



Article

Poly-D,L-Lactic Acid Filler Restores Hair Thickness and Shine by Ameliorating Age-Associated Follicular Decline

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Abstract

Hair aging, a complex physiological process involving progressive hair thinning and loss of luster, is primarily driven by functional decline of hair follicle components and sebaceous glands due to cumulative oxidative stress. This decline manifests as dermal papilla cell (DPC) senescence, with reduced insulin-like growth factor-1 (IGF-1) secretion, impaired hair matrix keratinocyte proliferation, and decreased keratin synthesis. We investigated the restorative potential of poly-D,L-lactic acid (PDLLA) filler, a biostimulatory polymer with antioxidant properties, against these age-related changes. PDLLA filler treatment significantly reduced oxidative stress—as indicated by decreased 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels—in hydrogen peroxide-induced senescent human DPCs, alleviated cell-cycle arrest, and significantly upregulated IGF-1 secretion. Conditioned medium from PDLLA filler-treated DPCs stimulated proliferation and pan-keratin expression in senescent hair follicular keratinocytes (HFKs). Intradermal PDLLA filler injection in aged mice significantly reduced 8-OHdG levels, restored DPC proliferative capacity (indicated by proliferating cell nuclear antigen [PCNA] positivity), increased IGF-1 expression within the dermal papilla, and enhanced HFK proliferation in the hair matrix. Consequently, PDLLA filler treatment robustly upregulated hair cortex keratins (K35, K85) and inner root sheath markers (AE15, K25, K71), leading to improved cuticle integrity and the attenuation of follicular miniaturization. Senescence within sebaceous glands was also mitigated, as evidenced by increased PCNA and peroxisome proliferator-activated receptor gamma (PPAR- γ) expression, accompanied by enhanced hair shaft reflectivity and shine. Overall, PDLLA filler ameliorated senescence-associated phenotypes and restored senescence-associated functional decline, supporting its potential as an intervention for age-related hair thinning and quality deterioration.

Keywords: hair aging; poly-D,L-lactic acid; hair shaft keratins; hair shine; hair follicle senescence



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1. Introduction

Hair aging is a ubiquitous and complex physiological process, primarily characterized by two major cosmetic and structural changes: progressive hair thinning and a noticeable loss of natural luster [1,2]. These visible features fundamentally depend on the synthesis and maintenance of the hair shaft, processes largely governed by two key components: keratin, which determines hair shaft thickness, and sebum, which provides lubrication and protection [3]. Effective anti-aging strategies require an understanding of age-related decline in molecular and cellular units responsible for producing these components.

Keratin, the primary structural element of the hair shaft, is produced by hair matrix keratinocytes (HMKs) [4]. HMKs reside in the hair matrix region at the base of the hair follicle, adjacent to the dermal papilla [4,5]. HMK proliferation and differentiation are dynamically regulated by dermal papilla cells (DPCs), specialized mesenchymal fibroblasts located within the dermal papilla at the follicle base [5]. DPCs function as a signaling hub, transmitting essential growth factors, including insulin-like growth factor-1 (IGF-1) and various fibroblast growth factors, to the overlying HMKs [6,7]. Upon activation, HMKs proliferate, migrate upward, and undergo terminal differentiation to synthesize structural keratins of the hair shaft (i.e., keratinization). Furthermore, a subset of HMKs differentiates into inner root sheath (IRS) cells, which synthesize the protective hair cuticle [1,2].

With advancing age, DPCs undergo functional senescence, characterized by increased oxidative stress and eventual cell-cycle arrest [8]. This senescent state impairs signaling capacity and leads to a substantial decrease in IGF-1 secretion [9]. The resulting reduction in trophic signaling diminishes HMK proliferation and reduces overall keratin synthesis. Concurrent impairment of HMK differentiation decreases the IRS cell population, leading to a less robust hair cuticle [10]. The combined effects of reduced keratin production and compromised cuticle integrity result in a measurable decrease in hair shaft diameter and onset of observable hair thinning [11]. Moreover, DPC aging represents a key pathological contributor to follicular miniaturization, further exacerbating reductions in hair thickness and density [12].

In parallel with follicular changes, the aging scalp exhibits sebaceous gland atrophy [13]. This involution directly leads to a lower rate of sebum secretion onto the scalp surface [14]. Because sebum provides a natural protective coating for the hair shaft, its quantitative reduction compromises external defense mechanisms, resulting in diminished shine and increased susceptibility to environmental damage [15]. Collectively, the age-related decreases in keratin and cuticle synthesis, together with decreased sebum production, contribute to a pronounced decline in overall hair quality, leading to unfavorable esthetic outcomes and associated psychosocial concerns.

Given the established association between age-related oxidative stress and follicular senescence, the present study investigated the potential of poly-D,L-lactic acid (PDLLA) filler as a strategy to mitigate these effects and to improve age-related hair structural decline. Previous studies have demonstrated that PDLLA filler, a well-characterized biostimulatory polymer, exhibits potent antioxidant properties by suppressing reactive oxygen species (ROS) accumulation and modulating related signaling pathways in dermal fibroblasts and other senescent cell types [16].

Redox homeostasis plays a pivotal role in the aging process, as an imbalance between ROS production and antioxidant activity disturbs redox-dependent signaling and leads to the accumulation of toxic oxidative by-products [17]. These oxidative insults activate key molecular pathways, primarily the p53/p21 and p16/Rb axes, which establish permanent cell cycle arrest [18,19]. Beyond these cell cycle regulators, several redox-sensitive transcription factors mediate survival and stress resistance, including the Forkhead box protein O (FOXO) family [20,21], nuclear factor erythroid 2-related factor 2 (NRF2) [22,23], hypoxia-inducible factor- α (HIF-1 α) [24,25], and nuclear factor- κ B (NF- κ B) [26,27]. Given that all aging hallmarks possess

a redox-regulated component, therapeutic strategies are increasingly focused on modulating these specific signaling pathways to alleviate the burden of senescent cells [28].

Current senotherapeutic approaches are broadly categorized into senolytics and senomorphics, which differ fundamentally in their mode of action. Senolytics are small molecules designed to selectively eliminate senescent cells by inducing apoptosis [29]. By clearing even a fraction of the senescent cell burden, senolytics can delay, prevent, or reverse age-related pathologies and extend health span [30]. In contrast, senomorphics aim to suppress senescent characteristics, specifically the senescence-associated secretory phenotype (SASP), without killing the senescent cells [31,32]. These agents work by blocking intracellular pathways related to SASP expression, such as p38 MAPK, PI3K/Akt, and mTOR, or by neutralizing specific SASP factors like interleukin (IL)-6 and IL-8 using antibodies [33–35]. While senolytics physically remove senescent cells, senomorphics focus on restoring the redox-regulated environment and attenuating inflammation, often by activating NRF2 or inhibiting NF- κ B to decrease the cellular ROS burden and subsequent SASP production [36].

By neutralizing chronic oxidative stress, a primary driver of DPC senescence and HMK functional decline [8,9], PDLA filler may interrupt age-related follicular deterioration. We therefore hypothesized that PDLA filler injection mitigates DPC and HMK senescence through the considerable reduction in intracellular oxidative stress, which could act as senomorphics. This mechanism is expected to restore cellular proliferative capacity and function, thereby enhancing keratin synthesis and preserving normal cuticle structure. Consequently, we speculated that PDLA filler treatment can produce measurable recovery of hair shaft diameter and restoration of natural hair luster. To test our hypothesis, we explored anti-senescence mechanisms using in vitro models, including hydrogen peroxide (H_2O_2)-induced senescent DPCs and hair follicular keratinocytes (HFKs), then evaluated the senomorphic effects of PDLA filler in an in vivo aging animal model.

