Pinpoint Accuracy – Taking Skin Rejuvenation to a New Level?

Lance B Setterfield

Abstract

Summary

Studies confirm that it is loss of structural integrity at the dermal-epidermal junction that causes rhytids. While there is some overlap between pathogenesis of intrinsic and extrinsic aging, the predominant mechanism for premature aging is triggering of MMP's via UV exposure. Targeting of the DEJ with treatment modalities may alleviate signs of aging optimally in the following ways:

- *Targets keratinocytes to promote cell cross-talk and gene expression* that regulates melanocyte and fibroblast function (amongst many other cells).
- *Targets gene expression at the dermal-epidermal junction* for the purpose of upregulation of *collagen types IV, VII and XVII* (in addition to I and III) and other components of the DEJ to *restore structure and adhesion.*
- Results in gene expression that downregulates MMP's, inflammatory growth factors, and cytokines (thus playing a *preventative* role in premature aging).
- Results in gene expression that upregulates anti-inflammatory growth factors and cytokines (thus *resulting in normal collagen* formation as opposed to scar tissue collagen that forms in the presence of inflammation).

A less-is-best approach offers benefits of reduction in downtime, reduced risk of side effects, and less pain (which will translate into greater compliance and best results).

Objective: To describe a new non-ablative approach to skin rejuvenation by targeting the epidermis and dermal-epidermal junction as opposed to the dermis.

Background

Are the results seen after microneedling for antiaging due to upregulation of genes that repair the DEJ? The present presupposition for rejuvenation is based on provoking and prolonging the inflammatory process in order to recruit fibroblasts to produce collagen type I and III in the repair process. Are we attributing results to the wrong cells? Are epidermal or dermal cells "to blame"?

What are we targeting? In other words, with rhytids, what is the pathology and at what depth is it found?

The photoaging model has long since been established. Lavker, 1979 [1] and Kligman et al., 1986 [2] reported histological findings of sun-exposed skin to include cellular atypia, loss of polarity, flattening of the DEJ, reduction of collagen, and increased dermal elastosis.

Genji Imokawa proposes a UVB-induced wrinkling mechanism as follows: "Repetitive UVB exposure causes keratinocytes to secrete IL-1α which triggers GM-CSF secretion in an autocrine fashion. Secreted IL-1α and GM-CSF penetrate into the dermis to stimulate the expression of skin fibroblast elastase, which then cleave elastic fibers surrounding the fibroblasts, leading to the deterioration of the three-

dimensional configuration of elastic fibers. This results in a loss of skin elasticity and subsequently to wrinkle formation." [3]

In a study by S. Amano, comparisons were made between intrinsic (or chronological) aging and photoaging. They found that the basement membrane (BM) **at the dermal–epidermal junction (DEJ) of sun-exposed skin was damaged and multilayered, even partly disrupted** compared with that of sun-protected skin. Given that one of the functions of the BM is to bind the epidermis to the dermis, such disruption destabilizes the skin and plays a role in premature aging. Offering three major environmental factors (UV radiation, dryness, and oxidation) as causes for accelerated aging, they sought solutions beyond sunblock, corneotherapy and antioxidants. [4]

Not surprisingly, they found that matrix metalloproteinases (MMPs) and urinary plasminogen activator, which are increased in UV-irradiated skin (even the cornified layer), caused BM damage. Herrmann *et al.*, 1993 [5] and Fisher *et al.*, 1996 [6] found MMPs-1, 2, 3, and 9 were increased by UV irradiation in experiments using human fibroblasts and human skin. This increase in MMPs occurred at *suberythemal* levels of UVB exposure. Thus, including MMP inhibitors and ingredients in products that increased BM components (which resulted in enhancement of BM repair mechanisms) formed the basis for their strategy in retarding the aging process. [4]

