Mesostructured Collagen from the Bovine Achilles Tendon as a Structural Unit of Collagen Films

Reporter: Jiaqi Li

Supervisor: Ying Pei (Professor)

College of Materials Science and Engineering, Zhengzhou University, China

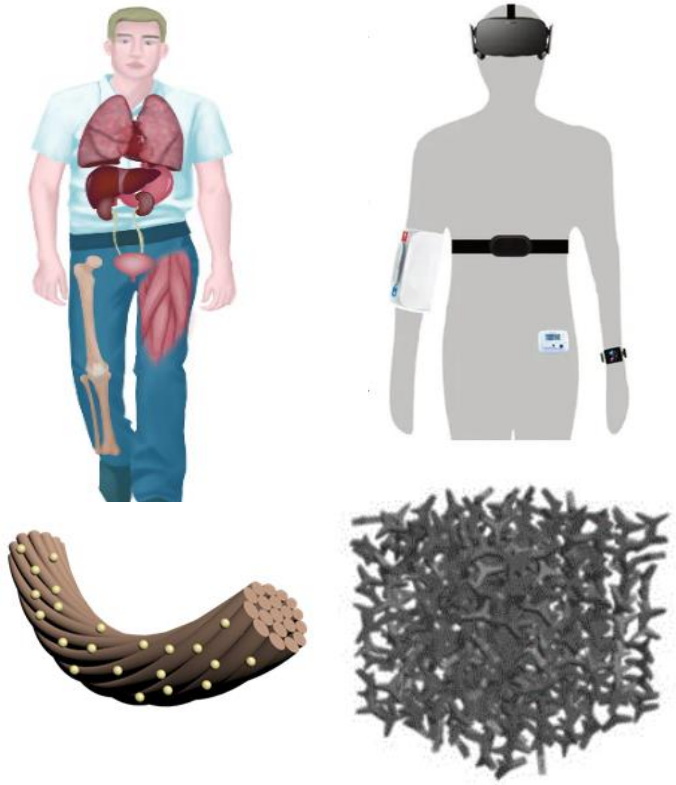
Henan International Joint Laboratory of Biomass Resources and Materials

