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A single-center clinical trial to evaluate the efficacy of a tripeptide/hexapeptide antiaging regimen

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Summary

Introduction: An antiaging regimen that aids in clearing the matrix of waste products and stimulating neocollagenesis and neoelastogenesis was tested among a group of subjects over the course of 12 weeks to assess its efficacy in women with mild to moderate wrinkles and skin sagging on the face.

Materials and methods: The efficacy of the product regimen was tested in 22 subjects using investigator clinical grading measurements, raking light imaging, 3D imaging, biopsies, and self-assessment questionnaires at baseline and weeks 4, 8, and 12.

Results: Clinical grading indicated that use of the antiaging regimen for 12 weeks produced a statistically significant improvement in scores for all evaluated parameters; the raking light image analysis demonstrated a statistically significant improvement in values for length, width, and area of wrinkles when compared with baseline values as did 3D imaging. Biopsy results in the 5 patients tested showed improvement in solar elastosis, collagen stimulation, and improvement in cornified layers in all 5 patients. Elastin stimulation was evident in 3 of 5 patients. Results from the self-assessment questionnaire analysis indicated favorable responses in a statistically significant proportion of subjects after 12 weeks of use for all inquiries.

Conclusion: Use of this facial antiaging regimen was effective in improving visual facial photoaging conditions and well-perceived when used by women with mild to moderate wrinkles and skin sagging on the face under the conditions of this study.

KEYWORDS
antiaging formulation, collagenesis, elastogenesis, extracellular matrix recycling, peptides

1 | INTRODUCTION

Photodamaged/aged facial skin is characterized by the presence of fine lines, wrinkles, and mottled pigmentation. In areas exposed to ultraviolet light, this "extrinsic" aging process is superimposed on underlying "intrinsic" aging mechanisms with direct cosmetic and structural consequences for the aging skin. Fragmentation of the elastin and collagenous protein components of the ECM transforms the formerly smoother, fine-lined appearance of intrinsically aged skin to the roughened, deeply wrinkled appearance of photodamaged skin, characterized histologically by the loss of elastin fibrillin microfibrils and fibulin from the papillary dermis together with collagen fragmentation and ECM dilution.[1]

A novel serum with a blend of synergistic peptides and active botanicals (ALASTIN Restorative Skin Complex with TriHex Technology®, ALASTIN Skincare®, Carlsbad, CA) comprehensively addresses these areas of degeneration by aiding in clearing the ECM of collagen and elastin agglutinated products, increasing the efficiency of protease and proteasome systems and stimulating regeneration of collagen, elastin, GAGs, and adipose tissue. ALASTIN Skincare™ with

TriHex Technology® products combine selected peptides and botanicals that target these ECM changes. In a sequence of events, these actives progressively break down the clumped collagen/gelatin elastin bundles and then stimulate replacement with new collagen and elastin, effectively recycling the ECM.[2,3]

The process of recycling the matrix begins with the release of gelatinase enzyme (MMP-2) by tripeptide 1, which reaches maximal levels at 18-22 days thus dissolving the gelatinous collagen/elastin bundles, thereby preparing the matrix for new collagen and elastin synthesis. The TriHex Technology® tripeptide/hexapeptide combination, created in a lipid moiety for improved absorption, is responsible for clearing this segment of the matrix and stimulating new protein synthesis in the form of collagen, elastin, and glycosaminoglycans.[3]

This single-center clinical trial was conducted by Stephens and Associates (Thomas J. Stephens & Associates, Inc, Texas) over the course of 12 weeks to assess the efficacy of an antiaging regimen, when used by women with mild to moderate wrinkles and skin sagging on the face.

2 | MATERIALS AND METHODS

A total of 22 subjects completed the study (Table 1). The study was IRB-approved consistent with the requirements in 21 Code of Federal Regulations (CFR) 50.25. Women between the ages of 45 and 65 years with Fitzpatrick Skin Types I-IV qualified for inclusion (inclusion and exclusion criteria listed below). Following full questioning and medical history taking, certain subjects were randomly assigned to have biopsy procedures.

