



# Poly-L-Lactic Acid Reduces the Volume of Dermal Adipose Tissue Through its Metabolite Lactate

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**Abstract** Poly-L-lactic acid (PLLA), a well-established biostimulator that induces collagenases, is widely used among clinical practice to treat skin aging. However, the precise regulatory effect of PLLA on different dermal cell subsets beyond fibroblast has not been fully elucidated. In this study, we constructed in vivo PLLA injection and in vitro PLLA-adipocyte co-culture models to analyze the regulatory effects of PLLA on the volume, differentiation, lipolysis, and thermogenic capacity of dermal adipocyte. We found that PLLA injection significantly reduced the

thickness of dermal fat in mice. In co-culture assay, PLLA showed no effect on adipogenesis, but stimulated the lipolysis activity. Interestingly, PLLA also enhanced the differentiation of fat cells into beige fat cells, which possess higher thermogenic capacity. In mechanical study, we blocked adipocyte lactate uptake with a monocarboxylate transporter (MCT1/4) inhibitor and found that the regulatory effect of PLLA on dermal adipocyte relies on its metabolite lactate. In summary, our results suggest that PLLA has complex regulatory effects on the dermal cells, and its ability to improve skin aging is not fully attributed to stimulating collagen synthesis, but also partially involves adipocytes.

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**Keywords** Poly-L-lactic acid · Dermal adipose · Lipolysis

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## Introduction

The skin, composed of the epidermis and dermis, is the largest organ of the human body, providing physical protection against external insults and also playing a crucial role in thermoregulation. However, due to sustained and irreversible skin aging process, which encompasses several phenotypes, such as, but not limited to, wrinkling, pigmentation, and telangiectasis [1–3], the protection effect of

the skin weakens and affects facial and body appearance. Skin aging not only affects the physical function of the human body, but also brings about psychological pressure and social anxiety [4]. Therefore, proper treatments should be offered for aging skin, to ensure that society's negative views not be unnecessarily reinforced, and maintain a realistic treatment expectation.

Skin aging is a continuous process, caused by both internal and external factors, characterized by a decrease in the production and content of collagen in the dermis [5]. Skin collagen, mainly secreted by fibroblast in the dermis, forms an abundant extracellular matrix (ECM), houses various cell types, such as adipocytes [6], and further supports the epidermis layer. Recently, it has been found that another major characteristic of skin aging, in addition to the reduction in collagen content, is the enlargement of dermal white adipose tissue (dWAT). Research based on model animals has found that in aged mice at 31-week-old [7, 8] or at 12-month-old [9], the content of dWAT significantly increases. Besides, dWAT also enlarges after ultraviolet radiation, the most important external factor leading to skin aging [10]. Recent data have discovered a paracrine effect of dWAT derived lipids in influencing metabolism of dermis fibroblast [11]. Hence, dermal adipocyte is now considered to be the prime targets in strategies aimed at counteracting skin aging [12].

In clinical practice to treat skin sagging and collapse caused by aging, the use of injectable dermal fillers has become an essential tool. Among the fillers, poly-L-lactic acid (PLLA), a biocompatible, biodegradable and bioresorbable polymer, has been used worldwide, associated with good long-term esthetic results [13]. PLLA is an alpha hydroxy acid polymer of the lactic acid L-enantiomeric structure that has been safely used in many applications and in medicine for more than 30 years [14, 15]. PLLA filler injected into the skin with a carrier solution immediately augments the volume at the injection site, which disappears rapidly as the carrier solution is absorbed by the tissue [16]. The remaining PLLA particles are degraded into lactic acid, which enhances collagen synthesis and gradually increases the dermal thickness [17, 18]. However, the effect of PLLA on the morphology and biological function of dWAT is still unclear.

Therefore, in this study, we performed in vitro and in vivo assay to analysis the regulatory effect of PLLA on dWAT. Moreover, using a monocarboxylate transporter (MCT1/4) inhibitor, we blocked adipocyte lactate uptake, to clarify whether the above effect of PLLA was dependent on its decomposition product, lactic acid.

## Materials and Methods

### Material

The PLLA was purchased from Changchun SinoBiom Medical Science & Technology Co., Ltd (Löviselle® PLLA Facial Filler, GXZZ No.20213130276, Specification: 340 mg/bottle). The Mct1/4 inhibitor 7ACC1 was purchased from MedChemExpress (#HY-D0067).

### Lactate Measurement

Lactate concentration, either in the medium or in the adipocyte, was determined using a colorimetric Lactate Assay Kit (#ab65331, Abcam). Briefly, the lactate containing sample was treated with lactate dehydrogenase to oxidize the lactate and generate a product which interacts with a probe to produce a color ( $\lambda_{\text{max}} = 450 \text{ nm}$ ), according to the manufacturer's protocol. As for the adipocyte cellular lactate content,  $2 \times 10^6$  cells were harvest and washed with cold PBS, followed by resuspending the cell pellet in 4\* volumes of lysis buffer offered in the kit. After centrifuging 3 min at 10000g, the supernatant was collected and used to measure lactate content, which were then normalized with the total protein concentration.

### Cell Viability Assay

The dermal fibroblast was seeded into 48-well trans-well plates and cultured to contact inhibition state. The cells were then differentiated into adipocyte. At the beginning of differentiation, the PLLA (200 $\mu\text{g/mL}$  medium) was added into the up-layer of the trans-well and co-cultured with adipocyte for indicated times. Cell viability was evaluated by the CCK8 assay (#CA1210-100T, Solarbio).

### Separation of Dermal Fibroblast

The skin samples were collected from the back of wild-type C57BL/6j mice, at the age of 3–4-day-old. Skin tissue was rinsed with 75% ethanol and cut into small pieces. Type I collagenase (2mg/ml, #SCR103, Sigma,) was pre-configured using DMEM basic medium (#C11995500BT, Gibco,) containing 2% volume of FBS (Gibco), 100 U/ml penicillin, 100  $\mu\text{g/mL}$  streptomycin, and stored in the dark after removal of bacteria by filtration using a 0.22- $\mu\text{m}$  filter. The volume ratio of collagenase to tissue was maintained at 3:1. The tissue was then digested in 37 °C water bath for 1h, during which the tube was reversed and mixed every five minutes. The digested fibroblast was filtered (70  $\mu\text{m}$ ) and then centrifuged at 100 g for 10 min. After resuspension with PBS, centrifuge at the same speed and

time. DMEM/F-12 (#MA0214, Meilunbio) containing 15% FBS (Gibco) and 1% penicillin-streptomycin was used to culture the primary dermal fibroblast, and then the cells were implanted into the pore plate of appropriate size. The medium was changed 24 h later and then every 48 h until the cells grew to contact inhibition state.