2. Results

2.1. Establishment of H_2O_2 -Induced Senescence Models and Determination of Optimal PDLA Filler Concentration for In Vitro Studies

In hDPCs, treatment with 150 μ M H_2O_2 resulted in significant increases in senescence markers (*p16* and *p21*), senescence-associated β -galactosidase (SA- β -gal) activity, and the oxidative stress marker 8-OHdG, while maintaining cell viability above 80% (Figure S1). This concentration was selected for subsequent experiments because it induced robust senescence marker expression while preserving relatively high cell viability, thus providing a reliable in vitro senescence model.

Subsequently, we sought the PDLA filler concentration that most effectively reduced oxidative stress without inducing cytotoxicity (Figure S2A). PDLA filler treatment induced a dose-dependent increase in the death of senescent hDPCs, with significant differences among concentration groups and a marked increase at 15 mg/mL and above (Figure S2B). Efficacy assessment across multiple concentrations demonstrated that 300 μ g/mL PDLA filler most effectively reduced the intracellular 8-OHdG levels (Figure S2C,D). Accordingly, 300 μ g/mL was selected as the optimal concentration for all subsequent in vitro experiments.

2.2. PDLA Filler Decreased Oxidative Stress in Senescent hDPCs

PDLA filler decreased the expression of *p16* and *p21* and SA- β -gal activity in the senescent hDPCs (Figure S3A–C). The expression of NRF2 and antioxidant enzymes, such as heme oxygenase-1 (*HO-1*), NAD(P)H:quinone oxidoreductase 1 (*NQO1*), superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), was evaluated to determine the antioxidant capacity of PDLA filler (Figure S3D–J).

2.3. PDLLA Filler Improved Proliferation and IGF-1 Secretion in Senescent hDPCs

In vitro analyses demonstrated that PDLLA filler treatment reactivated cell-cycle progression in H_2O_2 -induced senescent hDPCs (Figure 1A). Cell-cycle progression was promoted, as indicated by a reduced G0/G1-phase population and expanded S- and G2/M-phase populations compared with the untreated senescent controls (Figure 1B–D), resulting in the increased cell proliferation (Figure 1E). PDLLA filler treatment also increased the secretory function of senescent hDPCs, with significant upregulation of IGF-1 secretion (Figure 1F).

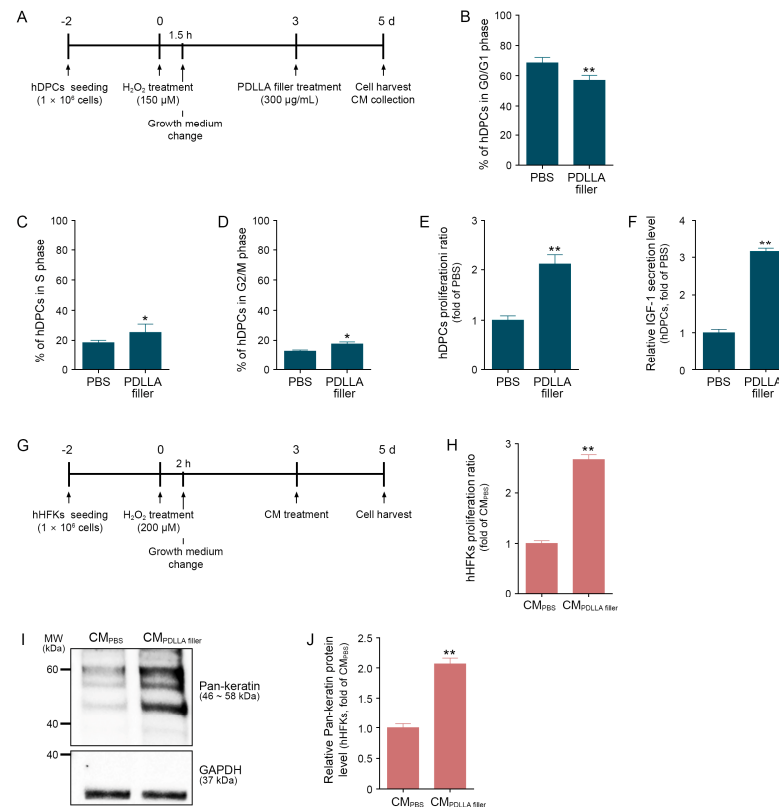


Figure 1. PDLLA filler restores cell-cycle activity and paracrine function in senescent hDPCs, thereby promoting keratin synthesis in senescent hHFKs. (A) Schematic overview of the hDPC experimental design. hDPCs (1×10^6 cells) were treated with H_2O_2 (150 μ M) for 1.5 h, cultured in fresh growth medium for 3 days, and subsequently treated with PDLLA filler (300 μ g/mL). Cells and CM were harvested 2 days after PDLLA filler treatment. (B–D) Cell-cycle distribution of senescent hDPCs analyzed by flow cytometry using PI staining. Percentages of cells in the G0/G1 (B), S (C), and G2/M (D) phases are shown. (E) Proliferation of senescent hDPCs assessed by proliferation assay after PDLLA filler treatment and expressed as fold change relative to PBS-treated senescent controls. (F) IGF-1 secretion level in CM from senescent hDPCs was quantified by ELISA and expressed as fold change relative to PBS-treated controls. (G) Schematic overview of the hHFK experimental design. Senescent hHFKs were cultured with CM derived from PBS-treated or PDLLA filler-treated senescent hDPCs. (H) Proliferation of hHFKs assessed after exposure to CM from PBS-treated (CM_{PBS}) or PDLLA filler-treated hDPCs (CM_{PDLLA filler}). (I) Western blot analysis of pan-keratin expression in senescent hHFKs. Molecular weight markers are indicated; pan-keratin bands were expected at 46–58 kDa. GAPDH served as the loading control. (J) Quantitative densitometric analysis of pan-keratin protein levels shown in (I), normalized to GAPDH and expressed as fold change relative to CM_{PBS}. Data are presented as mean $\pm$ standard deviation. Differences among groups were analyzed using the Kruskal–Wallis test, with Mann–Whitney U tests used for post hoc pairwise comparisons. *, $p < 0.05$ and **, $p < 0.01$ vs. PBS or CM_{PBS}. CM, conditioned medium; d, days; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; h, hours; hDPCs, human dermal papilla cells; hHFKs, human hair follicular keratinocytes; H_2O_2 , hydrogen peroxide; IGF-1, Insulin-like growth factor-1; PBS, phosphate-buffered saline; PDLLA, poly-D,L-lactic acid; PI, propidium iodide.

2.4. PDLLA Filler-Treated hDPCs Promote HFK Proliferation and Keratin Synthesis

Given that DPCs regulate hair growth via paracrine signaling, we examined the effects of PDLLA filler-treated DPCs on senescent HFKs. Because HMKs (the primary target cells for hair growth) are not commercially available, we utilized hHFKs as a representative epithelial model to evaluate paracrine signaling effects mediated by PDLLA filler-treated hDPCs.

Treatment of hHFKs with 200 μM H_2O_2 resulted in significant increases in senescence markers (*p16* and *p21*), SA- β -gal activity, and 8-OHdG levels while maintaining cell viability above 80% (Figure S4). Accordingly, 200 μM H_2O_2 was selected to establish a robust and viable senescence model.