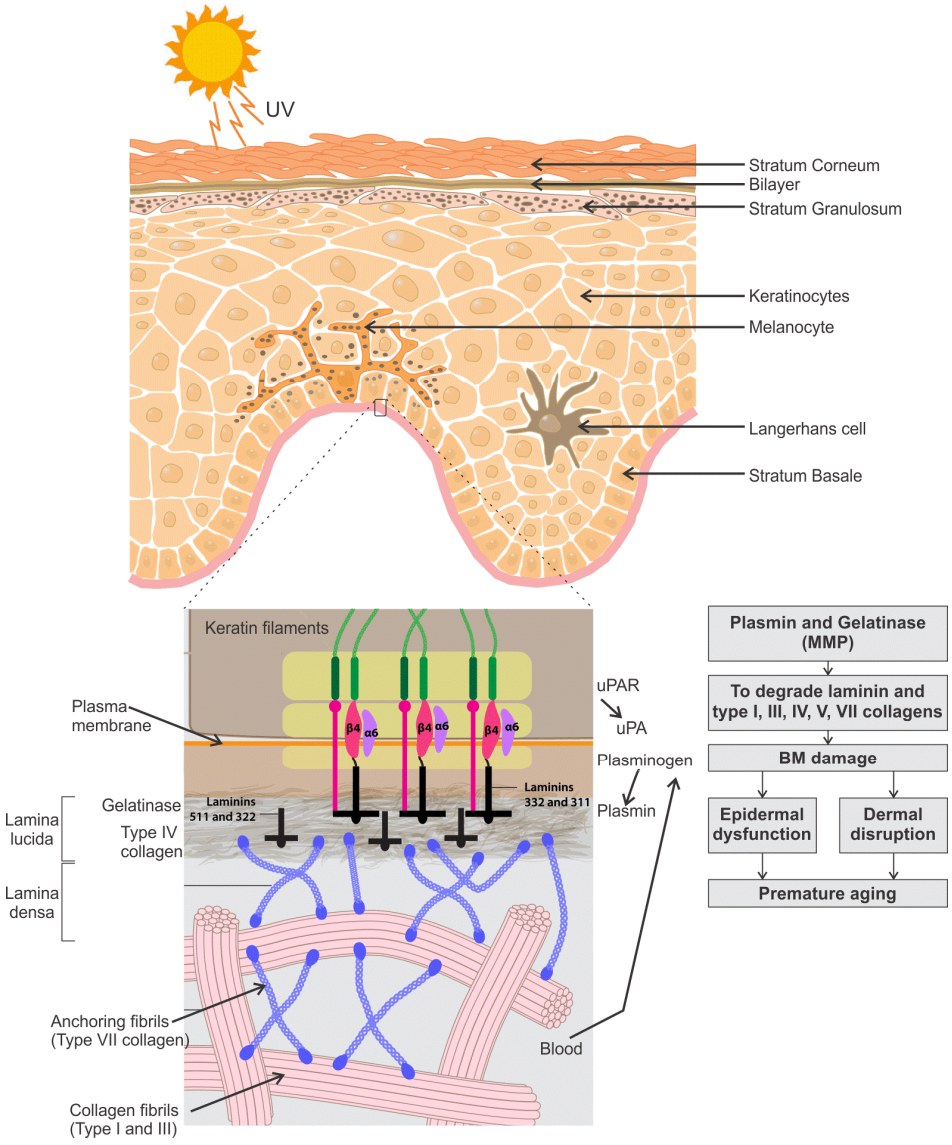

Figure 1. UVB causes disruption and reduplication of BM at the dermal–epidermal junction (DEJ) via elevation of plasmin and MMPs. (Laminin 332 (LN332) and type IV and VII collagens degraded via uPAR, urokinase-type plasminogen activator receptor.) [4]

General Observations Opposing Conventional Wisdom

Explaining Away Clinical Experience – Collagenesis Superficially Despite Injury at Greater Depths?

Schwartz and Laaf [7] noted that new collagen only formed in the top 0.6 mm of skin despite needling to a depth of 1.5 mm. [8] How can one explain this?

