Supporting Information

Co-assembled biomimetic fibrils from collagen and chitosan for performance-enhancing hemostatic dressing

by

Xingling Zeng [a, b], Zhaohui Sun [c], Lidan Chen [c], Xiaoxia Zhang [a, b], Xin Guo [a, b], Guoying Li [a, b *]

[a] *The Key Laboratory of Leather Chemistry and Engineering (Ministry of Education), Sichuan University, Chengdu 610065, PR China;*

[b] *National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065, PR China;*

[c] *Department of Laboratory Medicine General Hospital of Southern Theater Command, Guangzhou, Guangdong 510010, PR China;*

**Corresponding Author: Guoying Li.*

The Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University, Chengdu 610065, PR China

Tel: +86-28-85462568

Fax: +86-28-85405237

E-mail address: liguoyings@163.com

Methods

Monitoring the co-assembly process of collagen and chitosan with various degrees of deacetylation (DD) at different ratios

The freeze-dried collagen and three types of chitosan powders，characterized by varying degrees of deacetylation (50%, 70%, and 85% DD), were employed to formulate a series of blend solutions. Initially, both collagen and chitosan powders were dissolved in a 0.1 M acetic acid solution to achieve a concentration of 4 mg/mL. The collagen and chitosan solutions were then combined in specific mass ratios of 9:1, 8:2, 7:3, 6:4, and 5:5 (collagen to chitosan). The final collagen concentration was adjusted to 1 mg/mL using a phosphate-buffered saline (PBS) solution and distilled water, resulting in blends containing 10 mM phosphate and 110 mM sodium chloride. Subsequently, a 1 mol/mL NaOH solution was employed to adjust the pH to 7.2. Based on the difference in collagen to chitosan ratios, the samples were designated as Col/Chi (9/1), Col/Chi (7/3), Col/Chi (6/4), and Col/Chi (5/5), respectively. Additionally, a pure collagen solution was prepared as a control and labeled simply as Col. The self-assembly process of all samples was monitored at 37℃ using a UV-visible spectrophotometer (PerkinElmer Ltd, MA).

Standard curve of Hyp contents measurement

300 μL trans-4-hydroxy-L-proline with different concentrations (0,5, 7.5, 10, 15 and 20 μg/mL) were mixed with 600 μL isopropanol, respectively. Then 1.4% (w/v) chloramine-T solution dissolved in citric acid buffer and Ehrlich's aldehyde reagent (a mixture of p-dimethylamino-benzaldehyde diluted in 60% (v/v) perchloric acid and

isopropanol at a ratio of 3:13 (v/v)) were added, respectively. Finally, the solutions were incubated at 60 °C for 20 min followed by chilling down at ice for 2 min and the absorbance intensity of each solution was inspected at 560 nm by a spectrometer (PerkinElmer Ltd., Massachusetts, USA).

Results

Fibrillogenesis kinetics was assessed to investigate the modulation of collagen assembly by varying concentrations of chitosan with different DD (50%, 70%, 85%). The turbidity curve of collagen exhibited an S-shaped profile, characterized by three main phases: a lag phase with minimal turbidity increase, a growth phase with a rapid increase in turbidity, and an equilibrium phase where turbidity stabilized.[1] As shown in Fig. S1a-c, all turbidity curves, both in the presence and absence of chitosan, illustrated a typical "S" profile, indicating that the introduction of those three types of chitosan did not alter the nucleation mechanism of collagen. The results of the quantitative analysis of the turbidity profiles are presented in Table S1. The introduction of chitosan was found to reduce the lag time in collagen fibril formation compared to pure collagen (Col). Notably, the initial turbidity levels were higher in Col/Chi (6/4) and Col/Chi (5/5), likely due to the electrostatic interactions between collagen and chitosan, which leaded to the formation of collagen-chitosan polyelectrolyte complexes.[2] The effects of three types of chitosan at varying concentrations in the mixed systems on collagen self-assembly exhibited comparable trends. Specifically, the change in turbidity value (Δh) initially increased with rising chitosan concentration, reached a peak, and subsequently declined. The maximum Δh was observed at a collagen to chitosan ratio of 7:3. At ratios of 7:3 or lower, the growth rate was greater than that of Col, thereby accelerating collagen fiber formation. Conversely, when the ratio exceeded 7:3, the growth rate decreased, with some ratios showing rates lower than those of pure collagen, potentially hindering collagen fiber formation. Therefore, it could be concluded that the optimal

ratio of collagen to chitosan was 7:3, and this ratio was effective in promoting collagen fibril formation.