Senescent hHFKs cultured with conditioned medium from PDLLA filler-treated senescent hDPCs ($\text{CM}_{\text{PDLLA filler}}$) exhibited robust recovery of proliferative capacity compared with the controls (Figure 1G,H). Furthermore, Western blot analysis demonstrated that $\text{CM}_{\text{PDLLA filler}}$ significantly upregulated pan-keratin expression (Figure 1I,J), which serves as a comprehensive indicator of the increase in keratin structural proteins required for hair formation [37]. These findings confirm that PDLLA filler increased the function of DPCs, thereby stimulating HFKs to synthesize structural proteins essential for hair shaft formation.

2.5. PDLLA Filler Ameliorates Senescence and Restores DPC Function in the Animal

To validate the *in vitro* findings, we established a senescence model in female C57BL/6 mice, then performed intradermal PDLLA filler injection. Consistent with the antioxidant effects observed *in vitro*, PDLLA filler treatment significantly reduced the levels of the oxidative stress marker 8-OHdG within the hair bulb region of aged mice (Figure S5).

PDLLA filler treatment increased the proliferative capacity of DPCs. Because specific differentiation markers for DPCs are limited, we utilized proliferating cell nuclear antigen (PCNA) as a proliferation marker. The dermal papilla region, where DPCs are most densely localized, was delineated; PCNA-positive cells within this area were quantified. A significant increase in PCNA-positive cells within the dermal papilla region was observed in the PDLLA filler-treated group compared with the controls, indicating enhanced DPC proliferation (Figure 2A–C).

This functional recovery was accompanied by enhanced growth factor expression. IGF-1 expression was significantly upregulated within the dermal papilla region, as determined by immunohistochemistry (Figure S6). Furthermore, ELISA analysis of whole skin tissue confirmed a significant increase in IGF-1 levels after PDLLA filler treatment (Figure 2D).

2.6. PDLLA Filler Increases HMK Proliferation and Keratin Synthesis in the Animal

Because specific markers for HMKs are limited, we indirectly assessed proliferative activity by quantifying PCNA-positive cells in the hair matrix region (i.e., epithelial matrix surrounding the dermal papilla). A significant increase in PCNA-positive cells was observed, indicating enhanced cell division necessary for hair shaft formation (Figure 2E–G).

K35 (type I) and K85 (type II), the dominant hard keratins produced by HMKs and the principal constituents of hair shaft cortex macrofibrils [38], were selected for analysis. Immunostaining demonstrated robust upregulation of K35 and K85 in the hair cortex of the PDLLA filler-treated group, indicating enhanced synthesis of structural proteins essential for hair fiber formation (Figure 2H–K).

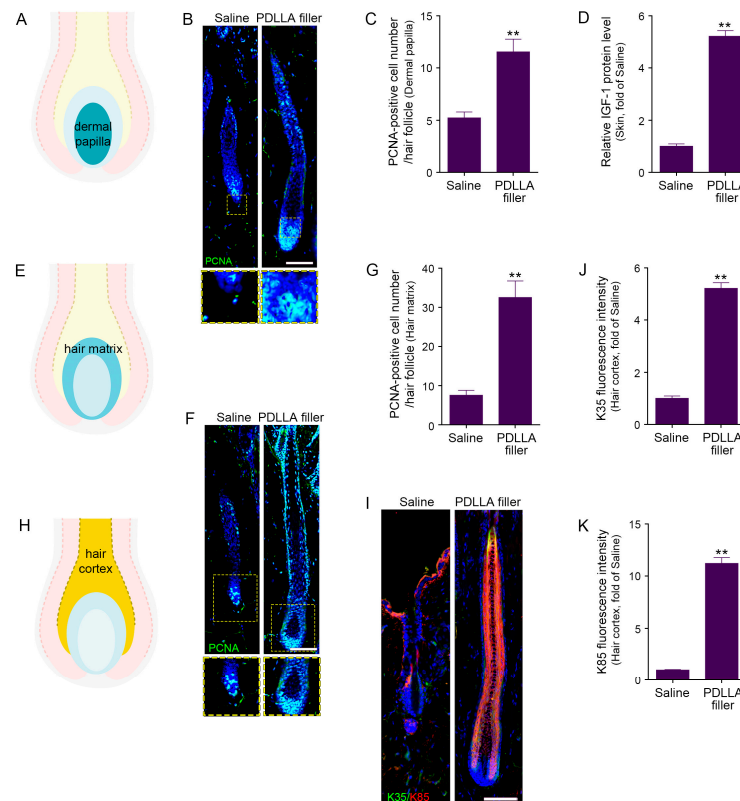


Figure 2. PDLLA filler restores dermal papilla cell proliferation and promotes HMK proliferation in the hair matrix, as well as hair shaft keratin formation, in vivo. (A) Schematic illustration indicating the dermal papilla region within the hair follicle, where DPCs are densely localized and were analyzed for proliferation. Darker shaded areas indicate analyzed regions (dermal papilla). (B) Representative immunofluorescence images showing PCNA (green) expression in the dermal papilla region of hair follicles from saline- and PDLLA filler-treated mice. Nuclei were counterstained with DAPI (blue). Dashed boxes indicate the dermal papilla region, shown at higher magnification in the bottom panels. Proliferating cells were quantified by counting PCNA-positive nuclei-colocalized with DAPI within defined regions of interest. Scale bar = 100 μ m. (C) Quantification of PCNA-positive cells in the dermal papilla of each hair follicle, expressed as the number of PCNA-positive cells per dermal papilla. (D) IGF-1 protein levels in whole skin tissue, measured by ELISA and expressed as fold change relative to the saline-treated control group. (E) Schematic illustration indicating hair matrix regions analyzed for HMK proliferation. Darker shaded areas represented the analyzed regions (hair matrix). (F) Representative immunofluorescence images showing PCNA expression (green) in the hair matrix region of hair follicles from saline- and PDLLA filler-treated mice. Nuclei were counterstained with DAPI (blue). Dashed boxes indicate the hair matrix region, shown at higher magnification in the bottom panels. Quantification was performed by counting PCNA-positive nuclei co-localized with DAPI within standardized regions of interest. Scale bar = 100 μ m. (G) Quantification of PCNA-positive cells within the hair matrix region, expressed as the number of PCNA-positive cells per hair follicle. (H) Schematic illustration indicating hair shaft cortex regions analyzed for keratin expression. Darker shaded areas indicate analyzed regions (hair cortex). (I) Representative immunofluorescence images showing the expression of hair shaft cortex-specific hard keratins K35 (type I; green) and K85 (type II; red) in hair follicles from saline- and PDLLA filler-treated mice. Nuclei were counterstained with DAPI (blue). Scale bar = 100 μ m. (J,K) Quantitative analysis of K35-positive (J) and K85-positive (K) fluorescence intensity within the hair shaft cortex region, expressed as fold change relative to the saline-treated control group. Data are presented as mean $\pm$ standard deviation (n = 5 per group). Group comparisons were performed using the Kruskal–Wallis test and Mann–Whitney U test. **, $p < 0.01$ vs. Saline. DAPI, 4',6-diamidino-2-phenylindole; DPCs, dermal papilla cells; ELISA, enzyme-linked immunosorbent assay; HMK, hair matrix keratinocytes; IGF-1, Insulin-like growth factor-1; PCNA, proliferating cell nuclear antigen; PDLLA, poly-D,L-lactic acid.

2.7. PDLLA Filler Increases IRS Cell Proliferation and Cuticle Formation in the Animal

We observed increased positive staining for the IRS marker AE15 [39] and the IRS type I keratins K25 and K71 within IRS layers after PDLLA filler treatment (Figure 3A–F). K25 and K71 are essential structural proteins involved in hair cuticle formation within the IRS [10].