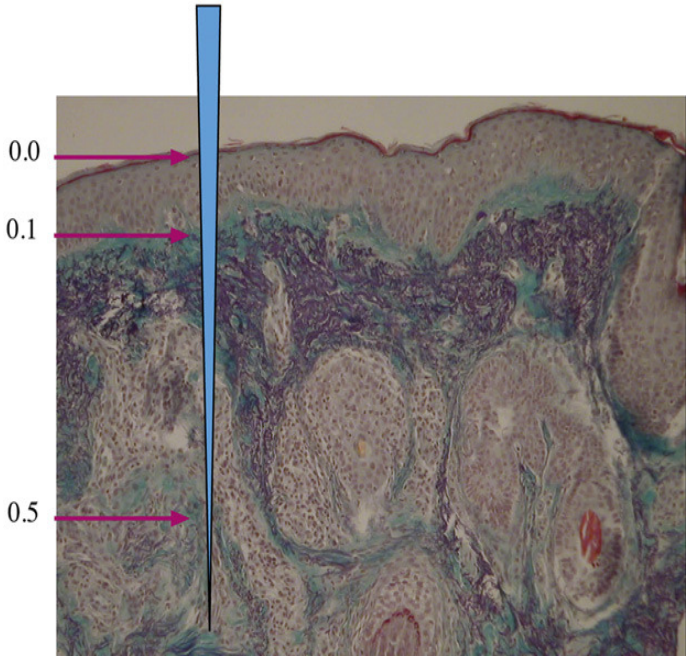

Figure 2. Biopsy from needled skin 6 weeks post-op; collagen stained purple with van Giesen. (1.5 mm needles) [7]

Explaining Away Clinical Experience – Results in Ehlers–Danlos Syndrome?

The author's very first microneedling patient in 2005 had this condition (type 2 and 3), and she responded to 0.3 mm and 0.5 mm treatments when, theoretically, nobody thought this possible. Genes affected by this condition involve mostly type I and III collagen, as shown in a partial classification below.

- Classic Type 1 and 2
  - Type 1 = severe skin involvement – COL5A1, COL5A2, COL1A1
  - Type 2 = mild skin involvement - COL5A1, COL5A2, COL1A1
- Hypermobility Type 3; COL3A1
- Vascular Type 4 - COL3A1

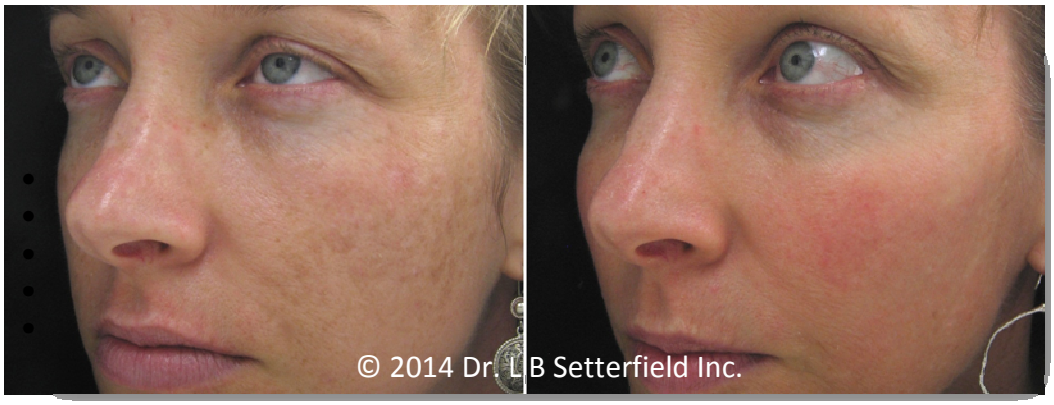

Figure 3. 36-44 years old, treated once a week with a 0.5 mm and 6 days a week with a 0.3 mm for 8 years.

Explaining Away Clinical Experience - Results Exceeding Expectations with Cosmetic Microneedling?