### Adipocyte Differentiation and PLLA Co-culture Assay

The plate fully covered cells then subjected to differentiation. As for beige adipogenesis, culture medium was added insulin (0.5 µg/ml, #I3536, Sigma), dexamethasone (5 µM, #D4902, Sigma), 3,3',5-triiodo-L-thyronine (T3, 1 nM, #T2977, Sigma), rosiglitazone (1 µM, #R2408, Sigma), isobutyl methylxanthine (0.5 mM, #I5879, Sigma). The above differentiation medium was changed 48 h later with medium only containing insulin, T3 and rosiglitazone and renewed every 48 h. Lipid droplet formation was observed at day 7–8, indicating successful induction of beige adipogenesis.

As for white adipogenesis, insulin (1 µg/ml), dexamethasone (1 µM), isobutyl methylxanthine (0.5 mM) were added into the same induction medium. The medium was changed 48 h later, only containing insulin to maintain the differentiation of adipocytes.

Co-culture of PLLA and adipocytes was performed using trans-wells. While inducing mesenchymal stem cells to differentiate into mature adipocytes, they were incubated with either PLLA (200 µg/mL medium) or a blank control chamber until differentiation was complete or at various time points indicated in each experiment.

Cells were incubated at 37 °C in a suitable atmosphere containing 5% CO<sub>2</sub>.

### Cellular Oil Red O Staining

Before oil red O staining, the cells were fixed with 4% paraformaldehyde for 15 minutes. Then, the saturated oil red O dye was mixed with ddH<sub>2</sub>O at a ratio of 3:2, and the dye was filtered with a 0.45-µm filter. The cells were stained for 15 minutes and then cleaned with PBS for 3 times. All cells were photographed by Olympus microscope.

The staining was quantified by extracting the Oil Red O stain with 100% isopropyl alcohol, after which the absorbance was measured using a spectrophotometer (Evolution One; Thermo-Fisher Scientific) at 510 nm.

### Triglyceride Content

Adipocyte was rinsed twice with PBS. Add cell lysis buffer (1 M Tris-HCl, 1 M MgCl<sub>2</sub>, 10% Triton X100) into the dish, and lyse the cells thoroughly through a freeze-thaw

cycle. The triglyceride content was determined using triglyceride test kit (#632-50991, LabAssay™ Wako) according to the instruction. The triglyceride content of each cell sample was then normalized to its corresponding protein content.

### Glycerol and NEFA Release Assay

The primary dermal fibroblast was separated and induced to differentiation into white adipocyte for 7 days, as described above. The mature adipocytes were then co-cultured with/without 200 µg/mL PLLA for 24h. Adipocyte was then rinsed twice with Krebs-Ringer-HEPES (KRH) buffer (121 mM NaCl, 4.9 mM KCl, 1.2 mM MgSO<sub>4</sub>, 0.33 mM CaCl<sub>2</sub>, 12 mM HEPES, pH 7.4), followed by incubated with KRH buffer containing 3% fatty acid-free bovine serum albumin (BSA), and 1 µM isoproterenol at 37°C, for indicated times. The amounts of glycerol and non-esterified fatty acids (NEFA) released into the medium were determined with a free-glycerol assay kit (#E1012, Applygen) and a NEFA kit (#633-52001, LabAssay™ Wako), respectively.

### Animal Model

Male C57BL/6j mice at 8-week-old were anesthetized with isoflurane and shaved. Insert the syringe from the midline of the abdomen, carefully move it subcutaneously to the lateral position of the abdomen, and inject PLLA and ensure no leakage of the filling material. The control mice received equal amount of saline. The mice were then raised normally for 2 more month, and the skin at the injection site was taken for subsequent analysis. The inguinal subcutaneous adipose tissue (sWAT) and epididymal adipose tissue (eWAT) were also isolated from mice.

### Histology

Skin tissue above the injection site, fixed with 4% paraformaldehyde overnight, paraffin-embedded sectioned at the thick of 5 µm, rehydrated and stained with hematoxylin and eosin (H&E). All sections were photographed by Olympus microscope. The diameter of adipocytes was calculated via ImageJ software, from 6 to 8 sections of dWAT obtained using three random mice within each group.

### Protein Extraction and Immunoblotting

Protein samples were obtained from fresh cells, lysed with ice-cold lysis buffer containing 50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1% NP-40, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, with complete protease inhibitor (1 tablet/10 mL; Roche). Protein content

was determined using BCA Protein Assay Kit (Takara, T9300A). Adjust the protein concentration to same level and boiled samples for 10 min. Samples are uniformly processed by SDS-Page gel electrophoresis at a loading amount of 30 µg. The protein was transferred to the methanol pre-activated PVDF membranes (Millipore Corp, USA) and then blocked with 5% skim milk, followed by incubation with corresponding first and second antibodies. The protein band was visualized using High-sig ECL (Tanon, 180-5001).

### RNA Extraction, and Quantitative PCR

Trizol reagent (Invitrogen) was used to extract total RNA from cells. 1 µg RNA was reverse-transcribed into cDNA by the reverse transcription kit HiScript II QRT SuperMix (Vazyme, R222-01). After mixing with ChamQ SYBR qPCR Master Mix (Vazyme, Q321-02) in proportion, quantitative PCR was performed using Light Cycler 480 II Sequence Detection System (Roche, Basel, Switzerland). The RNA sequences of the primers used are available in Table 1.

### Statistical Analysis

The data presented in this paper were expressed as mean ± SEM. Significant differences are assessed using a two-tailed Student t test or one-way ANOVA for multiple group comparison. All data analyses were performed using GraphPad Prism 8 (GraphPad Software).  $P < 0.05$  was considered statistically significant.

## Results

### PLLA Treatment Reduced the dWAT Cell Size in C57 Mice

To analyze the potential regulatory effect of PLLA on adipose tissue, we constructed a mouse-based PLLA injection model. After subcutaneously injection of PLLA

(Fig. 1A), the rodent was then fed normally for two months. PLLA injection did not affect the overall body weight of the mice (Fig. 1B), either changed tissue weight of iWAT or eWAT (Fig. 1C).

However, histological staining of the dWAT showed interestingly changes. Masson staining suggest that the PLLA treatment retains collagens within the dermis (Fig. 1D), consistent with its well-known biofunction of collagen stimulator [19]. Contrast to the increased collagen, the thickness of dWAT in the PLLA-treated mice significantly decreased, as shown by the H&E staining (Fig. 1D); a statistical classification of the adipocyte size found that the proportion of large cells in the PLLA group significantly decreased, while the proportion of small fat cells increased accordingly (Fig. 1E). Moreover, the dWAT thickness at the non-injection site of the mice was comparable to those of the control ones, indicating that the reduction of dWAT only occurs at the injection site.

