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Efficacy and Tolerability of a Novel Topical Treatment for the Neck: A Randomized, Double-Blind, Regimen-Controlled Study

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ABSTRACT

The neck plays a telling role as an age indicator. Due to its anatomy and function, neck skin ages differently than facial skin and special considerations need to be taken when providing treatment. A randomized, double-blind, regimen-controlled study was conducted to assess the efficacy and tolerability of a novel topical cosmetic cream (NCC) specifically tailored to address the signs of skin aging of the neck and décolletage. Twice daily application of NCC significantly improved skin sagging/laxity of the neck as well as the appearance of fine and coarse lines/wrinkles, crepiness, tactile roughness, overall skin texture, hyperpigmentation, skin tone evenness, and radiance. NCC also significantly improved the appearance of fine and coarse lines/wrinkles, tactile roughness, hyperpigmentation, skin tone evenness, and radiance of the décolletage. Investigator assessments were corroborated by objective cutometer measurements that demonstrated improved skin firmness and elasticity. In vitro analysis in human 3D skin models show that stimulation of neocollagenesis and neoelastogenesis as well as support of cellular proteostasis through proteasome and autophagy activation are potential mechanisms of action for the observed clinical outcomes.

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INTRODUCTION

The face is the primary concern for most patients seeking skin rejuvenation treatments; however, consumer focus is starting to include other body parts, including neck and décolletage. During the aging process, the neck experiences several anatomical changes including loss of elasticity, platysmal banding, and fat accumulation.[1] As the most superficial layer of the neck, the skin bears the cumulative burden of years of sun exposure resulting in loss of elasticity and firmness and the appearance of horizontal skin rhytides due to degeneration of dermal collagen and elastic fibers. Exposure to UVA/UVB rays and other environmental aggressors further causes hyperpigmentation, redness, and a telangiectatic mat referred to as poikiloderma of Civatte.[2,3] Typical aesthetic concerns of the décolletage include appearance of uneven skin tone, hyperpigmentation, and rough skin texture.

Neck skin is more flexible and extensible than facial skin to allow for head movements, experiences greater loss in elasticity with aging, and is more damage-prone as it is thinner and more delicate. Due to lower sebum secretion, neck skin shows more severe aging patterns and deeper wrinkles than facial skin. Furthermore, neck skin has fewer appendages, which can delay post-procedural healing and outcome.[4-7] A topical cream that provides clinically proven skin rejuvenation benefits could offer patients a reliable treatment option prior to or in combination with procedural modalities.

Understanding the key factors implicated in neck skin aging was crucial in formulating the novel topical neck cosmetic cream (NCC) described in the current study, allowing for a rational combination of ingredients designed to improve the signs of aging specific to skin of the neck and décolletage. Ingredients include shiitake mushroom extract and peptides to support extracellular matrix (ECM) quality and structure, Japanese lemon balm to enhance autophagy and promote collagen remodeling, rice extract to activate the proteasome system and maintain proteostasis, paracress extract to reduce muscle micro-contractions and support platysma muscle rejuvenation, and various potent antioxidants, including *Dunaliella salina* and *Physalis angulata*, to protect against environmental aggressors and reduce oxidative stress-induced damage.

MATERIALS AND METHODS

Human 3D Skin Model

In vitro studies were conducted using the EpiDermFT™ 3D full thickness human skin model (EFT-400, MatTek Corp). Tissues were cultured with EpiDermFT Assay Media (EFT-400-MM, MatTek Corp). EpiDermFT was irradiated with 200mJ/cm^2 ultraviolet (UV) light using UV-B filter UV lamp (Honle, Germany) to indicate an extrinsic aging model, followed by topical application of 25 µL of test product or dH_2O (control), and incubated at 37°C and 5% CO_2 for 24 hours. After incubation, five tissues of each condition were placed into RNA*later*® solution (ThermoFisher Scientific).