Over the years the author noted results beyond expectations with 0.3 mm or 0.5 mm in the group of patients where wrinkles were excessive and most likely due to dermal-epidermal crash.

Figure 4. 55 years old, treated with products for 18 months and 0.3 mm for 6 months. (6 months between pictures)

Structure of the Dermal-Epidermal Junction [9]

The dermal-epidermal junction may be divided into four morphological regions:

- The basal epidermal cell membrane with
  - Keratinocytes, connected to it via hemidesmosomes
  - Melanocytes, connected via focal adhesions
  - Merkel cells, connected via densifications of their plasma membrane in contact of the associated nerve end
- The lamina lucida, traversed by anchoring filaments rich in laminin 332, and 311, which adhere to α6 β4 integrin at the cell surface of keratinocytes to form an adhesion structure with the hemidesmosomes.
- The lamina densa is mainly composed of type IV collagen, but also contains laminin 322 and 511, nidogen, and heparan sulfate proteoglycans, a major one being perlecan. (The latter acts as the on-switch for FGF7.) The lamina densa is thicker in men than in women, while the lamina lucida has a similar thickness in both sexes.
- The fibrillar zone includes anchoring fibrils that are composed of type VII collagen, tethering the lamina densa to the papillary dermis.

Which Cells Are Responsible for Production of Basal Membrane Components?

"Keratinocytes synthesize BM components, except nidogen. Fibroblasts also produce BM components other than laminin 332. We recently found that increasing the production of BM components, such as laminin 332, collagen IV, and collagen VII, in keratinocytes and/or fibroblasts with/without inhibitors of gelatinases and/or serine proteinases is also effective in enhancing the repair and assembly of BM." [4]

