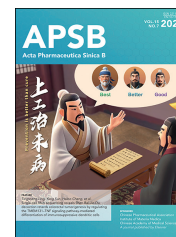




Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

A high clinically translatable strategy to anti-aging using hyaluronic acid and silk fibroin co-crosslinked hydrogels as dermal regenerative fillers



Jialing Cheng^{a,b,†}, Zhiyang Chen^{a,b,†}, Demin Lin^{a,b}, Yanfang Yang^{a,b}, Yanjing Bai^d, Lingshuang Wang^d, Jie Li^d, Yuchen Wang^a, Hongliang Wang^{a,b,*}, Youbai Chen^{c,*}, Jun Ye^{a,b,*}, Yuling Liu^{a,b,*}

^aState Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

^bBeijing Key Laboratory of Drug Delivery Technology and Novel Formulation, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

^cDepartment of Plastic and Reconstructive Surgery, Chinese PLA General Hospital, Beijing 100853, China

^dShenzhen Xiuli Medicine Technology Co., Ltd., Xinlan Community, Shenzhen 518110, China

Received 16 December 2024; received in revised form 8 April 2025; accepted 14 April 2025

KEY WORDS

Silk fibroin;
Hyaluronic acid;
Dermal filler;
Hydrogel;
Collagen regeneration;
Anti-aging;
Type III collagen;
Extracellular matrix remodeling

Abstract An ideal dermal filler should integrate filling, repair, and anti-aging effects, with immediate tissue augmentation, slow degradation, and progressive stimulation of collagen regeneration. However, commonly used hyaluronic acid (HA) hydrogels, while effective for rapid filling, suffer from limited duration of support, weak cell adhesion, and an inability to promote collagen regeneration. Silk fibroin (SF), a natural protein from silkworm cocoons, is known for its excellent cell adhesion and collagen-stimulating abilities. However, its limited gelation capability restricts its potential application as a standalone injectable hydrogel. Based on a complementary strategy, this study combines the rapid gelling properties of HA with the collagen regenerative properties of SF to create a co-crosslinked HA-SF hydrogel. The composite hydrogel merges HA's rapid filling effect with SF's strong tissue adhesion and collagen-stimulating abilities. The formulation, physicochemical properties, degradation, biocompatibility, and filling effects of the HA-SF hydrogel were systematically investigated. HA-SF hydrogel

*Corresponding authors.

E-mail addresses: wanghl@imm.ac.cn (Hongliang Wang), chenyoubai@301hospital.com.cn (Youbai Chen), yelinghao@imm.ac.cn (Jun Ye), ylliu@imm.ac.cn (Yuling Liu).

[†]These authors made equal contributions to this work.

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2025.04.020>

2211-3835 © 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

exhibits excellent mechanical properties and ensures long-term support while maintaining injectability. Interestingly, after intradermal injection in the UVB-induced photoaging model, HA-SF hydrogel not only enhances hydrogel–cell interaction but also continues to stimulate collagen regeneration, especially type III collagen. This dual action achieves the biological effects of repair and anti-aging while maintaining the filling effect. Proteomic analysis confirms that repair and anti-aging effects are enhanced by the regulation of skin fibroblasts and modulation of amino acid and lipid metabolism. This composite hydrogel holds strong promise for clinical applications, offering a safer, long-lasting, and more natural injectable filler that combines filling, repair, and anti-aging into one system.

© 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Skin aging, whether attributable to intrinsic chronological factors or extrinsic photoaging, is primarily characterized by dryness, an increase in wrinkles, and a reduction in facial soft tissue volume. Soft tissues serve as essential components of the skin, primarily consisting of collagen and elastin within the dermis, as well as muscle and water¹. With the progression of aging, collagen depletion results in a diminished structural framework, which manifests as epidermal wrinkles and facial depressions. Thus, loss of collagen is considered a significant intrinsic factor contributing to skin aging. Injectable dermal fillers have emerged as popular non-surgical interventions for volume restoration, contour enhancement, and aesthetic correction².

Current dermal fillers include those based on natural polymers, such as hyaluronic acid (HA) and collagen, as well as synthetic polymers, such as polycaprolactone (PCL) and poly-L-lactic acid (PLLA) microsphere fillers. HA, a natural polysaccharide, is abundant in the extracellular matrix (ECM) of skin, vitreous body, and joints^{3,4}. Advances in microbial fermentation, molecular weight (MW) control, and crosslinking have led to diverse HA hydrogel products. HA fillers are available in different MW ranges: low MW (for hydration and wrinkle reduction), medium MW (for soft tissue repair), and high MW (for structural contouring), making them widely useful in facial contouring and soft tissue repair⁵. While HA is valued for its biocompatibility and contouring effects, it has key limitations: short dermal longevity, minimal cell adhesion, and lack of collagen-regenerative capacity^{6–8}. HA fillers mainly provide temporary structural support and hydration, often requiring reinjection without promoting collagen synthesis, thus limiting their use in advanced anti-aging therapies⁹.

To address these limitations, synthetic fillers like polymethylmethacrylate (PMMA), PCL, and PLLA microspheres have been developed, providing prolonged maintenance (2–10 years) and ongoing collagen stimulation^{10–12}. However, these lack inherent biological functions, relying on external stimulation rather than true dermal regeneration. Additionally, PLLA degradation can lower pH and cause irritation¹⁰. Hydroxyapatite microspheres, valued for osteogenic properties, have been used mainly in bone treatments and were only recently FDA-approved as facial fillers, with limited research on their collagen effects^{13,14}. Fat-derived stem cell fillers, while generally safe, pose challenges including complex preparation, allergy risks, and potential for vascular occlusion^{15,16}. Thus, finding natural polymers that support controllable collagen regeneration and adaptable half-life remains difficult.

Silk fibroin (SF) is a promising natural polymer, derived from the inner proteins of silkworm cocoons after degumming. SF consists of 18 amino acids, forming heavy, light, and P25 chains. Due to its excellent biocompatibility and biodegradability, SF has expanded from traditional textiles to applications in biomimetic materials¹⁷, nanomaterials¹⁸, and optoelectronic materials¹⁹. Regenerated SF solutions can be processed into various SF-based materials suitable for tissue engineering scaffolds²⁰, controlled-release nanoparticles for drug delivery systems²¹, and micro-needles for wound dressings²².

In aesthetic fillers, SF offers several advantages, including safety from its protein composition, durable mechanical support due to its β -sheet structure²³, strong cell adhesion²⁴, and immunomodulatory functions²⁵. Despite its mechanical properties and bioactivity, the intrinsic gelation of SF is poor, which hinders its standalone application as a facial filler²⁶. To address this, SF can be chemically modified to include cross-linkable groups or combined with appropriate crosslinking agents or auxiliary polymers. Rather than modifying SF chemically, it is advantageous to combine it with complementary materials to overcome its limitations. As mentioned above, HA is known for its excellent gelation properties and clinical use but lacks regenerative and adhesive capabilities, whereas SF offers strong bioactivity but has limited gelation capacity. Therefore, based on the advantageous complementary strategy, the combination of SF and HA highlights their synergistic advantages and overcomes individual limitations, yielding hydrogels with superior bioactivity and gelation capacity suitable for dermal fillers. This composite HA-SF hydrogel (HA-SF gel) not only meets conventional filler requirements but also promotes collagen regeneration and enhances the dermal condition through both physical and biological modulations. Integration of “filling, repairing, anti-aging” into one: the first rapid filling, and then stimulate collagen regeneration, to achieve “safer, more natural, more long-lasting” effects. To our knowledge, there has been no research focusing on HA-SF dermal fillers.

In this study, HA-SF gel was developed as an injectable dermal filler for soft tissue restoration using a low-high temperature co-crosslinking method. The formulation was systematically analyzed for mechanical and physicochemical properties, including particle size, pore size, porosity, and mechanical stability. The supporting effects of composite hydrogel *in vitro* and *in vivo* and the biocompatibility with blood, cells, tissues, and organoids were systematically evaluated. Biological effect studies demonstrate that HA-SF gel promotes regeneration of type III collagen, the non-renewable “gold collagen” that increases skin elasticity and smoothness, effectively countering aging. This

anti-aging effect cannot be achieved by single HA or SF hydrogels. This study skillfully exploits material properties to design fillers that specifically address clinical problems, integrating physical repair with endogenous regeneration to achieve anti-aging through symptomatic relief and root cause targeting. At the same time, this ingenious preparation method supports further scale-up production, positioning HA-SF gel as a promising filler with significant clinical translation potential.

2. Methods and materials

2.1. Materials

HA (average Mn $\approx$ 800–1500 kDa) was purchased from Macklin (Shanghai, China). SF (average Mn $\approx$ 50–100 kDa) was generously provided by Huasi Biotechnology Co., Ltd. (Shenzhen, China). 1,4-Butanediol diglycidyl ether (BDDE) was purchased from Sigma–Aldrich. (St. Louis, USA). Fluorescein isothiocyanate (FITC) and IR-820 dyes were purchased from Macklin (Shanghai, China). Fetal bovine serum (FBS, No. FBS01) was purchased from Sino Biological (Beijing, China).

2.2. Cells and animals

L929 and RAW264.7 cells were obtained from the Cell Resource Center of the Peking Union Medical College (Beijing, China). Horse serum (HS), fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), L-glutamine, penicillin, streptomycin, and 0.25% trypsin were purchased from Thermo Fisher Scientific (MA, USA). The L929 cells were cultured in DMEM supplemented with 10% HS, 1% L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. The RAW264.7 cells were cultured in DMEM supplemented with 10% FBS, 1% L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. All cells were maintained at 37 °C in a humidified chamber with 5% CO₂. Female ICR mice (6–8 weeks old) were purchased from HFK Bioscience Co., Ltd. (Beijing, China). All animal experiments were conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee of the Chinese Academy of Medical Sciences & Peking Union Medical College.

2.3. Preparation and formulation screening of HA-SF gel

An injectable hydrogel was prepared using a chemical cross-linking method at two different temperatures. First, a NaOH solution containing 1% BDDE was prepared for the HA-SF gel. Next, 10% HA and 2% SF powders were thoroughly mixed, and the BDDE solution was added to this mixture. The mixture was initially crosslinked at 4 °C for 48 h and then further crosslinked at 37 °C for 3 h. The hydrogel was purified using PBS. The hydrogel was sieved through an 80-mesh screen to obtain granules. The granules were combined with 5% non-crosslinked sodium HA solution and PBS. The mixture was then sealed and stored at 4 °C, forming injectable HA-SF co-crosslinked hydrogel particles (HA-SF gel Particle).

The gelation performance and time were assessed as critical parameters for hydrogel formulation. Single-factor investigations explored the effects of various HA concentrations (2.5%, 5%, 7.5%, 10%, and 20%). Mechanical properties, specifically the storage modulus (G') and loss modulus (G''), were used as evaluative indicators. Single-factor investigations were performed to examine

the effect of varying BDDE concentrations (1%, 2%, 3%, and 4%). Appearance, storage modulus (G'), loss modulus (G''), and viscosity were evaluated as key parameters. Single-factor investigations were performed to assess the influence of varying SF concentrations (1%, 1.25%, 1.5%, 2%, 5%, and 10%). Mechanical properties were determined using a Kinexus Lab + rotational rheometer (Netzsch, Germany), with frequency sweep measurements at 1% stress and a frequency range of 0.01–1 Hz. The G' and G'' values were recorded at a frequency of 0.1 Hz.

2.4. Characterization of HA-SF gel

The transparency and uniformity in the appearance of the HA-SF gel were evaluated and compared with that of the HA gel to assess the gelation performance. The mechanical properties were determined using the same methodology as that for the BDDE concentration screening. For Fourier transform infrared spectroscopy (FTIR), approximately 2 mg of the freeze-dried hydrogel sample was ground with potassium bromide, pressed at 20 MPa, and analyzed using an iS10 FT-IR spectrometer (Thermo Fisher, USA) over a wavenumber range of 400–4000/cm. Shear thinning measurements were conducted using a Kinexus Lab + rotational rheometer with a shear rate ranging from 0.1 to 100/S, a Ramp time of 0:00–02:00, and a sample per decade of 10.

FITC-loaded gel: A 1% BDDE solution was prepared and mixed with 0.05 mg/mL FITC (Macklin, China); subsequently, SF and HA were dissolved using the same procedure. After sieving, the hydrogel was dispersed by ultrasonication for fluorescence microscopy. The gross morphology of the lyophilized hydrogels was observed using scanning electron microscopy (SEM) (Thermo Fisher, USA) at an accelerating voltage of 20 kV. Mercury porosimetry measurements were conducted using an AutoPore IV 9620 automated mercury porosimeter (Micromeritics, USA). The linear viscoelastic region of the hydrogel was quantified using a Kinexus Lab + rotational rheometer with an amplitude sweep low-viscosity dynamic rheometry (LVDR) program. The measurement was performed at shear stresses ranging from 0.1% to 100% and a frequency of 1000 Hz, 25 °C. Continuous alternating strain measurements were performed using a Kinexus Lab + rotational rheometer. The program consisted of five cycles, each lasting for 5 min, with a stress range of 1%–300%, 25 °C. G' and G'' were thus determined.

Push force measurement: The sample was filled into a 2.25 mL syringe, a 30 G injection needle was installed, a small amount of air at the front end of the syringe was discharged, and then installed on the universal material testing machine (Jinan Zhongzheng ZDW-T100, China), and the testing parameters of the universal material testing machine were set. One hour in advance, it was placed at RT for equilibrium and measurement, and the pushing speed was set at 30 mm/min.

