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Immediate and Long Term Clinical Benefits of a Novel Topical Micronized Collagen Face Cream

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Abstract

Collagen has been a component of skin care formulations for many years, and over this time, there have been numerous claims of its efficiency. Collagen protein is responsible for firm strong skin, but since collagen fibers are too large to penetrate the stratum corneum (SC), topical creams containing collagen fibers remain on the skin surface without affecting skin quality. To overcome the poor penetration of collagen fibers, we prepared in the past micronized collagen fibers that were proven to reach the epidermis layer while inserted in a cream. In the present paper, we have performed a clinical study that analyzes the effect of the micronized fibrillar collagen containing cream on skin. Fifty five healthy female volunteers were enrolled and completed the study. The anti-ageing, firming, elasticity and moisturization efficacy of the cream were measured using Profilometer, Cutometer and Corneometer respectively. The results showed a significant improvement in skin hydration firmness and elasticity, a significant reduction in fine lines and wrinkles was also observed.

Keywords

Micronized Collagen Face Cream, Clinical Studies, Skin Parameters, Penetration

1. Introduction

Collagen is the primary connective tissue protein that conforms skin and cartilage. The most abundant types of collagen in the skin are I and III, their fibrils being largely responsible for the skin's mechanical properties such as strength, texture and resilience [1].

From early adulthood, the production of collagen is decreased by about 1.0% -

1.5% a year. Many factors are involved in the reduction in age-related collagen production [2]. It is thus clear why collagen has been a component of skin care formulations for many years.

However, despite the wide distribution of collagen containing products in the market, clinical studies on skin rejuvenation following topical collagen treatment are lacking [3]. It has been assumed that topically applied collagen can replace the native one, but unfortunately collagen fibers are too large to penetrate the stratum corneum (SC) [4]. Since the effect of collagen is ascribed to its penetration depth [4] it can be assumed that a topically applied collagen cream has little to no effect on the skin.

To overcome this problem, hydrolyzed collagen has been introduced to many cosmetic formulations. The hydrolyzed collagen (mainly derived from bovine, or marine collagen) is collagen broken enzymatically or chemically to its amino acids and/or peptides components. Amino acids and peptides are small enough to penetrate the skin [5], but they differ totally from collagen characteristics [6].

To overcome the poor penetration of collagen into skin, we have recently prepared, using a new technology, micronized collagen (m-collagen) fibers, that have been shown to reach the epidermis layer [7].

The aim of this study was to analyze the short and long term effect on facial skin of female volunteers following application of the new facial cream prepared by Hava Zingboim Ltd. "Deep Skin Technology".

For evaluation of cream efficacy, Profilometer, Cutometer and Corneometer measurements were studied at predetermined time intervals. Irritation and sensitization potential of the cream were also evaluated.

The clinical results of this study demonstrate that this uniquely formulated m-collagen crème leads to significant improvements in many skin parameters.

2. Study Design and Methods

2.1. Cream Formulation

The m-collagen was inserted, at 0.2% concentration, in basic cream consisting of: aqua re-micronized hyaluronic acid, cetyl alcohol, silybum marianum ethyl ester, isoamyl laurate, hydrogenated soybean oil, steareth-21, cetearyl ethylhexanoate, castor isostearate succinate, theobroma cacao (cocoa) seed butter, hydroxyethyl urea, steareth-2, sodium PCA, sodium lactate, fructose, glycine, niacinamide, urea, inositol, tocopherol, 1,2-hexanediol, dimethicone, caprylyl glycol, xanthan gum, polyacrylate crosspolymer-6, trisodium ethylenediamine disuccinate, magnolia officinalis bark extract, ethylhexylglycerin, fragrance, phenoxyethanol.

2.2. Clinical Protocol

The study was performed by PCR Corp (Essex, United Kingdom), from May to June, 2021. Fifty five (55) healthy subjects aged 35 to 50 years (Fitzpatrick 2 - 5) were enrolled in the study. The study schedule is summarized in **Table 1**.