TABLE 1 Summary of demographic population

	All subjects
N	22
Age (y)	
Mean	54.8
Standard deviation	5.9
Minimum	45
Median	55.5
Maximum	65
	All subjects n (%)
Sex	
Female	22 (100.0)
Ethnicity	
Hispanic or Latino	3 (13.6)
Not hispanic or Latino	19 (86.4)
Race	
American Indian or Alaska Native	2 (9.1)
White	10 (81.8)
Multi-racial	2 (9.1)
Fitzpatrick Skin type	
II	7 (31.8)
III	12 (54.6)
IV	3 (13.6)
Biopsy Sample Collection	5 (22.7)

2.1 | Inclusion criteria (summarized)

- Women 45-65 years of age having general good health.
- Fitzpatrick skin type I-IV.
- Individuals with mild to moderate global face wrinkles and facial sagging skin (score of 3-6 on according to the modified Griffiths' scale1, where 0 = none and 9 = severe, half points are acceptable to qualify).
- Willing to withhold all facial treatments during the course of the study including botulinum toxin, injectable fillers, microdermabrasion, IPL, peels, facials, laser treatments and tightening treatments. Waxing and threading are allowed but not facial laser hair removal.

2.2 | Exclusion criteria

- Individuals diagnosed with known allergies to facial skincare products.
- Individuals who are nursing, pregnant, or planning to become pregnant during the study according to subject self-report.
- Individuals with a history of skin cancer.
- Individuals who used any of the following medications or had any of the listed procedures within the listed time frame prior to the study start date: Retin-A®, Retin-A Micro®, Renova®, Avita®, Tazorac®, Soriatane®, Differin®, or retinol over 1% within 4 months; Accutane® within 12 months; Prescription strength skin-lightening products (eg hydroquinone, tretinoin, AHA, BHA and polyhydroxy acids, 4-hydroxyanisole alone or in combination with tretinoin) within 4 months; Any antiwrinkle, skin-lightening products, or any other product or topical or systemic medication known to affect skin aging or dyschromia (products containing alpha/beta/polyhydroxy acids, vitamin C, soy, Q-10, hydroquinone; systemic or licorice extract (topically), Tego® Cosmo C250, gigawhite, lemon juice extract (topically), emblica extract, etc.), retinoids, or less than or equal to 1% retinols, within 4 weeks; Had a chemical peel, dermabrasion, microneedling, nonablative laser or fractional laser resurfacing of the face and neck within 6 months; Have undergone a regimen of Thermage treatments or an equivalent type of high-energy treatments, plastic surgery, or ablative laser resurfacing of the face and neck within 12 months; Have undergone a noninvasive professional ultrasonic skin tightening treatment (eg Ulthera) with 6 months; Had facial treatment with a botulinum toxin base injectable (Botox), injectable fillers, or a fat transfer within 6 months.

The antiaging regimen (Restorative Skin Complex with TriHex Technology®, Daily Moisturizer, Broad Spectrum Sunscreen (SPF 30+) and Gentle Cleanser, ALASTIN Skincare®, Carlsbad, CA) was applied to the entire face twice per day (each morning and evening) after cleansing, except the sunscreen was applied only in the morning.

Subjects were clinically graded for the following efficacy parameters at the indicated locations using a modified Griffiths' 10-point scale[4] according to the following numerical definitions (with half-point scores assigned as necessary to accurately describe the skin condition-0 = none-best possible condition; 1-3 = mild; 4-6 = moderate; 7-9 = severe-worst possible condition).