Mechanical strength assessment of hydrogels: The mechanical strength of the hydrogel samples was evaluated using a Universal TA texture analyzer (Shanghai Tengba Instrument Technology Co., Ltd., China) through a compression test. A 10 mm cylindrical probe was used, with a testing speed of 0.5 mm/s, a compression distance of 8 mm, and a trigger force of 0.049 N. The displacement-load stress curve was recorded for further analysis.

Particle size analysis of injected hydrogel: The prepared hydrogel was injected through a 30 G syringe needle, and 10 mg of the sample was dispersed in 15 mL of PBS. The particle size distribution (Dn10, Dn50, and Dn90) before and after injection was measured using a laser particle size analyzer (Malvern, Switzerland).

The unscreened dialyzed hydrogel (W_0) was immersed in PBS at 25 °C, and its weight change was recorded periodically until a steady state was reached (W_C). To determine the water-holding capacity of the hydrogel, it was placed at water equilibrium (W_C) in an oven at 37 °C for 12 h and the weight change (W_H) was measured according to Eqs. (1) and (2).

$$\text{Swelling ratio (SD)} = (W_C - W_0) / W_0 \times 100\% \quad (1)$$

$$\text{Water holding capacity (WHD)} = (W_C - W_H) / W_C \times 100\% \quad (2)$$

Further, the hydrogel (W_0) was immersed in PBS containing 200 U/mL HA to assess reversibility. The system was incubated at 37 °C for 10 h with periodic samples taken and weighed until the hydrogel was completely degraded (W). As shown in Eq. (3):

$$\text{Degradation rate} = (W / W_0) \times 100\% \quad (3)$$

2.5. Stability and degradation of HA-SF gel

The *in vitro* degradation rate of the gels was determined using a gravimetric method. Samples were incubated at 37 °C for 1, 3, 5, 10, and 15 days in PBS containing 76.1 ± 51.8 ng/mL HA enzymes (Sigma, USA), 26.84 ± 10.1 ng/mL Cathepsin XIV (Sigma, USA) (Human Protein Atlas, <https://www.proteinatlas.org>). The initial mass (dissolved mass W_1) and mass at pre-determined time points (W_2) were recorded according to Eq. (4).

$$\text{Degradation rate} = (W_2 / W_1) \times 100\% \quad (4)$$

The IR-820-stained gels were prepared using the same method as that of the FITC-stained gels, with an IR-820 concentration of 0.1 mg/mL. Healthy 4–6 weeks-old ICR mice were ear-tagged and injected subcutaneously with approximately 0.1 mL of HA-SF hydrogel and 0.1 mL of IR-820 solution (SOL). *In vivo* fluorescence imaging was used to assess the initial fluorescence intensity of the hydrogel 1 h after injection, followed by imaging at various time points.

2.6. Biocompatibility assessment of HA-SF gel

The hydrogel was prepared by mixing with 0.9% NaCl at a ratio of 0.05:10 ($M_{\text{hydrogel}}: V_{0.9\% \text{NaCl}}$). Physiological saline was used as the negative control and deionized water was used as the positive control for hemocompatibility assessment. Samples were incubated at 37 °C for 30 min, and then 250 µL of diluted anticoagulant blood was added. After mixing, the samples were incubated for 1 h at 37 °C. The supernatant was collected after centrifugation at 111.8×g for 5 min, and the optical density (OD) was measured at $\lambda = 540$ nm. As shown in Eq. (5):

$$\text{Hemolysis rate} = (\text{OD}_{\text{sample}} - \text{OD}_{\text{negative control}}) / (\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}}) \times 100\% \quad (5)$$

Cell compatibility was assessed using a CCK-8 assay. The hydrogel was soaked in DMEM at 37 °C for at least 72 h to prepare the hydrogel extract. The L929 cells were counted using a Countstar automated cell counter (Countstar Mira FL, Shanghai, China). The L929 cells were seeded at 5×10^4 cells/mL for 1 day, 2×10^4 cells/mL for 3 days, and 8×10^3 cells/mL for 7 days.

RAW264.7 cells were seeded at 8×10^4 cells/mL for 1 day, 4×10^4 cells/mL for 3 days, and 1×10^4 cells/mL for 7 days. The cells were incubated with 200 µL of hydrogel extract at 37 °C in a CO₂ incubator for 1, 3, and 7 days. Absorbance at 450 nm was measured after the addition of the CCK-8 detection solution (Solarbio, China). A sterile bottom-water hydrogel was prepared and preincubated with 100 µL of culture medium for 4 h. After removing the medium, the hydrogel was washed three times with PBS. Cells were seeded as per the previous CCK-8 method, with 6000 L929 cells per well and 8000 RAW264.7 cells per well. After 2 days of co-culture, wells were selected for LIVE/DEAD staining (Thermo Fisher, USA), and the samples were observed by fluorescence microscopy (Biotek, USA).

The L929 cells in the logarithmic growth phase were prepared at a density of 2500 cells/mL for the skin organoids. The cell suspension was added into ultra-low adhesion 96-well plates and centrifuged at 1000×g for 10 min at 25 °C. The plates were then incubated for 14 days. On Day 3, the medium was replaced with a 3% lyophilized hydrogel extract solution, ensuring no stirring of the culture plates before medium replacement. Observations and cross-sectional area measurements were performed on Days 3, 6, 9, and 14. After 14 days, the organoids were fixed and stained with hematoxylin and eosin (H&E).

In vivo, animal studies were performed using healthy female ICR mice. The mice were divided into four experimental groups: CTRL, commercial HA hydrogel (HA Product), homemade HA hydrogel (HA gel), and 2% SF HA-SF hydrogel (HA-SF gel). The HA product name: BIOHYALUX (Modified sodium hyaluronate gel for injection), 20 mg/1.0 mL. Batch number: 36926107. Product code: 06948060430686. Manufacturer: Bloomage Biotechnology Corporation Limited. Approximately 0.05 mL of hydrogel per 10 g body weight was injected subcutaneously. The mice were assigned to three time points: on Days 7, 14, and 21. At each time point, the mice were euthanized and blood samples were collected and centrifuged to separate the serum for future analysis. Subcutaneous tissues were collected for subsequent analyses.

The levels of inflammatory factors IL-6 and TNF- α in serum were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, China). Serum samples were collected from all groups on Days 7, 14, and 21. Pro-inflammatory cytokines levels in mouse serum were evaluated using a flow cytometry assay kit (BioLegend, USA), with serum samples collected on Days 7, 14, and 21.

2.7. Investigation of bioactivity of HA-SF gel

Cell Immunofluorescence: Sterile 48-well culture plates were prepared with the hydrogel, and the L929 cells were seeded at a density of 3000 cells per well. After fixation with 4% paraformaldehyde, permeabilization, and blocking with 1% BSA, the cells were incubated with vinculin and fibronectin antibodies (Abcam, USA) at 4 °C overnight. The cells were then washed and incubated with secondary antibodies (diluted in 1% BSA) at 25 °C. After washing, the cells were stained with DAPI and observed under an inverted fluorescence microscope.

Measurement of collagen I and III: Skin tissues were collected from the CTRL, HA product, HA gel, and HA-SF gel groups. The hydroxyproline content was determined using a hydroxyproline assay kit (Nanjing Jiancheng, China). Collagen I and III levels were quantified using ELISA kits (NOVUS, USA), according to the manufacturer's instructions.

Table 1 UVB irradiation dose schedule.

Time (Week)	1	2	3	4	5	6
Irradiation intensity (mJ/cm ²)	60	60	120	150	180	180
Irradiation time (min)	2	2	4	5	6	6

The L929 cells in the logarithmic growth phase were harvested, centrifuged, and resuspended. The cells were seeded at a density of 2500 cells/mL in 200 μ L in ultra-low attachment 96-well plates (Sigma, USA) and centrifuged at 1000 $\times g$ for 10 min at 25 °C. The plate was incubated for 7 days, with 100 μ L of fresh medium replaced on Day 3. Imaging was performed to observe morphology and measure area. Organoid-like structures were collected for H&E staining and immunofluorescence (IF) staining.

The skin tissues were soaked in polyformaldehyde at 25 °C for 24 h, dehydrated, and embedded in paraffin. Each tissue was then sectioned into five slices, 3- μ m thick, for histological staining. The staining techniques included H&E, Masson's trichrome, Sirius Red, and immunostaining with antibodies against collagen type I (Col-I) (Novus, USA), type III (Col-III) (Abcam, USA), vinculin (Abcam, USA), and fibronectin (Abcam, USA). The tissues were scanned for observational purposes.

2.8. UVB-induced skin photoaging model and treatment

Six-week-old female BALB/c nude mice were divided into six groups. The mice's dorsal skin was irradiated using a UVB phototherapy device (UV9B3H, Beijing Saibao Technology Co., Ltd.) at 312 nm. The exposure time per session ranged from 2 to 8 min over 6 weeks. The UV lamp was positioned 28 cm above the dorsal skin. The irradiation intensity, measured in minimal erythema dose (MED), was gradually increased from 1 MED (60 mJ/cm²) to 3 MED (180 mJ/cm²), controlled by a UV-B radiometer (UV-297 probe, Beijing Normal University Optoelectronic Technology Co., Ltd.)²⁷⁻²⁹. Mice were irradiated every other day, with the dosing regimen detailed in Table 1.

After 3 weeks of UVB irradiation, visible signs of skin aging, including increased wrinkling, confirmed the success of the photoaging model. At the wrinkled dorsal areas, HA-SF gel or other gels (7 mL/kg) were injected intradermally, followed by a 15-min saline wet compress. Additionally, 0.1% all-trans retinoic acid was topically applied once a week, while UVB irradiation continued for an additional 4 weeks to deepen the aging model. Throughout the study, skin hydration and ultrasound parameters were monitored using a skin analysis device (DermaLab Combo 4, Cortex Technology, Denmark). At the end of 6 weeks, tissues were collected, fixed, and cryopreserved for further analysis. Skin wrinkles and erythema were observed and recorded weekly from the start of irradiation, with evaluations detailed in Table 2. Skin Condition Assessment. The primer sequences for collagen-related assays are provided in Supporting Information Table S1.

2.9. D DIA proteomics analysis

Injected skin tissues from mice were used for proteomic assays commissioned by Novogene Co., Ltd. Shotgun proteomic analyses for transition library construction were performed using an EASY-nLCTM 1200 UHPLC system (Thermo Fisher, Germany) coupled with a Q ExactiveTM HF-X mass spectrometer (Thermo Fisher,

Table 2 Scoring scale for skin wrinkling and redness.

Grade	Characteristics
0	No sagging or wrinkles; normal skin texture visible
1	Fine wrinkles visible, mild skin flaking
2	Few shallow wrinkles visible, moderate skin flaking
3	Multiple shallow wrinkles visible, severe skin flaking
4	Skin surface roughened and thickened, a few deep wrinkles and mild sagging visible
5	Significant increase in deep wrinkles, skin damage with bleeding

Germany) in data-dependent acquisition (DDA) mode at Novogene Co., Ltd. (Beijing, China).

The DIA data were imported into the Spectronaut software (Biognosys) to generate a DDA library and extract ion-pair chromatographic peaks. Ion matching and peak area calculations were performed for qualitative and quantitative peptide analyses. iRT was used for retention time correction, with a precursor ion Q-value cutoff of 0.01. Statistical analysis was performed using Student's *t*-test for protein quantification. Proteins showing significant differences between experimental and control groups ($P < 0.05$, $|\log_2 \text{FC}| > *$ ($\text{FC} > *$ or $\text{FC} < *$ [FC]) were defined as differentially expressed proteins (DEP).

2.10. Statistical analysis

Quantitative data were expressed as mean $\pm$ standard error of mean (SEM) for $n \geq 3$. Statistical analysis was performed by one-way or two-way ANOVA followed by Tukey's test using GraphPad Prism 9.5.1 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Statistical significance was defined as $*P < 0.05$, with highly significant differences denoted as $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

3. Result and discussion

3.1. Preparation of HA-SF gel

The HA-SF co-crosslinked injectable hydrogel was prepared using a potent chemical crosslinking method (Fig. 1A) employing BDDE: a crosslinking agent widely used in commercial products. The gel-forming properties of SF are suboptimal, and several strategies can address this limitation. One approach is to modify SF properties directly, either by introducing gel-promoting functional groups or by genetically enhancing gel formation during SF extraction. For example, SF was modified to create SilMA, which can independently form a gel³⁰. However, this methacrylate-modified SF yields a hydrogel with excessively high mechanical strength, suitable for load-bearing joints but less appropriate for facial applications. Another study³¹ enhanced SF gel properties using high-reactivity L-DNA/Y-DNA in the extraction process, enabling gelation with mild HRP reactions. While effective, this method is complex and reliant on DNA, which is less practical for large-scale production. An alternative approach is to incorporate a complementary material to compensate for SF's limitations, such as sodium alginate or chitosan. Here, we selected HA, commonly applied in dermatology, due to its intrinsic gel-forming properties that balance SF's deficiencies. This method avoids complicated preparation processes by using a straightforward ring-opening

