ARTICLE
Biomedical Engineering Communications 2023;2(2):9. https://doi.org/10.53388/BMEC2023009

An anti-aging skin strategy: promoting repair and regeneration in UV-induced photoaging micro-environment using growth factors-rich platelet lysates composite Self-protection Collagen Hydrogel

Jian-Peng Zhang[1#], Zi-Duo Huang[1#], Bing-Qing Wang[1], Yi-Xin Chen[1], Qing-Qing Zhou[1], Hui-Qin Li[1], Xin-Sheng Peng[2*], Yan-Fang Zhou[1*]

[1]Department of Pathophysiology, Guangdong Medical University, Dongguan 523808, China. [2]Department of Pharmacy, Guangdong Medical University, Dongguan 523808, China.

[#]Jian-Peng Zhang and Zi-Duo Huang are the co-first authors of this paper.

***Corresponding to:** Xin-Sheng Peng, Department of Pharmacy, Guangdong Medical University, 1 Xincheng Road, Songshan Lake, Dongguan 523808, Guangdong, China. E-mail: xspeng@gdmu.edu.cn. Yan-Fang Zhou, Department of Pathophysiology, Guangdong Medical University, 1 Xincheng Road, Songshan Lake, Dongguan 523808, Guangdong, China. E-mail: yfzhou@gdmu.edu.cn.

Author contributions

Jianpeng Zhang, Ziduo Huang: Data analysis and writing. Ziduo Huang: Formal analysis. Xinsheng Peng, Yanfang Zhou: Supervision and funding acquisitioning. Bingqing Wang, Yixin Chen, huiqin Li: Investigation. Jianpeng Zhang, Qingqing Zhou: Methodology and conceptualization.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This research received funding from the Discipline construction project of Guangdong Medical University (4SG21277P, 2021ZDZX2039, 2DK21003, 4SG23060G). Our work described in the present manuscript was also supported by research grants from Guangdong Provincial Natural Science Foundation (2021A1515012437, 2021KTSCX040), Guangdong, China.

Peer review information

Biomedical Engineering Communications thanks Fu-Wen Yuan and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

ROS, Reactive oxygen species; Col, Collgen; SOD, Superoxide Dismutase, GSH, L-Glutathione; MDA, malondialdehyde; PL, platelet lysates; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; CTGF, connective tissue growth factor; EGF, epidermal growth factor; Col-I, Col type I; PPP, poor polar platelets; PRP, platelet-rich plasma; PBS, phosphate buffered saline; HE, Hematoxylin-eosin.

Citation

Zhang JP, Huang ZD, Wang BQ, et al. An anti-aging skin strategy: promoting repair and regeneration in UV-induced photoaging micro-environment using growth factors-rich platelet lysates composite Self-protection Collagen Hydrogel. *Biomed Eng Commun*. 2023;2(2):9. doi: 10.53388/BMEC2023009.

Executive editor: Zi-Yao Feng.

Received: 12 April 2023; **Accepted:** 16 May 2023; **Available online:** 19 May 2023.

Abstract

Skin photoaging is induced and sustained by UV-induced oxidative damage, and stimulating regeneration of the UV-induced aging has remained a great challenge due to high-level oxidative stress factor (ROS) -induced chronic oxidative damage and inactivation of bio-macromolecule-based regeneration in oxidative photoaging micro-environment. In this study, we designed a “seed and soil” strategy to pursue a safer and more efficient way to prevent and treat photoaging by simultaneously changing UV-induced ROS-rich micro-environment into a proregenerative one (the “soil”) and providing growth factor-rich platelet lysates (PL, the “seed”) using PL-impregnated, collagen-reinforce hydrogel (PL/Col). SD rats were used to establish photoaging model by 8 weeks of UV irradiation. The effectiveness of different treatments was evaluated by making pathological sections and detecting photoaging-related indicators. Rats treated with PL/Col demonstrated a significant acceleration in skin healing and enhancement in the quality of trauma repairing. After treated with PL/Col, the rats showed smooth yellowish appearance, integral structure of skin collagen fiber and epidermis, a decrease in inflammation and a reshaped active micro-environment with reduced levels of SOD enzyme activity, GSH enzyme activity and MDA toxic products. Treatment of PL/Col in skin photoaging has shown potential anti-oxidation and anti-aging effects and is worthy of further study in related field.

Keywords: photoaging; platelet derivatives; oxidative stress; growth factors; collagen; trauma repair

Biomedical Engineering Communications 2023;2(2):9. https://doi.org/10.53388/BMEC2023009

Introduction

Skin photoaging is a universal phenomenon mainly caused and sustained by UV-induced oxidative damage [1]. However, the development of industry and depletion of ozone layer in recent years intensify the ultraviolet radiation, further increase the risk of skin photoaging [2]. A recent study indicated that ultraviolet radiation had become the most harmful factor of skin photoaging [3]. UV is mainly composed of UVA (λ = 320–400 nm), UVB (λ = 280–320 nm) and UVC (λ = 100–280 nm). UVC ray, different from UVA and UVB rays, is completely absorbed by the ozone layers. Therefore, the skin structure is mainly damaged by the combined action of UVA and UVB radiations [4]. Short time exposure to ultraviolet light can damage the skin barrier, causing erythema, dry skin, pigmentation and skin lesion [5]. Chronical radiation to the skin may lead to skin photoaging even skin cancer, such as solar keratosis, elastic fiber, melanin cell carcinoma and basal cell tumor [6]. This process is mainly caused by the excessive oxidative stress reaction producing a large amount of active oxygen and free radicals (ROS), which would damage dermis collagen, elastic fibers, proteoglycan and epidermal cells [1]. High levels of ROS can also damage capillaries in epidermis, limit the delivery of oxygen and nutrients and lead to massive destruction of endogenous stem cells, growth factors and nucleic acids in the damaged tissue, limiting their regeneration potential greatly [7]. Long-term photoaging often leads to chronic inflammatory damage, and direct injection of nucleic acids or growth factors in the UV oxidative damage micro-environment can be easily destroyed by ROS [8]. Therefore, it is urgent to design an effective treatment for the UV-induced photoaging micro-environment.

In recent years, the research on the prevention and treatment of photoaging has developed rapidly, which mainly includes chemical stripping agent therapy and physical therapy [4, 9–11]. Physical therapy such as laser and intense pulse light, radio frequency surgical treatment and micro-needle array, has a confirmed efficacy to control and improve the appearance of skin, but it cannot quickly promote the regeneration of damaged tissue, and it have the risk of deterioration [9]. Chemical stripping treatment or chemical agent therapy like applying, injecting and filling chemical drug, have certain toxicity and side effects [10, 11]. Therefore, it is urgent to find safe and efficient natural products to prevent ultraviolet radiation to protect modern people against the harm of skin photoaging.