Quantitative Real-time PCR

mRNA was extracted from the human skin model tissues (Maxwell® RSC simplyRNS Tissue Kit; Promega) followed by cDNA synthesis (High-Capacity cDNA Reverse Transcription Kit; ThermoFisher Scientific) and quantitative real-time PCR (TaqMan Fast Advanced Master Mix, ThermoFisher Scientific). Gene expression analyses were performed using TaqMan Gene Expression Assays (ThermoFisher Scientific) with real-time PCR system QuantStudio7 Flex (ThermoFisher Scientific). The assay mix used for studies were: GAPDH (Cat#: Hs02758991_g1), COL1A1 (Cat#: Hs001640004_m1); COL3A1 (Cat#: Hs00943809_m1), COL6A1 (Cat#: Hs01095585_m1), COL7A1 (Cat#: Hs00164310_m1), TGFB1 (Cat#: Hs00998133_m1), DCN (Cat#: Hs00754870_s1), ELN (Cat#: Hs00355783_m1), FBN1 (Cat# Hs00171191_m1), FBLN5 (Cat#: Hs00197064_m1), MFAP1 (Cat#: Hs00195537_m1), LOXL1 (Cat# Hs00935937_m1), POMP (Cat#: Hs01106088_m1), PSMB5 (Cat#: Hs00605652_m1), PSMB6 (Cat#: Hs00382586_m1), ATG5 (Cat#: Hs00169468_m1), ATG7 (Cat#: Hs00893766_m1), ATG12 (Cat#: Hs04980076_s1), BECN1 (Cat#: Hs01007018_m1), HSPA8(Cat#: Hs03044880_gH).

Elastase Assay

The assay employed was based on methods previously described in literature.[8] In brief, human skin model tissue was homogenized and solubilized in 0.1% Triton-X 100, 0.2 M Tris–HCl (pH 8.0) buffer, followed by ultrasonication and subsequent centrifugation at 2000 x g for 10 minutes to obtain supernatants for enzyme assay. To measure elastase activity, 2 μL of substrate (62.5mM, N-succinyl-tri-alanyl-p nitroanilide from Sigma-Aldrich) was incubated with 100 μL lysate solution at 37°C for 2 hours. The amount of released nitroaniline was measured by determining absorbance at 410 nm using a spectrophotometer (Molecular Devices).

Clinical Study Design

Study Design

A randomized, double-blind, regimen-controlled study was conducted to assess the efficacy and tolerability of NCC as a topical treatment for the neck and décolletage. Criteria for study participation included female subjects aged 45–70 years, presenting moderate to severe overall skin texture on the neck and moderate to severe skin tone unevenness on the décolletage.

Institutional Review Board approval (IntegReview IRB, Austin, TX) was obtained prior to conduct of any study procedures. The conduct of the study followed all applicable guidelines for the protection of human subjects for research as per 21 CFR 50, in accordance with accepted standards for Good Clinical Practices (GCP) and International Conference on Harmonization (ICH). All subjects provided informed consent prior to study participation.

Subjects were not allowed to apply any other topical products than the ones provided (including skin brighteners, retinoids, alpha/beta/poly-hydroxy acids) nor undergo treatments to the test area. All subjects were randomized either to the NCC-treatment group, which used NCC twice-daily (once in the morning and night, after cleansing) in addition to standard skin care products (SkinMedica® Facial Cleanser and SkinMedica® Essential Defense Mineral Shield SPF 35 Sunscreen), or to the control group, which only used the provided standard skin care products. All subjects were provided with pre-weighed units of assigned products of which they were instructed to apply a thin layer to the neck and décolletage and blend in an upward motion. A total of 75 subjects were enrolled in the study with 46 subjects in NCC-treatment group and 29 subjects in control group.

Clinical Efficacy Assessment

Clinical efficacy was conducted at baseline, weeks 4, 8, and 12 by the investigator who was blinded to the treatment randomization of the subject. Subjects were instructed to cleanse their neck, face, and décolletage and remove all makeup at least 30 minutes prior to each scheduled visit. Clinical assessments and standardized photographs of the neck and décolletage were taken at each visit. Assessments of efficacy parameters were conducted at all study visits using a modified Griffiths' 10-point grading scale, where 0=none (best possible condition), 1–3=mild, 4–6=moderate, and 7–9=severe (worst condition possible), with half-point scores assigned as necessary to accurately describe the skin condition. Crepiness, laxity/sagging, and overall skin texture were only assessed on the neck and not décolletage. Efficacy parameters are listed below with description of grade 0 and 9 anchors:

Fine lines/wrinkles: 0=none, 9=numerous, long, deep fine lines/wrinkles

Coarse lines/wrinkles: 0= none, 9=numerous, long, deep coarse lines/wrinkles

Crepiness: 0=skin appears smooth with no wrinkles or "crinkliness", 9=skin appears thin and easily crinkled when lightly pinched

Tactile roughness: 0=smooth, even feeling skin texture, 9=rough, uneven feeling skin texture

Laxity/sagging: 0=firm, dense appearance, no sagging, 9=soft, droopy appearance, prominent skin folds

Overall skin texture: 0=smooth skin appearance and touch no roughness, laxity, nor crepey/crinkled appearance, 9=pronounced, extensive visible skin roughness, lines/wrinkles, laxity and crepey/crinkled appearance

Hyperpigmentation: 0=even skin color, no observable hyperpigmentation, 9= significant (severe) hyperpigmented appearance, involving most of the area, with very strong intensity

Skin tone evenness: 0=even, healthy skin color, 9=uneven, extensive discolored appearance (brown and red colors)

Radiance/Luminosity/Brightness: 0=radiant, luminous, or glowing appearance, 9=full/matte and/or sallow skin appearance

In addition, at weeks 4, 8, and 12, an expert grader assessed the *global improvement in overall skin texture (pronounced, extensive visible skin roughness, lines/wrinkles, laxity and crepey/crinkled appearance)* on the neck and *overall photodamage* on the décolleté using a grading scale, where 1=worse, 2=no improvement, 3=mildly improved, 4=moderately improved, and 5=markedly improved.