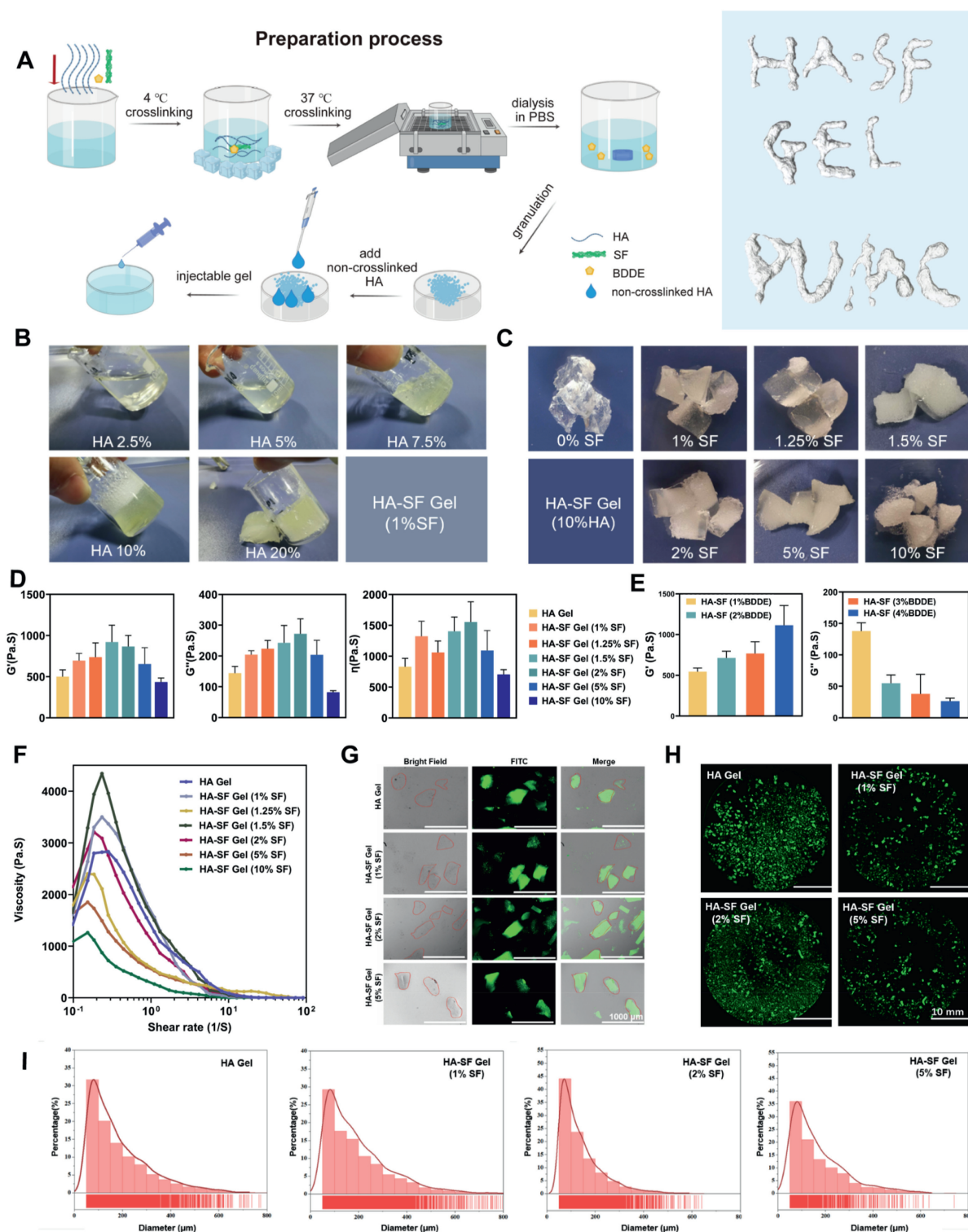


Figure 1 Physicochemical properties of HA-SF gel. (A) Schematic of the preparation process and appearance of HA-SF gel. (B) Appearance of HA-SF hydrogel (no sieving) with different HA concentrations (2.5, 5, 7.5, 10, and 20% HA) and 1% SF. (C) Appearance and (D) physicochemical properties, including G' , G'' , and viscosity of HA-SF gel with different SF concentrations (1, 1.25, 1.5, 2, 5, and 10% SF) and 10% HA. ($n = 3$ trials) (E) Physicochemical properties of HA-SF gel with varying BDDE concentrations (1, 2, 3, and 4% BDDE). ($n = 3$ trials) (F) Injectability through shear rate variation and rheological analysis (1, 1.25, 1.5, 2, 5, 10% SF and 10% HA). (G) Morphology, (H) particle size uniformity analysis, and (I) particle size of FITC-stained hydrogel particles of HA-SF gel (1, 2, 5% SF, and 10% HA) and HA gel (10% HA). All data are representative of at least three independent experiments. Data are presented as mean $\pm$ SEM.

reaction with BDDE (epoxy propyl) to form ether bonds with hydroxyl groups in SF or HA, tightly interlinking the two components into a robust three-dimensional network (Supporting Information Fig. S1). The resulting hydrogel meets the structural and performance requirements for facial applications. The formulation was simplified by minimizing the additional components. Preliminary crosslinking was achieved at a low temperature for 48 h. Subsequently, further crosslinking was performed at a higher temperature to avoid direct exposure to high temperatures (60 °C) that could introduce impurities resulting from BDDE decomposition^{32–34}.

Dialysis was performed to remove the residual BDDE and ensure the safety of the hydrogel. The hydrogel was then sealed to injectable HA-SF co-crosslinked hydrogel particles (HA-SF gel Particle). The particle size was reduced by physical sieving to enhance injectability. A co-crosslinked HA-SF injectable hydrogel was obtained by mixing the sieved hydrogel particles with a non-crosslinked HA solution and PBS, resulting in a biphasic system (HA-SF gel). The entire preparation process was straightforward as well as highly feasible and conducive to scaling up production.

3.2. Formulation screening of HA-SF gel

The hydrogel formulation comprised three main components: HA, SF, and BDDE. Different HA concentrations resulted in different gelation properties. Concentrations of 2.5% and 5% remained as sols even with prolonged crosslinking, whereas 7.5% produced an excessively soft hydrogel lacking filling support. Conversely, the 20% concentration exhibited strong mechanical properties, but was too hard and lacked elasticity (Fig. 1B). Therefore, 10% HA was selected as an optimal filling material.

The SF concentration was determined based on the hydrogel's appearance, mechanical properties, and viscoelasticity. Incorporating SF improved the appearance of the hydrogel, making it less transparent than pure HA gels and with reduced surface defects (Fig. 1C). Hydrogels with an SF concentration of 1.5%–2% exhibited optimal mechanical properties. Higher SF concentrations led to a decline in the mechanical properties (Fig. 1D). This may be attributed to excessive long-chain SF molecules competing with HA for crosslinking, which reduces the crosslinking efficiency and hydrogel structural density. Corresponding with existing research, increasing the connection “LDH” into chitosan (CS)/SF hydrogels decreased the covalent crosslinking density³⁵. Finally, an optimal HA-SF concentration ratio of 5:1, along with the addition of 1% BDDE as a crosslinking agent, was selected for hydrogel preparation.

As a crosslinking agent, BDDE facilitated the formation of a three-dimensional hydrogel structure through ring-opening reactions between HA and SF. Increasing the BDDE concentration led to a higher elastic modulus (G') and a lower viscous modulus (G'') of the hydrogel (Fig. 1E), indicating increased hydrogel hardness but decreased viscoelasticity. High BDDE concentrations result in excessively rigid and brittle gels. Therefore, it is crucial to avoid excessive BDDE concentrations. The data demonstrated that a 1% BDDE concentration was sufficient to satisfy the mechanical property requirements.

3.3. Excellent characterization and gelation benefits

3.3.1. Validation of crosslinking by FTIR

Cross-linking between HA and SF was achieved by the formation of ether bonds *via* BDDE. The C—O—C stretching vibration was

primarily observed in the range of 1310–1000/cm for asymmetric stretching and 1055–870/cm for symmetric stretching. A significant increase in the C—O—C peak was observed in the HA-SF gel compared to HA and SF, indicating successful crosslinking (Supporting Information Fig. S2). The 5% SF hydrogel showed a significantly weaker C—O—C stretching vibration peak compared to the other concentrations, suggesting that an excessively high SF concentration may hinder efficient crosslinking, which is consistent with the observed decrease in mechanical properties. The FTIR spectrum of the HA-SF gel exhibited several characteristic HA peaks. The peaks at 1407 and 3418/cm are attributed to —OH stretching, whereas the peaks at 2909 and 1630/cm are attributed to —COOH and —NH₂ groups, respectively^{36,37}.

The absorption peaks of Silk I appear at 1650–1655/cm (amide I), 1525–1540/cm (amide II), and 1266/cm (amide III). The absorption peaks of Silk II appear at 1620–1635/cm (amide I), 1531/cm (amide II), and 1230–1235/cm (amide III)^{38–41}. The HA-SF gel exhibited a prominent absorption peak at 1638/cm, indicating a predominant β -sheet structure. Additionally, the characteristic amide II peak at 1535/cm significantly diminished in the HA-SF gel, suggesting the transformation of the original random structure into a β -sheet form.

3.3.2. Particle size and compressive force assessment for injectability

The hydrogel particle size and rheological properties are critical factors for successful injection. The shear-thinning behavior of the HA-SF gels, which showed an increasing and then decreasing viscosity with increasing shear stress, indicated their shear-thinning properties for injectability (Fig. 1F). The sieved hydrogel particles stained with FITC exhibited irregularly fragmented shapes (Fig. 1G). The analysis indicated a uniform particle size distribution, with an average particle size of 150–200 μ m for both HA and HA-SF gels (Fig. 1H and I). We measured the injection force, which remained steady between 18 and 20 N, similar to existing HA products (22 N). The results (Supporting Information Table S2) show that the HA-SF gel has uniform particle size and meets the injectability requirements. The selected particle size range of 150–200 μ m for HA and HA-SF gels is appropriate for intermediate to deep dermal repair, aligning with the typical range of dermal fillers used for structural support. This choice is further validated by its consistency with commercial products and smooth injection performance, as well as literature findings highlighting the influence of particle size on filler integration and mechanical support. Physical sieving has limited control over particle size, suggesting the need for a hydrogel particle-sizing machine for industrial scale-up⁴².

The compression stress–strain properties of the hydrogel were assessed to determine whether it could maintain structural integrity during injection. As shown in Supporting Information Fig. S3 and Supporting Information Table S3, HA-SF hydrogel particles withstood the mechanical stress generated during injection without rupture, ensuring their effectiveness as a filler. Additionally, the impact of the injection process on hydrogel particle size was evaluated. The results indicated that both HA gel and HA-SF hydrogel maintained a particle size of approximately 190 μ m before and after injection, with only a slight reduction post-injection (Supporting Information Fig. S4 and Table S4). This suggests that the hydrogel retained its mechanical properties and particle integrity, further supporting its suitability for injection-based applications. The current research underscores the

importance of reducing the hydrogel particle size to ensure injectability, providing a theoretical foundation for further development.

3.3.3. Porous structure beneficial for cell proliferation

The microstructure and porosity of the gels are crucial for cell adhesion⁴³. SEM images of the freeze-dried gels showed a porous sponge-like structure for both HA and HA-SF gels at different concentrations (Fig. 2A). The non-crosslinked HA solution exhibited a denser structure, while the HA-SF gels had a network structure similar to that of the biological scaffold, facilitating dermal cell and tissue adhesion after injection⁴⁴.

Mercury intrusion porosimetry was used to analyze the pore distribution and porosity of the lyophilized gels. The predominant pore sizes for HA and HA-SF (1% SF) gels were 50–75 μm . When the SF concentration increased, the pore size distribution increased to approximately 150–200 μm (Fig. 2B). The strong hydrophilicity and flexibility of SF contribute to a loosely packed three-dimensional network with larger pores compared to the rigid HA structure. Additionally, the incorporation of SF alters the cross-linking density, leading to a more dispersed network with increased pore size due to the longer and more flexible nature of SF molecules. Differences in pore structures affect the promotion of various cell types. For dermal layer filling, a moderate pore size (150 μm) of the HA-SF composite hydrogel was considered favorable for skin cell proliferation⁴⁵.

The differences in testing principles and sample preparation contribute to variations in measured pore sizes, as SEM examines dried hydrogels, which may shrink, while the mercury intrusion method assesses pores in their original hydrated state. Additionally, the mercury intrusion method provides a broader measurement range, capturing larger pores that SEM might overlook due to resolution and magnification limits.

3.3.4. Reversibility reduce adverse reactions

In aesthetic medicine, the reversible degradation of HA gels allows for corrective measures using high-concentration enzymes. We investigated whether the addition of SF maintained this reversibility *in vitro* by simulating rapid degradation. The HA gel exhibited the fastest reversible degradation because of its higher affinity for HA enzymes. Reversibility experiments on the HA-SF gels (1%, 2%, and 5% SF) indicated slower degradation rates compared to the HA gel (Fig. 2C). However, all gels achieved complete degradation within 8 h, meeting the clinical requirements of rapid hydrogel degradation within 24 h. HA-SF gels also demonstrated reversible degradation, allowing for the correction of unsatisfactory injection results. These results confirm the reversibility of HA-SF gels and their potential for adjustment and reversal in aesthetic medicine.

3.3.5. Better performance in swelling and moisturizing

The swelling index and equilibrium water content of the hydrogel directly affect the nutrient and gas exchange³⁵. The HA gel exhibited the highest water-uptake capacity. The addition of SF reduced the swelling capacity and prevented excessive swelling (Fig. 2D), maintaining a moderate swelling ratio without competing with the skin for water molecules. The water retention capacity was significantly improved; the mixed gels containing 1%–5% SF demonstrated 1.5 times higher retention than the HA control. These results demonstrate the enhanced moisturizing capacity of the HA gel. The addition of SF significantly increased the hydrophilicity of the hydrogel, resulting in a tighter

association between SF and H₂O molecules and improved the moisturizing capacity of HA gel.

3.3.6. Strong stress–strain stability

Stress–strain stability tests were conducted to assess stability under *in vivo* stress conditions. The mechanical properties of hydrogel G' were tabulated by two measurement modes, amplitude sweep mode, and continuous alternate strain sweep mode. If G' is stable, it indicates that the internal structure of hydrogel is stable. The gels maintained stable G' values, indicating excellent structural stability and fulfilling of the stress requirements (Fig. 2E). These data confirm the stability of HA-SF and HA gel, supporting the reliability of the preparation method. The gels exhibited good mechanical performance under various stresses, making them suitable for tissue filling or biomedical applications and providing mechanical support to the surrounding tissues.

