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 ORIGINAL ARTICLE

A Clinical Histology Study Evaluating the Biostimulatory Activity Longevity of Injectable Poly-L-Lactic Acid for Facial Rejuvenation

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ABSTRACT

Background: Poly-L-lactic acid (PLLA) is an injectable filler used for restoring facial fat volume loss that improves skin quality.
Objective: To evaluate the histological changes underlying the observed improvement in skin quality after repeated PLLA injections.
Methods: Ten healthy women were enrolled in this randomized, placebo-controlled, single-center study. Eligible subjects received 3 treatments every 4 weeks with either PLLA (treatment group) or saline (control group) injections, into both sides of the face. Follow-up visits were at week 18 after the last treatment. Assessments included live ratings, patient questionnaires, three-dimensional microtopography imaging analysis, and histological analysis from biopsies taken before and after PLLA treatment.
Results: At the 18-week follow-up, there was a significant improvement in investigator- and subject-rated global aesthetic improvement (GAIS) scores, as well as a decrease in wrinkle severity in PLLA-treated but not placebo-treated patients. Skin quality parameters of erythema, pore size, and roughness were significantly improved from baseline and compared with placebo at the 18-week follow-up as assessed by microtopographic analysis and investigator ratings. Histologic analysis revealed increased tissue remodeling and angiogenesis in PLLA-treated tissues at the 18-week follow-up and decreased elastin fragmentation compared with baseline. No treatment-related adverse events occurred.
Conclusion: Repeated PLLA treatments may improve skin quality through tissue remodeling and neovascularization.

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INTRODUCTION

The aging process is a complex phenomenon that is influenced by both internal (genetic) and external (environmental) factors. Bone resorption, fat pad repletion, ligament/muscle atrophy, and changes in skin tone, color, and texture all contribute to the loss of a youthful appearance. Injectable soft tissue fillers and neurotoxins are the gold standard in aesthetic medicine for correcting the facial changes associated with aging. These treatments can be used in conjunction with energy-based devices and a daily skincare regime to achieve natural, long-lasting facial rejuvenation.[1]

Some fillers, such as hyaluronic acid, volumize the area of injection instantly. Others, such as poly-L-lactic acid (PLLA), are biostimulatory, meaning that they activate resident fibroblasts to produce autologous collagen over time. This leads to a more natural and long-lasting effect.[2]

The mechanism by which PLLA stimulates neocollagenesis is by triggering a foreign body reaction to the injected material demonstrated by neutrophil and macrophage infiltration from day 2 to day 10 after injection. This is followed by a cellular inflammatory response that leads to the formation of vascularized, connective tissue. PLLA is then hydrolyzed into lactate, converted to pyruvate, and oxidized into carbon dioxide. The inflammatory response subsides in 6 months, but the extracellular matrix production continues, leading to a gradual increase in dermal thickness that can last for at least 2 years.[3]

PLLA was approved by the US Food and Drug Administration (FDA) in 2004 for the treatment of HIV-associated lipoatrophy, and in 2009 for the correction of shallow to deep nasolabial folds and other facial wrinkles in healthy patients. In 2023, the

FDA expanded the approval of the product to the correction of fine lines and wrinkles in the cheek region.[4-7] It has also extensively been used off label for augmentation and aesthetic improvement of other areas such as the abdomen, gluteal region, and chest.[8-11]

Aside from volumizing the face and improving wrinkles, anecdotal evidence has suggested that patients' skin quality may also improve after PLLA injection, something referred to as the "Sculptra glow;" and this was demonstrated in a clinical trial using investigator and subject assessments of skin quality.[12,13] The study showed that global skin quality improvement, including radiance, smoothness, and pigment uniformity, as well as a decrease in erythema index and pore size, was noted following PLLA injections.

In order to bridge these observations with a histological explanation of the underlying pathophysiology, the objective of this study was to evaluate the histological changes underlying the observed improvement in skin quality after repeated PLLA injections.

