

An open observational trial of a novel peptide and hyaluronic acid based lip cosmetic



NEIL FISHER

• Neil Fisher¹

SUMMARY

Twenty Caucasian women aged from 18 to 60 years were enrolled into a 7-day open observational trial to assess the safety and effectiveness of a commercially available lip cosmetic. Physiological parameters assessed included: tolerability, lip volume, lip hydration, and lip elasticity. Application and testing were overseen by a third-party testing facility following the principles of good laboratory practice.

No adverse effects or tolerability issues were noted, all 20 enrolled participants finished the trial. The tested product (Lip Volumizer, Contrad Swiss SA, Switzerland) increased the mean moisture content of participant's lips by 24% relative to T0 at the 20-minute timepoint ($p < 0.01$) and by 13% at the 1-hour timepoint ($p = 0.013$).

Measurement of lip elasticity revealed no significant changes in any of the short-term timepoints (20m, 1h, 4h), but instead showed a significant 25.6% increase (absolute increase of 11.4N/m relative to T0) after 1 week of twice-daily usage ($p < 0.01$). Lip surface area was increased 1.3% by 20m after application (absolute increase of 1.4 mm² relative to T0, $p < 0.01$). This effect was maintained at the 1 hour timepoint (absolute increase of 1.2mm² relative to T0, $p < 0.01$), but by 4 hours the effect was no longer significant. After 1 week of sustained useage a significant mean 1.8% increase in lip area was recorded (absolute increase of 1.8mm² relative to T0, $p = 0.02$).

KEYWORDS

peptides, elasticity, volume, moisture, cosmetic, lip

ABBREVIATIONS

hyaluronic acid (HA), stratum corneum (SC), matrix metalloproteinase (MMP), extracellular matrix (ECM), ultraviolet (UV), good laboratory practice (GLP).

¹ "Medical Science Liaison"
Lugano, Swiss

INTRODUCTION

Despite a reported increase in demand for noninjectable short-term lip volumizing products (“lip plumpers”) in recent years, this has not been reflected in the scientific literature, with little documented evidence describing the effect and duration of these products [1-4]. Many commercially available lip products contain irritants, such as cinnamon or capsaicin, which cause temporary vasodilation of the lips [5, 6]. These substances can achieve this effect though inflammatory mediators such as prostaglandins and substance P, triggering non-immunological contact urticaria (NICU), or irritant contact cheilitis [6-8]. This is often reported as a “tingling” or “burning” sensation felt on the lips for several minutes following application.

The product tested in this study contained no irritants, instead relying on well-known skin conditioning agents including hydrolysed collagen and hyaluronic acid (HA), as well as a novel cosmetic agent, shpolypeptide-47. Hydrolysed collagen has been shown to increase collagen and glycosaminoglycan synthesis [9], improve wound healing [10], increase skin elasticity [10-13] and moisture content [10-12], and to counter signs of skin aging, potentially through increased collagen synthesis in the dermis [9-11]. The majority of these studies involved the oral consumption of hydrolysed collagen, although there is evidence to suggest that topical hydrolysed collagen from fish scales can effectively penetrate the stratum corneum, stimulate collagen synthesis, and increase the elasticity and moisture content of the skin [11].

HA is the major glycosaminoglycan in human skin and is well-

known for its water-binding and volumizing properties, which make it a common ingredient in cosmetic products. HA is widely used to increase the apparent volume of the lips, although this is often achieved by injection. High molecular weight HA has been shown to not only recapitulate the ordered state of the youthful extracellular matrix, but also to increase the passage of proteins over the intact stratum corneum (SC) [14] which is thinner in the skin of the lips than skin in other areas of the body [15] further potentiating the passage of macromolecules over the SC. Additionally, dermal penetration of topical HA formulations has been shown to be enhanced by the co-application of oligopeptides [16].

The use of peptides in cosmetics is gaining momentum with fewer than 10 publications per year in Pubmed (<https://pubmed.ncbi.nlm.nih.gov/>) prior to 2006, increasing to over 30 per year on average from 2017-2019. Sh-polypeptide-47 is a single chain synthetic human peptide, derived from an isomer of Collagen Alpha 1 [17]. Previously it was shown that collagen-derived peptide fragments were sufficient to inhibit the degradative actions of matrix metalloproteinase (MMP) activity while conversely stimulating production of extracellular matrix (ECM) components [18], which has been shown to reduce fine lines and wrinkles in the skin [19] as well as increase the density of elastin fibres [20].

Type I collagen is comprised of two alpha-1 polypeptide chains and one alpha-2 polypeptide chain, encoded by COL1A1 and COL1A2 respectively. Mutations in COL1A1 are known to be associated with osteogenesis imperfecta (OI) [21], Ehlers-Danlos syndromes [22], and osteoporosis [23], underlining the

role of the protein in the regulation of bone growth and connective tissue development, although little information is available as to the specific effects of Sh-polypeptide-47.

