Supplementary Materials for

Electro-assembly of a dynamically adaptive molten fibril state for collagen

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Supplementary Notes

1.1 Induction of a pH gradient by electrode reactions

The following half-reactions result in the generation of a pH gradient between the electrodes

Anode: \(2\text{H}_2\text{O} - 4e^- \rightarrow 4\text{H}^+ + \text{O}_2\);  
Cathode: \(4\text{H}_2\text{O} + 4e^- \rightarrow 4\text{OH}^- + 2\text{H}_2\).

The electrodes were applied with a constant current density (8 mA/cm\(^2\), on demand) for a predetermined period (15 min). The charge transfer density (Q) is calculated for collagen EDP according to the Equation (S1):

\[
\text{Cathodic charge density} \,(Q) = \int \, i \, dt; \, \text{C/cm}^2 \quad (\text{S1})
\]

Where \(i\) is the instantaneous electrode current (A/cm\(^2\)), and \(t\) is the operation time (sec).

1.2 D-banding space calculation

The positions of all the diffraction peaks observed in SAXS from each sample could be explained by a single periodic D-banding space (D), which is calculated according to the Bragg Equation (S2):

\[
\text{D banding space} \,(D) = \frac{n \, 2 \pi}{q}; \, \text{nm} \quad (\text{S2})
\]

Where \(n\) is the order of the peak 1, 2, 3, etc. And \(q\) is the peak’s position at the 1D SAXS profiles.

1.3 The deformation ratio of hysteresis

Plastic deformation of sample during dynamic loading-unloading test can be quantified by a deformation ratio (DR): The deformation ratio (DR) of each cycle is calculated according to the following Equation (S3):

\[
\text{DR} = \left(\frac{\varepsilon_y - \varepsilon_z}{\varepsilon_x - \varepsilon_z}\right) \times 100\% \quad (\text{S3})
\]

Where \(\varepsilon_x\), \(\varepsilon_y\), and \(\varepsilon_z\) are the strains after loading, the strain after unloading, and the strain before loading for each cycle.

1.4 Calculation of the toughness and the dissipated portion
The toughness is the area below the loading curves (red region, as shown in Fig. S1 (A)), which is calculated by the integral area of the loading curve from the 0% strain to the strain at fracture. And the dissipated portion is the area between the loading-unloading curves (yellow region, as shown in Fig. S1 (B)), which is calculated by the integral area of the loading curve minus the integral area of the unloading curve.

Fig. S1. Illustration for calculating the total toughness and its dissipative part. (A) The toughness is calculated by the area in red and (B) the dissipated portion of toughness is calculated by the area in yellow.

1.5 Calculation of the Herman’s orientation factor

The degree of alignment of collagen network is quantified by using the Herman’s orientation factor \( f_C \), which is defined as the following Equation S (5-6):

\[
 f_C = \frac{3<\cos^2\phi>-1}{2} \quad \text{(S5)}
\]

\[
<\cos^2\phi> = \frac{\int_0^{\pi/2} I(\phi) \cos^2\phi \sin\phi \, d\phi}{\int_0^{\pi/2} I(\phi) \sin\phi \, d\phi} \quad \text{(S6)}
\]

Where \( \phi \) is azimuthal angle, and \( I(\phi) \) is the 1D intensity distribution along with the azimuthal angle after subtracting the background intensity. The average \( \langle \cos^2\phi \rangle \) is calculated by integrating the intensity of specific 2θ diffraction peak along the \( \phi \), using the aforementioned equation. For an isotropic material, \( f_C = 0 \); and for an ideal uniaxially oriented material, \( f_C = 1 \).
Fig. S2. Characterization of acid-soluble collagen I. (A) Zeta potential and (B) CD spectrum of collagen I. Zeta potentials of collagen molecule (0.5 mg mL\(^{-1}\)) at different pH values (adjust by 0.1 M NaOH) were analyzed on a Nano ZS Zeta-sizer (Malvern). The results indicate the isoelectric point (pI) of collagen I is about 4.5 ~ 5, where the sol-gel phase transition can be observed. Circular dichroism CD spectrum of collagen solution (0.125 mg mL\(^{-1}\) at pH 3.5) was obtained using a JASCO J-815 spectrometer (quartz cell, 1 mm path length). The CD result indicates that the CoI I still remains a right-handed triple helix structure under an acidic condition (pH=3.5), the same pH value as the electrolyte.
Fig. S3. Schematic of the electro-assembly and solution assembly of Col I and the Optical comparison of the resultant EA-Col and SA-Col materials. (A) Electro-assembly device and the resultant electro-assembled collagen film (named “EA-Col”) with high optical transparency. (B) Solution-assembly of Col I and the resultant assembled collagen film (named “SA-Col”) with an opaque milky white appearance. The quantified comparison of (C) transmittance and (D) haze.
Fig. S4. Transparency stability of EA-Col evaluation. (A) Optical images of 0.1% glutaraldehyde crosslinked EA-Col incubated in simulated body fluid (i.e., SBF) or PBS for 1 to 4 weeks showed visual transparency. (B) The quantified transmittance of crosslinked EA-Col (500 μm thickness) after 4 weeks incubation, which indicate the transmittance more than 80%.

