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Biological Effects of Hyaluronic Acid-Based Dermal Fillers and Laser Therapy on Human Skin Models

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ABSTRACT

Injection of dermal fillers is one of the most frequently performed aesthetic procedures. The aim of the present study was to investigate the biological effects of different stabilized hyaluronan (HA) and poly-l-lactic acid fillers with and without subsequent additional fractional laser co-treatment on skin morphology and gene expression. Intradermal injection resulted in a significant enhancement of epidermal thickness detected by histological analysis. Combining HA fillers with ablative fractional CO2- or Er:YAG laser irradiation enhanced this effect. Gene expression profiling revealed an upregulation of modulators of tissue remodeling (eg TIMP3, SERPIN E1) and collagens (COL11A1). On the other hand, we detected a downregulation of differentiation markers (eg FLG, LOR, KRT1) and proinflammatory cytokines (eg IL-36, IL-1β). Interestingly, HA-based fillers revealed a specific upregulation pattern of chemokines such as CXCL5 and CCL20 suggesting a secondary effect of these fillers on the immune cells of the skin, especially monocytes and macrophages.

Taken together, our data show enhancing effects of dermal fillers on epidermal thickness and prove the proliferating effects of these products on epidermal cells on the molecular level. Moreover, our findings reveal synergistic effects of fractional ablative laser treatment and HA dermal filler injection suggesting a combination of both treatments.

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INTRODUCTION

Injection of hyaluronic acid (HA) dermal fillers is one of the most frequently performed aesthetic procedures.[1] HA fillers exist in many different formulations differing in HA concentration, particle size and cross-linking density. While HA fillers with high-density and large particles are recommended for deep dermal injections, fillers with low-density and small particles are more commonly used for fine lines.[1]

The direct biological effects of dermal fillers monotherapy and combination therapy with ablative fractional CO2- or Er:YAG laser irradiation on human skin cells are not completely understood. Organotypic three-dimensional (3D) skin equivalents have been established for standardized studies of the human skin.[2] The aim of the present study was to investigate the molecular effects of different stabilized HA and poly-l-lactic acid (PLLA)-based fillers with and without subsequent additional fractional laser co-treatment.

MATERIALS AND METHODS

In this comparative effectiveness research in vitro study different stabilized HA (Restylane Skinboosters Vital (HA1) and Vital Light (HA2) as well as Refyne (HA3)) and PLLA-based (Sculptra) fillers were injected intradermally into skin models as a monotherapy and in combination with ablative fractional CO2- or Er:YAG laser irradiation. Effects on skin morphology were assessed immediately after treatment and 5 days later by histological analysis. In addition, a transcriptomic gene expression profiling was performed on day 5 after treatment. Statistical analysis was performed using the Mann-Whitney U test. Values of $P<0.05$ were considered statistically significant.

RESULTS

Intradermal injection of the different fillers into skin models resulted in a significant enhancement of epidermal thickness detected by histological analysis (Figures 1A and 1B). On the molecular level, gene expression profiling by microarray analyses revealed an upregulation of modulators of tissue remodeling (eg, TIMP3, SERPIN E1) and collagens (COL11A1) on day 5 after injection of the different fillers (Figures 1C–E). On the other hand, we detected a downregulation of differentiation markers (eg, FLG, LOR, KRT1) and proinflammatory cytokines (eg, IL-36, IL-1β). Combining HA dermal fillers with ablative fractional CO2- or Er:YAG laser irradiation enhanced the effect of epidermal thickening (Figures 2A and 2B). Gene expression profiling of the combined treatment revealed an upregulation

FIGURE 1. (A) Representative HE stained sections of 3D skin models on day 0 and day 5 after intradermal injection of different HA-based fillers (HA 1: 20mg/ml non-animal stabilized hyaluronic acid (NASHA) gel with small particles; HA 2: 12mg/ml NASHA gel with finer particles; HA 3: 20mg/ml NASHA gel with bigger particle size) and a poly-l-lactic acid (PLLA)-based filler. (B) Measurement of epidermal equivalent thickness on day 5 after injection. Data are given as arithmetical means ± standard deviation; $^{*}P<0.05$, $^{**}P<0.01$. (C-E) Gene expression profiling in models that were injected with HA 2, HA 3, and PLLA on day 5 after injection.

