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Poly-lactic Acid Dermal Filler Enriched With InCube®-encapsulated Vitamin C and Glutathione: Preclinical Evidence of Superior Collagen Induction and Tissue Response Compared With Conventional PLA

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Abstract

Background/Aim: Poly-Lactic acid (PLA) fillers are widely used for facial and body volume restoration because of their collagen-stimulating properties. However, early inflammation, oxidative stress, and variable long-term remodeling remain as concerns. Vitamin C and glutathione support collagen synthesis and redox homeostasis but degrade rapidly in conventional systems. This study evaluated a PLA filler incorporating Ethyl ascorbic acid (Vitamin C) and glutathione stabilized within an InCube® microlattice (PLA-IC) focusing on antioxidant stability, collagen induction, and tissue response. *Materials and Methods*: PLA-IC were prepared by combining PLA microspheres with microlattice-encapsulated vitamin C and glutathione. Scanning and transmission electron microscopy and selected-area electron diffraction were used to characterize particle morphology and structure. Antioxidant stability was examined using high-performance liquid chromatography and DPPH radical-scavenging assays during long-term storage. *In vivo*, SKH mice received subcutaneous injections of PBS, commercial PLA filler, or PLA-IC. Surface volume changes were measured by Primos imaging, and histology was used to assess inflammation, neovascularisation, and collagen deposition over 12 weeks.

continued

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Results: Microlattice encapsulation preserved vitamin C and glutathione for 277 days and maintained strong DPPH-scavenging activity for up to 200 days. *In vivo*, PLA-IC showed significantly greater volumizing effect than standard PLA filler between weeks 4 and 12. Masson's trichrome staining demonstrated higher collagen density in the PLA-IC group, reaching nearly twice that of conventional PLA at 12 weeks. H&E sections revealed reduced inflammatory infiltration and minimal neovascularisation in PLA-IC-treated tissues.
Conclusion: Microlattice-based incorporation of vitamin C and glutathione enhances the antioxidants stability and overall biostimulatory performance of PLA filler. Compared with conventional PLA, PLA-IC demonstrated improved volume retention, increased collagen formation, and a more controlled tissue response, indicating potential advantages for aesthetic applications and warranting clinical evaluation of safety and efficacy.

Keywords: Poly-Lactic acid filler, PLA filler, antioxidant encapsulation, vitamin C and glutathione stability, neocollagenesis.

Introduction

Injectable poly-Lactic acid (PLA) has become an important biostimulatory filler in aesthetic plastic surgery (1). Its ability to induce gradual collagen formation allows sustained volumization and skin quality improvement, making it widely used for midface rejuvenation, contour enhancement, and correction of age-related lipoatrophy (2, 3). Despite its established clinical utility, variability in early inflammatory responses, inconsistent collagen induction, and patient-specific differences in tissue remodeling remain challenges that affect predictability of outcomes (4, 5).

A key factor contributing to this variability lies in the biological environment into which PLA is introduced. The degradation of PLA generates lactic acid oligomers, lowering the local pH and promoting oxidative stress (6, 7). Excess oxidative stress can impair fibroblast function, hinder collagen synthesis, and prolong inflammation influencing nodule formation, delayed edema, or suboptimal biostimulatory effects (8, 9). Therefore, strategies that stabilize the microenvironment and support cellular activity during the early phases of PLA remodeling may improve both safety and treatment consistency.

Vitamin C and glutathione are central to dermal homeostasis (10, 11). Vitamin C facilitates collagen hydroxylation and matrix organization, while glutathione maintains intracellular redox balance and regulates inflammatory signaling. Their combined activity has the potential to optimize the regenerative milieu around PLA particles. However, both molecules degrade rapidly when exposed to oxygen, moisture, or heat, making incorporation into injectable fillers difficult (12, 13). Conventional encapsulation methods such as hydrogels, emulsions, or polymer coatings extend stability only modestly and are often incompatible with long-term storage required for commercial facial fillers (14).

Microlattice encapsulation provides a structural solution to this limitation. The ordered lattice physically isolates sensitive molecules, minimizing oxidative degradation while allowing controlled release. Integrating vitamin C and glutathione in this stable microlattice, and subsequently combining them with PLA particles, may protect the antioxidants during storage and after injection, modulate the local biochemical environment, and enhance fibroblast-driven collagen synthesis. Such an approach could refine the tissue response to PLA and potentially improve clinical outcomes.

The objective of this study was to develop a PLA filler incorporating microlattice-encapsulated vitamin C and glutathione (PLA-IC) and to evaluate its physicochemical stability and biological effects. We assessed long-term antioxidant stability, characterized material morphology, and examined the volumizing effect, collagen deposition, and tissue reactions in a murine model. These findings

provide insight into whether antioxidant-enhanced PLA formulations can improve the consistency and quality of biostimulatory soft-tissue augmentation.

Materials and Methods

Preparation of PLA-IC. PLA-IC was prepared by mixing PLA particles (InCube 701®, LabInCube Co., Ltd., Cheongju, Republic of Korea) with microlattice-encapsulated vitamin C (InCube 301®, LabInCube Co., Ltd.), microlattice-encapsulated glutathione (InCube Glu®, LabInCube Co., Ltd.), and sodium hyaluronate (HA; AWA Biopharm Co., Ltd., Shandong, PR China). A phosphate-buffered aqueous solution (pH 7.0) was prepared from monobasic and dibasic sodium phosphate. The final suspension contained 82.9% PLA particles, 0.56% microlattice-encapsulated vitamin C, 0.17% microlattice-encapsulated glutathione, and 11.2% HA (w/w). The mixture was gently stirred until homogeneous and then frozen to dry.

Scanning electron microscopy (SEM). Particle morphology and size were examined using field-emission scanning electron microscopy (FE-SEM; JSM-7600F, JEOL Ltd., Tokyo, Japan) at the Chronic and Metabolic Diseases Research Center of Sookmyung Women's University. Two preparation methods were used: (1) dry powder samples were mounted on copper tape on aluminum stubs and sputter-coated with platinum; (2) powder samples were dispersed in saline, drop-cast on silicon wafers, vacuum-dried, and sputter-coated before imaging. For particle size analysis, at least 200 microspheres were randomly selected, and a log-normal distribution model was used to describe particle size distribution.

Transmission electron microscopy (TEM). The morphology and internal structure of the vitamin C and glutathione-free microlattice capsules were evaluated using TEM and selected area electron diffraction (SAED; JEOL Ltd.). Samples were dispersed in an organic solvent by sonication and dropped onto copper grids. After vacuum drying, TEM images and SAED patterns were acquired at 200 kV.

Glutathione content assay. Glutathione stability in the microlattice system was assessed by high-performance liquid chromatography (HPLC). Microlattice-encapsulated glutathione, approximately 7 g, was placed in a 15 ml conical tube and stored at 20±5°C and 60±5% relative humidity (RH) for 277 days. Glutathione content was measured immediately after loading and after 277 days of storage.

The HPLC system (Shimadzu Prominence HPLC-UVD, Shimadzu Corp., Kyoto, Japan) was equipped with a UV detector set at 210 nm and a 4.6×250 mm, 5 μm particle size C18 reversed-phase column maintained at 30°C. The mobile phase consisted of potassium dihydrogen phosphate and sodium 1-heptanesulfonate in water, adjusted to pH 3.0 with phosphoric acid and supplemented with methanol. The flow rate was set to achieve a glutathione retention time of approximately 5 min. The injection volume was 10 μl.