Standardized Photography

Digital images were taken of each subject's face, neck, and décolletage (center view, left side, and right side) using Stephens & Associates' photo station with a Canon Mark II 7D digital SLR camera (Canon Inc, Tokyo, Japan) with a Canon EF-S 60 mm f/2.8 macro lens under visible light. Unfiltered full-spectrum (white) light was provided using Profoto D1 (500W) studio strobes affixed to the photo station. Canon EOS utility software was used for image overlay to ensure post-baseline images match baseline images. Prior to photography, subjects were instructed to remove any jewelry from the area to be photographed, cleansed their face, and equilibrated in the clinic for at least 15 minutes.

Cutometer Measurements

A single measurement was taken of each subject's left side of the neck, in the center (vertically), approximately left of the laryngeal prominence ("Adam's apple") using Cutometer MPA 580 (Courage + Khazaka Electronic GmbH, Köln, Germany) to measure the viscoelastic properties of the skin. For each subject, the measured location was marked on a body diagram to ensure the same site was measured at week 12.

Subject Self-Assessment Questionnaire

Subjects completed a self-assessment questionnaire regarding various skin parameters and product texture and attributes at weeks 4, 8, and 12.

Statistical Analysis

Clinical grading scores at weeks 4, 8, and 12 were compared to baseline scores using Wilcoxon signed-rank test and comparisons between treatment groups using Wilcoxon rank sum test. Average percent change from baseline/placebo was calculated for all parameters at each follow-up visit. Comparisons between treatment groups were calculated for all parameters at each follow-up visit. All differences are considered statistically significant at the $P \leq 0.05$ level.

RESULTS

In vitro analysis

The efficacy of NCC in supporting skin rejuvenation was assessed at a molecular biology level using quantitative real-time PCR to provide an early indication of potential activity of the cosmetic product. Human 3D skin models were irradiated with ultraviolet (UV) light to mimic extrinsic skin aging prior to the application of NCC. Gene expression results for UV-irradiated, non-treated tissues and UV-irradiated, NCC-treated tissues were calculated as percentage increase compared to non-UV-irradiated, non-treated control tissues (set as 0%). Expression levels of genes encoding collagens (COL1A1, COL3A1, COL6A1, COL7A1), elastic fiber proteins (ELN, FBN1, FBLN5, MFAP1, LOXL1), TGFβ1 and decorin (DCN) were assessed (Figure 1A and 1B). Application of NCC resulted in significant upregulation of all genes tested compared to UV-irradiated, non-treated samples indicating that NCC stimulates ECM components for dermal structure improvement that could translate into firmer skin, as well as improvement of the dermal-epidermal junction (DEJ), which is important for skin elasticity. Additionally, elastase activity assays showed that NCC significantly reduced the level of elastase (an enzyme that breaks down elastin) following UV irradiation suggesting protection against elastic fiber degradation (Figure 1C).

