Science of Hyaluronic Acid Beyond Filling: Fibroblasts and Their Response to the Extracellular Matrix

Marina Landau, MD
Steven Fagien, MD
Holon, Israel; and Boca Raton, Fla.

Summary: Loss of viscoelasticity is one of the primarily signs of skin aging, followed by appearance of visible wrinkles. Hyaluronic acid (HA)-based fillers are widely used to fill wrinkles and compensate for volume loss. Recent clinical observations demonstrate persistence of the filling effect longer than the biological availability of the filler. Stimulation of new collagen by cross-linked HA and up-regulation of elastin have been suggested as possible explanation to this observation and have been supported experimentally. Cross-linked HA substitutes for fragmented collagen in restoring extracellular matrix required for normal activity of fibroblasts, such as collagen and elastin production. To restore extracellular matrix efficiently, serial monthly treatments are required. Boosting of facial and nonfacial skin through fibroblast activation is a new indication for HA-based products. Injectable HA has also been recently registered in Europe as agents specific for the improvement of skin quality (Restylane Skinboosters). Further explanation of the possible mechanisms supported by long-term clinical examples is presented herein. (Plast. Reconstr. Surg. 136: 188S, 2015.)

Changes in the mechanical properties of the skin is one of the first measurable signs of skin aging.1,2 In most cases, they are followed by visible wrinkling. Dermal fillers are widely used for skin rejuvenation for more than 20 years. Due to their efficacy and safety, hyaluronic acid (HA)-based fillers had become a “gold” standard and the commonest type of filler currently used.3 HA-based fillers have been used primarily for wrinkles and lines filling. Following better understanding of facial aging process, these products started to be utilized for 3D volumization, compensating for age-associated volume loss.4 Clinical observations on patients repetitively treated by HA-based fillers demonstrated some initially unexpected discoveries, including the improvement in mechanical properties of the skin following “field” implantation of HA and persistence of the results after retreatment longer than expected from injected HA longevity.5,6 Restoration of extracellular scaffold, stimulating formation of younger components of the skin by injectable cross-linked HA, provides possible explanation of the mechanism of this phenomena.6-13 Skin boosting is a new indication for clinical use of injectable cross-linked HA gels.

Skin aging is a complex biological process affected by both genetic and extrinsic factors. The most important structural components of the dermis are collagen, elastin, and ground substance,

Disclosure: Dr. Landau consults Galderma, Merz and Croma. She occasionally speaks and runs practical workshops on Skinboosters. Dr. Fagien is a consultant/investigator for Allergan, Galderma, Merz, Kythera, and Aquavit.

Supplemental digital content is available for this article. A direct URL citation appears in the text; simply type the URL address into any Web browser to access this content. A clickable link to the material is provided in the HTML text of this article on the Journal’s Web site (www.PRSJournal.com).
all produced mainly by fibroblasts. Each component plays a unique role in maintaining normal function of human skin. Type I collagen is by far the most abundant protein in the human skin comprising about 90% of its dry weight. For successful production of connective tissue components, fibroblasts need a stable collagen scaffold to which they can bind.14

This binding is carried out through specific receptors on the fibroblasts surface, called integrins.15,16 Integrins attach to molecules of collagen in the extracellular matrix and bridge them with tubular cytoskeleton inside the cell. While intracellular microtubules “pull” fibroblast skeleton inward, focal adhesions with extracellular stable matrix push the cells to spread. This results in increased mechanical tension inside the fibroblasts and stimulates production of new collagen and down-regulation of collagen degrading enzymes.

Due to the aging process, collagen fragmentation occurs.17,18 Fragmented collagen does not provide sufficient mechanical support to the fibroblasts required to counterbalance inward pulling by the internal microtubular skeleton. As a result, fibroblasts collapse. This collapse signals to down-regulation of the production of type I collagen, possibly through stimulation in production of prostaglandin E2.19 In addition, fibroblasts collapse up-regulates activity of collagen degrading matrix metalloproteinases.

**CLINICAL OBSERVATIONS**

It has been shown that correction of nasolabial folds by Restylane lasts 18 months after a single retreatment at 4.5 or 9 months.8 The same was found for Juvederm line products. Treated subjects sustained 18–21 months correction with a single repeat treatment.9

As these results do not correlate with the expected, if degradation was the primary determinant of the effect duration, the authors wonder whether another phenomenon is responsible for the correction longevity. These phenomena could be due to other biologic effects from injectable HA including, but not limited to, new collagen and elastin production.

