJ/cm$^2$ (two - MEDs) in Week 3; 180 mJ/cm$^2$ (three MEDs) in Week 4 and 240 mJ/cm$^2$ (four MEDs) from Week 5 to Week 14].

At the end of the 14-week dosing period, all animals were euthanized by intraperitoneal injection of a mixture of zoletil (Virbac, Carros, France) and rompun (Bayer Korea Ltd., Seoul, South Korea) at a ratio of 4:1.

### 2.3. Skin elasticity, TEWL, and wrinkle analysis

Skin elasticity (arbitrary units) and TEWL (g/h/m$^2$) of the euthanized animals were measured with a Cutometer® Dual MPA 580 (CK Electronic GmbH, Germany) and a GPSkin Barrier® Corneometer (GPSkin, South Korea), respectively. Following skin elasticity and TEWL measurements, skin replicas were created using a Visioline® Replica Full Kit (CK Electronic GmbH, Germany) and were subsequently analyzed for the following skin wrinkle parameters using a Visioline® VL 650 skin wrinkle analyzer (CK Electronic GmbH, Germany): wrinkle area (%), number of wrinkles, total and mean wrinkle length (mm), and total, mean and maximum wrinkle depth (μm).

### 2.4. Histomorphometry

Dorsal skin biopsy samples were collected and rapidly frozen before use. For the histomorphometrical analyses, a portion of the skin samples were fixed in 10% neutral buffered formalin solution (Sigma-Aldrich, USA) for 24 h, embedded in a paraffin block, and sectioned to a thickness of 3–4 μm using a Leica RM2255 microtome (Leica Biosystems, Germany). The slides were stained with either hematoxylin-eosin (Sigma-Aldrich) for measurement of epidermis thickness (μm) and dermal inflammatory cells (number/mm$^2$ of skin), with Masson's Trichrome (Abcam, United Kingdom) for analysis of collagen fiber occupied regions (%/mm$^2$ of skin), or with Toludine Blue (Sigma-Aldrich) to record the number of mast cells (number/mm$^2$ of skin). Histomorphometric analyses were performed under an Eclipse 80i (Nikon, Japan) microscope and analyzed using iSolution FL version 9.1 (iSolutions Inc., Canada) software.

### 2.5. Determination of MMP-1, MMP-2, and hyaluronic acid

The previously frozen skin biopsy samples were converted into skin extracts by mixing with protease inhibitors containing EzRIPA lysis buffer (Atto, Japan). MMP-1, MMP-2, and hyaluronic acid content were assessed using western blotting, gelatin zymography, or enzyme-linked immunosorbent assay (ELISA), respectively. For western blotting, a 20-μg protein sample was prepared by separation using sodium dodecyl sulfate polyacrylamide gel electrophoresis for 2 h before the proteins were transferred onto a polyvinylidene fluoride membrane. The membrane was then incubated with primary antibodies against MMP-1 (Abcam, United Kingdom) and horseradish peroxidase-linked secondary antibody (Invitrogen, Rockford, IL, USA). MMP-1 was detected with an enhanced chemiluminescence solution (ThermoFisher Scientific, USA) and visualized with the Gel Doc EZ System (Bio-Rad, USA). For gelatin zymography, a 20-μg protein sample was mixed with loading buffer containing 2% sodium dodecyl sulfate (SDS) without β-mercaptoethanol and separated by 10% SDS containing 1% gelatin (Koma Biotech, South Korea). After washing with 2.5% Tritox X-100 for 1 h, the gel was incubated with reaction buffer containing 10 mM calcium chloride and 0.15 M sodium chloride and finally visualized with a Coomassie brilliant blue R-250 stain (Biosesang, South Korea) followed by detaining with 30% methanol and 10% acetic acid. For determination of hyaluronic acid content in the skin samples, ELISA was carried out using the Hyaluronan DuoSet ELISA kit (R&D Systems, USA).

### 2.6. Statistical analysis

Nonirradiated vehicle controls were compared with UVB exposed controls to demonstrate the photoaging effects of UVB exposure on animals that did not receive the test article. Test article groups were compared with the UVB exposed controls to assess the efficacy of the test article in reducing UVB-induced photoaging effects. Statistical analyses were conducted using a one-way ANOVA, and post-hoc analysis was conducted using either the Dunnett's test or Duncan test. Analyses were performed using SPSS statistics 12.0 K (SPSS Inc., USA).