Fig. S5. Cross-sectional SEM image, FTIR and XRD of EA-Col and SA-Col. The SEM image in (A) shows compact layered stacked structure of EA-Col and (B) shows the loose and random organization of fibrils in SA-Col. (C) The FTIR result indicate the EA-Col and SA-Col show the same characteristic peaks of collagen. (D) XRD patterns indicate the molecular conformations in
EA-Col and SA-Col. One sharp and dominant peak at 7.5° (red box) is related to the intermolecular lateral packing distance among the three peptide chains, and its intensity is assigned to the triple helix content, indicating electro-assembled molten fibril network can preserve the triple helix structure well. The broad peak at 15°~ 22.5° (blue box) corresponds to the distance between amino acid residues along the helix.

![Diagram](image)

**Fig. S6. Thermal stability difference.** (A) The thermo-gravimetric analysis curves and (B) Digital thermos-gravimetric curves of EA-Col and SA-Col. Three main weight loss stages in the range of 50~100 °C, 190~250 °C and 300~350 °C were observed in EA-Col and SA-Col. These three stages are respectively relevant to the loss of physically absorbed water, structural water, and the degradation of collagen. Compared with the SA-Col, the EA-Col undergoes a significant weight loss at 200 °C, while the SA-Col show an obvious weight loss until 330 °C. The differences in thermal stability are considered to be related to the different internal binding in EA-Col and SA-Col networks (i.e. weak and reversible physical bonds in EA-Col vs strong and covalent bridges aldimide/ketoimine in SA-Col).
Fig. S7. Formation of higher hierarchical order structures in EA-Col. (A) The illustration of EA-Col incubated in 0.1M PBS at 37 °C using a constant temperature and humidity incubator, and the optical images of EA-Col indicate the appearance of EA-Col transformed from transparent to milky after 24 hours incubation. (B) The AFM and (C) TEM images of EA-Col before and after incubation, which indicate the formation of thicker fibrils with D-band (100~200 nm diameter fibrils was observed in the surface AFM images; and the 30~40 nm diameter fibrils was observed in the cross-section TEM images).
**Fig. S8. Response of Molten Fibril Network and Static Fiber Network to Mechanical Force.**

The enlarged stress–strain curves of (A) static fiber network and (B) molten fibril network which reveal the different mechanical response to external force. The static fiber network shows a typical “J-shaped” stress–strain curve of collagen materials. The curve usually can be divided into four regions: toe or low strain region (I), heel region (II), elastic or linear region (III), and failure (IV) as shown in enlarged view of **Fig. S8 (A)**, which related to the complex deformation mechanism of collagenous structures. Briefly, large strains lead to the stretching of the triple helixes and fiber slipping, by which fiber or fibrils can split into individual micro-fibrils. Once a certain number of micro-fibrils break up, the whole collagen network would rupture, it’s termed defibrillation. It can be confirmed that rigid fiber networks provide relatively sufficient covalent cross-linking (intermolecular and inter-fibrillar) to limit large deformation. In comparison, the molten fiber network doesn’t undergo a “J-shaped” deformation process, and shows the trend of ductile fracture, and consistent with a weak viscoelastic network with large deformation, as shown in **Fig. S8 (B)**. The (C) stress relaxation under the same initial strain (20%; 25 °C; stress normalized to initial value) shows the greater stress relaxation ability of molten fibril network.
Fig. S9. Observation of molten fibril network exposed to Hofmeister salt solutions (2 M) for 24 hours. Generally, the ions in the Hofmeister series are divided into kosmotropes and chaotropes. The kosmotive is describing ions that stabilize hydrophobic interactions in water, while chaotrope is that disrupts such interaction. As shown in Fig. S9, the molten fibril gels immersed in kosmotive solutes such as Na$_2$SO$_4$ and Na$_2$CO$_3$ show enhanced mechanical strengths. While the gels treated with chaotrope solute NaI became highly swollen. And the more significant chaotrope salt NaSCN even dissolved this gel.

