Supplementary Information

Extraction of Collagen from Bovine Tannery Solid Waste Preserving Original Conformation via Radical Initiation and Hydrogen Bond Reformation

Fang Luo[a], Zhuo Liu[a], Peng Zhou[b], Siqi Wang[a], Lingzhi He[a], Yi Wu[a], Lidan Du[a], Mengjie Jiao[a], Zhuwei Liao[b], Zhuqi Chen[a*]

[a] Key laboratory of Material Chemistry for Energy Conversion and Storage, Ministry of Education, Hubei Key Laboratory of Material Chemistry and Service Failure, School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan 430074, PR China

[b] Urban Construction Engineering Division, Wenhua College, Wuhan, China

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Text S1 Alkali alcoholate-based process

TBAH and DMSO were recovered using the classic alkali alcoholate-based process through the following procedure: twice the molar quantity of NaOH compared to TBAH was added to the supernatant. Then, the mixture was stirred at 25 °C for 2 hours. Crystalline precipitates were observed, and identified as NaCl through ICP-OES analysis. After stirring, the mixture was filtered and the supernatant was collected. Then, the ethanol was evaporated, and crystalline precipitates were observed again, which were identified as NaOH through ICP-OES analysis and pH meter.The mixture was filtered to obtain the supernatant, a slightly yellow transparent liquid identified as the recycled TBAH and DMSO mixture. This mixture was analyzed by IC and ICP-OES to determine the residual concentrations of Cl^- and Na+. Additionally, a small amount of the mixture was diluted tenfold in ultrapure water to measure its pH and determine the OH^- concentration in the mixture. Then, the recovery rate of TBAH and DMSO was calculated as follows:

$$\textit{Recovery rate of TBAH (\%)} = [n(OH^-) - n(Na^+)] / n(TBAH) \quad (1)$$

$$\textit{Recovery rate of DMSO (\%)} = \{m(Total) - \{[n(OH^-)-n(Na^+)]\times 259.5+n(Cl^-)\times 277.9+n(Na^+)\times 40\}\} / m(DMSO) \quad (2)$$

Where $n(OH^-)$ and $n(Na^+)$ are the molar masses of OH^- and Na^+ in the mixture, respectively. n(TBAH) is the molar mass of the TBAH initially added to the TDH solvent, m(Total) is the total mass of the mixture, m(DMSO) is the mass of the DMSO initially added to the TDH solvent.

Subsequently, the solution was adjusted to 80% DMSO, 10% TBAH, and 10% H_2O with fresh DMSO and ultrapure water. Thus, the recycled TDH solvent was prepared for recycling and analyzed by ^1H NMR.

Table S1 The solubility, recovery rate of DMSO, TBAH and the residual concentration of Na^+ in each cycle

Number of cycles	Solubility (%)	Recovery rate (%)		Residual concentration of Na^+(mg/L)
		DMSO	TBAH	
1	9.71	82.34	78.83	1.35×10^2
2	10.98	81.03	89.75	2.01×10^2
3	9.96	82.54	93.13	1.26×10^2
4	11.03	80.42	90.79	3.62×10^2
5	10.07	78.08	91.51	2.65×10^2

Text S2：Pre-treatment of collagen product before analysis

The obtained collagen, which was not soluble in water, necessitated an initial dissolution step employing an acid/enzymatic method. Specifically, the collagen product was dissolved in a mixture containing 5% acetic acid and 0.5 g/L pepsin. This mixture was stirred at room temperature for two days to prepare a collagen solution. Subsequently, the solution was dialyzed for two days, with the dialysis water being refreshed every six hours, to remove the acetic acid and pepsin. Finally, the dialyzed solution was lyophilized in a freeze-dryer to obtain the purified collagen for circular dichroism (CD) spectra analysis (Jasco J-810, Japan), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis (Bruker ultraflextreme, germany) and SDS-PAGE by gel electrophoresis apparatus (DYY-6C, Beijing 61 instrument factory, China).

Table S2 Proportion of soluble and insoluble collagen in collagen product obtained via IL-SDR method across various DMSO concentrations in TDH solvent

TDH solvent	Soluble collagen (%)	Insoluble collagen (%)
80% DMSO	75.59	24.41
60% DMSO	83.47	16.53
40% DMSO	92.41	7.59

Text S3 SDS-PAGE experimental procedure

1 Experimental equipment and reagents

Table S3 Experimental Equipment of SDS-PAGE experimental

Name	Manufacturer	Model
Benchtop High-Speed Refrigerated Centrifuge	Cence	TGL-16
Mini Centrifuge	SCILOGEX	S1010E
Vortex Mixer	SCILOGEX	MX-S
Electrophoresis apparatus	Beijing 61 instrument factory	DYY-6C

Table S4 Main Experimental Reagents

Reagent	Manufacturer	ID
Protein Marker	Thermo	26616
Coomassie Brilliant Blue	Scientifc Phugene	PH0360-10G

2 Experimental procedures

(1) Sample solution preparation

A sample solution of 30 μl was taken into a 200 μl PCR tube, to which 6 μl of 6x protein reducing loading buffer was added. The mixture was vortexed, briefly centrifuged, subjected to heating at 99℃ for 8 minutes, and then briefly centrifuged again.