## 3. Results

### 3.1. Clinical observations, body weights, and food consumption

There were no deaths and no clinical signs (data not shown). Body weight and food consumption were both unaffected by the test article, with values for test article-administered groups remaining comparable with those of the vehicle controls throughout the study; body weight and food consumption graphs have been provided as supplementary data (Supplementary Fig. 1 and Supplementary Fig. 2, respectively).

### 3.2. Skin elasticity, TEWL, and wrinkle analysis

Skin elasticity (Fig. 1a) was statistically significantly ($p < 0.01$) lower (-42%) for UVB controls compared with nonirradiated controls, demonstrating the UVB-induced physical damage to the skin of those animals. Conversely, skin elasticity was statistically significantly higher for both test article dose groups (18% and 17% higher at 200 and 600 mg/kg body weight/day, respectively) compared with UVB controls.

TEWL (Fig. 1b) was statistically significantly ($p < 0.01$) increased (+146%) for UVB controls compared with nonirradiated controls, demonstrating that UV exposure damaged the skin barrier integrity of controls that were exposed to UVB radiation. The test article reduced TEWL compared with UVB controls at both dose levels (−18% and −33% at 200 or 600 mg/kg body weight/day, respectively), but the difference was only statistically significant at the high dose ($p < 0.05$).

Results for wrinkle analysis are illustrated in Fig. 2a–d; dorsal skin

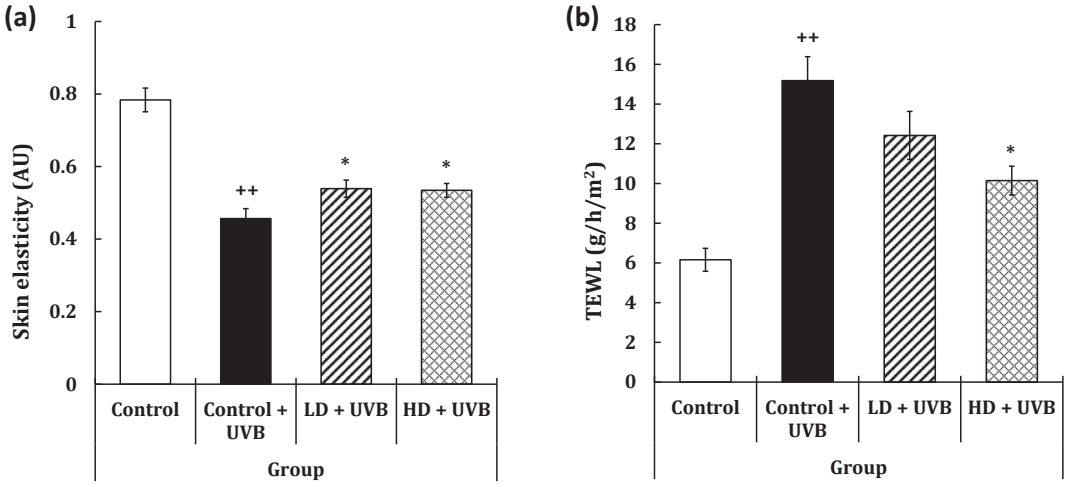


**Fig. 1.** Changes in (a) skin elasticity and (b) TEWL. AU, arbitrary units; HD, high-dose hydrolyzed chicken sternal cartilage extract; LD, low-dose hydrolyzed chicken sternal cartilage extract; UVB, ultraviolet B. For comparisons between nonirradiated controls and UVB-irradiated controls, ++ indicates $p < 0.01$. For comparisons between UVB-irradiated controls and test article groups, * indicates $p < 0.05$.

replica images are provided in Fig. 2e. There was a statistically significant ($p < 0.01$ to $p < 0.05$) higher total wrinkle area (+56%) and number of wrinkles (+38%) for UVB controls compared with nonirradiated controls. At the low dose of the test article (200 mg/kg body weight/day), total wrinkle length was statistically significantly ($p < 0.05$) reduced by 23.6% compared with UVB-exposed controls. Total wrinkle area (−28%), number of wrinkles (−18%), and total length (−28%) and depth (−22%) of wrinkles were all statistically significantly ($p < 0.01$ to $p < 0.05$) reduced for the high-dose group (600 mg/kg body weight/day) compared with UVB controls. There were no differences in mean wrinkle length, mean wrinkle depth, and maximum wrinkle depth between controls and test article groups.

