Supporting Information

Development of an Injectable Nitric Oxide Releasing Poly(ethylene) Glycol-Fibrin Adhesive Hydrogel

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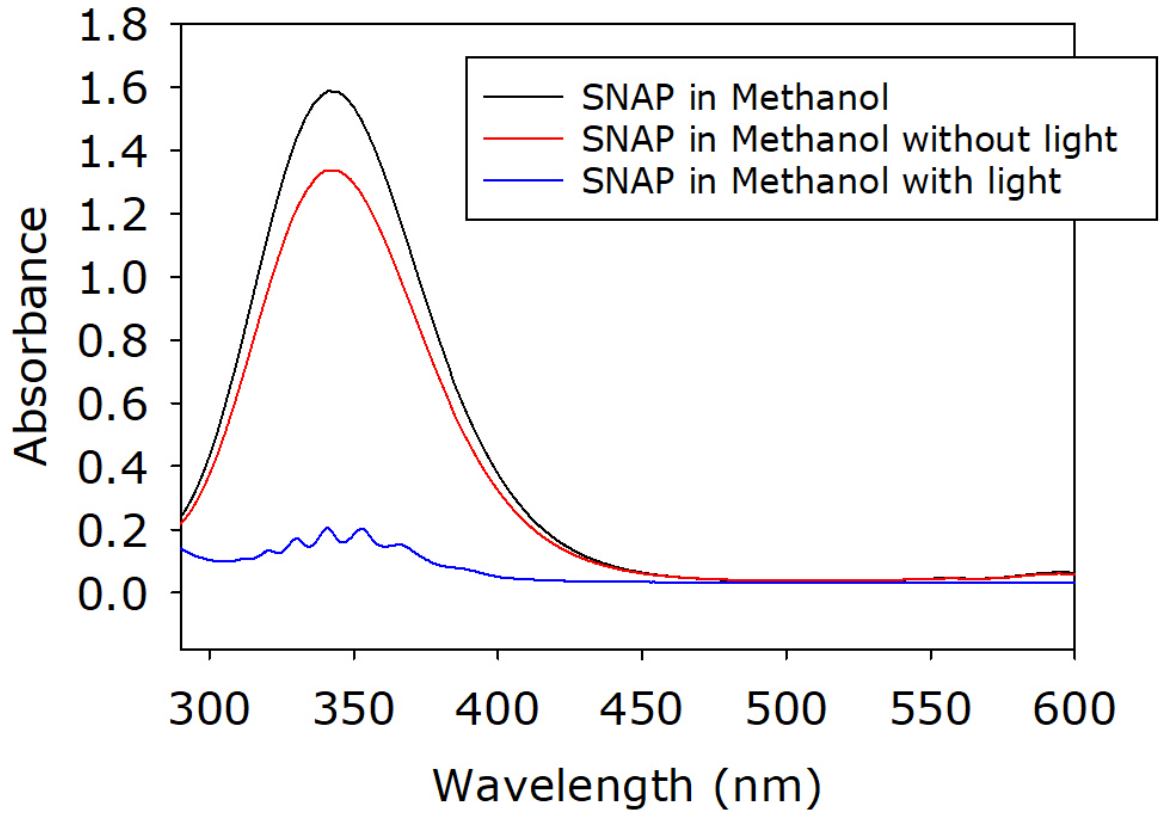

Figure S1. UV-Vis absorbance spectra from 600 nm to 290 nm for SNAP solution at 0.33 mg/ml concentration (SNAP in Methanol), the solution after being kept in the dark at 4 ^{o}C for 24 hours (SNAP in Methanol w/o light), and the solution after light exposure for 24 hours (SNAP in Methanol w/ light). UV-Vis spectra showed a peak absorbance at 340 nm which conforms to other literature [1]. The degradation of SNAP was confirmed with the significantly reduced absorbance at 340 nm after explosure to fluorescent light for 24 hours. Compared to the degraded solution, SNAP solution that was kept in the dark registered a negligible reduction in absorbance at 340 nm after 24 hours.