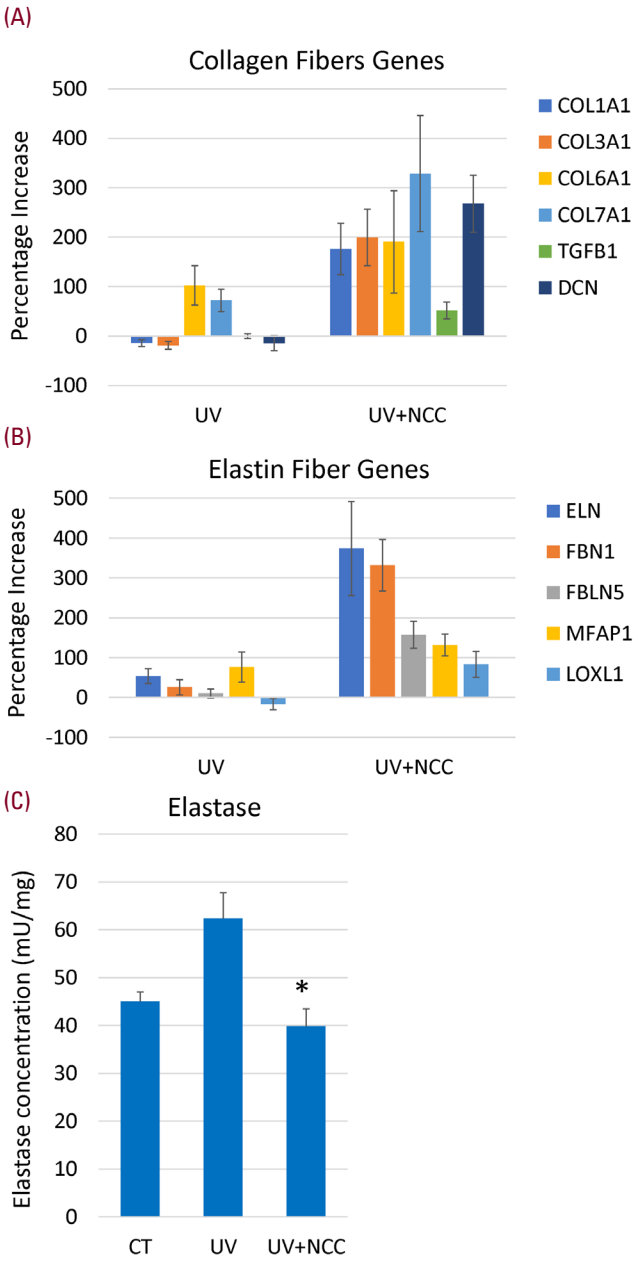
Aging alters essential processes involved in maintaining cellular proteostasis (protein homeostasis), including the proteasome and autophagy systems, leading to an accumulation in cellular debris, protein aggregation, and cellular damage.[9] Gene expression levels of key recycling genes involved in proteasome activity (POMP, PSMB5, PSMB6)[10] and autophagy (ATG5, ATG7, ATG12, BECN1) along with a heat shock protein that facilitates and stabilizes proper folding of proteins (HSPA8) were increased with NCC treatment (Figure 2), indicating stimulation of the various cellular recycling processes.

Altogether the in vitro pre-clinical data demonstrate the efficacy of NCC in providing overall skin rejuvenation benefits by boosting various ECM and DEJ components as well as supporting systems that help maintain proteostasis.

Clinical Efficacy of NCC

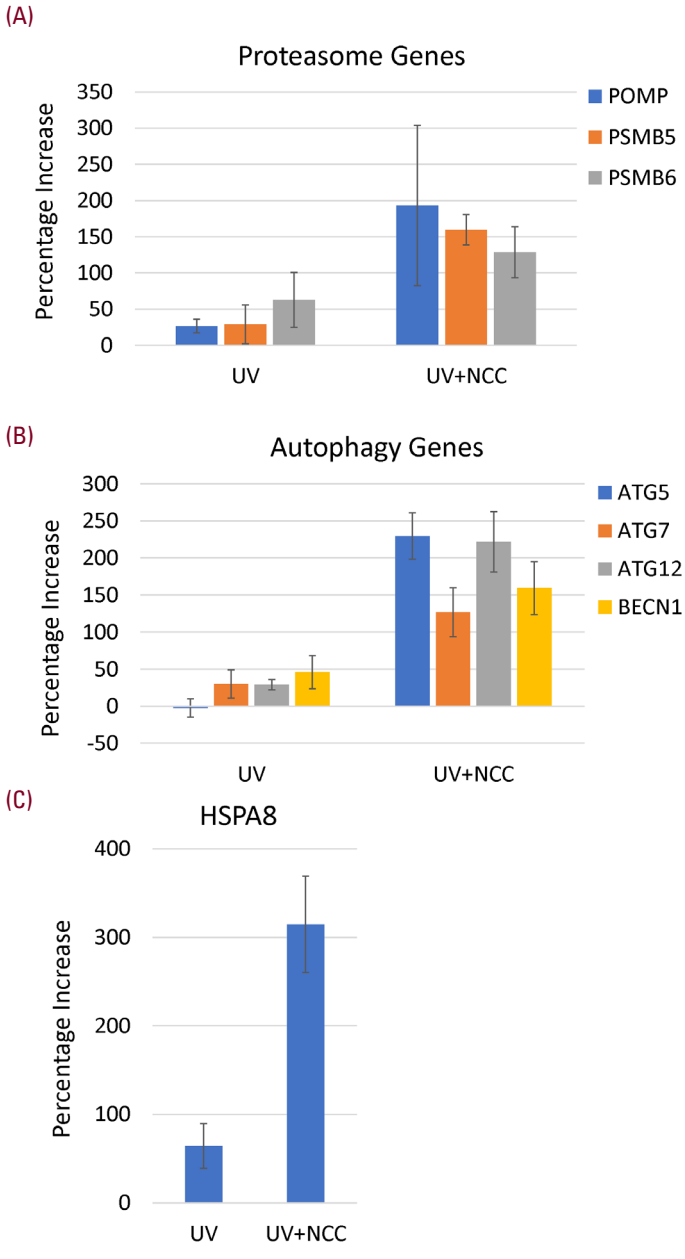
Sixty-nine subjects with Fitzpatrick skin types I–V, completed the 12-week, double-blind, randomized, regimen-controlled study (NCC- treatment group: n=42, control group: n=27). Overall results indicate that twice daily topical application of NCC was effective in improving neck and décolletage skin condition and firmness.

