Electronic Supplementary Material (ESI) for Materials Advances.

Supplementary Material

In Vitro Evaluation of the Biodegradability of Chitosan-Genipin Hydrogels

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Supplementary Methods

1 Gravimetric degradation study of hydrogel disks

The lysozyme-mediated degradation of chitosan-genipin hydrogel disks was investigated gravimetrically, using lysozyme concentrations ranging from 2 mg/ml to 6 mg/ml. The weight of an empty 70 µm cell strainer was first recorded. Upon gelation, hydrogels were removed from vials, transferred to a cell strainer, and weighed. The hydrogels were then immersed into 30 ml of PBS/lysozyme solution and their weight was recorded at regular time points, after draining solution from the strainer and removing excess solution from the hydrogel surfaces using filter paper. The experiment was performed in triplicate. The degradation rate (D) was calculated using the following equation: $Degradation\ (\%) = \left[\frac{W0 - Wd}{W0}\right] \times 100$, where $W0$ is the initial weight of the sample and Wd is the weight of the sample after degradation.

2 Volume change study

The volume change of chitosan-genipin hydrogels in PBS (pH 7.4±0.2) was investigated gravimetrically. The weight of an empty cell strainer was first recorded. Upon gelation, hydrogels were removed from vials, transferred to a cell strainer, and weighed. The hydrogels were then immersed into 30 ml of PBS and their weight was recorded at regular time points, after draining solution from the strainer and removing excess solution from the hydrogel surfaces using filter paper. The experiment was performed in triplicate and finished when no change in weight was measured over three adjacent time intervals, at which point it was assumed a state of equilibrium was met. The volume change was calculated the following equation: $Volume\ change\ (\%) = \left[\frac{Wt - W0}{W0}\right] \times 100$, where $W0$ is the initial weight of the sample and Wt is the weight of the sample after immersion into PBS.