The current application status of collagen

Traditional application fields

Leather, food

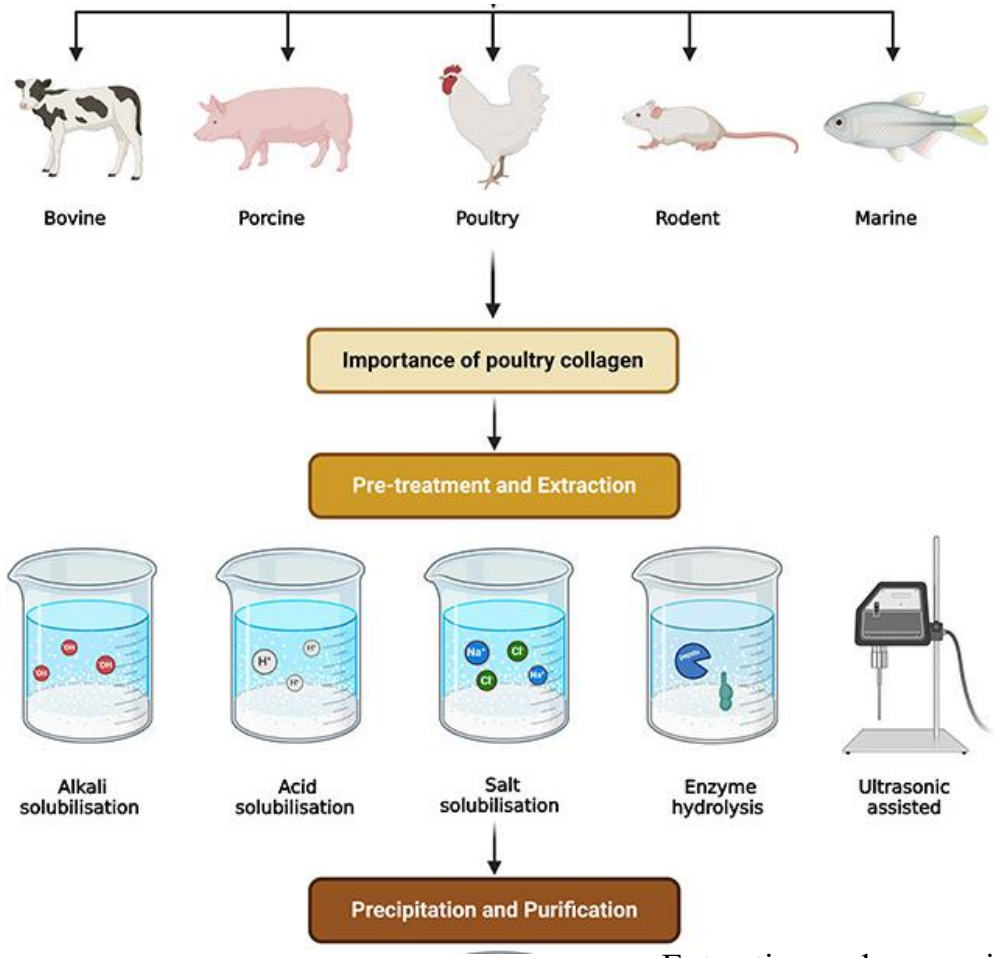
Emerging high value-added fields

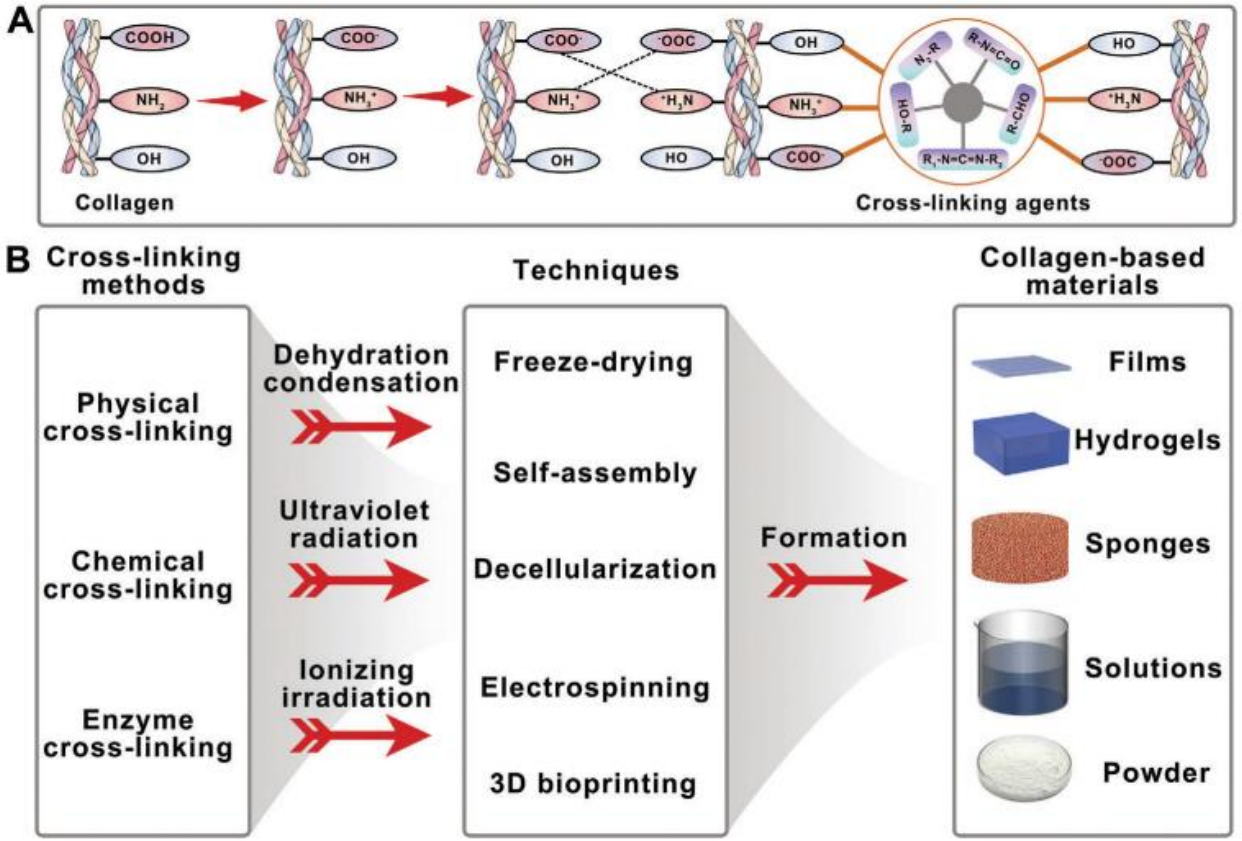


Tissue engineering, sensing, adsorption, catalysis, etc

- In the early days, collagen was mainly applied in traditional fields.
- Collagen materials are diverse in form, have excellent and controllable material properties, and are functionalized

Extraction and processing of collagen molecules





The processing procedure of collagen (molecular) materials（Adv. Healthcare Mater., 2023, 12, 2202042）

Extraction and processing of collagen (molecules)（Intl. J. Biol. Macromol., 2024, 273, 133004）

- **At present, collagen molecules are mainly extracted through methods such as acid hydrolysis and enzymatic hydrolysis.**
- **Meanwhile, crosslinking agents are needed in the process of processing collagen molecules into materials**

The limitations of collagen molecule dissolution processing

The material properties are not ideal (mechanical, thermal stability,)

Controllability of assembly at the macromolecular scale

The biosafety issues of crosslinking agents

The complexity of collagen extraction

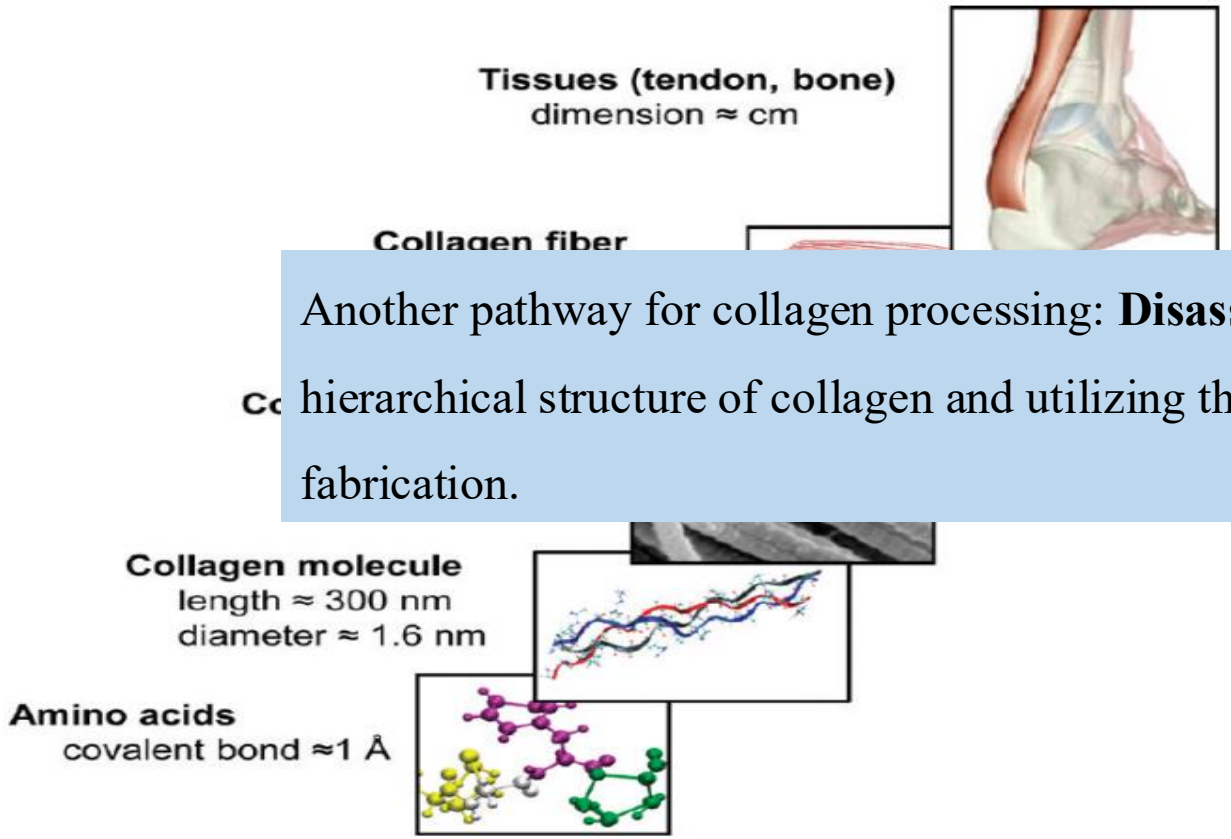
Compatibility of blended composite systems

Harsh storage conditions for collagen solution

- The performance advantage of collagen in aggregate structure is lost during the dissolution process.
- Constructing materials at the molecular scale is somewhat complex.