For sample preparation, 25 mg of microlattice-encapsulated glutathione was dissolved in 50 ml of distilled water. A glutathione standard solution (0.05 mg/ml) was prepared in the mobile phase. Glutathione content (%) was calculated using the following equation:

$$\text{Glutathione content (\%)} = [m_{STD} \times (As_{ample}/A_{STD}) \times 0.05/m_{Sample}] \times 100\%$$

Antioxidant stability of vitamin C and glutathione. Antioxidant stability was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays (ALLPASSBIO, Daegu, Republic of Korea). To examine antioxidant stability within the PLA-IC formulation, vials were stored at 20±5°C and 60±5% RH for up to 200 days. Samples were collected at 70, 120, and 200 days. For each test, one vial was dissolved in 4 ml of distilled water and mixed 1:1 (v/v) with 0.2 mM DPPH solution. After 30 min of incubation in the dark, absorbance at 517 nm was measured. L-ascorbic acid (1 mg/ml) was used as a positive control. DPPH scavenging activity (%) was calculated as:

$$\text{DPPH scavenging activity (\%)} = (1- A_{Sample}/A_{Control}) \times 100\%$$

Animal experiments. All animal procedures were approved by the Institutional Animal Care and Use Committee of

Seoul National University Bundang Hospital (approval number: BA-2502-409-004-01) and conducted in accordance with institutional and national guidelines. Five-week-old male SKH mice (20–25 g; BioOrient, Seongnam, Republic of Korea) were housed in a specific pathogen-free facility under a 12-h light/dark cycle at 24°C and 55% relative humidity, with free access to food and water.

A total of 45 mice were randomly allocated to three groups: PBS, commercial PLA filler, and PLA-IC. Each group included 15 animals. In each mouse, 100 μl of the assigned material was injected subcutaneously into the dorsal skin. At 0 (baseline, immediately after injection) and 2, 4, 8, 12 weeks, Primos images (Canfield Scientific, Parsippany, NJ, USA) were obtained for all animals, and three mice per group were euthanized at each time point for histological analysis. Thus, each group had n=15, 12, 9, 6, and 3 mice contributing to volume measurements at 0, 2, 4, 8, and 12 weeks, respectively.

H&E staining. Paraffin-embedded sections from each time point were deparaffinized in xylene and rehydrated through graded ethanol (100%, 95%, 90%, 80%, and 70%). Sections were rinsed in distilled water and stained with hematoxylin for 5 min, followed by a bluing reagent for 10-15 s. Eosin was applied for 30 s to stain cytoplasmic and extracellular components. Sections were dehydrated in 95% and 100% ethanol, cleared in xylene, and mounted with coverslips. Images were captured using an Olympus optical microscope (Olympus Corporation, Tokyo, Japan). Neovascularization and inflammatory cell infiltration were evaluated semi-quantitatively. Neovascularization was assessed by measuring the percentage of tissue area occupied by blood vessels. This was done by selecting 3 representative areas of each section and calculating the vessel area fraction using image analysis. Inflammatory cell infiltration was assessed by counting the number of inflammatory cells, such as lymphocytes, in 3 high-power (400×) fields per section and averaging these counts to compare among groups.

MT staining. Masson's trichrome staining was performed for tissue samples. Sections were incubated in Bouin's solution overnight at room temperature, rinsed, and stained with Weigert's hematoxylin for 10 min. Cytoplasm and muscle fibers were labeled with Biebrich scarlet–acid fuchsin, followed by differentiation in phosphomolybdic–phosphotungstic acid. Collagen fibers were stained with aniline blue and briefly rinsed in water. After treatment with 1% acetic acid, slides were dehydrated, cleared in xylene, and mounted. Collagen deposition was quantified using ImageJ software (NIH, Bethesda, MD, USA). For each mouse, 1 trichrome-stained section of the injection site was analyzed. Three non-overlapping fields of view within the implant region were selected, and a color threshold was applied to identify aniline blue–stained collagen. The collagen-positive area (% of field area) was measured in each field and averaged per section as an indicator of collagen density.

Statistical analysis. All data are presented as mean±standard error (SE), analyzed using GraphPad Prism 9 (GraphPad Software, Boston, MA, USA). Statistical analysis was performed with SPSS (v.20, IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro-Wilk test, along with kurtosis and skewness measures. The pairwise comparisons between groups at each time point for Primos volumetric and histological data were performed using Bonferroni-corrected *post hoc* tests following the two-way Analysis of Variance (ANOVA). Missing data due to scheduled euthanasia were handled by the mixed-model structure, which uses all available measurements for each animal without imputing later time points. Significance was defined as p<0.05 after correction.

Results

Characterization of filler particles. SEM images of PLA particles (InCube 701®) showed smooth, spherical microspheres with uniform morphology (Figure 1A). SEM analysis of PLA demonstrated a median particle size of 49.67 μm and a mean size of 50.39 μm, with most particles in the 40–60 μm range, indicating a relatively narrow size distribution (Figure 1B). SEM images of PLA-IC before