Supplementary Figures

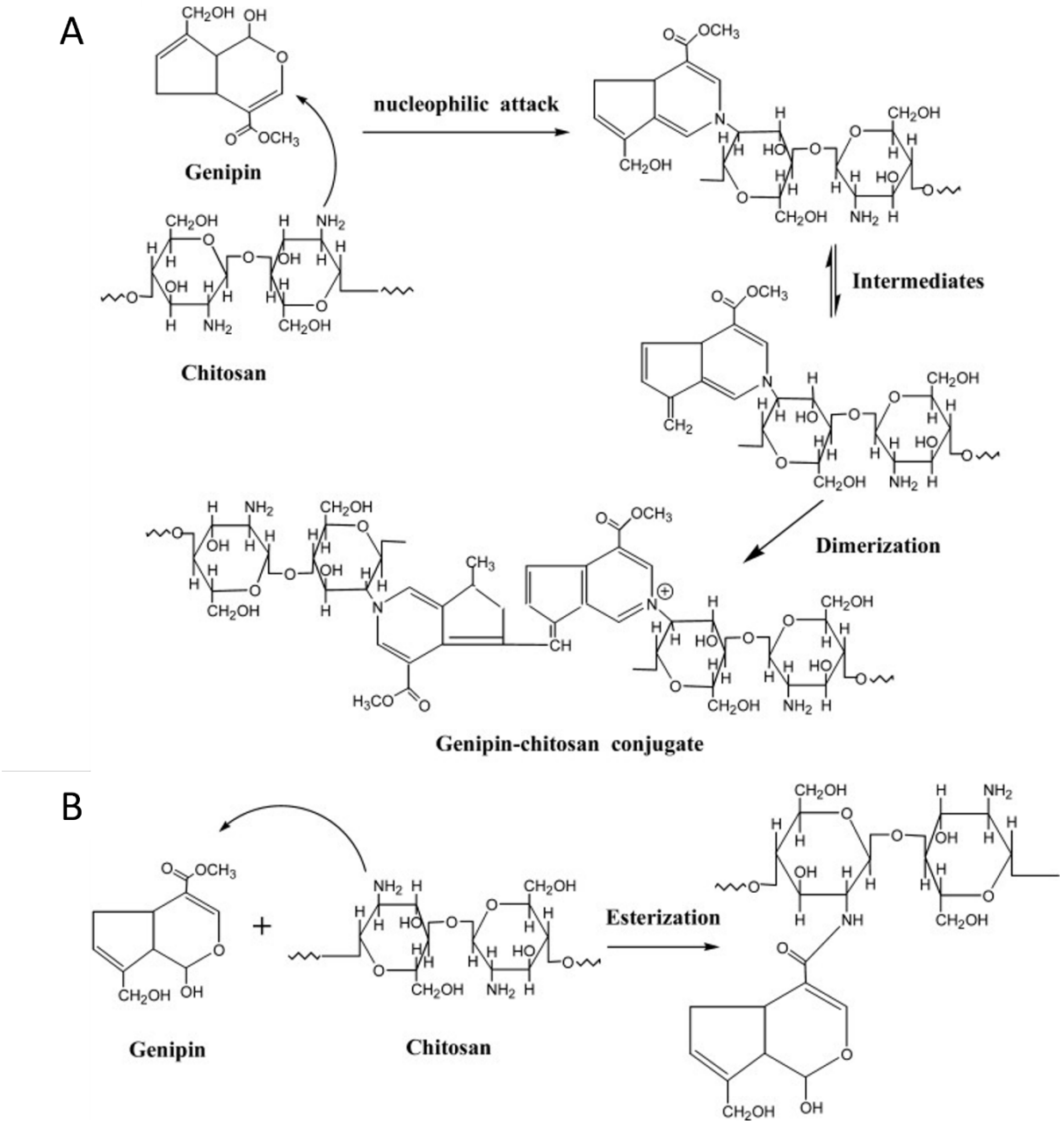

Figure S1. Reaction mechanism between chitosan and genipin. A, formation of highly conjugated genipin derivative; B, formation of secondary amide linkage. Extracted from [1].