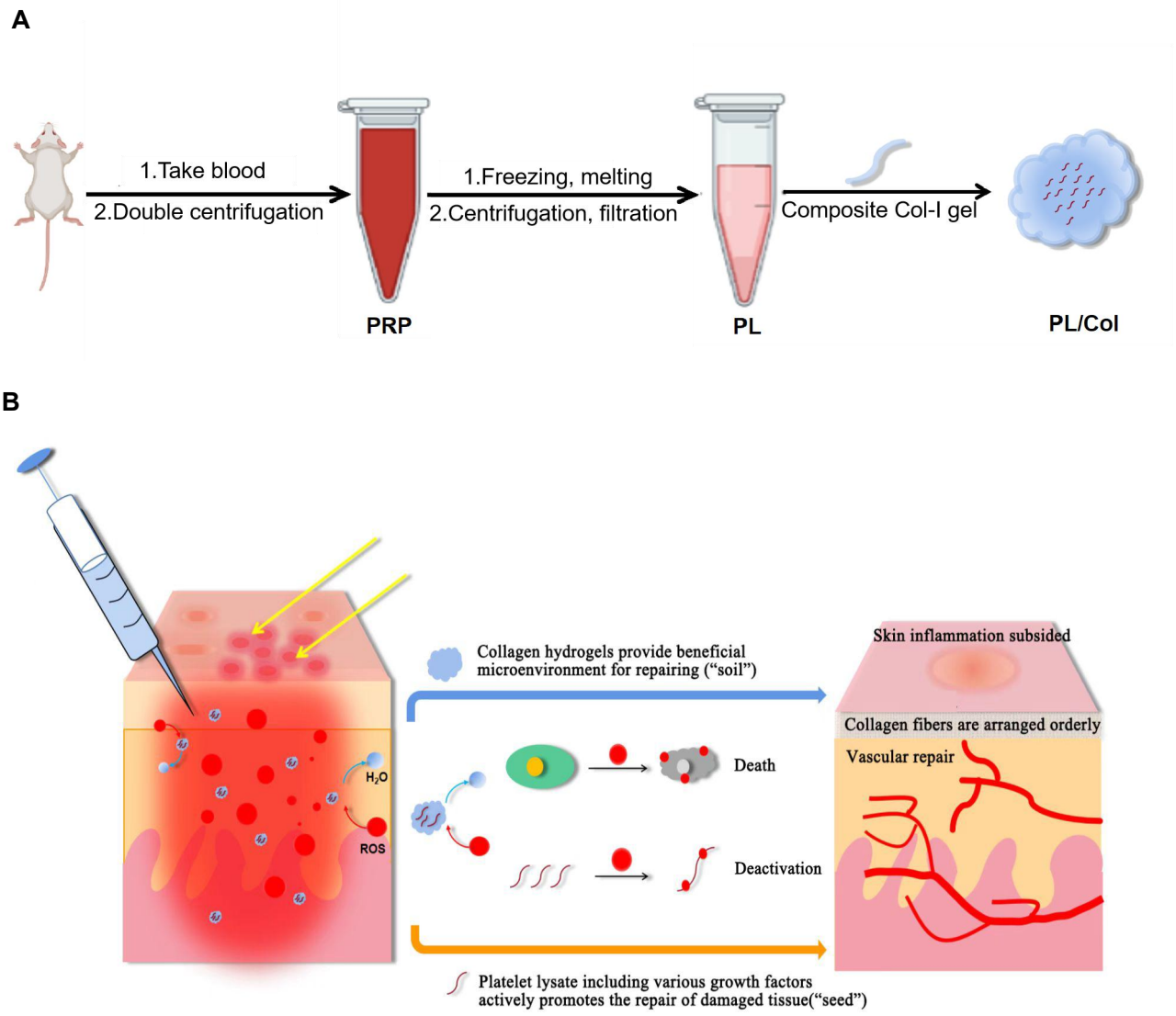

Figure 1 Schematic illustration of the fabrication process for PL/Col, and the strategy for restorative and regenerative skin photoaging treatment. Schematic illustration of the PL/Col-enabled strategy for simultaneous self-protection carrier of proregenerative PL (A); creation of active micro-environment for photoaging skin healing (B).

Platelet derivatives, active biological agents, are extracted from platelets *in vitro* and processed by a series of special processes [12]. They are rich in a variety of growth factors, which include platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF) and epidermal growth factor (EGF) [13]. They can rapidly promote wound healing, regeneration of defective tissues and vascular reconstruction [13]. Many experiments have proved that platelet derivatives play an important role in the repairing of injured tissues [12, 14]. However, their clinical application has not been widely promoted [15]. The main reasons may be related to the harsh storage conditions, strong immunogenicity and lack of unified extraction and preparation standards [12]. Some new studies reported a new generation of platelet derivatives, i.e. platelet lysates (PL), can effectively prolong the storage time and reduce the immunogenicity [16, 17]. This may be related to its optimizing extraction process: activating platelets to release growth factors and filtering out excess cell debris [15]. Nevertheless, an insurmountable obstacle is their rapid breakdown and inactivation in the hostile micro-environment, in which the excessive oxidative stress leads to the UV-induced skin damage [1]. In addition to the UV-induced photoaging, excessive ROS is tightly linked with a myriad of serious diseases, where PL could serve as a promising therapeutic approach [12, 16, 17]. Unfortunately, to the best of our knowledge, studies devoted to the design and construction of highly efficient PL carriers with self-protecting capacity in the hostile oxidative micro-environment have been rarely reported so far and the use of the PL has been faced with severe challenge [18, 19]. Meanwhile, engineering a friendly micro-environment is increasingly recognized as a novel paradigm for the successful healing of skin photoaging [20, 21]. Accordingly, natural materials applied to simultaneously deliver regenerative PL cue (the “seed”) in a self-protected way and rebuild the hostile UV-induce chronically oxidative micro-environment(the “soil”) are desired yet challenging for the treatment in photoaging [1, 2, 6, 20, 21]

Col type I (Col-I), as a natural material, possesses the advantages of good biocompatibility, low immunogenicity, repaid degradation and absorption [22, 23]. As a drug carrier, it can effectively maintain the stability of loaded drugs and facilitate the recovery of injured tissue [24, 25]. Furthermore, some studies have shown that Col-I is not only an excellent natural delivery matrix, but also a natural anti-oxidant providing beneficial growth micro-environment for tissue formation, cell attachment and proliferation in special ROS-induced damage. These effects may be linked to the hydrolysates and peptide fractions of Col-I and the enhancements of the content and activity of reductases *in vivo* [24–29]. PL and collagen are derived from natural biological agents, which have the advantages of low immunogenicity, small toxic side effects, low cost, and synergistic interaction [23, 28, 30, 31]. These beneficial intrinsic properties of Col-I make it superior to other conventional antioxidant molecules or enzymes. On the basis of these considerations, we introduced a unique “seed-and-soil” strategy: natural anti-oxidant Col-I as the “soil” provides active regeneration micro-environment, and growth factors-rich PL as the “seed” promotes UV-induced photoaging skin healing, aiming to design PL composite Col-I hydrogel (PL/Col) as a safe and efficient natural product to control and treat skin photoaging (Figure 1A, B).

Methods

Animal Groups

25 male SD rats within a month (SPF, Southern Medical University, China) were randomly divided into 5 groups with 5 rats in each group: (a) Control group, (b) Radiation group, (c) Radiation + PL/Col group, (d) Radiation + PL group and (e) Radiation + Col group (Table 1). The rats in group b to c were treated with daily UV-irradiation after shaving, while the rats in group a were only treated with daily shaving. The Ethics Comments of laboratory animal ethical committee (LAEC) approved the study, grant No. GDY2004022.

Establishment of the Photoaging Model

After one week of adaptive rearing, the rats were treated with UV-irradiation (UVA, 320–360 nm, Philips UVB, 290–320 nm Philips) at an initial dose (800 W/cm^2 UVA and 200 W/cm^2 UVB, 18 min). The dose of UV-irradiation increased 30–40% weekly, and the treatment of UV-irradiation last for total 8 weeks. Finally, photoaging model was established by cumulative exposure dose of UVA at 112.9 mJ/cm^2 and UVB at 33.0 mJ/cm^2.