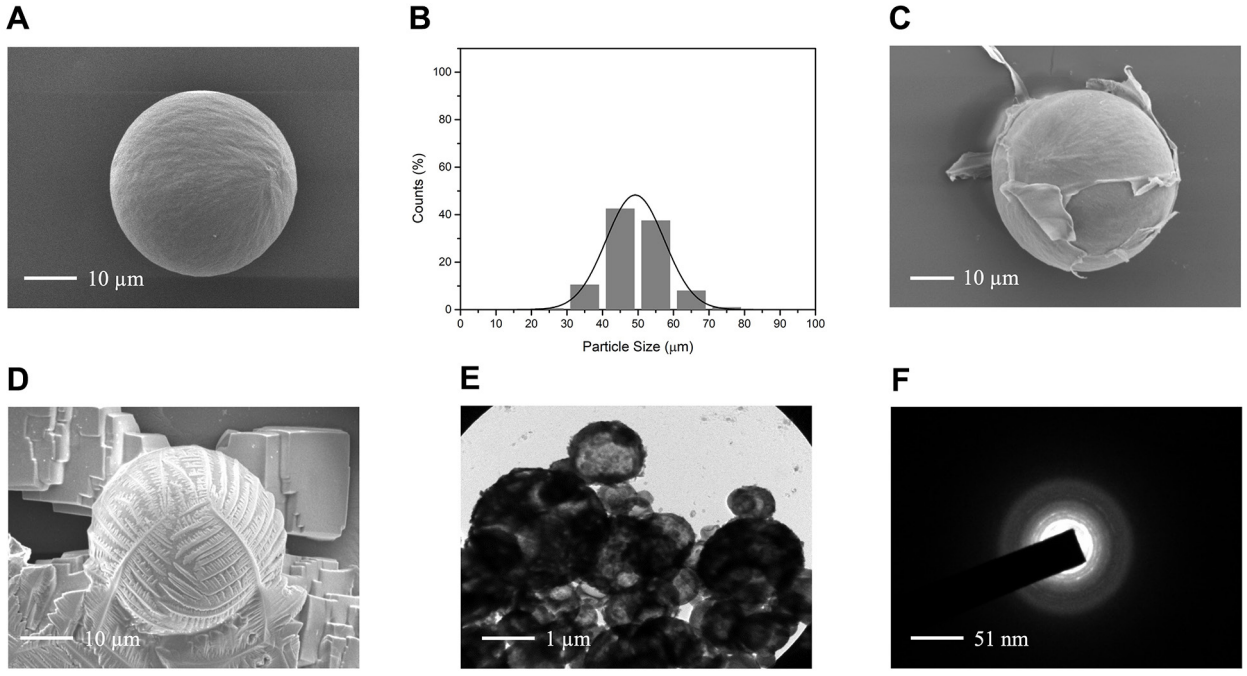

Figure 1. *Characterization of the filler particles. (A) SEM image showing the dry PLA microspheres with a uniform spherical morphology and smooth outer surface (scale bar: 10 µm). (B) Particle size–distribution analysis indicating a narrow diameter range, consistent with a relatively homogeneous particle population. (C) SEM image of PLA-IC particles before hydration, demonstrating intact spherical geometry (scale bar: 10 µm). (D) SEM image of PLA-IC particles after suspension in saline, illustrating preserved morphological integrity under hydrated conditions (scale bar: 1 µm). (E) TEM image showing the internal structural features of InCube® (scale bar: 1 µm). (F) SAED pattern exhibiting concentric diffraction rings, reflecting an ordered internal arrangement within the InCube® microstructure (scale bar: 51 nm). SEM, Scanning electron microscopy; TEM, transmission electron microscopy; SEAD, selected area electron diffraction; PLA, poly-Lactic acid; PLA-IC, PLA filler with microlattice-encapsulated vitamin C and glutathione.*

hydration revealed similarly smooth, spherical particles (Figure 1C), while images of hydrated particles in saline provided additional detail of surface structure, further confirming the uniformity and distinct morphology of the particles (Figure 1D). The microlattice capsules designed to carry vitamin C and glutathione exhibited well-defined morphology on TEM (Figure 1E). SAED patterns revealed distinct diffraction rings, indicating an ordered internal arrangement consistent with a microlattice structure (Figure 1F). This organization supports stable encapsulation of active molecules within the filler matrix.

Biopropertical analysis of PLA-IC. HPLC analysis demonstrated that glutathione content in the microlattice encapsulation remained essentially preserved after 277 days of storage at 20±5°C and 60±5% RH, indicating good chemical stability over a prolonged period (Figure 2A). DPPH assays showed strong radical scavenging activity of encapsulated vitamin C and glutathione (Figure 2B). For vitamin C and glutathione in the microlattice encapsulation, DPPH scavenging capacity remained high throughout the evaluated storage period. Within the PLA-IC formulation, antioxidant activity was maintained for up to 200 days, with minimal decline at 70, 120, and 200 days. These findings suggest that microlattice encapsulation effectively preserves antioxidant function during long-term storage in a filler-compatible format. As this was a single-run qualitative assessment, no statistical analysis or error bars were applicable.

Volumizing effect and collagen deposition. Primos volumetric analysis (Figure 3A, B) showed similar initial

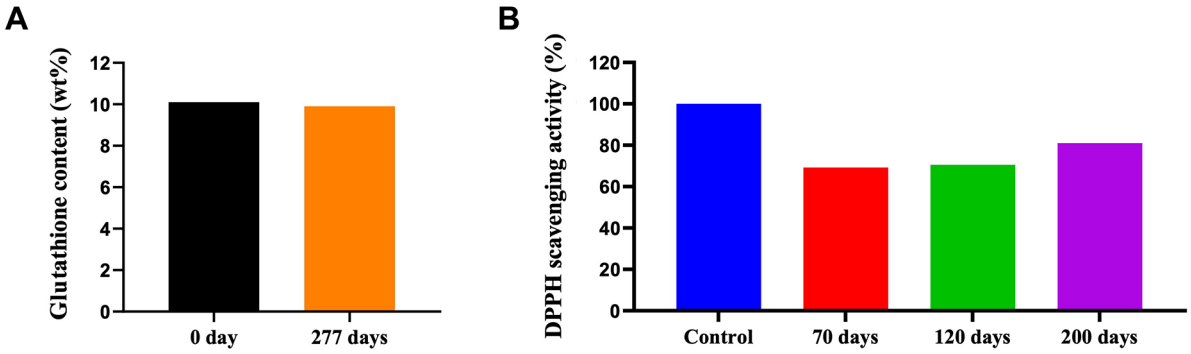

Figure 2. *The stability of glutathione content and DPPH scavenging activity of the filler over time. (A) Glutathione content remained stable after 277 days of storage under ambient environmental conditions, with no difference compared with baseline (0 day) (n=1). (B) DPPH radical scavenging activity of the PLA-IC formulation decreased at 70 and 120 days, but substantial activity was still present at 200 days (n=1). DPPH, 2,2-diphenyl-1-picrylhydrazyl; PLA-IC, poly-lactic acid filler with microlattice-encapsulated vitamin C and glutathione.*

volume elevation in all groups at day 0 ($p>0.05$). At 2 weeks, a reduction in volume was observed in each group. Subsequently, a gradual increase in skin surface elevation was noted over the 12-week period with the PLA-IC group showing the most pronounced increase in volume. Between weeks 4 and 12, the PLA-IC group exhibited significantly greater volume gain compared with the conventional PLA group ($p<0.01$). The mean volume increment from week 4 to week 12 was approximately +6.77 mm^3 in the PLA-IC group versus +4.76 mm^3 in the PLA group, indicating superior volume enhancement with the antioxidant-enhanced formulation.

Masson's trichrome staining revealed progressive collagen deposition in all groups (Figure 3C, D). The PBS group showed an early increase in collagen at 4 weeks, followed by a decline thereafter. The PLA group demonstrated a gradual, sustained increase in collagen density. The PLA-IC group exhibited the highest collagen intensity at every evaluated time point, with nearly two-fold higher collagen deposition compared with PLA at 12 weeks. These findings indicate that PLA-IC enhances collagen formation beyond that achieved by standard PLA filler.

Histological analysis. H&E staining allowed assessment of neovascularization and inflammatory cell infiltration. At baseline, neo-vessel formation presence was minimal in all groups. Over time, the PBS group showed little change in neovascularisation. The PLA group exhibited a marked increase in neo-vessel area at 2 and 4 weeks, exceeding 1.5% of the tissue area, followed by a gradual reduction at later time points. In contrast, the PLA-IC group showed consistently lower neovascularization at all time points compared with PLA, suggesting a more controlled vascular response at the injection site (Figure 4A, B).

Inflammatory cell counts were negligible at baseline in all groups. In the PBS group, inflammatory cells remained elevated at all time points. The PLA group showed a peak in inflammatory infiltration at 2 weeks, followed by gradual resolution. The PLA-IC group had fewer inflammatory cells at each time point compared with both PBS and PLA, indicating a milder and better-regulated tissue reaction to the filler (Figure 4C, D). These findings suggest that the PLA-IC filler promotes a more controlled tissue response, with minimal neovascularization and inflammation, compared to both the PBS and PLA groups.