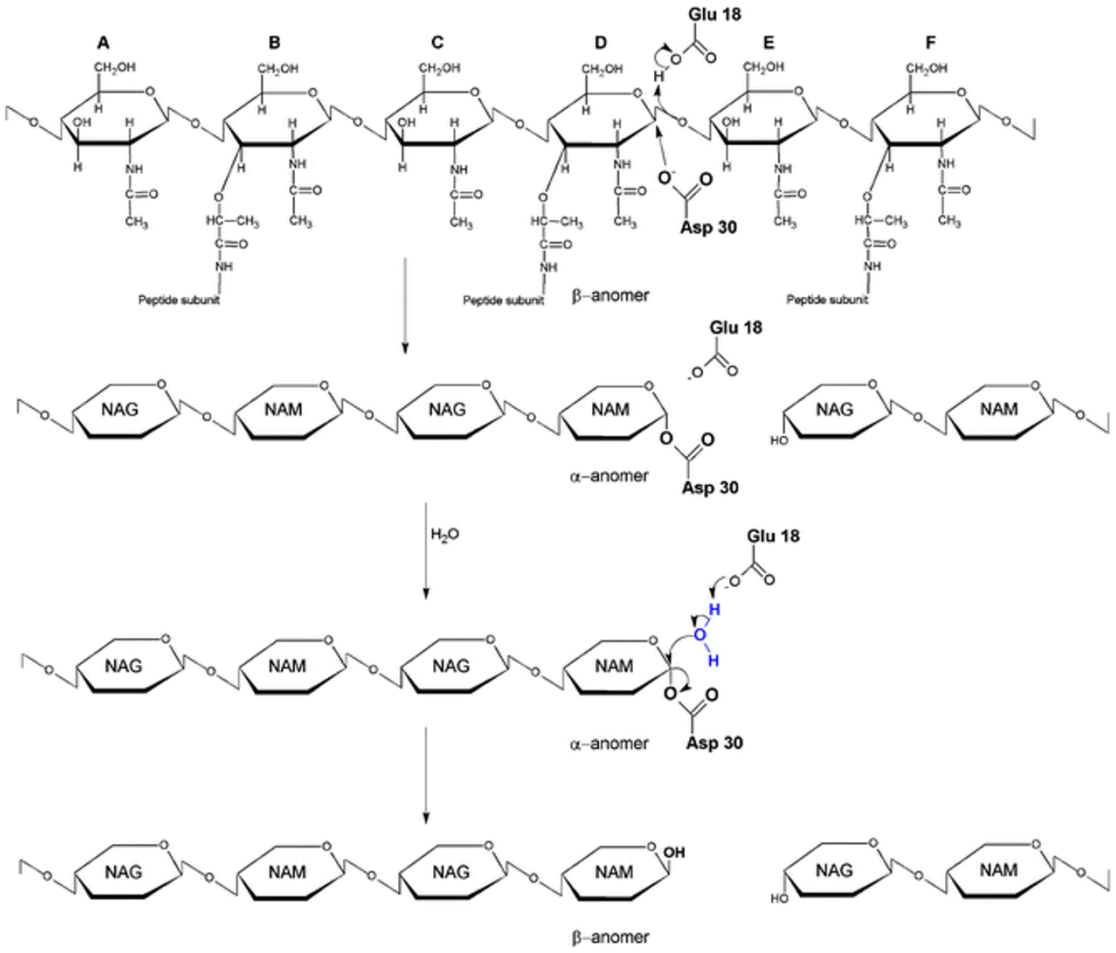

Figure S2. The Koshland mechanism of lysozyme hydrolysis of peptidoglycan. NAG, N-acetylglucosamine; NAM, N-acetylmuramic acid; Glu35, glutamic acid 35; Asp52, aspartate 52. Extracted from [2].

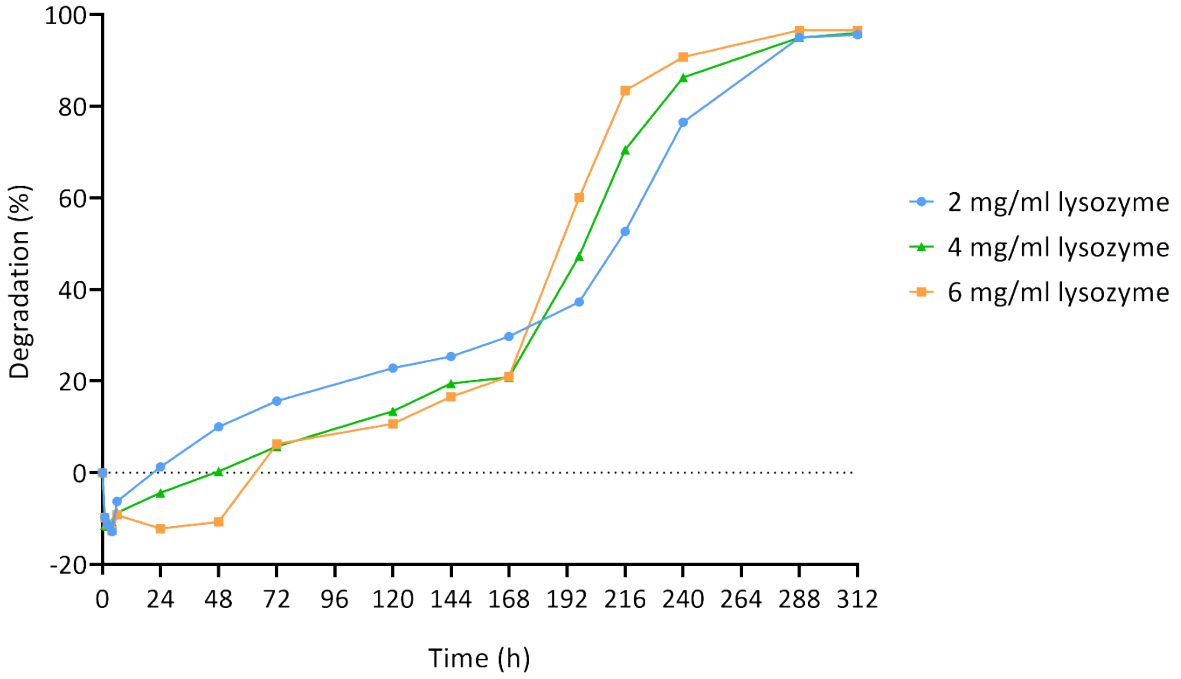

Figure S3. Gravimetric degradation study of chitosan-genipin discs containing 0.5% w/v genipin exposed to different concentrations of lysozyme solution.

