Supplementary Information

Silk degumming time controls horseradish peroxidase-catalyzed hydrogel properties

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Figure S1. (a) Polyacrylamide gel images of SF with reference protein ladder. (b) Standard curve was generated by plotting log scale MW from the reference ladder versus distance in pixel from the starting point in the lanes. The relation between the log (MW) and distance was determined by simple linear regression analysis ($R^2 = 0.9835$) and the resulting linear equation was used to convert distance on gel images in pixels to MW values on sample lanes. (c) MW distributions of 1 DT silk with highest intensity peak at 391 kDa and mean MW of 146 kDa.



Figure S2. (a) Polyacrylamide gel images of SF with reference protein ladder. (b) Standard curve was generated by plotting log scale MW from the reference ladder versus distance in pixels from the starting point in the lanes. The relation between the log (MW) and distance was determined by simple linear regression analysis ($R^2 = 0.9773$) and the resulting linear equation was used to convert distance in gel images in pixels to MW values on sample lanes. (c) MW distributions of 30 DT silk with highest intensity peak at 157 kDa and mean MW of 77 kDa. (d) MW distribution of of 120 DT silk with highest intensity peak of 79 kDa and mean MW of 69 kDa respectively.



Figure S3. (500 MHz, D₂O) ¹H-NMR spectroscopy of 1 DT silk.



Figure S4. (500 MHz, D₂O) ¹H-NMR spectroscopy of 30 DT silk.



Figure S5. (500 MHz, D₂O) ¹H-NMR spectroscopy of 120 DT silk.



Figure S6. Product ion mass spectra for dityrosine (m/z transition = $361.1 \rightarrow 315.3$) from LC-MS/MS analysis from the enzymatically crosslinked silk hydrogels with (a) 1 DT, (b) 30 DT, and (c) 120 DT.



Figure S7. Digital images of hydrogels with different DT during mechanical testing. Top, middle and bottom panel indicates images of hydrogel before, during and after compression respectively. scale bar: 1 cm.



Figure S8. Digital images of swelling (left) and contraction (right) of different MW hydrogels at different time interval. Scale Bar: 1 cm.



Figure S9. Quantification of (a) spread area and (b) circularity of L929 cells on SF hydrogels at D1. (n > 250, *p < 0.05, **p < 0.01 and ***p < 0.001)



Figure S10. % dye reduction of the cells cultured on SF hydrogel surfaces for 8 days determined using alamar Blue viability assay. (n=4, *p < 0.05, **p < 0.01 and ***p < 0.001)