The structure and properties of collagen

The multi-level structure of collagen



Nano Lett., 2011, 11, 757-766

Natural collagen aggregates have excellent properties

- **Young's modulus**

Natural collagen microfibers: 3.75-11.5 Gpa

Natural collagen microfibers: 60.3 ℃

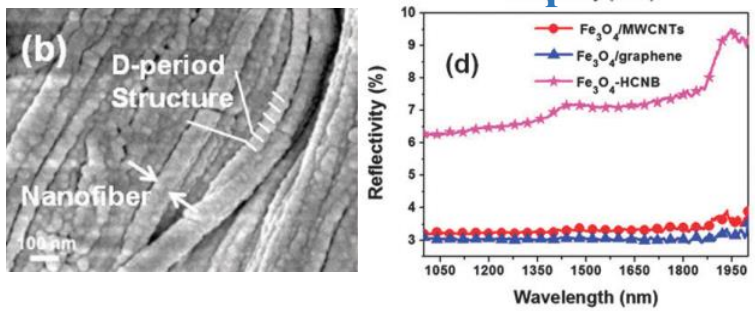
Biophys. J., 2007, 93(4), 1255
Int. J. Biol. Macromol., 2010, 46, 458
ACS Biomater. Sci. Eng. 2020, 6, 739

Another pathway for collagen processing: **Disassembling** the mesoscale **aggregates** within the hierarchical structure of collagen and utilizing them as structural building blocks for material fabrication.

- **Collagen has a multi-level structure, including molecules, aggregated states, and tissues.**
- **Collagen aggregates have a higher Young's modulus and thermal stability, similar to nanocellulose.**

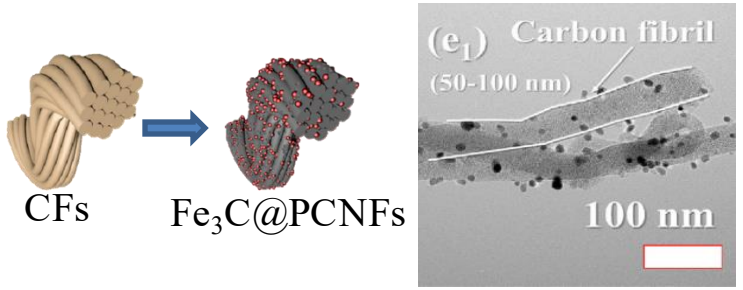
The advantages of collagen aggregates in material construction

- **Build absorbing materials: Increase microwave transmission paths**



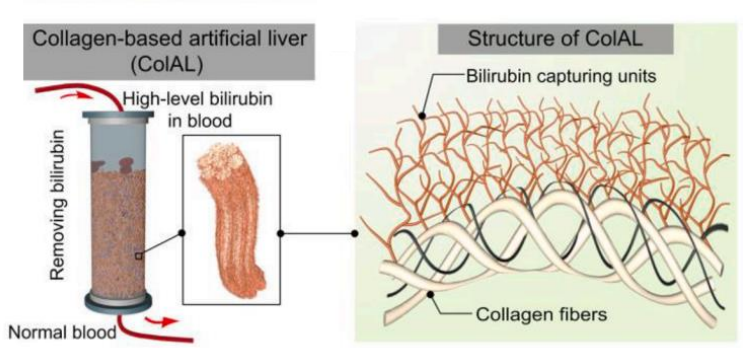
J. Mater. Chem. C, 2015, 3, 10146

- **Construction of catalytic materials: serving as a nitrogen-doped carbon template**



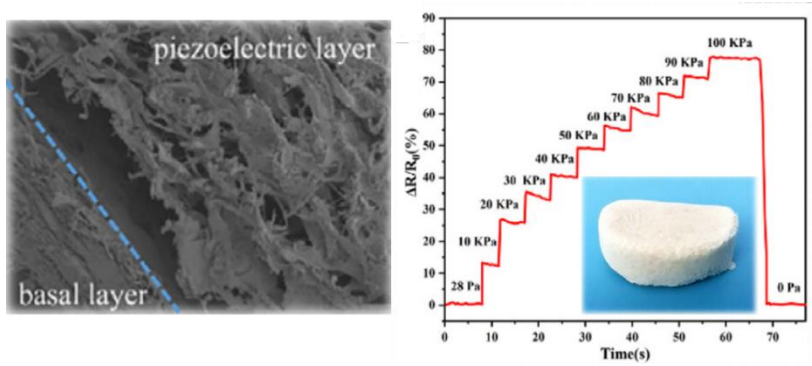
Carbon, 2018, 130, 645

- **Construction of adsorption materials: Provide multi-level adsorption sites**



Chem. Eng. J., 2021, 426, 130791

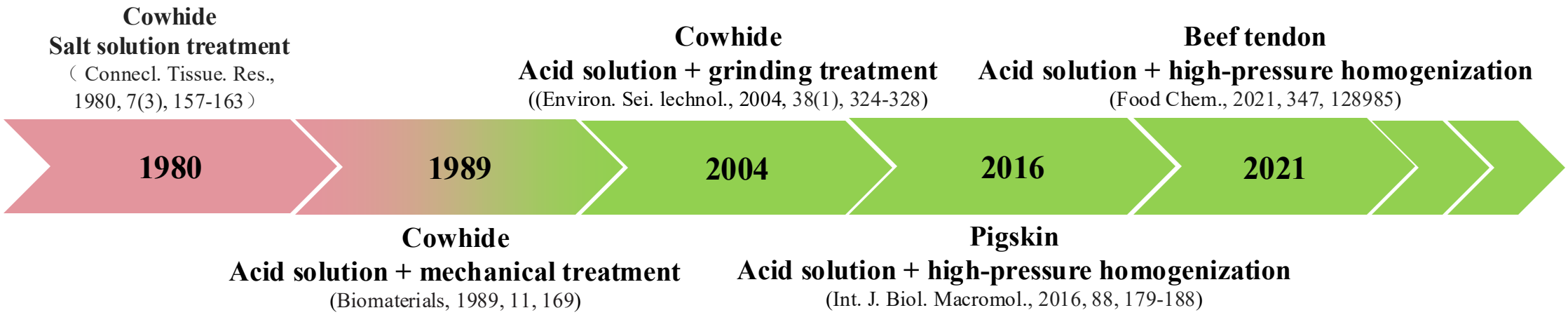
- **Constructing sensing materials: Forming a three-dimensional network structure**



Chem. Eng. J., 2020, 392, 123672

■ **Collagen aggregates have many advantages when constructing materials, such as providing multi-level adsorption sites, Forming a three-dimensional network structure, etc.**

The resolution of collagen aggregates



At present, the resolution of collagen aggregates mainly uses acid solutions and mechanical treatment:

- **The splitting method is single (acid solution + mechanical);**
- **The splitting mechanism is not clear.**
- **The controllable resolution of collagen aggregates was not involved.**