Extraction of the Platelet Lysate

Whole blood of 15 rats in the blood supply group was extracted from the celiac artery for vitro platelet extraction. About 6 mL of whole blood was extracted from each rat. The whole blood was collected into a 2 mL centrifuge tube and centrifuged at 1500 r/min for 10 minutes. Then, upper plasma and middle white film were taken to a 1.5 mL centrifuge tube, which was centrifuged again at 3000 r/min for 10 minutes. The upper 3/4 layer was discarded as poor polar platelets (PPP), and the lower 1/4 layer was mixed and quickly put into −20 ˚C for storage as platelet-rich plasma (PRP). After 24 h, PRP were taken out and incubated in 37 ˚C water bath for 5 minutes. Then PRP was centrifuged at 3000 r/min for 10 minutes in a 4 ˚C pre-cooled machine. The upper lysate was collected and filtered through a 0.22 μm [32] membrane, and then put into −20 ˚C refrigerator. The above process was repeated for 3 times. Finally, a part of the PL was taken out from the −20 ˚C refrigerator as a sample to detect the content of growth factor with ELISA kits (Jiangsu Jingmei Biological Technology Co., Ltd, China) , and the remaining PL was stored in a 4 ˚C refrigerator for use (Figure 2).

Extraction of Col-I

Col-I was extracted from bovine Achilles tendon by an acid-enzyme method. The extraction was then passed through a 0.22 μm [32] pore size membrane filter and finally stored at 4 ˚C refrigerator. After that, SDS-PAGE gel electrophoresis and UV-vis detection were used for qualitative analysis and concentration determination. The solubility of collagen was determined according to the method specified in General Rule 15 of Part 4 of the 2015 edition of the Chinese Pharmacopoeia. To observe the appearance and microstructure, collagen was imaged under a scanning electron microscopy and light microscopy. The structure of collagen was studied with FT-IR. PL/Col, consistent with platelet lysate and collagen (1:1), was analyzed by ELISA and UV-vis detection.

Skin Appearance Evaluation

During the 8 weeks of radiation, the conditions of back skin of rats were photographed and recorded, and the dose of UV irradiation was adjusted according to the changes of skin morphology.

Injection Therapy

After 8 weeks of radiation, the rats in different groups were given different treatments (Table 1).

Evaluation of Treatment Effect

After 1 week of treatment, the changes of back skin morphological of each rat were recorded. Then the rats were sacrificed and the skin tissue from the back was collected for pathological analysis (HE Staining, PH0516, PHYGENE, Masson Staining, G1340, Solarbio) and biochemical detection (MDA, A003-1, Nanjing Jiancheng, SOD, A001-2, Nanjing Jiancheng, GSH-Px, A005, Nanjing Jiancheng, BCA, P0010, Beyotime).

Results

Extraction Process of PL and Analysis Quality among Extracted Samples

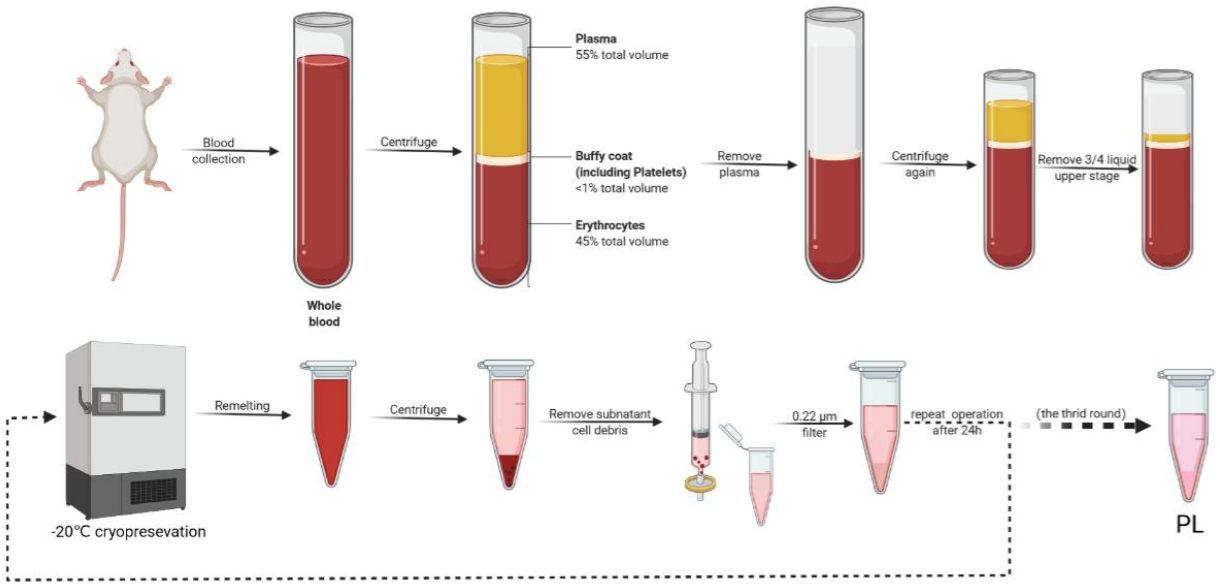

Figure 2 Schematic Representation of PL Extraction

Table 1 Different groups of the rats

Group	Treatment
Control group	Normal feeding (shaved only)
Radiation group	Injected with 1mL normal saline
Radiation + PL/Col group	Injected with 1 mL PL/Col
Radiation + PL group	Injected with 1 mL PL
Radiation + Col group	Injected with 1 mL of collagen

The facile process to extract PL begins with the whole blood extracted from the rats, followed by two-steps centrifugation (1500 r/min 10 min then 3000 r/min 10 min) for PRP preparation in 4 °C. After the first time centrifuging, the plasma, buff coat and erythrocytes were significantly separated, which were more remarkable after the removing of 3/4 upper part and the second time centrifugation (Figure 3A). It is necessary to preserve the buff coat between the plasma and erythrocytes since the buff coat is rich in platelets that can release various types of growth factors and natural products in favor of trauma repairing. The PRP passed through multi-step manufacturing processes to reduce cell debris and promote the release of related growth factors, which was beneficial to prolong the storage time and reduce immunogenicity. Notably, there were some cell debris pelleting in the bottom of the centrifuge tube after −20 °C/37 °C melting and centrifugation (Figure 3A). Then its surface part was taken out to filter through a 0.22 μm [32] membrane and the process was repeated two times. The final centrifuge tube image with few cell debris pelleting in the bottom (Figure 3A) indicated that the platelet had been completely released related-active molecules and the PL preparation had been completed.

Recent studies reported that platelet derivatives like PL could release various types of growth factors which would accelerate healing and regeneration [12, 13, 17]. Therefore, three type of growth factors content of PL were monitored to possess the quality of preparation. The detected results showed that the content of PDGF in PL was slightly higher than PRP, and the contents of VEGF and TGF-β in PL were scarcely lower than PRP, whereas statistical analysis revealed that there was no significant difference between the groups (Figure 3B, C, D and Table 2). Therefore, PL basically cut the mustard, and compared with PRP, PL can prolong the storage time and reduce immunogenicity, which will be shown in the following results.

Extracted Samples

Abstraction process of PL:extracted the whole blood from the celiac artery of rats and used multi-step process to extract PL (A). ELISA analysis of plate-derived growth factor (PDGF) content in extracted samples (B). Comparison of vascular endothelial growth factor (VEGF) content among the samples (C). Column showing the sum of transforming growth factor (TGF)-β content detected by ELISA (D).

