Histological changes associated with extracellular matrix-remodeling topical therapy

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BACKGROUND

Skin aging occurs through intrinsic processes (genetics, cellular metabolism, hormones and fibroblast 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].

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].

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. 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 thus undertaken a series of histological examinations in subjects in their 60s assessing changes related to pure topical application of product to the skin.

HISTOLOGICAL CHANGES following TOPICAL APPLICATION

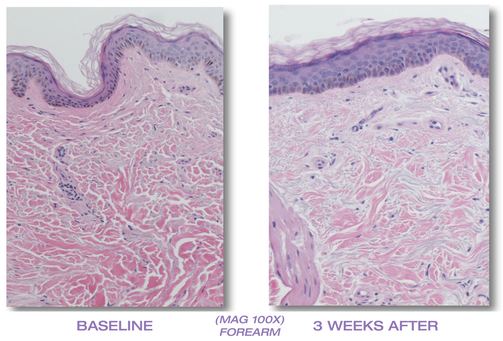

Figure 1: H/E staining (100X) showing ECM and epidermal changes following 3 week topical application of serum containing TriHex peptides and botanicals to the forearm (female 65 years). Baseline H/E stained sections from non-treated forearm skin shows thicker, mature collagen bundles, and thinner epidermis with flattened basal cells. Post-treatment, 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 neocollagenesis.

Formulations with TriHex peptides and selected botanicals simultaneously activates 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.

These peptides increase MMP-2 (gelatinase) levels in the ECM digesting the clumped gelatin fragments clearing the ECM of these mature bundles (Figures 1,2) followed by stimulation and replacement of freshened collagen and elastin (Figures 2-4).

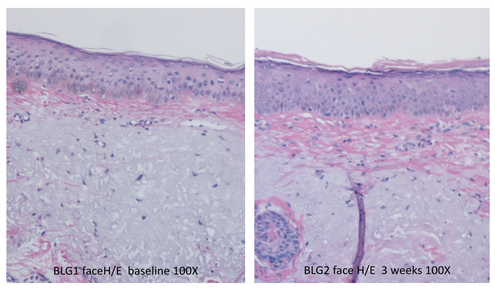

Figure 2: H/E stained sections (100X) from biopsies taken at baseline and following 3 week topical application of an anhydrous topical TriHex peptide gel to the pre-auricular region (male 60s). There is an increase in the number of layers in the epidermis (less epidermal atrophy), with recovery of normal 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.

One of the peptides contained in the TriHex formulation 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[4,5] (Figure 3).

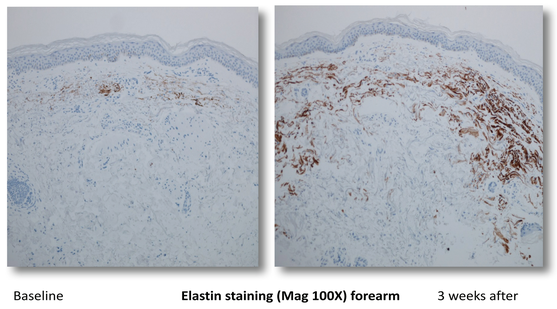

Figure 3: Elastin immunohistochemical staining of sections from biopsies taken at baseline and following 3-week topical application of the TriHex and botanical containing serum to the forearm (male 63 years) (100X) shows a striking increase in the amount of dermal Elastin staining post-treatment. (Abcam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

The dramatic stimulation of Elastin does not only manifest in those who demonstrate little dermal Elastin staining at baseline. In fact, some subjects show significant Elastin staining in the pre-treatment biopsy specimens. In these cases, topical application of the product often results in not only an increase in Elastin staining, but also significant, gradual, uniform redistribution of Elastin throughout the upper dermis (Figure 4).

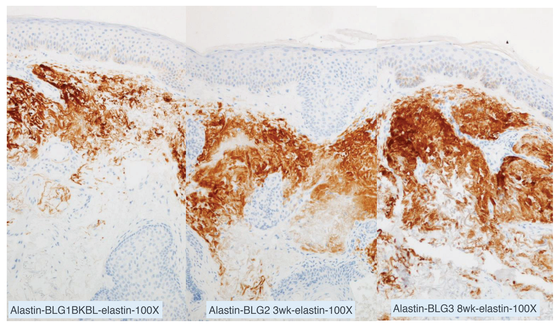

Figure 4: Topical application of TriHex peptide and botanical containing anhydrous gel to the pre-auricular region at baseline, 3 weeks and 8 weeks (male 60s) results in significant gradual increase in dermal Elastin staining (100X). (Abcam Anti-Elastin mouse monoclonal antibody [BA-4] 1:100 dilution)

Phosphatidylserine (PS) has been found to decrease expression of matrix metalloproteinase 1 and to increase procollagen formation[6] (Figure 5).

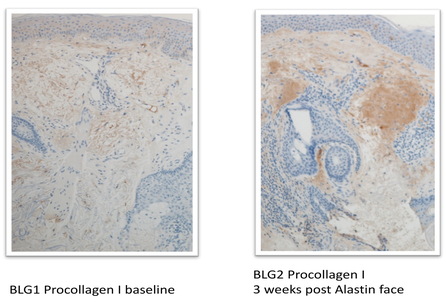

Figure 5: Sections stained with an immunohistochemical stain for Procollagen-1 show slight staining in the upper and mid dermis at baseline and increased staining in the upper and mid dermis at 3 weeks of topical application.

CLINICAL IMPLICATIONS

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. Thus the anhydrous gel with TriHex peptides and botanicals is used as a pre-conditioning aid before surgery to prepare the ECM and following surgery 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).

Figure 6
Unretouched photographs of subject at baseline and after Fraxel re:pair. Procedure Enhancement System- Invasive Kit was used two weeks pre- and post-care. Individual results may vary. Data on file at ALASTIN Skincare. Photos provided by Dr. Sabrina G Fabi.

In addition, the serum (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 (Figure 7A, B).

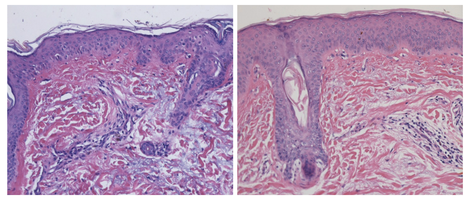

Figure 7A

Figure 7: (A) Topical application of the TriHex and botanical anti-aging line to the crows feet region at baseline and 12 weeks (female 61 years) results in significantly decreased solar elastosis with new collagen formation, improved cornified layer and epidermis - H/E staining (100X). (B) This manifests clinically as reduced wrinkles with improved texture and tone in the crows feet area.

Figure 7B

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, 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[7,8].

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