### PLLA Has no Significant Cytotoxicity in In Vitro Assay

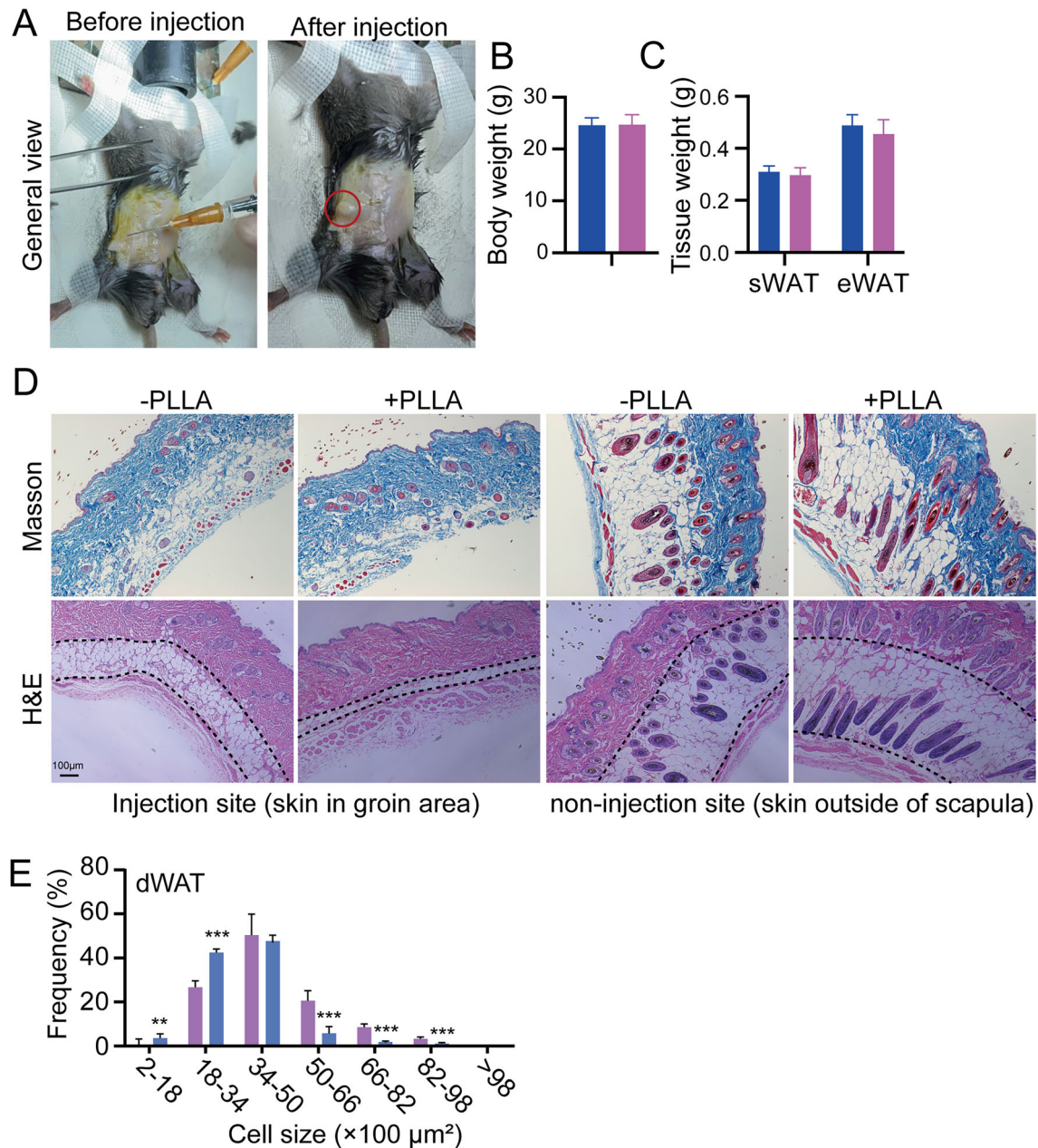
To explore the specific mechanism of PLLA (Fig. 2A) in reducing the size of dermal adipocytes, we constructed a co-culture model of PLLA and adipocytes derived from dermal fibroblast (Fig. 2B). Considering that the known biological effects of PLLA mainly rely on its degradation product—lactate, we first detected the level of lactic acid in the co-culture system. The results showed that PLLA co-culture can rapidly increase the level of lactate in the culture medium and stably persist for at least four days (Fig. 2C).

In order to clarify whether the mechanism of PLLA-lactate on adipocytes comes from the extracellular or intracellular, the cells were treated with 7ACC1, inhibitor that blocked the Mct1/4, the transporter of lactate. We found that the usage of 7ACC1 significantly reduced the level of lactate inside the adipocytes, but had no effect on the lactate level in the medium (Fig. 2C, D). The results of CCK8 assay showed that PLLA treatment did not significantly inhibit the viability of adipocytes (Fig. 2E),

**Table 1** Primers

Target gene	Forward	Reverse
Pparγ	GGAAGACCACTCGCATTCCTT	GTAATCAGCAACCATTGGGTCA
C/ebpα	GCGGGAACGCAACAACATC	GTCACCTGGTCAACTCCAGCAC
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAAG
Lipe	GATTACGCACGATGACACAGT	ACCTGCAAAGACATTAGACAGC
Pnpla2	ATGTTCCCGAGGGAGACCAA	GAGGCTCCGTAGATGTGAGTG
Abhd5	TGGTGTTCCACATCTACATCA	CAGCGTCCATATTCTGTTTCCA
Cidea	TGACATTCATGGGATTGCAGAC	CATGGTTTGAAACTCGAAAAGGG
Pgc1	TATGGAGTGACATAGAGTGTGCT	GTCGCTACACCACTTCAATCC
Ucp1	GTGAACCCGACAACCTCCGAA	TGCCAGGCAAGCTGAAACTC





**Fig. 1** PLLA treatment reduced the dWAT cell size in C57 mice. **A** PLLA injection model. After anesthesia, PLLA was injected under the skin of mice, as described in the Materials and Methods section. **B** The body weight of the mice, measured two months after PLLA injection. **C** The inguinal adipose tissue (iWAT) and epididymal adipose tissue (eWAT) weight of mice, measured two months