Preparation of Col-I and its Qualitative Detection

The quality of Type I collagen that extracted from bovine Achilles tendon by acid-enzyme extraction method was tested by UV-Vis and SDS-PAGE gel electrophoresis. The absorption of this collagen in

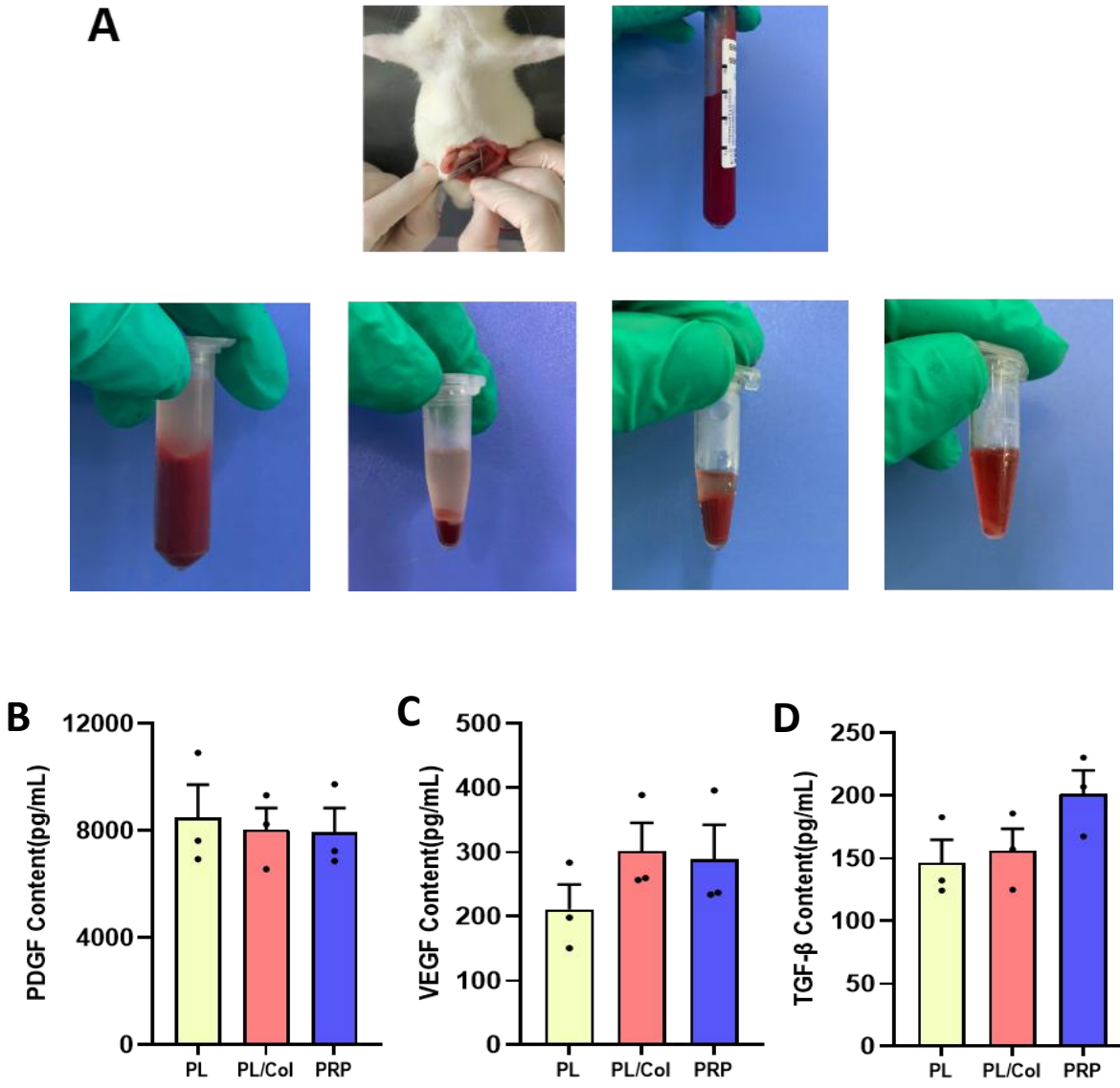

Figure 3 Extraction Process of PL and Analysis Quality among

Table 2 The detection of growth factors in the samples with ELISA kits (pg/mL)

	VEGF	PDGF	TGF-β
PL	231.731 ± 30.987	6794.856 ± 197.196	146.635 ± 31.744
PL/Col	356.387 ± 62.919	7707.490 ± 503.697	156.152 ± 30.400
PRP (after Ca^{2+} activation)	214.054 ± 55.842	9996.320 ± 822.949	201.784 ± 31.820

UV-Vis spectrum (rang 210 to 600 nm) has been shown in Figure 4A, and there was a maximum UV absorption peak near to 235 nm, without obvious strong peak near to 280 nm, which was consistent with the characteristics of Col-I. In the image of SDS-PAGE gel electrophoresis strip B (extraction of collagen), it can be clearly seen that α1, α2, β and γ subunit were separated by different migration rate in accord with the Col-I qualitative detection, which also can be seen in strip A (standard substance of type I collagen) in Figure 4B. Taken together, these results supported that the preparation of collagen meets the characteristics of type I collagen, and can be used to provide oxidative damage tissue actively regenerative micro-environment. Therefore, the concentration of collagen can be

determined by Coomassie Brilliant Blue G-250 method. After detecting the absorbance in 540 nm, the concentration of Col-I was calculated and the result was shown in Table 3.

Microscopic and Apparent Structures of Collagen

Collagen is white and odorless, and appears in the form of loose fibers or micro clumps (Figure 5A). These small units enable them to quickly adhere to various irregular shaped parts, creating a renewable microenvironment. As shown in Figure 5B, the FT IR spectra of collagen and Avit-Flour showed three characteristic peaks at 1652 cm^{-1}, 1539 cm^{-1}, and 1238 cm^{-1}, respectively, corresponding to amide bands I, II, and III. Amide I is mainly caused by the tensile vibration of protein amide C = O. Amide II consists of the bending vibration of amide N-H and the tensile vibration of C-N. Amide III is composed of planar bending N-H, planar stretching C-N, and CH2 groups of amide bonds that vibrate from the glycine backbone and proline side chains. Amide A is mainly caused by N-H tensile vibration, with vibration wave numbers between 3400 cm^{-1} and 3440 cm^{-1}. The peak at 3298 cm^{-1} is due to the N-H tensile vibration of the amide A band caused by C-H asymmetry, while the peak at 2937 cm^{-1} is due to the tensile vibration of the amide B band. In the range of 1450 cm^{-1}~1230 cm^{-1}, the ratio of collagen characteristic peaks is 0.95 for collagen microfibers and 0.90 for Avit-Flour, indicating the integrity of collagen's triple helix structure [33] (Table 4).

Solubility testing of collagen protein

The solubility test results showed that collagen is almost insoluble in pure water, phosphate buffered saline (PBS) solution, physiological saline and 0.5 mol/L acetic acid solvent, demonstrating its ideal performance as a drug for preventing photoaging. In addition, the sample rapidly expanded in the studied solution. Collagen has poor solubility, which enables it to provide a microenvironment for active regeneration of oxidative damaged tissues [33].

Comparison and Analysis Preserving Effect between PL/Col and PL Alone in -20℃ Environment after 14 Days

Recent studies indicted that collagen was beneficial to the preservation of biological active proteins, providing similar micro-environment *in vivo* and protecting these proteins against external destruction [24, 25, 27]. To assess the preserving effect, the contents of three main types of growth factors were detected. As exhibited in the columns, the concentrations of PDGF and VEGF in PL/Col were slightly higher than PL alone, although there was no significant difference between two groups (Figure 5A, B). The result in column C showed that the content of TGF-β in PL/Col was obviously higher than PL alone (Figure 5C). In addition, Table 2 clearly revealed the detailed data of the contents of growth factors as follows. These results supported that the preserving effect of PL/Col was better than PL alone. Thus we chose PL/Col as a more effective therapy for photoaging in the following animal experiment.

