EVALUATION OF THE ANTI-WRINKLE EFFECTS OF THE FOOD SUPPLEMENT COLLACTIVE™: A RANDOMISED, PLACEBO CONTROLLED STUDY

Authors

Correspondence

Synopsis

The aim of this study was to evaluate the anti-ageing effect of a commercial product based on collagen and elastin peptides: Collactive™. A randomized, controlled, single-blind study was conducted on healthy female subjects. Forty-four subjects aged 40 to 55 years old and with wrinkles on the crow's feet were included. Twenty-two subjects were supplied with the active product for 3 months and twenty-two with the placebo. Anti-wrinkle and moisturizing effects were assessed on crow's feet by objective methods, following 28 days and 84 days of products consumption; tolerance and global appreciation were directly evaluated by subjects at the end of the study, using self questionnaires. After 3 months of supplementation, the number of deep wrinkles decreased in the active group and a significant difference was seen between the two groups on this parameter ($p<0.05$). No intergroup difference was highlighted on microrelief furrows and average wrinkles but a difference limit to significance was observed on the total wrinkled surface between the active and placebo groups ($p<0.1$). No adverse effect was reported during Collactive™ supplementation. This study demonstrates the skin anti-ageing benefits of Collactive™: results suggest that Collactive™ prevents and diminishes wrinkles on crow's feet.

Key words

Collagen peptide, skin, wrinkle, (anti-)ageing

Introduction

Skin characteristics are known to be affected by endogenous and environmental factors. As a consequence, cutaneous ageing is the result of both intrinsic ageing, which causes changes in tissue structure, and extrinsic ageing, caused by environmental influences and in particular chronic exposure to solar ultraviolet exposure and smoking (Langton et al, 2010). Lifestyle factors are also involved in skin ageing and diet habits have been related to skin appearance (Boelsma et al, 2003; Cosgrove et al, 2007; Purba et al, 2001). In particular, Purba et al. illustrated that skin wrinkling in a sun-exposed site in older people of various ethnic backgrounds is influenced by the types of foods consumed and one third of the variance is predicted by food intake. Oils, fish, vegetables and cereals appeared to be protective whereas dairy, sugar products and meat were predictive of a greater degree of photoageing.
Further investigations on food effect on skin were conducted, namely on collagen, the most abundant protein of the human body. It has been reported that the oral ingestion of collagen

peptides affects various functions of the body. For example, lower bone mineral density in protein malnutrition and joint disease are improved by ingestion of collagen peptides (Koyama et al, 2001; Moskowitz, 2000). It has also been reported that hair thickness increased after prolonged ingestion of collagen peptides (Scala et al, 1976) and that nail disorders, such as brittle nails, were improved by collagen peptides intake (Tyson, 1950). Collagen peptides supplementation was also investigated in the dermis. Matsuda et al. (2006) showed on animal model that collagen peptide supplementation for 62 days induces the increase of fibroblasts density and the increase of collagen fibrils diameter and density of collagen fibrils in the dermis. These results demonstrated that the ingestion of collagen peptides improves the mechanical strength of the skin in a collagen-specific manner. Considering a previous publication showing that collagen peptide increase the water absorption potential of the stratum corneum in humans (Sumida et al, 2004), the authors conclude that the daily ingestion of collagen peptide improves the function of both the epidermis and the dermis. The effect of collagen supplementation was also evaluated on skin characteristics of women. In a pilot study from Matsumoto et al. (2006) 25 women who tend to have dry and rough skin were daily supplemented with fish type I collagen hydrolysate mixture. After 6 weeks of consumption, improvements were noted on skin surface parameters (skin smoothness and skin roughness), viscoelastic properties (pliability and elasticity) and moisture content of the skin. The supplementation with fish collagen hydrolysate was based on the type I collagen which is the major structural component of skin.
In this study, the anti-ageing effects of Collactive™, a marine ingredient is investigated. Collactive™ is issued from marine products (fish skin) and is composed of collagen and elastin peptides. Collactive™ collagen is type I collagen, and is therefore very similar to human skin collagen.