Discussion

In this study, we developed and characterized a PLA-IC microsphere system incorporating vitamin C and glutathione using the microlattice encapsulation (InCube®), and we evaluated its physicochemical stability, regenerative capacity, and tissue compatibility *in vivo*. The findings demonstrate several advantages of PLA-IC over

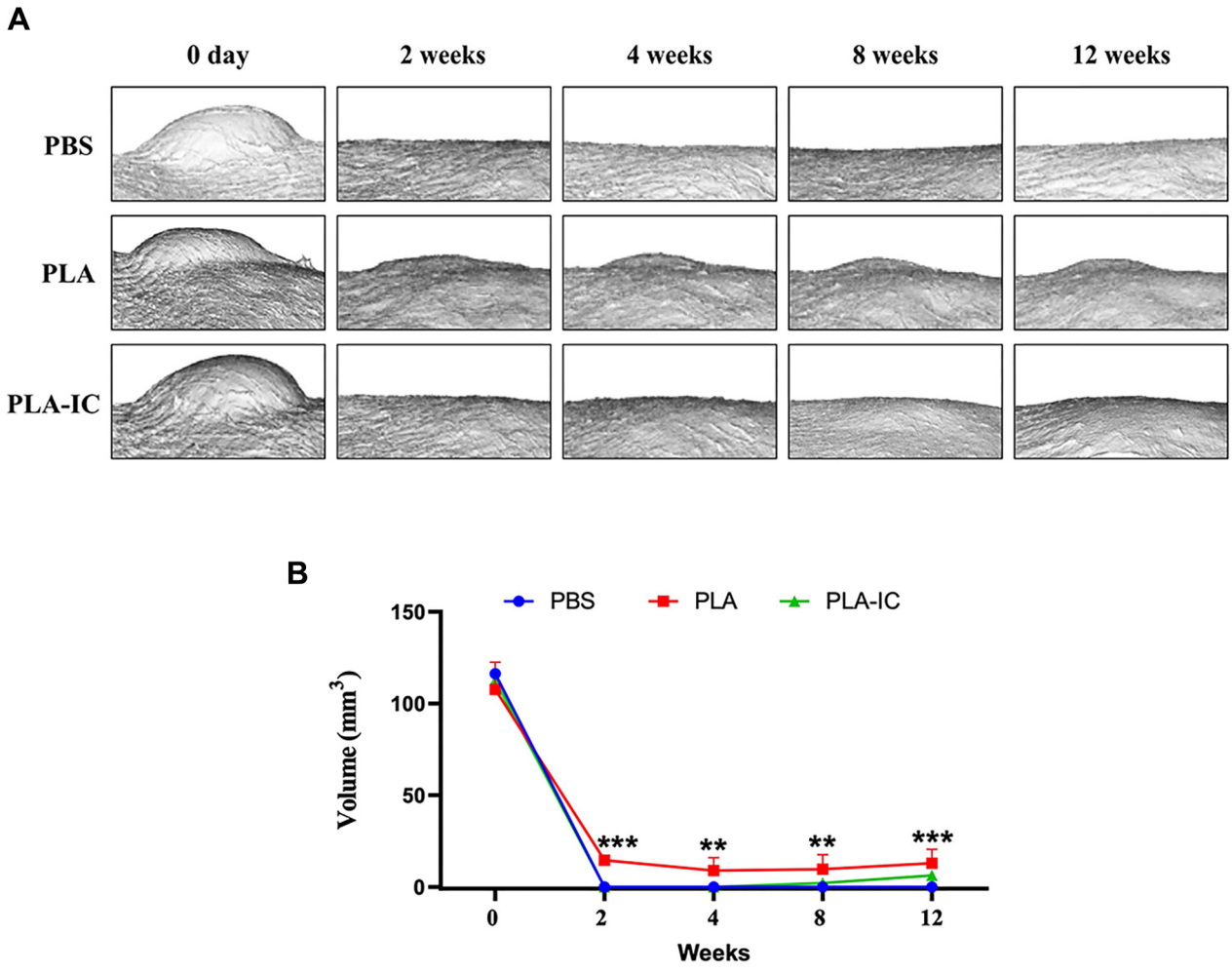

Figure 3. *Continued*

conventional PLA fillers and contribute to the growing evidence that biochemical augmentation of polymeric fillers can enhance their performance in soft-tissue remodeling: 1) microlattice encapsulation provided long-term chemical and functional stability of glutathione and vitamin C; 2) PLA-IC induced greater collagen deposition and superior collagen-associated volumizing effect compared with a conventional PLA filler; and 3) PLA-IC was associated with reduced neovascularization and a lower inflammatory cell burden in a murine model.

The long-term stability of encapsulated antioxidants is particularly relevant for clinical use (14, 15). PLA fillers are often stored and used over extended periods, and any incorporated bioactive molecules must remain effective during this time. However, vitamin C and glutathione are known to degrade rapidly in aqueous and even in polymeric matrices, with previous reports indicating substantial loss of activity within days to weeks due to oxidation and hydrolysis (16, 17). Studies employing liposomes, PLGA nanoparticles, or HA hydrogels have achieved modest improvements, but rarely beyond 30 - 60 days of stability (18-21). In contrast, the maintenance of glutathione content for 277 days and preserved DPPH activity for 200 days in this study indicates that the microlattice structure

