Supporting Information

Silk Hydrogels Crosslinked by Fenton Reaction

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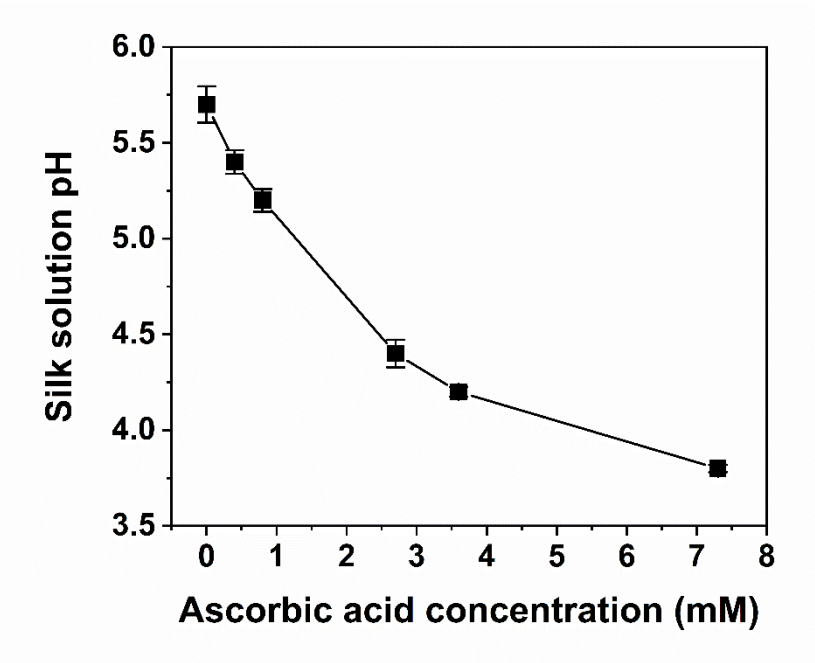

Figure S1. Effect of ascorbic acid concentration on pH of silk solutions. The pH of pristine silk solutions was 5.7 (0 mM ascorbic acid). The addition of ascorbic acid to the silk solutions resulted in a decrease of pH value. Since the silk solutions showed precipitation with turbidity or a sol-gel transition when the ascorbic acid concentration was above 2.7 mM, lower ascorbic acid concentrations (0.4 and 0.8 mM) were chosen for the present study.