Material and methods

Participants

Forty-four subjects were recruited at the clinical trial centre. All subjects were women aged 40-55 years having wrinkles on the crow's feet. Subjects having cutaneous pathology on the studied zone, having had injections and/or palpebral face lift or using anti-wrinkle during the last month were not included in the study. Pregnancy, lactation and allergy to cosmtic or dermopharmaceutical product were exclusion criteria too. The use of anti-wrinkle or

hydratant (dermopharmaceutical or cosmetic products) was not authorized on studied zone during the whole study.

All subjects were informed on the product use and restrictions related to the study, and gave their informed written consent before the beginning of the study.

Experimental design

The study was conducted in compliance with the Declaration of Helsinki, GCP and the French law 2004-806 concerning public Health.

The products to test were placebo (maltodextrin) and Collactive™ (Copalis, France), a combination of marine collagen and elastin peptides. Subjects were divided in two groups (N=22 per group) and randomly assigned to consume either the active or placebo product. Subjects from both groups were asked to consume 5 capsules per day in 2 portions (2 capsules in the morning and 3 capsules on evening) over a period of 84 days. Two grams of the active product Collactive™ were ingested daily. On D0, subjects came to the clinical trial centre without having applied any product on the face and forearms since the previous evening. After checking of inclusion and non-inclusion criteria, cutaneous zones were defined on the face (crow's feet) and on the forearm. Measurement of wrinkles on the crow's feet was performed and repeated on D28 and D84. At the last visit (D84), subjects answered the questionnaire of subjective evaluation.

Study evaluations

Ambient conditions during measurements were standardised for temperature and humidity conditions (24 ± 2 °C, relative humidity 40-60%).

Anti-wrinkle effect: A print of crow's feet was taken with Silflo®, a polymer silicone, and then studied using the Skin Image Analyser® (S.I.A.) (Monaderm, Monaco). Outcomes were the total wrinkled surfaces, the number and the average depth of cutaneous microrelief furrows, median wrinkles and deep wrinkles.

Global tolerance: Tolerance was assessed by clinical examination and self-reporting by subjects. Relevant elements related to functional, physical and clinical signs were recorded.

Subjective evaluation questionnaire: At the end of the study, subjects answered a 26-items questionnaire. The items concerned their global appreciation and the organoleptic characteristics of the products, the product efficacy, the product safety and the future use of the product by the subjects.

Statistical analysis

Results are presented as mean variations ± standard error of the meran (SEM) and in percentage of variation. Depending on whether the distribution of data was normal or not, the

statistical comparison between the two products was conducted running either a paired Student t test or U Mann-Whitney test. The intra-group comparisons were studied with the paired Student t test or a Wilcoxon ranks test according to the normality of the data. Differences were considered significant at $p<0.05$. The statistical analyses were performed with the software SAS 9.0 version 9.1 (SAS Institute Inc., Cary, NC, USA) and EXCEL version 2003.

Results

Twenty-one female subjects in the Collactive™ group and 20 female subjects in the placebo group completed the study. Three subjects were excluded between D28 and D84 for digestive intolerance: one from the Collactive™ group and two from the placebo group.

Anti-wrinkle effect

The different parameters of cutaneous relief, as recorded during the study, are presented in **Table I**.

As illustrated in **Fig 1**, a significant increase in the number of deep wrinkles is observed in the placebo group at D28 ($p<0.05$) and D84 ($p<0.01$) compared to baseline value. Conversely, this number significantly decreases in Collactive™ group after one month of supplementation ($p<0.01$). A decrease is still observed at D84 but with no significance. A significant difference between placebo and Collactive™ is however highlighted at both D28 ($p<0.001$) and D84 ($p<0.05$) on the number of deep wrinkles after products consumption. At D28, this reduction in the number of deep wrinkles was observed in 71% of subjects supplemented with Collactive™ *vs*. 18% of subjects supplemented with placebo.

Simultaneously, there was a significant increase in the depth of deep wrinkles at D28 compared to baseline in the Collactive™ group. This significance does not continue at the end of the study.

Looking at the total wrinkled surface, an increase of this parameter, limit to significance, is observed in the placebo group whereas this parameter remains constant in the Collactive™ group (**Fig 2**). A trend appears between the two groups at D84 ($p<0.1$).

None significant difference is observed on the other parameters of cutaneous relief (number and depth of microrelief furrows and average wrinkles, depth of deep wrinkles).