The measurement of hydroxyproline content in the sample supernatant serves as an indirect indicator of quantitation of collagen incorporated into fibrils. As illustrated in Fig. S1d, the hydroxyproline content in the supernatants of composite samples containing all three chitosan was lower than that of pure collagen (Col) when the ratio of Col to Chi was 7:3 or less. This finding further corroborated that the appropriate incorporation of chitosan could enhance the self-assembly of collagen. Conversely, when the Col/Chi ratio exceeded 7:3, the hydroxyproline content in the supernatant progressively increased beyond that of the control, suggesting an inhibitory effect on collagen self-assembly. This observation aligns with the turbidity curve analysis, which indicated that at a 7:3 ratio of collagen to chitosan, the three types of chitosan and their respective concentrations exerted similar effects on collagen fibrillation. Consequently, a fixed collagen to chitosan ratio of 7:3 was selected to mitigate variability introduced by differing concentrations, thereby facilitating a more focused investigation into the impact of chitosan deacetylation on collagen self-assembly.

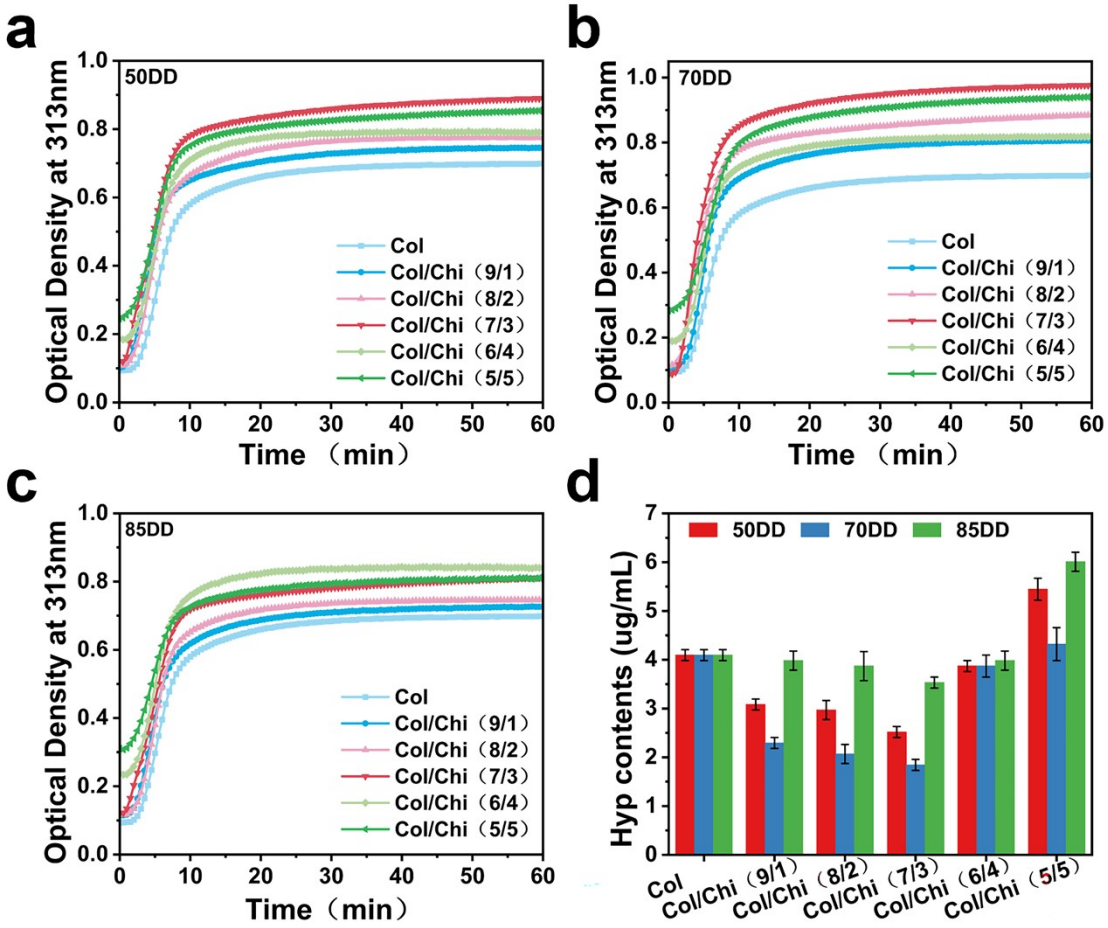

Fig. S1 Collagen self-assembly in the presence of different concentrations and degrees of deacetylation (DD, 50%, 70% and 85%) of chitosan. (a) Turbidity curves (DD, 50%). (b) Turbidity curves (DD, 70%). (c) Turbidity curves (DD, 85%). (d) The content of Hyp in the supernatant.