Method for splitting collagen aggregates 1

尿素/盐酸胍深共晶溶剂拆分体系

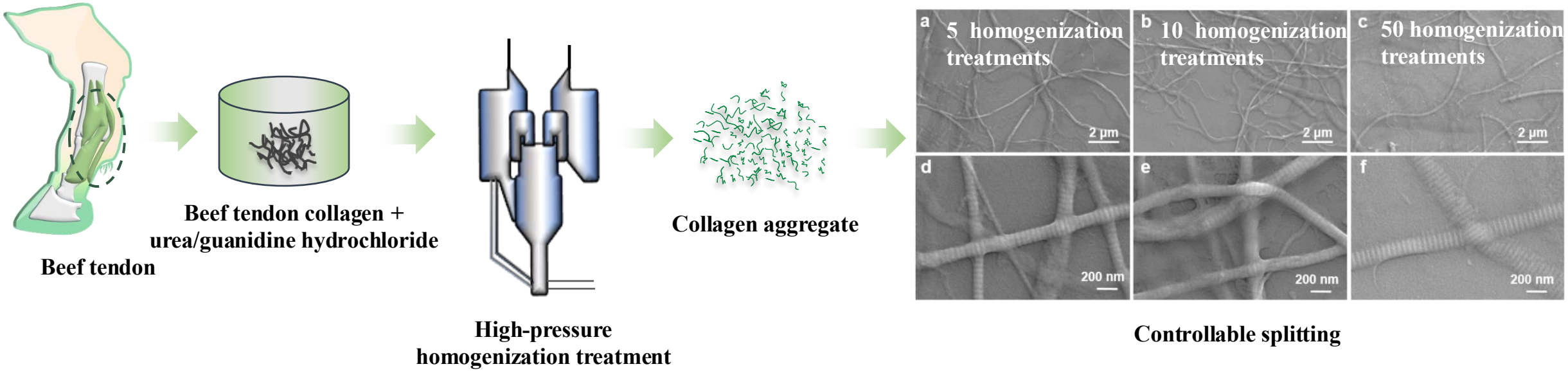


Figure 1. The splitting process of collagen aggregates.

- **Method 1: Use urea/guanidine hydrochloride combined with high-pressure homogenization to separate collagen aggregates**
- **The splitting process is controlled by the number of homogenizations**

Mechanism

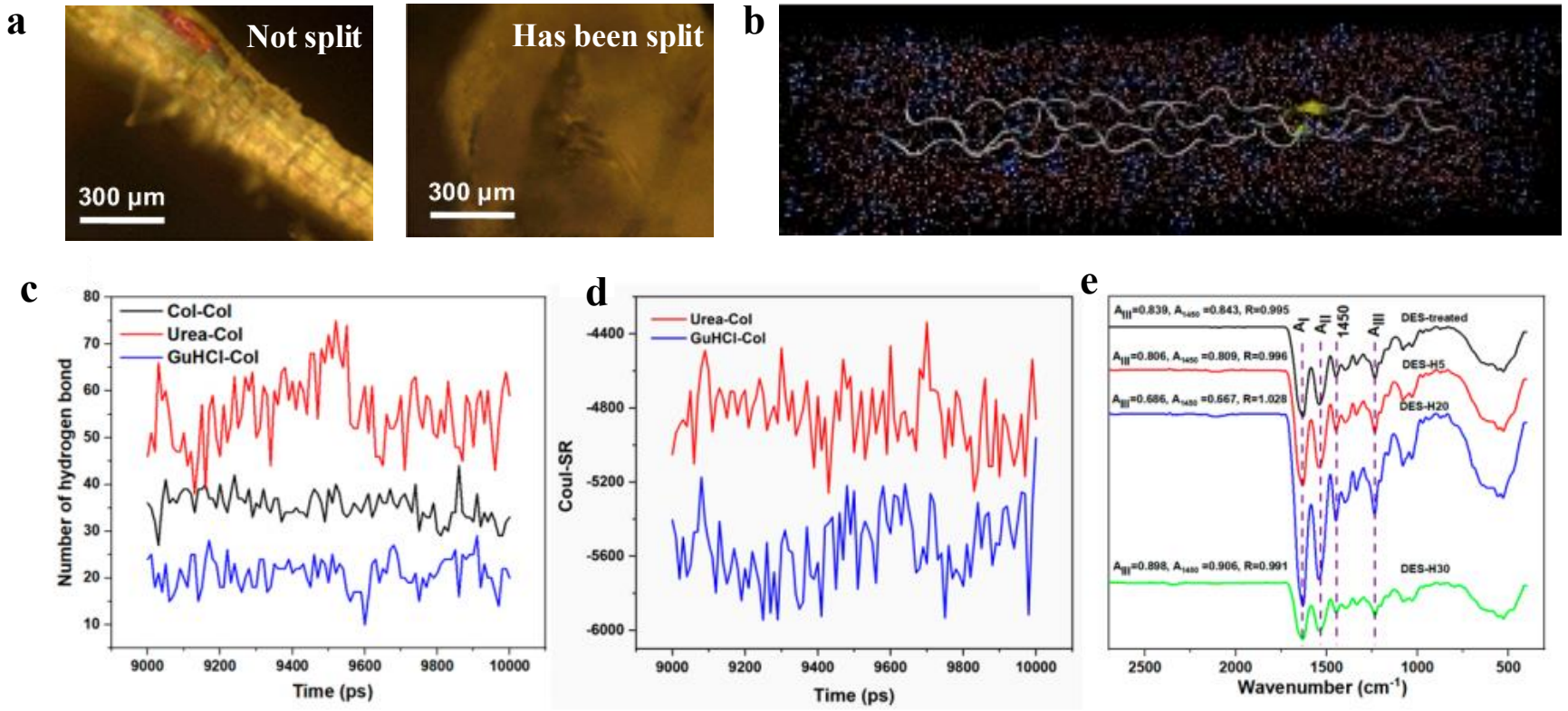


Figure 2. (a) POM images of collagen, (b) - (d) molecular dynamics simulations and (e) FT-TR spectra during the splitting process.