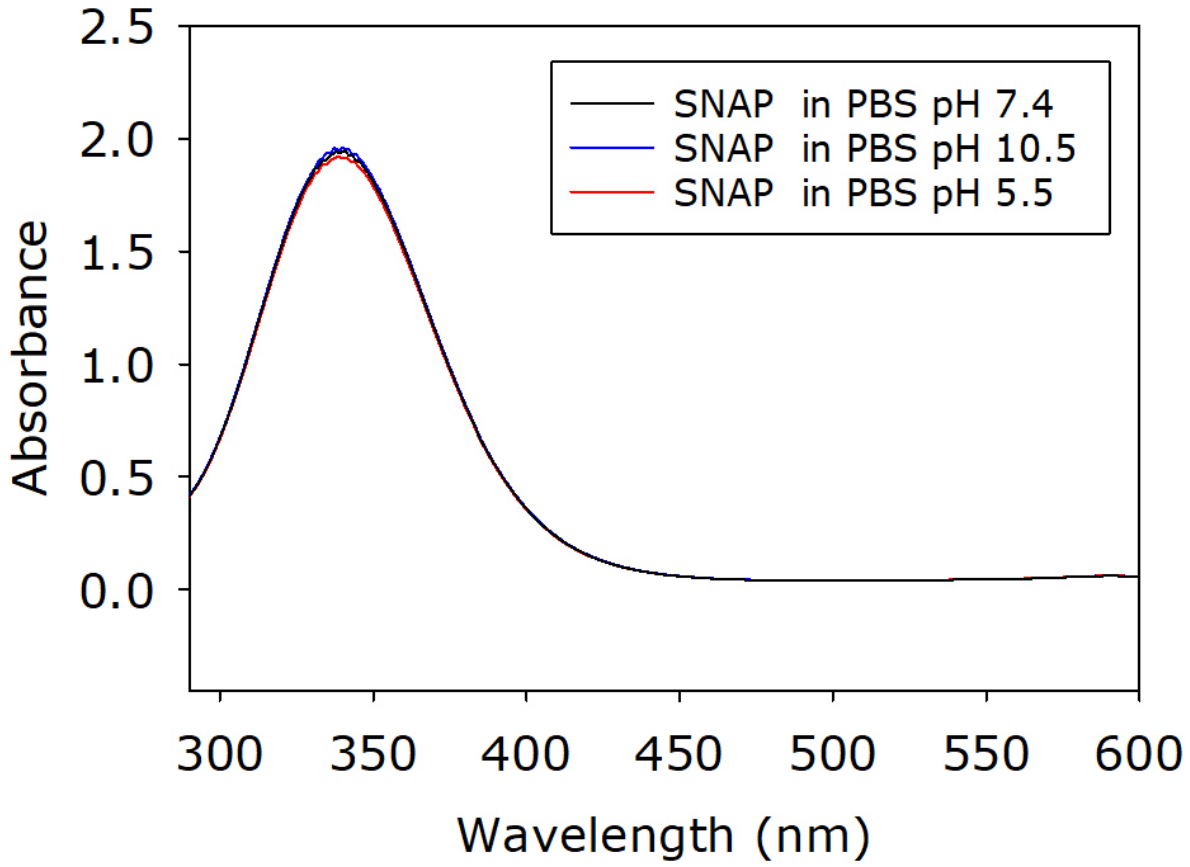

Figure S2. UV-Vis abosorbance spectra from 600 nm to 290 nm for SNAP solution at 0.35 mg/ml concentration in PBS at pH 7.4, 10.5, and 5.5. UV-Vis spectra showed a peak absorbance at 340 nm which conforms to other literature [1]. pH changes did not affect the SNAP absorbance peak suggesting that fibrin-SNAP microparticle preparation method does not degrade SNAP prematurely.