Table 1. Study schedule.

Visit number	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Time point	Wash out Phase	Baseline	24 Hour	2 weeks	4 weeks
Informed consent	X				
Pre-treatment questionnaire	X				
Profilometer		X		X	X
Cutometer		X		X	X
Corneometer		X (at 1, 3, 5, 12 H)	X	X	X
Visual/Tactile evaluation		X		X	X
Self-perception questionnaire				X	X

The following Exclusion criteria were applied:

Subject is pregnant, breastfeeding, or planning to become pregnant.

Subject is currently using concurrent medication likely to affect the response to the test article or confuse the results of the study including anti-depressants, and Botox/collagen fillers.

Heavy alcohol consumption in the opinion of the investigator.

A fever in the last 12 hours, prior to start of the study.

Significant past medical history of hepatic, cancerous, multiple sclerosis, high blood pressure, renal, Thrombosis/Phlebitis, cardiac, pulmonary, digestive, hematological, neurological, locomotor or psychiatric disease, which in the opinion of the investigator would compromise the safety of the subject.

Insulin-dependent diabetes.

Participating, at PCR or other clinical testing facility, in a study utilizing the same test site (Face) or product or with conflicting inclusion/exclusion criteria.

Pacemaker.

Photo Epilepsy for Light Therapy.

All subjects gave written informed consent and agreed to the following prohibitions and restrictions for the duration of the study.

Subject agrees to keep using only the bar of soap (Dove) given at the washout period for the duration of the study and to continue using the same make up for the duration of the study.

Subject agrees to not use any foundation or other facial make up products. Eye makeup (not to be used on the crow's feet or cheeks) and lipstick allowed.

Subject agrees to only use (as instructed) the test article provided for the duration of the study.

Avoid Area: Metal pins/plates or silicone implants in face, open cuts and abrasions, skin and eye infections, severe sunburn, conjunctivitis, styes, and in-flare eczema/psoriasis on face.

Subject agrees to attend all visits with clean face, free of makeup.

Subject agrees to not use any moisturizing products on the face.

Subject agrees to not bathe during the first 24 hour period of the study.

The study conformed to the requirements of the Declaration of Helsinki (including its subsequent amendments [8]. And in the spirit of the ICH Guidelines on Good Clinical Practice, 1996 [9] and other recognized guidelines.

All subjects were given the investigational product and instructed to apply it twice a day (to clean and dry skin). The participants were instructed to apply 1 pump of cream to the entire face including both sides of the eyes (without applying to upper and lower eyelids), massaged gently until fully absorbed.

The subjects were instructed to report any adverse effects.

2.3. Skin Profilometry

2.3.1. Site Identification, Relocation and Replica Generation

Profilometry of periobital skin (lateral canthus/smile lines) was obtained on replica.

A detailed procedure was used to identify replica location and facilitate relocation at subsequent visits: The subjects were positioned on their backs with their face to the side, looking over their shoulder. Test sites were located using "replica locating rings" ensuring that each ring lied flat on the skin (the skin wasn't stretched or pulled during ring placement). The ring was placed in the center of the crow's feet area with the tab directed towards the hairline. The foam and paper portions of each ring were aligned. Transparency film was placed over the subject's face and landmarks were traced onto the film. The film was removed from the face, labelled to identify the subject, and stored in a cool, dry location until next use.

For replica generation the subjects were placed in an identical manner to that described above, and landmarks on the transparency film were lined up with the subject's facial features. A skin marker pen was used to make dots through the film onto the face of the subject to enable exact location of the test sites. The ring was positioned and filled with Silflo® (JS Davis, Hart) material. Once the replica has set completely (~5 minutes) it was removed and allowed to dry (skin side up) for a few minutes.

2.3.2. Profilometric Analysis

Collimated light source was directed at a 25˚ angle from the plane of the replica. The replica was placed in a holder that fixes the direction of the tab position so that the replica can be rotated to align the tab direction normal or parallel to the incident light direction.