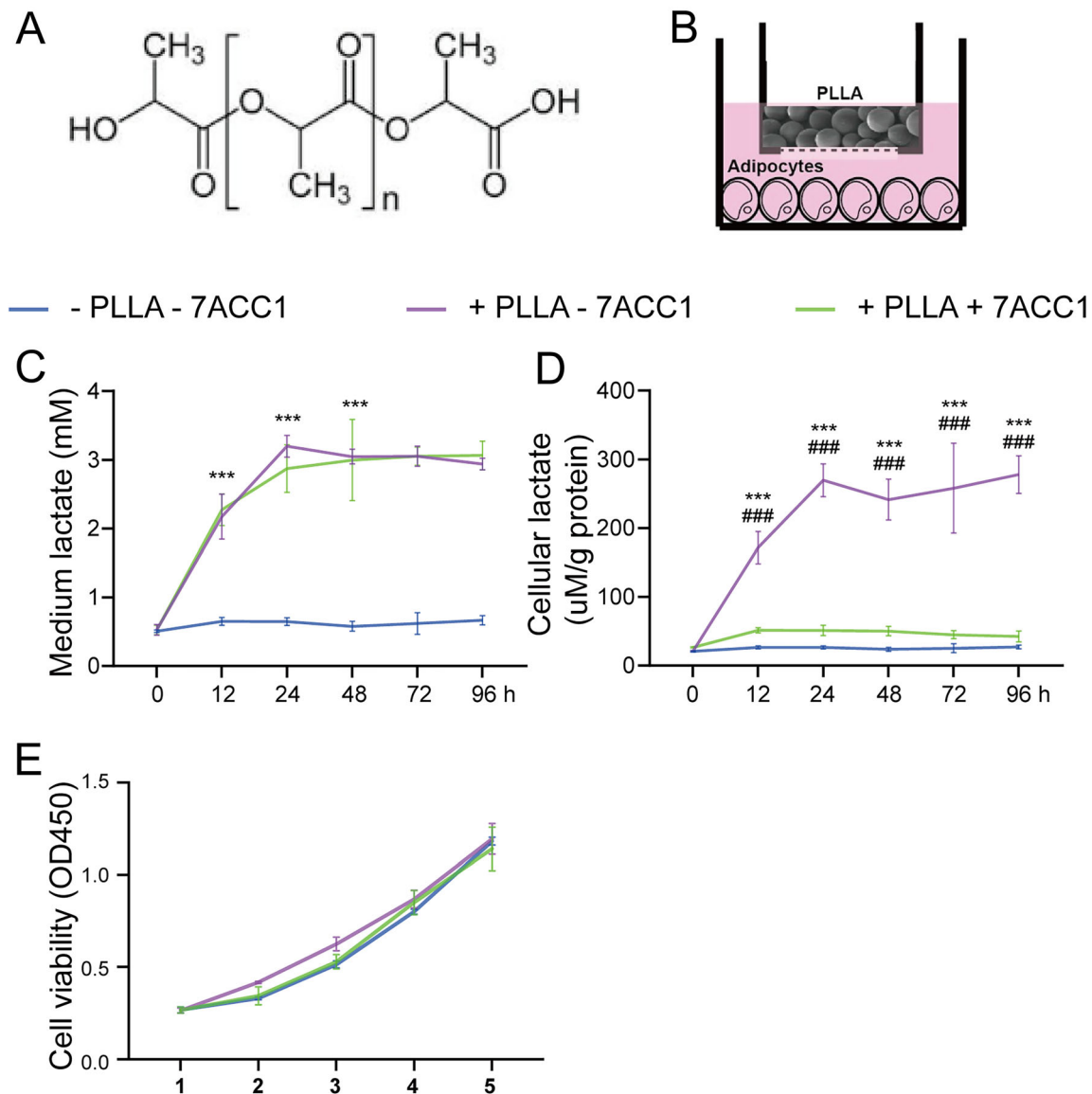
indicating that its effect on reducing adipocyte volume was not dependent on toxic effects.

#### PLLA Has no Effect on the Differentiation of Adipocytes and Fat Accumulation In Vitro

The in vitro effect of PLLA on adipogenesis was then explored. Neither PLLA only or PLLA plus Mct1/4

after PLLA injection. **D** Morphological staining of dermis. Top: Masson staining for collagen; bottom: H&E staining showing overall morphology. The dWAT is indicated between the two dashed line. **E** In dWAT, the percentage of adipocyte in relation to cell diameter were calculated. Values are represented by means +SEM. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

inhibitor treatment could dramatically altered the differentiation capacity of the adipocytes, as reflected by the number of lipid droplet stained by oil red O (Fig. 3A, B) and by the total triglyceride content within the fat cell (Fig. 3C). The expression levels of adipocyte marker genes, such as Ppar $\gamma$  (Fig. 3D), C/ebp $\alpha$  (Fig. 3E), and Fasn (Fig. 3F), were comparable between the three groups,



**Fig. 2** PLLA has no significant cytotoxicity on fibroblast and adipocyte. **A** The structural formula of PLLA. **B** The diagram of co-culture system using trans-well plates. **C**, **D** Lactate level in the medium (**C**) and adipocyte (**D**) were measured. The primary dermal fibroblast was induced differentiation into white adipocyte. After 4-days differentiation, the adipocyte was then co-cultured with / without PLLA for indicated time. The Mct1/4 inhibitor 7ACC1 were also added into the co-culture system at the start point.  $N=3$  for each

