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Histological Changes Associated with Extracellular Matrix-Remodeling Topical Therapy

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Abstract

Introduction: An innovative skincare product line incorporating a proprietary blend of selected peptides and botanicals in a skin-penetrating formulation has been developed with the aim of preparing skin surfaces for rejuvenating procedures enhancing healing and outcomes and for their long-term maintenance. The premise is to clear the extracellular matrix (ECM) of accumulated waste products improving cellular ECM cross talk enabling efficient collagenesis and elastogenesis to follow.

Methods: To validate this scientific narrative and examine efficacy without the use of rejuvenating devices, we have undertaken a series of histological examinations in 5 subjects in their 60s assessing changes following isolated topical application of product to the skin.

Results: From a histological perspective, our findings showed significant changes within the ECM, with new collagen formation and increased elastin, a 'healthier' epidermis with healthy cuboidal basal stem cells at the dermo-epidermal junction, a thickened epidermis and increased procollagen levels within the newly created ECM. This was evident in all patients in varying degrees.

Conclusion: It is apparent that in periods as short as 3 weeks, histological changes can be initiated purely by topical application of this proprietary blend of peptides and botanicals. This validates the use of these products as pre-conditioning for procedures and in conjunction with the use of rejuvenating devices

Keywords: Histology; Collagen; Elastin; Peptides; Extracellular matrix remodeling

Introduction

Through daily living we are subjected to aging processes that manifest in changes within multiple organ systems including the skin. Skin aging occurs through intrinsic processes (genetics, cellular metabolism, hormones and senescence) and extrinsic factors (photodamage, sunlight exposure, pollutants, chemicals, toxins) resulting in gradual loss of elasticity, fine lines and a slowed turnover of regenerating cells [1].

On a cellular, molecular level the area most affected by these changes is the extracellular matrix (ECM). The ECM governs cell-to-cell and cell-to-matrix interactions, signaling and cross talk. With aging of the cells, a gradual accumulation of damaged proteins occurs within the cells and ECM [1,2]. These proteins are modified by various post-translational mechanisms common with age 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 [2], whereas in older, damaged skin the systems become inefficient and 'clogged' with these protein fragments. UV irradiation can reduce collagen production by fibroblasts by approximately 80%. Fragmentation of this collagen in the ECM environment prevents good fibroblast attachment resulting in round, inefficient, senescent cells, thought to be the major cause of the reduced collagen production in both photoaged and chronologically aged human skin fibroblasts [3].

A proprietary formulation combining selected peptides and botanicals targets the ECM changes described above. 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.

As noted, breakdown products of aging and 'wear and tear' tend to accumulate and disrupt communication in the ECM in the following ways:

1) Photodamage releases enzymes (MMP-1, collagenase) that fragment collagen1 and neutrophil elastase, affecting the quality of existing elastic fibers, breaking down fibrillin components [1,3].

2) The fragments of collagen that remain, known as gelatin, adhere to the elastic fiber fragments causing an agglutination of gelatinous clumps interfering with normal ECM communication.

3) These fibers are recognized histologically as mature clumped batches within the ECM.

4) Senescence of basal stem cells in the epidermis manifests as flattened cells resulting in reduced epidermal turnover with overall thinning of the epidermal layer.

Tracking these changes within the skin and measuring efficacy of topical formulations can be challenging. To date the gold standard for *in vivo* 'proof of concept' and objective measurement of efficacy is the histological assessment of skin biopsy samples. Alterations manifesting as cellular changes and ECM remodeling are considered good evidence of efficacy.

The premise of the use of these products is to prepare the skin for

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rejuvenating procedures around 2 weeks prior to the procedures, thus clearing the ECM and improving the cross talk between fibroblasts and collagen fibers. Hastened healing and improved outcomes have been observed secondary to these ECM changes.

Methods

To validate the scientific narrative that has been developed for innovative skincare products incorporating a proprietary blend of selected peptides and botanicals (Regenerating Skin Nectar with TriHex Technology™, Restorative Skin Complex with TriHex Technology™, ALASTIN Skincare, Inc., Carlsbad, CA), we have undertaken a series of histological examinations in 5 subjects in their 60s assessing changes related to isolated topical application of product to the skin. The purpose of this informal study was to ascertain if topical application of product alone could initiate histological changes in a short period of time.