During the course of the study, subjects used the antiaging regimen on the face as directed. Subjects were evaluated for study eligibility at visit 1 (screening) and clinical evaluations were conducted at visit 2 (baseline), visit 3 (week 4), visit 4 (week 8), and visit 5 (week 12). Subjects participated in the following procedures at the indicated visits:

- Clinical grading of efficacy parameters

  At baseline and weeks 4, 8, and 12, subjects were clinically graded for the following parameters at the indicated locations: fine lines (forehead, crow's feet, under eye, cheek, nasolabial, and marionette lines), radiance (global face), skin firmness (global face), skin plumpness (global face), skin sagginess (jawline, cheek, and eye area), and wrinkles (global face).
- VISIA imaging

  At baseline and weeks 4, 8, and 12, full-face digital images were taken of each subject (right side, left side, and center views) using the VISIA CR photo-station (Canfield Imaging Systems, Fairfield, New Jersey) with a Canon Mark II 5D digital SLR camera (Canon Incorporated, Tokyo, Japan) under the following lighting conditions: standard 1 (visible [general purpose white light]), standard 2 (visible), standard 3 (raking light for crow's feet area), standard 4 (raking light for forehead area), cross-polarized, parallel polarized, and UV spots (blue). Left-view images were analyzed for crow's feet wrinkles upon study completion
- Primos^{lite} imaging

  At baseline and weeks 4, 8, and 12, images of the right crow's feet area were taken of each subject using Primos^{lite} 45 × 30 mm system which is a handheld 3D imaging device used to assess the microtopography of skin. Images were analyzed for crow's feet wrinkles upon study completion.
- Biopsy sample collection

  At baseline and weeks 8 and 12, 5 subjects had 1 punch biopsy (3 mm in size) collected from a location along the hairline (between the corner of the eye and top of the ear) on the right or left side of the face (according to randomization). Samples were shipped in accordance with Sponsor directions for analysis.
- Self-assessment questionnaires

  Subjects completed a Sponsor-provided self-assessment questionnaire at weeks 8 and 12.

2.3 | Self-assessment questionnaires

- Improved the overall condition of my skin
- Improved the overall appearance of my skin (week 12 only)
- Made my skin look more youthful
- Improved the appearance of my global facial fine lines and wrinkles
- Improved the appearance of my forehead fine lines and wrinkles
- Improved the appearance of my eye area fine lines and wrinkles
- My skin feels softer and smoother—Improved my overall skin tone
- Made my skin feel more elastic—Increased the radiance and clarity of my skin
- Made me feel more confident in the way my skin looks
- I would continue using this treatment regimen
- I would recommend this treatment regimen to others
- Overall satisfaction (week 12 only)

3 | RESULTS

Results of clinical grading of efficacy parameters indicated that use of the antiaging regimen for 12 weeks produced a statistically significant improvement in clinical grading scores for all evaluated parameters (fine lines on the forehead, crow's feet, under eye, cheek, nasolabial, and marionette lines; skin sagginess on the jawline, cheek, and eye area; and radiance, skin firmness, skin plumpness, and wrinkles on the global face—every listed parameter above $P < .001$) when compared with baseline scores.

After 12 weeks of use, the raking light image analysis showed that use of the antiaging regimen produced a statistically significant improvement in values for length, width, and area of wrinkles when compared with baseline values (Figure 1). Similarly, the Primos^{lite} 3D image analysis indicated that use of the antiaging regimen produced a statistically significant improvement in values for length, depth, area, and volume of wrinkles at week 12 when compared with baseline values (Figure 2). Overall wrinkle counts become relatively unimportant in these types of assessments as the count may well increase as the longer wrinkles break up into smaller ones. Thus, length, width, and area measurements are paramount in these image assessments.