### 3.3. Histomorphometry

Graphical illustrations and histological images for histomorphometrical analysis are provided in Fig. 3a–d. Statistically significant ($p < 0.01$) increases in epidermis thickness (+385%), number of dermal inflammatory cells (+392%), number of mast cells (142%), and collagen fiber occupied regions (94%) were observed for UVB control mice compared with nonirradiated control mice. The values for all of these parameters were statistically significantly ($p < 0.01$) lower for both groups administered with the test article, compared with those for UVB control mice.

### 3.4. MMP-1, MMP-2, and hyaluronic acid

Results for expression of MMP-1 and MMP-2 (enzymes responsible for degrading ECM components and contributing to photoaging) are illustrated in Fig. 4a and b, respectively. Statistically significant ($p < 0.01$) increases in the expression of MMP-1 (+44%) and MMP-2 (+217%) were observed for UVB controls compared with nonirradiated controls. In contrast, expression of MMP-1 and MMP-2 was statistically significantly ($p < 0.01$ to $p < 0.05$) reduced for the low-dose group (−19% and −65%, respectively) and high-dose group (−17% and −73%, respectively), compared with UVB controls. Hyaluronic acid content (Fig. 4c) was statistically significantly ($p < 0.01$) reduced by 37% for UVB-irradiated controls compared with nonirradiated controls. At the low dose (200 mg/kg body weight/day), hyaluronic acid content was statistically significantly ($p < 0.05$) higher (+24%) than that for UVB controls. Hyaluronic acid content was also 11% higher for mice in the high-dose group, but this increase was not statistically significant.

## 4. Discussion

Skin health is an important facet of psychological wellbeing and overall quality of life. It is widely known that a balanced diet is beneficial for maintaining healthy skin, which demonstrates that ingredients consumed orally can effectively improve skin health. Dietary supplements may be used if certain nutrients are not being provided in sufficient quantities from the normal diet, and dietary supplements specifically targeting the improvement of skin health are becoming increasingly popular (Szyszkowska et al., 2014). Hydrolyzed chicken sternal cartilage extract (BioCell Collagen®) is a dietary supplement comprising a unique complex of naturally-occurring primarily hydrolyzed type II collagen, hyaluronic acid, and chondroitin sulfate (extracted from a single source), which has been shown to reduce several signs of skin aging in humans (Schwartz & Park, 2012; Schwartz et al., 2019). However, several parameters were unaffected in these studies, which may be due to methodological limitations. To our knowledge, this is the first dermal efficacy study to examine whether subchronic (14-week) oral administration of hydrolyzed type II collagen (as opposed to the more widely studied type I form) in a complex with chondroitin sulfate and hyaluronic acid, reduces UVB-induced photoaging in hairless mice.

Once-daily administration of hydrolyzed chicken sternal cartilage extract for 14 weeks at up to 600 mg/kg body weight/day was well-tolerated, with no deaths or clinical signs observed and no differences in body weight or food consumption between hydrolyzed chicken sternal cartilage extract-dosed groups and vehicle controls. This was to be expected, given the results of previously conducted acute and subchronic toxicity studies with hydrolyzed chicken sternal cartilage extract, where no test article-related adverse effects were observed after a single dose at 5000 mg/kg body weight/day or after 90 days dosing at up to 1000 mg/kg body weight/day (Schauss, Merkel, Glaza, & Sorenson, 2007).

For all skin-related parameters measured as part of this study, UVB-irradiation of vehicle control animals consistently induced statistically significant changes (compared with nonirradiated controls) that are commonly associated with photoaging, including reduced skin elasticity and hyaluronic acid content, as well as increases in MMP expression, TEWL, dermal inflammatory cells, collagen fiber occupied regions, and wrinkles). This demonstrates the validity of the study and efficacy of the UVB-exposure process employed.

Administration of hydrolyzed chicken sternal cartilage extract induced a statistically significant increase in skin elasticity compared with UVB-irradiated vehicle controls. As skin elasticity inversely