Five (3 females, 2 males) patients were chosen for the study. Previous criticisms for biopsy results have been levelled at authors related to the choice of anatomic sites. Thus, to avoid criticism that the biopsy itself could have caused these results we elected to biopsy opposite volar forearms in non-sun exposed areas in 2 patients. To avoid critique that different areas may have different changes to start, we biopsied a further 2 patients in sun-exposed preauricular areas in the same site but adjacent to the previous biopsy. Finally, the last patient was followed and documented clinically as well as histologically over a longer period of time in the peri-ocular area. Representative histological results are presented on all these patients

Results

In this series of cases, topical application of product to various anatomical areas was undertaken twice a day for periods ranging from 3 weeks to 8 weeks. Patients underwent no procedures before or during the testing period and no other products were used. Baseline biopsies were taken before application at time intervals as indicated in individual results below. Changes in collagen nature and volume were documented in all 5 biopsies. Evidence of elastogenesis was evident in 4 of 5 cases. Special staining for procollagen levels undertaken in 1 case demonstrated increased levels. Detailed documented changes in individual cases follow.

Formulations with TriHex peptides and selected botanicals simultaneously activate the production of metalloproteinases and anti-proteases that remove damaged proteins from the ECM macromolecules while activating the synthesis of new proteins for rebuilding the ECM [4-7]. Tripeptide increases MMP-2 (gelatinase) levels in the ECM digesting the clumped gelatin fragments [8,9], clearing the ECM of these mature bundles (Figures 1 and 2) followed by stimulation and replacement of freshened collagen and elastin by tripeptide and hexapeptide (Figures 2-4).

One of the peptides contained in the TriHex peptide (hexapeptide) is an elastokine with a repeating amino acid sequence found in tropoelastin and containing the key sequence found at the binding site for the elastin protein to its cell surface receptor. Elastokines are among the most important matrikines because these elastin-derived peptides are chemotactic for fibroblasts and monocytes and have the capacity to stimulate the generation of elastin as demonstrated on the forearm of this patient [10,11] (Figure 3).

The dramatic stimulation of elastin does not only manifest in those who demonstrate little dermal elastin staining at baseline. In one subject, significant elastin staining was observed in the pre-treatment biopsy

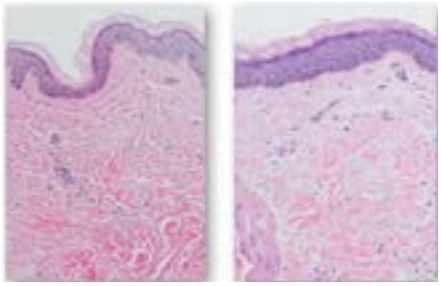

Figure 1: H/E staining (100X) showing ECM and epidermal changes following 3week topical application of product containing TriHex peptides and botanicals to the forearm (female 65 years). Baseline H/E stained sections from non-treated forearm (left) skin shows thicker, mature collagen bundles, and thinner epidermis with flattened basal cells. Post-treatment (right), the epidermis shows less atrophy, with increased number of layers, more cuboidal basal cells, and recovery of normal epidermal maturation. The ECM shows finer collagen bundles, likely representing neo-collagenesis.

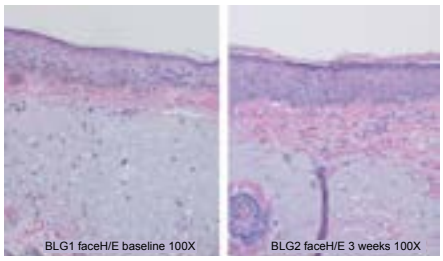

Figure 2: H/E stained sections (200X) from biopsies taken at baseline (left) and following 3 week (right) topical application of an anhydrous topical TriHex peptide gel to the pre-auricular region (male 60s). There is an increase in the number of layers in the epidermis (less epidermal atrophy), with recovery of normal epidermal maturation. In the dermis, newly formed (pink) collagen is present, replacing some of the severe solar elastosis. An increased number of fibroblasts is easily identified among the newly formed ECM in the papillary dermis.