3.4. Raising stability and support of HA-SF gel

3.4.1. Enhanced support effect in vitro

The supportiveness of the filler materials is critical for the evaluation of injectable hydrogels (Fig. 3A). The degradation rate of the HA-SF gel was monitored over time and compared with that of the HA gel (Fig. 3B). The hydrogel initially absorbed water in PBS, reaching its maximum swelling after 3 days, followed by linear degradation (Fig. 3C). The incorporation of 1%–2% SF notably reduced the degradation rate compared to HA gel, extending the complete degradation time to 30 days. SEM analysis showed that the HA-SF gel (2% SF) had smaller and denser pores compared to the HA gel at 15 and 20 days, due to increased crosslinking density from the addition of SF, resulting in a more compact structure (Fig. 3E). These results indicate that the presence of SF effectively prolongs the support time and stability of the injectable filler.

3.4.2. Prolonged subcutaneous support duration

Live animal fluorescence imaging was performed at different time points to compare the residence times of the IR-820 labeled gels. Results showed that CTRL (IR-820 Sol) diffused the fastest and had the shortest fluorescence quenching time (Fig. 3D and F). The HA gel had undetectable fluorescence after 40 days, while the composite hydrogel still exhibited fluorescence after 54 days. ROI analysis revealed prolonged residence time and increased support with the addition of SF to the hydrogel. The *in vivo* and *in vitro* results confirmed that the SF incorporation into facial fillers extends the support time, reduces the injection frequency, and improves patient compliance. This provides a potential solution to the multiple injections required by current HA gels.

3.5. Biocompatibility assessment of HA-SF gel

3.5.1. Extremely low hemolysis rate of the HA-SF gel

Evaluating the hemocompatibility of medical devices is crucial for assessing risks such as hemolysis and thrombus formation in medical aesthetics. The hemolysis rate results (Fig. 4A) showed that both the HA and HA-SF gels had a hemolysis rate of approximately 2%, which was significantly lower than the industry standard of 5%, confirming the excellent hemocompatibility and safety of the HA-SF gel.

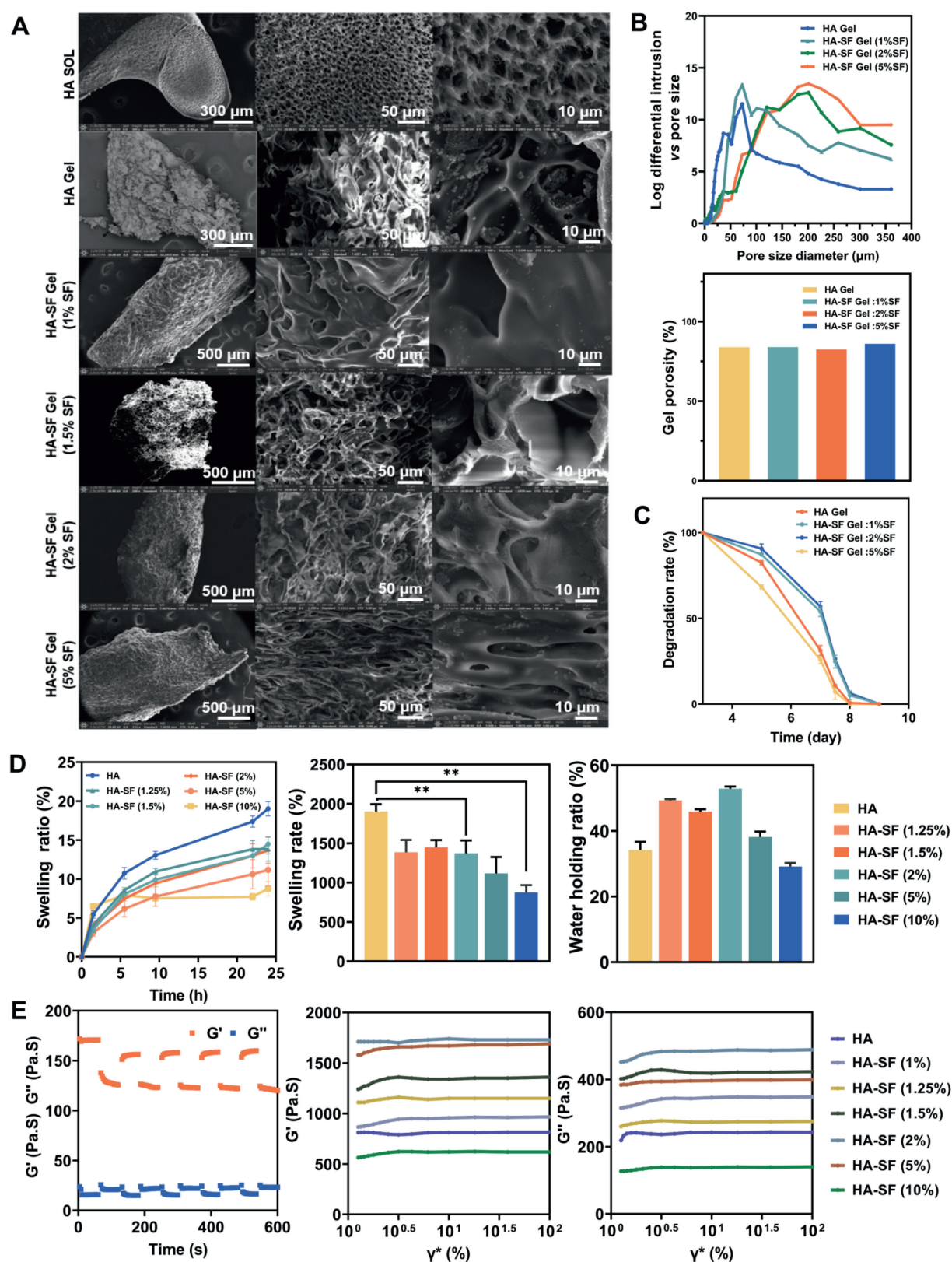


Figure 2 Physicochemical properties of HA-SF gel. (A) SEM images of HA gel and HA-SF gel with varying SF concentrations (1, 1.5, 2, 5% SF, and 10% HA). (B) Pore size distribution and porosity of lyophilized HA-SF gel (1, 2, 5% SF, and 10% HA) and HA gel (10% HA). (C) Rapid degradation curves in response to enzymatic activity (200 U/mL HA enzyme) of the hydrogel ($n = 3$). (D) Swelling curve, swelling rate, and water retention rate of HA-SF gel (1.25, 1.5, 2, 5, 10% SF and 10% HA) and HA gel (10% HA) ($n = 3$ trials). (E) The mechanical stability was assessed through a frequency sweep of HA-SF gel (2% SF, 10% HA) and continuous-discontinuous scanning in 1–100 γ^* of HA-SF gel (1, 1.25, 1.5, 2, 5, 10% SF and 10% HA) and HA gel (10% HA). Data are expressed by mean $\pm$ SEM.

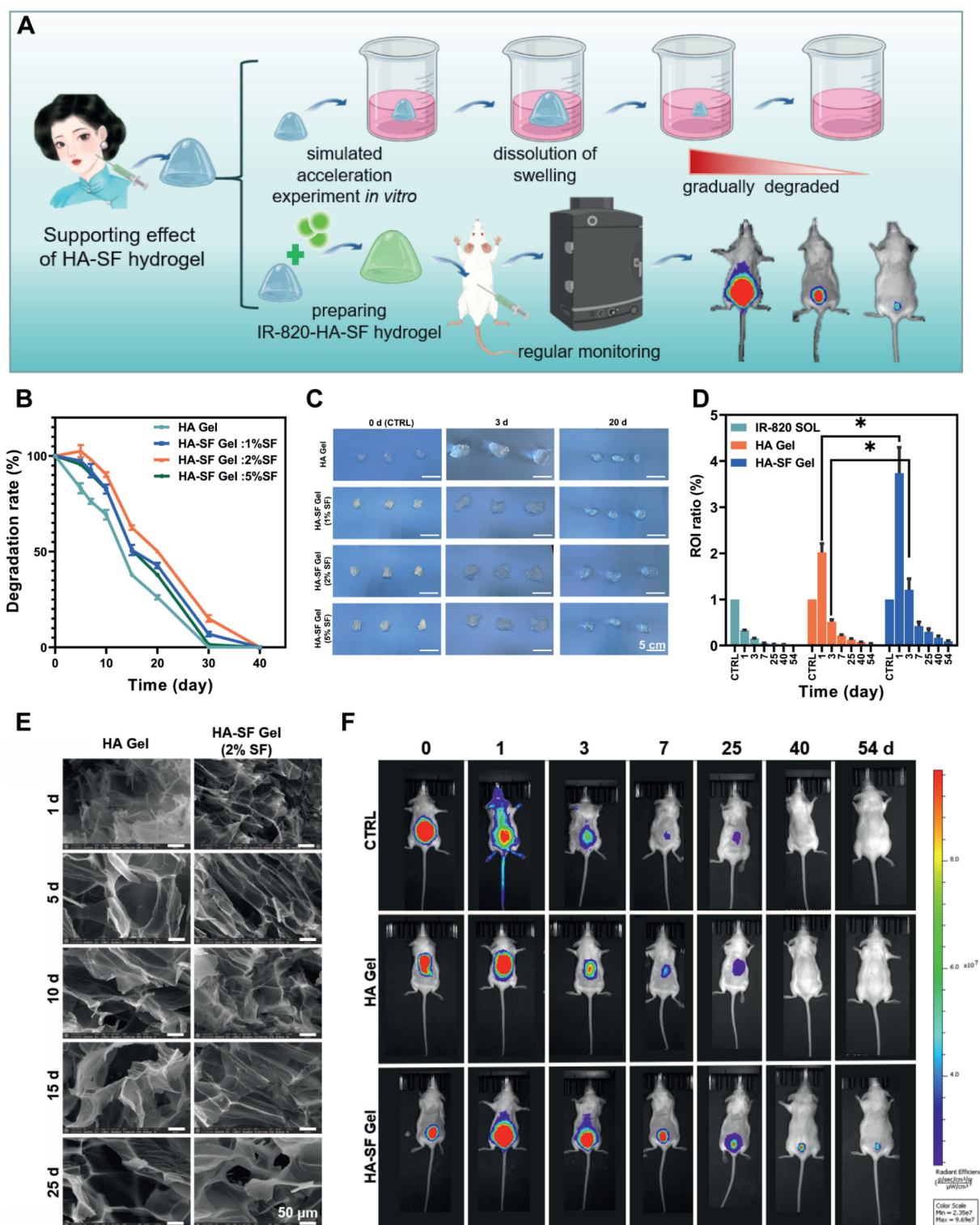


Figure 3 Enhanced stability and support effect of HA-SF gel. (A) Schematic of stability and support effect. (B) Degradation kinetics of HA-SF gel (1%, 2%, 5% SF and 10% HA) and HA gel in an accelerated skin microenvironment (76.1 ± 51.8 ng/mL HA enzyme and 26.84 ± 10.1 ng/mL Cathepsin XIV) ($n = 3$). (C) Micrographs illustrating degradation, and (E) SEM images of HA gel (10% HA) and HA-SF gel (10% HA, 2% SF) on Days 1, 5, 10, 20, and 25. (D) Fluorescence ROI intensity monitoring and (F) *in vivo* fluorescence imaging on Days 1, 3, 7, 25, 40, and 54 after subcutaneous injection of IR-820 HA-SF gel (10% HA, 2% SF) and HA gel (10% HA) in mice ($n = 5$). Data are expressed by mean $\pm$ SEM. * $P < 0.05$.