MATERIALS AND METHODS

Patients

A total of 10 subjects were enrolled in this trial. All subjects provided written informed consent prior to receiving any study-related procedures. Eligible subjects were healthy females (30-65 years of age, Fitzpatrick photo skin types I-IV), with shallow to deep nasolabial fold contour deficiencies or other facial wrinkles, who agreed not to have any procedures affecting facial wrinkles (eg, filler, botulinum toxin, radiofrequency, laser, intense pulsed light (IPL), ultrasound) or skin quality (microdermabrasion, peels, acne treatments, etc) for the duration of the study. Negative urine pregnancy test results were required for women with childbearing potential before enrollment. Previous therapy with botulinum toxin, fillers within 12 months of the baseline visit, or treatment with PLLA in the face at any time precluded women from participation. Subjects with dermatologic conditions including acne, rosacea, eczema, psoriasis, actinic keratosis, severe sun damage, scars, or a history of keloids were also excluded from the study.

Study Design

This was a randomized, controlled, double-blind single-center study conducted in accordance with the principles of the Declaration of Helsinki, current good clinical practice (GCP) guidelines, and Institutional Review Board (IRB) approval. The treatment phase consisted of a baseline visit and visits at week 4 and week 8, during which eligible subjects received injections of 5 cc of PLLA for the treatment group or saline for the control group on both sides of the face. In addition, subjects underwent a series of injections of Sculptra into non-lesional post-auricular skin, followed by 3-mm punch biopsies at specified intervals: at baseline prior to treatment 1, 4 weeks post-treatment 1, 48 hours post-treatment 3, and 18 weeks post-treatment 3. The contralateral site was not injected but served as a negative control for baseline biopsy studies. Each subject participated for a period of up to 28 weeks. The subject participation period included a screening period of up to 2 weeks, 3 injections at baseline, week 4, and week 8, and a follow-up visit at week 18 after the last injection.

Treatment

Twenty-four hours prior to injection, 5 cc of sterile water was added to the PLLA vial. One vial of PLLA was used per person: 2.4 mL reconstituted PLLA was injected on the left side, 2.4 mL on the right side, and 0.2 mL at the biopsy site. Injections were carried out using the tunneling technique into the deep dermis in a grid pattern with a 25-G needle (1.0 or 1.5 inches) at a 30° to 40° entry angle. To ensure even product distribution and prevent nodule formation, the injected area was massaged after every 2 to 3 injections and for longer at the end of treatment. Patients were instructed to massage the injected areas for 5 minutes, 5 times per day, for 5 days following the procedure.

Assessments

The degree of improvement was assessed by the investigator or the subject (GAIS) from screening in the treated areas by comparing the digital photographs of the subject's treatment area as follows: -3 = very much worse; -2 = much worse; -1 = worse; 0 = no change; +1 = minimally improved; +2 = much improved; +3 = very much improved.

Biopsies were histologically analyzed for procollagen 1, CD31 (vessels), elastin, and H/E. Standardized photographs were taken prior to all treatments and the week 18 follow-up visit. Live assessments were performed by blinded investigators during those time points. The assessment of wrinkle severity was performed before each treatment and at the week 18 follow-up.

The assessment of skin quality was performed by patients and a trained investigator, who was blinded to the visit number, at baseline and 18 weeks post-last treatment using a customized 10-point scale (Table 2).

Standardized Photography

Standardized photographs were taken at 0°, 45°, and 315° angles using the Visia® CR system (Canfield Imaging Systems, Fairfield). The PRIMOS digital fringe projection technology was used to determine the skin topography of the periorbital and mesolabial areas (ie, wrinkle depth, texture, and pore size).

Safety

Adverse events were monitored and recorded throughout the study. Patients were asked to report product-related adverse events.

Histological Analysis

All specimens were formalin-fixed and paraffin-embedded. Histological and immunohistochemical (IHC) evaluations were performed in a blinded manner by 2 dermatopathologist investigators. Immunohistochemical stains included those directed at procollagen 1, CD31 (vessels), and elastin. Quantitative image analysis was performed using Aperio ImageScope for areas occupied by collagen, areas occupied by inflammatory cells, areas possibly occupied by foreign material, and areas occupied by vessels (dermal vessel density).

Statistical Analysis

For the quantitative variables, the effectiveness, as well as the number of missing data, the number of knowledgeable data, the mean and the standard deviation, the median, the range, the minimum, and the maximum were reported. For the qualitative variables, the effectiveness, the number of missing data, the number of data populated, and the percentage of each modality were given. Summary tables (descriptive statistics and/or frequency tables) were provided for all baseline variables, efficacy, and safety variables, as appropriate.

