Improvements in Skin Quality Biological Markers in Skin Explants Using Hyaluronic Acid Filler VYC-12L

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Background: Hyaluronic acid (HA), both crosslinked and uncrosslinked, is used clinically to treat fine lines and provides additional improvements in skin quality attributes. The purpose of this study was to assess potential early differences in the expression of biological markers of skin quality in living human skin explants injected with uncrosslinked and crosslinked HA gels.

Methods: Living human skin explants injected with VYC-12L or noncrosslinked HA with mannitol (HYD) and noninjected controls were assessed via microscopy, histology, and immunohistochemistry on days 3 and/or 8 for biological markers of elasticity (collagen density, elastin, fibrillin-1) and hydration [aquaporin-3, acidic glycosaminoglycans (GAGs), HA]. Hydration was also assessed via a corneometer probe on days 0, 1, 2, and 8.

Results: On day 3 versus controls, VYC-12L moderately increased collagen density in the upper reticular dermis and clearly increased fibrillin-1 expression, with slight increases persisting on day 8. Increases with HYD were smaller and did not persist on day 8. Both VYC-12L and HYD increased aquaporin-3 expression and GAG content on days 3 and 8, but VYC-12L produced greater GAG increases in the reticular dermis. Day 8 instrument-assessed hydration increased by 49% and 22% for VYC-12L and HYD, respectively. Elasticin expression in oxytalan and elaunin fibers was unchanged. Upper-dermal HA reductions suggested HA injection-induced hyaluronidase expression.

Conclusion: VYC-12L produced greater, more lasting improvements in biological markers of skin quality than HYD. (Plast Reconstr Surg Glob Open 2020;8:e2723; doi: 10.1097/GOX.0000000000002723; Published online 25 March 2020.)

INTRODUCTION

Intrinsic processes, including aging and hormonal changes, and extrinsic factors, such as chronic sun exposure and smoking, contribute to structural and functional deficiencies in the skin.1–3 Structurally, the dermal extracellular matrix weakens, dermal collagen decreases, dermal elastic fibers become disorganized, and hyaluronic acid (HA) declines.1,5–7 Functionally, elasticity and hydration are reduced and barrier function is compromised.1,4,8 Over time, extrinsic and intrinsic forces can result in wrinkles, fine lines, dryness, and irregularities in tone and texture.8,9,10

Clinical dermatologic studies have examined elasticity and hydration to assess overall skin quality, particularly with regard to aging skin.10–12 HA, due to its natural abundance, stability, lack of toxicity and immunogenicity, and ability to attract and bind approximately 1,000 times its weight in water, has shown a wide range of benefits related to the quality of the skin, including wound healing, tissue regeneration, and skin repair.13,14 HA is involved in tissue hydration and preserving the integrity of the dermal extracellular matrix.15,16 Additionally, intradermal HA filler injections have demonstrated the ability to improve skin texture, luminance, hydration, and elasticity.12,17,18

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fillers come in different forms: noncrosslinked, which has been shown to provide a moisturizing effect, and crosslinked, which improves durability, mechanical properties, and hydration.15 Juvederm VOLITE [VYC-12L (Allergan, Annecy, France)] is a crosslinked HA (12.0 mg/mL) containing lidocaine developed using the Vycross technology platform (Allergan plc, Dublin, Ireland) and designed to treat superficial cutaneous depressions, such as fine lines, and provides additional improvements in skin quality attributes, such as elasticity and hydration.30 In a prospective, single-center, single-arm study in 131 subjects, VYC-12 (without lidocaine) was shown to be safe and effective for the treatment of superficial cutaneous depressions, such as fine lines, as measured by skin texture improvement, and for the improvement of skin quality in the face and neck, with improvements lasting up to 6 months and subject satisfaction with skin and improved hydration lasting up to 9 months (data on file; Allergan plc). Juvederm HYDRATE [HYD (Allergan)],12-20 a noncrosslinked HA (13.5 mg/mL) with mannitol, is indicated for injection into the superficial dermis and dermal–epidermal junction to improve skin hydration and elasticity. A 2-month multicenter clinical trial showed that 27 subjects who received HYD by depot injection in the face and/or neck or décolletage area had significant improvements in skin hydration.31 VYC-12L and HYD are Conformité Européenne marked,19,20 but have not been approved by the US Food and Drug Administration.