FIGURE 1. (A-B) Quantitative real-time PCR analysis of genes encoding extracellular matrix proteins in UV-irradiated, non-treated (UV) and UV irradiated, NCC-treated (UV+NCC) human skin models. (C) Quantification of elastase in non-irradiated, non-treated (CT), UV-irradiated, non-treated (UV) and UV irradiated, NCC-treated (UV+NCC) human skin models



*Statistically significant vs UV, $P \leq 0.01$; Student's t-test.

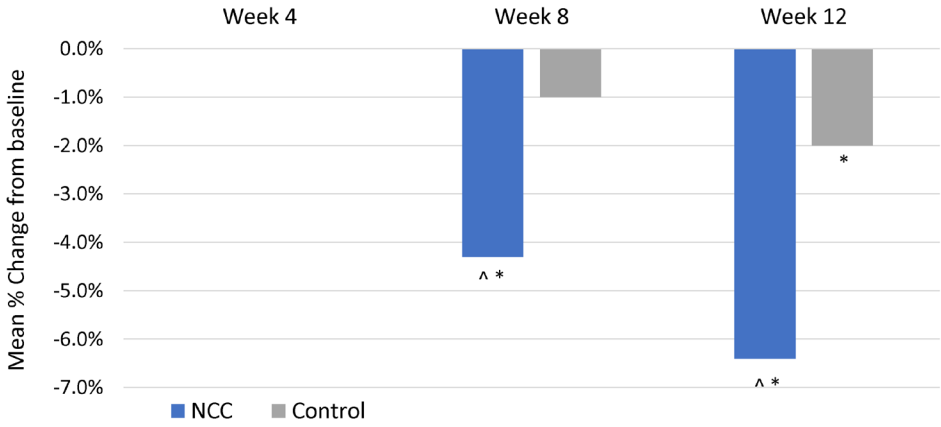
FIGURE 2. Quantitative real-time PCR analysis of genes encoding proteostasis- and cellular recycling-related proteins in UV-irradiated, non-treated (UV) and UV irradiated, NCC-treated (UV+NCC) human skin models.



NCC provided significant improvements in neck laxity/sagging at 8 weeks with continued improvements through week 12, compared to control (all $P \leq 0.006$; Wilcoxon rank sum test) and compared to baseline (all $P < 0.001$; Wilcoxon signed rank test). The control group only showed significance at week 12 compared to baseline in laxity/sagging ($P = 0.031$; Wilcoxon signed rank test; Figure 3).

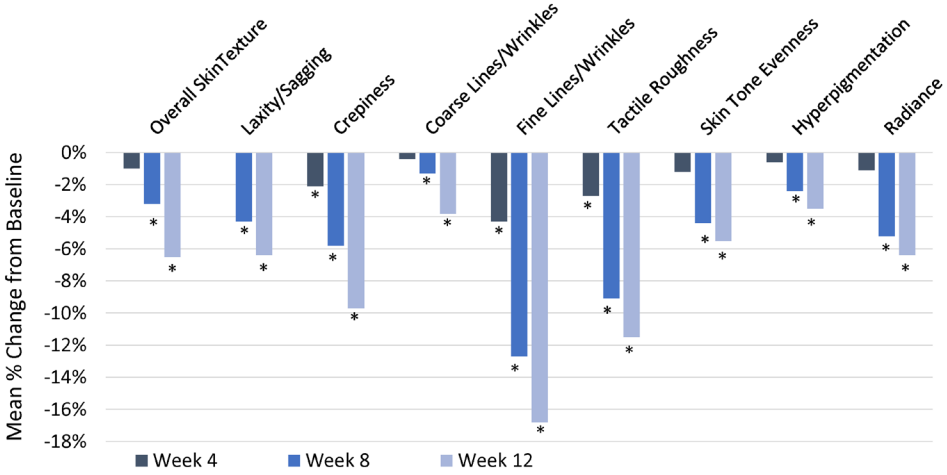
NCC also provided significant improvements in fine lines/wrinkles, tactile roughness, and crepiness at all follow-up visits and in coarse lines/wrinkles, hyperpigmentation, skin tone evenness, radiance, and overall skin texture at weeks 8 and 12, compared to baseline (all $P \leq 0.035$; Wilcoxon signed rank test; Figure 4).