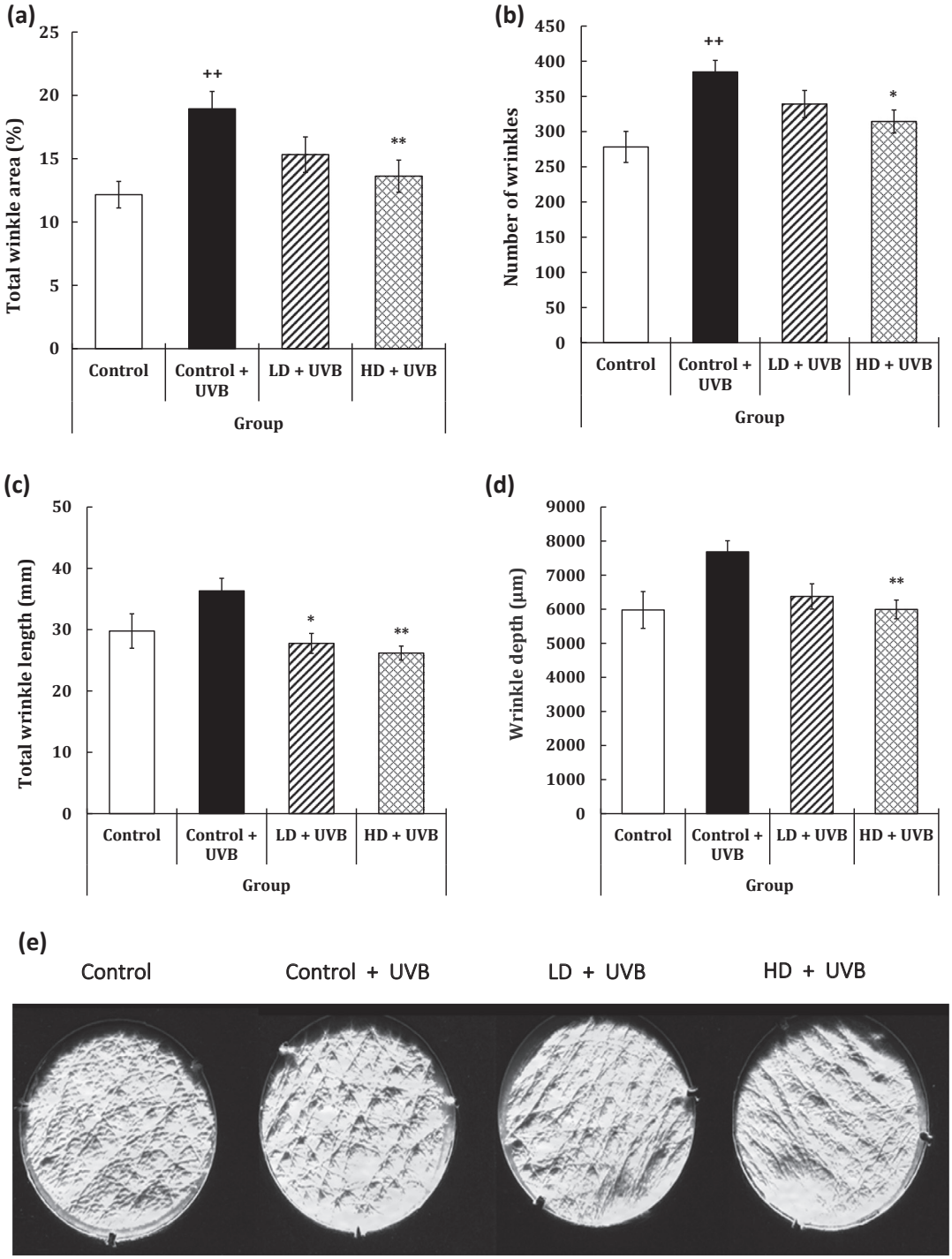


**Fig. 2.** Changes in (a) total wrinkle area, (b) number of wrinkle, (c) total wrinkle length, (d) wrinkle depth, and (e) dorsal skin photograph. HD, high-dose hydrolyzed chicken sternal cartilage extract; LD, low-dose hydrolyzed chicken sternal cartilage extract; UVB, ultraviolet B. For comparisons between nonirradiated controls and UVB-irradiated controls, + + indicates $p < 0.01$. For comparisons between UVB-irradiated controls and test article groups, * indicates $p < 0.05$ and ** indicates $p < 0.01$.

correlates with wrinkle formation, the reductions in the number, area, length, and depth of wrinkles were observed in the high-dose group were also expected. These effects are likely attributed in part to the hydrolyzed type II collagen component of the test article, as collagen hydrolysates have been shown to improve skin elasticity in numerous studies in humans (Inoue et al., 2016; Kim et al., 2018; Proksch et al., 2014; Pyun et al., 2012) and animals (Oba et al., 2013; Zhuang et al., 2009). Although the precise mechanism for collagen hydrolysate skin elasticity improvement is yet to be fully elucidated, it is believed that metabolism of collagen hydrolysates into smaller peptides (following oral consumption) is key to exertion of this beneficial effect (Liu et al., 2019). Collagen-derived peptides (in particular Gly-Pro-Hyp and Pro-Hyp) have been shown to be absorbed into the systemic circulation and transported to the skin following consumption of collagen hydrolysates (Shigemura et al., 2018; Watanabe-Kamiyama et al., 2010; Yazaki et al., 2017). Ingested collagen hydrolysate directly affects gene expression in the skin, upregulating genes involved in epidermal cell development and downregulating those involved in collagen dermal cell degradation