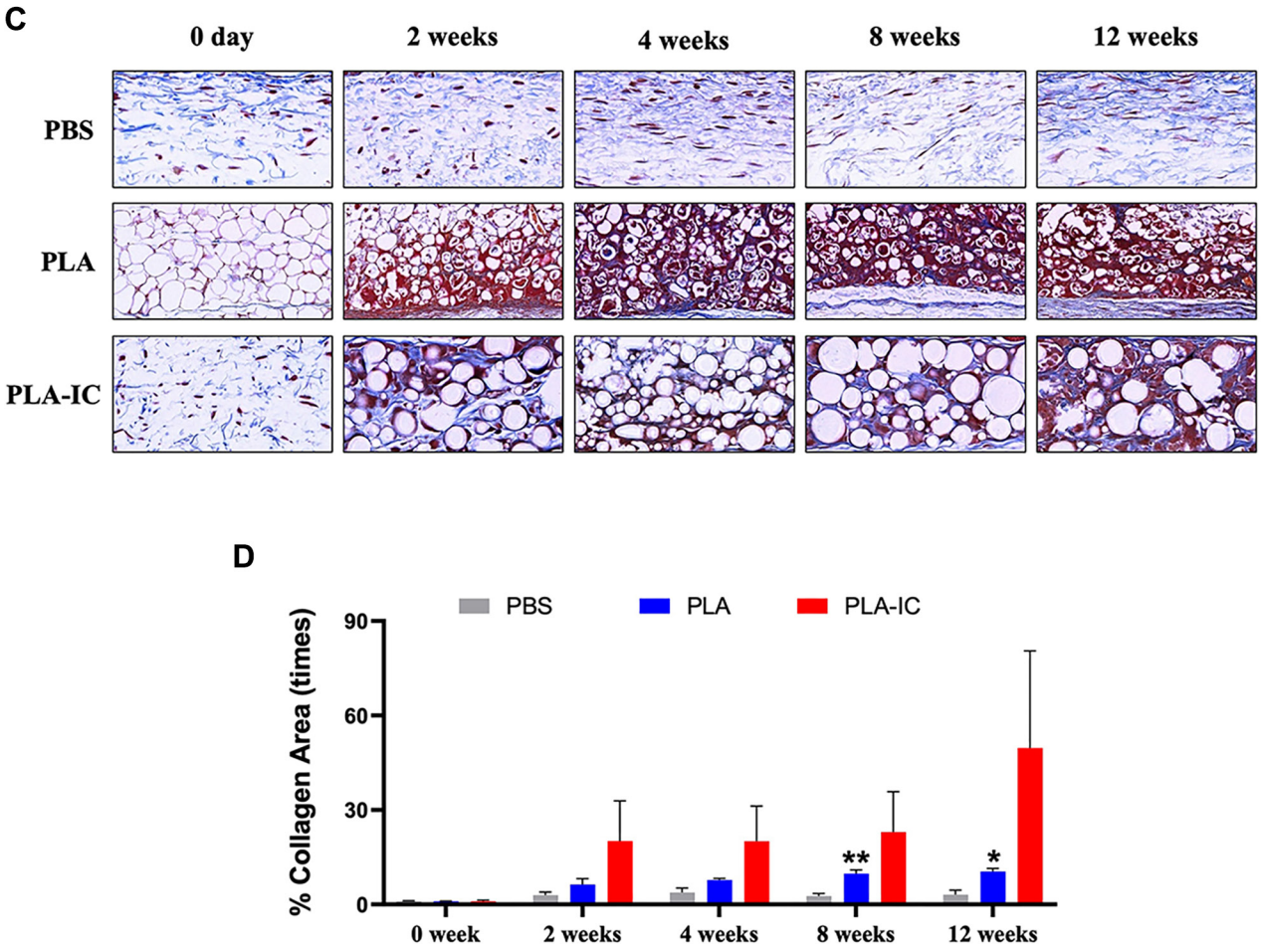

Figure 3. *The volumetric and histological analyses of filler injections over a 12-week period. (A) Representative Primos three-dimensional skin-surface images at 0 day and at 2, 4, 8, and 12 weeks after subcutaneous injection of PBS, PLA, or PLA-IC. (B) Time course of surface volume changes [mean±standard error (SE)]. All groups showed similar initial volumes at day 0 and a pronounced decrease at 2 weeks. From 4 to 12 weeks, the PLA-IC group exhibited a secondary increase in surface volume after the initial decline, which is characteristic of this formulation. (C) Representative Masson's trichrome–stained sections at each time point, illustrating the progressive accumulation of collagen (blue) around the injection sites in the three groups. (D) Quantitative analysis of collagen-positive area, expressed xas fold-change relative to baseline (0 week). Collagen area increased over time in all groups, with the largest values observed in the PLA-IC group at later time points. *p<0.05, **p<0.01, and ***p<0.001 compared with control group using two-way ANOVA. MT staining images were taken at 400´ magnification. PBS, Phosphate-buffered saline; PLA, poly-lactic acid; PLA-IC, PLA filler with microlattice-encapsulated vitamin C and glutathione.*

effectively shields these molecules from environmental degradation. These results exceed the stability ranges reported for conventional antioxidant-delivery systems and suggest that the microlattice architecture effectively isolates sensitive molecules from environmental degradants. For aesthetic practice, this suggests that a PLA filler prepared with microlattice-encapsulated antioxidants can be manufactured and stored under routine conditions without rapid loss of function.

In vivo, PLA-IC induced a markedly more favorable tissue response than traditional PLA fillers. Previous investigations have shown that PLA microspheres generate a transient inflammatory reaction involving macrophages and multinucleated giant cells, which contributes to collagen

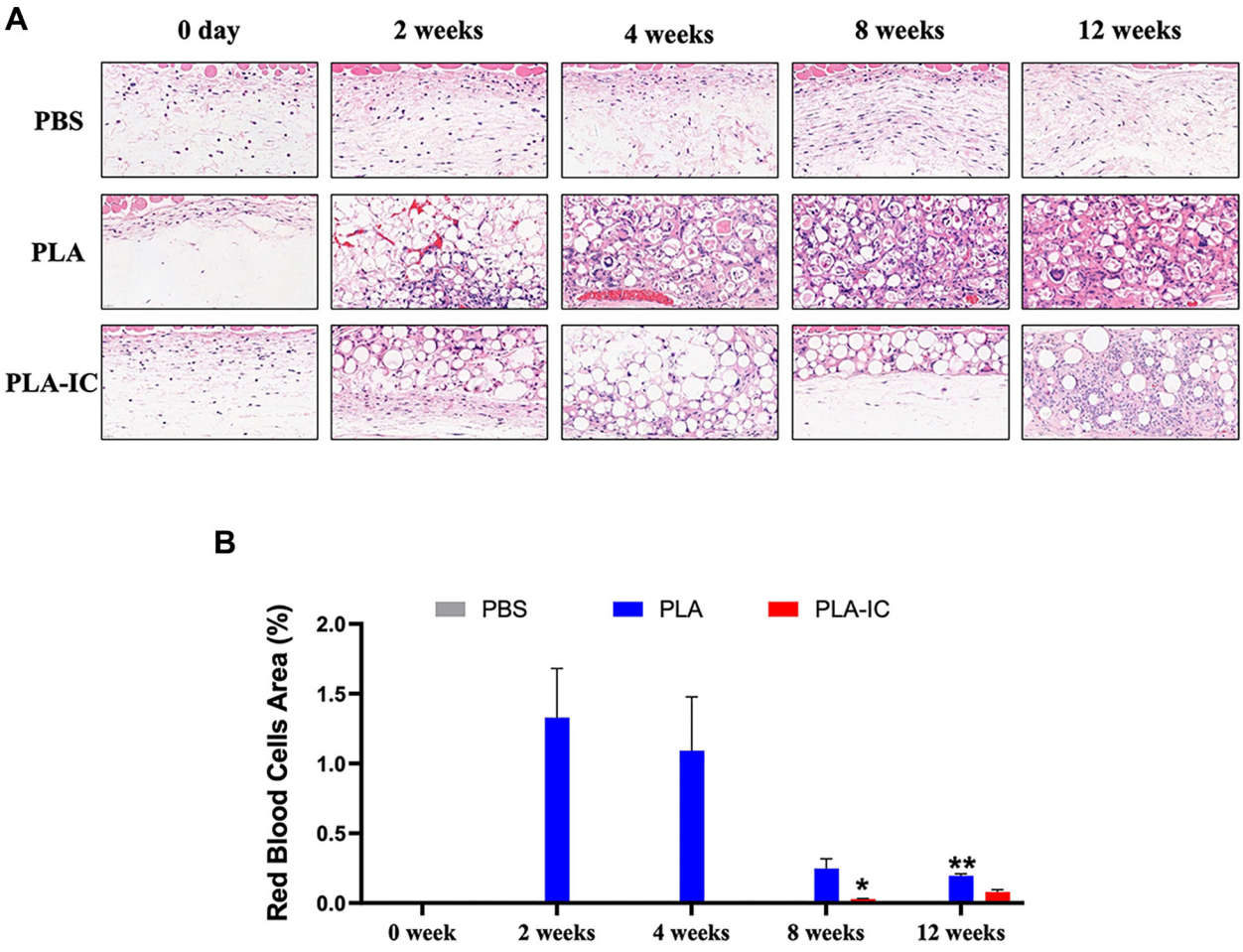

Figure 4. *Continued*

synthesis but may also lead to swelling, nodularity, or inconsistent clinical outcomes (22-24). In contrast, the PLA-IC group exhibited lower inflammatory cell infiltration and reduced neovascularization at all time points, consistent with a less active local inflammatory response (25, 26). TEM and TEM-SAED analyses further confirmed that PLA-IC possesses an ordered microlattice architecture capable of efficiently loading, protecting, and releasing vitamin C and glutathione in a controlled manner, supporting its function as a stable delivery platform. Such controlled release likely attenuates local oxidative stress and dampens downstream inflammatory cell recruitment, thereby contributing to the overall milder tissue response observed *in vivo*.