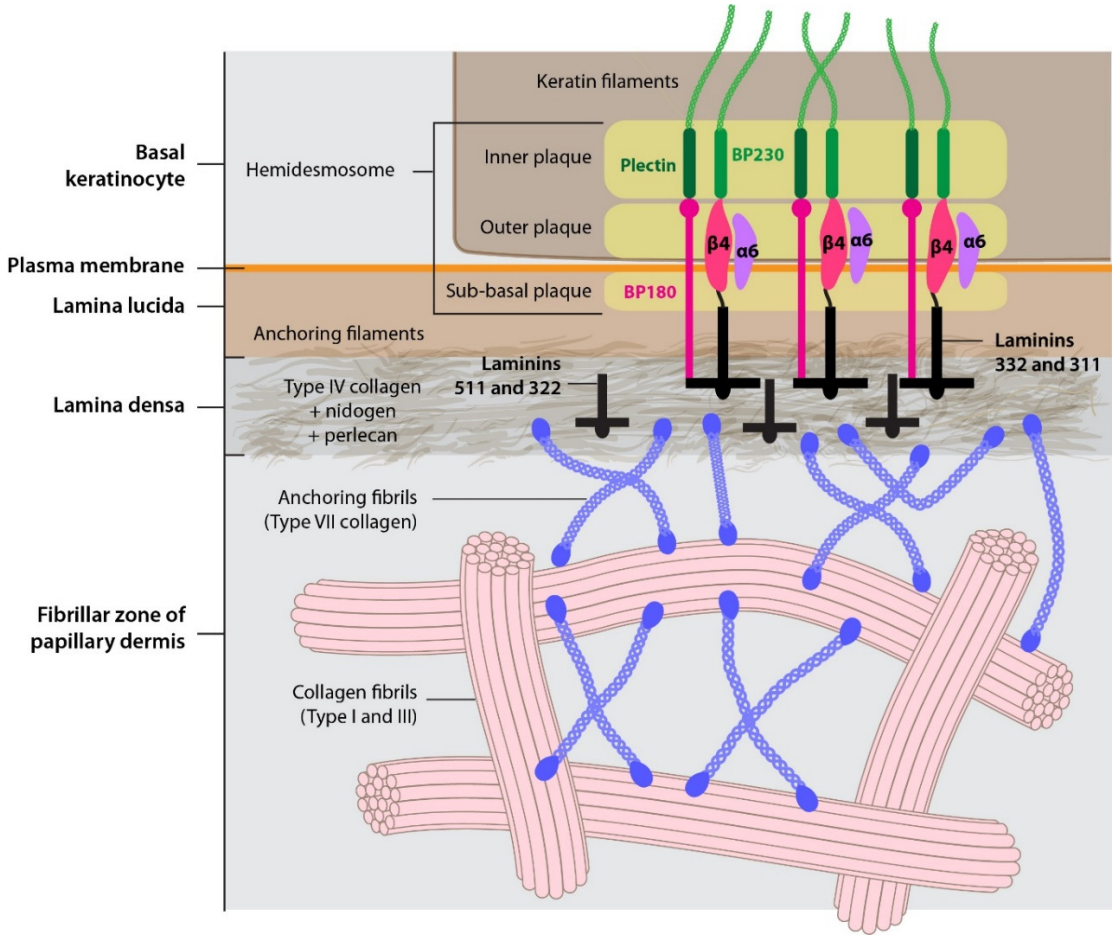

Figure 5. The adhesion of the epidermis to the dermis is made through the interactions between keratin intermediate filaments (K5 and K14), BP230 (BPAG1), plectin, integrins α6 β4, BP 180, laminin-332, lamina densa (type IV collagen, nidogen, perlecan, laminin 511 and 322), anchoring fibrils (type VII collagen), and collagen fibers (type I and III collagens) of the papillary dermis. [9]

Components of the DEJ and Associated Genes Showing Influence of Microneedling

Hemidesmosomes

- Keratin intermediate filaments (K5 192% and K14 184% upregulation) [10]
- BPAG1 and plectin
- Integrins α6 β4

Anchoring Filaments

- BP 180 - gene human collagen XVII gene, COL17A1 = 5% upregulation [10]
- Laminin-332 - (Altered function of laminin-332 (α3β3γ2) consequent to mutations in the LAMA3, LAMB3, and LAMC2 genes cause junctional epidermolysis.)

Lamina Densa

- Type IV collagen - COL4A1 gene = 26% upregulation [10]
- Nidogen – gene N1D1
- Perlecan – HSPG2 gene = 14% downregulation [10]
- Laminin 511 and 322
- Anchoring fibrils (type VII collagen) – *COL7A1 gene = 210% upregulation* [10] (In the skin, type VII collagen protein is synthesized by keratinocytes and considerably less by dermal fibroblasts. [11]
- Collagen fibers (type I and III collagens) of the papillary dermis – COL1A1 = 3% downregulation and COL3A1 = 117% upregulation [10]

Why cosmetic needling may improve wrinkles

The author noted results beyond expectations with 0.3 mm or 0.5 mm in the group of patients where wrinkles were excessive and most likely due to dermal-epidermal crash.

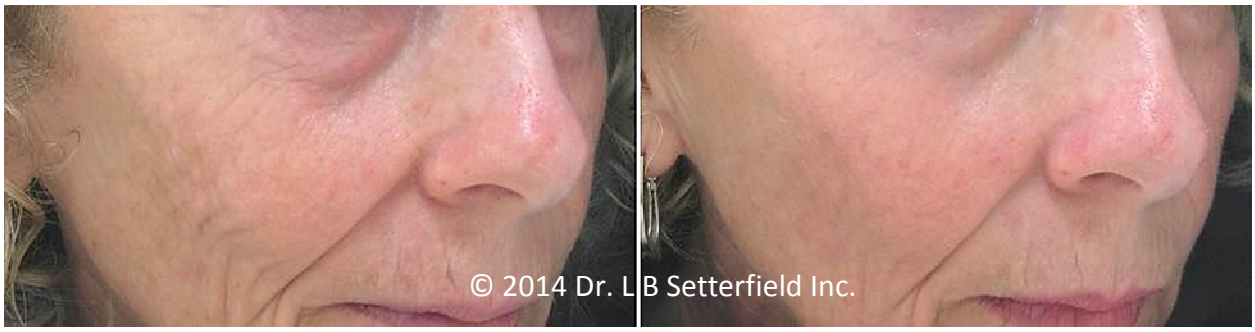

Figure 6. 64 years old, treated once a week with a 0.5 mm and 6 days a week with a 0.3 mm for 5 months.