The general background gradient of light intensity was adjusted by applying a 1st order correction in the direction of the light propagation. The shadow texture produced by the oblique lighting of the negative replica was analyzed by measuring the luminance along a set of 10 equal length parallel lines (passes) running across the replica parallel to the lighting direction. The variations in luminance were treated as indicative of the roughness and analyzed by traditional surface roughness statistics. The generated Rz value reports the average maximum difference in luminance value for five equal length segments in each of the

10 lines traversing the sample. Unless specified otherwise, 2 sets of measurements were produced for each replica: Normal (N) and Parallel (P). Analysis of the results depends on the direction of the Tab relative to features of the skin surface.

The topography of the skin was analyzed by a SONY solid state B&W camera, 50 mm lens/30mm extension, Coreco TCI Ultra frame grabber and OPTIMAS v6.5, Microsoft EXCEL 2007, StatSoft STATISTICA 7 on IBM compatible Pentium III 500 Mhz with 1 GB memory running under Windows XP Professional.

2.4. Skin Firmness (R0) and Elasticity (R2, R5, and R7)

The firmness and elasticity were measured using the Cutometer® MPA 580 (Courage and Khazaka, Germany). The measuring principle is based on the suction method. Negative pressure is created in the device and the skin is drawn into the aperture of the probe. Inside the probe, the penetration depth is determined by a non-contact optical measuring system. This optical measuring system consists of a light source and a light receptor, as well as two prisms facing each other, which project the light from the transmitter to receptor. The light intensity varies due to the penetration depth of the skin. The measurements for passive stretching were recorded. This measurement was displayed as curves at the end of each measurement using Windows® based software.

The software of the Cutometer® MPA 580 allows to calculate the below mentioned parameters from the different portions of the measurement curve.

R0: Stretchability/firmness, the maximum amplitude of the curve (measured in mm).

R2: Visco-elasticity, the resistance to the mechanical force versus ability of returning (measured in %).

R5: Net elasticity, the elastic portion of the suction part versus the elastic portion of the relaxation part (measured in %).

R7: Portion of the elasticity compared to the complete curve (measured in %).

Measuring mode 1 was used with constant suction of 400 mbar for five seconds followed by relaxation time of 3 seconds with three repetitions.

2.5. Skin Hydration (Corneometer)

Moisture levels in the skin were measured by testing changes in skin electrical properties. The Corneometer CM 825 (Courage and Khazaka, Cologne, Germany) was used to measure the electrical capacitance/hydration of the skin.

This instrument probe works on the principle that water has a higher dielectric constant than most other substances which affects capacitance. The measuring capacitor of the probe shows changes of capacitance according to the moisture content of the samples.

Three replicate measurements were taken on designated test sites at each interval.

2.6. Visual Assessment

Skin sagging on cheek, global wrinkles, and global fine lines were evaluated vi-

sually and graded. A tactile evaluation of skin firmness/tightening and skin elasticity was performed.

The grading scale for each parameter is presented in Table 2.

2.7. Self-Perception Questionnaire (SPQ)

Subjects completed a self-perception questionnaire (SPQ) on Day 14 (Week 2) and on Day 28 (Week 4). The questionnaire consists of 34 questions (some repetitive to ensure correct answer).

2.8. Irritation and Sensitization Potential of the Cream

The objective of this study was to investigate the irritation and sensitization potential of the cream. A repeated cutaneous occlusive patch applications based on the modified Draize method of Jordan and King [10] to support claims such as "Dermatologically Tested", "Hypoallergenic", "Clinically Tested", "Kind to Skin" and "Safe for Skin" were performed.

The study was conducted single blind, at a single center.