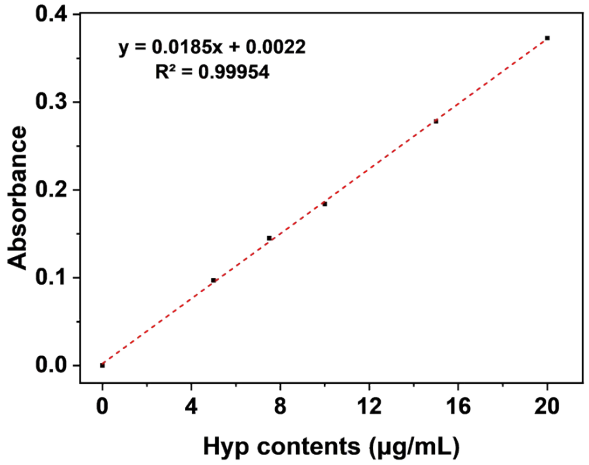

Fig. S2 The standard curves of Hyp.

Table S1 The quantitative data on collagen turbidity curves and content in the presence of chitosan at different concentrations and degrees of deacetylation: lag time, growth rate, change in turbidity value (Δh) and assembly degree.

	Samples	Lag time (s)	Growth rate (v× 10^{-3})	Δh	Assembly degree (%)
	Col	170.87±8.99	1.41±0.01	0.576±0.011	98.49±0.04
50DD	Col/Chi（9/1）	87.23±8.90	1.60±0.02	0.622±0.009	98.87±0.04
	Col/Chi（8/2）	117.06±9.59	1.56±0.04	0.643±0.009	98.91±0.07
	Col/Chi（7/3）	52.36±2.17	1.55±0.02	0.764±0.007	99.07±0.04
	Col/Chi（6/4）	123.15±9.33	1.42±0.03	0.590±0.007	98.58±0.04
	Col/Chi（5/5）	118.91±9.46	1.28±0.03	0.581±0.008	98.00±0.08
70DD	Col/Chi（9/1）	131.43±8.34	1.69±0.07	0.686±0.005	99.16±0.04
	Col/Chi（8/2）	90.98±8.66	1.76±0.04	0.733±0.017	99.24±0.04
	Col/Chi（7/3）	72.11±7.34	2.05±0.08	0.872±0.011	99.32±0.04
	Col/Chi（6/4）	144.88±9.80	1.52±0.04	0.725±0.010	98.58±0.04
	Col/Chi（5/5）	151.01±9.64	1.24±0.02	0.649±0.011	98.41±0.12
85DD	Col/Chi（9/1）	120.58±8.93	1.43±0.04	0.596±0.008	98.53±0.07
	Col/Chi（8/2）	127.97±8.16	1.48±0.06	0.617±0.005	98.58±0.11
	Col/Chi（7/3）	81.57±12.44	1.43±0.01	0.663±0.016	98.70±0.04
	Col/Chi（6/4）	127.38±8.32	1.41±0.04	0.590±0.007	98.53±0.07
	Col/Chi（5/5）	101.69±9.08	1.09±0.02	0.492±0.006	97.79±0.07