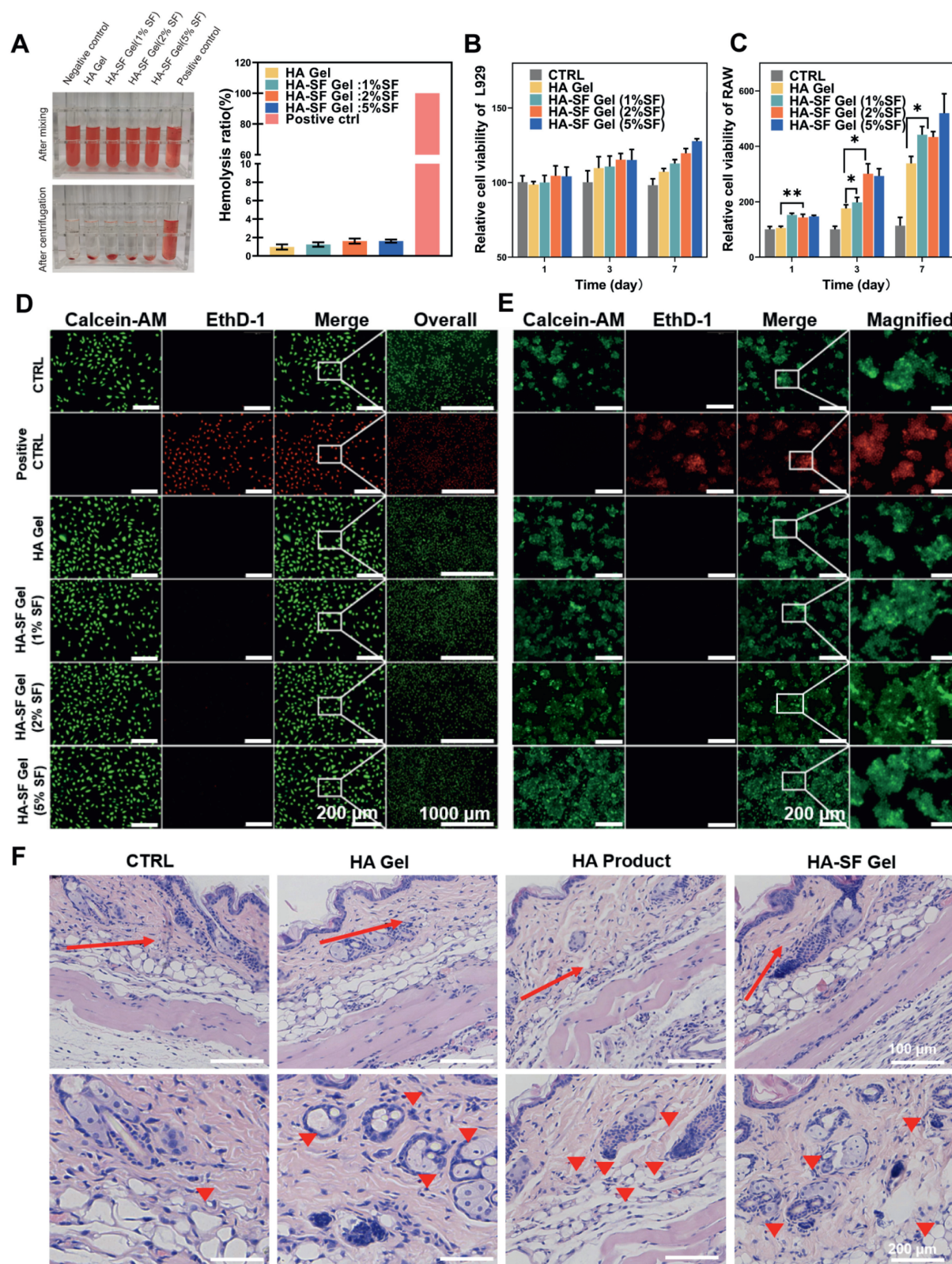


Figure 4 Biocompatibility assessment of HA-SF gel. (A) Hemolysis rate evaluation of HA-SF gel (1%, 2%, 5% SF and 10% HA) and HA gel (10% HA) ($n = 3$ trials). Viability assessment of (B) L929 cells and (C) RAW 264.7 cells co-cultured with the HA-SF gel (1%, 2%, 5% SF and 10% HA) and HA gel (10% HA) for 1, 3, and 7 days by CCK-8 assay. Calcein-AM/EthD-1 staining of (D) L929 cells and (E) RAW 264.7 cells co-cultured for 3 days. Live cells are stained green and dead cells are stained red. (F) H&E of dermal tissues in mice injected with HA-SF gel, HA gel, and HA Product on Day 14. Red arrows indicate dermal layers and red triangles highlight neutrophils. Data are expressed by mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$.

3.5.2. HA-SF gel promotes cell proliferation

In this study, L929 and RAW264.7 cells were used to assess material safety. A CCK-8 assay kit was used to evaluate the *in vitro* cell compatibility of the hydrogel at 1, 3, and 7 days, and the results (Fig. 4B and C) showed that the addition of SF significantly promoted the growth and proliferation of both cell types, especially RAW264.7.

To investigate the biocompatibility of the hydrogels, calcein-AM/EthD-1 (live/dead) staining was performed on co-cultured L929 (Fig. 4D) and RAW264.7 (Fig. 4E) cells, with live cells stained green and dead cells stained red. Green fluorescence indicated excellent cell morphology and growth after co-incubation with the HA-SF gel. The HA-SF gel significantly enhanced the proliferation of L929 and RAW264.7, as confirmed by increased cell density, consistent with the CCK-8 results. No evident cell death (red fluorescence) was observed, highlighting the non-cytotoxicity of the HA-SF gel to fibroblasts and macrophages, ensuring a favorable proliferative environment. The potential modulation of LPS-induced inflammatory responses was also investigated. The results revealed that both HA and HA-SF gels suppressed the secretion of NO (an inflammatory marker) in LPS-induced RAW264.7, protecting against potential adverse reactions (Supporting Information Fig. S5).

3.5.3. HA-SF promotes proliferation of skin 3D organoids

Organoids can mimic real organs in terms of both structure and function by simulating the tissue architecture and activities found *in vivo*. Previous 2D cell experiments demonstrated that SF significantly promotes cell proliferation. To further validate the efficacy of SF, we established a 3D skin fibroblast organoid model designed to mimic the functionality of the dermal layer. SF was co-cultured with organoids, and their growth was monitored over 2 weeks. The organoid volume in the SF group was significantly greater than that in the HA group between 9 and 13 days (Supporting Information Fig. S6). HE staining of the fixed organoids revealed no necrosis in the SF group; the cells were healthy, well concentrated, adhered well, and showed high density. This supports the benefits of SF in skin fibroblast proliferation, indicating that fibroblasts play a critical role as the main producers of collagen and elastic fibers in the dermal layer. The increased number of fibroblasts induced by SF may enhance collagen production, increase elasticity, reduce wrinkles, and improve key indicators of skin youthfulness.

3.5.4. Compatibility and reliability post-injection

The *in vivo* tissue compatibility of the gels was evaluated by injecting the hydrogel into the subcutaneous dermis tissue (Fig. 5A and D). IL-6 and TNF- α were measured using ELISA at 7, 14, and 21 days. The results showed consistently low levels of inflammatory factors in the three experimental groups compared with those in the CTRL group. Further analysis of the 13 pro-inflammatory factors revealed the highest inflammation on Day14 after injection. Similarly to many situations, material debris implanted *in vivo* can cause non-infectious inflammation⁴⁶. HA-SF gel exhibited inflammation levels similar to CTRL and lower than HA gel and commercial HA Product, particularly in reducing Eotaxin, IP-10, MIP1- β , and BLC. This indicated that the HA-SF gel maintained good biocompatibility and did not induce significant inflammation (Fig. 5B and C).

Furthermore, histological staining, including H&E staining, was performed on the filled dermal sections. Representative images are shown in Fig. 4F. After 14 days, H&E staining revealed

mild inflammatory reactions and a few neutrophils in all groups. The CTRL group had the highest safety score "A", followed by the HA-SF gel, HA gel, and commercial HA gel, all with a "B" rating. This indicates that the skin was stratified and structurally complete, representing a healthy state. In conclusion, the HA-SF gel not only matches the safety of the CTRL group but also shows the potential to reduce certain inflammatory factors, further improving the safety profile.

3.6. Investigation of bioreactivity of HA-SF gel

3.6.1. Enhancement of adhesion-related protein expression

In addition to safety, the future direction of aesthetic dermal fillers will focus on bioreactivity. Insufficient adhesion of the injected hydrogel can lead to its isolation in the dermis, thereby increasing hydrogel displacement in extreme adverse reactions. The addition of SF improved the adhesion of the filler materials to tissues. During growth and maturation, cells express adhesion-related proteins such as vinculin and fibronectin. Vinculin links actin filaments to the plasma membrane and is associated with trans-membrane protein induction. Fibronectin plays a vital role in cell adhesion, and cell polarity, and is involved in cell proliferation, migration, hemostasis, and tissue repair^{47,48}.

IF analysis of the adhesion functionality was performed using both cellular and histopathological methods. After co-incubation on the hydrogel for 3 days, results showed that Vinculin (Fig. 6A) and Fibronectin (Supporting Information Fig. S7) exhibited excellent adhesion to the HA-SF gel, with large spreading areas and prominent filamentous junctions. The expression of vinculin and fibronectin was significantly increased, especially fibronectin, which showed a remarkable increase in the HA-SF gel compared to that in CTRL (Fig. 6B).

Histopathological sections of mouse dermal tissue stained with adhesion antibodies (Fig. 6C and D) revealed an increased fluorescence intensity of fibronectin and vinculin in the dermis compared to that in CTRL, indicating that SF in the hydrogel enhanced adhesion. However, this enhancement was less pronounced at the cellular level, possibly because of the greater individual variability in mice, where protein expression is influenced by multiple factors (Supporting Information Fig. S8).

Overall, both cell-based and animal studies demonstrated that the HA-SF gel exhibited superior adhesive functionality compared with the HA gel. Improving tissue adhesion to filler materials offers potential advantages for future applications, underscoring the importance of bioactivity and safety in the development of filler materials.

3.6.2. Stimulation of collagen type III regeneration in dermis

Current fillers rely primarily on the physical filling of concave areas, which, although safe, lack inherent bioactivity. An exciting prospect lies in HA-SF gels, which can stimulate fibroblasts in the dermis, increase collagen secretion, and promote skin rejuvenation at the source, reducing the need for frequent injections. Skin collagen primarily consists of types I and III collagen. Type I collagen, which accounts for 80%, supports the skin structure and tissue integrity, whereas type III collagen (15%) provides skin elasticity, stretchability, and suppleness. Infants have abundant type III collagen (30%–60%) in their skin, which decreases to 10%–20% in adults with age^{49,50}. We hypothesized that SF would stimulate collagen regeneration and selectively increase type III collagen levels. Based on this hypothesis, the hydrogel may not

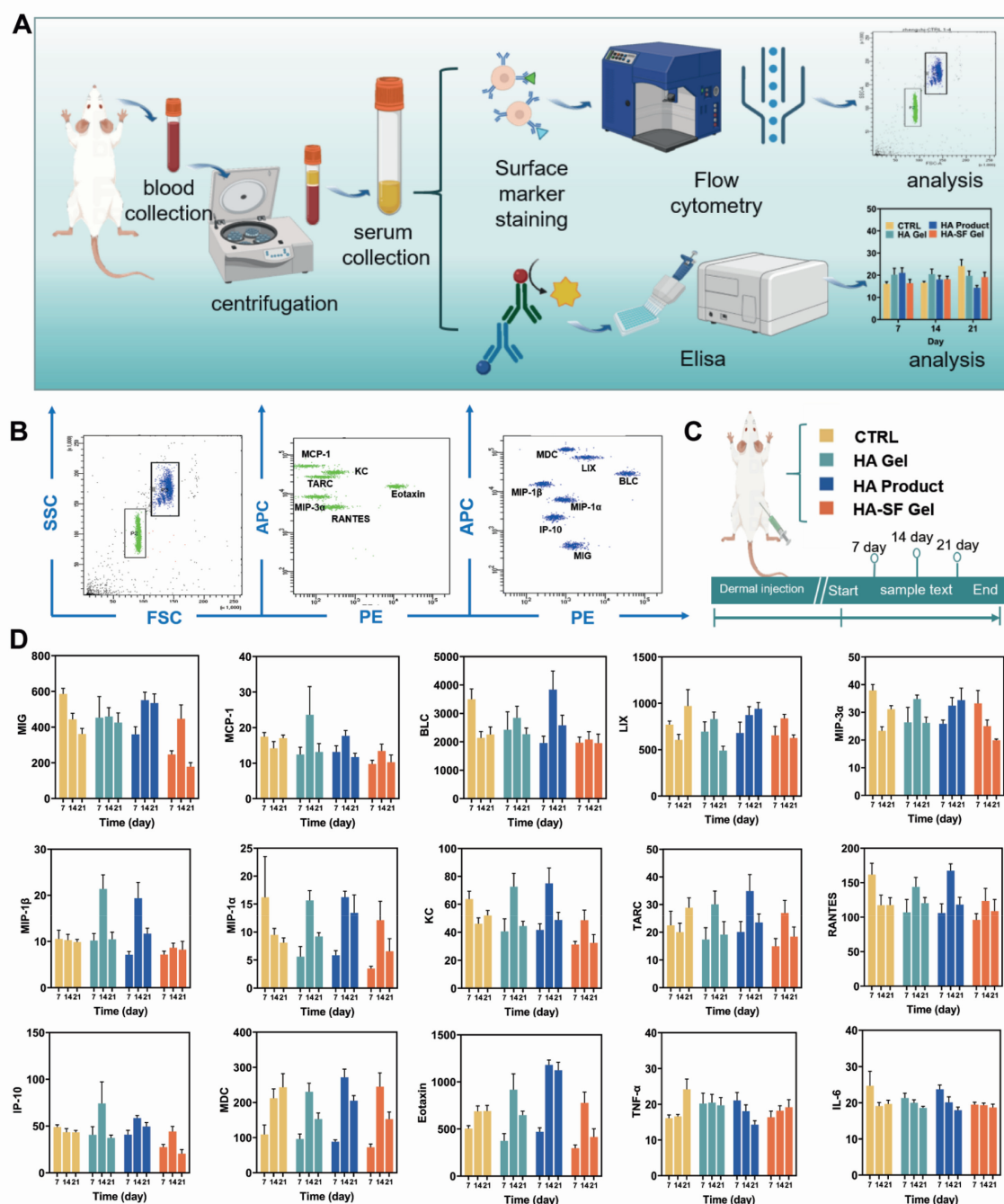


Figure 5 Biocompatibility evaluation of HA-SF gel. (A) Descriptive diagram of biocompatibility. (B) Distribution of inflammatory factors by flow cytometry analysis. (C) Diagram of intradermal injection experiment. (D) Quantification of inflammatory factors of Eotaxin, MIG, MCP-1, BLC, LIX, MIP-3α, MIP-1β, MIP-1α, KC, TARC, RANTES, IP-10, and MDC in the serum of hydrogel-injected mice, including HA-SF gel, HA gel, and HA Product on Days 7, 14 and 21 ($n = 6$ mice per group). Measurement of TNF-α and IL-6 levels in the serum of hydrogel-injected mice, including HA-SF gel (2% SF and 10% HA), HA gel (10% HA), and HA Product on Days 7, 14, and 21 ($n = 6$ mice per group). Data are expressed by mean ± SEM.

only serve as a physical filler but also actively promote rejuvenation by influencing the ECM.