Indicators of skin quality attributes may be evaluated in ex vivo models using biological markers. The density of dermal collagen, for example, is an indicator of skin elasticity because of collagen’s role in maintaining the strength of the skin, helping it to resist mechanical deformation.10,21,22 Other biological markers of elasticity are fibrillin-1, a glycoprotein that supports the integrity of the dermal elastic fiber network,3 and elastin, an essential protein of the elastic fibers of the skin, such as oxytalan and elaunin.23,24 Well-studied biological markers of skin hydration include aquaporin-3 (AQP3), water- and glycerol-transporting membrane proteins expressed in the epidermis,25,26 and glycosaminoglycans (GAGs), which are linear polysaccharide molecules, including HA, that bind water and help regulate the hydration of the dermis.37 The impact of these markers is often measured clinically using probes, such as the corneometer, which evaluates epidermal capacitance as a measure of hydration in the stratum corneum.27,28

The present study evaluated the early effects of VYC-12L injection compared with HYD or no injection on biological markers of hydration and elasticity in living human skin explants.

METHODS

Human Skin Explant Collection and Preparation

Two studies were performed at Laboratoire BIO-EC, Longjumeau, France. The first study, performed on explants from 1 donor, included all measurements described below. The second study was performed on explants from 3 different donors to confirm the hydration (corneometry) results obtained in the first study. Explants of living human skin were obtained during abdominal surgery from a total of 4 donors. Eighteen 11-mm round explants in study 1 (donor 1) were prepared for the analysis of biological markers of skin elasticity (collagen density, elastin, and fibrillin-1 expression) and skin hydration (AQP3 expression, GAG content, and HA). Eleven 1.5- by 2-cm rectangular explants from donors 1, 2, 3, and 4 were prepared for the analysis of epidermal capacitance via corneometry (n = 2 in study 1; n = 9 from study 2). Explants were stored in the BIO-EC survival culture medium at 37°C in 5% CO2-humidified air (Fig. 1). Half of the culture medium (1 mL) was refreshed on days 1, 2, 5, and 7.

This study was conducted on explants derived from discarded abdominoplasty tissue; all subjects provided written informed consent before the use of these tissues. The only subject data obtained included age, race, and sex.

Product Application

In both studies, the product was injected via a needle into the dermis of the round 11-mm explants on day 0. In study 1, explants were injected with either 50 μL of VYC-12L (VYC-12 with lidocaine), 50 μL of noncrosslinked 13.5 mg/mL HYD, or no injection (control). (Three of the 21 original explants in study 1 were designated as untreated controls for testing after day 0.) In parallel, 2 explants from study 1 consisting of 1.5- by 2-cm rectangular samples were injected on day 0 with either 3 × 50 μL of VYC-12L or HYD, or left untreated (control). Nine 1.5- by 2-cm rectangular explants from study 2 were also injected on day 0 with either 3 × 50 μL of VYC-12L or of HYD, or left untreated (control). Both studies utilized the same batches of VYC-12L and HYD products. All image analyses were conducted on 9 to 12 analyses for each batch of staining, with the histopathologist blinded to treatment.