Fig. S10. The static fiber network treated with 2 M (NH$_4$)$_2$SO$_4$ experienced a brittle fracture when loading a 500 gram weight.
Fig. S11. Dynamic mechanical test of strengthened molten fibril network. (A) Tension loading–unloading tests of molten fibril network treated with varying (NH₄)₂SO₄ concentrations. (B) The calculated total and dissipated toughness, as well as the dissipation coefficient of molten fibril networks. (C) Tension loading–unloading tests of molten fibril hydrogels treated with 2M (NH₄)₂SO₄ with varying strain (40%, 80%, 120%, 160%). (D) The calculated dissipated toughness with different cycles. The loading-unloading results indicate the (NH₄)₂SO₄ treated molten fibril network allows for high energy dissipation and large hysteresis loops. Generally, the area of hysteresis loops in loading–unloading curves represented the energy dissipation per unit volume upon deformation. The ε values in (B) and (D) represent the maximum strains used for the tests in (A) and (C).

As show in Fig. S11 (A), the areas became more prominent as the (NH₄)₂SO₄ concentration increased, which indicate the dissipated energy of molten fibril network increased sharply with (NH₄)₂SO₄ concentration. Fig. S11 (B) quantitatively described the hysteresis energy per unit volume of molten fibril network increased from 0.03 MJ m⁻³ to 2.96 MJ m⁻³ (more than 98 folds) with the increase of (NH₄)₂SO₄ concentration at ε=100% and the dissipation coefficient (dissipated
portion as a percentage of total toughness) is basically kept above 90% of the total toughness. In addition, the areas of hysteresis loops became more prominent as the strain increased (see Fig. S11 C-D). And in the three loading-unloading cycles, the energy dissipated in the first cycle is the largest, while the energy dissipated in the subsequent load-unloading cycles decreases sharply, indicating that the recovery of the internal dissipated non-covalent interaction (i.e. hydrogen bond and hydrophobic interactions) isn’t instantaneous. These results suggest that the weak associations (i.e. hydrogen bond and hydrophobic interactions) between the fibrils in the molten fibril state enable to be enhanced by the Hofmeister effect (along with a certain degree of water content reduction, as shown in Table S1). Such physically crosslinked domains in the hydrogel network can de-crosslink and dissociate to dissipate the large amounts of energy that have been exerted on the hydrogels.

**Fig. S12. Dynamic mechanical test of strengthened static fiber network.** (A) Tension loading–unloading tests of static fiber networks treated with varying (NH₄)₂SO₄ concentrations. (B) The calculated total and dissipated toughness, as well as the dissipation coefficient of static fiber networks. Due to the strong internal crosslinking of static fiber network, the fracture strain of the network decreases with the increase of (NH₄)₂SO₄ concentrations (as shown in Fig. 3C). Fig. S12 (A) shows a very small hysteresis area appears at 20% strain. Fig. S12 (B) quantitatively describes a narrow variation of the hysteresis energy per unit volume of static fiber network in the range of 0.005 MJ m⁻³ to 0.067 MJ m⁻³ (about 13.4 folds) with (NH₄)₂SO₄ concentration increasing at ε = 20%. The dissipation coefficient of the initial static fiber network (the percentage of the dissipative part in the total toughness) is 38%. With the increase of the concentration of (NH₄)₂SO₄, the dissipation coefficient gradually increased but did not exceed 70%. These results indicate that the
internal interaction and microstructure of the network significantly affect its mechanical response to Hofmeister effect.

**Fig. S13. In vivo application of strengthened molten fibril band.** (A) Stress–strain curves of molten fibril network with various Na$_2$CO$_3$ concentrations show that the Na$_2$CO$_3$ can also serve as a kosmotropic salt to strengthen the molten fibril network. (B) A stiff molten fibril band treated by 2 M Na$_2$CO$_3$ for 24 hours gradually became soft when exposing to simulated body fluid SBF for another 24 hours, indicating the strengthen by Na$_2$CO$_3$ is reversible with the ions leaching. (C) Doppler ultrasonography of arterial vessels before and right after pulmonary constriction surgery using strengthened molten fibril band (2 M Na$_2$CO$_3$ treated) and (D) the detection of blood flow velocity and Pressure gradient. Results indicate a reduction of blood flow velocity from initial 119 cm/s to 93.1 cm/s and a significant reduction of pressure gradient from 6 mmHg to 3 mmHg.
Fig. S14. **In vitro degradation evaluation of strengthened molten fibril materials.** (A) Optical images of strengthened molten fibril films incubated in simulated body fluid (i.e., SBF) with or without collagenase (100 U/mL) for 0 to 36 hours showed the difference of degradation. (B) The quantified residual mass of strengthened molten fibril films at different time points, which indicate the molten fibril film was gradually degraded by collagenase.