- **The results from POM images and molecular dynamics simulations demonstrate that the dissociation of collagen aggregates is due to hydrogen bonds and Coulomb forces between urea and collagen, as well as van der Waals forces between guanidine hydrochloride and collagen.**

Method 2 for splitting collagen aggregates

NaOH/尿素水溶液拆分体系

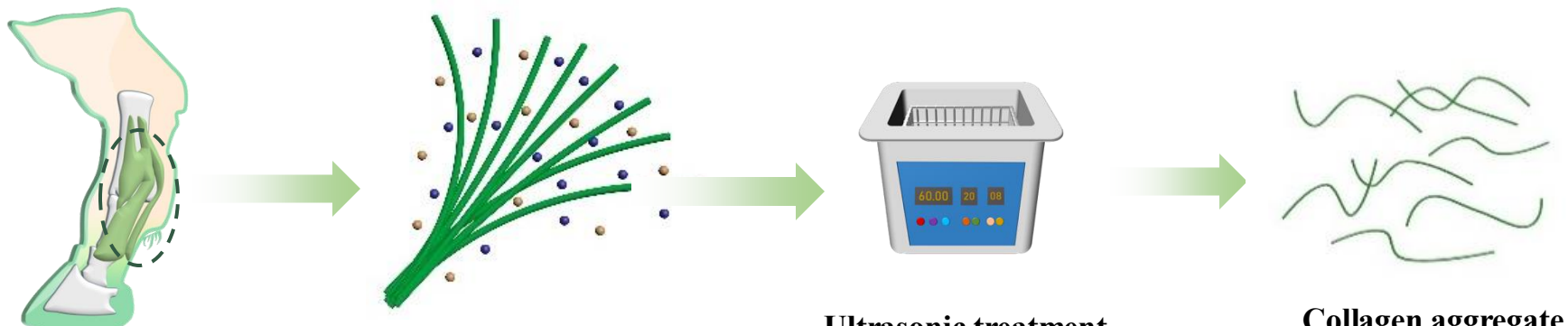
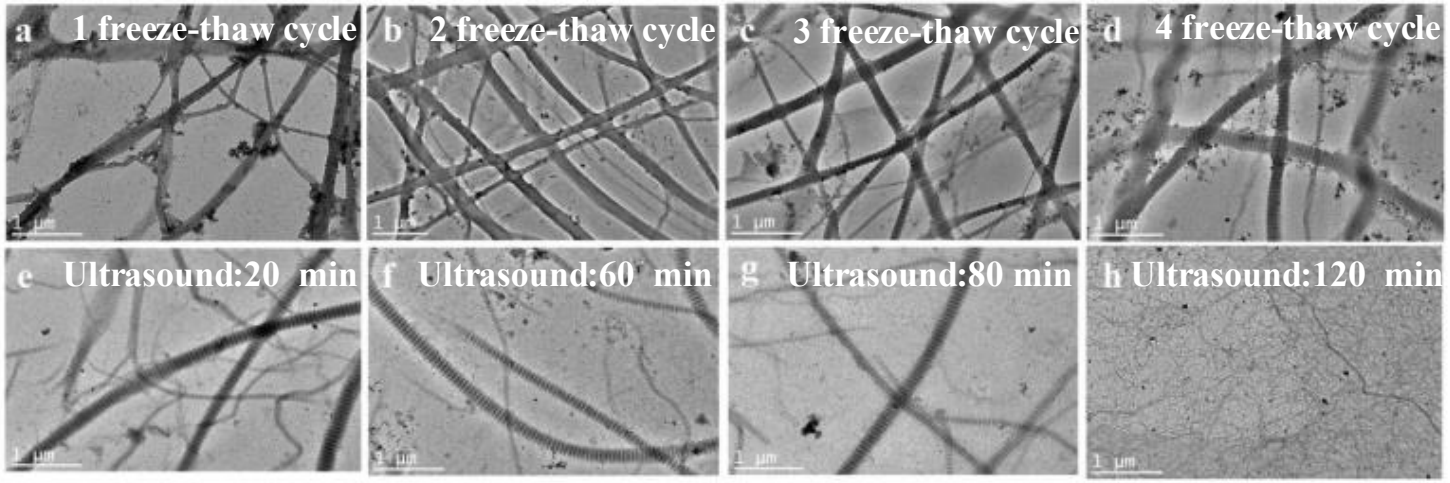


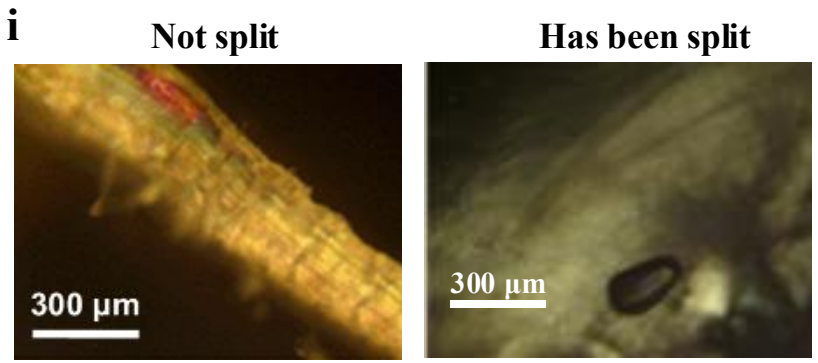
Figure 3. The separation process of collagen aggregates.

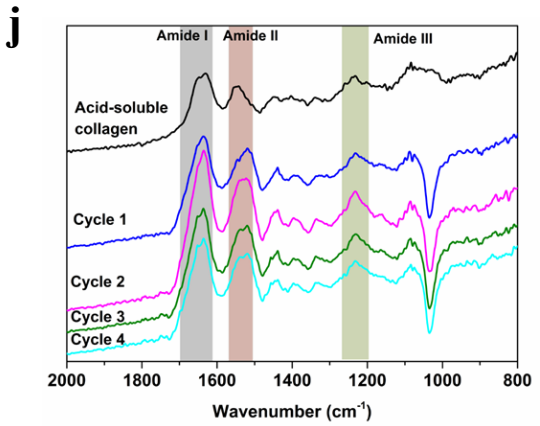
■ **Method 2: Bovine tendons were subjected to freeze-thaw cycles in Sodium hydroxide/urea solution and combined with ultrasound to obtain collagen aggregates from separation**

Controllable resolution of collagen aggregates



- **Controllable splitting: Collagen aggregates with diameters of 26-300 nm and aspect ratios of 16-1080 were obtained by changing the number of freeze-thaw cycles and ultrasound time.**





- **The splitting process did not significantly damage the triple helix structure of collagen.**

Figure 4. The splitting process of collagen aggregates (a) - (h) SEM images, (i) POM images and (j) FT-TR spectra.