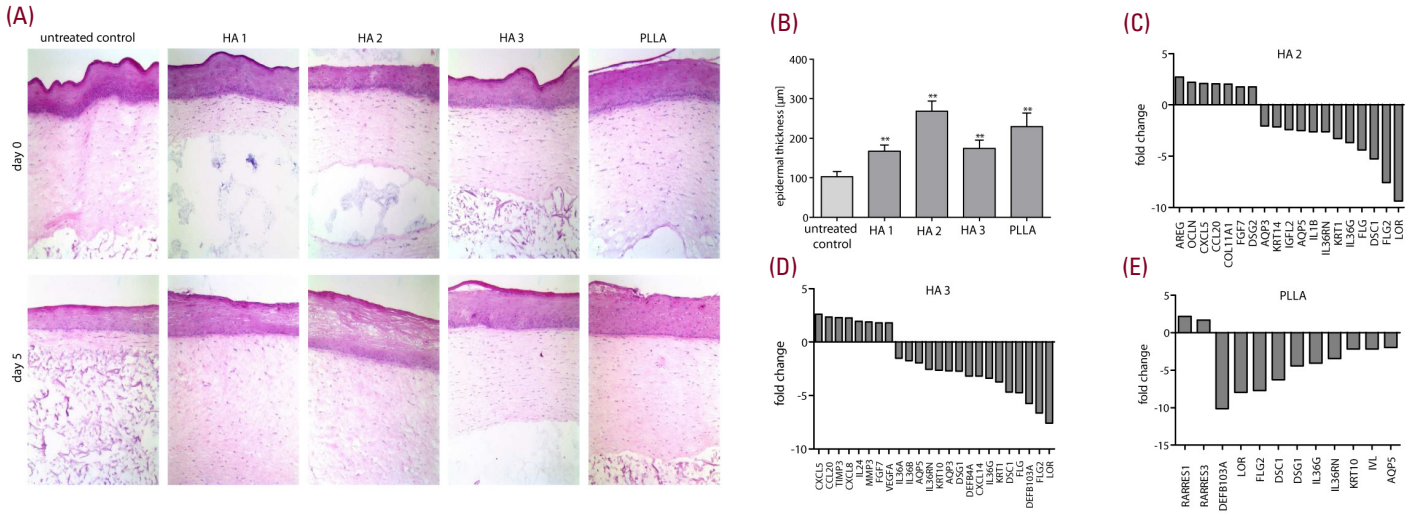
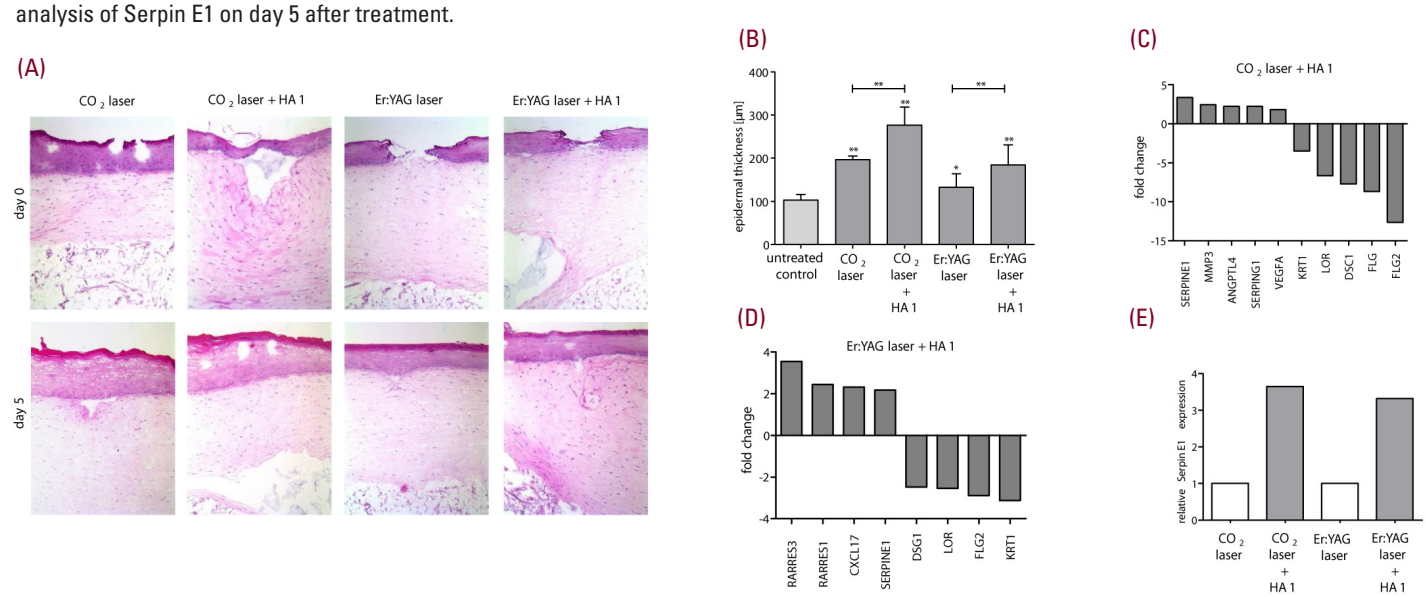