RESULTS

Ten female participants completed the study. Mean (SD) age of was 45.2 (9.6) and 50% were Fitzpatrick skin type III. Seven patients received PLLA parallel and perpendicular to the nasolabial fold and 3 received the placebo.

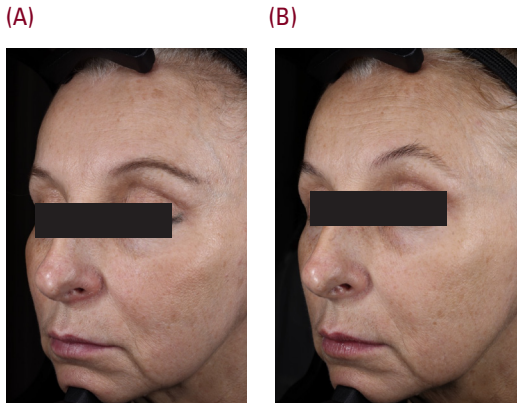
Responder Rates

At the 18-week follow-up, PLLA-treated patients had a 1.34 increase in investigator-rated GAIS score (improved/much improved) compared with baseline, while there was no change in GAIS score in the placebo-treated patients. Similar results were noted with the subject-reported GAIS where all 7 patients in the PLLA group noted a >1 point improvement in GAIS score at all follow-up visits. Patients treated with PLLA had a 1-point decrease in wrinkle severity at the first 2 follow-up visits (after Treatment 1 and Treatment 2), and a 2-point decrease in wrinkle severity at the 18-week follow-up post Treatment 3. There was no change in wrinkle severity in patients treated with saline (Figures 1 and 2).

All skin quality assessments (radiance, smoothness, pigmentation, erythema, pore size) were improved from baseline and compared with placebo at the 18-week visit post-treatment 3 according to the blinded investigator ratings (Table 1). Analysis of the subject-evaluated skin quality mean scores showed a similar trend for all assessments to be increased at the 18-week follow-up in the PLLA group compared with saline (data not shown).

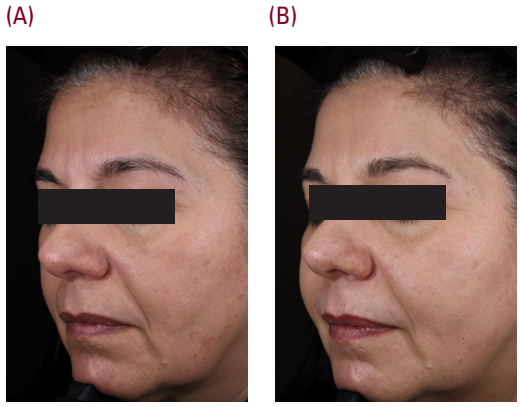
On 3D images and topography using PRIMOS, skin roughness was evaluated using values of Ra (arithmetic average skin roughness) and Rz (average maximum height of the wrinkle). Results of the roughness analysis showed that there was a reduction in both Ra and Rz values at all timepoints after baseline compared with placebo-treated patients (Figure 3).

FIGURE 1. Representative clinical photographs of a 59-year-old subject (A) before and (B) after 18 weeks past the 3rd PLLA treatment.



Reduction of pore size, increased radiance, and smoother more even tone are noted post-PLLA treatment.

FIGURE 2. Representative clinical photographs of a 48-year-old subject (A) before and (B) after 18 weeks past the 3rd PLLA treatment.



Reduction of pore size, increased radiance, and smoother more even tone are noted post-PLLA treatment.

Analysis of all PRIMOS parameters for skin roughness, erythema, coloration, wrinkle count, pore count, and mean thickness improved significantly over the course of the study (Table 1).

Histological Analysis

Histological analysis showed no evidence of the filler, indicating that it likely dissolved during tissue processing. There was no evidence of an inflammatory reaction in response to the injection, or evidence of damage to any of the skin structures, including the epidermis, hair follicles, glands, or vessels. PLLA-treated but not placebo-treated tissues had well-formed capillaries in the

TABLE 1.