Establishment of Rats Photoaging Model and Assessment via Appearance and Pathological Section Evaluation

Recently, some studies reported that UVA and UVB working together mainly contributed to chronic skin injury that induced photoaging (although the penetration of UVA alone is too weak to cause sun-induced skin damage) [3, 6]. Therefore, our team decided to choose UVA and UVB to simulate sunlight radiating to the rats for 2 months (two months in rats lifespan are close to 6.66 years of human life) to establish rat sun-induced photoaging. As expected, after 60 days of UV exposure, the back skin appearance of the rats in the radiation group presented remarkable skin photoaging characteristics: erythema and inflammation, which resulted from robust UV-induced oxidative damage (Figure 6A).

HE and Masson's trichrome stainings of the back skin of rats in the radiation group revealed significant thickening of epidermis and loss of epidermal ridge (yellow bracket sign) (Figure 6B, C). Indeed, some inflammation in epidermis and glandular hyperplasia can be observed in HE and Masson stainings (Figure 6B, C). Taken together, these findings suggested that the rat photoaging model was successfully established and endowed with the capacity to explore the effects of different treatments in photoaging.

PL/Col Reshapes Oxidative Stress Micro-environment

SOD enzyme and GSH enzyme are vital reductase *in vivo*. They can protect against the oxidative stress damage. Some studies indicated that collagen can effectively increase the concentration and activity of reductase and then reshape oxidative stress micro-environment which is beneficial to injury repairing. As it is shown in the columns, the concentrations of SOD enzyme and GSH enzyme in the group treated with PL/Col were remarkably higher than that in the untreated group. Compared with the untreated group, the concentrations of SOD enzyme and GSH enzyme in the group treated with PL or collagen alone were increased without statistical significance (Figure 7A, C). For the content of the lipid peroxidation product malondialdehyde (MDA), the treatment of PL/Col showed decrease effect without statistical significance, even though the treatment of PL alone showed better decrease effect with statistical significance (Figure 7B). In summary, these data demonstrated that the ROS-modulating hydrogel PL/Col exerted robust cellular defending effect against ROS accumulated damage.

PL/Col Accelerated Healing and Regeneration in Photoaging Skin

The images in Figure 8A showed the representative skin recovery after one week of therapy in each group. Erythema and inflammation can be observed in the untreated group. Indeed, the photoaging rats treated with PL or collagen alone also exhibited the characteristics of keratinization and pigmentation. In the contrast, the rats treated with PL/Col displayed fewer characteristics of keratinization and pigmentation than the above three groups. The photoaging skin of rats treated with PL/Col performed smooth yellowish appearance which was close to the control group. In addition, after one week of treatment, the comparision of representative HE staining images revealed various effects in different groups: the PL/Col group presented decrease of inflammation and regeneration of skin collagen fiber and epidermal, while the PL group and Col group remained a certain level of glandular hyperplasia and inflammation (Figure 8B). To determine the recovery effect in each group, 5 representative sites of the histological slide were taken, and the thickness of epidermis was measured. After statistical analysis, the epidermal thickness of PL/Col group is significantly thinner than the untreated group, demonstrating the recovery effect of PL/Col (Figure 8C). Taken together, these results indicated that PL/Col promoted the active rebuilding and regeneration in UV-induced injury photoaging skin.

Discussion

Anti-aging research has always been a hot-bottom research topic. Establishing a more objective, practical and mature experimental model of photoaging model lays a foundation for the further exploration of the anti-aging and damage repair mechanism of a drug. In this experiment, UVA and UVB were used to establish the rat photoaging model. After 8 weeks of radiation, the morphological observation and histopathology examination of the back skin of rats were obviously different from the control group, indicating that the rat photoaging model was successfully established.

Platelelet derivatives, a kind of biological agents derived from blood, is an emerging therapy in the current regeneration research due to its rich growth factors. However, its harsh storage conditions and potential immune response lead to the limitation of clinical application. As a third-generation platelet derivatives product, the platelet lysate is a kind of platelet ruptured adhesive liquid rich in various biological macromolecules, and has the advantages of low antigenicity and easy preservation. However, there are various methods to prepare platelet lysate. In this experiment, the platelet lysate was prepared through multi-step manufacturing processes (two-step centrifugation and repeated frozen thaw method), aiming to prolong its storage time and reduce its immunogenicity effectively.

Table 3 The concentration of collagen by Coomassie Brilliant Blue G-250 method

A1	c1	A2	c2	A3	c3	Average	Concentration
0.141	2.828	0.133	2.665	0.159	3.196	0.144	2.896

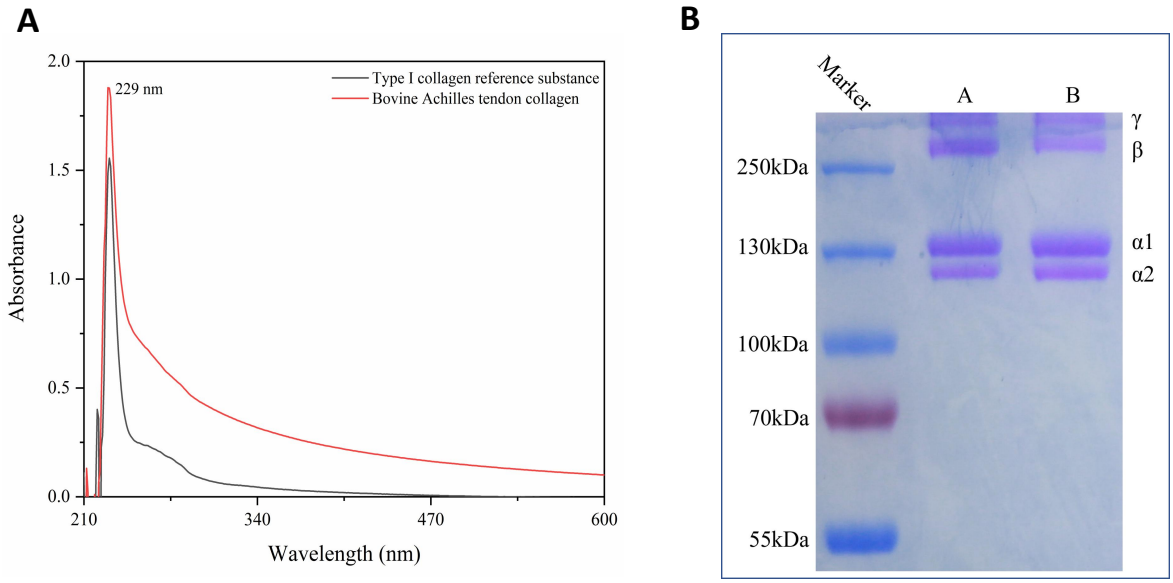

Figure 4 Preparation of Col-I and its Qualitative Detection. The UV-vis absorption spectrum of the extraction of collagen rang 210 to 600 nm (A). The result of SDS-PAGE gel electrophoresis of Col-I (strip A: standard of Col-I and, strip B: preparation of collagen) in accord with the characteristic of Col-I (B).