The enhanced collagen deposition observed with PLA-IC is also notable. Whereas conventional PLA fillers rely predominantly on a foreign-body reaction to stimulate fibroblast activity (27), the inclusion of vitamin C and glutathione appears to synergize with PLA-mediated biostimulation (28). Vitamin C facilitates collagen hydroxylation and fibrillar organization (29, 30), while glutathione prevents oxidative impairment of fibroblast function (31, 32). This synergism likely contributed to the significantly greater collagen density and improved volume retention observed in PLA-IC compared with standard PLA. The magnitude of improvement, nearly two-fold higher collagen deposition at 12 weeks, is

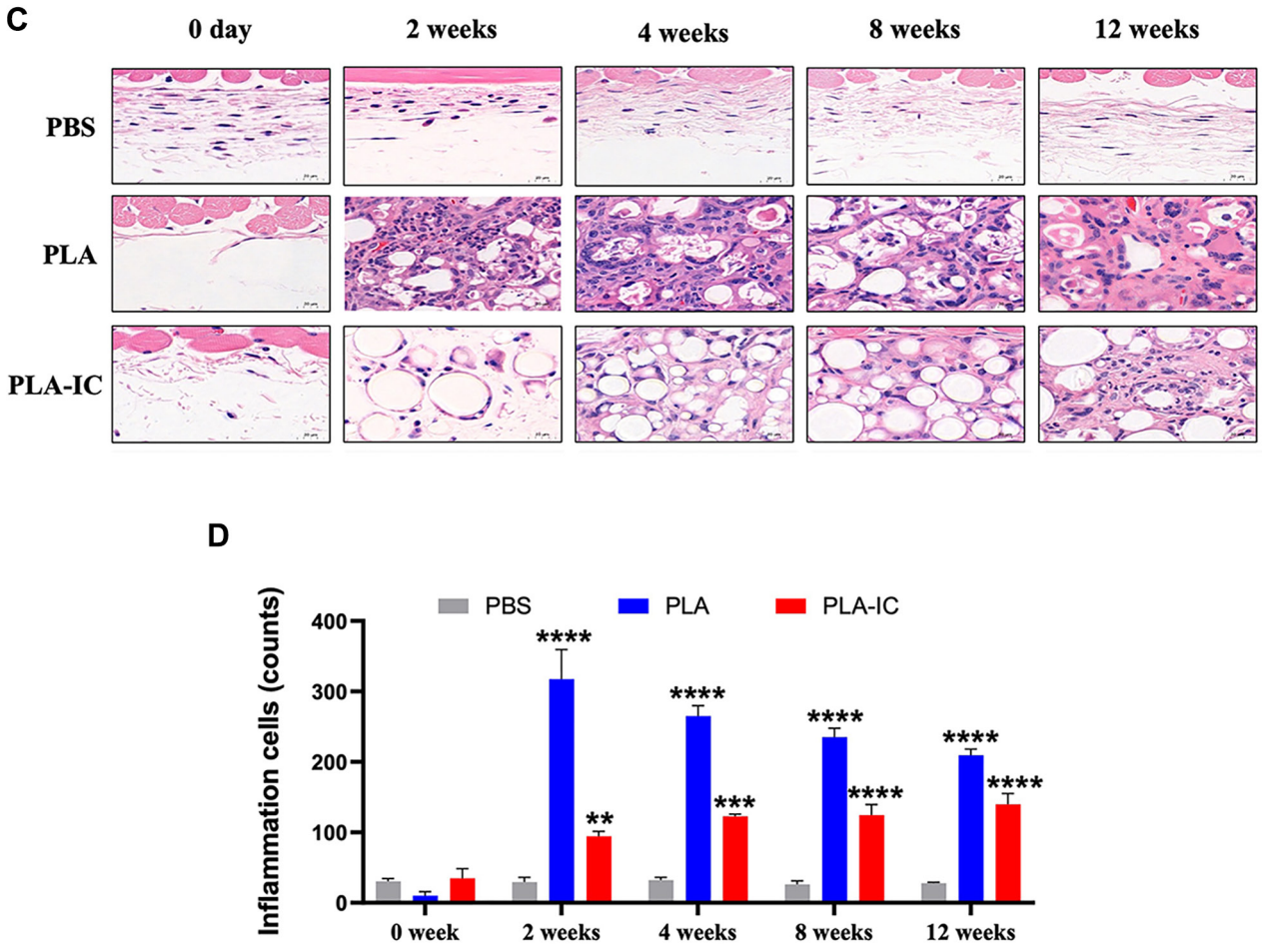

Figure 4. *Histological analysis of tissue samples stained with H&E. (A) Representative H&E-stained sections showing neovascularization at 0 day and at 2, 4, 8, and 12 weeks in each group. (B) Quantification of neo-vessel area (%). The PLA group showed pronounced neovascularization at 2 and 4 weeks, followed by a decline at later time points. The PLA-IC group exhibited lower neo-vessel area throughout the observation period. (C) Representative H&E-stained sections illustrating inflammatory cell distribution at each time point. (D) Quantification of inflammatory cell counts. Inflammatory cells increased at 2 and 4 weeks in the PLA group and subsequently decreased. The PLA-IC group showed consistently lower inflammatory cell counts over time compared with PLA. Data are presented as mean±standard error (SE). *p<0.05, *p<0.01, and ***p<0.0001 compared with the control group using two-way ANOVA. Images were taken at 400´ magnification for (A) and 800´ for (C). PBS, Phosphate-buffered saline; PLA, polylactic acid; PLA-IC, PLA filler with microlattice-encapsulated vitamin C and glutathione.*

greater than what has been reported for hybrid fillers incorporating HA (33), calcium hydroxyapatite (34), or bioactive ceramics (35). In addition, the newly formed collagen fibers were well-organized and exhibited a morphological pattern closely resembling native dermal collagen at 12 week through H&E staining, suggesting a regenerative rather than purely volumizing response. Clinically, this indicates potential for long-term tissue integration, improved dermal quality, and more durable aesthetic outcomes resulting from biologically driven remodeling rather than simple space-filling. These findings indicate that biochemical reinforcement can enhance both the quality and the consistency of long-term tissue remodeling.

Observations from other biomaterial studies provide additional context for our findings and support the

notion that modest alterations in material architecture can lead to discernible differences in tissue responses. Pantermehl *et al.* demonstrated that hyaluronic acid–based fillers with comparable chemical composition but differing structural or rheological features exhibit distinct cytocompatibility profiles, indicating that relatively small formulation changes may influence cellular tolerance and early material–tissue interactions (36). Likewise, Jung *et al.* reported that collagen scaffolds with different microstructural configurations provoke variable inflammatory and remodeling patterns, underscoring the relevance of matrix organization in guiding host responses (37). These findings are consistent with our observation that incorporating a microlattice-encapsulated antioxidant system into PLA, without altering the base polymer, was sufficient to modify the local inflammatory milieu and support a more ordered pattern of collagen deposition. Differences in tissue behavior across implantation sites reported by Burckhardt *et al.* further emphasize that biocompatibility outcomes depend not only on the material itself but also on the biological environment, as the same scaffold elicited distinct inflammatory and vascular responses in subcutaneous versus calvarial settings (38). Additionally, the angiogenic analyses by Salvante *et al.* showed that collagen-rich scaffolds tend to promote controlled neovascularization, whereas synthetic polymers are more likely to induce inflammation-associated vascular responses unless appropriately engineered (39). Taken together, these studies align with our results and suggest that targeted microarchitectural modification of PLA, such as through antioxidant-loaded microlattice structures, may enhance tissue compatibility and contribute to more favorable soft-tissue remodeling outcomes.