Table S5 Loading volumes of samples

Number	Loading volumes
1	20ul
2	20ul
3	20ul
4	20ul

(2) SDS-PAGE electrophoresis

(2.1) The glass plates were cleaned.

(2.2) Gel preparation and loading

(2.3) The glass plates were aligned and secured in a gel casting assembly to prevent leakage.

(2.4) The separation gel was prepared as required by the experiment, and TEMED was added. Immediately after mixing, the gel was poured until it reached three-quarters of the glass plate's height. To level the surface of the separating gel, the remaining space was filled with anhydrous ethanol. Approximately 30 minutes later, the ethanol layer above the gel was removed, and any remaining

ethanol was absorbed with filter paper.

Table S6 The detail dosage of reagent of separation gel in SDS-PAGE

Reagent	Separation gel
H_2O (ml)	3.3
30% Acrylamide/Bis (29:1) (ml)	4
1.5M TRIS-Hcl(PH 8.8) (ml)	2.5
10％SDS (ml)	0.1
AP (ml)	0.1
TEMED (ul)	10
Total volume (ml)	10

Table S7 The detail dosage of reagent of concentration gel in SDS-PAGE

Reagent	Concentration gel
H_2O (ml)	3.4
30% Acrylamide/Bis (29:1) (ml)	0.83
1.5M TRIS-Hcl(PH 8.8) (ml)	0.63
10％SDS (ml)	50
AP (ml)	50
TEMED (ul)	5
Total volume (ml)	5

(2.5) A 5% concentration gel was prepared as described in the table above, and immediately after adding TEMED, the gel was mixed and poured. The remaining space was then filled with concentration gel before the comb was inserted.

(2.6) After sufficient electrophoresis buffer was added, samples were loaded for electrophoresis. The samples were introduced into the wells and electrophoresis was performed; the concentration gel was run at 100V, and the separating gel at 120V, until the bromophenol blue reached approximately 1 cm from the bottom of the glass plate, at which point the electrophoresis was terminated.

3 Coomassie brilliant blue staining and subsequent experimental steps

(3.1) The gel was placed in a glass dish and Coomassie Brilliant Blue staining solution was added before staining on a shaker for 180 minutes；

(3.2) After decanting the Coomassie Brilliant Blue staining solution, the gel was rinsed with water, destaining solution was added, and the gel was placed on a destaining shaker overnight；

(3.3) The destaining solution was removed, the gel was rinsed with water, and photographs of the target bands were taken.

Text S4 Water Peak Identification in TDH Solvent

As shown in Figure S9 and Figure S11, the addition of 5% water into the TDH solvent notably enhanced the peak around 4.00 ppm, with the peak area increasing from 0.43 to 9.73. This indicates that this peak is attributed to water and OH^-, deviating from the typical chemical shift of the water peak observed when DMSO-d_6 is utilized as the solvent. This is obviously a phenomenon caused by an alkaline environment.

Text S5 Extraction procedure of natural collagen

The natural collagen used as a reference was extracted from bovine tendon tissues purchased from Zaozhuang Luyi Department Store Co., Ltd., using the acetic acid/pepsin method.[1] Specifically, the tendons were carefully cut into pieces smaller than 1×1×1 cm^3 via scissors, washed four times with ultrapure water for one hour each, and then weighed. These tissue pieces were immersed in 0.5 M acetic acid and stirred at 500 rpm for 48 hours, then sieved through a 100-mesh screen to obtain swollen tissue piece gel. Fresh 0.5 M acetic acid was then replaced, along with the addition of pepsin (in a mass ratio of tissue pieces to pepsin of 100:1), and the mixture was stirred for an additional 48 hours. Then, the mixture was sieved again via a 100-mesh screen and Collagen solution was obtained. 0.9 M NaCl was added to the collagen solution, which was then allowed to sit overnight at 4 °C. The crude collagen was dissolved in 1 M acetic acid, which was then centrifuged at 8000 rpm for 10 minutes to obtained the concentrated collagen solution. Subsequently, 0.9 M NaCl was added to the concentrated collagen solution and it was allowed to settle overnight at 4°C.The mixture was sieved via a 100-mesh screen to obtain collagen. Then, the collagen was dialyzed against 0.05M acetic acid for 72 hours, changing the 0.05M acetic acid every 8 hours. Finally, the collagen solution was

lyophilized to produce the final natural collagen product.

Text S6 Cytotoxicity test

1. Experimental Instruments and Reagents:

The experiments were conducted using a CO_2 incubator (WIGGENSWCI-180, Beijing Songyi Experimental Instrument Research Institute, China), a clean bench (SW-CJ-2FD, Shanghai Taisite Instrument Co., Ltd., China), a centrifuge (TD5, Shanghai Luxiangyi Centrifuge Instrument Co., Ltd., China), and a microplate reader (SPARK10M, TECAN, Switzerland). Reagents included FBS (Catalog No.: 164210-50, PronoCell, USA), MEM medium (Catalog No.: 10-010-CV, Corning, USA), PBS (Catalog No.: WH0112201911XP, Procell, China), trypsin (Catalog No.: BL512A, Biosharp, China), and CCK8 (Catalog No.: IV08-100, Invigentech, USA). The L929 cell line was purchased from PronoCell (USA).