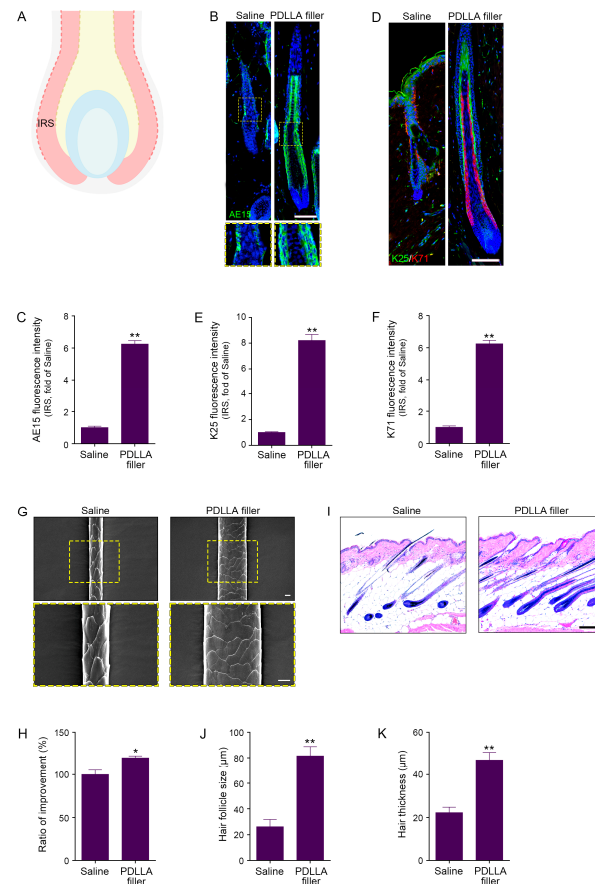


Figure 3. PDLLA filler enhances inner root sheath differentiation, improves hair cuticle integrity, and restores hair thickness in vivo. **(A)** Schematic illustration indicating the IRS region of the hair follicle analyzed for IRS-specific keratin expression. Darker shaded areas represented the analyzed regions (IRS). **(B)** Representative immunofluorescence images showing AE15 expression (green) in the IRS of hair follicles from saline- and PDLLA filler-treated mice. Nuclei were counterstained with DAPI (blue). Dashed boxes indicate the IRS region, shown at higher magnification in the bottom panels. Scale bar = 100 μ m. **(C)** Quantitative analysis of AE15-positive fluorescence intensity in the IRS region, expressed as fold change relative to the saline-treated control group. **(D)** Representative immunofluorescence images showing IRS-specific type I keratins K25 (green) and K71 (red) in the IRS region of hair follicles from saline- and PDLLA filler-treated mice. Nuclei were counterstained with DAPI (blue). Scale bar = 100 μ m. **(E,F)** Quantitative analysis of K25-positive **(E)** and K71-positive **(F)** fluorescence intensity in the IRS region, expressed as fold change relative to the saline-treated control group. **(G)** Representative SEM images of hair shafts from saline- and PDLLA filler-treated mice, highlighting differences in hair cuticle surface morphology. Dashed boxes indicate regions shown at higher magnification in the bottom panels. Scale bar = 10 μ m. **(H)** Quantitative assessment of hair cuticle integrity based on SEM images using a five-point SEM-based grading scale. **(I)** Representative H&E-stained skin sections from saline- and PDLLA filler-treated mice. **(J)** Quantification of hair follicle size based on H&E-stained sections. **(K)** Quantification of hair shaft thickness measured from SEM images. Data are presented as mean $\pm$ standard deviation. Group comparisons were performed using the Kruskal–Wallis test and Mann–Whitney U test. *, $p < 0.05$ and **, $p < 0.01$ vs. Saline. DAPI, 4',6-diamidino-2-phenylindole; H&E, hematoxylin and eosin; IRS, inner root sheath; PDLLA, poly-D,L-lactic acid; SEM, scanning electron microscopy.

Scanning electron microscopy (SEM) demonstrated that hair shafts from the PDLLA filler-treated group exhibited a smoother cuticle surface with reduced damage relative to the rough, lifted cuticles observed in the controls. Quantitative assessment using an SEM-based five-point wide-range grading scale revealed a substantial improvement in cuticle integrity in the PDLLA filler-treated group compared with the saline-treated group (Figure 3G,H). In this scale, grade 1 indicates almost intact, regularly overlapped cuticles, grades 2–4 represent progressively lifted and chipped cuticles, and grade 5 denotes near-complete cuticle loss with exposed cortex [40].

Histological examination via Hematoxylin and Eosin (H&E) staining showed that PDLLA filler treatment effectively attenuated hair follicle miniaturization, resulting in the recovery of overall follicular size and hair shaft caliber (Figure 3I–J), accompanied by increased hair thickness (Figure 3G,K).

2.8. PDLLA Filler Improves Hair Quality and Modulates Sebaceous Gland Activity in the Animal

We also investigated the effects of PDLLA filler on sebaceous glands, which frequently exhibit functional alterations in aging skin. PCNA staining of sebaceous glands indicated that PDLLA filler treatment mitigated the senescence-associated reduction in sebocyte proliferative capacity (Figure 4A,B).

Expression of peroxisome proliferator-activated receptor gamma (PPAR- γ), a key regulator of sebaceous lipogenesis [41,42], was evaluated to assess sebum production. PDLLA filler treatment significantly increased PPAR- γ expression (Figure 4C,D). Histological analysis via H&E staining demonstrated that sebaceous gland size was larger in the PDLLA filler-treated group than in the senescent controls (Figure 4E,F).

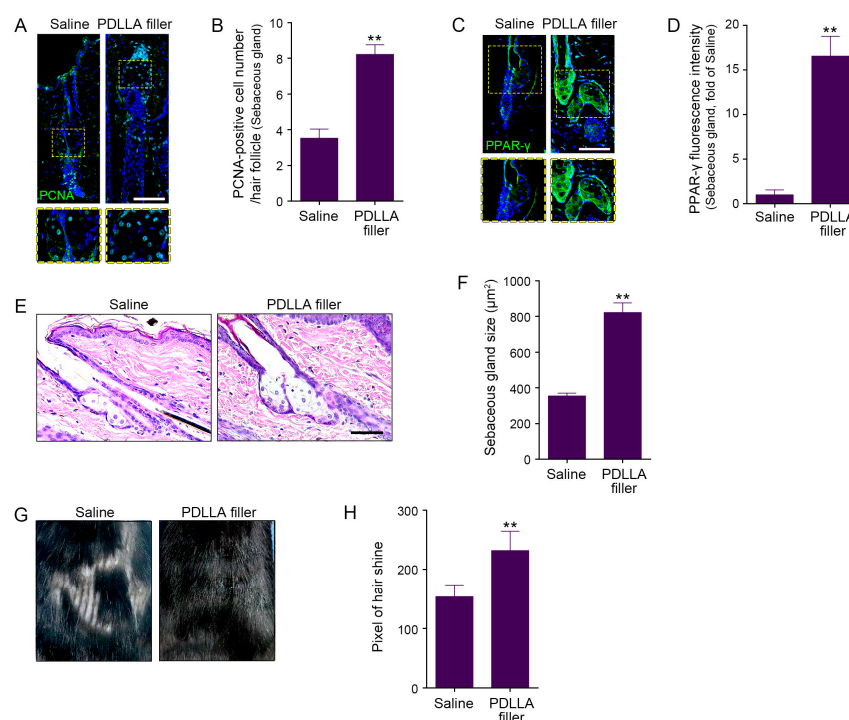


Figure 4. PDLLA filler restores sebaceous gland activity and improves hair shine in vivo. (A) Representative immunofluorescence images showing PCNA (green) expression in sebaceous glands from saline- and PDLLA filler-treated mice. Nuclei were counterstained with DAPI (blue). Dashed boxes indicate the sebaceous gland region, shown at higher magnification in the bottom panels. Scale bar = 100 μm. (B) Quantification of PCNA-positive cells within the sebaceous gland region, expressed as