FIGURE 3. NCC provides significant improvements in neck sagging compared to baseline and to control.



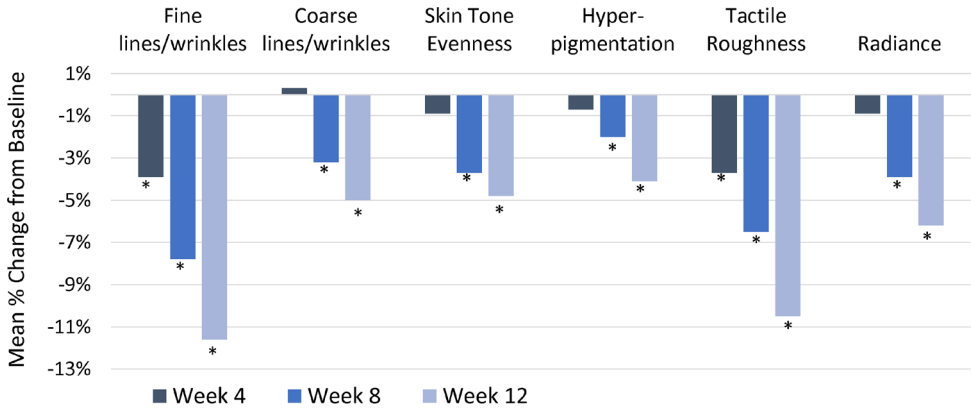
*Statistically significant vs baseline, all $P \leq 0.031$; Wilcoxon signed rank test, ^Statistically significant vs. control, all $P \leq 0.006$; Wilcoxon rank sum test.

FIGURE 4. NCC provides significant improvements in all neck efficacy parameters.



*Statistically significant vs baseline, all $P \leq 0.035$; Wilcoxon signed rank test.

FIGURE 5. NCC provides significant improvements in all décolletage efficacy parameters.



*Statistically significant vs baseline, all $P \leq 0.035$; Wilcoxon signed rank test.

FIGURE 6. Improvement in appearance of neck laxity/sagging and crepiness, age 69, standard lighting. (A) baseline, (B) 8 weeks, (C) 12 weeks.

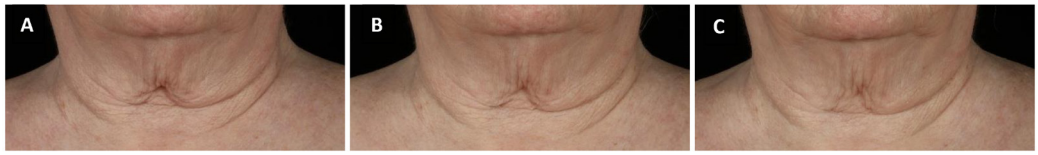


FIGURE 7. Improvement in appearance of neck laxity/sagging and crepiness, age 63, standard lighting. (A) baseline, (B) 4 weeks, (C) 8 weeks.

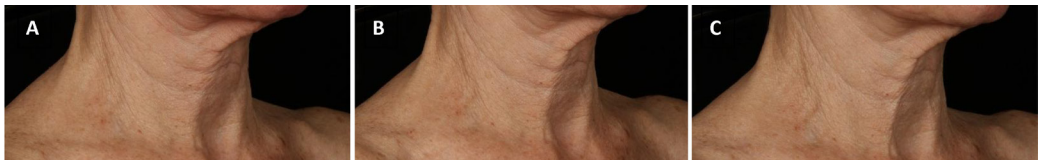


FIGURE 8. Improvement in appearance of lines/wrinkles, skin tone evenness and crepiness of the décolletage and the neck, age 70, standard lighting. (A) baseline, (C) 8 weeks, (C) 12 weeks.

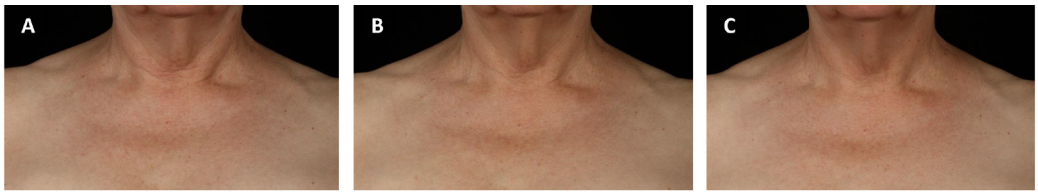


FIGURE 9. Improvement in appearance of skin tone evenness of the décolletage, age 61, standard lighting. (A) baseline, (B) 8 weeks.