2 Collagen solution sample

The natural collagen and the collagen produced via the IL-SDR process were separately dissolved in ultrapure water to prepare 2 mg/mL collagen solutions, which was then sterilized by UV irradiation. This solution was subsequently diluted with the culture medium to final concentrations of 0.2 mg/mL, 0.05 mg/mL, and 0.01 mg/mL.

3 CCK8 Assay

Logarithmically growing L929 cells were counted and adjusted to a concentration of $8x10^3$ cells per well in a 96-well plate. The cells were incubated at 37 °C with 5% CO_2 for 24 hours. After incubation, the medium was removed and the wells were washed three times with PBS. Subsequently, 100 μL of

culture medium containing 10% CCK8 was added to each well. The cells were incubated for another 2 hours at 37 °C with 5% CO_2. The absorbance at 450 nm was measured using a microplate reader.

4 Calculation of relative viability

The relative viability was calculated based on the absorbance values of each group:

Relative viability (%) = [(OD value of experimental group - background OD value) / (mean OD value of control group - background OD value)] × 100 *(3)*

where the background OD value corresponds to the absorbance of wells containing only CCK8 reagent and culture medium.

Text S7：Moisture retention rate test

The natural collagen and the collagen produced via IL-SDR process were separately dissolved in ultrapure water at a ratio of collagen to water of 40/1000 to prepare collagen gels. Then, 1.4 - 1.5 g collagen gel was carefully and evenly spread onto a culture dish with a diameter of 3 cm. The dishes were placed in a desiccator with a maintained humidity of 26%. The weight of the gels was recorded every two hours.

The initial moisture mass in each culture dish was calculated as follows:

$$M_{im} = M_{it} \times (1000\ mg/1040\ mg) \quad (4)$$

where M_{im} and M_{it} are the initial moisture mass and the initial total mass of the collagen gel, respectively.

At each recorded time point (M_t), the mass of water lost (M_l) was calculated as follows:

$$M_l = M_{im} - M_t \quad (5)$$

Finally, the moisture retention rate (%) was calculated as follows:

$$Moisture\ Retention\ rate\ (\%) = [(M_{im} - M_l) / M_{im}] \times 100\% \quad (6)$$

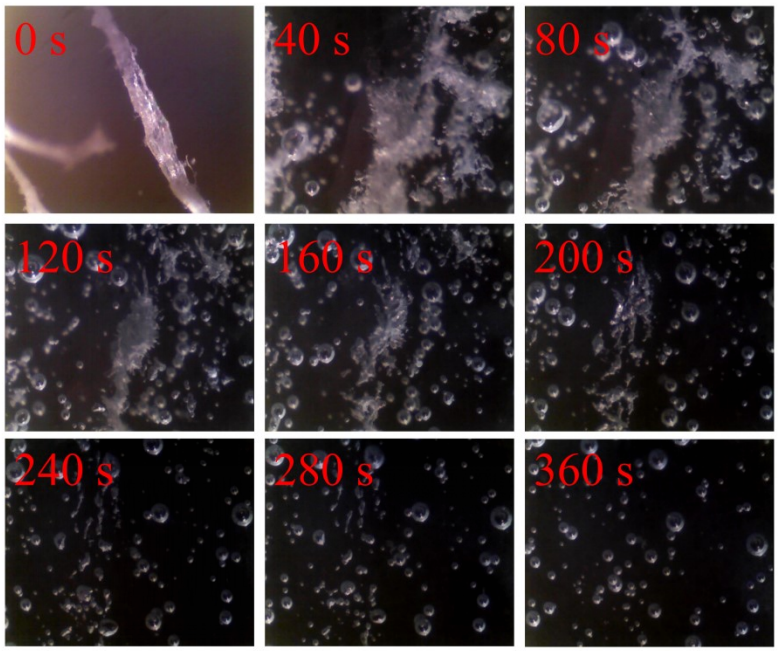


Figure S1 In situ images of a TSW fiber in TBAH aqueous solution (40% TBAH and 60% H_2O) observed by a polarized optical microscope at 25 °C.