Mechanism

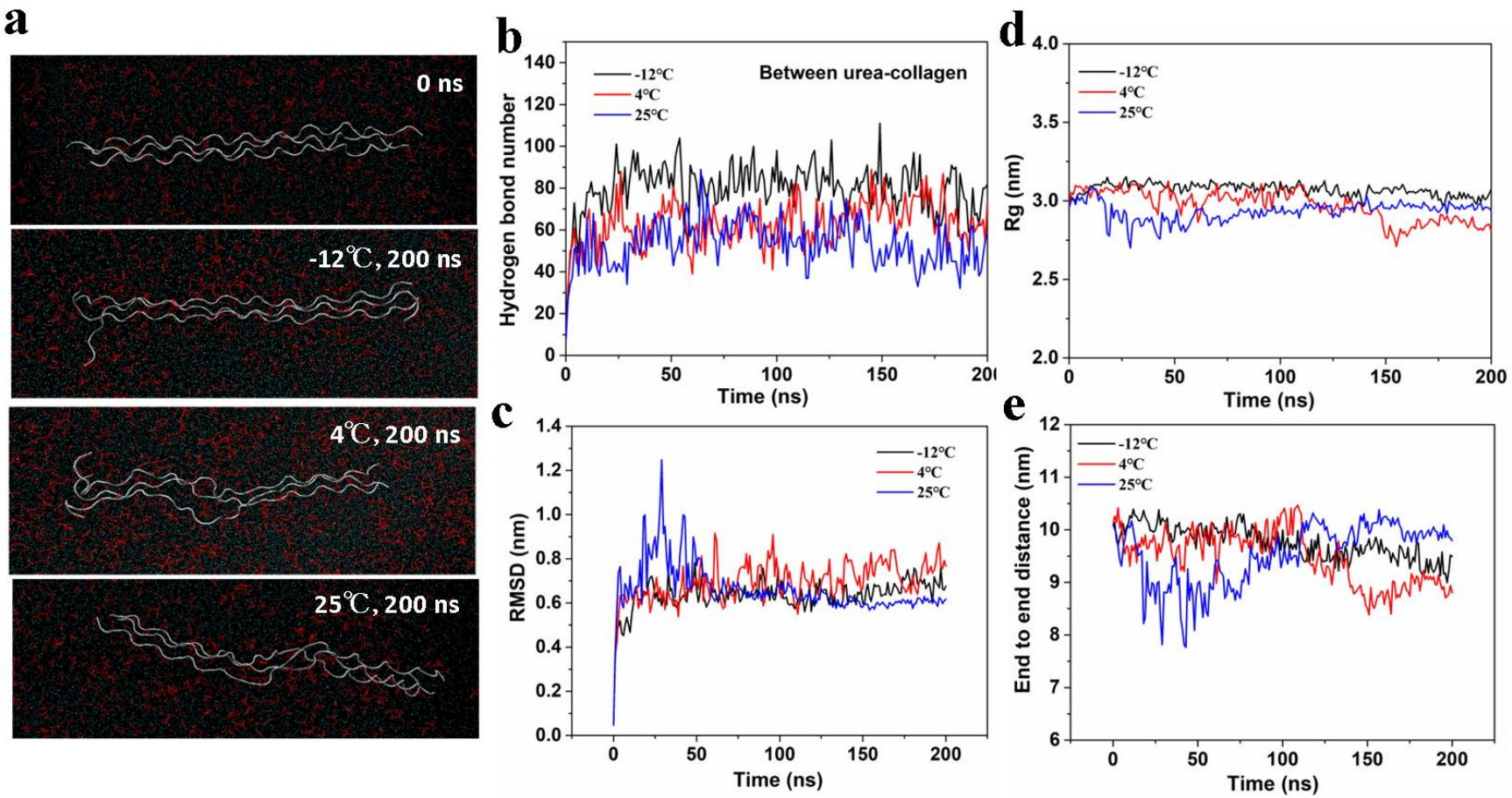


Figure 5. Molecular Dynamics simulation.

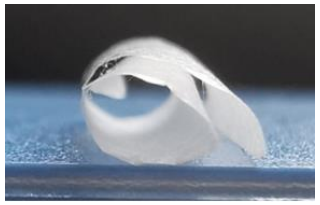
- **Sodium hydroxide causes the fibers of beef tendons to swell;**
- **Urea forms new hydrogen bonds with collagen;**
- **Temperature changes the number of hydrogen bonds.**

Collagen aggregate films and their mechanical properties-Verify the performance of collagen aggregates

a

Collagen suspension

NaOH/ urea

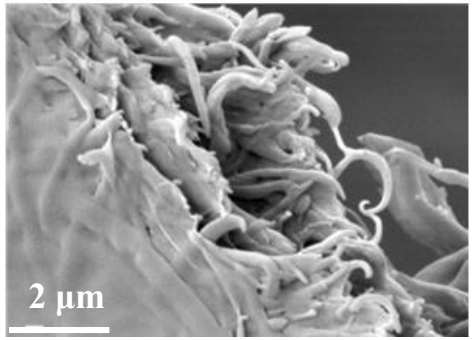


Urea/guanidine hydrochloride

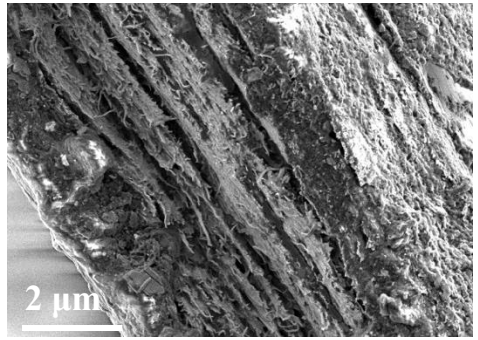
Collagen membrane

b

NaOH/ urea

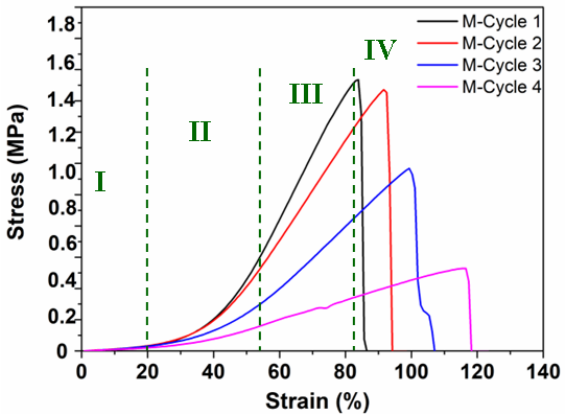


Urea/guanidine hydrochloride

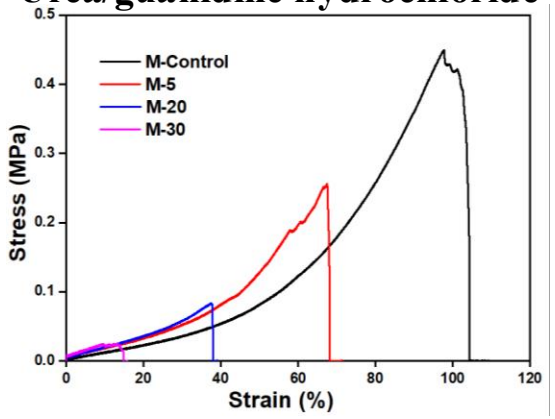


c

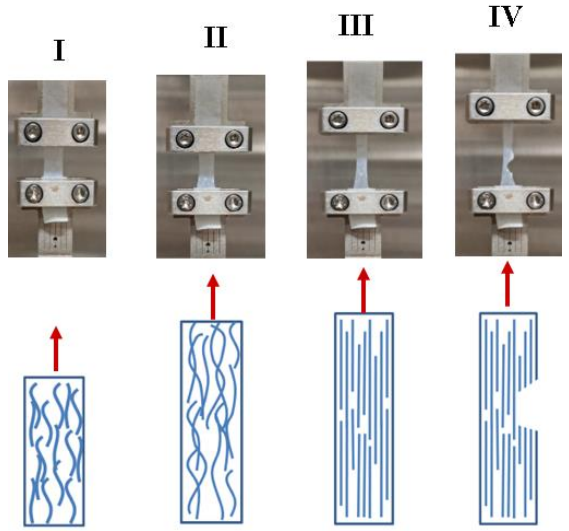
NaOH/ urea



Urea/guanidine hydrochloride



d



- The dense structure endows the collagen membrane with high mechanical properties.
- The mechanical properties of the wet film obtained in this work (breaking strength: 500-1700 kPa, elongation at break: 86-116%) are higher than those of the wet film prepared with acid sol (breaking strength: 5-1490 kPa, elongation at break: 20-40%).