Cream was patched under occlusive conditions using Finn chambers or equivalent occlusive patches. A total of nine inductions patches worn for 47 hours or 71 hours (patching occurred Mondays, Wednesdays and Fridays) for three weeks (a makeup day was allowed to ensure subjects had all 9 induction patches). Subjects had a rest period of 14 days. Challenge patches were applied for 48 hours, and readings were made 1 hour and 48 hours post removal.

2.9. Statistical Analysis

Statistical analyses were performed using t-test. Statistical significance was set at 0.05.

3. Results

No adverse events or reactions were reported, all 55 subjects enrolled, completed the study.

3.1. Skin Profilometry

The newly prepared cream significantly reduced fine lines and wrinkles as measured by profilometry (Figure 1) Rz values ($p < 0.05$). A within-treatment analysis

Table 2. Clinical grading scale: Best possible condition (!!) and worse possible condition (*).

Descriptors	Type of Grading	0	1	2	3	4	5	6	7	8	9
Skin Sagging on cheek	Visual	(!!)		Mild			Moderate			(*)	
Global Wrinkles	Visual	(!!)		Mild			Moderate			(*)	
Global Fine lines	Visual	(!!)		Mild			Moderate			(*)	
Skin Firmness/tightening	Tactile	(*)		Moderate			Mild			(!!)	
Skin Elasticity	Tactile	(*)		Moderate			Mild			(!!)	

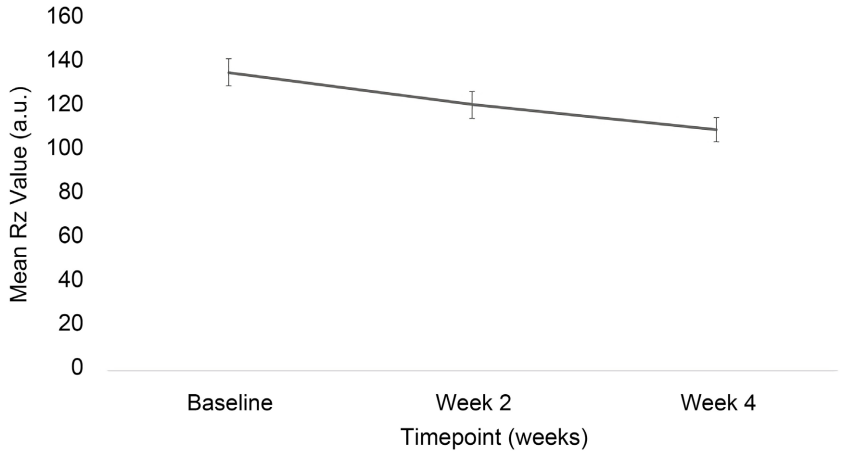

Figure 1. Skin profilometry.

showed data from weeks 2 and 4 were significantly ($p < 0.05$) reduced by 10.91 and 19.24% respectively, compared to baseline readings.

3.2. Skin Elasticity (R2, R5, and R7) and Firmness (R0)

Application of the cream significantly enhanced skin elasticity (Cutometer R2, R5 and R7 values), (**Figure 2(a)**) and skin firmness as measured by Cutometer R0 value (**Figure 2(b)**) ($p < 0.05$). A within-treatment analysis showed data from weeks 2 and 4 were significantly improved when compared to baseline readings ($p < 0.05$).

3.3. Skin Hydration (Corneometer)

Skin hydration/moisture were calculated using Corneometer CM 825 (Courage and Khazaka, Cologne, Germany). The Corneometer measures the changes in electrical capacitance of the skin which is related to the moisture content of the skin. The results clearly showed that hydration levels were improved following application of the tested cream both immediately after use and at each sequel time point (at 1, 3, 5, 12, 24 hours and at 2, 4 weeks). A within treatment analysis presented the measured data to be significantly improved when compared to baseline readings ($p < 0.05$). Mean percentage changes can be found in **Table 3**.

3.4. Visual and Tactile Assessment

Visual and tactile data showed positive trends with means improving at both weeks 2 and 4 when compared to baseline means (**Figure 3**).