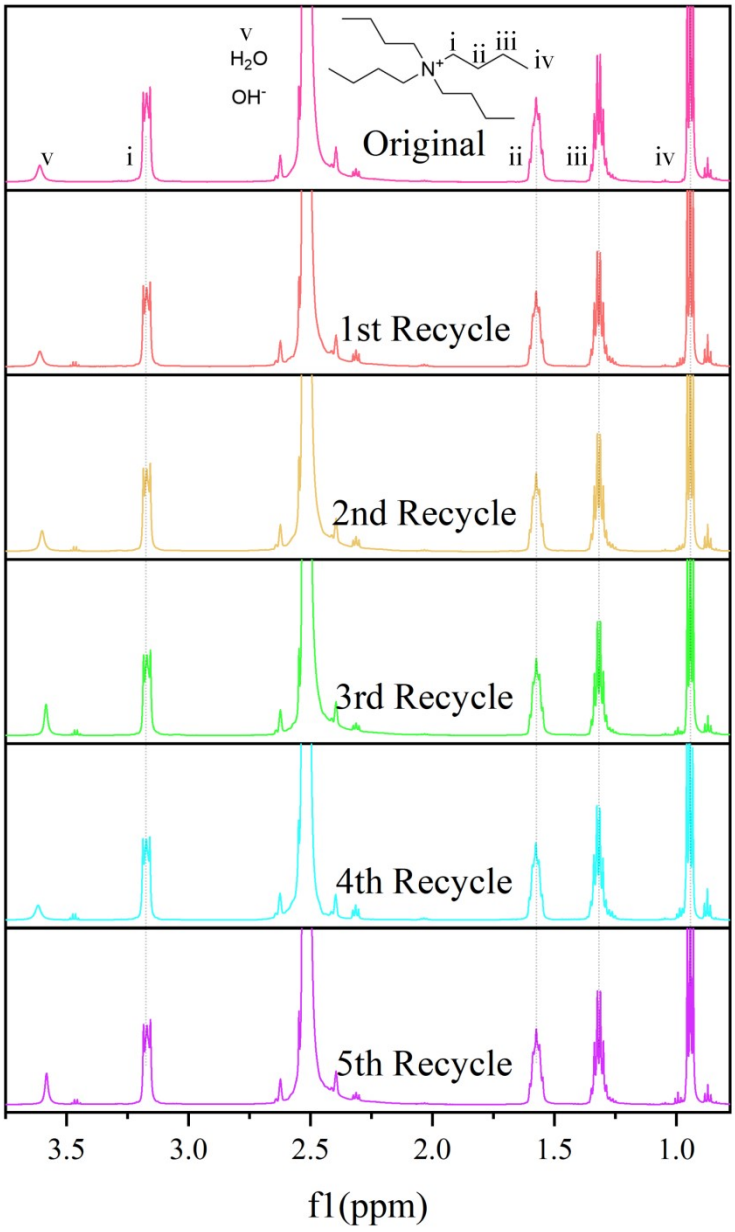


Figure S2 ^{1}H NMR spectra of recycled TBAH from consecutive cycles.

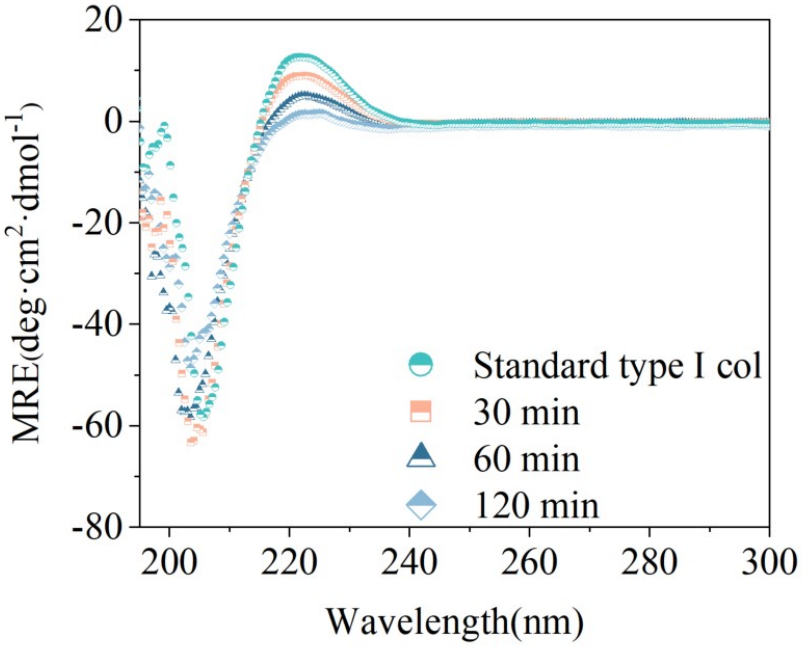


Figure S3 CD spectra of the collagen product obtained by IL-SDR method with different dissolution time.

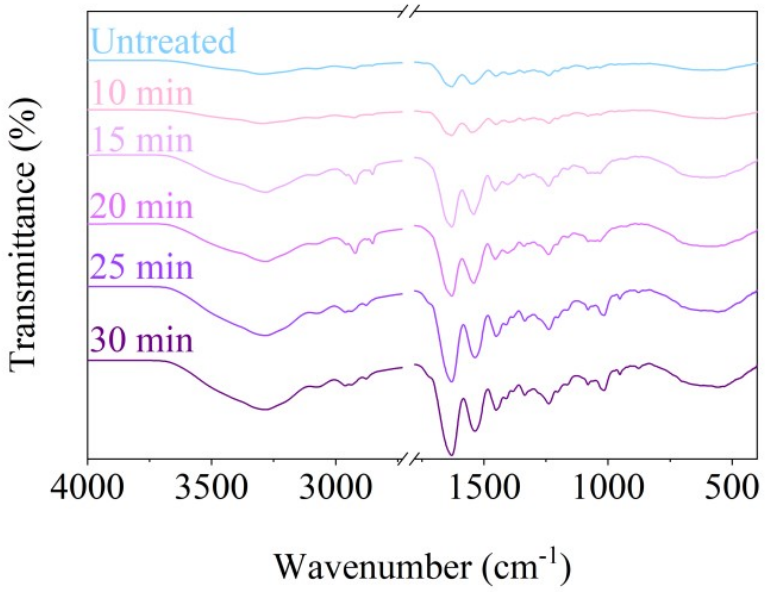


Figure S4 FT-IR spectra of the regenerated collagen with different dissolution time