Histologic Evaluations

In study 1, on days 3 and 8 after injection, 3 round samples from each treatment group were collected and cut into 2 parts, with 1 part frozen, cryofixed, and cryosectioned, and the other part formalin fixed, paraffin embedded, and sectioned. The samples were observed under a Leica DMLB microscope (Leica Microsystems GmbH, Wetzlar, Germany) or a BX43 Olympus microscope.
Assessment of General Morphology and Markers of Elasticity

To assess general morphology, including collagen density, paraffinized sections were stained according to Masson’s trichrome (Goldner variant) and examined at ×20 magnification. To evaluate elastin expression, frozen sections were stained with diluted rabbit anti-elastin polyclonal antibody and nuclei were post-stained with propidium iodide. Sections were examined at ×40 magnification. To evaluate the fibrillin-1 expression, frozen sections were stained with diluted mouse anti-fibrillin-1 monoclonal antibody, and nuclei were post-stained with propidium iodide. Sections were examined at ×10 magnification. Evaluation of elastin and fibrillin-1 expression was based on the qualitative microscopic observation of the stained sections.

Assessment of Markers of Hydration

To assess AQP3 expression, paraffinized sections were stained with a rabbit anti-AQP3 polyclonal antibody and examined at ×40 magnification. To measure GAG content, paraffinized sections were stained by Alcian blue/periodic acid–Schiff for acidic GAGs and examined at ×10 magnification. To visualize HA, paraffinized sections were stained using a diluted, biotinylated antibody against HA binding protein and examined at ×10 magnification. Evaluation of HA staining, AQP3 expression, and GAG content was based on the qualitative microscopic observation of the stained sections. Evaluation of HA was also made by an analysis of images of the stained sections. The staining intensity was measured in these images using CellD data scoring software (CellD, Roquemaure, France) and expressed as a percentage of the preselected skin layer of interest.

Table 1. Biological Markers of Skin Quality

| Attribute          | Marker       | Definition                                                                 | Rationale for Study                                                                 |
|--------------------|--------------|----------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Elasticity         | Collagen     | Concentration of collagen, a structural protein in the skin and other connective tissues | Collagen strengthens the skin, helping it to resist mechanical deformation          |
|                    | density       |                                                                             | Elastin constitutes about 90% of the elastic fibers in the skin                    |
|                    | Elastin       | Protein in the skin and connective tissue that maintains elasticity and flexibility | Fibrillin-1 is an essential component of the network of fibers that imparts elasticity to the skin |
|                    | Fibrillin-1   | Glycoprotein that supports the integrity of the dermal elastic fiber network and is expressed in dermal–epidermal junctions |                                                                                  |
| Hydration          | AQP3         | Water- and glycerol-transporting membrane proteins expressed in the epidermis | AQP3 is a major protein with a critical role in maintaining hydration in the skin   |
|                    | GAGs         | Linear polysaccharide molecules, including hyaluronic acid, that bind water and help regulate the hydration of the dermis | GAGs hold and maintain water, and skin aging is associated with reduced GAG expression |
|                    | Hyaluronic    | Linear polysaccharide molecule that maintains the hydration and structural integrity of skin and other connective tissues | HA has an immense capacity to retain water; a primary function in the skin is to regulate moisture homeostasis |
|                    | acid (HA)     |                                                                             |                                                                                  |
|                    | Epidermal     | A measure of the humidity level of the outermost cutaneous layers of the stratum corneum | Epidermal capacitance is a well-established measure of skin hydration in dermatological studies |
|                    | capacitance   |                                                                             |                                                                                  |

AQP3, aquaporin-3; GAGs, glycosaminoglycans.

RESULTS

Subject Data

The abdominoplasty tissue was obtained from 4 White female donors, aged 43 years old (study 1) and 42, 53, and 59 years old (study 2).

Elasticity Markers

Microscopic examination revealed elevated collagen density and associated extracellular matrix improvement in explants after injection of VYC-12L and HYD, but increases in collagen density with VYC-12L on day 3 were generally larger in magnitude than those observed with HYD (Fig. 2). Explants injected with VYC-12L displayed moderate increases in collagen density in the upper reticular dermis compared with controls on day 3, whereas the increases with HYD were slight. Improvements in the relief
of the dermal–epidermal junction were also detected with both VYC-12L and HYD. Improvements in collagen density and associated extracellular matrix persisted on day 8 for VYC-12L but not for HYD.