the number of PCNA-positive cells per hair follicle. (C) Representative immunofluorescence images showing PPAR- γ (green) expression in sebaceous glands from saline- and PDLLA filler-treated mice. Nuclei were counterstained with DAPI (blue). Dashed boxes indicate the sebaceous gland region, shown at higher magnification in the bottom panels. Scale bar = 100 μ m. (D) Quantitative analysis of PPAR- γ -positive fluorescence intensity in the sebaceous gland region, expressed as fold change relative to the saline-treated control group. (E) Representative H&E-stained skin sections showing sebaceous gland morphology in the saline- and PDLLA filler-treated mice. Scale bar = 50 μ m. (F) Quantification of sebaceous gland size based on H&E-stained sections. (G) Representative macroscopic images of hair shafts from saline- and PDLLA filler-treated mice used for hair shine assessment. (H) Quantitative analysis of hair shine based on grayscale image-derived pixel intensity, expressed as integrated reflective pixel intensity relative to the saline-treated control group. Data are presented as mean $\pm$ standard deviation. Group comparisons were performed using the Kruskal–Wallis test and Mann–Whitney U test. **, $p < 0.01$ vs. Saline. DAPI, 4',6-diamidino-2-phenylindole; H&E, hematoxylin and eosin; PCNA, proliferating cell nuclear antigen; PDLLA, poly-D,L-lactic acid; PPAR- γ , peroxisome proliferator-activated receptor gamma.

Image-based quantification of hair shine, performed by measuring grayscale pixel intensity under standardized imaging conditions, revealed a significant increase in shine index (integrated reflective pixel intensity) in the PDLLA filler-treated group compared with the controls (Figure 4G,H).

3. Discussion

Scalp hair undergoes characteristic age-related changes even in otherwise healthy individuals. In clinical settings, patients frequently report progressive hair thinning, reflected by reduced hair shaft diameter and density, along with a loss of luster that substantially alters perceived hair volume and cosmetic appearance. Large cross-sectional studies have shown that hair fiber diameter in women increases to a peak around the fourth decade of life and subsequently declines; postmenopausal women exhibit significantly lower hair fiber diameter, frontal scalp density, and growth rates compared with premenopausal women [43,44]. These changes are accompanied by alterations in curvature, tensile properties, and surface characteristics of the hair fiber, which collectively contribute to frizz, increased breakage, and an aged hair appearance [45].

Hair thickness and gloss are largely determined by the quantity and organization of structural keratins within the cortex and cuticle, as well as lipids derived from hair matrix cells and sebaceous gland sebum that form the hydrolipidic film on the fiber surface. Age-related alterations in hair lipids affect greasiness, shine, softness, and smoothness. Sebum production is low before puberty, greatly increases at puberty, remains elevated until approximately 45–50 years of age, and subsequently declines, particularly in women [46]. Menopause-associated sebaceous gland atrophy and reduced sebum secretion further contribute to drier, less manageable hair [45].

At the follicular level, the hair shaft is generated by rapidly proliferating HMKs at the base of the follicle, which differentiate upward to form the cortex and cuticle of the hair fiber and to generate the IRS that shapes and guides the emerging shaft. This process occurs during the anagen phase and depends on complex epithelial–mesenchymal crosstalk between DPCs and hair keratinocytes [11]. DPCs regulate hair cycle dynamics, as well as hair shaft diameter and shape, by secreting a range of growth factors and morphogens that control HMK proliferation and differentiation. Through these signals, HMKs only form the central keratinized hair shaft and generate progeny that contribute to the IRS, including IRS-specific keratins essential for proper cuticle formation and hair shaft anchorage [11].

Increasing evidence indicates that oxidative stress promotes cellular senescence in multiple hair follicle cell types [47]. In the present study, we induced DPC senescence by oxidative stress, then evaluated the ability of PDLLA filler to decrease senescence-

associated functional decline. Previous research has shown that aging-associated chronic oxidative stress induces DPC senescence, resulting in the reduced secretion of essential growth factors such as IGF-1, which is critical for hair growth [2,9].

In this context, the reduction in 8-OHdG observed after PDLLA filler exposure is best interpreted not simply as a descriptive marker change, but as evidence that the formulation attenuates oxidative DNA damage, which is a key upstream trigger for checkpoint activation (p53/p21 and p16) and stable cell-cycle arrest. Consistent with a senescence-ameliorating senomorphic-like mechanism, our findings support the model that rebalancing redox stress can preserve (or restore) DPC function—most notably IGF-1, a well-established paracrine cue implicated in hair growth and regeneration [8].

Redox restoration in aging tissues has been linked to engagement of the NRF2 antioxidant network and downstream enzymes (e.g., HO-1, NQO1, SOD, catalase, and GSH-Px), which have been discussed as modulators of cellular senescence and the SASP in broader aging biology [48]. Taken together, these considerations place our data within a coherent framework in which PDLLA filler improves a redox-regulated stress state and thereby mitigates senescence-associated functional decline, rather than implying direct senescent-cell elimination.

Our conditioned-medium experiments demonstrate that epithelial recovery is driven by a PDLLA filler-associated shift in the DPC secretome rather than a direct effect on keratinocytes. This confirms the role of DPCs as a functional bottleneck that regulates follicular behavior through paracrine mediators like IGF-1. While the complexity of the DPC secretome warrants further profiling, the concordance between our human-cell and aged-mouse data supports the biological plausibility of this paracrine axis. However, given the species differences, these findings should be viewed as proof-of-concept evidence requiring future human scalp validation.

The hair shaft exhibits a hierarchical structure consisting of the protective cuticle, structural cortex, and variable medulla [49]. The cuticle, which forms the outermost layer, contains 18-methyl eicosanoic acid and highly cross-linked proteins [50]. Its negatively charged surface interacts with positively charged peptides [51,52] and protects the fiber from external damage. The cuticle also contains the highest proportion of cystine within the A layer and exocuticle [53]. The cortex, which represents 70–90% of the fiber mass, provides mechanical strength and maintains fiber shape through its high content of cystine residues [54] organized into intermediate filaments comprising type I and type II keratins [55] embedded in a matrix of keratin-associated proteins. This cortical matrix contains the highest concentration of disulfide bonds, which are essential for mechanical stability and hair perming [3,56]. Notably, cuticle integrity preservation and robust synthesis of keratins and keratin-associated proteins within the cortex are critical determinants of hair quality and strength [57].

Among the hair cortex keratins, K35 (type I) and K85 (type II) are particularly informative because they represent the earliest pair expressed in hair matrix and precortical cells during the initial stage of cortex differentiation, indicating an early step in the keratinization program [37]. Consistent with this role, we detected increased expression of K35 and K85 after PDLLA filler treatment, indicating enhanced structural protein synthesis at the hair shaft level.