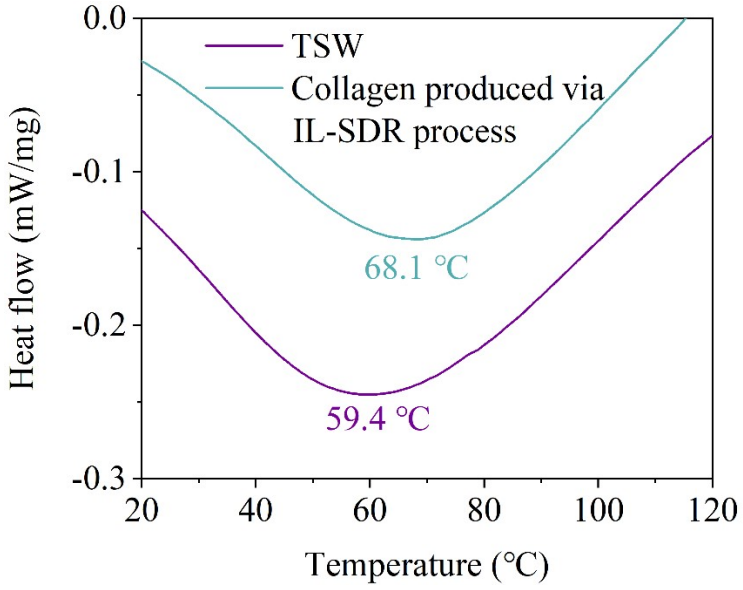


Figure S5 DSC curves of TSW and collagen produced via IL-SDR process

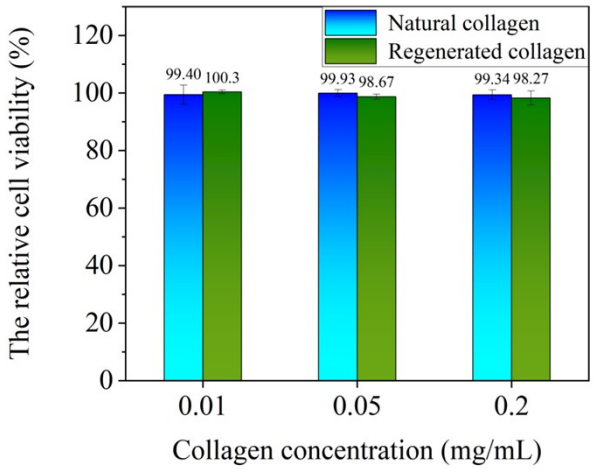


Figure S6 Cell viability of collagen at different concentrations for 24 h was examined against L929 cell lines by CCK-8 assay.

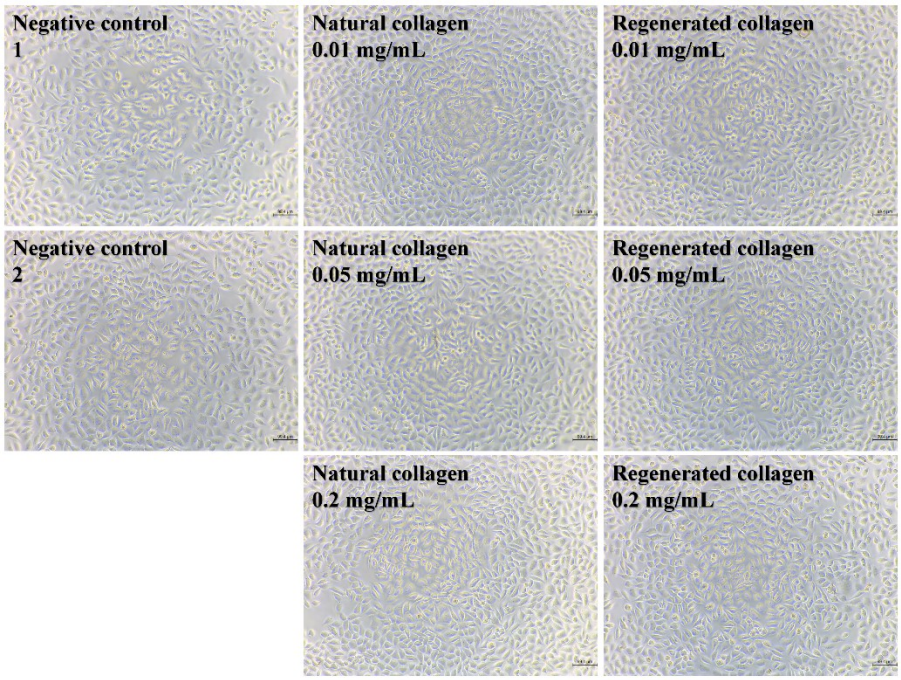


Figure S7 Morphological changes of L929 cells after treatment with collagen at different concentrations for 24 h (×100)