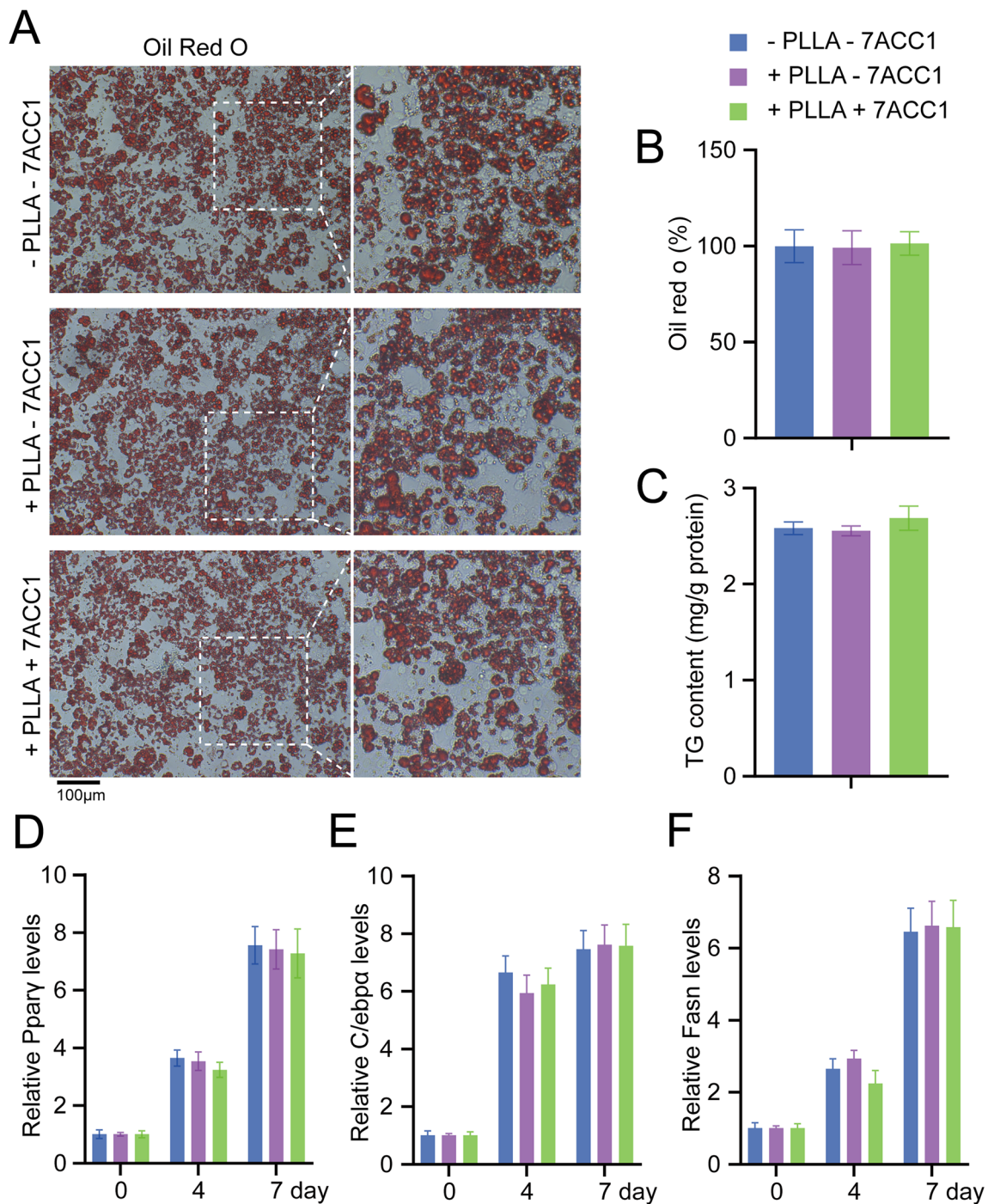
group. **E** The adipocyte viability was measured at indicated time point after PLLA treatment. The primary dermal fibroblast separated and differentiated into white adipocyte. At the beginning of differentiation, the cells were co-cultured with / without PLLA for indicated time.  $N=6$  for each group. Values are represented by means  $\pm$  SEM. + PLLA - 7ACC1 compared with - PLLA - 7ACC1,  $*p<0.05$ ;  $**p<0.01$ ;  $***p<0.001$ . + PLLA + 7ACC1 compared with + PLLA - 7ACC1,  $\#p<0.01$ ;  $\###p<0.001$

suggesting the adipogenesis were not affected by PLLA treatment.

### PLLA Enhances Dermis Adipocyte Lipolysis In Vitro

Next, we analyzed the potential impact of PLLA on lipolysis. Under the state of norepinephrine-stimulated lipolysis, the PLLA co-cultured adipocytes had a stronger fat mobilization ability, manifested by releasing more

lipolytic products, such as glycerol (Fig. 4A) and fatty acids (Fig. 4B), into the medium. Protein (Fig. 4C) and mRNA (Fig. 4D–F)-based expression assay suggested that PLLA significantly increased the expression of key lipolytic enzymes, such as p-HSL (Lipe), ATGL (Pnpla2), and Abdh5, indicating that these adipocytes have a stronger potential for fat decomposition. Interestingly, when blocking Mct1/4-mediated lactate uptake, the promotion effect of PLLA on lipolysis was almost completely abolished (Fig. 4A–F). The above data suggest that PLLA can



**Fig. 3** PLLA has no effect on the differentiation of adipocytes and fat accumulation in vitro. The primary dermal fibroblast was induced differentiation into white adipocyte. At the beginning of differentiation, the cells were co-cultured with / without PLLA. *N*=3 for both groups. **A** Oil red O staining of lipid droplets at day 8. **B** The

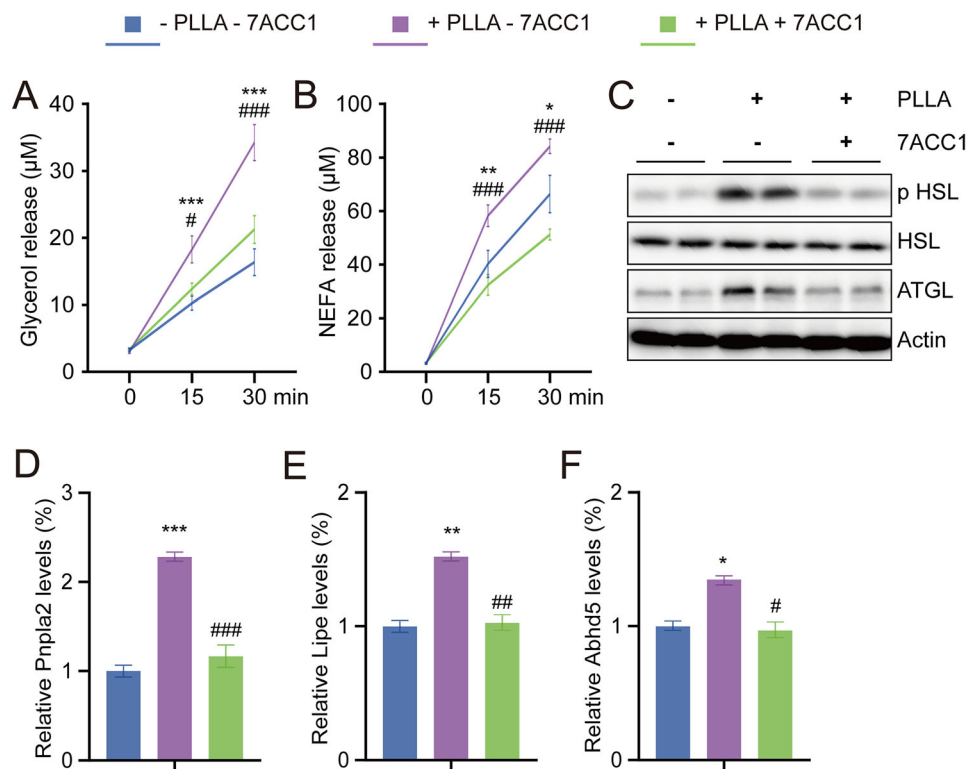
quantified measurement of Oil Red O staining. **C** Triacylglycerol (TG) content within the adipocyte were measured at day 8. **D–F** Quantitative PCR analyzing the mRNA level of white adipose marker gene, *Pparγ* (**D**), *C/ebpα* (**E**), and *Fasn* (**F**), at day 8. Values are represented by means +SEM

significantly promote lipolysis in adipocytes, and this effect is mainly dependent on the intracellular action of its metabolite, lactate, while the Gpr81 receptors have little role in this process.