FIGURE 2. (A) Representative HE stained sections of 3D skin models on day 0 and day 5 after ablative fractional laser treatment with either a CO_2 (80 mJ/cm^2) or an Er:YAG laser (N10%; 4 pulses, energy per impulse 40 J) and a combination of both laser treatments with intradermal injection of HA filler 1 (20mg/ml NASHA gel with small particles). (B) Measurement of epidermal equivalent thickness on day 5 after treatment. Data are given as arithmetical means ± standard deviation; $^{*}P<0.05$, $^{**}P<0.01$. (C-D) Gene expression profiling in models that were treated with a combination of laser irradiation and intradermal injection of HA 1 on day 5 after treatment in contrast to laser irradiated models. (E) Independent Real-Time PCR analysis of Serpin E1 on day 5 after treatment.



of genes that are associated with tissue remodeling (eg, Serpin E1, MMP3) as well as a downregulation of differentiation markers (eg, KRT1, FLG) (Figures 2C and 2D), reflecting the enhanced cell proliferation in the epidermal layer of the 3D skin model. Moreover, qRT-PCR analysis confirmed the stimulating effects of the combined treatment on Serpin E1 (Figure 2E).

DISCUSSION

This study aimed at understanding more precisely the biological effects of HA-based fillers, their combination with laser treatment and their differences to PLLA-based fillers. Our data show enhancing effects of dermal fillers on epidermal

thickness and prove the proliferating effects of these products on epidermal cells on the molecular level. Interestingly, HA-derived products revealed a specific upregulation pattern of chemokines such as CXCL5 and CCL20, in contrast to PLLA-based injectable dermal fillers. These data suggest a secondary effect of the HA-based compounds on the immune cells of the skin, especially monocytes and macrophages. Moreover, we detected that primarily HA fillers with fine particles promote collagen synthesis, which in previous studies was associated with increased TGF-β signaling.[3]

So far, scientific knowledge about the combined use of HA fillers and laser treatment is still limited.[4] Our results revealed synergistic effects of fractional ablative laser treatment and injection of HA dermal fillers supporting a combination of both treatments. This combined treatment promotes tissue modulators such as Serpin E1, which plays a key role in the cutaneous wound repair program.[5]

The present study showed that skin models are a reliable tool to investigate the molecular effects of dermal fillers and their combination with laser treatment.

DISCLOSURES

The authors have no relevant conflicts to report.

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