Ingestion of BioCell Collagen®, a novel hydrolyzed chicken sternal cartilage extract; enhanced blood microcirculation and reduced facial aging signs

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Abstract: Skin aging and its clinical manifestation is associated with altered molecular metabolism in the extracellular matrix of the dermis. In a pilot open-label study, we investigated the effect of a dietary supplement, BioCell Collagen® (BCC), which contains a naturally occurring matrix of hydrolyzed collagen type II and low-molecular-weight hyaluronic acid and chondroitin sulfate, in 26 healthy females who displayed visible signs of natural and photoaging in the face. Daily supplementation with 1 g of BCC for 12 weeks led to a significant reduction of skin dryness/scaling (76%, \( P = 0.002 \)) and global lines/wrinkles (13.2%, \( P = 0.028 \)) as measured by visual/tactile score. Additionally, a significant increase in the content of hemoglobin (17.7%, \( P = 0.018 \)) and collagen (6.3%, \( P = 0.002 \)) in the skin dermis was observed after 6 weeks of supplementation. At the end of the study, the increase in hemoglobin remained significant (15%, \( P = 0.008 \)), while the increase in collagen content was maintained, but the difference from baseline was not significant (3.5%, \( P = 0.134 \)). This study provides preliminary data suggesting that dietary supplementation with BCC elicits several physiological events which can be harnessed to counteract natural photoaging processes to reduce visible aging signs in the human face. A controlled study is necessary to verify these observations.

Keywords: BioCell Collagen, chicken sternal cartilage extract, hydrolyzed collagen type II, low-molecular-weight hyaluronic acid, skin aging

Introduction

In the human body, the skin is the largest organ and is involved in various functions, including sensation of temperature and pressure and protection from external insults such as pathogens and injury. The epidermis of the skin is composed of stratified squamous epithelium consisting of proliferating basal and differentiated keratinocytes. The dermis is composed of connective tissue consisting of diverse extracellular matrix (ECM) components, including collagen and elastin fibers and glycosaminoglycans (GAGs), which are synthesized by dermal fibroblasts.1

The structural integrity of the dermis is vital for the normal function and youthful appearance of the skin, as this layer lends structural support for the epidermis as well as for the vasculature and appendages of the skin. The structure of the dermis is defined by the ECM, which is primarily composed of type I collagen fibrils, making up 70% to 80% of the dry weight of the skin.2 GAGs in the skin are primarily composed of hyaluronic acid (HA) and dermatan sulfate (DS), and embedded as a ground substance into the collagen fiber network. HA can retain a large number of water molecules, creating turgidity to resist external pressure, while DS proteoglycans such as decorin are involved in interconnecting collagen fibrils to transmit force.3
The skin undergoes natural (chronological) aging, which occurs concurrently with the photoaging process in exposed areas such as the face and arms. Skin changes associated with natural aging are generally characterized by fine wrinkling and laxity, which can be worsened by chronic ultraviolet (UV) light exposure.

Clinical signs of photoaging include dryness, deep furrows, irregular pigmentation, elastosis, and a leathery appearance. The face is a site at which these changes are prominent.

Key molecular and histochemical features underlying age-dependent phenotypic alterations of the human skin include decreased collagen production in dermal fibroblasts, leading to the fragmentation and disarray of collagen fibrils in the dermis. This structural disturbance results in various physiological consequences, including interference with the mechanical properties of skin and impairment of resident cell functions in the dermis, resulting in disruption of the normal interactions of cells with the ECM microenvironment.

Additionally, both natural and photoaging processes appear to cause an imbalance between HA synthases and hyaluronidases, decreasing the integrity and amount of HA. Eventually, a cycle between defective stimulation of senescent dermal fibroblasts and deterioration of the dermal ECM occurs in association with elevated oxidative stress and heightened activity of ECM-degrading enzymes such as hyaluronidase and matrix metalloproteinases (MMPs), including MMP-1, to perpetuate skin aging.