### PLLA Enhances Beige Adipogenesis In Vitro

Due to its spatial distribution, dWAT can effectively receive external temperature stimuli and differentiate into thermogenic beige fat. PLLA treatment significantly





**Fig. 4** PLLA enhance dermis adipocyte lipolysis in vitro. The primary dermal fibroblast was induced differentiation into white adipocyte. Fully differentiated dermis adipocyte (at day 6 after differentiation) were co-cultured with / without PLLA for 24h.  $N=3$  for both groups. **A, B** Glycerol (**A**) and non-esterified fatty acid (**B**) release was measured with equal amount of dermis adipocyte ( $1 \times 10^5$ ) in the presence of isoproterenol at different time points.

**C** Immunoblotting measurement of the lipolysis enzyme, Hsl and ATGL, **D–F** Quantitative PCR analyzing the mRNA level of key lipolysis enzymes, Pnpla2 (**D**), Lipe (**E**), and Abhd5 (**F**). Values are represented by means  $\pm$  SEM. + PLLA - 7ACC1 compared with -PLLA - 7ACC1, \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . + PLLA + 7ACC1 compared with +PLLA - 7ACC1, #:  $p < 0.05$ ; ##:  $p < 0.01$ ; ###:  $p < 0.001$

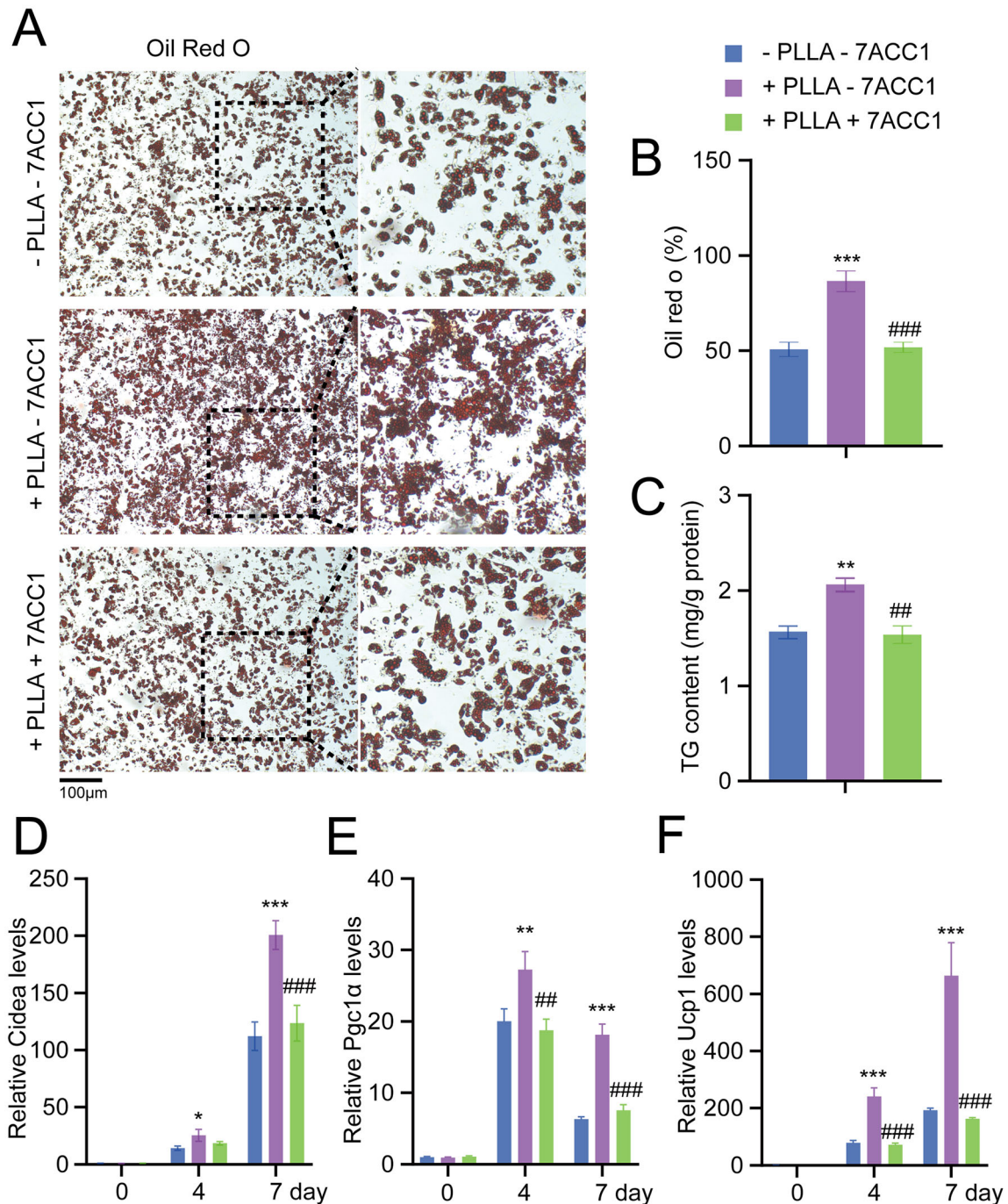
enhanced the beige adipocyte differentiation process, as reflected by the increase of lipid droplets (Fig. 5A, B) and total triglyceride content (Fig. 5C) within the adipocyte. Consistently, during the beige adipocyte differentiation, PLLA-stimulated cells exhibit higher expression levels of beige marker genes (Fig. 5D–F). Consistent with the results of the lipolysis assay, blocking Mct1/4-mediated lactate transport completely reversed the effect of PLLA on beige adipogenesis. The above results suggest that PLLA can activate the beige adipogenesis of dWAT through its metabolite lactate, and this process is dependent on Mct1/4.

## Discussion

As the aging population grows and becomes increasingly concerned with physical appearance, the number of patients seeking non-surgical rejuvenation treatments for the face and body is steadily rising. Women wanting to maintain a younger appearance and attractiveness account for 92% of all cosmetic procedures [19]. Men are keen to maintain physical characteristics associated with

masculinity [20]. Among the various treatment approaches, several minimally invasive techniques have been developed and rapidly applied in esthetic plastic treatment. PLLA, a well-established tissue filler and biostimulator that induces collagenases, is widely used among clinical practice to treat skin aging. PLLA was approved in esthetics in 1999 in Europe and in 2004 in Europe and in USA by the US Food and Drug administration (FDA). Since then, the worldwide adoption of PLLA has been driven by its ability to provide good long-term esthetic results, attributed to its biostimulatory-collagen effect. However, the precise regulatory effect of PLLA on different dermal cell subsets beyond fibroblast has not been fully elucidated. In this study, we constructed in vivo PLLA injection and in vitro PLLA-adipocyte co-culture models to analyze the regulatory effects of PLLA on the volume, differentiation, lipolysis, and thermogenic capacity of dermal adipocyte, one of the major cell types within the skin. Our results indicate that PLLA can exert a wide range of biological functions through its metabolite lactate, such as activating dWAT lipolysis and thermogenesis. Therefore, PLLA should not only considered as a collagen stimulator for