Acta Biomater., 2010, 6, 403-408
Food Chem., 2020, 318, 126404
Biomaterials, 2007, 28, 1385-1392

Figure 6. Construction of collagen membranes and their mechanical properties. (a) Flowchart of collagen membrane preparation, (b) SEM image, (c) typical stress-strain curve, and (d) the arrangement process of collagen aggregates when the membrane is subjected to force.

The application potential of collagen membranes -pH responsiveness

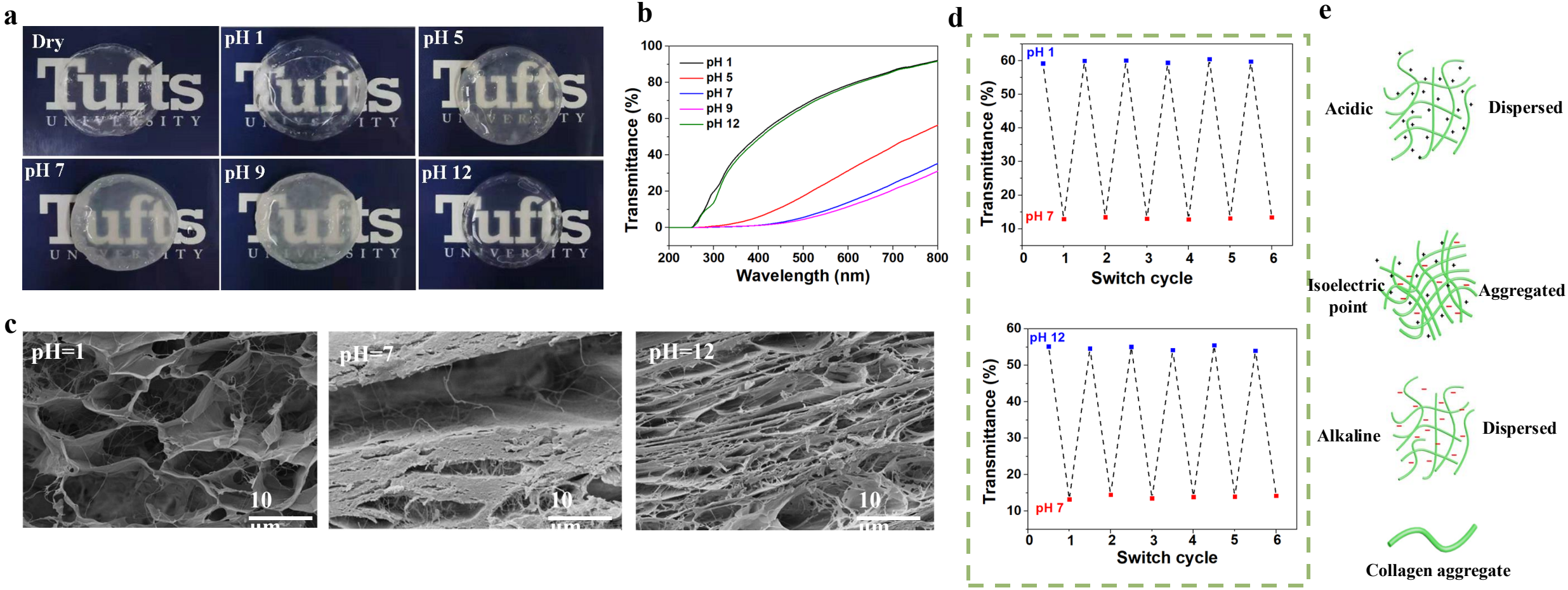


Figure 7. pH responsiveness of M-cycle3. (a) Digital photos after soaking in aqueous solutions with different pH values, (b) changes in optical transmittance, (c) SEM, (d) Cycling responses of aqueous solutions at pH= 1,7 and 12, (e) arrangement of collagen aggregates with pH changes.