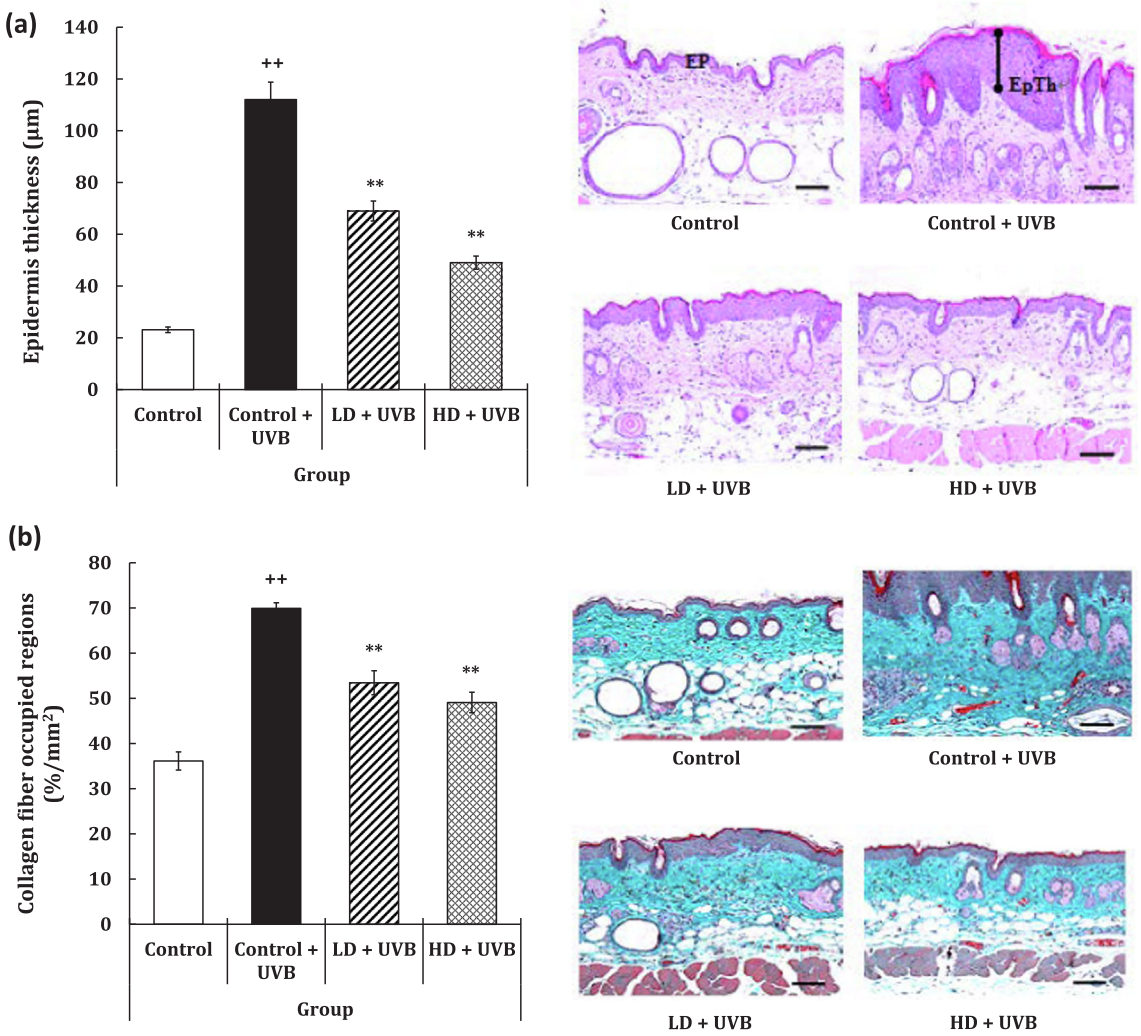


**Fig. 3.** Changes in (a) epidermis thickness, (b) collagen fiber occupied regions, (c) number of mast cells, and (d) number of dermal inflammatory cells. Scale bars = 80 μm. EP, epidermis; EpTh, epidermal thickness; HD, high-dose hydrolyzed chicken sternal cartilage extract; LD, low-dose hydrolyzed chicken sternal cartilage extract; UVB, ultraviolet B. For comparisons between nonirradiated controls and UVB–irradiated controls, ++ indicates $p < 0.01$. For comparisons between UVB-irradiated controls and test article groups, ** indicates $p < 0.01$.