3.1 | Biopsy sample collection and results

At baseline and weeks 8 and 12, 5 subjects had 1 punch biopsy (3 mm in size) collected from a location along the hairline (between the corner of the eye and top of the ear) on the right or left side of

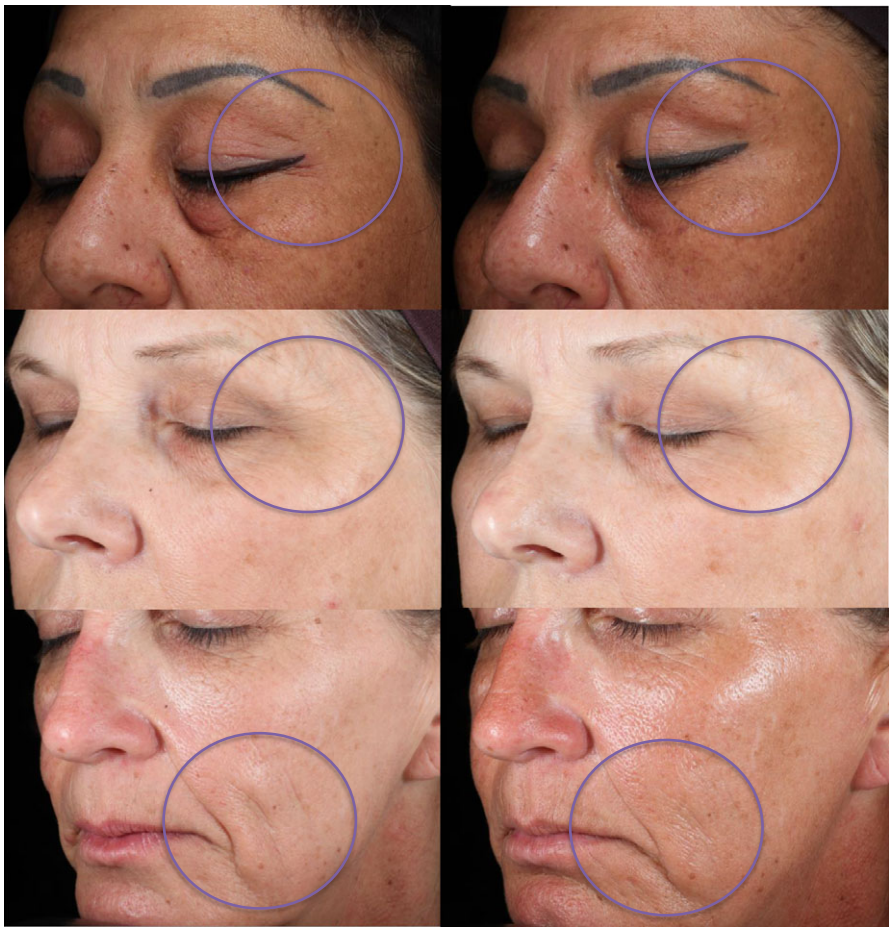

FIGURE 1 Clinical imaging using Visia system examining crow's feet and cheek changes after 12 wk of topical application (left before, right after 12 wk)

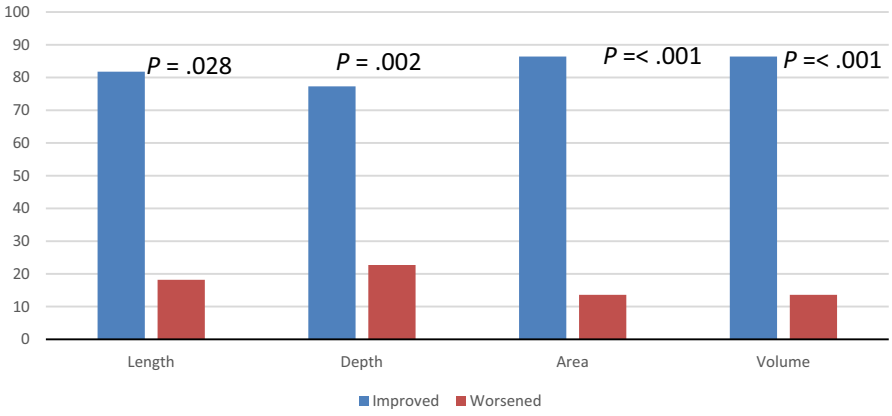

FIGURE 2 Change from baseline statistics for primos[lite] image analysis of wrinkles —12-wk data. Calculated from Wilcoxon signed-rank test for count and Paired t-test for the other parameters. Testing hypothesis is that the mean change from baseline is equal to zero

the face (according to randomization). Areas examined included changes within the dermal extracellular matrix, collagen arrangement and neocollagenesis, reversal/improvement in solar elastosis and evidence of new elastin fibers, improvement in epidermal status and corneocyte layer—Results: Table 2; Figure 3.