There is growing interest in hydrolyzed collagen (or collagen hydrolysate) as a nutraceutical and nutricosmetical solution to these issues because collagen-derived peptides harbor a variety of interesting biological properties implicated in various health benefits. First, hydrolyzed collagen derived from both bovine collagen type I or chicken sternal cartilage collagen type II was shown to stimulate chondrocytes in vitro to produce type II collagen and proteoglycans. Second, ingestion of hydrolysates derived from chicken sternal cartilage or porcine skin led to the appearance of various di- and tri-peptides in human serum, with the proline-hydroxyproline (Pro-Hyp) dipeptide as the major form. Dipeptide-stimulated human dermal fibroblasts and chondrocytes in vitro synthesize HA, which is responsible for holding water molecules in the skin and making up the synovial fluid in the synovial joint. Third, studies involving animal models suggest that ingestion of hydrolyzed collagen enhances the number of fibroblasts together with the density and thickness of collagen fibrils, protecting mice against UV-induced damage in the skin such as epidermal hypertrophy, dermal dehydration, and loss of type I collagen.

Derived from the chicken sternal articular cartilage, BioCell Collagen® (BCC; BioCell Technology, LLC Newport Beach, CA) is a dietary supplement containing a soluble, naturally occurring matrix of hydrolyzed collagen type II and low-molecular-weight HA and chondroitin sulfate. To investigate its effect on skin aging, a pilot open-label study was conducted in 26 females who had undergone natural and photoaging processes in their faces. Daily BCC supplementation for 12 weeks enhanced microcirculation in the dermis, while reducing visible age-dependent signs such as wrinkles in the face.

Material and methods

Study design
This was a pilot open-label human study examining the effect of BCC ingestion on skin aging. The study protocol was reviewed and approved by an Institutional Review Board (Table 1). One group of 29 subjects was enrolled, and 26 subjects completed the study. Visits occurred at 0 (baseline), 6, and 12 weeks. Facial skin condition was evaluated visually utilizing standard ordinal and visual analogue scales (VAS), as well as instrumentally. The effect of BCC was assessed by comparing baseline values of efficacy parameters at week 0 with those obtained at week 6 and at week 12, respectively.

Study subjects: inclusion and exclusion criteria
Twenty-nine female subjects aged 35 to 59 years who showed signs of age-associated natural and photoaging in their faces were enrolled. Subjects completed a brief medical/personal history and read and signed an informed consent document prior to receiving any study instructions. Subjects who provided informed consent and pre-qualified based on their medical history were given Camay™ facial soap (Procter and Gamble, Cincinnati, OH) and were instructed to use it for all facial cleansing during the 1-week washout period and during the 12-week study period. Subjects were also instructed to discontinue use of all other topical facial treatment products, including moisturizers, for the duration of the washout period and the study. Subjects underwent baseline visual assessments and were enrolled based on the following inclusion/exclusion criteria.

All enrolled subjects were healthy females of any ethnic origin and had mild to moderate photodamaged skin and mild to moderate fine lines and wrinkles in the crow’s feet region of the left eye scoring 1–6 centimeters on 0–10 cm VAS. They were free of any dermatologic disorders and willing to use no skin firming, antiaging, antiwrinkle, skin
lighten, or any topical or systemic medication known to affect skin aging or dyschromia (products containing α/β/poly-hydroxy acids, vitamin C, soy, Q-10, hydroquinone, systemic or topical retinoids, etc) during the study.

Subjects were excluded if they had known allergies to test product ingredients or an uncontrolled disease such as diabetes, hypertension, hyperthyroidism, or hypothyroidism. Those who were pregnant, breast-feeding, or currently planning a pregnancy were also excluded. Those who had used a dietary supplement containing collagen, HA, chondroitin sulfate, or any of their combination within the last 3 months prior to enrollment and those who had superficial to mid-deep chemical peels or dermabrasion within 6 weeks, a deep facial chemical peel, nonablative laser or fractional laser resurfacing within 12 months, facial plastic surgery or ablative laser resurfacing for photoaging within 3 years, or a regimen of Thermage treatments or an equivalent type of high-energy treatments on the face within 12 months prior to enrollment were also excluded.