(Oba et al., 2015). Recent research suggests that collagen peptides promote procollagen synthesis by activation of the transforming growth factor-β (TGF-β)/small mother against decapentaplegic (Smad) pathway, in addition to reducing collagen degradation by suppression of MMP protein expression (Liu et al., 2019).

Suppression of MMP-1 and MMP-2 was also observed in the groups administered with hydrolyzed chicken sternal cartilage extract, which correlates with these previous reports of reduced MMP protein expression following the consumption of collagen hydrolysates. Reduction of MMP-1 expression in this study was greater for the low-dose group compared with the high-dose group, an unexpected finding for which the etiology is unclear. In a similarly conducted study where a collagen tripeptide supplement was assessed for its efficacy in reducing photoaging in hairless mice, MMP-2 and MMP-9 activities were reduced to almost the same extent in groups administered either 167 or 333 mg/kg body weight/day (Pyun et al., 2012), which is also unexpected given the 2-fold difference in dosage each group received. It is unclear whether there is a potential threshold for reduction of these enzyme activities. Another potential reason could be the method of analysis (gelatin zymography), which was used in this study and the study by Pyun et al. (2012). In another similarly designed study investigating the anti-photoaging effects of a pomegranate juice powder in mice, reverse transcription-quantitative polymerase chain reaction was used for MMP analysis and the results showed a clear dose response reductions in MMP-1, MMP-9, and MMP-13 (Kang et al., 2017). While this is a compositionally different ingredient (which may also be a reason for the different results) a potential follow-up study using an alternative method for MMP analysis would provide clarity on the conflicting results for MMP expression observed in this study.

The other components of the test article (chondroitin sulfate and hyaluronic acid) are likely to have also played key roles in the reduction in photoaging observed in this study. While collagen undoubtedly plays a vital role in skin elasticity, chondroitin sulfate is required for collagen and elastic fiber formation (Kielty, Whittaker, & Shuttleworth, 1996); thus the chondroitin sulfate component of hydrolyzed chicken sternal cartilage extract will have also been instrumental in the beneficial effects on skin elasticity and wrinkles in this study. In a recently conducted clinical study using this same hydrolyzed chicken sternal cartilage extract, skin hydration was unaffected by consumption of the test article (Schwartz et al., 2019). However in this mouse study, reduction of TEWL and increased hyaluronic acid content were observed for both groups given hydrolyzed chicken sternal cartilage extract, compared with UVB-irradiated vehicle controls, demonstrating that hydrolyzed chicken sternal cartilage extract improved the skin barrier integrity and skin moisture that is compromised by UVB exposure. Interestingly, the low-dose group in this study slightly outperformed the high-dose group