Subjective evaluation questionnaire

Self-evaluation of the products efficacy and tolerance reveals a good appreciation of the Collactive™ product by the subjects (data not shown). Among the 21 subjects of the Collactive™ group who finished the study, 81% rated the product as neither pleasant nor unpleasant and 19% as pleasant. 52% of subjects supplemented with Collactive™ reported an improvement of their skin state and aspect, and they namely noticed a skin smoother, suppler and more moisturized and luminous after 84 days of supplementation. Endly 43% of the Collactive™ group subjects noticed a positive change in the number and the depth of wrinkles.

Tolerance

Tolerance was assessed throughout the study and no adverse event was reported above the 44 subjects. One subject in Collactive™ group and two subjects in placebo group dropped-out before the end of study for digestive disorders. No clinical signs were observed by the dermatologist. A general good tolerance of Collactive™ product was reported during the use of the product.

Discussion

The results obtained in this study demonstrate a potential beneficial anti-wrinkle effect of Collactive™. Product supplementation during 84 days leads to an improvement in skin ageing parameters, namely on deep wrinkles and wrinkled surface of crow's feet. This study is one of the first in vivo studies examining the effect of collagen and elastin peptides alone on ageing-parameters of skin after oral ingestion.

Market of skin care is predominantly dominated by cosmetic with topical application however more and more food supplements appear to be effective alternative products in skin care management. Collagen and elastin are two important proteins present in the skin. Collagen is the main constituent of the extracellular matrix and accounts for 70% of the dermis. It gives dermis its resistance to strain and traction whereas elastin has a structure role by binding collagen fibres, which allows skin elasticity. With advancing age, changes occur at skin level, in relation to intrinsic and extrinsic factors, such as hormonal changes, lifestyle influence and exposure to ultraviolet exposure (Farage et al, 2008). The reduction in collagen and elastin levels observed in both sex, as well as the disorganization of theses structural proteins are involved in marks of skin ageing and namely appearance of wrinkles, increased skin roughness, loss of firmness and elasticity… (Callaghan et al, 2008; Shuster et al, 1975).

Research has demonstrated that collagen peptides ingested orally reach blood flow few hours after ingestion (Iwai et al, 2005; Ohara et al, 2007) and may act at skin level on dermal extracellular matrix components and cell proliferation (Ohara et al, 2010). Many authors therefore investigated the useful of collagen or other skin components-like compounds in a food supplement matrix, to improve skin properties. As an example, Distante et al. (2002) tested a food supplement based on polysaccharides derived from fish cartilage and natural antioxidants. The consumption of the product for 2 months had significant effect on dermal thickness and skin wrinkling, in comparison to placebo. An other mixture based on fish cartilage and presented as food supplement show a positive effect on skin hydration, in addition to cutaneous smoothness and roughness, and the depth of furrow (Primavera & Berardesca, 2005). Authors of both studies concluded to the interest of combination of treatments, i.e topical and oral administration, for skin care. It is noteworthy that, contrary to Collactive™ collagen, fish cartilage is characterised by its type II collagen. This former is extensively studied for its application in joint care, namely for osteoarthritis and relief of joint pain. New interest for its potential cosmetic effect leads research in skin anti-ageing management via oral supplementation.

Glycosaminoglycans (GAGs), an other dermis component, represent a supplementary approach for skin health management. GAGs, like hyaluronic acid and chondroion sulfates, already have notable health application in osteoarthritis treatment (McAlindon et al, 2000; Richy et al, 2003), due to their abundance in connective tissue. In dermis, the main function of GAGs is the support and the maintenance of collagen and elastin. They also have the ability to bound large amount of water and consequently act as a natural moisturising ingredient responsible for the skin's plumpness and moisture. In relation to these properties and potential anti-ageing capacities, oral supplementation with GAGs was investigated (Segger & Schönlau, 2004; Udompataikul et al, 2009). As expected, data showed positive effect of GAGs supplementation on skin elasticity and skin roughness, as well as an anti-wrinkle effect