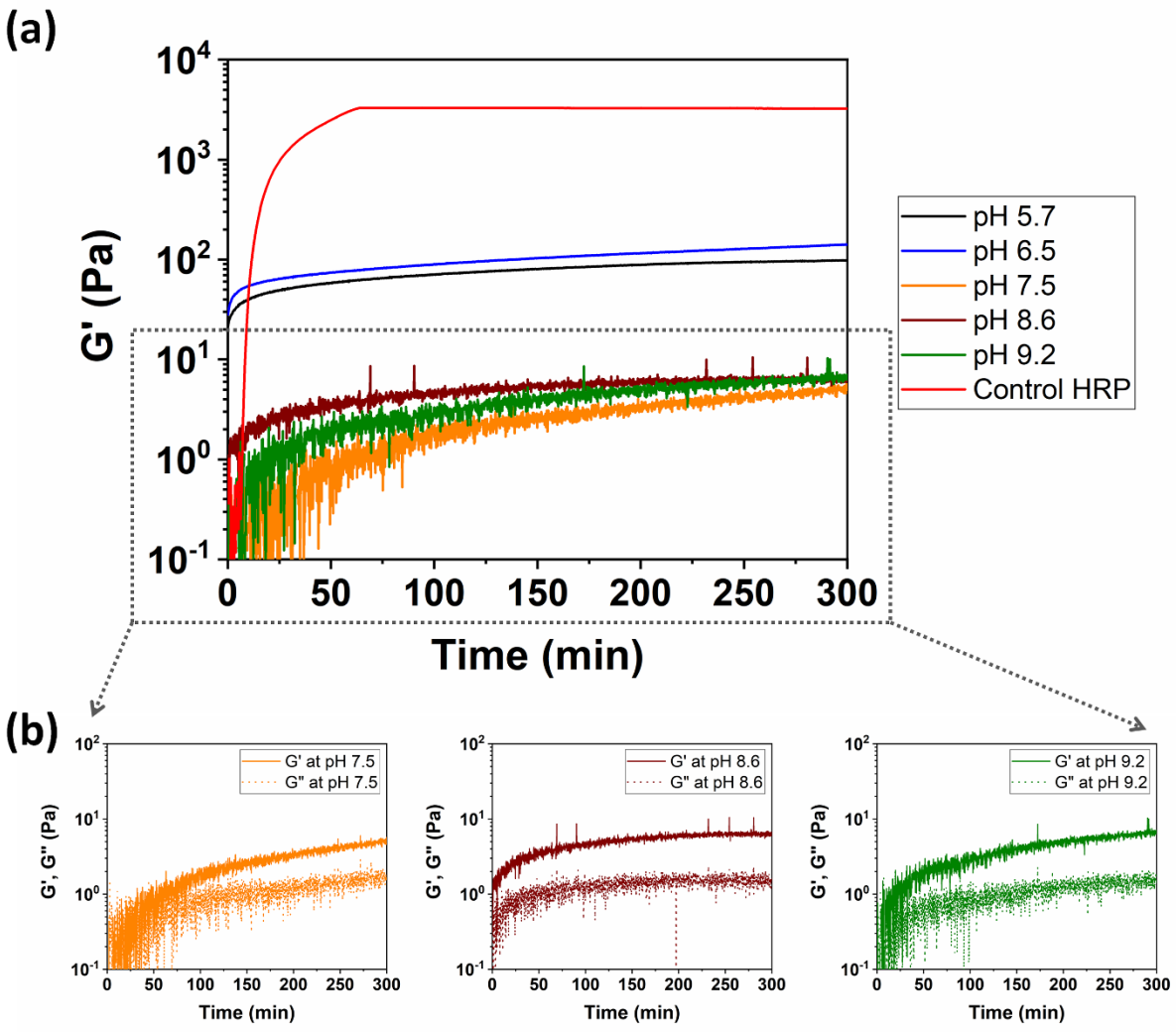

Figure S2. Effect of pH on the preparation of hydrogels crosslinked by Fenton reaction. (a) The evolution of the storage modulus (G') and loss modulus (G'') was monitored for 300 min. Below pH 5, the reactions were hard to control because of the acceleration of the sol-gel transition of silk fibroin. The soft hydrogels were formed at pH 5.7 and 6.5. (b) Rheological properties of the samples from the gray dotted rectangle in (a). The activity of Fenton reaction was significantly decreased at pH 7.5, 8.6, and 9.2. In this case, although G' was larger than G'' for all samples, the vial inversion tests at 300 min for all samples showed the sol state. We note that optically transparent hydrogels were formed at pH 9.2 after ~2 days. The dityrosine bonds of the hydrogels formed at pH 9.2 were analyzed, as shown in Figure 6 of the manuscript.