Supplements and doses

The dietary supplement used in the study was BioCell Collagen® (BioCell Technology, LLC), a hydrolyzed chicken sternal cartilage extract composed of a naturally occurring matrix of hydrolyzed collagen type II (molecular weight range of 1–2.5 kDa), low molecular weight HA, and chondroitin sulfate. Each capsule contained 500 mg of BCC, providing a naturally occurring composition of hydrolyzed collagen (300 mg), depolymerized chondroitin sulfate (100 mg), and HA (50 mg). The remaining 50 mg derived from other natural components of the sternal cartilage was not characterized further.

All the subjects were instructed to take two capsules (1 g) daily in two equally divided doses, one capsule in the morning and one in the evening. This dose was considered to be safe, as a 2-g dose of BCC had been shown to be well-tolerated without associated adverse events in osteoarthritic patients enrolled in a previous clinical trial.16

Baseline visit

Subjects arrived at the study site without make-up on their faces. They underwent baseline visual assessments after their skin was allowed to equilibrate to room temperature and humidity for 15 minutes before evaluation. Subjects were given the product, instructions, and a daily diary to record product consumption times, and advised to inform the laboratory immediately if any adverse reactions were noted. They were then dismissed from the site with instructions to return for their 6- and 12-week visit(s).

Protocol violation

Two protocol violations were recorded during this study. One subject did not undergo NOVA meter (Nova Technologies, Irvine, TX) recording at baseline, and subsequent data points were not used for comparison. Subjects were considered in compliance if they missed no more than three product dosing days during the study. The principal investigator evaluated compliance on a case-by-case basis if a subject missed more than three doses. One subject was found to be noncompliant at the 6-week visit, and was discontinued from the study. Another subject withdrew consent after enrollment.

Tolerability and efficacy variables

If a subject experienced a reaction during the study, she was to report for further instructions. Product use could be discontinued at the investigator’s or study coordinator’s discretion. However, no adverse events were recorded during the study.

To measure dryness/scaling, the facial skin of the subjects was evaluated at baseline and at subsequent visits using the ordinal scales as follows: 0 = None: Smooth, no evidence of dryness; 1 = Mild: Slight dry skin; powdery, ash appearance; 2 = Moderate: Uplifting cell layers; 3 = Moderate to Severe: Peeling, flaking cell layers; 4 = Severe: Severe flaking and peeling; possible fissures.

The facial skin of subjects was evaluated at weeks 0, 6, and 12 for facial fine lines/wrinkles, hyperpigmentation, and skin tone and texture using VAS. VAS is a method by which a numerical assessment value can be evaluated for subjective characteristics or attitudes that cannot be directly measured. VAS scales are widely used for dermatologic research, such as studies examining skin itch sensation and skin eczema.17,18 When responding to a VAS item, an evaluator specifies his/her level of agreement to a statement by indicating a position along a line (10 cm) between two end-points or anchor responses. Responses are categorized as follows: Mild = 1–3.9; Moderate = 4–6.9; or Severe = 7–10.

Facial skin was also evaluated employing the following bioinstrumentation, and subjects’ faces were equilibrated to indoor ambient temperature and humidity for at least 15 minutes prior to instrumental evaluations.

NOVA meter

The relative degree of skin hydration was assessed using the dermal phase meter (DPM) 9003, which is known as a NOVA meter (Nova Technologies). The NOVA meter is used to measure the hydration level of the stratum corneum.19,20
NOVA meter measurements were conducted by applying an alternating voltage to the skin with a closely spaced pair of electrodes and measuring impedance. Changes in water content alter the impedance of the capacitive circuit. The DPM device produces values of skin surface impedance expressed as arbitrary units (range 90–999), which in the skin is a correlate of capacitance. Measurements were made on one side of the face, and the same side of the face was measured at each visit.