To test this hypothesis, Masson's trichrome staining of a hydrogel-filled dermal section was performed. Collagen fibers were stained blue and muscle fibers were red. The results showed that both commercially available HA Product, HA gel and HA-SF

gel, promoted collagen fiber formation compared with the CTRL group, with no significant differences observed between them (Fig. 7A and C). Masson's trichrome staining provides an overall view and cannot differentiate between type I and type III collagen.

Picrosirius red staining (PSR) was performed using polarized light to scan the sections. PSR allowed the identification of Type I

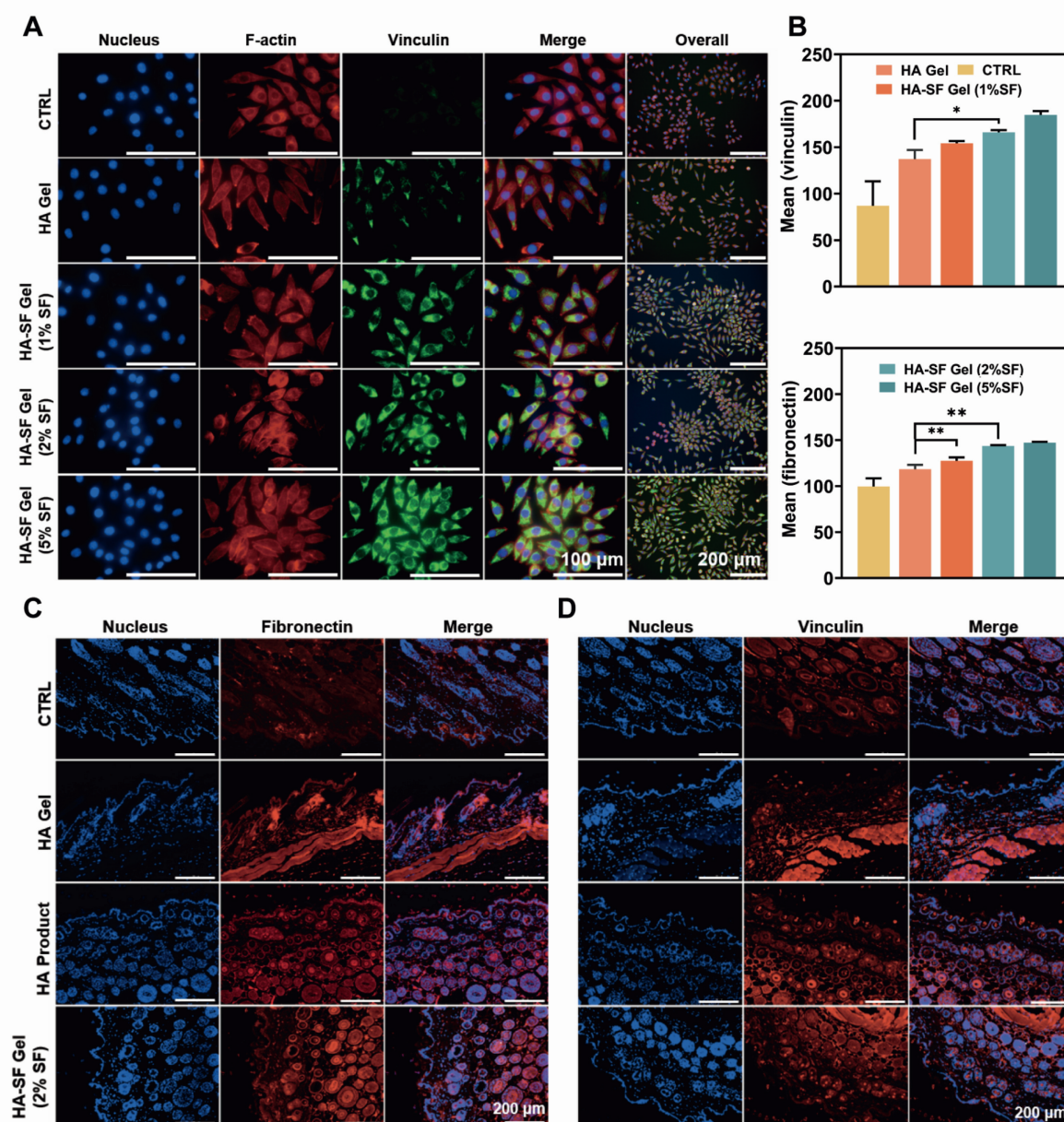


Figure 6 Expression of adhesion-related proteins. (A) IF staining of vinculin after co-culture of HA-SF gel (2% SF and 10% HA), HA gel (10% HA) with L929 cells. (B) Semi-quantitative analysis of fluorescence intensity for vinculin and fibronectin ($n = 6$ slices). (C, D) IF staining of mouse dermal tissue sections after injection of hydrogel, including HA-SF gel, HA gel, and HA product on Day 21 focusing on fibronectin and vinculin expression ($n = 3$ slices). Data are expressed by mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$.

collagen (yellow) and Type III collagen (green). The results showed that the dermis consisted primarily of type I collagen, with type III collagen dispersed in a star-like pattern among the type I collagen (Fig. 7A and D). Quantification revealed that the HA-SF gel exhibited significantly higher type III collagen content, suggesting that SF may facilitate type III collagen secretion.

The quantitative results of IF double staining for collagen I and III in pathological sections further confirmed this finding (Fig. 7B and E). Precise quantification of both collagen types in tissues was achieved by ELISA, confirming higher secretion of type III collagen in the skin of mice injected with the HA-SF gel (Fig. 7F).

Additionally, results of IF in skin organoids following co-culture with HA-SF gel also confirmed that the secretion of collagen I in the HA group was close to in HA-SF group (Supporting Information Fig. S9), but there was more red fluorescence in the HA-SF group, suggesting that type III collagen was secreted more due to SF.

Additionally, we validated two aging-related skin markers. SF treatment significantly enhanced the expression of collagen IV (Supporting Information Fig. S10), a key component of the basement membrane that maintains skin structural stability, cell adhesion, and barrier function. Age-related collagen IV reduction

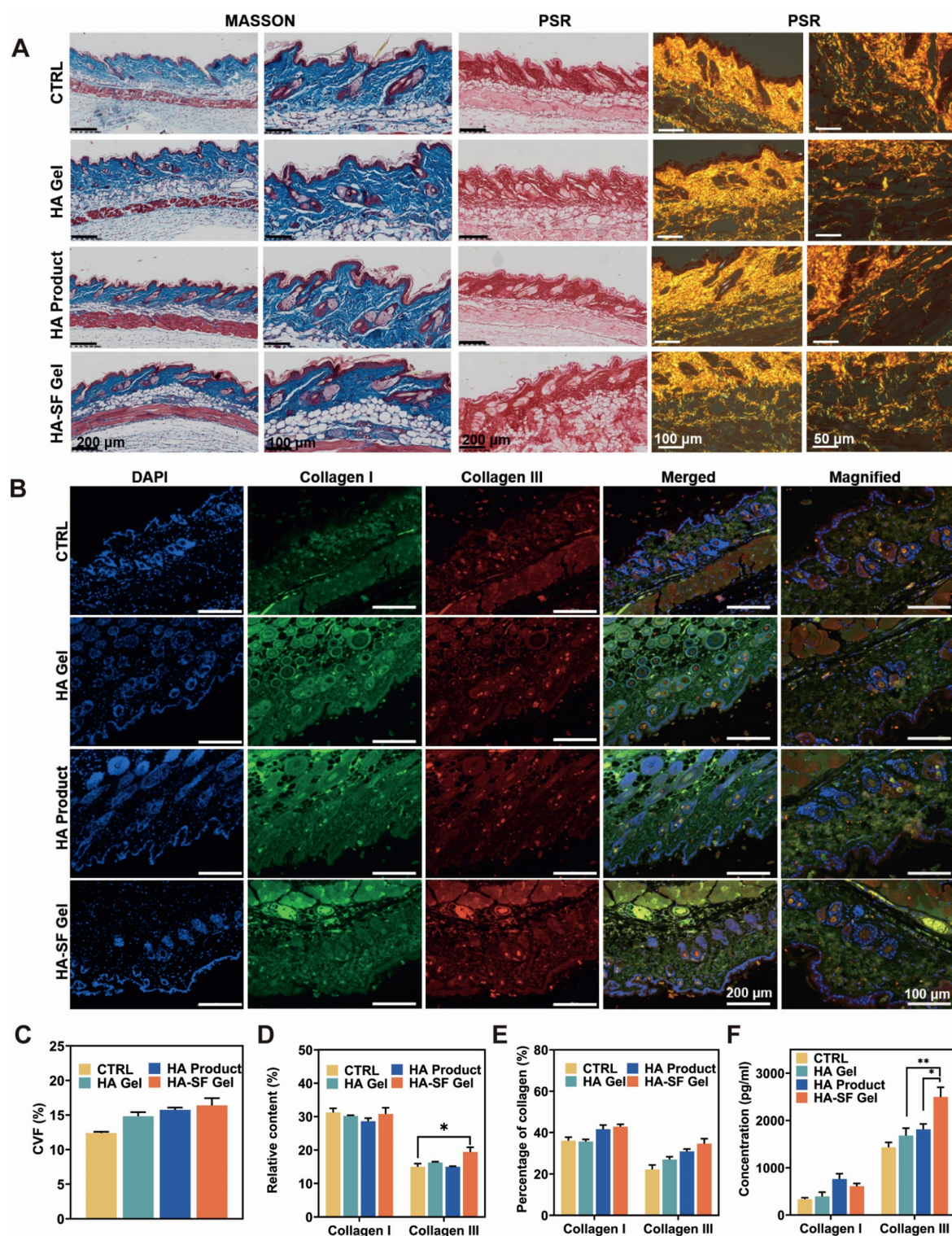


Figure 7 Stimulation of collagen type III regeneration in the dermis. (A) Masson and PSR staining, and (B) IF staining of collagen type III and type I in skin sections of mice injected with HA-SF gel (2% SF and 10% HA), HA gel (10% HA), and HA product on Day 21. (C) Calculation of collagen volume fraction of Masson staining in Fig. 7A ($n = 3$ slices). (D) Semi-quantitative analysis of collagen type III and I in PSR in Fig. 7A ($n = 3$ slices) (E) IF quantitative analysis of collagen type III and I in Fig. 7B ($n = 6$ slices). (F) ELISA quantification of collagen in dermal tissue of mice injected with HA-SF gel, HA gel, and HA product on Day 21 ($n = 6$ slices). Data are expressed by mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$.

compromises basement membrane integrity, and our findings suggest that SF may help counteract this aging process. Analysis revealed that SF treatment promoted elastin expression (Supporting Information Fig. S11), contributing to improved skin elasticity. Since elastin degradation is a major factor in skin laxity and wrinkle formation, this finding further highlights the potential anti-aging benefits of SF.

Taken together, SF enhances collagen secretion and specifically favors the generation and transformation of type III collagen. These promising results have significant implications for future applications of skin-filling materials, potentially providing an effective means for improving skin rejuvenation. However, we also recognize certain limitations that require reflection. We used the ICR mouse model to simulate human skin regeneration, recognizing, however, key differences in skin structure and collagen metabolism between mice and humans⁵¹. Mouse skin has a thinner epidermis, higher follicle density, and faster collagen turnover, which may influence the hydrogel's effectiveness, particularly in adult human skin where collagen type III synthesis differs. In the discussion, we address these model limitations and recommend further studies in human skin models to better predict clinical outcomes.

3.6.3. Enhancing type III collagen in the UVB photoaging SKIN

The UVB-induced photoaging model successfully simulates the progressive nature of skin aging. Over time, accumulated UVB exposure increases the tolerance levels of BALB/c nude mice, leading to gradually worsening skin damage (Fig. 8A). Changes range from mild erythema and scaling to more severe roughness and hemorrhage, in Supporting Information Table S5. Additionally, the model group (UVB) showed significant weight loss over the 6 weeks irradiation period (Fig. 8B). Following HA-SF gel injections, signs of delayed skin aging were observed, with improvements in skin smoothness, hydration, elasticity, and wrinkle scores approaching those of the unirradiated CTRL group (CTRL).

Skin conditions were carefully monitored throughout the experimental period. UVB exposure led to significant skin dehydration in UVB group, while HA-SF gel injections gradually restored hydration, reaching nearly normal levels by 6 weeks (Fig. 8C). Ultrasound imaging showed changes in dermal collagen density, with collagen (high-intensity regions) marked in white and yellow (Fig. 8D and E). Initially, collagen was abundant but decreased over time with UVB exposure. Notably, collagen levels in the HA-SF gel group increased significantly between 5 and 6 weeks, while the HA gel and HA Product groups showed moderate increases by 6 weeks.

Molecular analysis of skin samples, including gene and protein assessments on Week 6, revealed a notable increase in collagen in the HA-SF gel group, particularly in type III collagen (Fig. 8F and G). In contrast, the HA gel and commercial HA products produced only minor increases in type I collagen with negligible impact on type III collagen. Histopathological analysis with HE staining showed variations in dermal thickness, with the UVB group displaying substantial skin thickening and coarseness, while the HA-SF gel group showed reduced thickness. Masson staining demonstrated the collagen fiber density, showing sparse blue-stained fibers in the UVB group and a more compact fiber network in HA-SF gel (Fig. 8H and I).

All-trans retinoic acid (at-RA), the only FDA-approved anti-aging agent for skin, was used as a positive control. At-RA enhanced skin turnover and collagen synthesis, confirmed by

Western blot, PCR, and histopathological analyses, though its effect on type III collagen was minimal. Notably, it caused considerable adverse effects, including substantial peeling, erythema, and marked weight loss (Fig. 8H and I), with histopathology indicating skin thinning, consistent with known clinical side effects. In comparison, HA-SF gel demonstrated a balanced promotion of endogenous collagen production, effectively counteracting age-related dehydration and wrinkle formation, contributing to smoother, more youthful skin.