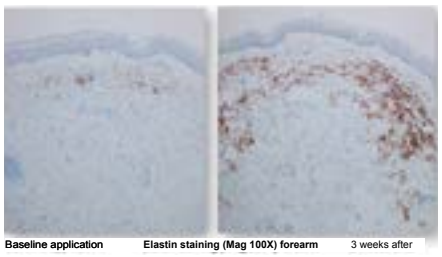

Figure 3: Elastin immunohistochemical staining of sections from biopsies taken at baseline and following 3-week topical application of the TriHex and botanical containing serum to the forearm (male 63 years) (100X) shows a striking increase in the amount of dermal Elastin staining post-treatment. (Abcam Anti-Elastin mouse monoclonal antibody [BA-4] 1:100 dilution)

specimens. In this case, topical application of the product resulted in not only an increase in elastin staining, but also significant, gradual, uniform redistribution of elastin throughout the upper dermis (Figure 4).

A key objective in altering the destructive ECM milieu is to prevent corrosive enzymes and their end products from causing protein fragmentation, misfolding, abnormal cross linkages and amorphous elastic fiber clumps. To this end, phosphatidylserine (PS), a highly enriched membrane phospholipid component, is known to have several physiological roles, such as activating signaling enzymes and antioxidant activity [12]. PS has been found to decrease expression of matrix metalloproteinases causing collagen and elastin degradation in a dose dependent manner, to increase procollagen formation and to

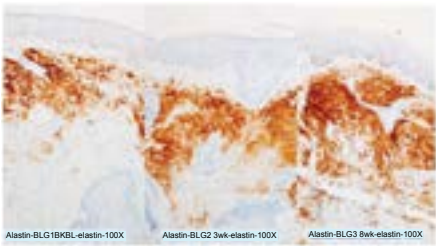

Figure 4: Topical application of TriHex peptide and botanical containing anhydrous gel to the pre-auricular region at baseline, 3 weeks and 8 weeks (male 60s) results in significant gradual increase in dermal Elastin staining (100X). (Abcam Anti-Elastin mouse monoclonal antibody [BA-4] 1:100 dilution).

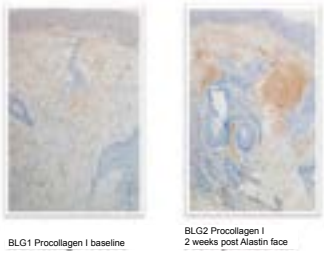

Figure 5: Sections stained with an immuno-histochemical stain for Pro-collagen-1 show slight staining in the upper and mid dermis at baseline and increased staining in the upper and mid dermis at 3 weeks of topical application in pre-auricular region (Female 62 years).

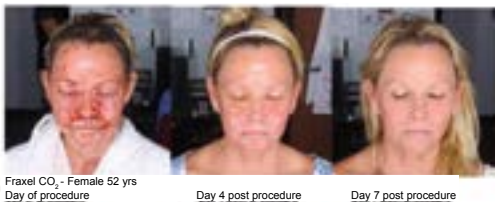

Figure 6: Example of the use of product before, during and after invasive resurfacing procedure demonstrating hastened healing with improved symptomatic relief (redness, exudate, pain, itching.)

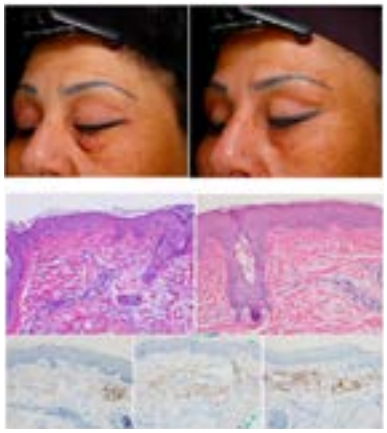

Figure 7A: Topical application of the TriHex and botanical anti-aging line to the Crow's feet region at baseline and 12 weeks (Female 61 years) showing reduced wrinkles with improved texture and tone in the Crow's feet area.
Figure 7B: Histologically the same patient demonstrates significantly decreased solar elastosis with new collagen formation, improved cornified layer and epidermis – (above) H/E staining (100X) and Elastin IHC staining shows significant, progressive, increase in Elastin from time points 1 to 2 to 3 - the changes correlate with the H&E findings and the VVG stained slides (below).

possibly act as a substrate for glycation end product (AGE) targets, thus reducing the damage from glycation effects [12-14]. Procollagen staining was added to the histological examination in one case - the biopsy of this 62-year-old female following 3 weeks of topical application demonstrates increased procollagen levels (Figure 5).