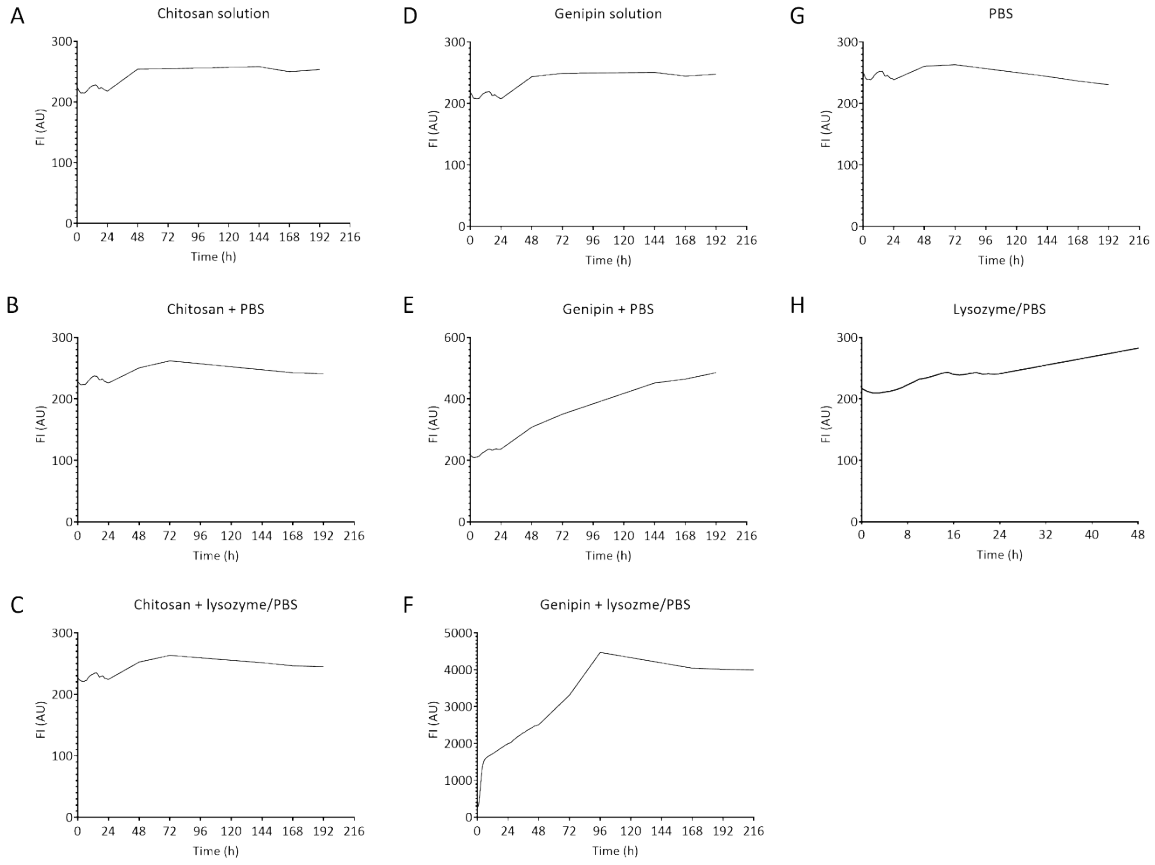

Figure S4. Fluorescence intensity of controls.