Collagen found in the DEJ region is Type IV, VII and XVII. We already know that reticular fibroblasts are not that responsive to needling. [12] Given that Collagen VII is due to COL7A1, and produced by both keratinocytes and fibroblasts, is it possible that the aesthetic industry has always attributed results to the wrong cell…..i.e. the reticular fibroblast (and Collagen type I or III). Instead, the injury of keratinocytes on the way down to the dermis may be responsible, via other types of collagen. (It would also explain why PRP results are limited for skin rejuvenation.)

In a study by Fan and Owen [10], they looked at skin treated with saline versus microneedling injury equivalent to 0.2 mm Dermaroller®. The 22,000 gene Microarray analysis reported the composite gene expression of both the keratinocytes and the fibroblasts within the total tissue sample. The results showed **keratinocyte-related dominant gene expression**, instead of what one would expect in traditional wound healing theorems i.e. collagen gene up-regulation for types I and III. (Their explanation for this was as follows. "As this experiment views the DNA from a much larger number of keratinocytes compared to fibroblasts, this is expected.") They reported that "collagen VII (COL7A1) and Collagen VI (COL6A1) upregulation are very significant." Also, "The significant upregulation of the KRT genes, and in particular KRT5 would also be expected to aid in epidermal-dermal adhesion. This protein is critical in anchoring the keratinocyte to its basement membrane."

COL1A1	-3%	IL1RAP	-12%	TGFB1	2%
COL3A1	117%	IL5	-19%	TGFB2	-2%
COL4A1	26%	IL6	-25%	TGFB3	-3%
COL7A1	210%	IL10	-14%	IGF1	-14%
COL17A1	5%	IL11	8%	PDGFA	11%
MMP1	-31%	IL16	-9%	VEGF	126%
MMP2	119%	IL26	-7%	EGF	-21%
MMP9	-45%	KRT5	192%	HSPA1A	562%
TIMP1	77%	KRT14	184%	FGF7	26%

Figure 7. The table above shows significant upregulation of Collagen III, IV and VII, TIMP1, HSPA1A, FGF7, KRT5, KRT14 and VEGF 24 hours after 0.2mm microneedling injury. Significant downregulation of MMP1 and MMP9, as well as most IL's and EGF. [10] These findings are predominantly positive and desirable. MMP2 degrades type IV collagen and elevation of this enzyme is expected to peak 24 hours after injury to the lamina densa where this form of collagen resides.

While the following statement encapsulates the essence of how microneedling works, it reflects the standard presupposition that it is the direct injury of dermal cells that facilitate a result. "Indeed, it is this stratum corneum that microneedles must effectively penetrate to allow direct stimulation, enhanced delivery of passive topicals, or active delivery via electro-assist or other methods." [10]

They concluded that the "use of microneedle arrays which are of a depth to interact with at least the basal layer **stimulate significant metabolically positive genes without a concurrent up-regulation of pro-inflammatory genes.** These benefits in particular aid the age related effects of epidermal-dermal adhesion. [10]

Speaking of pro-inflammatory genes, it is known that inflammation is absent or highly limited in embryo wound healing [13], so it is not a prerequisite for collagen production.

"Collagen VI is believed to be responsible for forming the scaffolding necessary for proper dermis strength, elasticity and flexibility. Collagen VI expression is believed to occur in both papillary and reticular fibroblasts. This enhanced dermal strength through collagen VI interaction with Collagen III provides a tough substrate for epidermal adhesion." [10]

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