Efficacy Evaluation of a Topical Hyaluronic Acid Serum in Facial Photoaging

Zoe Diana Draelos, MD;[1] Isabel Diaz, BA;[2] Jin Namkoong, PhD[3]; Joanna Wu, PhD[3]; Thomas Boyd, PhD[3]

[1]Dermatology Consulting Services, PLLC, High Point, NC; [2]Dermal Clinical Research, Colgate-Palmolive, Piscataway, NJ, USA [3]Early Research, Colgate-Palmolive, Piscataway, NJ, USA

Background

Improvement in overall facial skin appearance is most rapidly achieved through enhanced moisturization. Moisturization occurs by increasing the water content of the epidermis thereby improving skin texture and reducing the appearance of fine lines and superficial wrinkles. The skin naturally achieves hydration through dermal glycosaminoglycans (GAGs), such as hyaluronic acid. Hyaluronic acid (HA), also known as hyaluronan, is a linear carbohydrate polysaccharide found in all living organisms. The human body contains 15 grams of HA, with one-third residing in the skin.(1)

HA can penetrate into the stratum corneum, if of the correct size. HA with a low molecular weight of 20-300 kDa passes through the stratum corneum while high molecular weight HA of molecular weight 1000-1400 kDa is largely impermeable making proper formulation important to achieving facial moisturization in all Fitzpatrick skin types.(2)

Objective

The objective of this study was to evaluate the ability of a facial serum to deliver skin benefits by promoting skin plumpness and hydration while minimizing fine lines/wrinkles and improving the overall global assessment of facial appearance in all Fitzpatrick skin types.

Methods

In Vivo

40 females 30-65 years of age with Fitzpatrick skin types I-VI diagnosed by the dermatologist investigator as possessing poor skin plumpness and hydration along with photoaging were enrolled in this single site study to evaluate the efficacy of a facial serum. Following completion of an IRB approved informed and photography consent (Allendale Institutional Review Board, Old Lyme, CT) and after meeting all inclusion criteria and none of the exclusion criteria, subjects with lack of skin hydration and plumping and global photoaging were enrolled. Subjects were asked to wash their face at the research center. Subjects were provided with the study facial serum (PCA Skin Hyaluronic Acid Boosting Serum, Colgate-Palmolive Co. and PCA Skin, Piscataway, NJ) for twice daily use and a sunscreen (Neutrogena Clear Face Broad Spectrum SPF 55, Johnson & Johnson, Skillman, NJ) for use as needed during the study.

The dermatologist investigator evaluated lack of smoothness, lack of plumping, poor hydration, fine lines/wrinkles, and global appearance issues. The following 5-point ordinal scale was used: 0=none, 1=minimal, 2=mild, 3=moderate, 4=severe. Subjects assessed product tolerability in terms of stinging, itching, and burning. The following 5-point ordinal scale was used: 0=none, 1=minimal, 2=mild, 3=moderate, 4=severe. Corneometry (Dermalab Combo Pin Probe, Cortex Technologies, Hadsund, Denmark) was conducted for all subjects in triplicate of the left cheek after acclimating to the study environment for 20-30 minutes.

VISIA CR4.3 (Canfield Scientific, Parsippany, NJ) photography was conducted of the front, right, and left face with visible light for a subset of 15 subjects. The face was then swabbed on the right cheek for these same 15 subjects for HA levels. All subjects applied the study product and sat 10-15 minutes. Investigator assessments were completed for immediate plumping and hydration. Post-application corneometry was conducted for all subjects in triplicate of the left cheek. Subjects returned for the same assessments at weeks 2, 4, and 6.

Figure 1. Investigator Assessments

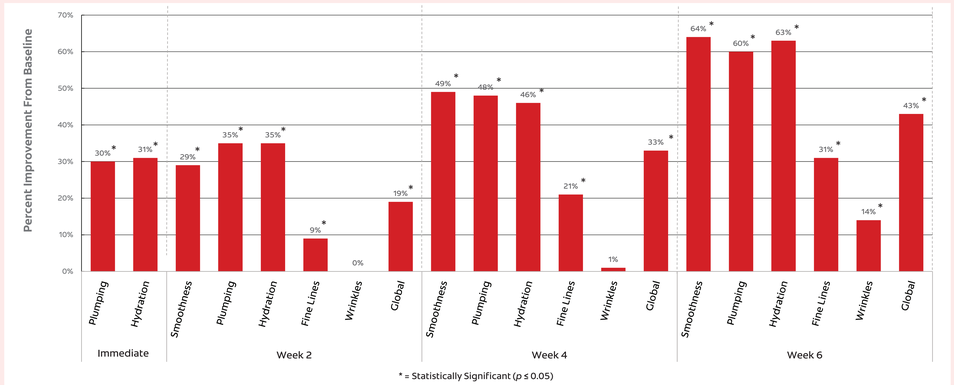


Figure 2: Clinical Effects of HA in Skin of Color (baseline vs. week 12). Fitzpatrick Skin Type V.

Figure 3: Clinical Effects of HA in Skin of Color (baseline vs. week 12). Fitzpatrick Skin Type V.

Figure 4: Clinical Effects of HA in Skin of Color (baseline vs. week 12). Fitzpatrick Skin Type V.