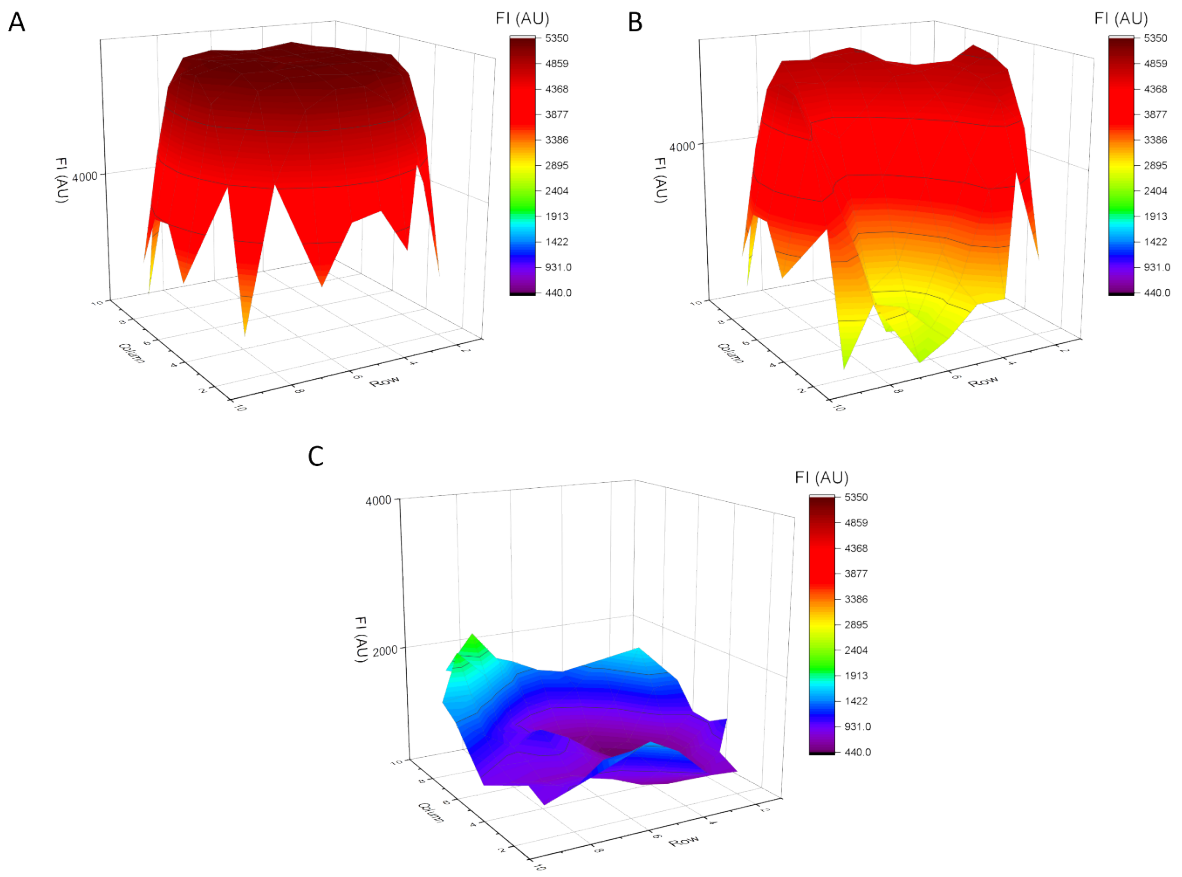

Figure S5. Pattern of lysozyme degradation of chitosan-hydrogel films formed with 0.5% w/v genipin using a 10 x 10 well scan. A, 0 hours post lysozyme exposure; B, 168 hours post lysozyme exposure; C, 192 hours post lysozyme exposure.