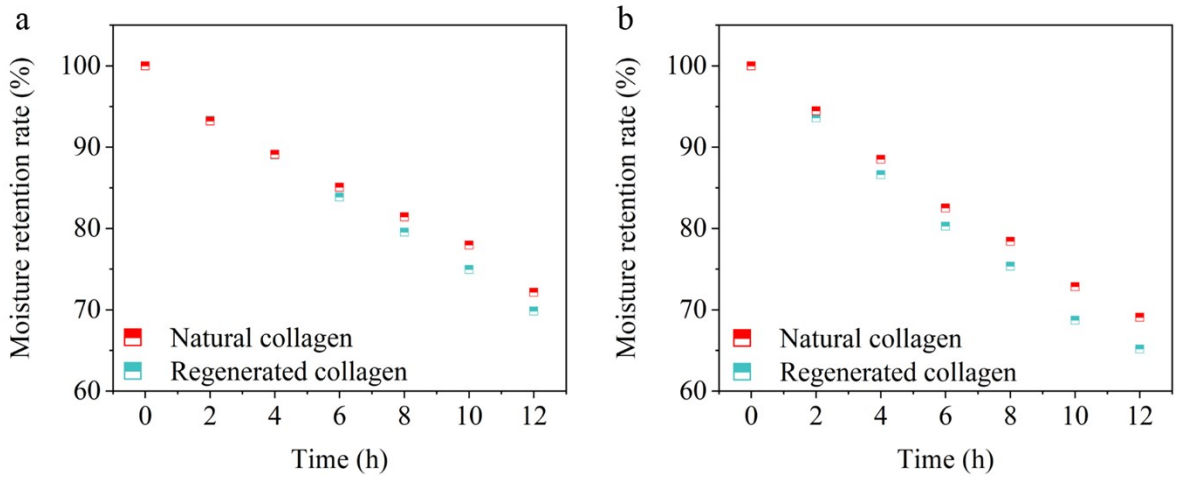


Figure S8 Moisture retention rate of natural and regenerated collagen at 37% (a) and 27%(b).

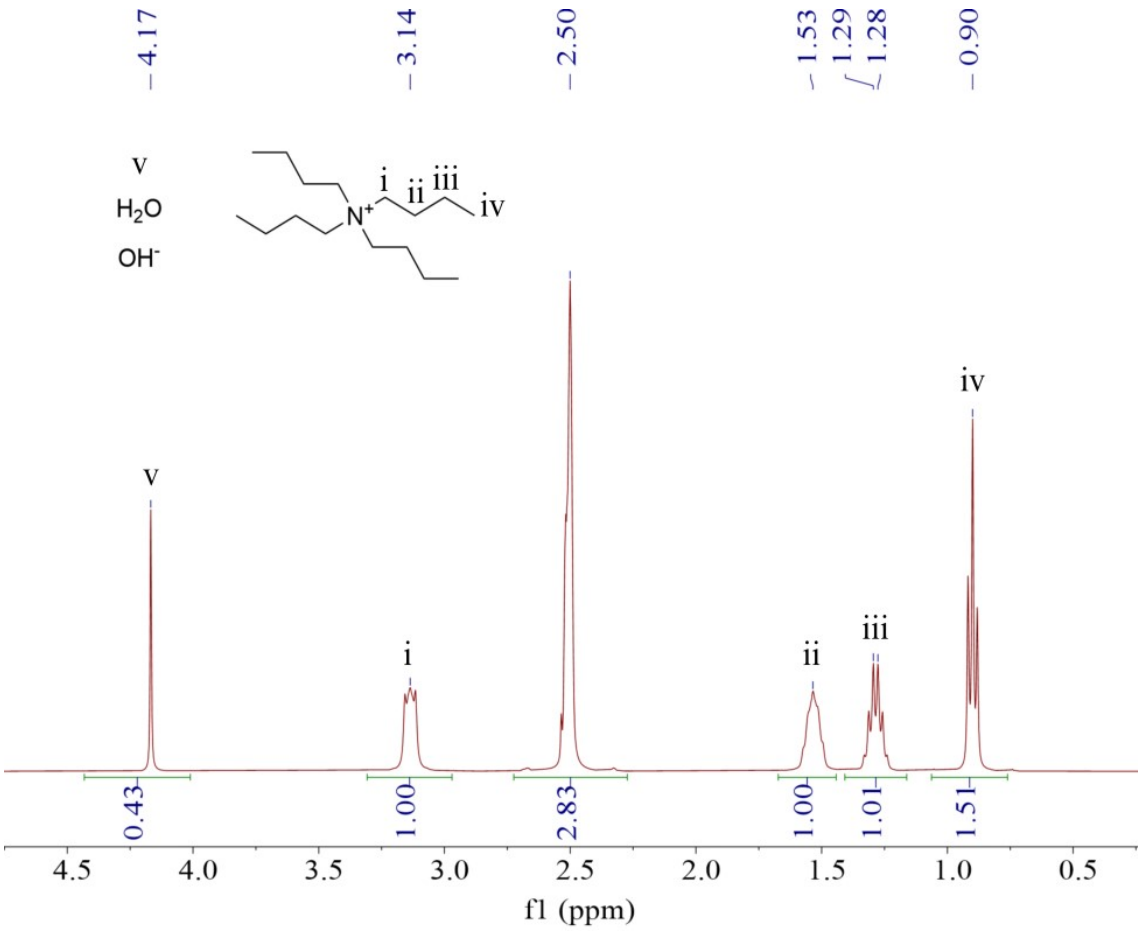


Figure S9 ^{1}HNMR spectra of TDH solvent (80% DMSO, 10% TBAH and 10 H_2O)