**Cosmetics™ SIAscope**

Noninvasive spectrophotometric intracutaneous analysis (SIA; Astron-Clinica, Cambridge, UK) was employed to measure structural molecule content in the dermis. Previous studies have demonstrated that this method can be used to measure blood and dermal melanin.\(^{21,22}\) The technique is based on a unique combination of dermatoscopy and contact remittance spectrophotometry. The hardware consists of a handheld imaging probe attached to a laptop computer. The unit is placed in contact with the skin surface, and a high-intensity light emission diode illuminates the skin with wavelengths of 400–1000 nm, spanning the visible spectrum and a small range of the near-infrared spectrum. A digital image is captured for each waveband. Custom SIA algorithms are then used to evaluate the complex relationship between red–green–blue “color-space” and melanin–hemoglobin–collagen “histology-space”, using a sophisticated model of cutaneous light-transport.\(^{23,24}\) Since there is a proven one-to-one mapping between color space and chromophore parameters, individual chromophore parameter values can be retrieved from the model, giving the color vector obtained from each point as a color skin image.\(^{25,26}\) The magnitude of each chromophore parameter can be displayed at each pixel location in a separate image, giving three parametric maps: epidermal melanin, dermal hemoglobin, and collagen (a dermal melanin map is also provided as a diagnostic criterion for melanoma). Thus, the SIA technique can be used to obtain a high-resolution white-light image of the skin over a 12 × 12 mm area and four additional maps displaying the concentration of epidermal melanin and hemoglobin, collagen, and melanin in the papillary dermis, pixel by pixel. Evaluation of the skin was performed on one side of the face at 0, 6, and 12 weeks.

**Statistical methods**

Efficacy and instrumental data mean scores were calculated and analyzed at each time point. Values from 6 and 12 weeks were compared to baseline values using the paired samples t-test, analyzed for change as a function of time and treatment. Mean percent change from baseline was reported. Change was considered significant when \(P \leq 0.05\).

**Results**

**Basic demographics and facial skin features**

Demographics of the 26 subjects who completed the study are summarized in Table 2. Their ages spanned from 35 to 59 years, with an average of 50.65, and most were Caucasian (57.7%), followed by African American (23.1%) and Hispanic (15.4%). All subjects had mild-to-moderate photo-damaged skin and mild-to-moderate fine lines and wrinkles in the crow’s feet region of the left eye as manifested by the scores of 1–6 centimeters on 0–10 cm VAS scoring.

**Reduction of global facial lines and wrinkles**

Skin aging in the face culminates with the appearance of visible signs such as wrinkles and lines. Measurement of these aging signs using visual/tactile scores showed that 12 weeks of

<table>
<thead>
<tr>
<th>Table 1 Study design</th>
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<tbody>
<tr>
<td><strong>Procedure</strong></td>
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<tr>
<td>Obtain informed consent</td>
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<tr>
<td>Evaluation of inclusion/exclusion criteria</td>
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<tr>
<td>Relevant medical history</td>
</tr>
<tr>
<td>Dispensal of investigational product</td>
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<tr>
<td>Dispensal of patient diary</td>
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<tr>
<td>Skin evaluation using visual analog scale</td>
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<tr>
<td>Skin evaluation with bioinstruments</td>
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<tr>
<td>Gather adverse event reports</td>
</tr>
<tr>
<td>Recovery of patient diary</td>
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<tr>
<td>Recovery of investigational product</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Baseline characteristics of the study subjects</th>
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</thead>
<tbody>
<tr>
<td>Number of subjects</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Mean ± sd (years)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>African American 6 (23.1%)</td>
</tr>
<tr>
<td>Hispanic 4 (15.4%)</td>
</tr>
<tr>
<td>Other 1 (3.8%)</td>
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</table>

**Note:** ± standard deviation.
supplementation with BCC led to significant improvement of facial lines and wrinkles with an average reduction of 13.2% \((P = 0.028)\) from baseline (Table 3). An initial increase was noted at week 6 (22.4%, \(P < 0.001\)). Another facial aging sign, crow’s feet, displayed a similar trend with a significant increase during the first 6-week period (50.7%), followed by a significant decrease (−30.4%) in visible aging signs between weeks 6 and 12. Unlike facial lines and wrinkles, however, crow’s feet were not affected significantly by the end of study compared to the baseline level.

**Reduction of skin dryness/scaling**

Facial dryness and scaling may be associated with the water content in the skin and the skin aging process. Skin dryness or scaling was measured using visual/tactile scores; its mean value at week 12 decreased significantly by 76\% \((P = 0.002)\) from the baseline level (Table 3), suggesting that BCC ingestion reduces skin dryness and scaling.

