Dermatology Case Report
Histological Changes Associated with Extracellular Matrix-Remodeling Topical Therapy

Antoanella Calame MD \(^1\) and Alan D Widgerow MBChB; MMed; FCS; FACS\(^2,3\)

\(^1\)Compass Dermatopathology, Chief of Dermatology, Scripps Memorial Hospital, La Jolla, UC San Diego, USA
\(^2\)Center for Tissue Engineering, Department of Plastic Surgery, University of California, Irvine
\(^3\)Alastin Skin Care Carlsbad, CA, USA

Abstract

**Introduction:** An innovative skincare product line incorporating a proprietary blend of selected peptides and botanicals formulation has been developed with the aim of preparing skin surfaces for rejuvenating procedures 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...
rejuvenating procedures around 2 weeks prior to the procedures, thus clearing the ECM and improving the cross talk between fibroblasts and collagen fibers. Fastened 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 undergoing 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 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 increase 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 matrixes 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 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
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 botanicaIs 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 in 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.

References

1. Baumann L (2007) Skin ageing and its treatment. J Pathol 211: 241-251.
2. Lu P, Takai K, Weaver VM, Werb Z (2011) Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harbor perspectives in biology 3: 12.
3. Fisher GJ, Varani J, Voorhees JJ (2008) Looking older: Fibroblast collapse and therapeutic implications. Arch Dermatol 144: 666-672.
4. Pickart L, Vasquez-Soltero JM, Margolina A (2015) GHK peptide as a natural modulator of multiple cellular pathways in skin regeneration. BioMed Research International 64108.
5. Pickart L, Vasquez-Soltero J, Margolina A (2015) GHK-Cu may prevent oxidative stress in skin by regulating copper and modifying expression of numerous antioxidant genes. Cosmetics 2: 236-247.
6. Pickart L, Margolina A (2016) GHK–copper peptide in skin remodeling and anti-aging. SFPW-Journal Cosmetics 136(6):10-18.
7. Pickart L (2007) The human tri-peptide GHK and tissue remodeling. J Biomater Sci Polymer Edn 19: 969-988.
8. Dufour A, Overall C (2013) Missing the target: Matrix metalloproteinase anti-targets in inflammation and cancer. Trends in Pharmacological Sciences 34:233-243.
9. Philips N, Auler S, Hugo R, Gonzalez S (2011) Beneficial regulation of matrix metalloproteinases for skin health. Enzyme research 2011:427285.
10. Floquet N, Hery-Huynh S, Dauchez M, Derreumaux P, Tamburro AM, et al. (2004) Structural characterization of VGVAPG, an elastin-derived peptide. Biopolymers 76: 266-280.
11. Blanchevoye C, Floquet N, Scandolera A, Baud S, Maurice P, et al. (2013) Interaction between the Elastin Peptide VGVAPG and Human Elastin Binding Protein. The Journal of Biological Chemistry 288(2):1317-1328.
12. Lee SH, Yang JH, Park YK, Han JJ, Chung GH, et al. (2013) Protective effect and mechanism of phosphatidylserine in UVB-induced human dermal fibroblasts. European Journal of Lipid Science and Technology 115: 783-790.
13. Draelos Z, Pugliese P (2011) Glycation and skin aging: A review. Cosmetics & Toiletries Magazine. June 2011: 1-6.
14. He M, Kubo H, Morimoto K, Fujino N, Suzuki T, et al. (2011) Receptor for advanced glycation end products binds to phosphatidylserine and assists in the clearance of apoptotic cells EMBO Rep 12: 358-364.
15. Widgerow A (2016) Topical skin restoration technology – Advances in age management strategies. Modern aesthetics 2016 (May/June):1-8.
16. Chilukuri S, Day D, Sog F, Jennings J, Rif P, et al. (2016) Recycling the Matrix – ALASTIN Skincare™ with TriHex Technology™ provides a new approach to optimizing rejuvenating procedure outcomes and treating aging skin. Aesthetic Guide (Sept Supplement): p: 1-8.
## Conferences by Country

| Country | Country | Country |
|---------|---------|---------|
| USA     | Malaysia | Singapore |
| Australia | South Africa | New Zealand |
| UAE     | Germany | Philippines |
| Italy   | UK      | Poland |
| Italy   | Japan   | Austria |
| Italy   | Brazil  | Turkey |
| Brazil  | South Korea | Finland |
| Netherlands | Norway | China |
| Spain   | Canada  | Mexico |
| France  | Switzerland | Denmark |
| India   | America: OMICS International Journals / Authorproofs  
5716 Corsa Ave, Suite 110, Westlake  
Los Angeles, CA 91362-7354, USA  

**America:** Tel: +1-888-843-8169, 1-650-268-9744, Fax: +1-650-618-1417  
**UK:** Tel: +1-800-216-6499; **Europe:** Tel: + 0805-080048  

## Clinical & Medical Journals

| Specialty | Specialty |
|-----------|-----------|
| Anesthesiology | Neurology |
| Cardiology | Nursing |
| Clinical Research | Nutrition |
| Dentistry | Oncology |
| Dermatology | Ophthalmology |
| Diabetes & Endocrinology | Orthopaedics |
| Gastroenterology | Pathology |
| Haematology | Pediatrics |
| Health Care | Physicaltherapy & Rehabilitation |
| Immunology | Psychiatry |
| Infectious Diseases | Pulmonology |
| Medicine | Reproductive Medicine |
| Microbiology | Surgery |
| Nephrology | Toxicology |