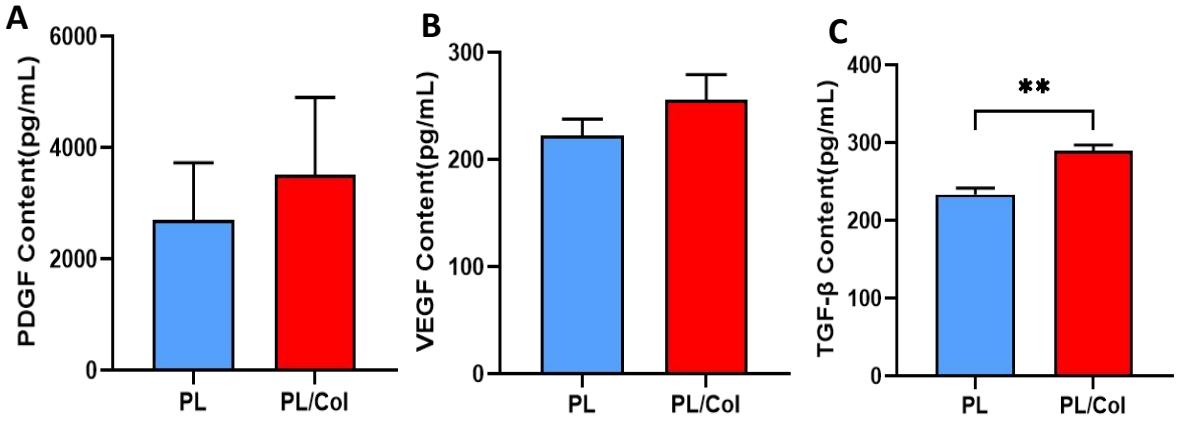

Figure 5 Comparison and Analysis Preserving Effect between PL/Col and PL Alone in −20 ℃ Environment after 14 Days. The comparison of plate-derived growth factor (PDGF) content detected by ELISA after 14 days of storage (A). The contents of vascular endothelial growth factor (VEGF) between the samples detected by ELISA after 14 days of storage (B). The results of transforming growth factor (TGF)-β content detected by ELISA: PL/Col is significantly higher than PL alone (C).

The data of the kit test and animal experiment showed that the PL/Col was rich in growth factors that accelerate the repairing and regeneration in photoaging skin, demonstrating that the preservation of PL/Col was better than PL alone

Collagen is an important part of human tissues and organs with the characteristics of good biocompatibility, low immunogenicity and biodegradable properties. At present, it has been developed and applied in the research field like photoaging treatment, wound hemostasis, wound healing, tissue defect filling, drug carrier and tissue engineering etc. However, the current preparation process of collagen is rough, and the efficacy of collagen is uneven under different preparation methods. Moreover, most of the collagen can not pass through the sterilizing filter film, and it is difficult to meet the injection standard. In this experiment, Col-I was extracted from the bovine Achilles tendon and the terminal triple helix was cut to greatly reduce the immunogenicity. Col-I was assessed by SDS-PAGE gel electrophoresis and UV-Vis spectroscopy, and the results showed that Col-I was accorded with the characteristics of type I collagen.

Collagen and PL are important natural products for the prevention and treatment of photoaging. In this experiment, Col-I reduced the degradation of the growth factors and prolonged the preservation of growth factors-rich PL, facilitating the repairing and regeneration of aging skin. The results showed that the preservation effect of PL/Col was better than PL, and the treatment with PL/Col was better than

Biomedical Engineering Communications 2023;2(2):9. https://doi.org/10.53388/BMEC2023009

Table 4 The detailed data of growth factor content comparison after 14 days of storage

	VEGF	PDGF	TGF-β
PL	222.424 ± 27.005	2696.438 ± 1796.546	233.998 ± 14.356
PL/Col	256.230 ± 40.369	3515.754 ± 2413.664	290.090 ± 13.103

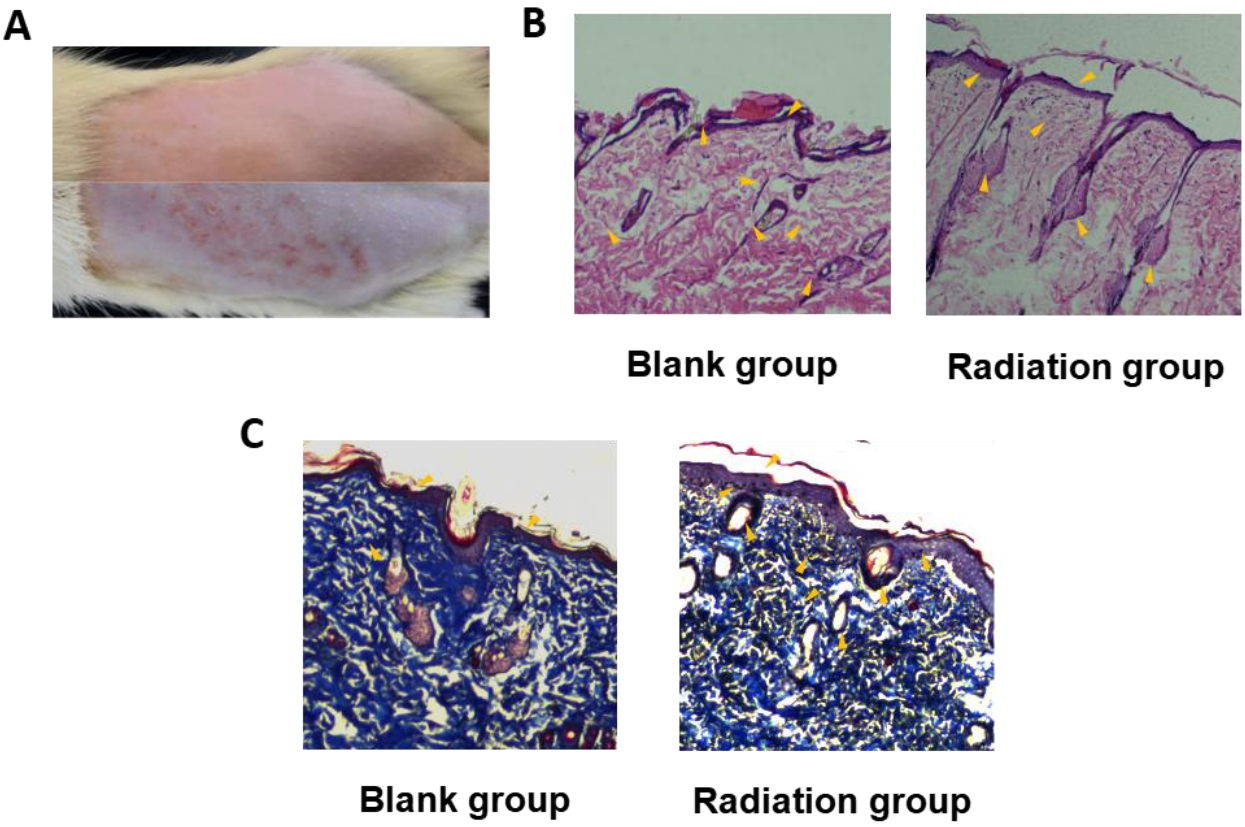

Figure 6 Establishment of Rats Photoaging Model and Assessment via Appearance and Pathological Section Evaluation. The comparison of skin appearance of rats in two groups: the control group in the upper picture and the irradiation group in the lower picture (A). Hematoxylin-eosin (HE) staining of representative back skin tissue from two groups (B). Representative pictures of Masson's trichrome staining from two groups (C).