A negative trend was observed in skin sagging on cheeks, fine lines and wrinkles, which means positive results. A 21, 25 and 35 percent decrease in skin sagging, wrinkles and fine lines, respectively was observed after only 2 weeks of treatment, after 4 weeks the effect was even more pronounced with more than 50% reduction in all visible parameters measured. An almost linear correlation ($R2 = 0.9853$, 0.9894, and 0.9992 respectively), between elapsed time and elimination of sagging, fine lines and wrinkles was observed.

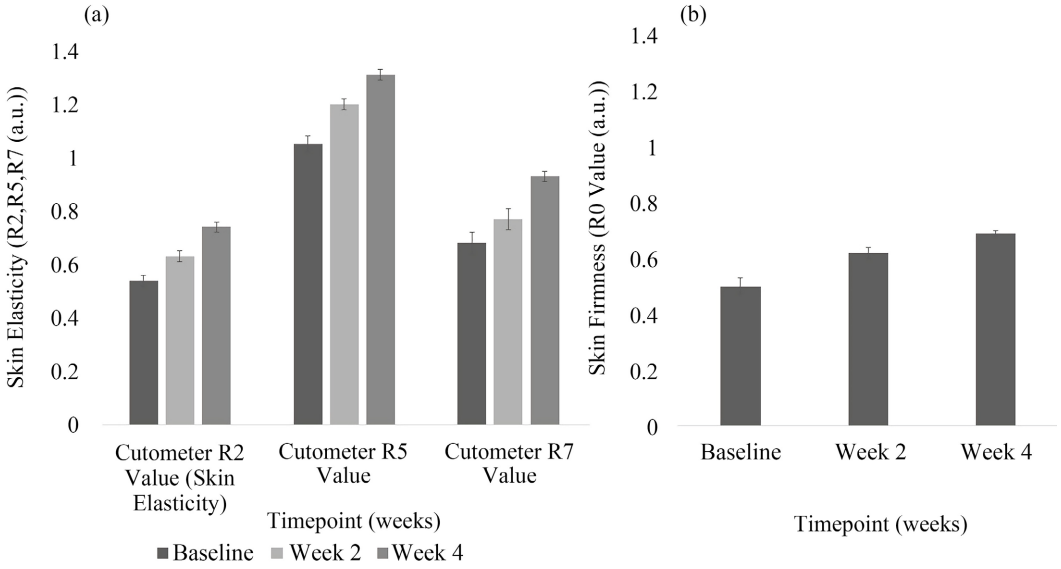

Figure 2. Cutometer measurements of: (a) elasticity (R2, R5, and R7) and (b) skin firmness (R0).

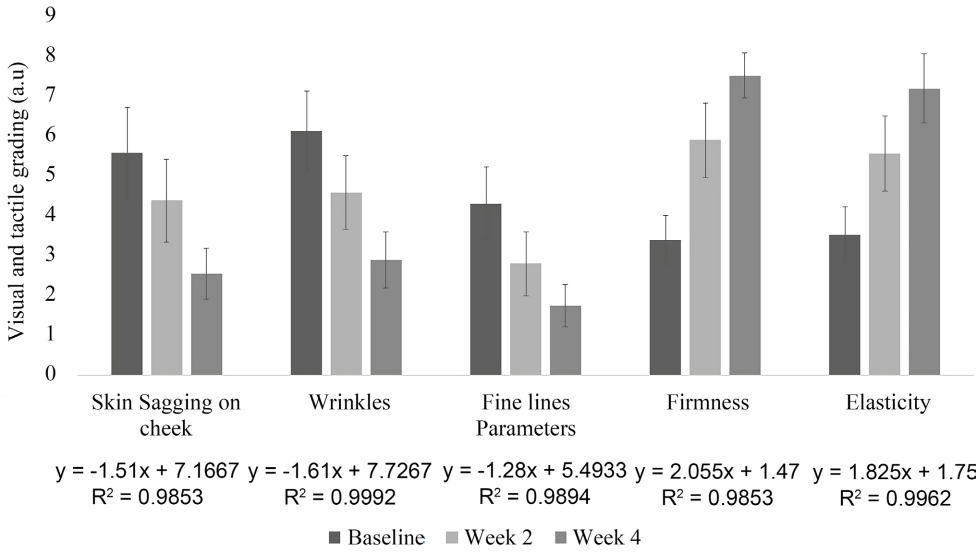

Figure 3. Visual and tactile assessment of skin parameters.