Study limitations. First, all *in vivo* data were obtained from a small animal model with a limited follow-up period of 12 weeks. Although this timeframe is sufficient to detect differences in early collagen formation and tissue response, PLA fillers in clinical practice are expected to provide effects for more than one year (40). Longer-term and large-animal studies are needed to determine how antioxidant stability influences late-phase degradation, collagen maturation, and potential delayed adverse events. Second, only one formulation and injection volume were evaluated. Dose-response relationships, different injection planes, and combination treatments with other fillers or energy-based devices were not explored. Third, due to the small sample size (n=3 per group at each time point), tests of normality had low power, and results should be interpreted cautiously. This is an inherent limitation of the study's exploratory nature. We acknowledge that non-parametric analyses might be more appropriate for such small samples. However, the observed group differences were large and consistent, and they aligned with the trends identified by parametric tests.

Finally, clinical trials will be required to translate these findings into practice. Comparative studies between PLA-IC and existing PLA fillers should assess not only objective outcomes such as volume enhancement and skin elasticity but also patient-reported satisfaction and rates of adverse events, including nodules and prolonged edema. These data will be essential before broad adoption in medical practice.

Conclusion

A PLA filler incorporating microlattice-encapsulated vitamin C and glutathione demonstrated favorable physicochemical and biological properties in a murine model. The formulation maintained long-term antioxidant stability, enhanced collagen deposition, improved collagen-associated volumizing effect, and elicited a more controlled inflammatory and vascular response compared with a conventional PLA filler. These preclinical results suggest that PLA-IC may offer advantages as a biostimulatory filler for aesthetic soft-tissue augmentation. Further work in larger animals and clinical studies will be necessary to confirm safety and efficacy in patients.

Conflicts of Interest

AB, SMK and HSL are employees of LabInCube Co. Ltd (Cheongju, Republic of Korea) which is developing the product. All other Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization: KMC, PNC, CYH; Data Collection and Curation: JYJ, CJJ, ZYD, YXJ, AB, SMK; Funding Acquisition: CYH; Investigation: AB, SMK, HSL; Methodology: JYJ, AB, SMK, HSL, PNC; Project Administration: KMC, PNC, CYH; Software: YXJ; Supervision: KMC, PNC, CYH; Validation: PNC, CYH; Writing – Original Draft: JYJ, CJJ, ZYD,YXJ; Writing – Review and Editing: KMC, PNC, CYH.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

References

1 Haykal D, Haddad A, Cartier H, Avelar L: Poly-L-lactic acid in aesthetic dermatology: a decade beyond volume restoration toward regenerative biostimulation. Aesthet Surg J 45(10): 1065-1072, 2025. DOI: 10.1093/asj/sjaf121

2 Xu Q, Sun X, Yang W, Bai Y, Tan X: Comprehensive systematic review of Poly-L-lactic acid in facial clinical application. Aesthetic Plast Surg 48(23): 5115-5135, 2024. DOI: 10.1007/s00266-024-04098-8

3 Arruda S, Prieto V, Shea C, Swearingen A, Elmadany Z, Sadick NS: A clinical histology study evaluating the biostimulatory activity longevity of injectable Poly-L-Lactic acid for facial rejuvenation. J Drugs Dermatol 23(9): 729-734, 2024. DOI: 10.36849/jdd.8057

4 Nowag B, Schäfer D, Hengl T, Corduff N, Goldie K: Biostimulating fillers and induction of inflammatory pathways: A preclinical investigation of macrophage response to calcium hydroxylapatite and poly-L lactic acid. J Cosmet Dermatol 23(1): 99-106, 2024. DOI: 10.1111/jocd.15928

5 Signori R, Barbosa AP, Cezar-Dos-Santos F, Carbone AC, Ventura S, Nobre BBS, Neves MLBB, Câmara-Souza MB, Poluha RL, De la Torre Canales G: Efficacy and safety of Poly-l-Lactic acid in facial aesthetics: a systematic review. Polymers (Basel) 16(18): 2564, 2024. DOI: 10.3390/polym16182564

6 Singhvi MS, Zinjarde SS, Gokhale DV: Polylactic acid: synthesis and biomedical applications. J Appl Microbiol 127(6): 1612-1626, 2019. DOI: 10.1111/jam.14290

7 Fitzgerald R, Bass LM, Goldberg DJ, Graivier MH, Lorenc ZP: Physiochemical characteristics of Poly-L-Lactic acid (PLLA). Aesthet Surg J 38(suppl_1): S13-S17, 2018. DOI: 10.1093/asj/sjy012

8 Lemperle G, Gauthier-Hazan N, Wolters M, Eisemann-Klein M, Zimmermann U, Duffy DM: Foreign body granulomas after all injectable dermal fillers: part 1. Possible causes. Plast Reconstr Surg 123(6): 1842-1863, 2009. DOI: 10.1097/PRS.0b013e31818236d7

9 Stein P, Vitavska O, Kind P, Hoppe W, Wieczorek H, Schürer NY: The biological basis for poly-l-lactic acid-induced augmentation. J Dermatol Sci 78(1): 26-33, 2015. DOI: 10.1016/j.jdermsci.2015.01.012

10 Pullar JM, Carr AC, Vissers MCM: The roles of vitamin C in skin health. Nutrients 9(8): 866, 2017. DOI: 10.3390/nu9080866

11 Dilokthornsakul W, Dhippayom T, Dilokthornsakul P: The clinical effect of glutathione on skin color and other related skin conditions: A systematic review. J Cosmet Dermatol 18(3): 728-737, 2019. DOI: 10.1111/jocd.12910

12 Lykkesfeldt J, Carr AC, Tveden-Nyborg P: The pharmacology of vitamin C. Pharmacol Rev 77(2): 100043, 2025. DOI: 10.1016/j.pharmr.2025.100043

13 Vašková J, Kočan L, Vaško L, Perjési P: Glutathione-related enzymes and proteins: a review. Molecules 28(3): 1447, 2023. DOI: 10.3390/molecules28031447

14 Maurya VK, Shakya A, McClements DJ, Srinivasan R, Bashir K, Ramesh T, Lee J, Sathiyamoorthi E: Vitamin C fortification: need and recent trends in encapsulation technologies. Front Nutr 10: 1229243, 2023. DOI: 10.3389/fnut.2023.1229243

15 Gaucher C, Boudier A, Bonetti J, Clarot I, Leroy P, Parent M: Glutathione: antioxidant properties dedicated to nanotechnologies. Antioxidants (Basel) 7(5): 62, 2018. DOI: 10.3390/antiox7050062

16 Jahani R, Behnamian M, Dezhsetan S, Karimirad R, Chamani E: Chitosan nano-biopolymer/Citrus paradisi peel oil delivery system enhanced shelf-life and postharvest quality of cherry tomato. Int J Biol Macromol 225: 1212-1223, 2023. DOI: 10.1016/j.ijbiomac.2022.11.182