Moreover, the number of protective cuticle layers is positively correlated with hair thickness, such that thicker fibers exhibit more cuticle layers relative to thinner fibers [58]. This relationship may be associated with a higher growth rate in thicker hairs, which permits a more rapid supply of cells from the hair follicle [59]. Because cuticle layers serve as the primary protective barrier for the underlying hair cortex against external physical and chemical stressors (e.g., water, ultraviolet radiation, mechanical abrasion, and

pollutants), a lower number of layers diminishes this protective capacity. Accordingly, beyond the reduction in hair density associated with thinning, the thinning process itself contributes to weakened structure and increased fragility, particularly in older individuals. Therefore, robust formation and maintenance of multiple cuticle layers are essential to preserve hair strength and resilience [11]. In the present study, PDLLA filler treatment increased the expression of IRS-associated markers (AE15, K25, and K71) and improved cuticle morphology, as determined by SEM-based grading. Given that cuticle and cortex architecture are fundamental determinants of fiber robustness and appearance, these IRS and SEM findings provide a plausible structural basis for the observed increases in hair thickness and overall hair quality [60].

Beyond the hair shaft, age-related loss of luster has been associated with reduced sebum secretion and alterations in the hydrolipidic layer, particularly among older women, in whom menopause is accompanied by sebaceous gland atrophy and drier hair [47]. Consistent with this concept, PDLLA filler treatment increased PCNA positivity in sebaceous glands, enhanced PPAR- γ expression, and increased sebaceous gland size on H&E staining. PDLLA filler also improved hair shine, as demonstrated by macroscopic imaging and quantitative pixel-intensity analysis, supporting a functional association between adnexal unit health and visible hair quality.

Despite the utility of aged murine skin as an *in vivo* proof-of-concept platform, several species differences between mice and humans should be considered when translating the present findings to clinical settings. Murine dorsal skin is dominated by densely packed pelage follicles, and hair cycling can occur in regionally coordinated wave-like patterns across the skin, meaning that hair-cycle stage and synchronization procedures may substantially influence follicle morphology [61]. In contrast, human scalp follicles generally cycle asynchronously (mosaic cycling) with markedly longer phase durations, and age-related thinning in humans often reflects chronic, long-term remodeling processes rather than short-cycle dynamics typical of mouse pelage follicles [62,63]. Therefore, improvements in murine follicle size, proliferation indices, or keratin marker expression after an intervention should be interpreted as supportive evidence of biological plausibility rather than a direct quantitative predictor of clinical efficacy in humans. In addition, commonly used murine strains do not fully recapitulate key human hair-loss contexts (e.g., androgen-dependent miniaturization), and thus the current aged mouse model is best viewed as representing intrinsic/age-associated follicular decline rather than a complete surrogate for human scalp disorders [63].

Species differences may also affect sebaceous gland-associated outcomes. Sebum composition is species-specific, and characteristic human sebaceous lipids such as squalene and wax esters contribute to the surface hydrolipidic film and optical properties of hair fibers, which underline the perception of shine [64]. Furthermore, cross-species transcriptomic studies suggest that mouse and human sebaceous glands can operate with partially distinct lipid-synthesis programs and metabolic features, which may influence how sebaceous changes translate between species [64–66]. Collectively, these considerations emphasize the need to validate PDLLA filler-associated effects in human scalp-relevant platforms and clinical studies.

Although our findings indicate that PDLLA filler can counteract age-related hair follicle deterioration by mitigating oxidative stress and restoring DPC function, some limitations warrant consideration. First, the *in vivo* data were obtained from a senescent mouse model; clinical studies are required to confirm translational efficacy and determine optimal dosing in humans. Furthermore, significant physiological differences in skin thickness and hair cycle dynamics between mice and humans—specifically the synchronized hair cycle of murine models versus the independent, mosaic pattern of human hair follicles—present

inherent limitations in the direct clinical translation of these findings. Therefore, the efficacy and longevity of PDLLA filler observed in this study should be further validated through controlled clinical trials on the human scalp. Second, long-term safety and sustained efficacy after chronic PDLLA filler exposure were not evaluated. Third, although we have shown that PDLLA filler increases the expression of NRF2 and antioxidant enzymes (SOD, catalase, and GSH-Px), the direct causal link between NRF2 activation and the observed reduction in 8-OHdG remains to be fully elucidated. As our current findings are based on protein/mRNA expression levels rather than pathway inhibition or perturbation studies, further research employing NRF2 inhibitors or gene-silencing techniques is needed to confirm whether the antioxidant effects of PDLLA filler are exclusively mediated through this specific signaling axis. Fourth, the use of hHFKs as a surrogate for HMKs in paracrine assays provides only an indirect assessment of keratin synthesis; future studies should incorporate primary HMKs or organoid models for direct validation. Finally, it should be noted that the PDLLA filler used in this study is a clinical formulation containing a small amount of HA as a suspending agent. Since our research was designed to evaluate the integrated therapeutic efficacy of the final product to reflect clinical applications, the absence of an HA-only control group may be considered a limitation in distinguishing the individual biological contributions of the PDLLA polymer from those of HA. Although the antioxidant effects observed here align closely with the established biostimulatory properties of PDLLA microspheres, future investigations incorporating an HA-only comparison group will be necessary to fully delineate the independent or synergistic roles of each component in hair follicle rejuvenation.

4. Materials and Methods

4.1. Preparation of PDLLA Filler

PDLLA (MW 80,000–200,000 g/mol; VAIM Co., Ltd., Seoul, Republic of Korea) was dissolved in an ethylene carbonate/dimethyl sulfoxide mixture (1:9, *v/v*; both from Sigma-Aldrich, St. Louis, MO, USA) and precipitated by spraying into cold *n*-hexane at -20°C . The precipitate was washed, dried, and sieved to obtain particles with diameters of 10–30 μm . These particles were blended with 0.6% hyaluronic acid (HA; intrinsic viscosity 3.0–5.5 dL/g; VAIM Co., Ltd.) at a weight ratio of 17:3 (PDLLA:HA), lyophilized, and sterilized with ethylene oxide gas before use [16,67,68].

4.2. In Vitro Study

4.2.1. Cell Culture

Human DPCs (hDPCs) were purchased from PromoCell GmbH (Heidelberg, Germany) and cultured in Follicle Dermal Papilla Cell Growth Medium (PromoCell) supplemented with the provided growth supplement mix and 1% penicillin/streptomycin, in accordance with the manufacturer's instructions.

Human HFKs (hHFKs) were obtained from ScienCell Research Laboratories (Carlsbad, CA, USA) and maintained in Keratinocyte Medium (ScienCell Research Laboratories) supplemented with keratinocyte growth supplement and 1% penicillin/streptomycin. All cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 , with medium replacement every 2–3 days. Cells at passages 3–6 were used for all experiments. Each experimental condition was assessed using at least three independently prepared culture wells, and all in vitro experiments were repeated at least three times.

4.2.2. Induction of Cellular Senescence

H_2O_2 treatment is widely used as an in vitro model of cellular senescence because H_2O_2 -induced oxidative stress reproduces characteristic features of senescent cells [69–71]. To induce

senescence in hDPCs, cells were exposed to 50–300 μM H_2O_2 (Sigma-Aldrich) for 1.5 h, washed with Dulbecco's phosphate-buffered saline (PBS) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), and cultured in fresh growth medium for 72 h. Cell viability, expression of senescence-associated markers (*p16* and *p21*), and SA- β -gal activity were assessed across this dose range to determine the working concentration. Treatment with 150 μM H_2O_2 induced a senescence-like phenotype in hDPCs while limiting cell death to approximately 10% of the total cell population; this condition was used in subsequent experiments.