Endly, some food supplements are developed considering marine active components, in particular marine proteins. In a review of literature, three published studies demonstrated the interest of commercial products in skin aging. Béguin first evaluated the effects of a supplement containing marine protein in addition to marine lipids and natural compounds (tocopherols, flavonoids and carotenes) on dermal structure parameters and skin surface (Béguin, 2005). After 3 months of consumption, the food supplement positively influenced signs of skin aging, considering various parameters: increase in dermis intensity,

improvement of epidermis and dermis thickness, decrease of furrows… The relevance of these results is confirmed by objective assessment however they are limited by the absence of significant effect in comparison to placebo. In a publication from Skovgaard et al. (2006), skin effect of a dietary supplement based on soy extract, fish protein polysaccharides, botanical extracts and vitamins was investigated on face, décolletage and hand. Clinical and objective assessments demonstrated significant differences between active group and placebo on face wrinkles, hyper pigmentation and overall appearance at the end of the 6-month supplementation. The last food supplement was based on marine proteins, completed with various minerals, vitamins and antioxidants (Thom, 2005). Objective skin measurements showed a significant increase in skin thickness and elasticity in the active group after 6 months of supplementation, with significant differences compared with placebo. These results were confirmed by clinical evaluation of skin-ageing symptoms and self-evaluation by subjects.

In complement to these previous researches, the present study gives additional data on the skin anti-ageing properties of collagen and elastin from marine extracts. Additionally the composition of Collactive™ allows the extrapolation of the study results to these two main constituents of the dermis extracellular matrix. In conclusion this study demonstrates the anti-wrinkle action of Collactive™ supplementation for 3 months and fully justifies its application in anti-ageing related formulations.

References

Béguin A. A novel micronutrient supplement in skin aging: a randomized placebo-controlled double-blind study. J Cosmet Dermatol. 2005 Dec;4(4):277-84.

Boelsma E, van de Vijver LP, Goldbohm RA, Klöpping-Ketelaars IA, Hendriks HF, Roza L. Human skin condition and its associations with nutrient concentrations in serum and diet. Am J Clin Nutr. 2003 Feb;77(2):348-55.

Callaghan TM, Wilhelm KP. A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part 2: Clinical perspectives and clinical methods in the evaluation of ageing skin. Int J Cosmet Sci. 2008 Oct;30(5):323-32.

Distante F, Scalise F, Rona C, Bonfigli A, Fluhr JW, Berardesca E. Oral fish cartilage polysaccharides in the treatment of photoageing: biophysical findings. Int J Cosmet Sci. 2002 Apr;24(2):81-7.

Farage MA, Miller KW, Elsner P, Maibach HI. Intrinsic and extrinsic factors in skin ageing: a review. Int J Cosmet Sci. 2008 Apr;30(2):87-95.

Iwai K, Hasegawa T, Taguchi Y, Morimatsu F, Sato K, Nakamura Y, Higashi A, Kido Y, Nakabo Y, Ohtsuki K. Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. J Agric Food Chem. 2005 Aug 10;53(16):6531-6.

Koyama Y, Hirota A, Mori H, Takahara H, Kuwaba K, Kusubata M, Matsubara Y, Kasugai S, Itoh M, Irie S. Ingestion of gelatin has differential effect on bone mineral density and body weight in protein undernutrition. J Nutr Sci Vitaminol (Tokyo). 2001 Feb;47(1):84-6.

Langton AK, Sherratt MJ, Griffiths CE, Watson RE. A new wrinkle on old skin: the role of elastic fibres in skin ageing. Int J Cosmet Sci. 2010 Jun 21. [Epub ahead of print]

Matsuda N, Koyama Y, Hosaka Y, Ueda H, Watanabe T, Araya T, Irie S, Takehana K. Effects of ingestion of collagen peptide on collagen fibrils and glycosaminoglycans in the dermis. J Nutr Sci Vitaminol (Tokyo). 2006 Jun;52(3):211-5.

Matsumoto H, Ohara H, Ito K, Nakamura Y, Takahashi S. Clinical effects of fish type I collagen hydrolysate on skin properties. ITE Letters. 2006;7(4):386–390.

McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. JAMA. 2000 Mar 15;283(11):1469-75.

Moskowitz RW. Role of collagen hydrolysate in bone and joint disease. Semin Arthritis Rheum. 2000 Oct;30(2):87-99.

Ohara H, Ichikawa S, Matsumoto H, Akiyama M, Fujimoto N, Kobayashi T, Tajima S. Collagen-derived dipeptide, proline-hydroxyproline, stimulates cell proliferation and hyaluronic acid synthesis in cultured human dermal fibroblasts. J Dermatol. 2010 Apr;37(4):330-8.