**NEOCOLLAGENESIS AND NON-HA FILLERS**

New collagen stimulation secondary to fillers implantation is not a completely unknown phenomenon. Injectable silicon is well-known for its ability to induce stimulation of collagen, through a process, similar to foreign body reaction.20 Poly-lactic acid induces slow and significant collagen stimulation through usually controlled inflammatory process.21 Calcium hydroxylapatite stimulates collagen formation and dermal thickening.22 Restoration of extracellular matrix as a mechanism for “rejuvenation” of dermal components and their function has been shown for HA-injected skin exclusively.10–13

**FIBROBLASTS AND THEIR RESPONSE TO EXTRACELLULAR MATRIX**

Until 2007, it was an axiom that HA-based fillers act by directly adding dermal or subdermal volume, which can be further augmented by tissue water attraction by HA.

The first report on intradermal changes induced by injectable HA was published in 2007.10 Wang et al10 assessed the effect of relatively new at that time nonanimal origin stabilized HA (NASHA, Restylane) on human skin in vivo. They injected 0.7 mL of Restylane or normal saline into photodamaged skin of the arm of 11 volunteers. The injected sites were biopsied and studied 4 and 13 weeks after the injection. The investigators found increased intracellular and extracellular dermal staining for collagen I in NASHA-treated samples, especially in the skin areas adjacent to the filler. The increase in collagen production was mediated by higher levels of transforming growth factor-β. In addition, in NASHA-treated skin, there was increased expression of tissue inhibitors of collagen degrading enzymes. As all the increased compounds are produced by dermal fibroblasts, the investigators looked closely at these cells. They found that fibroblasts embedded in connective tissue surrounding NASHA appeared different morphologically from fibroblasts in saline-treated skin. Adjacent to NASHA, fibroblasts were stretched with abundant endoplasmic reticulum. Unlike theoretically expected, no direct binding of fibroblasts to NASHA was demonstrated. Therefore, the proposed mechanism of collagen stimulation was not a direct effect of HA on fibroblasts but rather indirect mechanical phenomenon. NASHA-mediated hydration of extracellular matrix induces mechanical stretching of fibroblasts, thus, initiating production of connective tissue components, such as collagen type I.

Young extracellular matrix maintains mechanical tension due to intact collagen fibers, providing stable scaffold. Fragmentation of the scaffold causes fibroblasts to collapse and reduces their procollagenic function. Clinically, impaired fibroblast function, coupled with reduced collagen
synthesis, translates into atrophy and wrinkling of aged skin. When a stiffer injected cross-linked HA “invades” thinned skin, stable scaffold is restored and fibroblasts become stretched and active, enhancing skin appearance and function.

Quan et al.\(^\text{11}\) investigated interaction of NASHA with fibroblasts and extracellular matrix in chronologically aged skin. Similar to Wang et al.\(^\text{10}\) they found that enhancement of structural support of the extracellular matrix by injectable HA (Restylane) causes fibroblasts elongation with subsequent increase in procollagen I and collagens I and III production for at least 12 weeks after HA injection. This stimulation is mediated by up-regulation of type II transforming growth factor-\(\beta\) receptor and connective tissue growth factor. Enhanced structural support of extracellular matrix by injected HA was found to increase keratinocyte and fibroblast proliferation as well, resulting in epidermal thickening and expenditure of dermal vasculature. These findings support the importance of stable dermal scaffold for maintaining normal dermal and probably epidermal function and emphasize again the potency of injected cross-linked HA on age-related cutaneous changes.

Stimulation of production of extracellular matrix components, such as collagen and elastin, by cross-linked injectable HA, other than NASHA, has been demonstrated in human and animal models in additional publications.\(^\text{12,13}\)

**SKIN-BOOSTING CONCEPT**

The first product designed specifically for skin-boosting purposes was Restylane Vital. The product comprises small particles stabilized smooth and relatively thin NASHA gel (20 mg/mL). Restylane Vital Light skin booster is less concentrated (12 mg/mL) and injected in more delicate areas, such as a neck (Fig. 6). Both products contain Lidocaine hydrochloride 3 mg/mL.