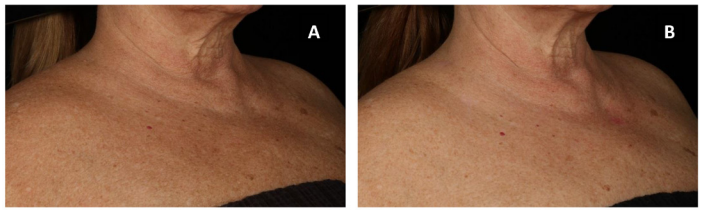
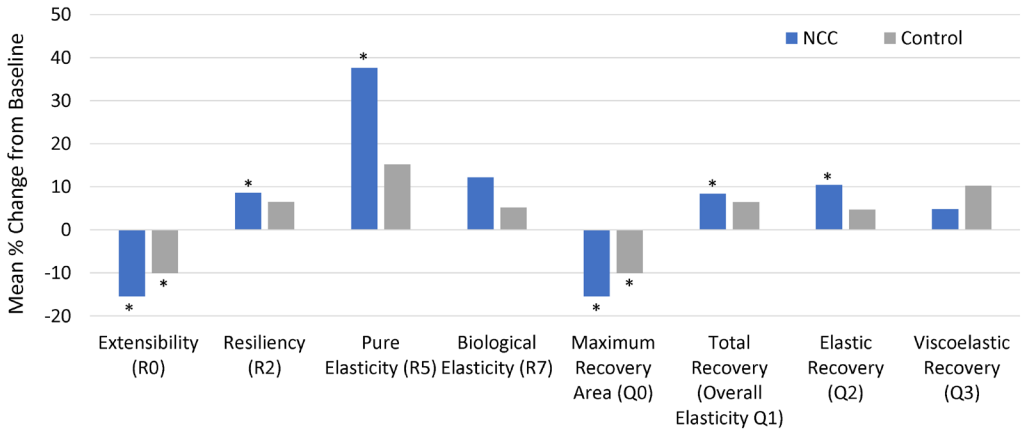


FIGURE 10. Cutometer values change compared to baseline of various elasticity parameters at week 12.



*Statistically significant vs. baseline, all $P \leq 0.035$; Wilcoxon signed rank test.

For the décolletage, NCC provided significant improvements in fine lines/wrinkles and tactile roughness at all follow-up visits, and in coarse lines/wrinkles, hyperpigmentation, skin tone evenness, and radiance at weeks 8 and 12, compared to baseline (all $P \leq 0.035$; Wilcoxon signed rank test; Figure 5).

Investigator's global improvement assessment showed significant improvements over control at weeks 8 and 12 in neck skin laxity/sagging and overall skin texture (including pronounced, extensive visible skin roughness, lines/wrinkles, laxity and crepey/crinkled appearance) ($P \leq 0.009$; Wilcoxon rank sum test). Compared to baseline, the NCC-treatment group showed significant global improvements in overall skin texture on the neck and in overall photodamage on the décolletage at all follow-up visits (all $P \leq 0.002$; Wilcoxon signed rank test). Improvements were observed in the control group but did not reach significance until week 12 for skin texture ($P \leq 0.016$; Wilcoxon signed rank test), and week 8 for overall photodamage on the décolletage ($P \leq 0.031$; Wilcoxon signed rank test). Representative photographs of treatment responses observed by investigator and subjects are shown in Figures 6–9.

Three subjects reported potential treatment-related mild/moderate adverse events (erythema/rash/burning sensation): two subjects in the NCC-treatment group and one subject in the control group. The adverse events (AE) all resolved without sequelae. One of the subjects in the NCC-treatment group discontinued due to the AE and was not included in the intent to treat analysis.

Cutometer measurements demonstrated statistically significant improvements in extensibility (decreased extensibility indicates reduced ability of skin to be extended or stretched), maximum recovery area (decreases with increased firmness of the skin), resiliency, pure elasticity, total recovery (overall elasticity), and elastic recovery for the NCC-treatment group at week 12 when compared to baseline (all $P \leq 0.035$; Wilcoxon signed rank test; Figure 10). NCC-treatment group showed consistently greater improvements numerically over control. The control group showed significant improvements in extensibility and maximum recovery area at week 12, while biological elasticity and viscoelastic recovery did not change for either group (Figure 10). Altogether, these results indicate an overall increase in skin elasticity and firmness.