**Fig. 5** PLLA enhances beige adipogenesis in vitro. The primary dermal fibroblast was induced differentiation into beige adipocyte. At the beginning of differentiation, the cells were co-cultured with / without PLLA. *N*=3 for both groups. **A** Oil red O staining of lipid droplets at day 8. **B** The quantified measurement of Oil Red O staining. **C** Triacylglycerol (TG) content within the adipocyte were

measured at day 8. **D–F** Quantitative PCR analyzing the mRNA level of white adipose marker gene, Cidea (**D**), Pgc1α (**E**), and Ucp1 (**F**), at day 8. Values are represented by means +SEM. + PLLA - 7ACC1 compared with -PLLA - 7ACC1, \**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001. + PLLA + 7ACC1 compared with + PLLA - 7ACC1, ##: *p*<0.01; ###: *p*<0.001

clinical application, its regulatory effect on dermal adipocytes suggests that it may have more clinical application scenarios.

Skin is the most widely distributed and abundant tissue type in the human body. Over time, our skin exhibits aging changes, characterized by a reduction in skin thickness, which is mainly attributed to the loss of collagen.

Interestingly, recent model animals-based studies have found that dermal adipocytes actually increased during skin aging [8], and thus, this specific subgroup of adipocytes have been elevated to the prime targets in strategies aimed at counteracting skin aging [12]. In this study, we analyzed the regulatory effect of PLLA, the most widely used skin fillers, on the physiological function of dermal adipocytes both *in vivo* and *in vitro*. Our data revealed that in addition to its classic role in promoting collagen production, PLLA can activate lipolysis and thermogenesis of dermal adipocytes, but has no significant effect on the differentiation of dermal fat.

During aging, the skin and adjacent adipose tissue exhibit three distinct morphological changes: a moderate reduction in dermal thickness, a significant expansion of dWAT located at the dermal-subcutaneous tissue junction, and a significant reduction in sWAT [12, 21–23]. Interestingly, dWAT and sWAT exhibit opposite behaviors in chronological aging, suggesting that dWAT is a group of cells with unique functions that are significantly different from classical lipid-rich subcutaneous white adipocytes [8, 12]. In our study, subcutaneous injection of PLLA in mice significantly reduced the volume of dWAT, manifested as the decrease of the thickness of dWAT layer and reduced adipocyte size. The size of adipocyte mainly depends on the size of the lipid droplets they contain, so the lipid accumulating processes, such as adipogenesis, can increase the size of fat cells, while lipolysis, which reducing lipid droplets, shows opposite effect. We believed that the effect of PLLA on reducing dWAT adipocyte volume is mainly achieved by activating lipolysis and has no significant effect on adipogenesis. Interestingly, PLLA had no such effect on the sWAT. Further studies are required to fully delineate the function and mechanism of PLLA in the discriminatory regulation of dWAT and sWAT.

Fibroblasts and dermal adipocytes together constitute the main cellular components of the dermis and can carry out cell-cell communication through signaling molecules such as metabolites. Ablation of dermal adipocytes in murine model *in vivo* demonstrated a significant reduction of dermal fibroblast genes related to FFA oxidation [11]. This supports the notion that dermal adipocytes can supply FFAs as metabolites to adjacent dermal fibroblasts. In our study, PLLA-treated dermal adipocytes showed higher lipolysis rate, which on the one hand led to a reduction in fat volume, and on the other hand, there was also a possibility that PLLA indirectly regulated the function of fibroblasts by promoting dermal adipocyte secreting free fatty acids. This suggests that PLLA may have an indirect regulatory pathway dependent on adipocyte-fibroblast

communication in addition to its classical direct regulatory effect on fibroblasts. This hypothesis remains to be further confirmed in subsequent studies.

As the main metabolite of PLLA, lactate could affect the adipocyte function via two pathways, one is activating Gpr81 on the membrane as a ligand [26], the other one is being ingested by the cell via MCT1/4 and then act as a core metabolite [27]. In this study, we blocked the entry of lactate into dermal adipocytes with Mct1/4 inhibitor and found that it reversed the effects of PLLA, suggesting that the main effect of PLLA depends on the intracellular effect of lactate. However, considering the high expression of GPR81 in adipocytes and the fact that lactate activates adipocyte thermogenesis and beigeing via GPR81 [28], which is highly consistent with the regulatory effect of PLLA on dermal adipocyte, our study cannot completely exclude the potential involvement of GPR81. Generating adipocyte-specific GPR81 knock out mice will help to reveal whether GPR81 pathway also mediates the regulatory effect of PLLA on physiological functions of dermal adipocytes in the future.

In summary, our study revealed that PLLA possess regulatory effects on dermal adipocyte, such as reducing cell volume, enhancing lipolysis, and promoting beige adipogenesis. When evaluating the effect of PLLA on improving skin aging, the potential effects of adipocytes should not be ignored.

**Author Contributions** Gang Chen contributed to data curation, visualization, and validation; Wen Jin contributed to formal analysis; Kai Li contributed to funding acquisition, resources, writing—original draft and writing—review & editing; Wen Jin, Wei Che, Guanqun Qiao, and Yuequ Deng contributed to investigation; Kai Li and Wei Cai contributed to methodology; Wei Cai supervised the study and contributed to project administration; and Wen Jin and Gang Chen contributed to software. All authors have read and agreed to the published version of the manuscript.

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## Declarations

**Conflict of interest** The authors declare that they have no conflicts of interest to disclose.

**Ethical Approval** All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University (protocol code: 1911030). And, all applicable institutional and national guidelines for the care and use of animals were followed.