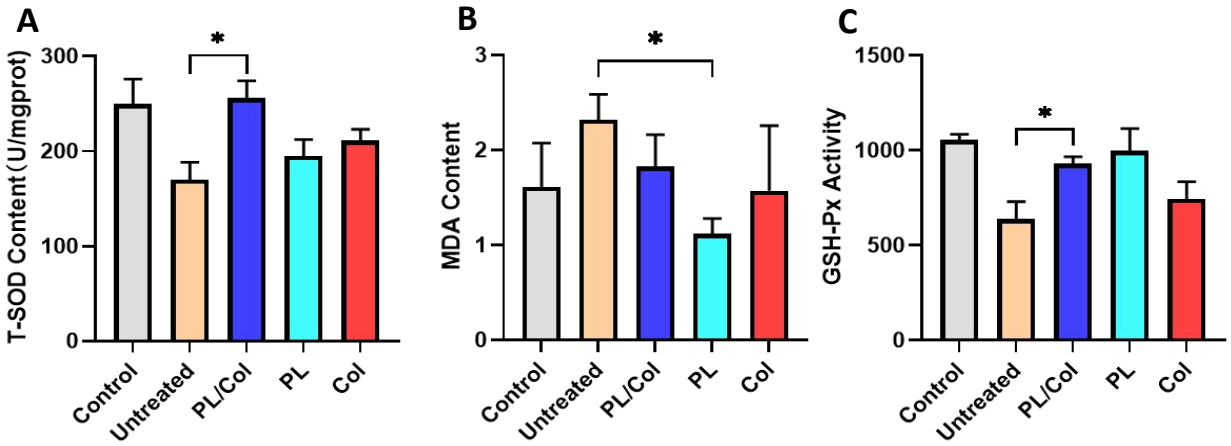

Figure 7 PL/Col Reshapes Oxidative Stress Micro-environment. The content of T-SOD enzyme of different groups (A). The concentration of lipid peroxidation product malondialdehyde (MDA) of different groups (B). The activity of GSH-Px enzyme of different groups (C).

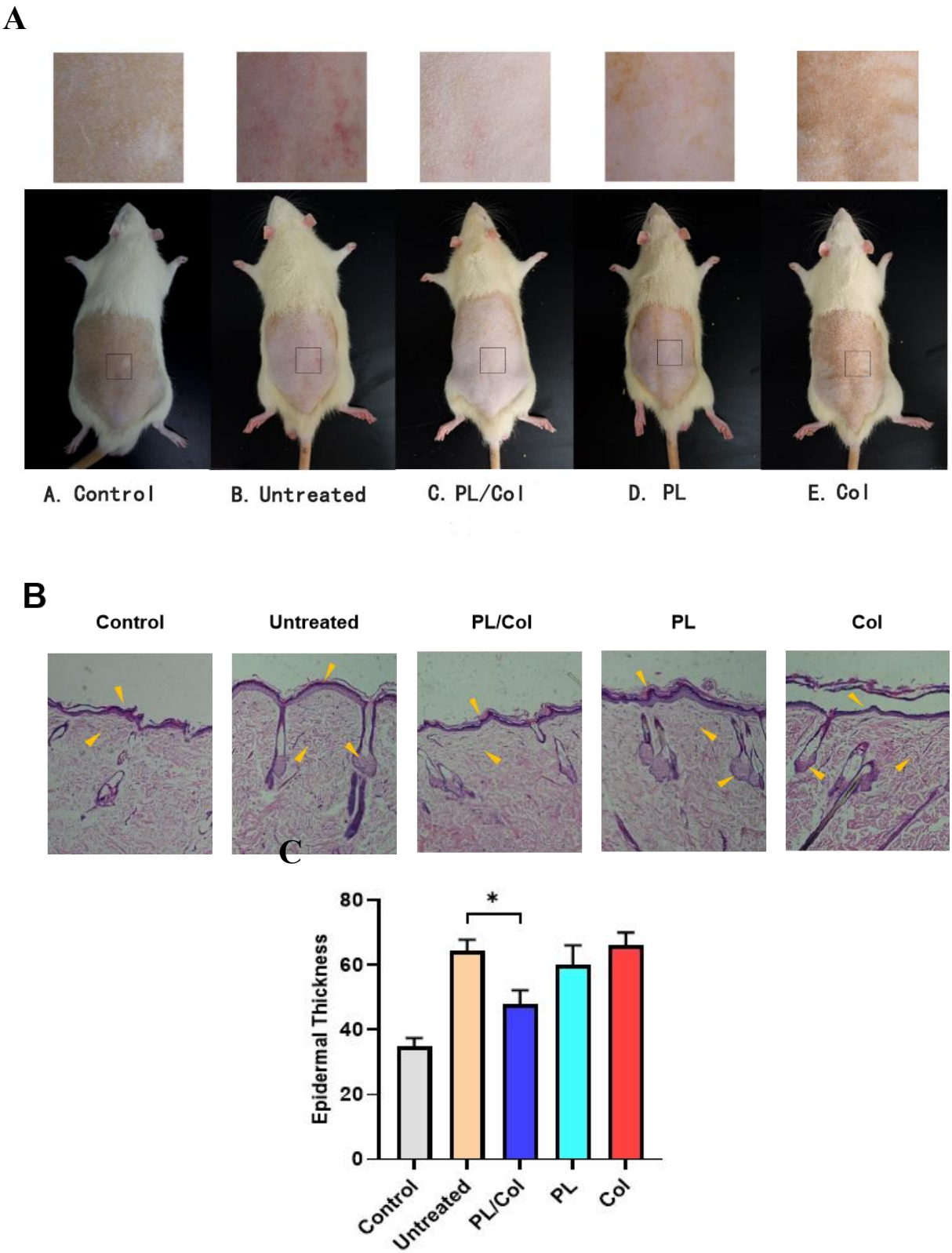

Figure 8 PL/Col Accelerated Regeneration of Photoaging Skin. Observe the back skin appearance of rats in different groups (A). Histologcal analysis of the back skin of rats in each group by HE staining (B). The measurement and analysis of epidermal thickness of back skin of rats with different treatments (C).

collagen or PL alone for preventing photoaging. PL/Col provides a more economical and safer prevention and control strategy for photoaging. Since that GSH-Px enzyme is only one of the various reductases *in vivo*, it is worthy of further research in the fields of anti-aging, antioxidant and regeneration.

Conclusion

This experiment presented a “seed and soil” strategy to prevent and treat UV-induced photoaging damage (“collagen” as “soil”to provide

proregenerative micro-environment for oxidative damaged tissue, and "growth factor-rich platelet lysate" as "seed" to promote the healing of damaged tissue) and explored a better method for PL preservation. The PL/Col not only reshaped the hostile oxidative micro-environment, but also ensured the integrity of encapsulated growth factor-rich platelet lysate in the oxidative environment. The results showed that the PL/Col provide a more affordable and safer treatment strategy in treating photoaging, and is worthy of further research in anti-aging, anti-oxidative and regenerative fields.