The idea of skin quality enhancement by injectable HA, without targeting wrinkles or volume loss, was introduced by Kerscher et al.\(^\text{6}\) who injected microdoses of small particles stabilized HA (Restylane Vital) into the dermis of the lower

---

**Fig. 1.** A 62-year-old man presented for blepharoplasty. He was not aware of deep horizontal forehead lines until after blepharoplasty. He was happy with surgery but now bothered by the forehead lines. A 0.6 mL of reconstituted Juvederm (Juvederm Ultra diluted with 1% lidocaine with epinephrine to a concentration

---

**Fig. 1. (Continued)** of 16 mg/mL by adding 0.5 mL anesthetic to a 1-mL syringe) was injected directly into the depth of the furrow in all areas at the approximate level of the mid-dermis in a serial threading technique. Above, Before blepharoplasty and reconstituted Juvederm to deep horizontal forehead lines. Center, After upper and lower blepharoplasty with noted persistent forehead lines. Below, 1 year after reconstituted Juvederm to deep horizontal forehead lines.
Fig. 2. A 46-year-old woman before (left) and 1 month after (right) a single session of 2 mL (1 mL per cheek) of Restylane Vital Skinbooster treatment. The product was injected using micropuncture techniques by “smart-click” system of the syringe.

Fig. 3. A 57-year-old woman before (left) and 1 month after (right) 2 monthly sessions of Restylane Vital Skinbooster treatment (1 mL per each cheek per treatment). The product was injected using micropuncture technique. Visible improvement of cheek smile lines is noted due to better skin elasticity.
Fig. 4. A 56-year-old woman before (left) and after (right) 2 monthly sessions of Restylane Vital Skinbooster 2 mL per session.

Fig. 5. A 57-year-old woman before (left) and after (right) 3 monthly sessions of Restylane Vital Skinbooster treatment (1 mL per each cheek per treatment). The product was injected by using micropuncture technique.
cheeks of 19 female patients. The procedure has been carried out in 3 monthly sessions. The investigators found that micropuncture injections of Restylane Vital significantly increased skin elasticity and created positive impact on skin surface roughness. Since greatest improvement in these parameters was evident 24 weeks after the last injection session, the authors concluded that placement of HA into the dermis enhanced biosynthesis of new dermal compounds and is not only due to better skin hydration, as initially expected.

Following our current better understanding of the biological process induced by injected cross-linked HA, field treatment, as suggested by Kerscher et al., restores extracellular dermal scaffold in a more diffused and homogenous fashion, than localized injections for wrinkles or volume correction. Furthermore, to create sufficient stimulation, a serial treatment is usually required. One monthly treatment followed by 2–3 repetitions improves skin quality in clinically evident fashion (Figs. 1–5).

Additional studies and case reports substantiated “skin-boosting” concept as a novel treatment for skin improvement by injectable cross-linked HA. Interestingly, non-cross-linked HA injected following the same principles (meso-therapy/biorevitalization therapy) demonstrate controversial results, probably due to insufficient or nonsustained effect on extracellular scaffold stabilization.

Restylane Vital is currently packaged in European countries in “smart-click” syringe. The system dosed the product and delivers 0.01 mL of it per each click. The procedure is carried usually out in 3 monthly sessions. During each session, the targeted area is treated by multiple intra/subdermal micropuncture injections of a dosed amount of Restylane Vital Skinbooster spaced approximately 1 cm from each
other. The injections are either perpendicular to the skin surface punctures or carried out as linear repetitive “clickings,” leaving two to three 0.01-mL product deposits along the intradermal channels. Restylane Vital Skinbooster syringe can be also used in regular continuous mode (without clicking) for skin defects correction and not as a skinbooster. In the United States, Restylane Vital has been recently (June 13, 2014) registered as Restylane Silk without smart-click delivery system. Detailed video clip demonstrating Restylane Skinbooster treatment technique is attached. (See Video, Supplemental Digital Content 1, which demonstrates Restylane Skinbooster treatment technique, available in the “Related Videos” section of the full-text article on PRSJournals.com or, for Ovid users, at http://links.lww.com/PRS/B425.)

In summary, improvement of skin quality by injectable cross-linked HA has evolved in the last years, as an additional indication (after wrinkles filling and volumization) of HA-based dermal fillers use. The procedure hydrates the dermis and creates stable extracellular matrix to support intradermal fibroblasts structure that is essential for their normal function. We postulate that multiple processes that improve the structural integrity and aesthetic appearance of aging skin are in play, and further research will identify the relative role of each component, so we can establish best practices, products selection, and techniques for optimal results.

Marina Landau, MD
Dermatology Unit
Edith Wolfson Medical Center
62 Halochamim Street
Holon 58100, Israel
mlandau@zahav.net.il

PATIENT CONSENT
Patients provided written consent for the use of their images.

REFERENCES