Fig. S15. **The continuous shear experiment of gold treated molten fibril network and static fiber network.** The instantaneous viscosity modulus of gold treated molten fibril networks was measured under a wide range of shear rate from 0.1 s\(^{-1}\) to 100 s\(^{-1}\). The shear cycle reveals that this network exhibits shear-thinning behavior that recovers upon a decrease of shear rate from 100 s\(^{-1}\) to 0.1 s\(^{-1}\). This shear-thinning and recovery is consistent with a self-healing of the gold-treated molten fibril network through the cleavage and reformation of reversible non-covalent crosslinking interactions. In comparison, the gold treated static fiber network shows relatively higher viscosity under low shear (~ 4500 Pa s), underwent shear-thinning but this network was not restored to the initial state upon removal of the shear. Presumably, the gold treated static fiber network would have been broken under the shear force because of the un-regenerated covalent bonding.
Fig. S16. Programmable 3D self-shaping and the biocompatibility of gold-collagen composite film. (A) Shape transformations of the patterned cross-linked Janus collagen films by 0.1% glutaraldehyde. (I) middle area patterned, (II) ends of the rectangular strip area patterned, (III) inner circle area patterned and (IV) outer ring area patterned. (B) CCK-8 assay quantitative analysis of L929 cells proliferation with the Janus collagen-AuNPs composite film and (C) live/dead cell staining with fluorescence imaging show that during 5 days cell culture, the population of viable cells (green) on the surface of collagen-AuNPs composite film increased over time and no dead cells (red) were observed, indicating the collagen-AuNPs composite film has good biocompatibility.

Fig. S17. Aligned stable fibril network (abbreviated as “ASF”) processed by stretching molten fibril network and crosslinking it by UV/riboflavin. (A) Visual images of ASF
network. (B) Stress-strain curves of molten fibril network before and after UV/riboflavin crosslinking.

Table S1. Water Content of networks with kosmotropic (NH₄)₂SO₄ concentration.

| (NH₄)₂SO₄ concentration [M] | Molten Fibril Network [%] | Static Fiber Network [%] |
|----------------------------|---------------------------|--------------------------|
| 0.0                        | 89.68 ±2.14               | 88.69 ±1.03              |
| 1.0                        | 76.47 ±1.94               | 79.04 ±3.02              |
| 2.0                        | 56.37 ±1.86               | 57.58 ±4.65              |
| 2.5                        | 48.93 ±3.47               | 50.76 ±2.69              |
| 4.0                        | 38.77 ±2.85               | 46.69 ±1.03              |

Table S2. Summary of the Mechanical properties of collagen networks under various (NH₄)₂SO₄ concentrations.

| (NH₄)₂SO₄ concentration [M] | Strength [MPa] | Elongation [%] | Young’s Modulus [MPa] | Toughness [MJ/m³] |
|----------------------------|----------------|----------------|-----------------------|-------------------|
| Molten Fibril network      |                |                |                       |                   |
| 0.0                        | 0.13 ±0.03     | 220.41 ±5.07   | 0.32 ±0.11            | 0.19 ±0.02        |
| 1.0                        | 0.83 ±0.16     | 185.53 ±13.62  | 0.58 ±0.08            | 0.67 ±0.15        |
| 2.0                        | 2.48 ±0.43     | 178.16 ±15.93  | 3.23 ±0.96            | 1.87 ±0.20        |
| 2.5                        | 3.95 ±0.77     | 171.35 ±8.75   | 8.35 ±2.25            | 3.22 ±0.36        |
| 4.0                        | 5.85 ±0.65     | 151.13 ±5.12   | 16.42 ±2.28           | 3.33 ±0.50        |
| Static Fiber network       |                |                |                       |                   |
| 0.0                        | 0.78 ±0.15     | 53.00 ±10.28   | 1.15 ±0.03            | 0.16 ±0.01        |
| 1.0                        | 0.95 ±0.16     | 42.41 ±8.87    | 1.45 ±0.11            | 0.24 ±0.03        |
| 2.0                        | 1.21 ±0.33     | 38.92 ±5.45    | 2.90 ±0.55            | 0.21 ±0.03        |
| 2.5                        | 1.52 ±0.37     | 34.33 ±5.84    | 4.50 ±1.35            | 0.24 ±0.07        |
| 4.0                        | 1.81 ±0.42     | 28.58 ±3.27    | 8.49 ±1.08            | 0.24 ±0.04        |
Table S3. Summary of the Stimulated Mechanical Properties of Protein Networks reported in references.