**Informed Consent** For this type of study, informed consent is not required.

## References

- Gilchrest BA (1989) Skin aging and photoaging: an overview. *J Am Acad Dermatol* 21(3):610–613. [https://doi.org/10.1016/s0190-9622\(89\)70227-9](https://doi.org/10.1016/s0190-9622(89)70227-9)
- Yaar M, Eller MS, Gilchrest BA (2002) Fifty years of skin aging. In: *Journal of investigative dermatology symposium proceedings*, 7(1):51–8. <https://doi.org/10.1046/j.1523-1747.2002.19636.x>
- Wong QYA, Chew FT (2021) Defining skin aging and its risk factors: a systematic review and meta-analysis. *Sci Rep* 11(1):22075. <https://doi.org/10.1038/s41598-021-01573-z>
- Gupta MA, Gilchrest BA (2005) Psychosocial aspects of aging skin. *Dermatol Clin* 23(4):643–648. <https://doi.org/10.1016/j.det.2005.05.012>
- Mays PK, Bishop JE, Laurent GJ (1988) Age-related changes in the proportion of types I and III collagen. *Mech Ageing Dev* 45(3):203–212. [https://doi.org/10.1016/0047-6374\(88\)90002-4](https://doi.org/10.1016/0047-6374(88)90002-4)
- Hosseini M, Koehler KR, Shafiee A (2022) Biofabrication of human skin with its appendages. *Adv Healthc Mater* 11(22):e2201626. <https://doi.org/10.1002/adhm.202201626>
- Kruglikov IL, Scherer PE (2016) Dermal adipocytes and hair cycling: is spatial heterogeneity a characteristic feature of the dermal adipose tissue depot? *Exp Dermatol* 25(4):258–262. <https://doi.org/10.1111/exd.12941>
- Zhang Z, Shao M, Hepler C, Zi Z, Zhao S, An YA et al (2019) Dermal adipose tissue has high plasticity and undergoes reversible dedifferentiation in mice. *J Clin Invest* 129(12):5327–5342. <https://doi.org/10.1172/jci130239>
- Bilkei-Gorzo A, Drews E, Albayram Ö, Piyanova A, Gaffal E, Tuetting T et al (2012) Early onset of aging-like changes is restricted to cognitive abilities and skin structure in *Cnr1*<sup>−/−</sup> mice. *Neurobiol Aging* 33(1):200.e11–22. <https://doi.org/10.1016/j.neurobiolaging.2010.07.009>
- Wang Y, Marshall KL, Baba Y, Gerling GJ, Lumpkin EA (2013) Hyperelastic material properties of mouse skin under compression. *PLoS ONE* 8(6):e67439. <https://doi.org/10.1371/journal.pone.0067439>
- Zhang Z, Kruglikov I, Zhao S, Zi Z, Gliniak CM, Li N et al (2021) Dermal adipocytes contribute to the metabolic regulation of dermal fibroblasts. *Exp Dermatol* 30(1):102–111. <https://doi.org/10.1111/exd.14181>
- Kruglikov IL, Scherer PE (2016) Skin aging: are adipocytes the next target? *Aging (Albany NY)* 8(7):1457–1469. <https://doi.org/10.18632/aging.100999>
- Goldberg D, Guana A, Volk A, Daro-Kaftan E (2013) Single-arm study for the characterization of human tissue response to injectable poly-L-lactic acid. *Dermatol Surg* 39(6):915–922. <https://doi.org/10.1111/dsu.12164>
- Fitzgerald R, Bass LM, Goldberg DJ, Graivier MH, Lorenc ZP (2018) Physiochemical characteristics of poly-L-lactic acid (PLLA). *Aesthet Surg J* 38(1):S13–S17. <https://doi.org/10.1093/asj/sjy012>
- Lorenc ZP (2012) Techniques for the optimization of facial and nonfacial volumization with injectable poly-L-lactic acid. *Aesthetic Plast Surg* 36(5):1222–1229. <https://doi.org/10.1007/s00266-012-9920-3>
- Moyle GJ, Lysakova L, Brown S, Sibtain N, Healy J, Priest C et al (2004) A randomized open-label study of immediate versus delayed poly(lactic acid) injections for the cosmetic management of facial lipotrophy in persons with HIV infection. *HIV Med* 5(2):82–87. <https://doi.org/10.1111/j.1468-1293.2004.00190.x>
- Oh S, Lee JH, Kim HM, Batsukh S, Sung MJ, Lim TH et al (2023) Poly-L-lactic acid fillers improved dermal collagen synthesis by modulating M2 macrophage polarization in aged animal skin. *Cells* 12(9):1320. <https://doi.org/10.3390/cells12091320>
- Brady JM, Cutright DE, Miller RA, Barristone GC (1973) Resorption rate, route, route of elimination, and ultrastructure of the implant site of polylactic acid in the abdominal wall of the rat. *J Biomed Mater Res* 7(2):155–166. <https://doi.org/10.1002/jbm.820070204>
- Christen MO (2022) Collagen stimulators in body applications: a review focused on poly-L-lactic acid (PLLA). *Clin Cosmet Investig Dermatol* 15:997–1019. <https://doi.org/10.2147/ccid.S359813>
- Wat H, Wu DC, Goldman MP (2018) Noninvasive body contouring: a male perspective. *Dermatol Clin* 36(1):49–55. <https://doi.org/10.1016/j.det.2017.09.007>
- Kruglikov IL, Zhang Z, Scherer PE (2019) Caveolin-1 in skin aging - from innocent bystander to major contributor. *Ageing Res Rev* 55:100959. <https://doi.org/10.1016/j.arr.2019.100959>
- Salzer MC, Lafzi A, Berenguer-Llargo A, Youssif C, Castellanos A, Solanas G et al (2018) Identity noise and adipogenic traits characterize dermal fibroblast aging. *Cell* 175(6):1575–90.e22. <https://doi.org/10.1016/j.cell.2018.10.012>
- Zhang W, Qu J, Liu GH, Belmonte JCI (2020) The ageing epigenome and its rejuvenation. *Nat Rev Mol Cell Biol* 21(3):137–150. <https://doi.org/10.1038/s41580-019-0204-5>
- Liu C, Wu J, Zhu J, Kuei C, Yu J, Shelton J et al (2009) Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. *J Biol Chem* 284(5):2811–2822. <https://doi.org/10.1074/jbc.M806409200>
- Lagarde D, Jeanson Y, Barreau C, Moro C, Peyriga L, Cahoreau E et al (2021) Lactate fluxes mediated by the monocarboxylate transporter-1 are key determinants of the metabolic activity of beige adipocytes. *J Biol Chem* 296:100137. <https://doi.org/10.1074/jbc.RA120.016303>
- Ahmed K, Tunaru S, Tang C, Müller M, Gille A, Sassmann A et al (2010) An autocrine lactate loop mediates insulin-dependent inhibition of lipolysis through GPR81. *Cell Metab* 11(4):311–319. <https://doi.org/10.1016/j.cmet.2010.02.012>
- Kobayashi M, Narumi K, Furugen A, Iseki K (2021) Transport function, regulation, and biology of human monocarboxylate transporter 1 (hMCT1) and 4 (hMCT4). *Pharmacol Ther* 226:107862. <https://doi.org/10.1016/j.pharmthera.2021.107862>
- Yao Z, Yan Y, Zheng X, Wang M, Zhang H, Li H et al (2020) Dietary lactate supplementation protects against obesity by promoting adipose browning in mice. *J Agric Food Chem* 68(50):14841–14849. <https://doi.org/10.1021/acs.jafc.0c05899>

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