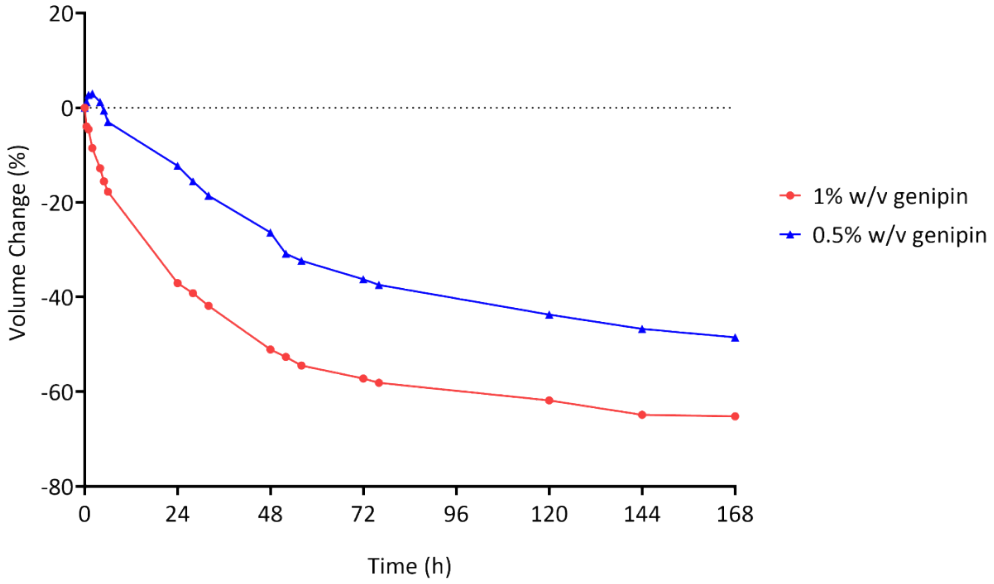

Figure S6. Gravimetric volume change study of chitosan-genipin hydrogel discs containing 0.5% w/v and 1% w/v genipin in 0.5 mg/ml PBS solution.

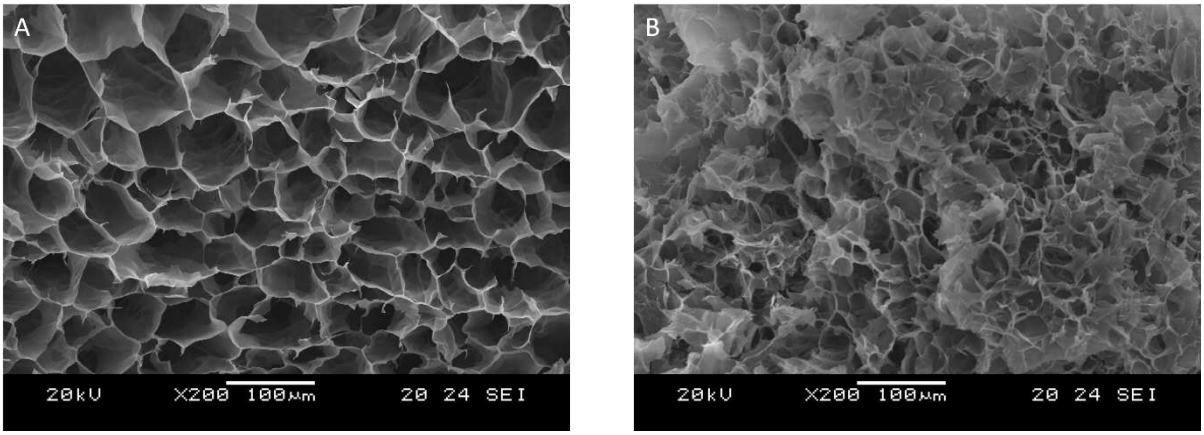

Figure S7. SEM images of horizontal cross sections of chitosan-hydrogel disks formed with 1% w/v genipin (magnification of ×200). A, hydrogel formed at 37°C for 24 hours; B, formed hydrogel immersed in PBS for 1 week. No lysozyme is added.