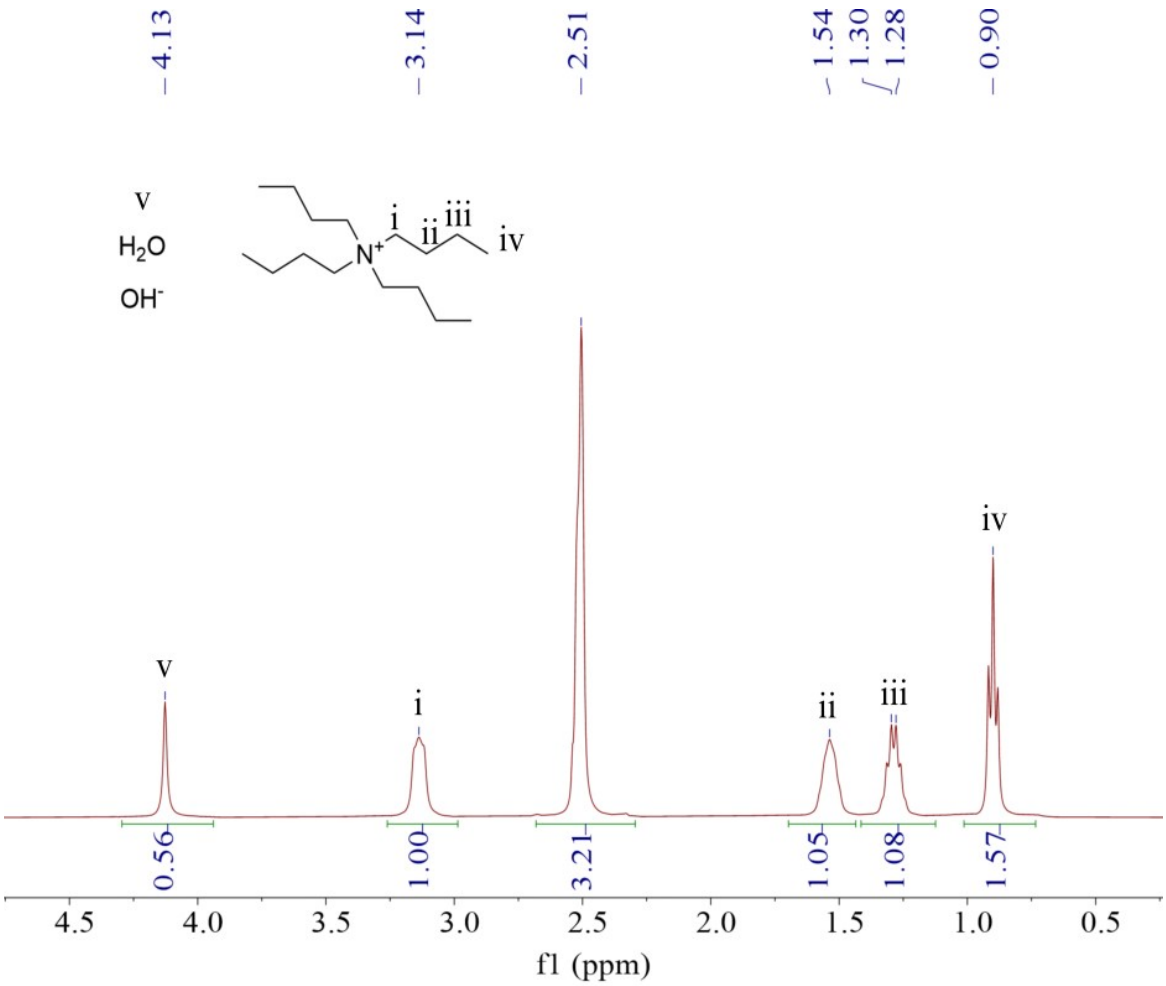


Figure S10 ^{1}HNMR spectra of TDH solvent (80% DMSO, 10% TBAH and 10 H_2O) dissolving 4% TSW