References

1. Kammeyer A, Luiten RM. Oxidation events and skin aging. *Ageing Res Rev* 2015;21:16–29. http://doi.org/10.1016/j.arr.2015.01.001
2. Bosch R, Philips N, Suárez-Pérez J, et al. Mechanisms of Photoaging and Cutaneous Photocarcinogenesis, and Photoprotective Strategies with Phytochemicals. *Antioxidants (Basel)* 2015;4(2):248–68. http://doi.org/10.3390/antiox4020248
3. Gromkowska-Kępka KJ, Puścion-Jakubik A, Markiewicz-Żukowska R, Socha K. The impact of ultraviolet radiation on skin photoaging — review of in vitro studies. *J Cosmet Dermatol* 2021;20(11):3427–31. http://doi.org/10.1111/jocd.14033
4. Decean H, Fischer-Fodor E, Tatomir C, et al. Vitis vinifera seeds extract for the modulation of cytosolic factors Bax-α and NF-kB involved in UVB-induced oxidative stress and apoptosis of human skin cells. *Med Pharm Rep* 2016;89(1):72–81. http://doi.org/10.15386/cjmed-508
5. Kim JC, Park TJ, Kang HY. Skin-Aging Pigmentation: Who Is the Real Enemy? *Cells* 2022;11 (16):2541. http://doi.org/10.3390/cells11162541
6. Sanches Silveira JEP, Myaki Pedroso DM. UV light and skin aging. *Rev Environ Health* 2014;29(3). http://doi.org/10.1515/reveh-2014-0058
7. Benndorf RA, Schwedhelm E, Gnann A, et al. Isoprostanes Inhibit Vascular Endothelial Growth Factor–Induced Endothelial Cell Migration, Tube Formation, and Cardiac Vessel Sprouting In Vitro, As Well As Angiogenesis In Vivo via Activation of the Thromboxane A2 Receptor. *Circ Res* 2008;103(9):1037–46. http://doi.org/10.1161/CIRCRESAHA.108.184036
8. Namkoong J, Kern D, Knaggs H. Assessment of Human Skin Gene Expression by Different Blends of Plant Extracts with Implications to Periorbital Skin Aging. *Int J Mol Sci* 2018;19(11):3349. http://doi.org/10.3390/ijms19113349
9. Yamada M, Prow TW. Physical drug delivery enhancement for aged skin, UV damaged skin and skin cancer: Translation and commercialization. *Adv Drug Delivery Rev* 2020;153:2–17. http://doi.org/10.1016/j.addr.2020.04.008
10. Lautenschlager S, Wulf HC, Pittelkow MR. Photoprotection. *The Lancet* 2007;370(9586):528–37. http://doi.org/10.1016/S0140-6736(07)60638-2
11. Zaid AN, Al Ramahi R. Depigmentation and Anti-aging Treatment by Natural Molecules. *Curr Pharm Des* 2019;25(20):2292–2312. http://doi.org/10.2174/1381612825666190703153730
12. De Pascale MR, Sommese L, Casamassimi A, Napoli C. Platelet Derivatives in Regenerative Medicine: An Update. *Transfus Med Rev* 2015;29(1):52–61. http://doi.org/10.1016/j.tmrv.2014.11.001
13. Scopelliti F, Cattani C, Dimartino V, Mirisola C, Cavani A. Platelet Derivatives and the Immunomodulation of Wound Healing. *Int J Mol Sci* 2022;23(15):8370. http://doi.org/10.3390/ijms23158370
14. Chen X, Yan J, Jiang Y, et al. Platelet-Activating Biominerals Enhanced Injectable Hydrogels With Superior Bioactivity for Bone Regeneration. *Front Bioeng Biotechnol* 2022;10. http://doi.org/10.3389/fbioe.2022.826855
15. Cecerska-Heryć E, Goszka M, Serwin N, et al. Applications of the regenerative capacity of platelets in modern medicine. *Cytokine Growth Factor Rev* 2022;64:84–94. http://doi.org/10.1016/j.cytogfr.2021.11.003
16. Losi P, Barsotti MC, Foffa I, et al. In vitro human cord blood platelet lysate characterisation with potential application in wound healing. *Int Wound J* 2019;17(1):65–72. http://doi.org/10.1111/iwj.13233
17. Meftahpour V, Malekghasemi S, Baghbanzadeh A, et al. Platelet lysate: a promising candidate in regenerative medicine. *Regen Med* 2021;16(1):71–85. http://doi.org/10.2217/rme-2020-0065
18. Wang L, Xie R, Fan Z, et al. The contribution of oxidative stress to platelet senescence during storage. *Transfusion (Paris)* 2019;59(7):2389–2402. http://doi.org/10.1111/trf.15291
19. Lannan KL, Refaai MA, Ture SK, et al. Resveratrol preserves the function of human platelets stored for transfusion. *Br J Haematol* 2015;172(5):794–806. http://doi.org/10.1111/bjh.13862
20. Forbes SJ, Rosenthal N. Preparing the ground for tissue regeneration: from mechanism to therapy. *Nat Med* 2014;20(8):857–69. http://doi.org/10.1038/nm.3653
21. Hao D, Lopez JM, Chen J, Iavorovschi AM, Lelivelt NM, Wang A. Engineering Extracellular Microenvironment for Tissue Regeneration. *Bioengineering* 2022;9(5):202. http://doi.org/10.3390/bioengineering9050202
22. Ricard-Blum S. The Collagen Family. *Cold Spring Harb Perspect Biol* 2010;3(1):a004978–a004978. http://doi.org/10.1101/cshperspect.a004978
23. Naomi R, Ridzuan PM, Bahari H. Current Insights into Collagen Type I. *Polymers* 2021;13(16):2642. http://doi.org/10.3390/polym13162642
24. Geanaliu-Nicolae RE, Andronescu E. Blended Natural Support Materials—Collagen Based Hydrogels Used in Biomedicine. *Materials* 2020;13(24):5641. http://doi.org/10.3390/ma13245641
25. Dong C, Lv Y. Application of Collagen Scaffold in Tissue Engineering: Recent Advances and New Perspectives. *Polymers* 2016;8(2):42. http://doi.org/10.3390/polym8020042
26. Zhao Y-L, Liu W-W, Liu W, et al. Phorbol ester (PMA)-treated U937 cells cultured on type I collagen-coated dish express a lower production of pro-inflammatory cytokines through lowered ROS levels in parallel with cell aggregate formation. *Int Immunopharmacol* 2018;55:158–64. http://doi.org/10.1016/j.intimp.2017.12.013
27. Mu H, Wang Y, Wei H, et al. Collagen peptide modified carboxymethyl cellulose as both antioxidant drug and carrier for drug delivery against retinal ischaemia/reperfusion injury. *J Cell Mol Med* 2018;22(10):5008–19. http://doi.org/10.1111/jcmm.13768
28. do Amaral RJFC, Zayed NMA, Pascu EI, et al. Functionalising Collagen-Based Scaffolds With Platelet-Rich Plasma for Enhanced Skin Wound Healing Potential. *Front Bioeng Biotechnol* 2019;7:371. http://doi.org/10.3389/fbioe.2019.00371
29. Ao J, Li B. Amino acid composition and antioxidant activities of hydrolysates and peptide fractions from porcine collagen. *Food Sci Technol Int* 2012;18(5):425–34. http://doi.org/10.1177/1082013211428219
30. Böhm S, Strauß C, Stoiber S, Kasper C, Charwat V. Impact of Source and Manufacturing of Collagen Matrices on Fibroblast Cell Growth and Platelet Aggregation. *Materials* 2017;10(9):1086. http://doi.org/10.3390/ma10091086

31. Bonferoni MC, Rossi S, Sandri G, et al. Bioactive Medications for the Delivery of Platelet Derivatives to Skin Wounds. *Curr Drug Deliv* 2019;16(5):472–83. http://doi.org/10.2174/1381612825666190320154406
32. Hiroyuki H, Kouta N,Tohey M. Membrane filter (pore size, 0.22 – 0.45 μm; thickness, 150 μm) passing-through activity of Pseudomonas aeruginosa and other bacterial species with indigenous infiltration ability. *FEMS Microbiol Lett* 2003;223(1):41-46. http://doi.org/10.1016/S0378-1097(03)00327-6
33. Zhou Y, Liu W, Gan B, et al. Non-cross-linked collagen type I microfibers for improved hemostasis and wound healing. *J Mater Sci* 2022;57(28):13570–85. http://doi.org/10.1007/s10853-022-07447-7