Subject self-assessment questionnaires support the investigator-observed improvements in neck and décolletage with a significant proportion of subjects agreeing that NCC made their neck look and feel firmer, reduced the appearance of lines and wrinkles, and improved skin texture at all follow-up visits (all $P \leq 0.007$; binomial test). Furthermore, NCC product attributes were highly rated by subjects (100% of subjects agreed) for texture, ease of application, skin absorption, as well as skincare regimen compatibility and convenience.

DISCUSSION

The neck is gaining attention as it plays a telling role as an age indicator, and patients are increasingly seeking treatment options for a younger-looking neck to achieve an overall more aesthetically pleasing look.[11] Because of differences in structure and function compared to facial skin, neck skin ages differently and special considerations should be taken when providing treatment. Though mostly similar procedural modalities can be used to treat both neck and facial skin,[12] neck skin is not prioritized in skincare and the limited topical treatment options available often have little (pre-)clinical data to support anti-aging claims specific to the signs of skin aging on neck and décolletage. Utilizing a formulation optimized through pre-clinical research, the NCC was clinically proven to be effective and well-tolerated in significantly improving multiple key skin quality parameters of the neck and décolletage including laxity/sagging, crepiness and skin tone evenness, supported by blinded investigator assessments as well as objective cutometer measurements.

It is well established that intrinsic and extrinsic aging lead to significant degradation of ECM components such as collagen and elastic fibers. Because of its anatomy, neck skin is more prone to the degradation of these structural fibers losing its elasticity faster with age compared to facial skin, which results in accelerated sagging and deeper wrinkle formation.[4] Supporting ECM components production as well as protection against further degradation while specifically addressing the dermal elastic fibers and DEJ that play vital roles in maintaining skin elasticity and firmness[13] is required to provide robust visible neck skin rejuvenation effects. The presented in vitro data demonstrated that NCC reduces elastase levels and induces the gene expression of key ECM components including elastin, fibrillin 1, fibulin 5, microfibrillar-associated protein 1, and lysyl oxidase-like protein 1 (ELN, FBN1, FBLN5, MFAP1, LOXL-1), which govern the formation of healthy and functional elastic fibers. Collagen type VII (COL7A1) forms anchoring filaments in the basement membrane of the DEJ. Collagens type I and III (COL1A1 and COL3A1) constitute most of the dermis providing skin its firmness and strength for a more youthful appearance, and decorin (DCN) is a proteoglycan that is important for proper collagen fiber alignment. Collagen type VI (COL6A1) is a major component of skeletal muscle ECM and thus, is involved in health and function of muscle fibers.[14,15] Supporting COL6A1 expression can be of particular relevance to improving the overall appearance of aged neck skin since accentuation of platysma muscle protrusion and platysmal bands on the neck mid-line could be tied to weakening and loss of tissue and fibers that connect muscle to the overlying skin.

The proteasome and autophagy systems are part of the cellular recycling mechanism that ensures orderly degradation of damaged, dysfunctional, and misfolded proteins and cellular components to maintain cellular proteostasis. Aging alters the activity of these systems leading to accumulation of damaged

cellular components and an exacerbated intrinsic aging process.[16,17] NCC treatment resulted in upregulation of various genes related to proteasome assembly and function (POMP, PSMB5, PSMB6), different stages of autophagy (ATG5, ATG7, ATG12, BECN1), and protein folding and stabilization (HSPA8) suggesting that NCC helps maintain proteostasis and mitigate intrinsic aging processes to support a healthier and more youthful appearance of the neck and décolletage.

CONCLUSION

Rejuvenation of neck skin requires a differentiated approach that is tailored to the neck's specific characteristics. Designed to address the signs of skin aging of the neck and décolletage, NCC significantly improved skin laxity/sagging, firmness and elasticity of the neck, as well as overall appearance and skin quality of both the neck and décolletage area. The clinical findings from this double-blind, regimen-controlled study were corroborated by in vitro analysis in human 3D skin models demonstrating stimulation of neocollagenesis and neoelastogenesis as well as support of cellular proteostasis, providing insights on possible mechanisms of action for the observed clinical outcomes.

DISCLOSURES

Financial support for these studies was provided by Allergan Aesthetics, Inc., an AbbVie Company. Ms. Makino, Dr. Kadoya, Ms. Chung, Dr. Mikati, and Dr. Mehta are employees of Allergan Aesthetics, Inc., an AbbVie Company.

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Data Sharing

AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual, and trial-level data (analysis data sets), as well as other information (e.g. protocols and clinical study reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications.

This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and statistical analysis plan and execution of a data sharing agreement. Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

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