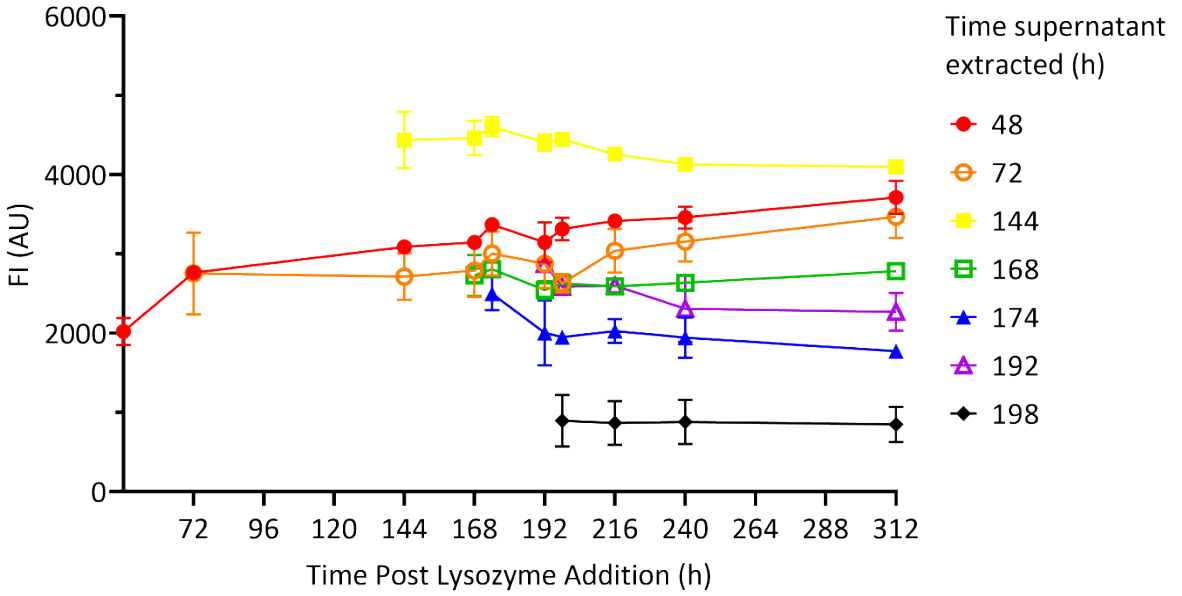

Figure S8. FI of lysozyme degradation solutions over time. Data are presented as the mean ± standard deviation where n = 3.

Supplementary Tables

Table S1. Concentration of constituents of chitosan-genipin hydrogels and NH_2 group calculations

Sample	Concentration (mol/ml)		Number of NH_2 groups[b]	Number of NH_2 groups remaining after crosslinking
	1.5% w/v Chitosan[a]	Genipin		
0.5% w/v genipin	5.1×10^{-8}	3.68×10^{-6}	5.9×10^{-5}	5.16×10^{-5}
1% w/v genipin	5.1×10^{-8}	7.37×10^{-6}	5.9×10^{-5}	4.43×10^{-5}

[a]The average molecular weight of chitosan used in the calculation is 245,000 g/mol

[b]The number of NH_2 groups in 1 mole of 80% deacetylated chitosan was calculated as 1157

To calculate the molar concentration of each constituent in the hydrogel, fist calculate molarity. Multiply molarity by the volume of the component added to the hydrogel mixture, then divide this value by the total volume of the mixture.

Table S2. Peak height ratios between amide I and amide II bands

Sample	**Amide I peak height (%)**	**Amide II peak height (%)**	**Peak height ratio amide I: amide II**
Chitosan powder	10.62	11.19	1: 1.05
Formed hydrogel disk	17.01	28.49	1: 1.68
Partially degraded hydrogel disk	14.49	11.74	1: 0.81
Hydrogel film degradation solution	13.35	10.64	1: 0.80

Supplementary References

1. Chen H, Ouyang W, Lawuyi B, Martoni C, Prakash S. Reaction of chitosan with genipin and its fluorogenic attributes for potential microcapsule membrane characterization. J Biomed Mater Res A. 2005;75A(4):917–27.

2. Herreweghe JMV, Michiels CW. Invertebrate lysozymes: Diversity and distribution, molecular mechanism and in vivo function. J Biosciences. 2012;37(2):327–48.

Reference for graphical abstract

The structural representation of lysozyme in the graphical abstract has been extracted from Vaney MC, Maignan S, Ries-Kautt M, Ducruix A. THE 1.33 A STRUCTURE OF TETRAGONAL HEN EGG WHITE LYSOZYME. 1995.