17 Wansapura PT, Díaz-Vásquez WA, Vásquez CC, Pérez-Donoso JM, Chasteen TG: Thermal and photo stability of glutathione-capped

cadmium telluride quantum dots. J Appl Biomater Funct Mater 13(3): 248-252, 2015. DOI: 10.5301/jabfm.5000221
18 Caritá AC, Fonseca-Santos B, Shultz JD, Michniak-Kohn B, Chorilli M, Leonardi GR: Vitamin C: One compound, several uses. Advances for delivery, efficiency and stability. Nanomedicine 24: 102117, 2020. DOI: 10.1016/j.nano.2019.102117
19 Koo SH, Lee JS, Kim GH, Lee HG: Preparation, characteristics, and stability of glutathione-loaded nanoparticles. J Agric Food Chem 59(20): 11264-11269, 2011. DOI: 10.1021/jf2024648
20 Dziadek K, Drozdowska M, Kruk T, Piasna-Słupecka E, Nowak-Nazarkiewicz N, Grzebieniarz W, Jamróz E: Enhancing glutathione delivery and efficacy: a novel microcapsule approach. J Mater Sci 60(15): 6657-6670, 2025. DOI: 10.1007/s10853-025-10845-2
21 Yu J, Du G, Yao Y, Liao Q, Zhou T, Cui H, Hussain S, Hayat K, Zhang X, Ho CT: Microencapsulation of glutathione through water/oil emulsification and complex coacervation: Improved encapsulation efficiency, physicochemical stability, and sustained release effect. Food Res Int 202: 115723, 2025. DOI: 10.1016/j.foodres.2025.115723
22 He T, Zhang Z, Zhang X, Niu H, Wang S, Wang Q, Lai C: Effects of Poly-L-Lactic acid fillers on inflammatory response and collagen synthesis in different animal models. J Cosmet Dermatol 24(2): e70000, 2025. DOI: 10.1111/jocd.70000
23 Narins RS: Minimizing adverse events associated with Poly-l-lactic acid injection. Dermatol Surg 34 Suppl 34(s1): S100-S104, 2008. DOI: 10.1111/j.1524-4725.2008.34250.x
24 Oh S, Lee JH, Kim HM, Batsukh S, Sung MJ, Lim TH, Lee MH, Son KH, Byun K: Poly-L-Lactic acid fillers improved dermal collagen synthesis by modulating M2 macrophage polarization in aged animal skin. Cells 12(9): 1320, 2023. DOI: 10.3390/cells12091320
25 Ianhez M, de Goés E Silva Freire G, Sigrist RMS, Colpas PT, de Faria IA, Parada MOAB, Miot HA: Complications of collagen biostimulators in Brazil: Description of products, treatments, and evolution of 55 cases. J Cosmet Dermatol 23(9): 2829-2835, 2024. DOI: 10.1111/jocd.16343
26 Kern JA, Kollipara R, Hoss E, Boen M, Wu DC, Groff W, Goldman MP: Serious adverse events with injectable fillers: retrospective analysis of 7,659 patient outcomes. Dermatol Surg 48(5): 551-555, 2022. DOI: 10.1097/dss.0000000000003409
27 Ray S, Ta HT: Investigating the effect of biomaterials such as Poly-(l-Lactic Acid) particles on collagen synthesis in vitro: method is matter. J Funct Biomater 11(3): 51, 2020. DOI: 10.3390/jfb11030051
28 Meister A: On the antioxidant effects of ascorbic acid and glutathione. Biochem Pharmacol 44(10): 1905-1915, 1992. DOI: 10.1016/0006-2952(92)90091-v
29 Ryu TK, Lee H, Yon DK, Nam DY, Lee SY, Shin BH, Choi GW, Jeon DS, Oh BB, Kim JH, Yoon Y, Kim HJ, Duteil L, Bruno-Bonnet C, Heo CY, Kang SM: The antiaging effects of a product containing collagen and ascorbic acid: In vitro, ex vivo, and pre-post intervention clinical trial. PLoS One 17(12): e0277188, 2022. DOI: 10.1371/journal.pone.0277188
30 Pinnell SR: Regulation of collagen biosynthesis by ascorbic acid: A review. Yale J Biol Med 58(6): 553-559, 1985.
31 Deveci M, Gilmont RR, Dunham WR, Mudge BP, Smith DJ, Marcelo CL: Glutathione enhances fibroblast collagen contraction and protects keratinocytes from apoptosis in hyperglycaemic culture. Br J Dermatol 152(2): 217-224, 2005. DOI: 10.1111/j.1365-2133.2004.06329.x
32 Jones SA, McArdle F, Jack CI, Jackson MJ: Effect of antioxidant supplementation on the adaptive response of human skin fibroblasts to UV-induced oxidative stress. Redox Rep 4(6): 291-299, 1999. DOI: 10.1179/135100099101535133
33 Fan Y, Choi TH, Chung JH, Jeon YK, Kim S: Hyaluronic acid-cross-linked filler stimulates collagen type 1 and elastic fiber synthesis in skin through the TGF-β/Smad signaling pathway in a nude mouse model. J Plast Reconstr Aesthet Surg 72(8): 1355-1362, 2019. DOI: 10.1016/j.bjps.2019.03.032
34 Nowag B, Casabona G, Kippenberger S, Zöller N, Hengl T: Calcium hydroxylapatite microspheres activate fibroblasts through direct contact to stimulate neocollagenesis. J Cosmet Dermatol 22(2): 426-432, 2023. DOI: 10.1111/jocd.15521
35 Huang J, Xue J, Huang J, Zhang X, Zhang H, Du L, Zhai D, Huan Z, Zhu Y, Wu C: An injectable hyaluronic acid/lithium calcium silicate soft tissue filler with vascularization and collagen regeneration. Bioact Mater 44: 256-268, 2024. DOI: 10.1016/j.bioactmat.2024.10.014
36 Pantermehl S, Foth A, Meyer E, Barbeck M, Jung O: In vitro cytocompatibility analysis and comparison of different hyaluronic acid fillers for minimally invasive esthetics. In Vivo 38(4): 1621-1635, 2024. DOI: 10.21873/invivo.13612
37 Jung O, Burckhardt K, Stammkoetter S, Barrientos MM, Reinholdt C, Andreeva T, Stošić MR, Stojanović S, Najman S, Krastev R, Walther F, Pantermehl S, Barbeck M: In vitro, ex vivo and in vivo characterization of cytocompatibility and biocompatibility of a cross-linked fish collagen wound membrane. In Vivo 39(5): 2517-2533, 2025. DOI: 10.21873/invivo.14051
38 Burckhardt K, Jung O, Stošić MR, Stojanović S, Najman S, Pantermehl S, Schnettler R, Barbeck M: Comparative evaluation of biocompatibility data from the subcutaneous and the calvaria implantation model of a novel bone substitute block. In Vivo 39(6): 3116-3127, 2025. DOI: 10.21873/invivo.14113
39 Salvante ERG, Popoiu AV, Barb AC, Cosma AA, Fenesan MP, Saxena AK, Popoiu TA, Boia ES, Stanciulescu MC, Caplar BD, Dorobantu FR, Cimpean AM: Artificial intelligence (AI) based analysis of in vivo polymers and collagen scaffolds inducing vascularization. In Vivo 38(2): 620-629, 2024. DOI: 10.21873/invivo.13481
40 Han WY, Kim HJ, Kwon R, Kang SM, Yon DK: Safety and efficacy of Poly-L-Lactic acid filler (Gana V vs. Sculptra) injection for correction of the nasolabial fold: a double-blind, non-inferiority, randomized, split-face controlled trial. Aesthetic Plast Surg 47(5): 1796-1805, 2023. DOI: 10.1007/s00266-023-03600-y