Table 3. Skin hydration percent.

Time point	Percent Change
Immediately post application	198.95%
1 Hour	207.74%
3 Hours	197.63%
5 Hours	186.05%
12 Hours	142.96%
24 Hours	41.84%
Week 2	166.18%
Week 4	197.35%

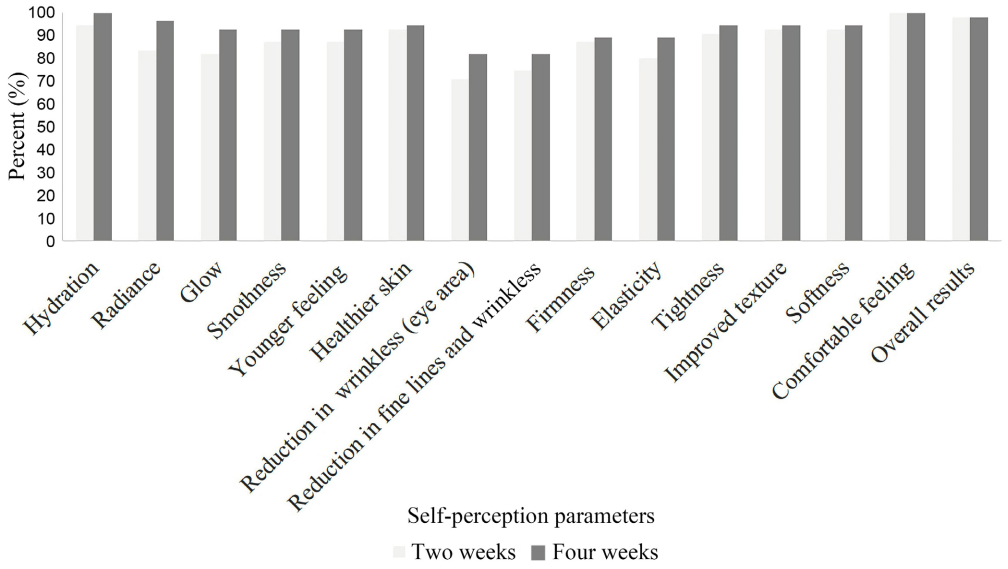

Figure 4. Results of selected questions from Self-perception questionnaire.

A tactile assessment of skin revealed around 2.0 fold elevation in firmness and in elasticity.

3.5. Self-Perception Questionnaire (spq)

All volunteers did not report any adverse reactions. Vast majority of the volunteers expressed a highly positive judgment regarding the performances of the tested cream (**Figure 4**); 98% were satisfied with simplicity of application, 96% reported that their skin felt more hydrated and moisturized right after the first application of the cream. 92.73% were pleased with the results after 4 weeks. Moreover, in a follow up question 94.55% were amazed by the results. A 96.36% answered that they never had results like these after 4 weeks.

3.6. Irritation and Sensitization Potential

Total 111, male (41) and female (70), subjects (age~32) were enrolled into the safety study. 109 subjects completed the study (2 subjects withdrew for personal reasons).

No adverse events or reactions were reported.

There were no deviations that occurred during the conduct of the study.

There were no questionable reactions observed during the Challenge Phase (Days 38 and 40) by any of the subjects. These results support that the tested cream demonstrates low potential of irritation or sensitization.