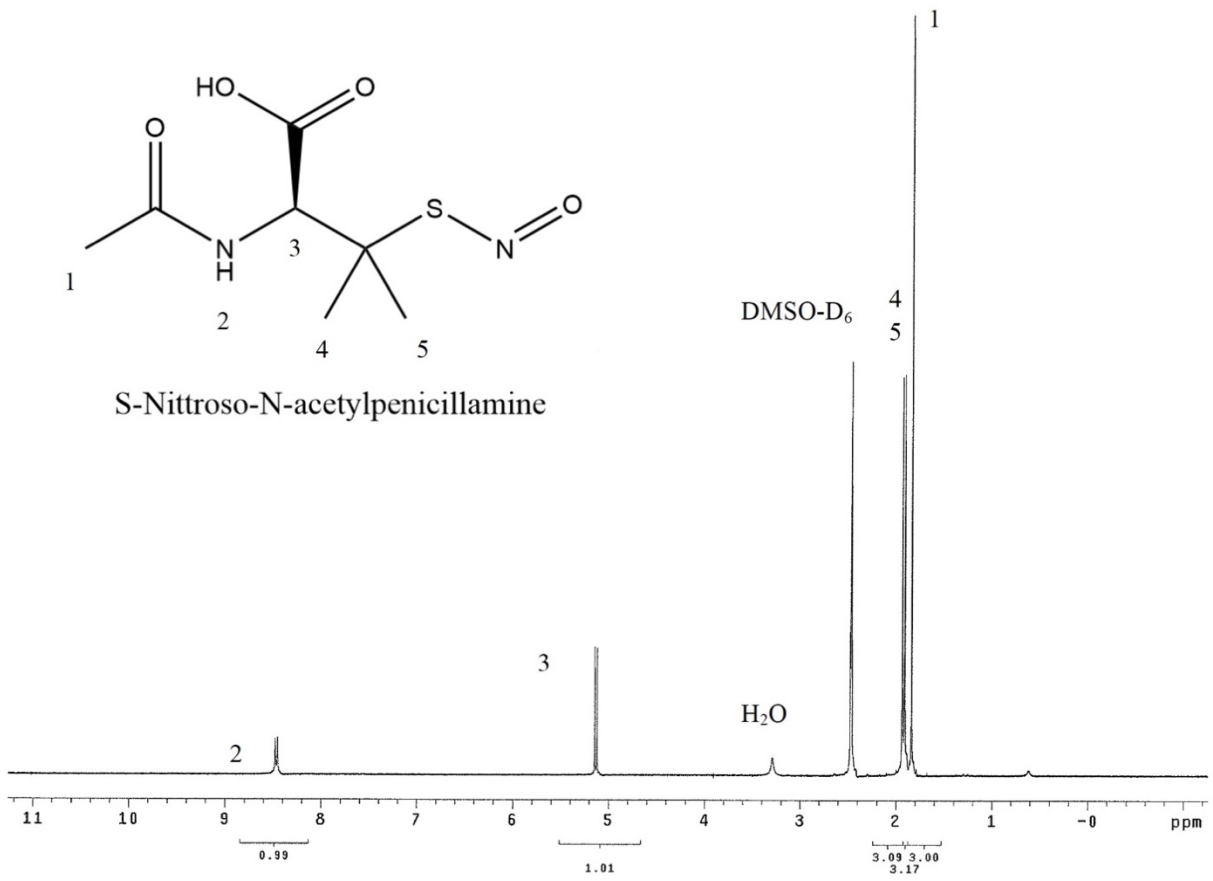

Figure S3. NMR spectra for SNAP in DMSO-D_6 (4mg/ml). NMR results fit with the predicted peak position and proton ratio from peak integration.