Investigator Rated Skin Quality Score				
Group	**Baseline**	**4 Wks Post Tx 1**	**4 Wks Post Tx 2**	**18 Wks Post Tx 3**
Radiance				
Saline	5.2 (0.1)	6.2 (1.2)	5.8 (1.4)	5.9 (1.1)
PLLA	5.8 (0.2)	6.2 (0.4)	6.3 (0.2)	7.6 (0.5)
Radiance				
Saline	4.1 (1.1)	4.3 (0.8)	4.4 (1.2)	4.1 (1.3)
PLLA	4.8 (0.2)	5.2 (0.4)	5.3 (0.2)	6.6 (0.5)
Pigmentation				
Saline	5.6 (1.2)	6.1 (0.8)	5.5 (1.3)	5.9 (0.5)
PLLA	5.3 (1.6)	6.0 (0.2)	6.5 (0.3)	6.9 (0.4)
Erythema				
Saline	4.1 (1.3)	4.8 (1.4)	4.7 (1.2)	4.9 (1.4)
PLLA	4.8 (0.2)	5.2 (0.4)	5.3 (0.2)	6.6 (0.5)
Erythema				
Saline	5.5 (0.3)	5.3 (0.7)	5.9 (1.1)	5.7 (0.8)
PLLA	5.8 (0.2)	6.2 (0.4)	6.5 (0.2)	7.1 (0.5)

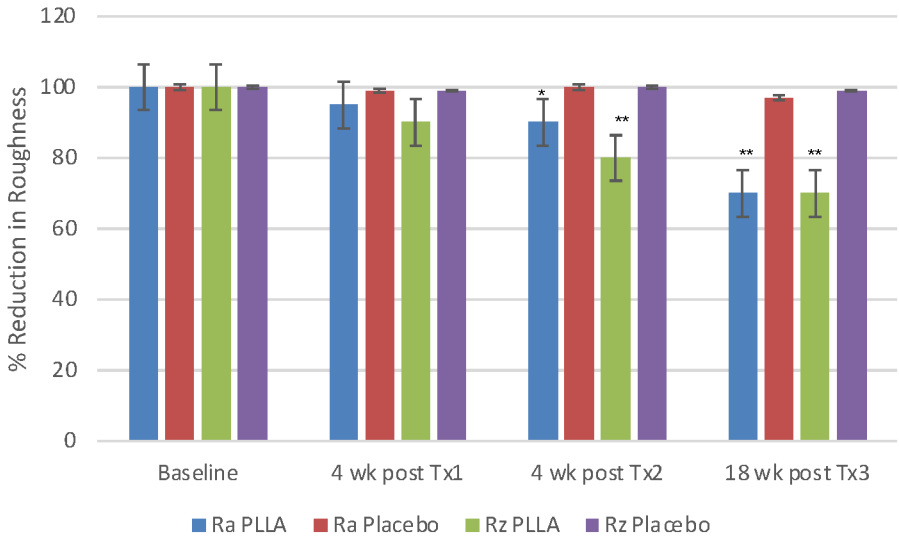
Footer

upper dermis and fewer telangiectasias overall, indicating that PLLA stimulated vascularization (Figure 4A and B). H&E-stained sections from placebo-treated tissue showed thicker, mature collagen bundles, and thinner epidermis with flattened basal cells vs the PLLA-treated tissue that had reduced epidermal atrophy; with increased number of layers, more cuboidal basal cells, and recovery of normal epidermal maturation, likely representing neocollagenesis (Figure 4C and D). Finally, PLLA-treated tissue exhibited reduced elastin fragmentation and increased numbers of elastic fibers in the papillary and reticular dermis (Figure 4E and F).

Safety

No serious adverse events or unexpected side effects were observed or reported for any of the subjects in either the PLLA or placebo groups. All adverse effects were injection-related (bruising, erythema) and resolved within 2 weeks of treatment. There were no reports of post-inflammatory pigmentation following bruising.

FIGURE 3. Reduction in skin roughness in PLLA- and placebo-treated patients over time as assessed by Ra (arithmetic average skin roughness) and Rz (average maximum height of the wrinkle).



*P<0.05; **P<0.01.

FIGURE 4. CD31 Angiogenesis marker is increased (A) post-PLLA Tx vs (B) placebo. H/E showed improved tissue remodeling (C) post-PLLA Tx vs (D) placebo. Decreased elastin fragmentation (E) post PLLA Tx vs (F) placebo.