To observe the effect of BCC on skin hydration level, a NOVA meter was used to compare water content of the stratum corneum before and after BCC consumption. Table 4 shows that water content increased by 8\% at week 12, although this value was not significant \((P = 0.210)\). Similarly to facial aging signs shown in Table 3, hydration level during the first 6 weeks decreased by 5.4\%, but this value was not significant \((P = 0.420)\). A significant increase of 12.5\% \((P = 0.003, \text{data not shown})\) occurred between weeks 6 and 12.

**Enhancement of hemoglobin content**

The Cosmetics™ SIAscope was used to measure hemoglobin content in the dermis to investigate how microvascular blood circulation is affected by daily ingestion of BCC. The amount of hemoglobin significantly increased by 17.7\% \((P = 0.018)\) by week 6 and by 15.0\% \((P = 0.008)\) by week 12 (Table 4), suggesting that intake of BCC enhances dermal microcirculation in the face.

The SIAscope was also used to evaluate how the metabolism of key molecules in the skin such as collagen and melanin are affected by BCC supplementation. Table 4 shows that dermal collagen content significantly increased by 6.36\% \((P = 0.002)\) by week 6 from baseline, but the 3.45% increase by week 12 was not significant \((P = 0.134)\). Melanin content was not affected significantly.

**Adverse events**

During the 12-week study period, no adverse events were reported, suggesting that daily ingestion of 1 g of BCC was well-tolerated by the subjects (data not shown).

**Discussion**

Skin aging in the face involves physiological changes in the underlying tissues and is manifested as visible signs such as dryness, laxity, and wrinkles. The results from this study enrolling women who had undergone both natural and photoaging showed that daily supplementation with BCC counteracts some of these changes, as their facial skin presented with fewer visible aging signs.

Daily ingestion of BCC for 12 weeks led to a significant decrease in facial lines and wrinkles as well as in skin dryness and scaling. Improvement of these visible aging signs appears to be correlated with physiological changes in the metabolism of molecules comprising the epidermal and dermal tissues. First, the amount of hemoglobin was elevated in the dermis, implying that blood circulation

### Table 3 Effect of BioCell Collagen ingestion on facial skin appearance

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Week 0 (mean ± SD)</th>
<th>Week 6 (mean ± SD)</th>
<th>% change</th>
<th>Week 12 (mean ± SD)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wk 6-wk 0</td>
<td>P-value</td>
<td>Wk 12-wk 0</td>
</tr>
<tr>
<td>Global lines/wrinkles</td>
<td>4.01 ± 1.00</td>
<td>4.91 ± 1.12</td>
<td>22.4</td>
<td>&lt;0.001</td>
<td>3.48 ± 1.56</td>
</tr>
<tr>
<td>Crow’s feet</td>
<td>2.88 ± 0.74</td>
<td>4.34 ± 0.95</td>
<td>50.7</td>
<td>&lt;0.001</td>
<td>3.02 ± 1.28</td>
</tr>
<tr>
<td>Dryness/scaling</td>
<td>0.50 ± 0.51</td>
<td>0.38 ± 0.57</td>
<td>−24.0</td>
<td>0.416</td>
<td>0.12 ± 0.43</td>
</tr>
</tbody>
</table>

**Abbreviation:** SD, standard deviation.

### Table 4 Effects of BioCell Collagen ingestion on epidermal and dermal components

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Week 0 (mean ± SD)</th>
<th>Week 6 (mean ± SD)</th>
<th>% change</th>
<th>Week 12 (mean ± SD)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wk 6-wk 0</td>
<td>P-value</td>
<td>Wk 12-wk 0</td>
</tr>
<tr>
<td>Hydration</td>
<td>155.7 ± 44.8</td>
<td>147.3 ± 45.9</td>
<td>−5.4</td>
<td>0.420</td>
<td>168.2 ± 48.5</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>55.5 ± 15.0</td>
<td>65.3 ± 32.9</td>
<td>17.7</td>
<td>0.018</td>
<td>63.8 ± 39.4</td>
</tr>
<tr>
<td>Collagen</td>
<td>276.8 ± 128.0</td>
<td>294.4 ± 142.3</td>
<td>6.4</td>
<td>0.002</td>
<td>286.4 ± 149.6</td>
</tr>
<tr>
<td>Melanin</td>
<td>217.4 ± 117.9</td>
<td>212.6 ± 120.6</td>
<td>−2.2</td>
<td>0.169</td>
<td>216.7 ± 121.9</td>
</tr>
</tbody>
</table>