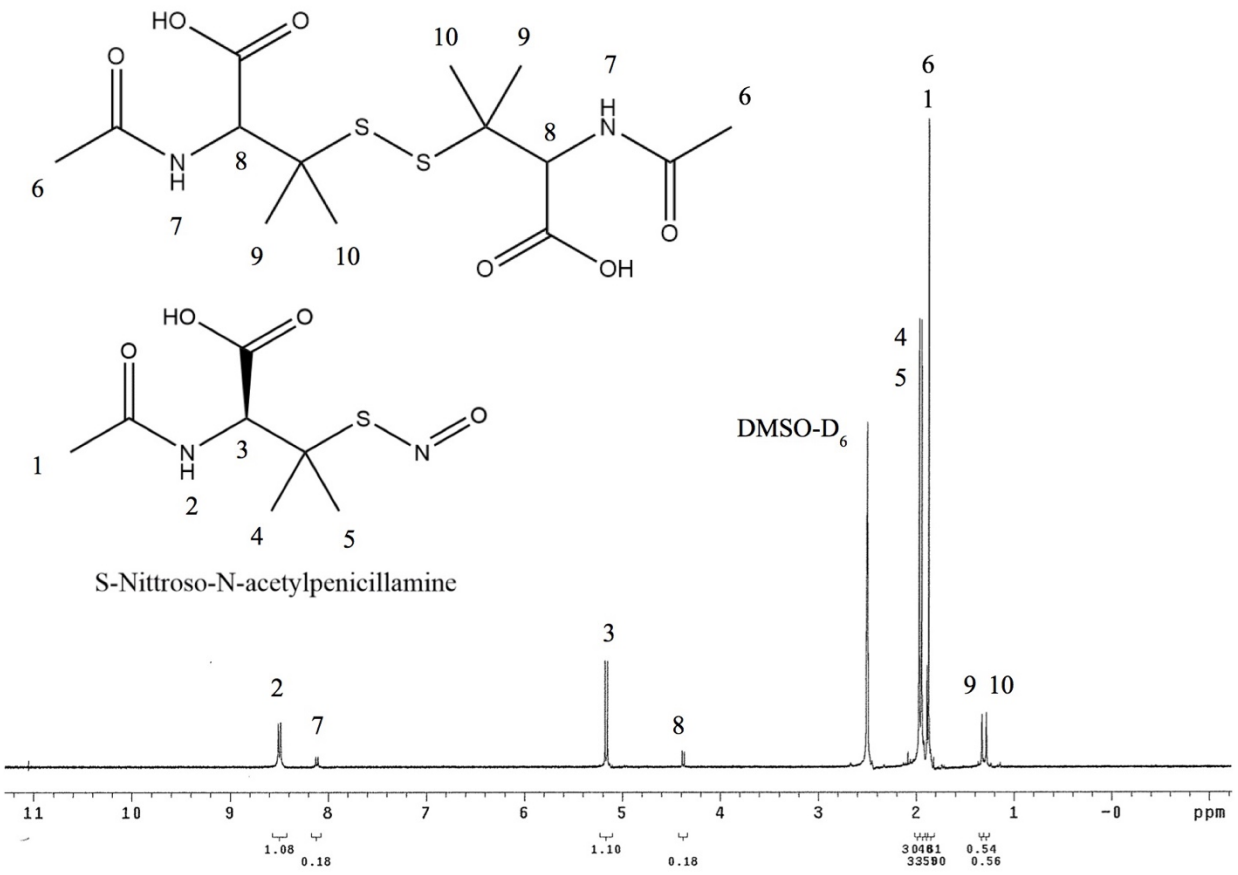

Figure S4. NMR spectra for SNAP in DMSO-D_6 (4mg/ml) after 24 hour incubation at 37 ^{o}C. Based on the ratio of the integral values of peaks 3 and 8, it was estimated that ~20% of the SNAP degraded under this incubation condition.