For hHFKs, senescence was induced using a protocol analogous to that used for hDPCs, with modifications to H_2O_2 concentration and exposure duration. Briefly, hHFKs were treated with 100–400 μM H_2O_2 (Sigma-Aldrich) for 2 h, rinsed with Dulbecco's PBS (Gibco, Thermo Fisher Scientific), and maintained in fresh growth medium for 72 h. Cell viability and expression of senescence-associated markers were assessed across this concentration range. H_2O_2 at 200 μM was selected as the working concentration because it induced a clear senescence-like phenotype in hHFKs while limiting cell death to approximately 10% of the total cell population.

For both cell types, control cells were treated with PBS instead of H_2O_2 and cultured under identical conditions for comparative analyses.

4.2.3. Determination of PDLLA Filler Concentration in Senescent hDPCs

To establish an appropriate PDLLA filler concentration for ROS modulation, PDLLA filler was applied to hDPCs rendered senescent by H_2O_2 treatment as described above. After senescence induction, cells were exposed to PDLLA filler at 0–20 mg/mL for cytotoxicity assessment and at 0–400 $\mu\text{g}/\text{mL}$ to determine the optimal concentration for the modulation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels for 48 h. For comparison, two PBS-treated control groups were included: non-senescent cells without H_2O_2 exposure and senescent cells following H_2O_2 treatment. Analysis of 8-OHdG indicated that 300 $\mu\text{g}/\text{mL}$ PDLLA filler achieved the most favorable reduction in oxidative DNA damage without inducing cell death; this concentration was used in subsequent experiments.

4.2.4. SA- β -gal Staining

SA- β -gal activity was assessed using a commercial staining kit (Cell Signaling Technology, Danvers, MA, USA), in accordance with the manufacturer's instructions. Cells exhibiting blue staining under light microscopy were classified as SA- β -gal-positive. Images were acquired from at least five randomly selected fields per sample, and the percentage of positive cells was calculated.

4.3. In Vivo Study

4.3.1. Mouse Model

C57BL/6 mice (6 weeks old) were obtained from Orient Bio (Seongnam, Republic of Korea) and housed under standard laboratory conditions (temperature, 20–24 $^{\circ}\text{C}$; humidity, 45–55%) with free access to food and water. After a 1-week acclimation period, mice were bred; 17-month-old female mice were used for the experiments.

All animal procedures were conducted in accordance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee of Gachon University (IACUC No. LCDI-2024-0105). This study complied with the ethical standards of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International, Frederick, MD, USA) and adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Each mouse was injected and analyzed individually throughout the study.

4.3.2. Material Injection in Mice

Anesthetized 17-month-old female mice underwent initial dorsal hair removal using electric clippers, followed by complete depilation with a depilatory agent. Animals exhibiting irregular hair follicle cycles were excluded, and only mice in the telogen phase were included; these inclusion and exclusion criteria were defined a priori before group allocation. Mice were randomly assigned to two groups ($n = 5$ per group; total of 10 mice). Sample size was determined with reference to published guidelines for animal studies [72]. Group 1 received 500 μL of sterile saline as a vehicle control, whereas Group 2 received an equivalent volume of PDLLA filler. Injections were evenly administered at five dorsal sites within the depilated area using a 27-gauge needle. Three weeks after injection, all mice were anesthetized by inhalation of isoflurane (HANA Pharm Co., Ltd., Seoul, Republic of Korea) delivered in 1.5% O_2 and subsequently euthanized in accordance with the American Veterinary Medical Association guidelines. Skin tissues were harvested specifically from the injected dorsal sites. All histological, immunofluorescence, molecular, and SEM analyses were performed using samples obtained from these injection sites. No animals were excluded from the analysis after group allocation.

4.3.3. Hair Shine Scoring

Hair shine was assessed by capturing images from the same anatomical location under identical imaging conditions. Images were quantitatively analyzed by pixel intensity measurement using ImageJ software (version 1.53s; National Institutes of Health, Bethesda, MD, USA). Images were converted to grayscale, regions of interest corresponding to hair shafts were selected, and the mean gray values were calculated for each region and averaged per mouse for analysis as an indicator of hair shine.

4.4. Cell Viability Assay

Cells were treated with H_2O_2 as described above. After senescence induction, cells were seeded in 96-well plates (SPL Life Sciences, Pocheon, Republic of Korea), exposed to H_2O_2 , and treated with PBS or PDLLA filler. Cell viability was assessed via Cell Counting Kit-8 (CCK-8; TransGen Biotech Co., Ltd., Beijing, China), in accordance with the manufacturer's instructions. Absorbance was measured at 450 nm using a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific).

4.5. Enzyme-Linked Immunosorbent Assay (ELISA)

Proteins were extracted from cells and skin tissues using EzRIPA buffer (ATTO Corporation, Tokyo, Japan). Protein concentrations were determined via the bicinchoninic acid assay (Thermo Fisher Scientific). Protein samples diluted in carbonate–bicarbonate coating buffer (pH 9.6) were added to 96-well plates (SPL Life Sciences) and incubated overnight at 4 °C. Plates were washed with PBS containing 0.1% Tween-20, blocked with 5% skim milk (LPS Solution, Daejeon, Republic of Korea) in PBS for 1 h at room temperature, and incubated with anti-IGF-1 antibody (1:500; Bioss Antibodies Inc., Woburn, MA, USA) or 8-OHdG (1:500; Abcam, Cambridge, UK) overnight at 4 °C. After the plates had been washed with PBS containing 0.1% Tween-20, horseradish peroxidase-conjugated secondary antibodies (Vector Laboratories, Burlingame, CA, USA) were applied for 1 h at room temperature. Color development was achieved via 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution (Sigma-Aldrich), and the reaction was terminated with 1 M sulfuric acid. Absorbance was measured at 450 nm using a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific). The activities of SOD (Thermo Fisher Scientific), catalase (Thermo Fisher Scientific), and GSH-Px (Thermo Fisher Scientific) were measured using commercially available assay kits according to the manufacturer's instructions.

4.6. Quantitative Gene Expression Analysis

Total RNA was extracted using RNAiso (Takara Bio Inc., Kusatsu, Japan), in accordance with the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Complementary DNA was synthesized using a reverse transcription kit (Takara Bio Inc.) following the manufacturer's protocol.

Quantitative polymerase chain reaction analyses were performed using SYBR Green chemistry (Takara Bio Inc.) on a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific). Amplification conditions comprised initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative gene expression levels were calculated using the comparative Ct ($\Delta\Delta C_t$) method; β -actin (*ACTB*) served as the reference gene. Primer sequences for each target gene are listed in Table S1.

4.7. Flow Cytometry for Cell Cycle Analysis

Cell cycle distribution was analyzed by flow cytometry using propidium iodide (PI) staining. Cells (1×10^6) were harvested, pelleted by centrifugation, and washed once with 1 mL PBS at room temperature. The pellet was gently resuspended in residual PBS; the suspension was slowly added, with vigorous vortexing, to 4 mL of ice-cold absolute ethanol (−20 °C) for fixation. Cells were maintained in ethanol at −20 °C for 10 min, then collected by centrifugation. After removal of the ethanol, the pellet was loosened by gentle tapping and resuspended in 5 mL PBS at room temperature, then rehydrated for 15 min.