On the basis of these known effects of several constituents of the lip cream, we hypothesised that application could have a moisturizing and softening effect and that the increased moisture could translate to increased volume. Accordingly, this study assessed the volumizing, moisturising, elasticising effects of a lip plumping product using widely-implemented standardised objective measures. The primary endpoints were safety and tolerability, the secondary endpoints were the moisturizing, elasticizing and volumizing effect on the lips.

Materials and methods

Twenty healthy female Caucasian participants aged between 18 and 60 years of age (mean age: 31 years old, median age: 22 years old) were enrolled in a 7-day open observational study during November and December 2019. Participants were enrolled from the study centre database of volunteers after meeting all inclusion criteria and none of the exclusion criteria (**Table 1**). Participants were informed of the aims of the study, the procedures and the possible risks involved in the study, and freely gave their written informed consent following the principles outlined in the Declaration of Helsinki.

During the study, the following withdrawal criteria were applied: development of illness or accident or condition which could affect the outcome of the study, intolerance to the tested product, participants that no longer wished to participate in the study. For the duration of the study, the subjects are instructed to continue their normal lip hygiene practice and not to apply the prod-

TABLE 1. INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria

- Caucasian
- female gender
- aged 18-60 years
- good general health
- capable of following the study protocols
- agreed to avoid use of UV tanning beds for the duration of the study

Exclusion criteria

- pregnancy, or breastfeeding
- history of skin reactions to cosmetic products, detergents, or components thereof
- use of medication that could affect the response of the skin (anti-inflammatories, cortisone, etc.)
- signs of skin irritation or skin disorders
- use of local or systemic lip treatments within the previous 40 days
- participation in another similar study within the previous 30 days

TABLE 2. COMPONENTS OF THE TESTED PRODUCT (INCI NOMENCLATURE).

INCI nomenclature

Aqua, Cetearyl Alcohol, Dimethicone, Cyclopentasiloxane, Hydrolyzed Collagen, C12-20 Acid Peg-8 Ester, Glycerin, Cetareth-12, Aloe Barbadensis Leaf Juice, Prunus Amygdalus Dulcis Oil, Cetareth-20, Cetearyl Isononanoate, Sodium Stearoyl Glutamate, Persea Gratissima Oil, Helianthus Annuus Seed Oil, Argania Spinosa Kernel Oil, Panthenol, Sodium Hyaluronate, Tocopherol, Sh-Polypeptide-47, Tocopheryl Acetate, Retinyl Palmitate, Carbomer, Sodium Polyacrylate, Sodium Hydroxide, Disodium EDTA, Citric Acid, Phenoxyethanol, DMDM Hydantoin, Sodium Benzoate, Potassium Sorbate.

uct in question in parts other than those prescribed. Similarly, throughout the study, the subjects were instructed not to use different lip care products or expose themselves to UV rays, wherever possible. Twenty participants were screened, enrolled and all twenty completed the study; there were no screen failures or withdrawals.

The tested product was an odourless cosmetic lip cream (ACTV8SKN™ Lip Volumizer, Contrad Swiss SA, Switzerland), the composite ingredients of which are reported in **Table 2**. All participants were instructed on how to apply the product (approximately 60mg per application) to clean lips twice a day (morning and evening) for the 7-day study period. All participants were assessed for each of the assessed parameters both before, and after 20 minutes, 1 hour, 4 hours and 1 weeks use of the product. All measurements were performed by trained staff. Data was collected under controlled environmental conditions where temperature and humidity were monitored and maintained constant at each measurement time point (24°C +/-2°C, 50% +/-10% relative humidity). There was no formal acclimation time. Volunteers are asked not to wash and/or wet their lips for at least two hours before taking the measurements and not to apply any cosmetics for at least 12 hours before the acquisition of the initial measurements. This study was conducted in accordance with quality assurance requirements and with the principles of good laboratory practice (GLP), as established in the principles of the Declaration of Helsinki.

Measurements of hydration, elasticity and lip area/volume were performed prior to application of the product (T0), and then 20 minutes, 1 hour, 4 hours after the first application and after 1 week of treatment. Tests were not blinded.

Hydration of the lips was assessed using a MoistureMeterSC (Delfin Technologies, Kuopio, Finland), which produces an arbitrary value proportional to the moisture content of the SC. Elasticity was assessed as the instant skin elasticity (N/m), using an ElastiMeter (Delfin Technologies) with a 0.3mm long measuring probe to assess the stiffness of the skin. The surface area of the lips was measured using a high definition Antera 3D camera (Miravex Limited, Dublin, Ireland) and CS software (Miravex) running the "Spoton" algorithm to combine multiple photographs of the lips to calculate the surface area (mm²). The assessed area of the lips was the tissue within the vermilion border.

The data was analysed using R statistical software [24]. Shapiro-Wilk tests for normality dictat-

ed use of parametric (paired t-test) or non-parametric tests (Wilcoxon). Correction for multiple comparisons was performed using the Holm method [25]. Homoscedasticity was assessed with Bartlett's test [26].