4. Summary and Discussion

Collagen is a protein responsible for the elasticity and regeneration of our skin. It keeps skin firm, plump, smooth and young-looking. While aging our natural production of collagen reduces and unfortunately when applied topically it cannot enter deep skin layers, because of its large fibers.

In this clinical study, we used objective instrumental methodologies as well as personal perception. The anti-aging effects of the novel cream formulated with m-collagen, that had been proven to penetrate skin [7] were clinically demonstrated. Women older than 35 years were selected for the study since there are notable changes in skin visibility due to decrease in collagen production in this group [2].

In the present clinical study, our penetrable micronized collagen (m-collagen) cream has been shown to have a significant reduction in fine lines and wrinkles by 20%, skin firmness (Ro) increased significantly by ~38%, and elasticity R7 R5 and R2 by approximately 39%, 25% and 37% respectively, following 4 weeks of treatment. Skin hydration achieved remarkable increase, almost 200% following the treatment. To assess whether the results are also meaningful to the subjects, self-perception questionnaire (spq) was administered. In order to avoid an "automatic response", subjects were addressed by several similar questions with different wording. The results of the spq correlated with the instrumental data.

To the best of our knowledge, the new facial m-collagen cream is the only topical solution that allows administration of fully functional collagen fibers into deeper layers of the skin.

Fund

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Avila Rodríguez, M.I., Rodríguez Barroso, L.G. and Sánchez, M.L. (2018) Collagen: A Review on Its Sources and Potential Cosmetic Applications. *Journal of Cosmetic Dermatology*, **17**, 20-26. https://doi.org/10.1111/jocd.12450

[2] Reilly, D.M. and Lozano, J. (2021) Skin Collagen through the Lifestages: Importance for Skin Health and Beauty. *Plastic and Aesthetic Research*, **8**, 2. https://doi.org/10.20517/2347-9264.2020.153

[3] Sionkowska, A., Skrzyński, S., Śmiechowski, K. and Kołodziejczak, A. (2017) The Review of Versatile Application of Collagen. *Polymers for Advanced Technologies*, **28**, 4-9. https://doi.org/10.1002/pat.3842

[4] Coapman, S.D., Lichtin, J.L., Sakr, A. and Schiltz, J.R. (1988) Studies of the Penetration of Native Collagen, Collagen Alpha Chains, and Collagen Cyanogen Bromide Peptides through Hairless Mouse Skin *in Vitro*. *Journal of the Society of Cosmetic Chemists*, **281**, 275-281.

[5] Aguirre-Cruz, G., León-López, A., Cruz-Gómez, V., Jiménez-Alvarado, R. and Aguirre-Álvarez, G. (2020) Collagen Hydrolysates for Skin Protection: Oral Administration and Topical Formulation. *Antioxidants*, **9**, 181. https://doi.org/10.3390/antiox9020181

[6] León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V.M., Vargas-Torres, A.,

Zeugolis, D.I. and Aguirre-Álvarez, G. (2019) Hydrolyzed Collagen-Sources and Applications. *Molecules*, **24**, 4031. https://doi.org/10.3390/molecules24224031

[7] Lubart, R., Yariv, I., Fixler, D. and Lipovsky, A. (2022) A Novel Facial Cream Based on Skin-Penetrable Fibrillar Collagen Microparticles. *Journal of Clinical and Aesthetical Dermatology*, **15**, 59-64.

[8] Association, W.M. (2013) World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*, **310**, 2191-2194. https://doi.org/10.1001/jama.2013.281053

[9] International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Note for Guidance on Good Clinical Practice, Consolidated Guideline. Step 4, Consolidated Guideline, 1/5/96. CPMP/ICH/135/95.

[10] Jordan, W.P. and King, S.E. (1977) Delayed Hypersensitivity in Females. The Development of Allergic Contact Dermatitis in Females during the Comparison of Two Predictive Patch Tests. *Contact Dermatitis*, **3**, 19-26. https://doi.org/10.1111/j.1600-0536.1977.tb03582.x