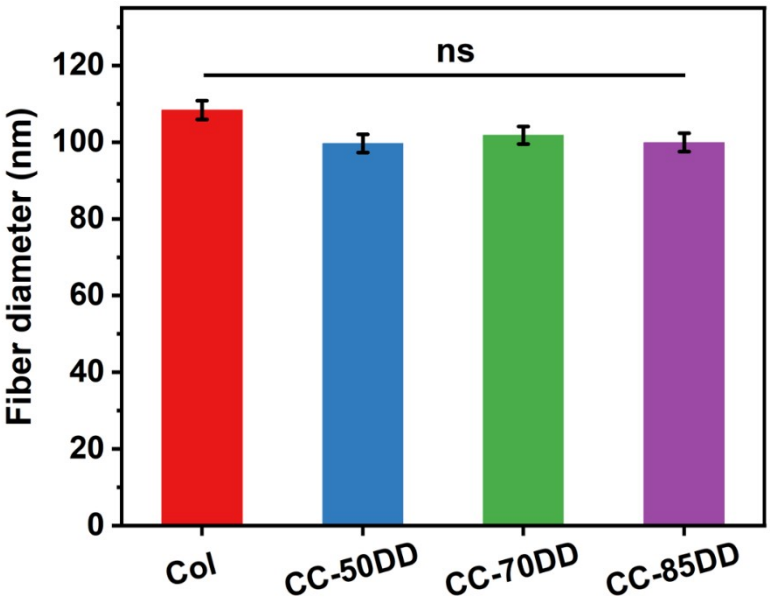

Fig. S3 Statistical analysis of fibril diameter of collagen and collagen/chitosan (7/3) assemblies with different DD of chitosan.

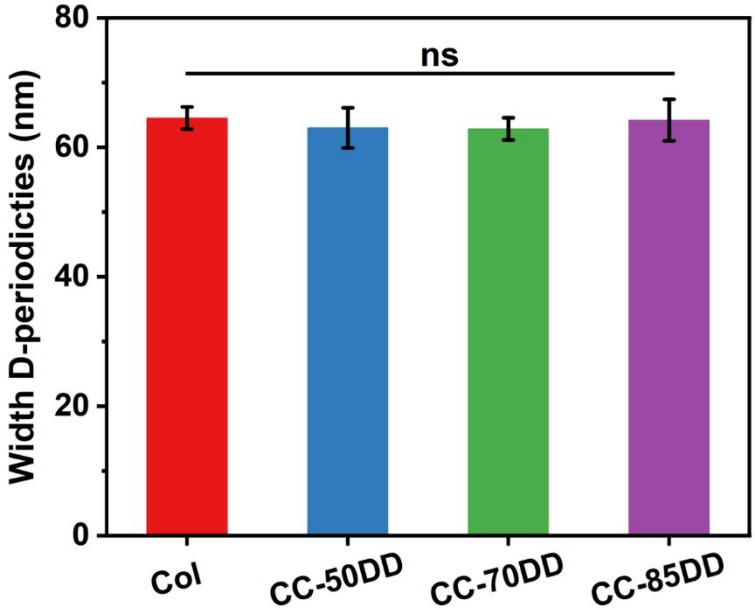

Fig. S4 Statistical analysis of width *D*-periodicity of collagen and collagen/chitosan (7/3) assemblies with different DD of chitosan.

References:

1. X. Zhang, C. Yang, X. Guo, C. Yang and G. Li, *Biomaterials Science*, 2023, **11**, 7408-7422.
2. X. Wang, L. Sang, D. Luo and X. Li, *Colloids and Surfaces B: Biointerfaces*, 2011, **82**, 233-240.