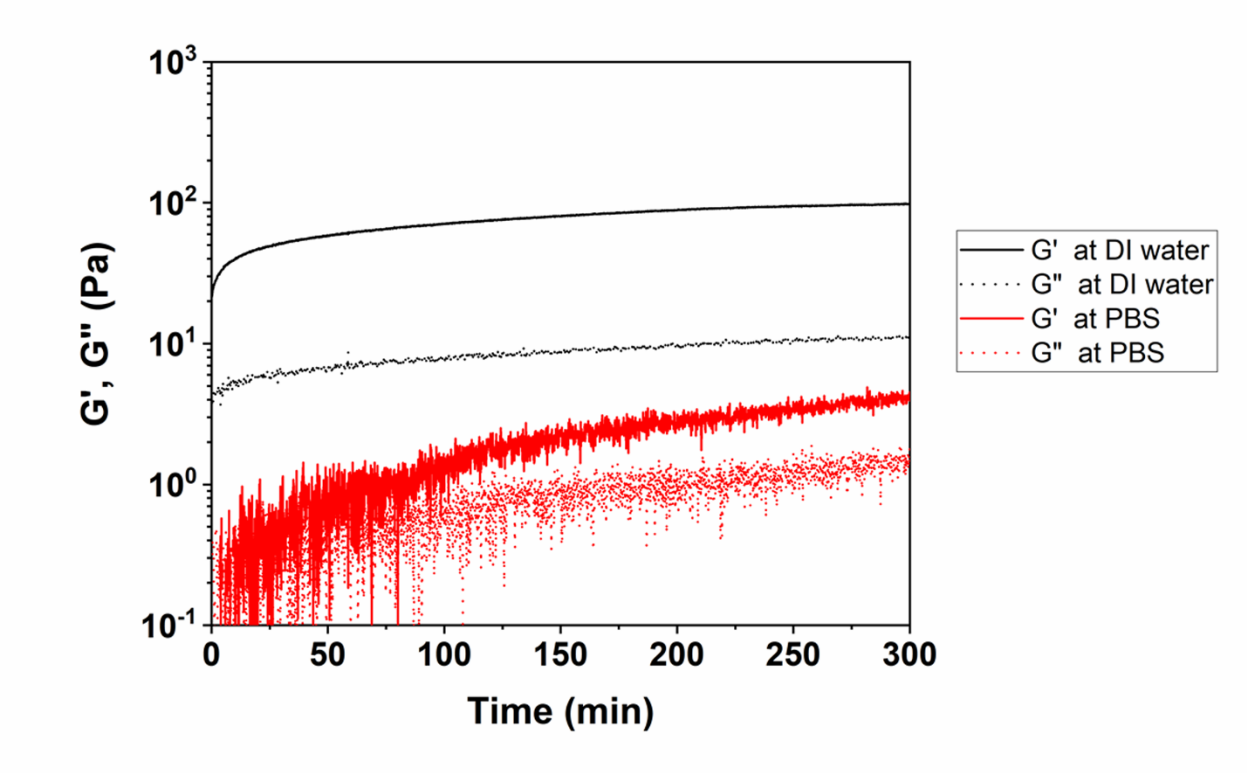

Figure S3. Effect of buffer solution on the preparation of hydrogels crosslinked by Fenton reaction. The activity of Fenton reaction was decreased in the presence of PBS (1x) media, which is attributed to binding of phosphate ions to iron ions, hindering the generation of •OH radicals.[1] Although G' was larger than G'' at 300 min for the samples prepared in PBS media, the vial inversion tests at 300 min showed the sol state.

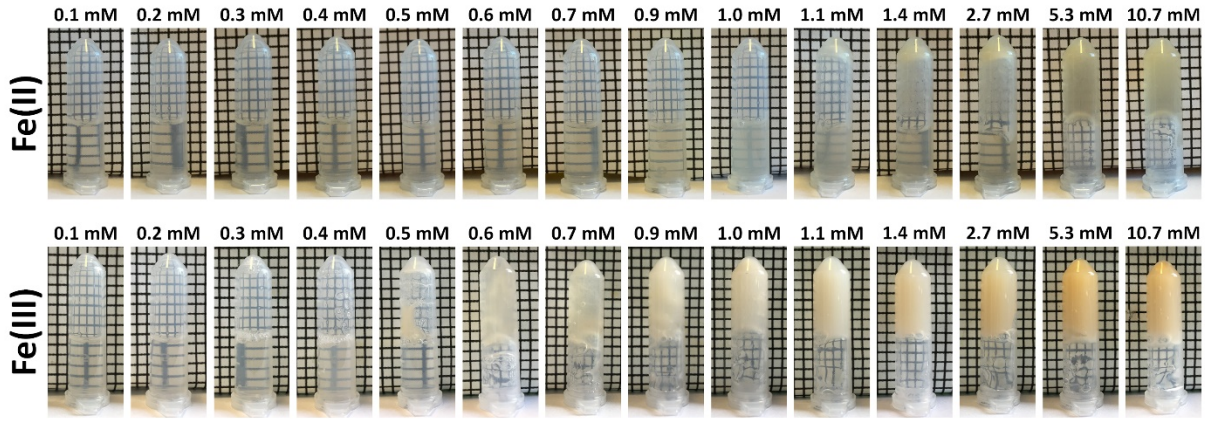

Figure S4. Effect of iron ions on gelation of silk fibroin. For Fe(II) ions, the precipitations of silk fibroin were observed at 1.1, 1.4, and 2.7 mM. The samples at 5.3 and 10.7 mM Fe(II) showed the gelation with an opaque greenish color. For Fe(III) ions, the gelation was observed above 0.5 mM, but the different final level of gelation was obtained in the range of 0.5 and 1.0 mM, suggesting that this concentration range is insufficient for inducing complete gelation. Gels containing Fe(III) ions showed an opaque yellowish brown color.