Primavera G, Berardesca E. Clinical and instrumental evaluation of a food supplement in improving skin hydration. Int J Cosmet Sci. 2005 Aug;27(4):199-204.

Purba MB, Kouris-Blazos A, Wattanapenpaiboon N, Lukito W, Rothenberg EM, Steen BC, Wahlqvist ML. Skin wrinkling: can food make a difference? J Am Coll Nutr. 2001 Feb;20(1):71-80.

Richy F, Bruyere O, Ethgen O, Cucherat M, Henrotin Y, Reginster JY. Structural and symptomatic efficacy of glucosamine and chondroitin in knee osteoarthritis: a comprehensive meta-analysis. Arch Intern Med. 2003 Jul 14;163(13):1514-22.

Scala J, Hollies N, Sucher KP. Effect of daily gelatin ingestion on human scalp hair. Nutr Rep Int 1976;13:579-92.

Segger D, Schönlau F. Supplementation with Evelle improves skin smoothness and elasticity in a double-blind, placebo-controlled study with 62 women. J Dermatolog Treat. 2004 Jul;15(4):222-6.

Shuster S, Black MM, McVitie E. The influence of age and sex on skin thickness, skin collagen and density. Br J Dermatol. 1975 Dec;93(6):639-43.

Skovgaard GR, Jensen AS, Sigler ML Effect of a novel dietary supplement on skin aging in post-menopausal women. Eur J Clin Nutr. 2006 Oct;60(10):1201-6. Epub 2006 May 3.

Sumida, E., Hirota, A., Kuwaba, K., Kusubata, M., Koyama, Y., Araya, T. Irie, S. and Kasugai, S. 2004. The effect of oral ingestion of collagen peptide on skin hydration and biochemical data of blood. Journal of nutritional food, 7(3): 45-52.

Thom E. A randomized, double-blind, placebo-controlled study on the clinical efficacy of oral treatment with DermaVite on ageing symptoms of the skin. J Int Med Res. 2005 May-Jun;33(3):267-72.

Tyson TL. The effect of gelatin on fragile finger nails. J Invest Dermatol. 1950 May; 14(5):323-5.

Udompataikul M, Sripiroj P, Palungwachira P. An oral nutraceutical containing antioxidants, minerals and glycosaminoglycans improves skin roughness and fine wrinkles. Int J Cosmet Sci. 2009 Dec;31(6):427-35.

Figures

Figure 1: Number of deep wrinkles.

Evaluation of the number of deep wrinkles on crow's feet using the Skin Inage Analyser®.

The same crow's feet was analysed throughout the study for a subject.

means significant different compared to D0 ($p<0.05$)

* means significant different compared to D0 ($p<0.01$)

† means significant different compared to placebo ($p<0.05$)

§ means significant different compared to placebo ($p<0.001$)