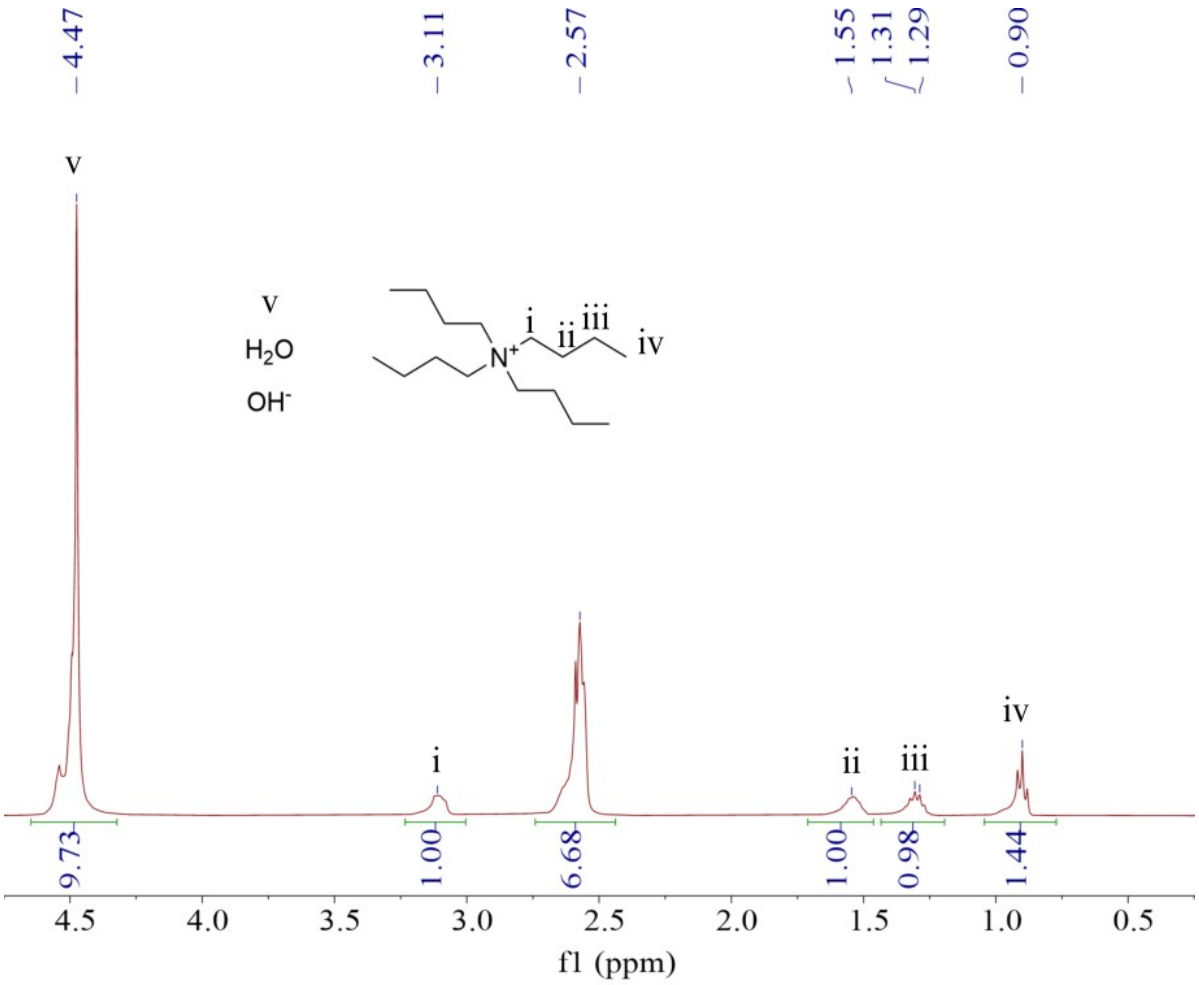
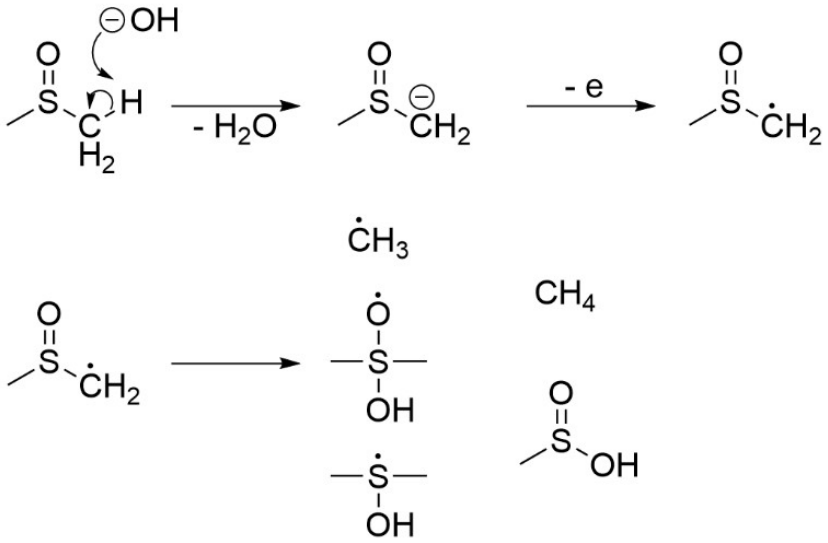


Figure S11 ^{1}HNMR spectra of TDH solvent (80% DMSO, 10% TBAH and 10 H_2O) with an additional 5% water added



Scheme. S1 The complete reported DMSO free radical reaction

References

1. A. Sorushanova, I. Skoufos, A. Tzora, A. M. Mullen and D. I. Zeugolis, *J Mater Sci Mater Med*, 2021, **32**, 12.