3.7. Regulate skin metabolism and improve skin vitality of SF

To further explore the mechanisms underlying the cellular behavioral properties of the HA-SF gel, a proteomic analysis was performed. We compared the influence of CTRL (A), HA-product (B), HA gel (C), and HA-SF gel (D) using principal component analysis (PCA) (Fig. 9A). Significant differences were observed between the CTRL and HA-SF groups as well as between HA-gel and HA-Pro. The HA-SF gel samples exhibited different protein expression levels compared to the HA gel and commercial HA gel, and subsequent analyses were performed.

In Fig. 9B, the heatmap shows the differential protein expression in groups A–D, with 7423 proteins identified. The volcano map showed 1342 differentially expressed proteins, including 508 upregulated and 834 downregulated proteins marked in red and green, respectively (Fig. 9C). In the C–D group (Supporting Information Fig. S12), 7447 proteins and 145 differentially expressed proteins were identified, including 88 upregulated and 58 downregulated proteins. In groups B–D (Supporting Information Fig. S13), 7545 proteins and 340 differentially expressed proteins were identified, including 217 upregulated and 122 downregulated proteins. The distribution of samples within group C showed significant variability, precluding further analyses. ($P < 0.05$, fold change (FC) > 1.2).

Among the 10 most significantly enriched gene ontology (GO) terms in each category for the A–D comparison (Fig. 9D), terms such as biosynthetic process of peptide, cellular macromolecule, cellular, and organic substance in biological process (BP) category; nucleic acid binding, tropomyosin, voltage-gated ion channel activity in the molecular function (MF) category; and intracellular organelle, cytoskeletal part, cytoskeleton, and ribosome in cellular component (CC) category suggested the beneficial biosynthesis effects and cell proliferation of HA-SF gel on the dermis (Fig. 8D).

In the B–D comparison, proteins were enriched in biosynthetic processes, metabolic processes, and transmembrane transport of alpha-amino acids in BP, cytoskeletal part, keratin filament, and intracellular organelle in CC, and structural molecule activity, receptor activity, and transferase activity in MF (Supporting Information Fig. S14) that are closely related to cell adhesion and metabolism. Both *in vitro* and *in vivo* experiments demonstrated that HA-SF effectively promoted cell growth, proliferation, and adhesion.

We performed Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis to further evaluate the pathways affected by HA-SF gel. In A–D comparison, proteins were enriched in amino acid metabolism, carbohydrate metabolism, protein metabolism, and other general cellular metabolism, such as “Ribosome,” “DNA replication,” “Mismatch repair,” “RNA transport,” all closely related to adhesion (Fig. 9E).

Notably, the analysis revealed potential associations between the HA-SF gel and cardiac diseases such as dilated

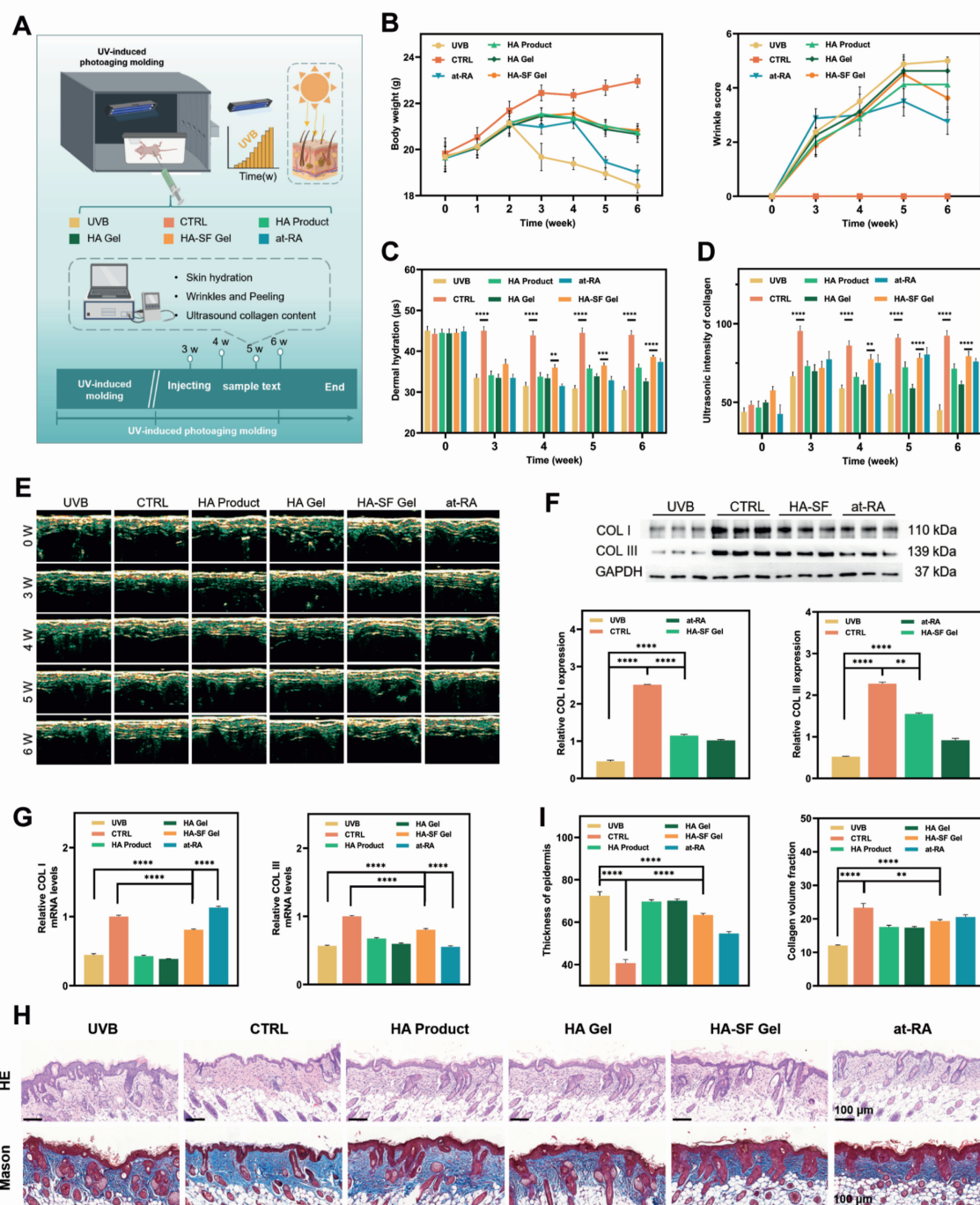


Figure 8 HA-SF gel reduces skin photoaging damage and increases collagen. (A) Schematic representation of the UVB-induced photoaging model and experimental design of HA-SF gel (2% SF and 10% HA). (B) Body weight and skin wrinkle score index of nude mice during the modeling period ($n = 8$ mice per group). (C) Statistics of skin hydration measured by electrical conductivity ($n = 4$ trials). (D) Statistical analysis of collagen intensity in the dermis measured by ultrasound ($n = 4$ trials). (E) Ultrasound imaging (yellow and white regions represent high-intensity collagen, while blue and green regions represent low-intensity substances such as skin fat, blood, and moisture). (F) Western blot analysis of collagen type I and type III expression in skin on Week 6 ($n = 3$ trials). (G) qPCR analysis of collagen type I and collagen type III gene expression levels in skin on Week 6 ($n = 8$ trials). (H) HE and Masson staining of skin on Week 6 (the deep purple margin at the top of HE staining represents epidermal thickness). (I) Statistical analysis of epidermal thickness from HE staining ($n = 8$ slices) and collagen deposition percentage from Masson staining ($n = 4$ slices). All data are representative of at least three independent experiments. Data are presented as mean $\pm$ SEM.

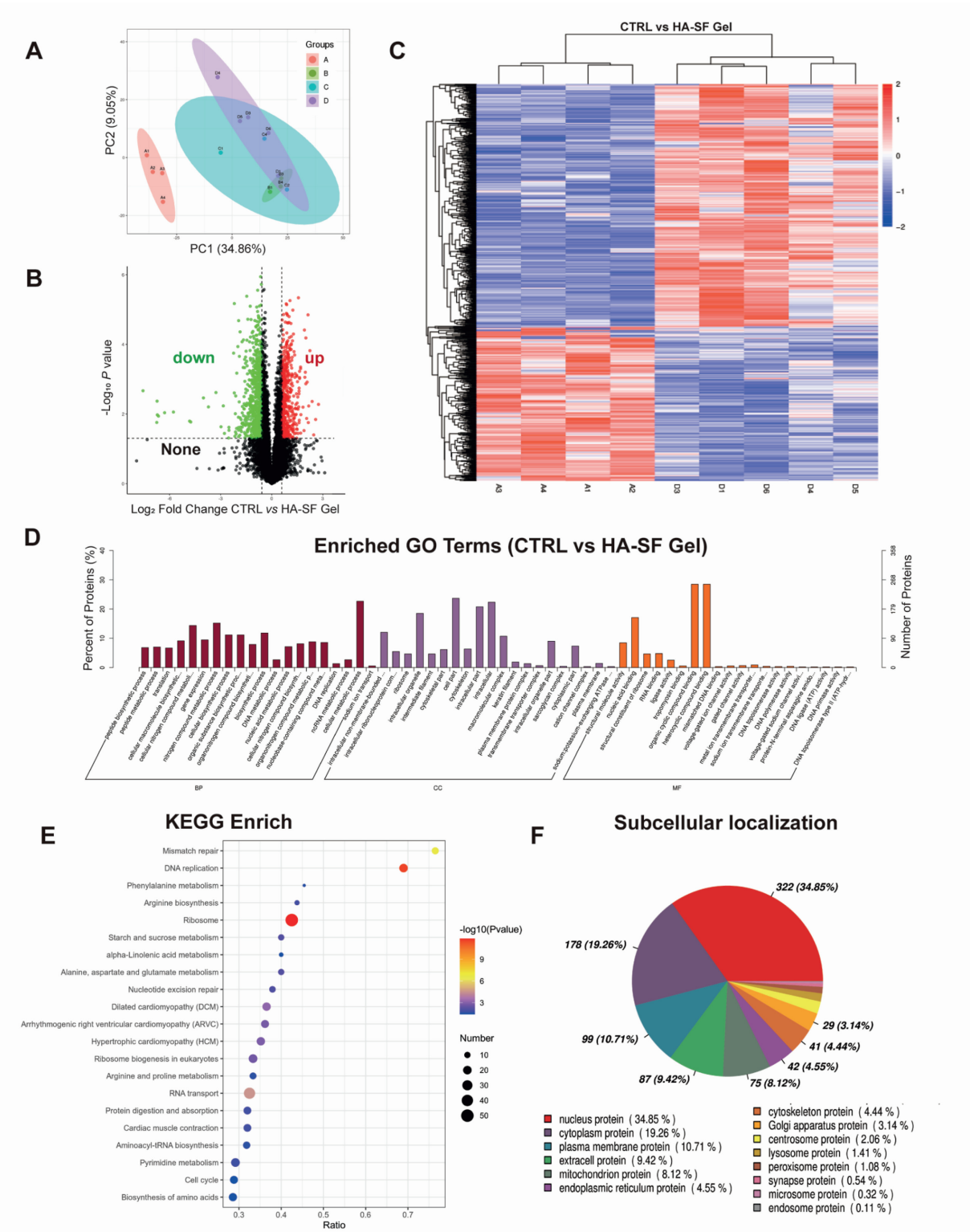


Figure 9 Analysis of differential protein expression in mice dermal tissue. (A) Principal component analysis (PCA) of various groups. A, CTRL; B, HA-product; C, HA gel and D, HA-SF gel. (B) Identification of Differentially Expressed Proteins (DEPs) in skin samples of CTRL vs HA-SF gel. The x-axis represents protein differences (log2-transformed fold changes), and the y-axis shows the corresponding $-\log_{10}$ -transformed P values. Red dots indicate significantly upregulated proteins, green dots represent significantly downregulated proteins, and gray dots indicate proteins with no significant change. (C) Cluster analysis chart of the identified DEPs of CTRL vs HA-SF gel. Higher red and blue intensities signify a greater degree of upregulation and downregulation, respectively. (D) Functional Gene Ontology (GO) classification of DEPs of CTRL vs HA-SF gel. The x-axis represents pathway annotation entries, and the y-axis shows the number of DEPs enriched in each pathway. (E)

cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC). We hypothesized that HA-SF gel may enhance cardiac myofilament contractility, thereby increasing myocardial strength and potentially reducing the risk associated with these conditions. Thus, this study provides a novel perspective on the role of SF in the treatment of heart disease (Fig. 9F).

KEGG enrichment analysis in the B–D comparison indicated that proteins were enriched in lipid metabolism, including arachidonic acid and linoleic acid metabolism; amino acid metabolism; alanine, aspartate, and glutamate metabolism; and phenylalanine metabolism (Supporting Information Fig. S15). This suggests that the addition of SF may benefit the metabolism of the organism, with potential improvements in key physiological processes such as lipid, amino acid, and protein metabolism.

In conclusion, our study systematically analyzed the proteomic data of HA-SF gels. Differentially expressed proteins were predominantly enriched in various physiological processes related to cell proliferation, including amino acid and lipid metabolism. Consequently, the incorporation of this gel into the skin results in improved cellular conditions. In the long term, it positively affects skin vitality and contributes to a more youthful complex.