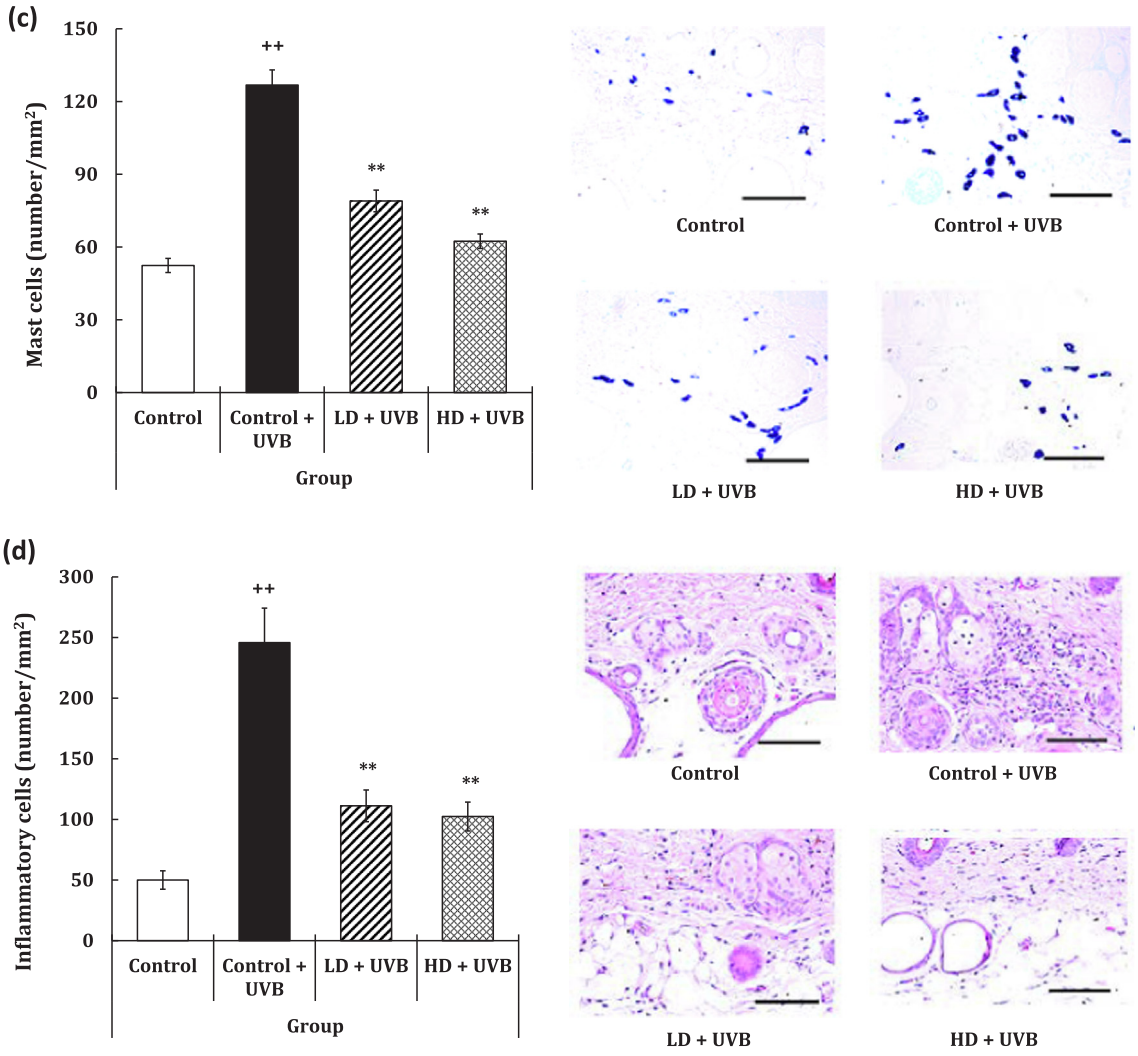


**Fig. 3.** (*continued*)

in terms of hyaluronic acid content and the reasons for this are unclear. Photoaging has been associated with reduced expression of hyaluronic acid synthase-1 (HAS-1) and increased expression of hyaluronidases (HYAL-1, -2, and -3) in the skin, which all contributes to an overall reduction in hyaluronic acid content following exposure of the skin to UV irradiation (Tzellos et al., 2009). As the key molecule involved in retaining skin moisture, the hyaluronic acid component of hydrolyzed chicken sternal cartilage extract likely played a key role in maintaining skin moisture and barrier integrity in mice that had been administered hydrolyzed chicken sternal cartilage extract in this study.

Exposure of skin to UV irradiation contributes to the accumulation of ROS and free radicals (by depletion of antioxidants and antioxidant enzymes), which in turn stimulates the production and secretion of pro-inflammatory mediators, inducing an inflammatory response via increased infiltration of inflammatory cells to the skin (Pillai, Oresajo, & Hayward, 2005). Dermal inflammatory cells (such as neutrophils) and mast cells not only release their own ECM-degrading enzymes, but also stimulate activation of MMPs (Pillai et al., 2005), further contributing to the breakdown of the ECM and ultimately many of the effects associated with photoaging. This is also corroborated by the results in this study, where in the UV-irradiated control group, degradation of the ECM led to the breakdown of collagen cross links and an increase in regions occupied by non-cross-linked collagen. The number of collagen-occupied regions was reduced by administration of hydrolyzed chicken sternal cartilage extract, which is similar to results observed in the previously mentioned study assessing the anti-photoaging effects of pomegranate juice powder in mice (Kang et al., 2017). In terms of the mechanism of its antiphotoaging effects, it is unclear whether the hydrolyzed chicken sternal cartilage extract directly reduces ROS production or increases the activities of free radical scavengers. It has been previously proposed that collagen peptides from bovine bone may increase the activities of the antioxidants superoxide dismutase and catalase (Song, Zhang, Zhang, & Li, 2017) and further research is required to determine whether his is how hydrolyzed chicken sternal cartilage extract reduces photoaging. Nonetheless, it appears evident that the reductions in dermal inflammatory cells and mast cells observed for groups administered hydrolyzed chicken sternal cartilage extract are key mechanisms in how this novel dietary supplement reduces photoaging in mice.