The following parameters were examined in histological sections based on anticipated results relating to actives within the test product (most attention was given to the 12-week result vs baseline).

- Solar elastosis: This reflects as a loss of eosin staining on H&E sections resulting in a bluish color (basophilic degeneration) of the upper dermis with accumulation of irregularly thickened elastic fibers. Reversal with extracellular matrix (ECM) clearance of elastin and collagen clumping within the extracellular matrix results in eosinophilic pinking of the matrix with regeneration. All 5 patients showed improvement of solar elastotic changes
- Corneocyte layer/barrier function: Strengthened thickened layer is sought; again, on H/E, this was demonstrated in all cases
- Neocollagenesis: Stimulation of new fresh, young collagen fibers particular in upper dermis and beyond. In keeping with reversal of basophilic degeneration seen in all cases, evidence of neocollagenesis was seen in all 5 biopsy specimens at the 12-week period

TABLE 2 Histological changes related to solar elastosis

	Solar elastosis (H/E)		Corneocyte layer (H/E)	Neocollagenesis (H/E)			Epidermal thickening (H/E)		Elastin fibers (IHC, VVG)		
	Improved	Worse	Strengthened	+	–	No diff	Yes	No	Increase	Less	No diff
1	✓		✓	✓			✓		✓		
2	✓		✓	✓			✓		✓		✓
3	✓		✓	✓			✓		✓		
4	✓		✓	✓			✓				✓
5	✓		✓	✓			✓		✓		

Histological changes related to solar elastosis (in the 5 subjects who were subjected to biopsies).

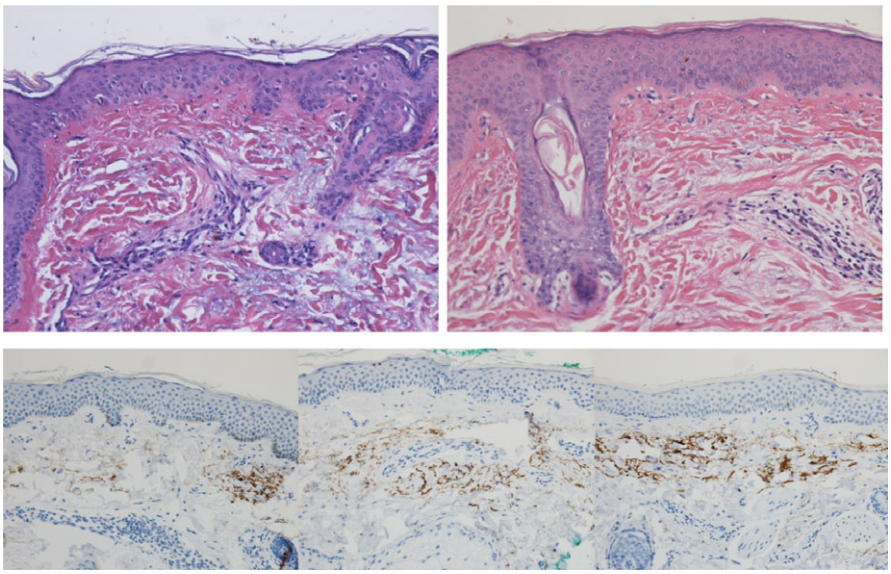

FIGURE 3 H/E staining 100× showing neocollagenesis, epidermal thickening, corneocyte improvement and Elastin IHC patient staining shows significant, progressive, increase in Elastin from time points 1 to 2 to 3 (below)—the changes correlate with the H&E findings