Discussion

The science behind this novel skincare formulation with TriHex technology is based on the concept of approaching photo-damaged and aged skin as we would a chronic wound. The photo-damage and aging process too, is a chronic one, with disturbances in ECM constitution, senescent cells and an imbalance of proteolytic mechanisms. Invasive resurfacing procedures are designed to treat aging skin by denaturing collagen and proteins, producing more protein fragments that normally stimulate collagen regeneration. However, in a background of excessive existing photo-induced protein fragmentation, clearance of these fragments prior to the procedure may facilitate the regenerative phase and hasten healing. Thus, in order to stimulate matrix regeneration, improve skin health maintenance and to optimize healing from rejuvenative procedures, a sequence of 'skin bed preparation' and matrix modulation has been introduced. This takes the form of ECM modulation by aiding in the removal of protein degradation products, balancing inflammatory mediators and proteases, and stimulating basal keratinocytic stem cells and fibroblasts, setting the stage for regeneration of collagen within the ECM. Thus the anhydrous gel with TriHex peptides and botanicals is used as a pre-conditioning aid before the rejuvenating procedure to prepare the ECM and following the procedure to optimize healing. This has been shown to manifest clinically as hastened healing with improved symptomatic relief (less redness, exudate, pain, itching, etc.) following invasive resurfacing procedures (Figure 6). This short period of time was used to demonstrate improved healing and symptomatology when the topical is used as pre-conditioning and immediately after the rejuvenating procedure.

In addition, the Complex product (anti-aging line) uses the same TriHex peptide and botanical technology to clear the matrix and stimulate new collagen and elastin production, with added ingredients to create some plumping of the skin (Figures 7A and 7B).

Overall, from a histological perspective, our findings show significant changes within the ECM in all subjects tested with new collagen formation and increased elastin, a 'healthier' epidermis with more cuboidal basal stem cells at the dermo-epidermal junction and a thickened epidermis. In the subject tested for procollagen effects, increased procollagen staining was evident within the newly created ECM. Thus, a process of recycling of the ECM appears to have been initiated within weeks of application of this topical formulation. This provides evidence for the premise of optimizing rejuvenating procedure outcomes and long-term skin maintenance, findings supported by several clinical case studies [15,16].

A major limitation of this study is the small sample size. However, the aim of the study was to demonstrate that even with topical application alone (no device used) it was possible in a short period of time to initiate changes in the ECM, thus justifying a pre-conditioning period before a rejuvenating procedure. The different sites for biopsies were chosen to demonstrate that changes were possible in sun-exposed (face) as well as sun-protected (volar forearm) areas. In addition, in some cases the same site was chosen for repeat biopsy to show changes in this same site. Then to avoid critique that the wounding of the biopsy initiated some change, we also did biopsies on opposite sides of the body (both forearms). Patients in the 60-year-old range were chosen

because established solar elastosis could be demonstrated and changes in solar elastosis as observed after topical application of product, are highly significant related to the ECM changes that were sought. Thus, even with the small sample, we tried to limit many possible variables.

These observed histological changes are in keeping with a clearance and recycling of this matrix from old collagen and elastin to new proteins. This transformation of the ECM allows for improved cross-talk between fibroblasts and the ECM proteins allowing for more efficient regeneration when procedures are performed (especially if pre-conditioned for 2 weeks before) and for longer term anti-aging maintenance. The advantage of applying the Regenerating Nectar with TriHex Technology is the sequential mode of action that is produced by this formulation. First MMP2 is released which breaks down collagen/elastin clumping, then once these old waste products are cleared, new collagen and elastin is stimulated providing a freshened matrix for regeneration. Now the procedure is performed, the laser/RF etc denatures collagen which stimulates new collagen and elastin formation. This process is accomplished much more efficiently in an environment of a cleared ECM. In addition, anti-inflammatories, anti-oxidants and an anhydrous solution ensure that the patient endures far less discomfort at the time of the procedure than is the case with typical bland standard of care ointments often used post-resurfacing procedure.

Conclusion

Biopsy and histological assessment of topical formulations have long been regarded as the 'gold-standard' for *in vivo* confirmation of efficacy. Using this time-tested analysis in 5 subjects, we have been able to demonstrate significant changes within the ECM and related cellular structures that validate the scientific narrative of ECM recycling and skin bed preparation for peri-procedure use and long-term skin maintenance.

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