In conclusion, once-daily oral gavage administration of hydrolyzed chicken sternal cartilage extract at 200 or 600 mg/kg body weight/day for 14 weeks improved several signs of UVB-induced photoaging in hairless mice (increases in skin elasticity and hyaluronic acid content, as well as reductions in MMP expression, TEWL, dermal inflammatory cells, collagen fiber occupied regions and the number, area, length and depth of wrinkles, compared with UVB-exposed vehicle controls). These results were likely a synergistic effect of the natural occurring components of this novel hydrolyzed chicken sternal cartilage extract and they demonstrate that this extract is an efficacious functional ingredient for the improvement of skin health

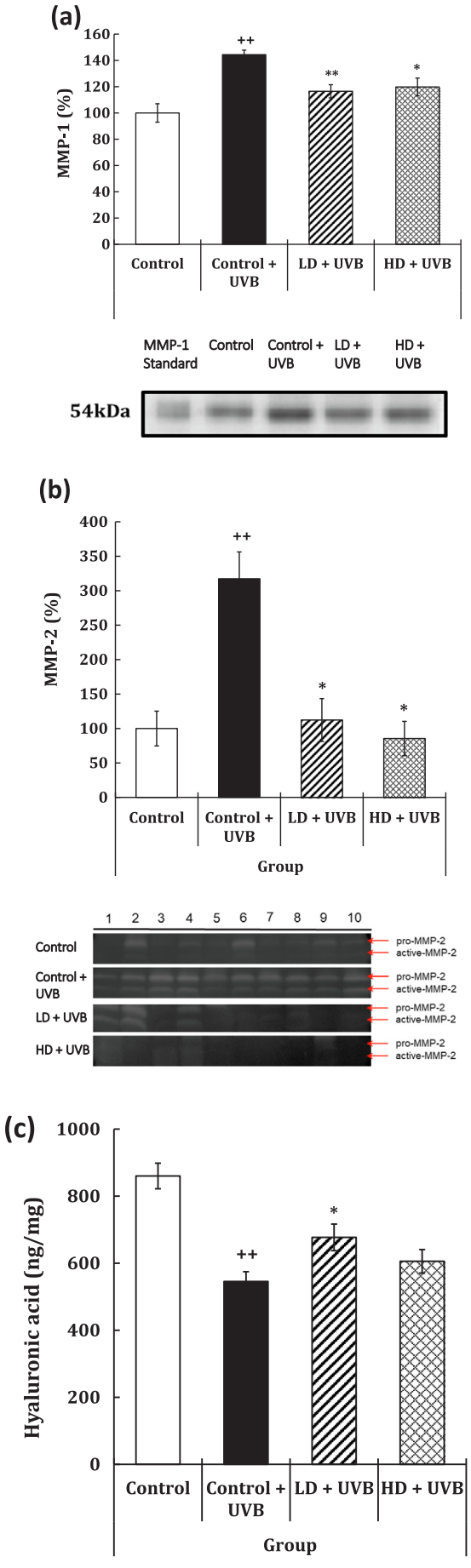


**Fig. 4.** Changes in (a) MMP-1 expression, (b) MMP-2 secretion, and (c) skin hyaluronic acid. HD, high-dose hydrolyzed chicken sternal cartilage extract; LD, low-dose hydrolyzed chicken sternal cartilage extract; MMP, matrix metalloproteinase; UVB, ultraviolet B. For comparisons between nonirradiated controls and UVB-irradiated controls, + + indicates $p < 0.01$. For comparisons between UVB-irradiated controls and test article groups, * indicates $p < 0.05$ and ** indicates $p < 0.01$.

## CRediT authorship contribution statement

**Kirt R. Phipps:** Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Han Youl Lee:** Conceptualization, Writing - review & editing. **Hoon Kim:** Conceptualization, Methodology, Validation, Resources, Investigation, Data curation, Resources, Writing - review & editing. **Boram Jeon:** Formal analysis, Investigation, Writing - review & editing.

## Declaration of Competing Interest

The study was conducted at Kyung Hee University, Technology Subsidiary Company, Skin Biotechnology Center (South Korea); Hoon Kim and Boram Jeon are employees of Kyung Hee University. BioCell Technology LLC sponsored the study and supplied the test article. Kirt R. Phipps and Han Youl Lee are employees of Intertek, which has provided consultancy services to BioCell Technology LLC.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.103870.

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