4. Conclusions

In this study, natural SF and HA were chemically crosslinked to form an injectable hydrogel. Based on its physicochemical properties, the preparation method and formulation were optimized to determine the optimal substance composition. The HA-SF gel exhibited enhanced basic properties such as swelling rate, moisture retention, stress stability, porosity, and reversibility compared to single HA gel. This resulted in longer support duration, safety, and an inflammation-modulating capacity. Besides providing physical support, HA-SF gel demonstrated multiple biological activities, enhancing cell adhesion, boosting skin collagen (especially type III), and providing anti-aging benefits. Developed to address clinical concerns, the HA-SF gel exemplifies the complementary strengths of HA and SF, resulting in a dual-function filler that promotes bioactive effects. This filler not only addresses facial soft tissue loss but also replenishes collagen, supporting endogenous skin renewal and reducing signs of aging.

Limitations and future directions: while this study conducted extensive characterization of the hydrogel, the underlying mechanisms by which SF exerts its beneficial effects—particularly its role in skin improvement and anti-aging—remain unexplored. In future research, we will systematically investigate the mechanisms through which SF enhances skin quality and combats aging, providing deeper insights into its biological functions. Additionally, while this study was conducted on mouse models—where relevant similarities to human skin exist—further research is necessary to verify the effects on human skin. Beyond the current findings, this cross-linking approach applies to other polysaccharides (e.g., sodium alginate) and proteins (e.g., gelatin), expanding the hydrogel's potential use. Beyond dermatologic aesthetics, HA-SF gel could be beneficial for acute and chronic skin wounds, bone and joint treatments, and male genital fillers.

Further studies will explore HA gel in combination with filipin protein microspheres, seeking to refine its structural support and better understand its bioactivity mechanisms.

Acknowledgments

This work was financially supported by National Natural Science Foundation (No. 82304393, China), Beijing Natural Science Foundation (Nos. L242027 and L212059, China), National Key R&D Program (2023YFC2706100, China), CAMS Innovation Fund for Medical Sciences (No. 2021-I2M-1-026, China), and Beijing Nova Program (Nos. Z211100002121127 and 20220484219, China).

Author contributions

All authors contributed to the study conception, design, data analysis, and manuscript writing. All authors reviewed and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2025.04.020>.

References

- Samizadeh S, Samizadeh S. Dermal fillers: understanding the fundamentals. In: Samizadeh S, editor. *Non-surgical rejuvenation of Asian faces*. Cham: Springer International Publishing; 2022. p. 253–65.
- Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. *Acta Biomater* 2013;**9**:7081–92.
- An C, Li H, Zhao Y, Zhang S, Zhao Y, Zhang Y, et al. Hyaluronic acid-based multifunctional carriers for applications in regenerative medicine: a review. *Int J Biol Macromol* 2023;**231**:123307.
- Sekar MP, Suresh S, Zennifer A, Sethuraman S, Sundaramurthi D. Hyaluronic acid as bioink and hydrogel scaffolds for tissue engineering applications. *ACS Biomater Sci Eng* 2023;**9**:3134–59.
- Luo Y, Tan J, Zhou Y, Guo Y, Liao X, He L, et al. From crosslinking strategies to biomedical applications of hyaluronic acid-based hydrogels: a review. *Int J Biol Macromol* 2023;**231**:123308.
- Kim H, Jeong H, Han S, Beack S, Hwang BW, Shin M, et al. Hyaluronate and its derivatives for customized biomedical applications. *Biomaterials* 2017;**123**:155–71.
- Ding YW, Wang ZY, Ren ZW, Zhang XW, Wei DX. Advances in modified hyaluronic acid-based hydrogels for skin wound healing. *Biomater Sci* 2022;**10**:3393–409.
- Alauzun JG, Young S, D'Souza R, Liu L, Brook MA, Sheardown HD. Biocompatible, hyaluronic acid modified silicone elastomers. *Biomaterials* 2010;**31**:3471–8.
- Ballin AC, Brandt FS, Cazzaniga A. Dermal fillers: an update. *Am J Clin Dermatol* 2015;**16**:271–83.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway classification of DEPs of CTRL vs HA-SF gel. The x-axis indicates the enrichment factor (Ratio), i.e., the number of DEPs annotated to each pathway divided by all identified proteins annotated to the same pathway. The larger the value, the greater the proportion of DEPs annotated to each pathway. Dot sizes represent the number of DEPs annotated to each pathway. (F) Subcellular localization of DEPs.

10. Oh H, Lee S, Na J, Kim JH. Comparative evaluation of safety and efficacy of a novel hyaluronic acid-polynucleotide/poly-L-lactic acid composite dermal filler. *Aesthetic Plast Surg* 2021;**45**:1792–801.
11. Sedush NG, Kalinin KT, Azarkevich PN, Gorskaya AA, Hano C. Physicochemical characteristics and hydrolytic degradation of polylactic acid dermal fillers: a comparative study. *Cosmetics* 2023;**10**:110.
12. Gao Q, Duan L, Feng X, Xu W. Superiority of poly(L-lactic acid) microspheres as dermal fillers. *Chin Chem Lett* 2021;**32**:577–82.
13. Ielo I, Calabrese G, De Luca G, Conoci S. Recent advances in hydroxyapatite-based biocomposites for bone tissue regeneration in orthopedics. *Int J Mol Sci* 2022;**23**:9721.
14. Shi J, Dai W, Gupta A, Zhang B, Wu Z, Zhang Y, et al. Frontiers of hydroxyapatite composites in bionic bone tissue engineering. *Materials* 2022;**15**:8475–83.
15. Li P, Guo X. A review: therapeutic potential of adipose-derived stem cells in cutaneous wound healing and regeneration. *Stem Cell Res Ther* 2018;**9**:302.
16. Surowiecka A, Strużyna J. Adipose-derived stem cells for facial rejuvenation. *J Personalized Med* 2022;**12**:117–25.
17. Reizabal A, Costa CM, Pérez-Álvarez L, Vilas-Vilela JL, Lanceros-Méndez S. The new silk road: silk fibroin blends and composites for next generation functional and multifunctional materials design. *Polym Rev* 2023;1–64.
18. Liu J, Xie X, Wang T, Chen H, Fu Y, Cheng X, et al. Promotion of wound healing using nanoporous silk fibroin sponges. *ACS Appl Mater Interfaces* 2023;**15**:12696–707.
19. Zhou J, Lu F, Cong J, Wu Z. Effect of plasma jet on electrochemical properties of silk fibroin hydrogel doped with PEDOT:PSS. *Energy Sci Eng* 2023;**11**:2521–34.
20. Yang J, Li Z, Li S, Zhang Q, Zhou X, He C. Tunable metacrylated silk fibroin-based hybrid bioinks for the bioprinting of tissue engineering scaffolds. *Biomater Sci* 2023;**11**:1895–909.
21. Chen Y, Lyu R, Wang J, Cheng Q, Yu Y, Yang S, et al. Metal-organic frameworks nucleated by silk fibroin and modified with tumor-targeting peptides for targeted multimodal cancer therapy. *Adv Sci (Weinh)* 2023;**10**:e2302700.
22. Qi Z, Yan Z, Tan G, Jia T, Geng Y, Shao H, et al. Silk fibroin microneedles for transdermal drug delivery: where do we stand and how far can we proceed?. *Pharmaceutics* 2023;**15**:355.
23. Lee J, Park S, Lee S, Kweon HY, Jo YY, Kim J, et al. Development of silk fibroin-based non-crosslinking thermosensitive bioinks for 3D bioprinting. *Polymers* 2023;**15**:3567.
24. Shen Y, Wang X, Li B, Guo Y, Dong K. Development of silk fibroin-sodium alginate scaffold loaded silk fibroin nanoparticles for hemostasis and cell adhesion. *Int J Biol Macromol* 2022;**211**:514–23.
25. Zhang Y, Sheng R, Chen J, Wang H, Zhu Y, Cao Z, et al. Silk fibroin and sericin differentially potentiate the paracrine and regenerative functions of stem cells through multiomics analysis. *Adv Mater* 2023;**35**:e2210517.
26. Grabska-Zielinska S, Sionkowska A. How to improve physico-chemical properties of silk fibroin materials for biomedical applications?—blending and cross-linking of silk fibroin—a review. *Materials* 2021;**14**:1510.
27. Bachelor MA, Bowden GT. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. *Semin Cancer Biol* 2004;**14**:131–8.
28. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, et al. Mechanisms of photoaging and chronological skin aging. *Arch Dermatol* 2002;**138**:1462–70.
29. Gilchrist BA. A review of skin ageing and its medical therapy. *Br J Dermatol* 1996;**135**:867–75.
30. Shen C, Wang J, Li G, Hao S, Wu Y, Song P, et al. Boosting cartilage repair with silk fibroin-DNA hydrogel-based cartilage organoid precursor. *Bioact Mater* 2024;**35**:429–44.
31. Zhou Z, Song P, Wu Y, Wang M, Shen C, Ma Z, et al. Dual-network DNA–silk fibroin hydrogels with controllable surface rigidity for regulating chondrogenic differentiation. *Mater Horiz* 2024;**11**:1465–83.
32. Baek J, Fan Y, Jeong SH, Lee HY, Jung HD, Kim HE, et al. Facile strategy involving low-temperature chemical cross-linking to enhance the physical and biological properties of hyaluronic acid hydrogel. *Carbohydr Polym* 2018;**202**:545–53.
33. Cui YJ, Wang WG, Li P, Zhao YL, Gu YN, Wan JL, editors. The comparison of physicochemical properties of four Cross-linked sodium hyaluronate gels with different cross-linking agents. *Adv Mater Res* 2012:396–8.
34. Ruhr D, John M, Reiche A. Determination of the effective degree of cross-linking of porous cellulose membranes cross-linked with bifunctional epoxides. *Carbohydr Polym* 2021;**251**:117043.
35. Lv Z, Hu T, Bian Y, Wang G, Wu Z, Li H, et al. A MgFe-LDH nanosheet-incorporated smart thermo-responsive hydrogel with controllable growth factor releasing capability for bone regeneration. *Adv Mater* 2023;**35**:e2206545.
36. Lv S, Sheng J, Zhao S, Liu M, Chen L. The detection of brucellosis antibody in whole serum based on the low-fouling electrochemical immunosensor fabricated with magnetic Fe₃O₄@Au@PEG@HA nanoparticles. *Biosens Bioelectron* 2018;**117**:138–44.
37. Lim EK, Kim HO, Jang E, Park J, Lee K, Suh JS, et al. Hyaluronan-modified magnetic nanoclusters for detection of CD44-overexpressing breast cancer by MR imaging. *Biomaterials* 2011;**32**:7941–50.
38. Zhang Q, Li M, Xu W, Li J, Yan S. A novel silk fibroin scaffolds with oriented multichannels. *Mater Lett* 2013;**105**:8–11.
39. Yu Q, Meng Z, Liu Y, Li Z, Sun X, Zhao Z. Photocuring hyaluronic acid/silk fibroin hydrogel containing curcumin loaded chitosan nanoparticles for the treatment of MG-63 cells and ME3T3-E1 cells. *Polymers* 2021;**13**:2302.
40. Yan S, Li M, Zhang Q, Wang J. Blend films based on silk fibroin/hyaluronic acid. *Fibers Polym* 2013;**14**:188–94.
41. Wang L, Lu C, Zhang B, Zhao B, Wu F, Guan S. Fabrication and characterization of flexible silk fibroin films reinforced with graphene oxide for biomedical applications. *RSC Adv* 2014;**4**:40312–20.
42. Salwowska NM, Bebenek KA, Źądło DA, Wcisło-Dziadecka DL. Physicochemical properties and application of hyaluronic acid: a systematic review. *J Cosmet Dermatol* 2016;**15**:520–6.
43. O'Brien FJ, Harley BA, Waller MA, Yannas IV, Gibson LJ, Prendergast PJ. The effect of pore size on permeability and cell attachment in collagen scaffolds for tissue engineering. *Technol Health Care* 2007;**15**:3–17.
44. da Silva LP, Cerqueira MT, Sousa RA, Reis RL, Correlo VM, Marques AP. Engineering cell-adhesive gellan gum spongy-like hydrogels for regenerative medicine purposes. *Acta Biomater* 2014;**10**:4787–97.
45. Dou Z, Li B, Wu L, Qiu T, Wang X, Zhang X, et al. Probiotic-functionalized silk fibroin/sodium alginate scaffolds with endoplasmic reticulum stress-relieving properties for promoted scarless wound healing. *ACS Appl Mater Interfaces* 2023;**15**:6297–311.
46. Wu J, Zheng K, Huang X, Liu J, Liu H, Boccaccini AR, et al. Thermally triggered injectable chitosan/silk fibroin/bioactive glass nanoparticle hydrogels for *in-situ* bone formation in rat calvarial bone defects. *Acta Biomater* 2019;**91**:60–71.
47. Dzamba BJ, Jakab KR, Marsden M, Schwartz MA, DeSimone DW. Cadherin adhesion, tissue tension, and noncanonical Wnt signaling regulate fibronectin matrix organization. *Dev Cell* 2009;**16**:421–32.
48. Le Clainche C, Carlier MF. Regulation of actin assembly associated with protrusion and adhesion in cell migration. *Physiol Rev* 2008;**88**:489–513.

49. Balasubramanian P, Prabhakaran MP, Sireesha M, Ramakrishna S. Collagen in human tissues: structure, function, and biomedical implications from a tissue engineering perspective. In: Abe A, Kausch HH, Moller M, Pasch H, editors. *Polymer composites - polyolefin fractionation - polymeric peptidomimetics - collagens*; 2013. p. 173–206.
50. Reilly DM, Lozano J. Skin collagen through the lifestages: importance for skin health and beauty. *Plastic and Aesthetic Research* 2021;**8**:2.
51. Lin M, Liu X, Wang X, Chen Y, Zhang Y, Xu J, et al. A comparative study of skin changes in different species of mice in chronic photo-aging models. *Int J Mol Sci* 2023;**24**:10812–24.