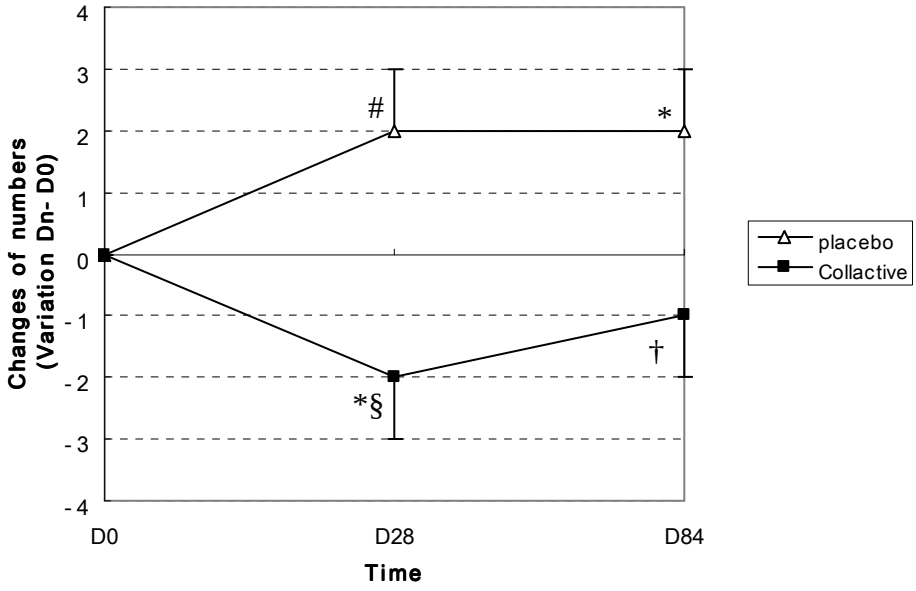
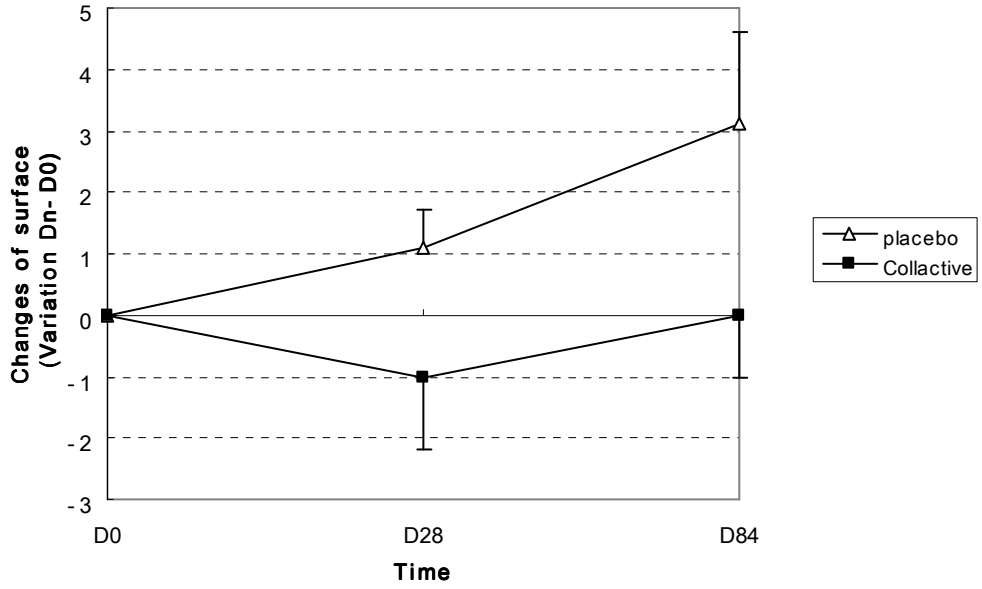


Figure 2: Total wrinkled surface (mm²).

Evaluation of the total wrinkled surface on crow's feet using the Skin Inage Analyser®. The same crow's feet was analysed throughout the study for a subject.



Tableaux

Table I: Cutaneous relief

	placebo			**Collactive™**			**Difference of change Collactive™-placebo**	
	baseline	**D28-D0**	**D84-D0**	**baseline**	**D28-D0**	**D84-D0**	**D28-D0**	**D84-D0**
	N=22	N=22	N=17	N=22	N=22	N=21		
Number of microrelief furrows	22 ± 3	3 ± 2	-4 ± 2	19 ± 3	1 ± 2	1 ± 2	0.524	0.108
Depth of microrelief furrows (µm)	44.7 ± 0.4	-0.2 ± 0.6	0.8 ± 0.6	45.9 ± 0.5	-1.0 ± 0.6	-0.3 ± 0.4	0.293	0.104
Number of average wrinkles	33 ± 4	-5 ± 3	-2 ± 3	36 ± 4	-3 ± 3	0 ± 2	0.557	0.749
Depth of average wrinkles (µm)	71.8 ± 1.1	0.5 ± 1.2	1.0 ± 1.4	73.5 ± 0.9	-0.5 ± 0.9	-0.3 ± 0.8	0.533	0.441
Number of deep wrinkles	6 ± 1	2 ± 1#	2 ± 1*	10 ± 1	-2 ± 1*	-1 ± 1	0.000§	0.011#
Depth of deep wrinkles (µm)	180.0 ± 8.1	2.8 ± 9.5	-2.2 ± 10.8	168.4 ± 6.4	7.3 ± 3.2#	4.4 ± 4.3	0.827	0.305
Total wrinkled surface (mm²)	13.1 ± 1.3	1.1 ± 0.6	3.1 ± 1.5	17.6 ± 1.7	-1.0 ± 1.2	0.0 ± 1.0	0.140	0.081

§ $p<0.001$

* $p<0.01$

# $p<0.05$