The threshold for statistical significance was set at 5% ($P < 0.05$). Changes seen over the course of 30 days were calculated as percentage change $((\text{new value} - \text{old value}) / (\text{old value})) \times 100$ for each individual; the mean of the percentage change was used. Contemporaneous differences between different treatments were calculated as percentage difference $((\text{new value} - \text{old value}) / (\text{mean of new and old values})) \times 100$; the mean of the percentage difference was used.

Results

All 20 enrolled participants completed the 7-day study, no adverse reactions or tolerability issues were reported. The mean moisture content of participants' lip SC was significantly increased by 24% relative to T0 at the 20-minute timepoint ($p < 0.01$, $n = 20$) and by 13% at the 1-hour timepoint ($p = 0.013$, $n = 20$) (Fig. 1). By the following timepoint, 4 hours after the single application, the increase in moisture content was no longer significantly higher than T0. No cumulative effect was noted after repeated application of the product over 1 week. Measurement of lip elasticity revealed no significant changes in any of the short-term timepoints (20m, 1h, 4h), but instead showed a significant 25.6% increase (absolute increase of 11.4N/m relative to T0) after 1 week of twice-daily usage ($p < 0.01$, $n = 20$) (Fig. 2). Lip surface area was increased 1.3% by 20m after application (absolute increase of 1.4 mm² relative to T0, $p < 0.01$, $n = 20$) (Fig. 3). This effect was maintained at the 1 hour timepoint (absolute increase of

1.2mm² relative to T0, $p < 0.01$, $n = 20$), but by 4 hours the effect was no longer significant. After 1 week of sustained use a significant mean 1.8% increase in lip area was recorded (absolute increase of 1.8mm² relative to T0, $p = 0.02$, $n = 20$).

Discussion

Contrary to the majority of cosmetic "lip plumpers" on the market which contain irritants such as capsaicin, we describe the moisturising, volumizing and elasticising effects of a novel cosmetic lip product which does not contain irritants and therefore does not function by causing local vasodilation of the lips and surrounding tissues.

Following application of the product to the lips, a short-term 24% increase in the moisture levels of the lip SC was observed; this effect was maintained for between 1 and 4 hours. This is likely attributable to the glycerin, dimethicone and hydrolysed collagen which are known skin conditioning agents.

A significant increase in lip volume was observed 20 minutes after application and was maintained for between 1 and 4 hours. Additionally, a significant increase in lip volume (relative to baseline) was seen after 1 week of continued use suggesting a possible cumulative effect on the lips with repeated use.

Lip elasticity was significantly increased by 25.6% after 1 week of continued use relative to baseline. There was no significant difference at any of the earlier timepoints relative to baseline; this could be attributed to the effect of collagen fragments and peptide signalling molecules on the stimulation of ECM component production [18], as well as increased elastin fibre density [20] that would not be detectable within the shorter, 1-4 hour timepoints. It

Fig. 1

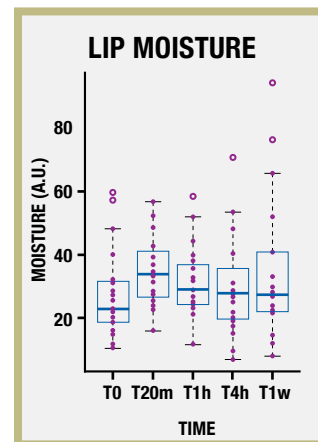


Fig. 2

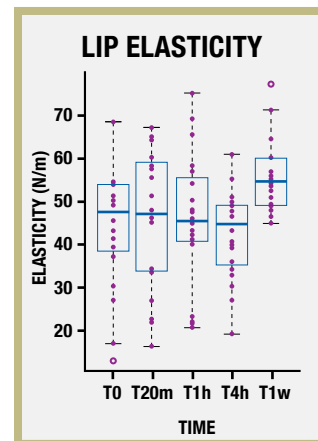
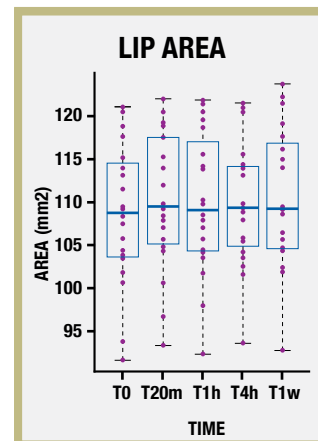


Fig. 3



should be noted that the large increase in lip elasticity likely came at the expense of increased lip area: the elastic modulus of a material is proportional to its stiffness. Therefore, the elasticity of the lips will increase at the expense of the increase in volume and vice versa.

Conclusions

In conclusion, we present evidence of the physiological effects of a novel lip cosmetic product that significantly increases the elasticity, moisture content, and volume of the lips.

Conflict of interest: Neil Fisher is employed by Contrad Swiss SA, which manufactures the product described in this manuscript.

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