Methods (cont'd)

In Vitro

Full-thickness human skin equivalents from MatTek (EFT-400, Ashland, MA) were used to evaluate whether the study HA serum product could increase hyaluronan expression. After equilibration overnight after receiving EFT-400, 10uL products were applied on top of the skin equivalents for further incubation at 37°C with 5% CO_2. Triplicates were used for untreated samples and duplicates for the serum treated samples. After 24 hours of treatment, the media was collected and tissues were rinsed with PBS three times and collected. The epidermis and the dermis were separated from tissues for protein extraction using TissueLyser II (Qiagen, Carlsbad, CA). Protein concentration was determined using Micro BCA Protein Assay Kit (Thermo Scientific, Waltham, MA) according to the Manufacturer's protocol. IL-1a release and hyaluronan expression were determined using ELISAs (R&D Systems) according to the manufacturer's protocols. For hyaluronan ELISA, protein lysates were diluted by 1,000-fold (epidermis) and 10,000-fold (dermis) and normalized to the protein concentration.

Results

In Vivo

40/40 subjects of Fitzpatrick skin types I-VI successfully completed the 6 week study. The subjects noted no statistically significant tolerability issues. No product stickiness was identified. The investigator assessed hydration and plumping visually immediately after product application and all criteria at weeks 2, 4, and 6 (Figure 1). Representative subject before and after photographs are presented in Figures 2, 3, and 4 (baseline vs. week 12).

The investigator noted improvement was probably due to the enhanced water holding capacity of the topically applied HA, which was effective in all Fitzpatrick skin types regardless of degree of photodamage. Immediately after application, there was a 134% increase in skin water content ($p<0.001$) as measured by corneometry. After 2 weeks of application, a 15% increase ($p=0.025$) was seen with continued improvement in water content at 4 weeks of 29% ($p<0.001$) and 55% increase in skin water content ($p<0.001$) at week 6.

Facial swabs from baseline, week 2, week 4, and week 6 were analyzed. IL-1a was measured to determine if the HA moisturizer produced any irritation. There was no statistically significant increase in IL-1a indicating no irritation. Filaggrin, which is broken down into natural moisturizing factor (NMF), was also measured. There was no statistically significant change in filaggrin indicating the HA moisturizer helped maintain NMF. An examination of HA present on the skin surface was conducted. There was a statistically significant increase in HA. The change from baseline p-values were p=0.02 at week 2, p=0.04 at week 4 and p=0.04 at week 6 (Figure 5).

In Vitro

After 24 hours of treatment with serum, the full-thickness skin equivalents were rinsed with PBS. The epidermis and the dermis of the full-thickness skin equivalents were separated by pulling followed by protein extraction. Samples were analyzed for the presence of hyaluronan by ELISA. More hyaluronan was present in the dermis compared to the epidermis, which is consistent with the literature. While no changes were observed between the untreated and serum-

Figure 5. In Vivo Clinical Hyaluronic Acid Amount (ng/ml) HA was deposited in the skin from the Hyaluronic Acid Boosting Serum (HABS).

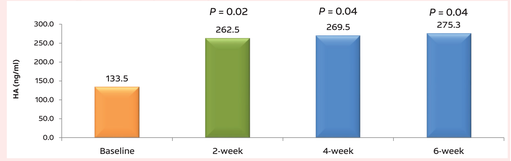
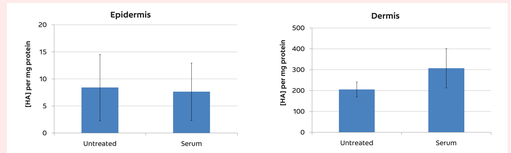


Figure 6. In Vitro Hyaluronic Acid Amount (per mg protein) The increase in dermal hyaluronan was from the boosting effects of the Hyaluronic Acid Boosting Serum (HABS).



Results (cont'd)

treated samples in the epidermis, an increase was shown in the dermis from serum-treated samples. Based on this finding, the increase in dermal hyaluronan is from the boosting effects of the HA serum, not residual hyaluronan from the study product, since there was no difference between the untreated and serum treated epidermis (Figure 6).

Discussion

HA is a valuable ingredient to improve facial appearance in women with poor skin plumpness, decreased skin hydration, and photoaging.(3) The current formulation contained hydrolyzed 50 kDa HA and 10-1000 kDa sodium hyaluronate. This size HA allowed penetration into and through the stratum corneum.(4) These humectant ingredients produced an immediate increase in the water holding capacity of the skin, as demonstrated by the investigator assessments and corneometry 15 minutes post application. In addition, the improvement was cumulative with increasing results over the 6 weeks of study product application.

HA increased significantly after 2 weeks of product application with slight continued increases through weeks 4 and 6 (Figure 5). These results indicate there was a significant amount of HA deposited on the skin from the study serum. The HA boost from the study product was observed as a result of accumulation from product usage. Thus, this research confirmed the clinical findings of increased skin hydration by documenting the increase in HA through skin swabbing. This increased skin hydration resulted in facial appearance improvement.

Summary

Skin hydration is one of the major concerns for skin health. Among ingredients that would improve skin hydration, HA stands out with its ability to retain moisturization. With an appropriately selected HA based on efficacy, this well formulated HA serum can visually improve skin plumping and mechanistically improve skin hydration by 55% as measured by corneometry due to an increase in dermal hyaluronan in all Fitzpatrick skin types.

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Disclosure

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Isabel Diaz, BA, Jin Namkoong, PhD, Joanna Wu, PhD, and Thomas Boyd, PhD, are employees of Colgate-Palmolive Company (Piscataway, NJ, USA)

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