As qualitatively assessed, explants injected with VYC-12L exhibited clear increases in fibrillin-1 expression compared with controls on day 3 (Fig. 3). These increases corresponded to the observed improvement in the dermal–epidermal junction on day 3, consistent with the presence of fibrillin-rich elastic fibers at the dermal–epidermal junction. The fibrillin-1 expression on day 3 was slightly higher in magnitude for VYC-12L than for HYD, and slight increases in fibrillin-1 expression with VYC-12L persisted on day 8. In contrast, there was no modification in expression with HYD on day 8.

Neither VYC-12L nor HYD was observed to affect elastin expression within oxytalan or elaunin fibers at day 3 or 8 relative to noninjected controls.

Hydration and Hydration Markers

Both VYC-12L and HYD slightly increased AQP3 expression on day 3. On day 8, the increase was greater (Fig. 4). The increases on both days, however, were clearly visible with VYC-12L. VYC-12L produced a strong increase in acidic GAG content in the upper and lower reticular dermis on day 3, which was sustained on day 8. The effect was less pronounced with HYD (Fig. 5).

On day 3, explants injected with HYD and VYC-12L showed significantly reduced HA in the epidermis above the injection site and in the papillary and upper reticular dermis versus controls, with a concurrent HA increase in the lower reticular dermis. For example, on day 3, the average area of the epidermis that was positive for the presence of HA in controls was 68.6% versus 30% and 6.6% (P < 0.01) in explants injected with HYD and VYC-12L, respectively. On day 8, the average area of the lower reticular dermis positive for HA in controls was 6.4% versus 8.5% (P < 0.1) and 12.0% (P < 0.01) with HYD and VYC-12L, respectively. After 8 days, HA levels recovered to approximately the same level as in controls with VYC-12L for all skin layers; for example, on day 8, the average epidermal area positive for HA was 69.9% for controls and 72.4% for VYC-12L; for HYD, it was 35.8%.

Corneometry readings for hydration in study 2 revealed significant increases on days 1, 2, and 8 for VYC-12L-injected explants relative to control and HYD-injected explants. The corneometry readings for HYD were significantly increased at day 8 compared with control. Relative to control, the average corneometry-measured skin hydration increase in explants from the 3 donors was 49% in the VYC-12L-injected explants on day 8, compared with 22% in the HYD-injected explants, a significant increase (Fig. 6).

DISCUSSION

An increasing array of injectable HA fillers are used in aesthetic medicine to restore facial volume loss and to smooth wrinkles. Numerous reports exist in the literature that describes HA fillers on the basis of their various biophysical characteristics, including cohesivity, elasticity, and viscosity. Most of these studies were conducted in vitro, and their results have helped to predict clinical outcomes and guide physicians to select the
most relevant products to optimize the intended aesthetic objectives.29-33

In the present analyses, biological markers of skin hydration and elasticity in living human skin explants were studied ex vivo. This model has been used previously to evaluate early treatment effects on these markers,34 as the tissue maintains viability for 7 to 14 days (maximum) in culture.35-37 This model allows injection of the product directly into human skin, allowing for exposure of the cells and tissue to the hydrogel composition and hydrogel physical properties, both of which can impact tissue response. Improvements were seen following VYC-12L injection, supporting the benefits of VYC-12L with respect to specific skin quality attributes. Collagen density, fibrillin-1 and AQP3 expression, and acidic GAG content increased following VYC-12L injection. The observation of increases in these markers persisted from day 3 to day 8 after injection. Microscopic evaluation of collagen density revealed associated extracellular matrix and dermal–epidermal junction improvements with VYC-12L, demonstrating the potential of the product to improve microscopic structural properties of the skin in this model. Elastin expression did not increase in injected explants, whereas fibrillin-1 expression clearly improved, which may reflect a delayed synthesis of elastin or maintenance of a lower ratio of elastin to fibrillin in the oxytalan and elaunin elastic fibers studied.38,39