- Epidermal changes: Aged skin manifests as thinned epidermal layer with flattening of cells at the dermo-epidermal junction. At 12 weeks, thickening and improvement of the epidermal layer was evident in all cases
- Elastin fibers: elastogenesis is very difficult to consistently identify—therefore, 2 IHC and VVG stains were undertaken to try to accurately identify new healthy elastin fibers. At 12 weeks, there appeared to be increased elastin fibers in at least 3 of the 5 cases. Two specimens were equivocal as changes were more apparent in 1 stain than the other and a vast increase seen in one posttreatment case at 8 weeks could have been due to a biopsy site variation. However, no cases demonstrated decreased elastin staining in comparison to baseline and only 1 case out of 5 showed no difference between baseline and 12 weeks.

Table 2: Histological changes after 12-week use of test product compared to baseline.

Results from the self-assessment questionnaire analysis indicated that a statistically significant proportion of subjects selected favorable responses compared to unfavorable responses regarding the test materials after 12 weeks of use for all inquiries results from the self-assessment questionnaire analysis indicated that a statistically significant proportion of subjects selected favorable responses compared to unfavorable responses regarding the test materials after 12 weeks of use for all inquiries, including improvements in overall condition and appearance of skin ($p<.001$), appearance of fine lines and wrinkles on the global face, forehead and eye area ($p<.001$), overall skin tone ($p<.001$), more youthful appearance ($p<.003$), skin feel more elastic ($p<.001$), softer and smoother ($p<.001$), increasing radiance and clarity of skin ($p<.001$), and willingness to continue using this treatment regimen ($p<.001$), recommending to others, ($p<.001$) and overall satisfaction ($p<.001$), including improvements in overall condition and appearance of skin, appearance of fine lines and wrinkles on the global face, forehead, and eye area, overall skin tone, more youthful appearance, skin feel more elastic, softer and smoother, increasing radiance and clarity of skin, and willingness to continue using this treatment regimen, recommending to others, and overall satisfaction.

4 | DISCUSSION

Skin aging is the obvious external manifestation of a natural process occurring in tissues and organs throughout the body. Intrinsic

processes (genetics, cellular metabolism and senescence, hormones) present with a gradually advancing loss of elasticity, fine lines and a slowed turnover of regenerating cells. Cumulatively, these present as a recognized pattern of structural and physiologic changes that manifest as aged skin.

In particular, the changes occurring within the extracellular matrix (ECM) have profound effects on cell to cell and cell to matrix signaling and cross talk. One of the main mechanisms responsible for intrinsic and extrinsic aging of the cells is the accumulation of damaged proteins in the cells and ECM.[5,6] These proteins are modified by various posttranslational mechanisms common with aging such as oxidation, glycation, and conjugation with products from lipid peroxidation. In young healthy skin, the proteolytic systems can effectively prevent the accumulation of damaged proteins both intracellularly and within the ECM,[6] whereas in older, damaged skin the systems become inefficient and "clogged" with these protein fragments.

Previous reports have established the link between extracellular matrix collagen fragmentation and oxidative stress.[7] The loss of cell shape and mechanical tension is closely associated with increased transcription factor AP-1, which stimulates MMP production and decreases type I procollagen expression.[8] UV irradiation can reduce collagen production by approximately 80%, with changes occurring within 24 hours following a single acute exposure and then subside to normal during the following 48-72 hours.[9] However, even daily minimal to moderate sun exposure results in cumulative damage and manifests in the abnormalities described above. The insoluble elastin then becomes resistant to neutrophil elastase digestion.[10,11] The remaining insoluble elastin is degraded to soluble fragments predominantly by MMPs-2,9.[10,12]

Very few skincare formulations available today pay attention to these important background changes taking place after years of wear and tear and toxic exposures. The comprehensive skincare regimen tested in this study addresses ECM recycling in its entirety by incorporating agents that support both intracellular and extracellular protein renewal. This is accomplished by addressing the following areas[2]:

- Modulation and clearance of metabolic byproducts of photodamage and aging both in the extracellular matrix (tripeptide 1 via MMP-2 stimulation) and within the cells (UPS and autophagic system stimulation—tripeptide and phosphatidylserine)
- Control of the corrosive damaging proteases (decreasing MMP1/collagenase—phosphatidylserine)
- Stimulation of new collagen and elastin formation (tripeptide, hexapeptides, dipeptides)
- Volumizing the ECM and subdermis to optimize fibroblast function (hexapeptide 38, ornithine)
- Incorporating antioxidant activity against oxygen radicals (niacinamide, phytoene, phytofluene)

One "Hex" component of the TriHex formulation has the repeating amino acid sequence found in tropoelastin and the key sequence found at the binding site for the elastin protein to its cell surface receptor. Matrikines that predominantly activate elastin formation, elastokines, are among the most important matrikines yet described. This is because these elastin-derived peptides are chemotactic for fibroblasts and monocytes and have the capacity to stimulate the generation of elastin.[13,14] Thus, the overall strategy is one of ECM clearance, predominantly by tripeptide, followed by replacement with neocollagenesis and elastogenesis by tripeptide, hexapeptide combinations.

Volumizing of the dermis and subdermal areas improves the density of these areas allowing stretching of the fibroblast at its focal adhesion sites resulting in spindle-shaped functional cells. This heralds new collagen, elastin and GAG production. Volumizing results from this neocollagenesis, GAG and elastin production but can also be contributed to in large degree by fatty tissue, the process of adipogenesis.

The Alastin proprietary formulation includes a PGC1α stimulator (peroxisome proliferator-activated receptor-gamma—PPARγ—coactivator 1 alpha). PGC1α is a transcriptional coactivator that plays a central role in adipogenic activity. It is intimately involved in thermogenesis conserving and donating energy as heat in response to environmental conditions such as cold stress.[15-17] The formulation incorporates a phospholipid delivery system to facilitate penetration and absorption of the materials through the stratum corneum.

In the process of adipogenesis, PGC1α expression is strongly induced in differentiation of preadipocytes of mesenchymal origin into white adipocytes. This differentiation of preadipocytes to white adipocytes occurs under the influence of PPARγ.[18,19] The young adipocytes formed under these conditions appear to be small and active, and this size and activity have been seen to be synergistic and in line with good elastin formation.[19,20] In other words, large, mature adipocytes have been associated with diminished elastin—manifesting as aged sagging skin—whereas younger, smaller, newly synthesized adipocytes are accompanied by increased elastin levels. In addition, adiponectin secreted by small adipocytes (found in normal nonobese subjects) increases hyaluronic acid synthesis, whereas palmitic acid secreted by enlarged adipocytes (obese subjects) decreases collagen and elastin and fibroblast function.[19,20] Fibroblasts cultured with enlarged adipocytes have shown decreased elastin gene expression. PGC1α stimulators, such as those used in ALASTIN Skincare™ formulations, are thus useful candidates for increasing adipogenesis, providing small active adipocytes through this PPARγ activation pathway.

The results of this trial suggest that the required improvement or reversal of solar elastosis has been achieved and manifests as improved skin tone and firmness, decreased sagginess, diminished lines and an overall "plumpness" with a more youthful global appearance. Biopsy data validate the statistically significant positive investigator assessments and image analyses. In addition, clinical results (Figures 1 and 2) correlated with clinical grading, image analysis and biopsy results.

5 | CONCLUSION

Overall results of this single-center clinical trial conducted over the course of 12 weeks indicate that use of a skincare regimen including a novel serum with a blend of synergistic peptides and active botanicals was effective in improving visual facial photoaging conditions and well-perceived when used by women with mild to moderate wrinkles and skin sagging on the face under the conditions of this study.

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