**Abbreviation:** SD, standard deviation.
through the microvasculature in the facial skin improved. As blood carries oxygen, nutrients, and growth factors to the tissue while removing metabolic wastes, improved microcirculation may lead to enhanced homeostasis of the skin. For example, efficient delivery of oxygen and nutrients to the dermis can nourish resident cells, including the dermal fibroblasts, while the structure of dermal ECM may resist various stresses caused by the oxidative aging process.

During the first 6 weeks, there was a significant increase in global fine lines and wrinkles. The cause of this initial worsening of facial aging signs was not clear. Potential factors involved include the physiology (eg, menstruation cycle) of the subjects, seasonal change, or other confounding factors. A placebo-controlled clinical trial is required to exclude these factors in revealing the true effect of BCC.

Second, a significant decrease in skin scaling and dryness appeared to be accompanied by an increase in the water content of the stratum corneum by 8.0% at week 12 from baseline values. However, this increase in hydration level was not significant, suggesting that multiple factors contribute to skin scaling and dryness. Interestingly, although the retention of water molecules in skin tissues is primarily mediated by HA, there is evidence that collagen fibrils may help hydrate the skin, as intake of hydrolyzed collagen protected mice against dermal dehydration. It can be speculated that improved health of the epidermis may be due to the effect of daily use of Camay soap. Camay bar soap is primarily used in clinical studies for subject compliance reasons, as it properly cleanses the skin without over-drying or irritation to the skin. A blinded, controlled study should be conducted to normalize any effect from the use of Camay.

Third, ingestion of BCC was accompanied by increased collagen content in the dermis. Unlike other efficacy parameters, however, its increase was significant at week 6, but not at week 12. It was not clear why the effect of BCC dampened as the study progressed. Seasonal or dietary factors may have been involved. A controlled study including more subjects is needed to verify the effects of BCC not only on collagen content but also on the content of diverse skin molecules and facial aging signs.

Despite its limited effect on collagen content, BCC supplementation appeared to affect metabolic balance between biosynthesis of collagen type I and/or type III by dermal fibroblasts and their degradation by MMPs. This is intriguing because collagen present in BCC is predominantly type II, as it is extracted from chicken sternal cartilage which belongs to articular or hyaline cartilage. One of the major constituents of BCC is hydrolyzed collagen type II. Interestingly, both hydrolyzed collagen type II derived from chicken sternal cartilage, and hydrolyzed collagen type I derived from bovine skin, stimulate chondrocytes to produce collagen type II in vitro, which was likely due to a high level of homology in their amino acid sequences. Additionally, the Pro-Hyp dipeptide, a major peptide form present in the human blood following the ingestion of either the hydrolyzed chicken cartilage extract or hydrolyzed collagen derived from bovine skin, was shown to stimulate human dermal fibroblasts to produce HA in vitro. It remains unknown whether the dipeptide or other biologically active compounds derived from hydrolyzed chicken cartilage extract can directly stimulate dermal fibroblasts for de novo biosynthesis of collagen type I, which is most abundant in the skin dermis.

This study provided preliminary evidence that ingestion of BCC, a hydrolyzed chicken sternal cartilage extract, affects aging-associated physiological processes and reduces visible aging signs in the face. A controlled, long-term human study as well as in vitro studies is necessary to verify the antiaging effect of BCC supplement and to better understand its potential mechanisms.

Acknowledgments/disclosure

The supplement, BioCell Collagen®, was provided by GMP Laboratories of America, Inc (Anaheim, CA). The study was funded by BioCell Technology LLC (Newport Beach, CA). At the time of manuscript submission, JP was an employee of BioCell Technology LLC (Newport Beach, CA); SRS has no financial interest to report.

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