| List                  | Polymer Species                  | External Stimulus          | Original Modulus ($E_0$) | Highest-Stimulated Modulus ($E_1$) | Highest Toughness | Stimulus Sensitive coefficient ($E_1/E_0$) |
|-----------------------|----------------------------------|----------------------------|--------------------------|------------------------------------|-------------------|---------------------------------------------|
| This Work Molten fibril network | Collagen Type I                  | Hofmeister Salts: [(NH₄)₂SO₄] | 0.32 MPa                 | 16.42 MPa                          | 3.33 MJ/m³        | 51.31                                        |
| Static fiber network  | Collagen Type I                  | Hofmeister Salts: [(NH₄)₂SO₄] | 1.15 MPa                 | 8.49 MPa                           | 0.24 MJ/m³        | 7.45                                         |
| (41) Static fiber network  | Gelatin                          | Hofmeister Salts: [(NH₄)₂SO₄] | 0.0235 MPa               | 16.42 MPa                          | 3.33 MJ/m³        | 51.31                                        |
| (42) Static fiber network  | Whey protein isolate (WPI)       | Hofmeister Salts: [NaH₂PO₄] | 0.029 MPa                | 0.7583 MPa                         | NA                | 32.26                                        |
| (43) Bovine Serum Albumin (BSA) | Mental ions: [Zn²⁺]           | 0.011 MPa                  | 0.191 MPa                | 0.24 MJ/m³                         | 7.45              | 15.96                                        |
| (44) Polyelectrolytes: Polyethyleneimine (PEI) | 0.01 MPa | 0.064 MPa Soaking in 10% w/w NaH₂PO₄ | 2.8×10⁻³ MJ/m³          | 17.36                              | 6.40              |
| (45) Elastin-like polypeptides (ELPs) | Mental ions: [Zn²⁺]           | 0.01 MPa                  | 0.16 MPa Soaking in 1 M Zn²⁺ | 1.3×10⁻³ MJ/m³ | 16.00                                      |
| (46) Synthetic peptides | Temperature (Tem.)               | 5×10⁻³ MPa                | 0.304 MPa                | 1.33 MJ/m³                         | 10.12             |
| (47) Silk/Elastin      | Temperature (Tem.)               | ~0.005 MPa                | ~0.025 MPa               | ~ 0.07 KJ/m³                       | ~5                |
| (48) Silk/Elastin      | Temperature (Tem.)               | ~0.005 MPa                | ~0.025 MPa               | ~ 0.07 KJ/m³                       | ~5                |

Table S4. Summary of the Mechanical properties of ASF Networks

|                | Parallel direction (⊥) | Perpendicular direction (∥) |
|----------------|------------------------|-----------------------------|
|                | Young’s Strength [MPa] | Young’s Modulus [GPa]       |
| Young’s Modulus [MPa] | Perpendicular direction (∥) |
| ASF- 0 %       | 1.10 ± 0.17            | 4.83 ± 1.63                |
| ASF-50 %       | 1.98 ± 0.31            | 4.83 ± 1.63                |
| ASF-100 %      | 3.21 ± 0.49            | 11.08 ± 0.68               |
| ASF-200 %      | 11.08 ± 0.68           | 11.08 ± 0.68               |

Table S5. Summary of the Mechanical properties of Dried Samples

|                | Strength [MPa] | Young’s Modulus [GPa] |
|----------------|---------------|-----------------------|
| Natural tendon tissue | 128 ± 14     | 0.89 ± 0.12           |
| Biomimetic tendon film | 107 ± 6      | 0.80 ± 0.06           |
| Static fiber film     | 40 ± 8       | 0.37 ± 0.10           |
Supplementary Movies

Movie S1. Bending movement of Janus collagen strip.

Movie S2. Self-shaping process of a flower-like Janus collagen film.

Movie S3. Self-shaping process of patterned Janus collagen film (fixed the right half area).