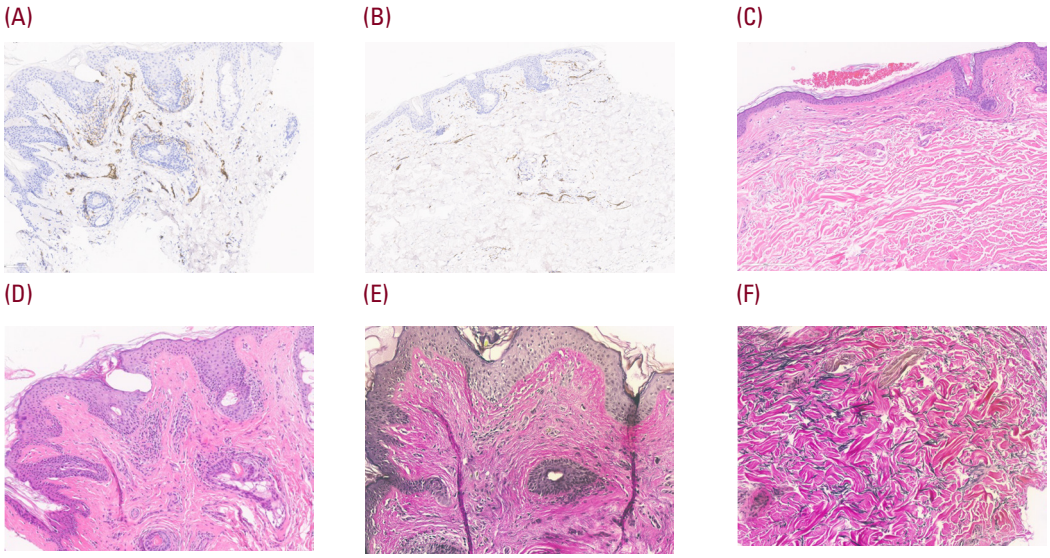


TABLE 2.

Customized Ten-Point Scale for Rating of Skin Quality
Radiance 1 2 3 4 5 6 7 8 9 10 I----------I----------I----------I----------I----------I----------I----------I----------I----------I No radiance maximum radiance
Smoothness 1 2 3 4 5 6 7 8 9 10 I----------I----------I----------I----------I----------I----------I----------I----------I----------I Very rough Very smooth
Pigmentation 1 2 3 4 5 6 7 8 9 10 I----------I----------I----------I----------I----------I----------I----------I----------I----------I Very irregular pigmentation Very uniform pigmentation
Erythema 1 2 3 4 5 6 7 8 9 10 I----------I----------I----------I----------I----------I----------I----------I----------I----------I Intense redness No redness
Pore size 1 2 3 4 5 6 7 8 9 10 I----------I----------I----------I----------I----------I----------I----------I----------I----------I Very large pores Minimal pores

DISCUSSION

This single-center prospective study demonstrates that repeated PLLA injections improve skin quality through dermal remodeling processes, which include decrease in elastin fragmentation, dermal reorganization, and neovascularization. A comprehensive set of measurements, including digital analysis of skin topography and investigator- and subject- assessments, as well as histological analysis of pre- and post-treatment tissues, were used to investigate the biological process underlying the improvement of skin quality in this set of patients.

Skin quality and wrinkle parameters showed significant improvement, as assessed by both investigators and subjects at each post-treatment evaluation. The global aesthetic improvement peaked at the 18-week follow-up.[12,14]

Results from the main histologic analysis evaluating vascularization, skin structure, and elastin fibers showed that PLLA-treated but not placebo-treated tissue exhibited morphologic changes consistent with those expected in a process of skin rejuvenation. A more detailed histological analysis of these tissues that will evaluate markers of inflammation and of regenerative capacity is ongoing to further elucidate the breadth of PLLA biostimulatory properties.

Limitations of the study include the small number of subjects, the fact that only female subjects were enrolled, and the relatively short period of follow-up. How the treatments affect males, a variety of ethnic skin types and the synergism with other aesthetic treatments such as microneedling and neurotoxins is an important topic of future research. Finally, assessing the longevity of the biological response to PLLA and the appropriate intervals for repeat injections will be further evaluated at a later timepoint.

In sum, the results presented herein demonstrate that repeat treatments of PLLA improve skin quality by stimulating dermal remodeling processes in the tissue over time.

DISCLOSURES

The authors have no conflicts of interest to disclose.

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