For DNA staining, a 3 μ M PI solution was prepared by diluting a 1 mg/mL (i.e., 1.5 mM) PI stock solution 1:500 in staining buffer (100 mM Tris-HCl, pH 7.4; 150 mM NaCl; 1 mM CaCl_2 ; 0.5 mM MgCl_2 ; and 0.1% Nonidet P-40). Rehydrated cells were pelleted, the supernatant was discarded, and cells were resuspended in 1 mL PI staining solution; they were subsequently incubated for 15 min at room temperature in the dark. Samples were analyzed by flow cytometry to generate DNA content histograms and determine the proportion of cells in each cell cycle phase.

4.8. Western Blot Analysis

Proteins extracted using the method described for ELISA were mixed with equal volumes of 4× lithium dodecyl sulfate sample buffer and 10× reducing reagent (Thermo Fisher Scientific), then denatured at 70 °C for 10 min. Samples were separated at 200 V for 25 min on 10% sodium dodecyl sulfate–polyacrylamide gels using 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (Thermo Fisher Scientific) and transferred to polyvinylidene fluoride membranes (Millipore, Burlington, MA, USA) using a semi-dry blotting system (ATTO Corporation) at 1 A for 10 min.

Membranes were blocked for 1 h using 5% skim milk (LPS Solution) in Tris-buffered saline containing 0.1% Tween-20 at room temperature with gentle agitation. After three washes in Tris-buffered saline containing 0.1% Tween-20, membranes were incubated overnight at 4 °C with Pan-keratin antibody (1:1000; Cell Signaling Technology). Horseradish peroxidase-conjugated secondary antibodies (Vector Laboratories) were then applied for 1 h at room temperature, followed by additional washes with Tris-buffered saline containing 0.1% Tween-20.

Membranes were detected using ECL Detection Reagents (Cytiva, Marlborough, MA, USA) on a ChemiDoc imaging system (Bio-Rad, Hercules, CA, USA). A prestained protein molecular weight marker (TransGen Biotech Co., Ltd.) was run in parallel to determine the apparent molecular weights of detected bands. Band densities were quantified using ImageJ software (version 1.53s; National Institutes of Health); GAPDH served as the loading control.

4.9. Paraffin Block Preparation and H&E Staining

Skin tissues were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 72 h, dehydrated through a graded ethanol series, and infiltrated with paraffin using an automated tissue processor (Leica, Wetzlar, Germany). Paraffin blocks were sectioned into 7- μ m slices using a microtome, mounted on glass slides, and baked overnight at 60 °C to ensure adhesion.

Paraffin-embedded sections were deparaffinized in xylene, rehydrated through a graded ethanol series, and stained with hematoxylin (KPNT, Cheongju, Republic of Korea) followed by eosin (KPNT). After dehydration and clearing, sections were mounted for histological evaluation and scanned using a slide scanner (Motic, Beijing, China). Hair follicle size and thickness were measured from scanned images using ImageJ software (version 1.53s; National Institutes of Health).

4.10. Immunofluorescence

Rehydrated sections were rinsed in PBS and permeabilized with 0.5% Triton X-100 in PBS for 10 min. Nonspecific binding sites were blocked with 5% normal serum for 1 h at room temperature. Sections were incubated with primary antibodies (Table S2) overnight at 4 °C in a humidified chamber. After sections had been washed with PBS, Alexa Fluor-conjugated secondary antibodies (Thermo Fisher Scientific) were applied for 1 h at room temperature in the dark. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; 1 μ g/mL; Sigma-Aldrich), and slides were mounted using an antifade mounting medium (Vector Laboratories). Fluorescence images were acquired using an LSM-710 confocal microscope (Carl Zeiss, Oberkochen, Germany) at the core facility for cell-to-in vivo imaging. For quantitative analysis, regions of interest (ROIs) were defined within the hair follicle area. For PCNA staining, proliferating cells were quantified by manually counting PCNA-positive nuclei co-localized with DAPI staining within the defined ROIs at a consistent magnification. For all other immunofluorescence markers, fluorescence intensity was measured within the same ROI at a consistent magnification, and values were normalized to the control group. In each experimental group, five randomly selected hair follicle fields per section were analyzed and quantified using ZEN imaging software (version 5.1; Carl Zeiss).

4.11. SEM Imaging

Hair samples were randomly selected and consistently obtained from the same anatomical location. Specimens were fixed for 24 h in Karnovsky's fixative (2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4), then washed twice for 20 min each in 0.1 M phosphate buffer (pH 7.4). Samples were post-fixed with 1% osmium tetroxide (OsO₄) in 0.1 M phosphate buffer for 2 h and dehydrated through a graded ethanol series (50–100%). The dehydrated specimens were dried using a critical point dryer (EM CPD300; Leica). Dried samples were carbon-coated using an ion sputter coater (EM ACE600; Leica) and examined with a field-emission scanning electron microscope (MERLIN, Carl Zeiss). Energy-dispersive X-ray spectroscopy elemental mapping was performed using an EDS system (Bruker, Billerica, MA, USA) equipped with an XFlash[®] 5060 FlatQUAD detector. Mapping was conducted at an accelerating voltage of 15 kV, magnification of $\times 2000$, and acquisition time of 300 s.

4.12. Statistical Analysis

Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). All statistical analyses were conducted using non-parametric methods. The Kruskal–Wallis test was used for comparisons among multiple independent groups, and the Mann–Whitney U test was used for comparisons between two independent groups.

Data are presented as mean $\pm$ standard deviation. Statistical significance was set at $p < 0.05$, and statistically significant differences are indicated in the figures using standardized significance symbols (*, $p < 0.05$; **, $p < 0.01$).

5. Conclusions

This study demonstrated that PDLLA filler effectively mitigates age-related structural deterioration of hair by acting as a potent antioxidant that decreased cellular senescence. PDLLA filler treatment promoted the intrinsic function of senescent DPCs, as evidenced by reduced oxidative stress and subsequent upregulation of IGF-1 secretion. This enhanced HMK proliferation, promoted robust synthesis of hard keratins (K35 and K85), and improved cuticle integrity. Furthermore, PDLLA filler ameliorated senescence within sebaceous glands, resulting in measurable recovery of hair luster. Collectively, these findings support PDLLA filler as a promising biostimulatory strategy to counteract age-associated hair thinning and quality degradation.

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Informed Consent Statement: Not applicable.

Data Availability Statement: All datasets generated or analyzed in this study are included in the article.

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Abbreviations

The following abbreviations are used in this manuscript:

ARRIVE	Animal Research: Reporting of In Vivo Experiments
CCK	Cell Counting Kit
CM	Conditioned medium
DAPI	4',6-diamidino-2-phenylindole
DPC	Dermal papilla cell

ELISA	Enzyme-linked immunosorbent assay
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HA	Hyaluronic acid
HFKs	Hair follicular keratinocytes
HMKs	Hair matrix keratinocytes
HO-1	Heme oxygenase-1
H&E	Hematoxylin and eosin
H ₂ O ₂	Hydrogen peroxide
IGF-1	Insulin-like growth factor-1
IRS	Inner root sheath
NQO1	NAD(P)H:quinone oxidoreductase 1
NRF2	Nuclear factor erythroid 2-related factor 2
PBS	Phosphate-buffered saline
PCNA	Proliferating cell nuclear antigen
PDLLA	Poly-D,L-lactic acid
PI	Propidium iodide
PPAR- γ	Peroxisome proliferator-activated receptor gamma
SA- β -gal	Senescence-Associated β -Galactosidase
SEM	Scanning electron microscopy
SOD	Superoxide dismutase
TMB	3,3',5,5'-tetramethylbenzidine
8-OHdG	8-hydroxy-2'-deoxyguanosine

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