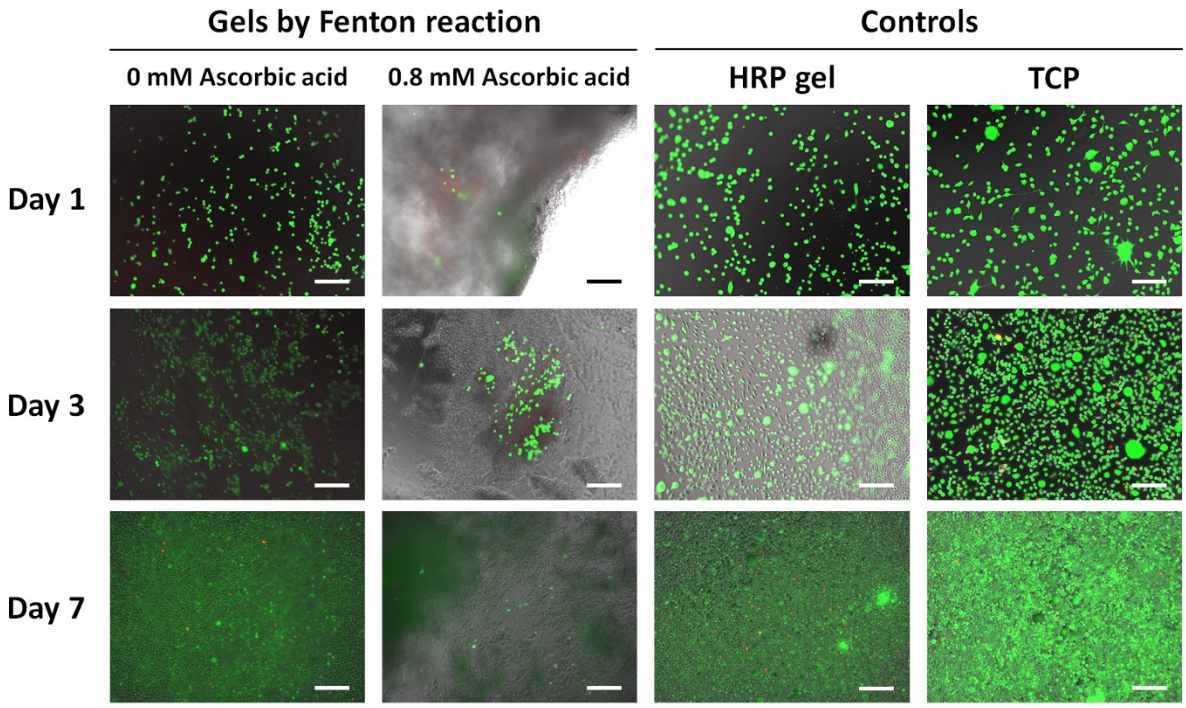

Figure S5. Fluorescence images of live (green) and dead (red) L929 mouse fibroblasts cultured in 2D. The hydrogels prepared by both Fenton reaction (0 mM ascorbic acid) and Fenton reaction with 0.8 mM ascorbic acid showed cell viability and proliferation similar to that of controls, indicating the cytocompatibility. This finding was further confirmed with Alamar Blue assay, as shown in Figure 7b of the manuscript. It is noted that the cells cultured on the surface of the hydrogels crosslinked by Fenton reaction were embedded within the hydrogels over time. In particular, in the case of the hydrogels crosslinked by Fenton reaction with 0.8 mM ascorbic acid, most of the cells were embedded within the hydrogels. Therefore, live/dead imaging was performed after sectioning the hydrogel surface using a blade. Scale bars, 200 μm.

References

[1] Y. Yoshimura, Y. Matsuzaki, T. Watanabe, K. Uchiyama, K. Ohsawa, K. Imaeda, *J. Clin. Biochem. Nutr.* **1992**, *13*, 147.