Furthermore, VYC-12L injection in the dermis produced strong increases in acidic GAG content in both the upper and lower reticular dermis, correcting hydration levels in layers of the skin deeper than the direct site of injection. Corneometry results also showed a significantly increased improvement in hydration with VYC-12L than with HYD, a noncrosslinked HA formulation that has a similar HA content and has been shown to increase hydration clinically.12 Injection with VYC-12L generally resulted in more pronounced and longer-lasting increases in skin quality biomarkers than HYD. These results with VYC-12L support the role of VYC-12L as a dermal filler with additional potential for improving skin elasticity and hydration.

This is the first evaluation in living human skin explants of the impact of an injectable crosslinked HA filler...
(VYC-12L) on markers of hydration and elasticity versus an injectable noncrosslinked HA formulation (HYD) or no injection. Quan et al. examined fibroblast behavior and collagen production in cultured skin specimens from healthy elderly volunteers (mean age 81 years) biopsied 1, 2, 4, and 12 weeks after injection with crosslinked HA and vehicle and determined that the HA-injected skin samples exhibited heightened pro-collagen fibroblast function and extracellular matrix stability. Sundaram et al. demonstrated that a topically applied crosslinked HA product was more effective than a noncrosslinked HA topical for demonstrating that a topically applied crosslinked HA product was more effective than a noncrosslinked HA topical formulation in improving measures of hydration and skin barrier function in living human skin explants analyzed on day 9 after treatment. The results of our analyses are consistent with such findings, but they are also unique, deriving from studies conducted after direct HA injection into explants and showing improvements in various biological markers of skin quality that were sustained over 8 days. Although interpretations of our data are limited by the short study duration due to tissue degradation over time and by the inability to replicate the conditions of live human skin in vivo, such limitations are common to all studies using human skin explants. Moreover, the ability to inject an HA gel directly into a living skin explant allows physical interaction between HA and the tissue and cells. As the primary effect of HA gels is to provide a filling effect, and the physical interaction (ie, stretch effect) has been shown to impact collagen and elastin production, this model provides a good opportunity to evaluate HA fillers in an in situ tissue environment with similarities to clinical HA injection conditions. As a result, living human skin explants provide a realistic model for the effects that might be expected in human skin in vivo. Further evaluation of these results in longer-term clinical studies would help to confirm the longer-term time course of these effects. A recently published prospective clinical study by Niforos et al. evaluated the same formulation and reported skin smoothness up to 6 months and hydration lasting 9 months, consistent with and expanding upon the results of the current study. The reduction in HA content with HYD and VYC-12L at day 3 was consistent with effects previously observed by the laboratory with injected HA, suggesting increased expression of hyaluronidase in response to the influx of HA; by day 8, HA levels had increased and normalized with VYC-12L, but not to the same extent with HYD, which may reflect a more durable cutaneous response to VYC-12L than HYD, consistent with effects observed for the biological markers of collagen density, fibrillin-1 and AQP3 expression, and acid-based GAG content. The more sustained response to VYC-12L compared with HYD in this study may be related to the crosslinking of VYC-12L, which increases persistence in tissue relative to noncrosslinked HYD, as observed in the increased GAG staining in the lower reticular dermis.

CONCLUSIONS

VYC-12L improved biological markers of skin quality in human living skin explants. Increases in biological markers of skin elasticity and hydration were generally greater and persisted longer with VYC-12L compared with HYD. The data suggest that VYC-12L could improve microscopic structural aspects of the skin, consistent with previous observations investigating the physical interaction; however, as these analyses were conducted ex vivo over early time points, additional in vivo or clinical investigations, such as that of Niforos et al., will be valuable for offering further insight on additional effects following treatment.
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