■ **Collagen films have great application potential.**

The application potential of collagen membranes - Conductive composite films

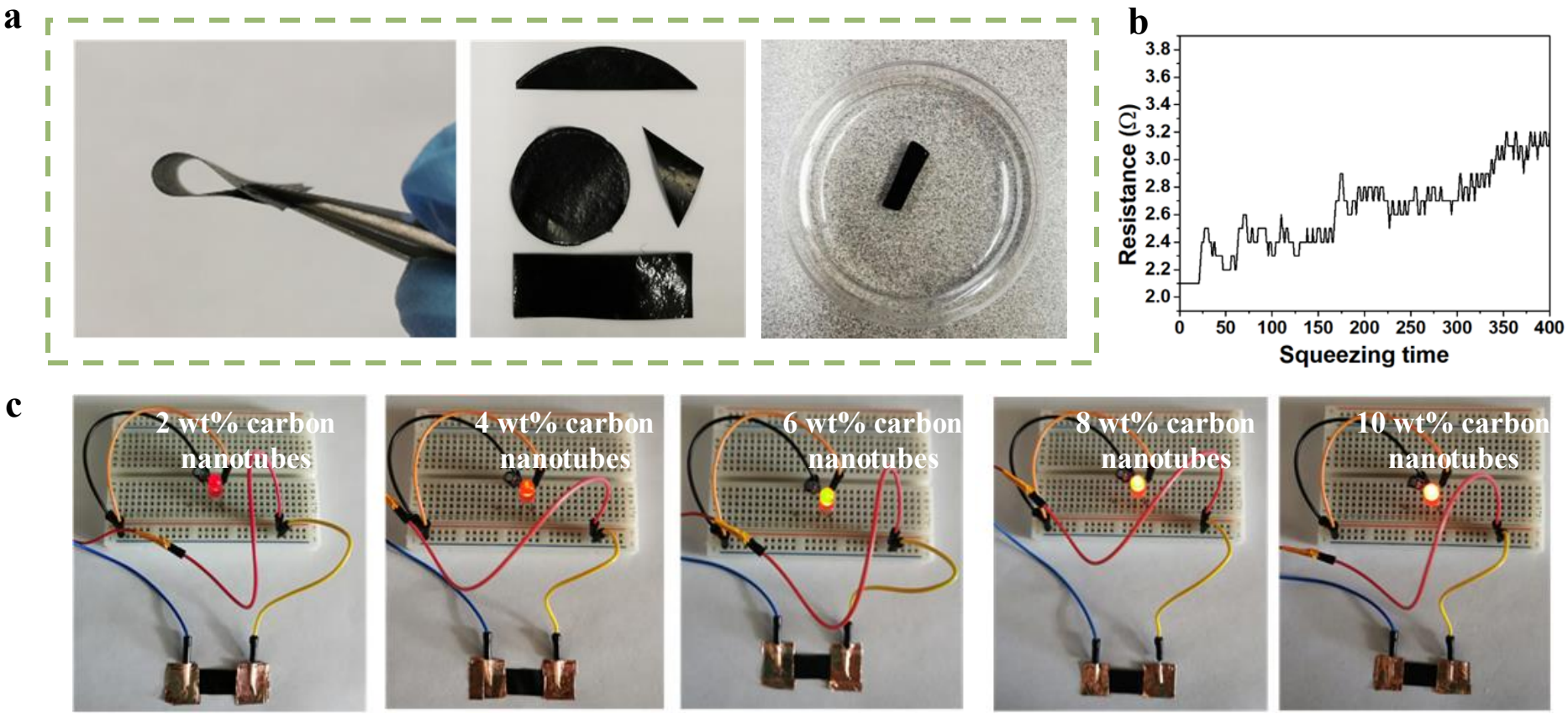
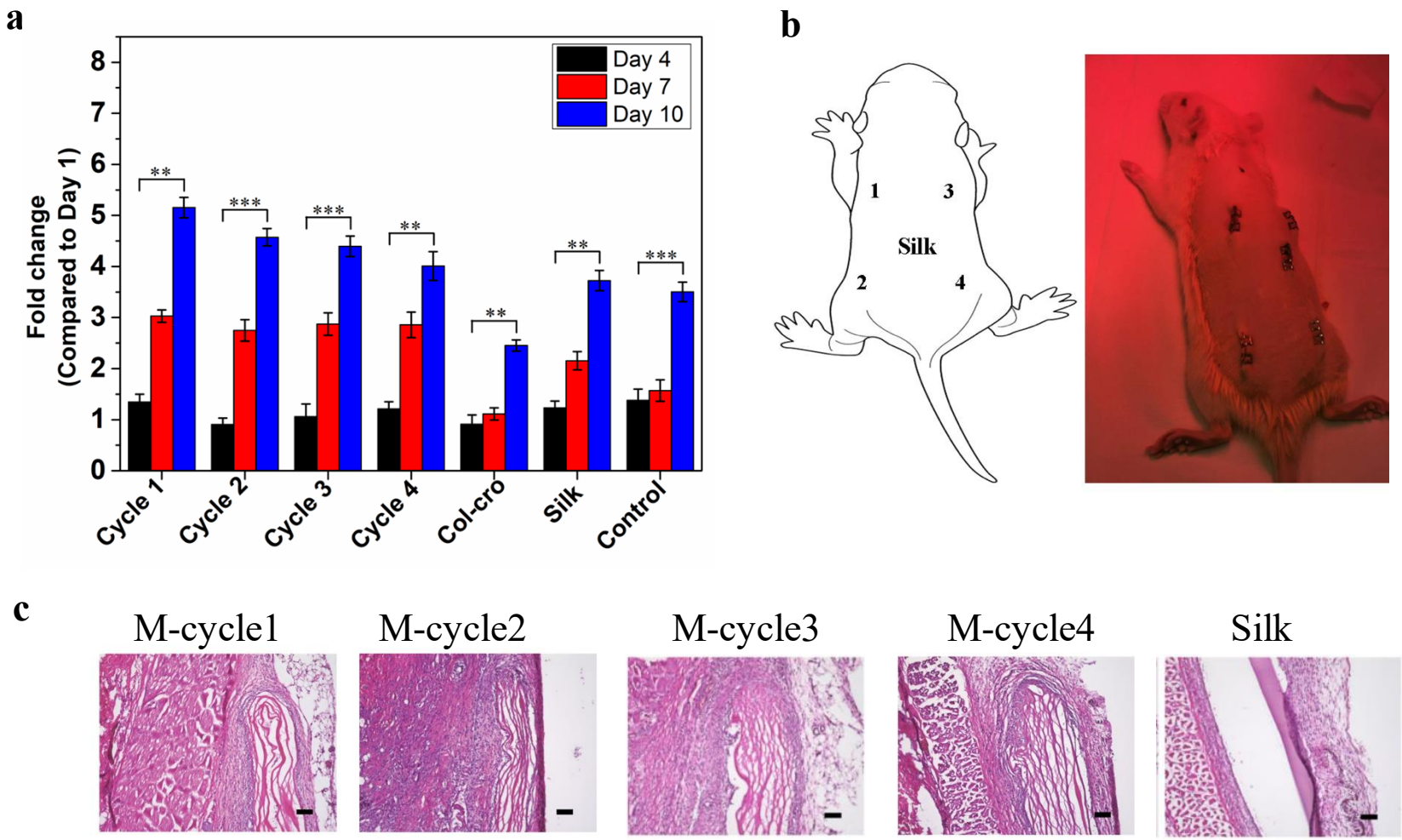


Figure 8. (a) Digital photo of M-cycle3/ carbon nanotube composite film. (b) Resistance change curve of the composite film after 400 bending cycles when the carbon nanotube content is 10 wt%. (c) Digital photo of the conductivity change of the carbon nanotube composite film with different contents.

The application potential of collagen membranes - biocompatibility



- **Good cell compatibility.**
- **It has better biocompatibility compared with silk.**

Figure 9. Biological evaluation (a) multiple changes in metabolic activity on days 4, 7, and 10 compared with day 1 (b) Collagen membrane on the back of rats and the implantation site of control silk (c) Hematoxylin and eosin (H&E) staining images of tissues one week after subcutaneous implantation (scale: 100 μm).

(1) Achieve controllable separation of collagen aggregates;

(2) Compared with the films prepared from collagen solutions, the membrane materials with collagen aggregates as structural units have superior mechanical properties and environmental stability;

(3) The unique structure of collagen aggregates enables collagen membrane materials to exhibit excellent application potential in optics, electronics and biomedicine.

Acknowledgements: The XXXVIII IULTCS CONGRESS; College of Materials Science and Engineering, Zhengzhou University; Henan International Joint Laboratory of Biomass Resources and Materials; Ying Pei (Professor)

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Thanks!

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