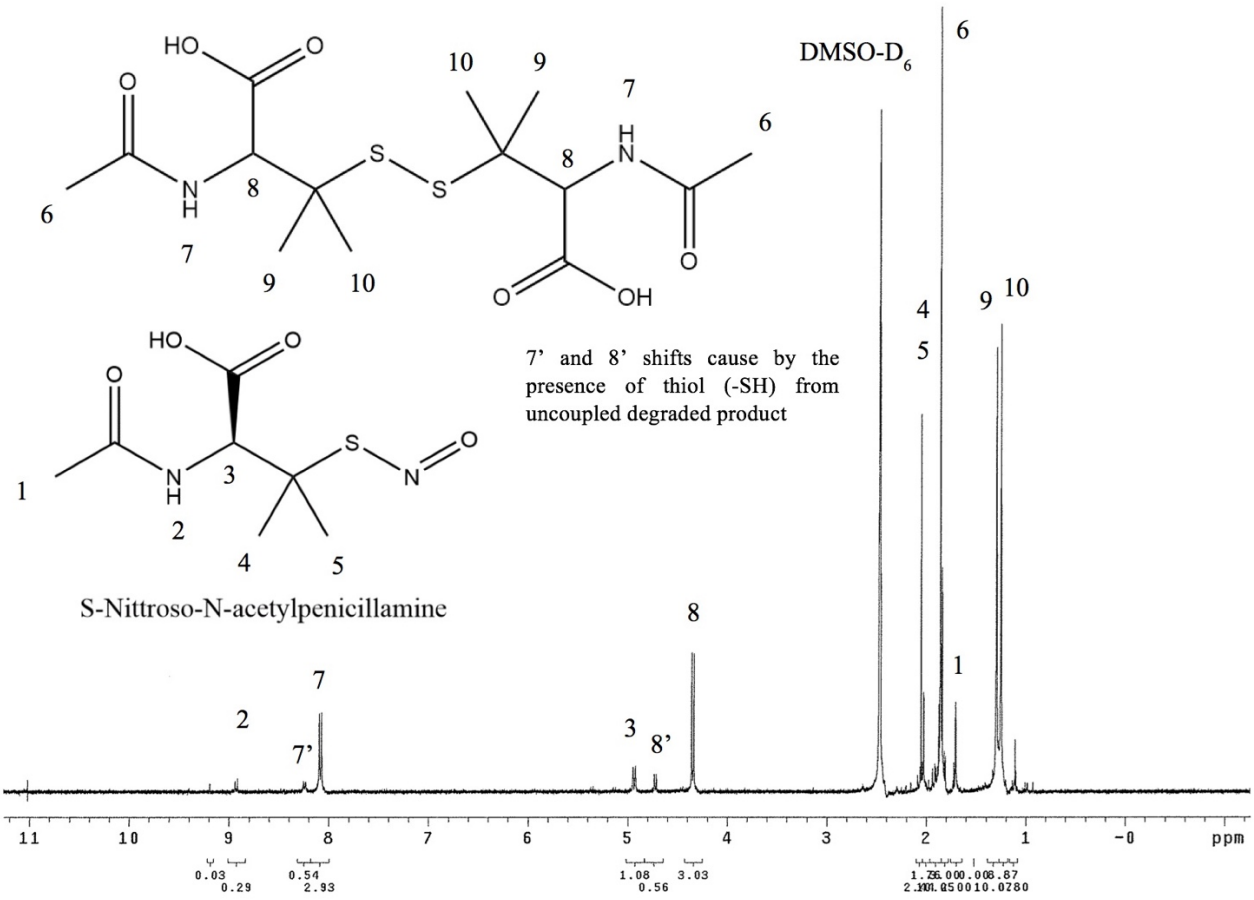

Figure S5. NMR spectra for SNAP in DMSO-D_6 (4mg/ml) after 24 hour incubation at 60 ^{o}C. Shifts 7' and 8' (observed peaks at 8.12 and 4.75ppm) are caused the presence of thiol (-SH) from the uncoupled degraded product. The spectra suggested a complete depletion of SNAP with peak shifting similar to the experiment reported by Ketchum [1]. Based on the ratio of the integral values of peaks 3, 8 and 8', it was estimated that ~80% of the SNAP degraded under this incubation condition.

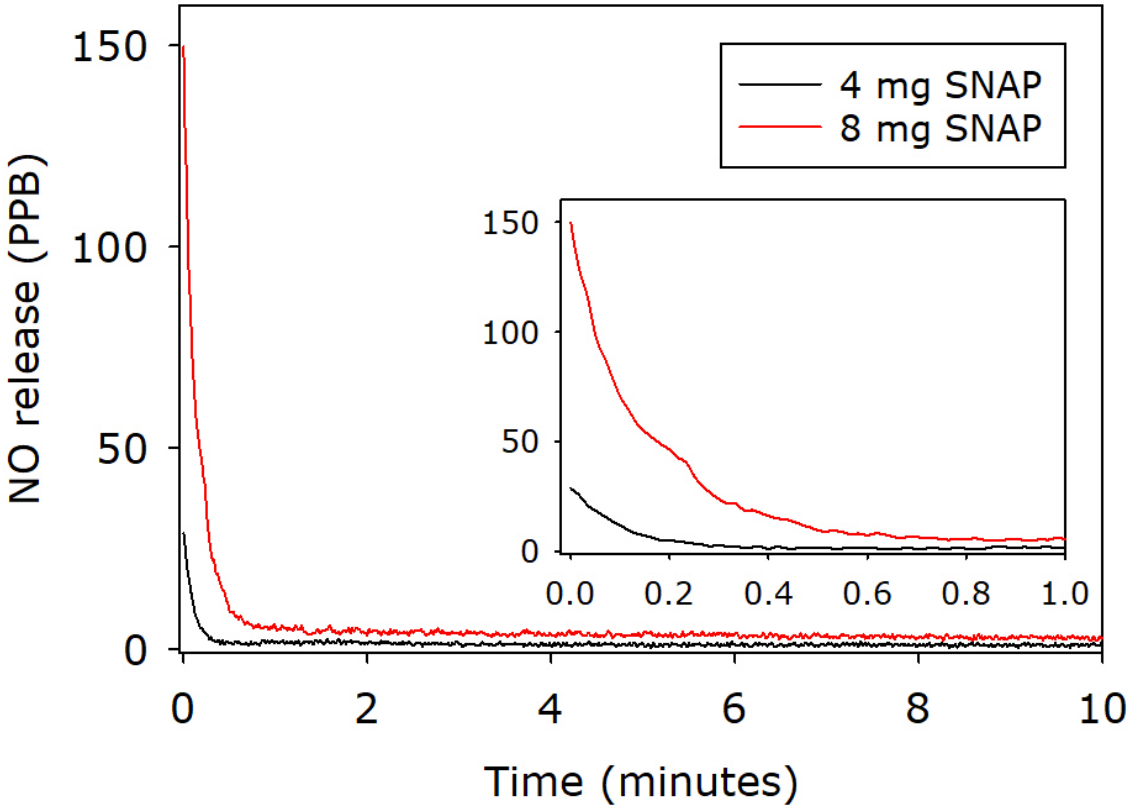

Figure S6. Nitric oxide release measured via an NO analyzer for a 10 minute runtime. For nitric oxide release, 4 and 8 mg of fibrin-SNAP microparticles (n=3) were tested using an NO analyzer at a UV strength of 1 Watt for 24 hours to ensure depletion of the SNAP. The release data were recorded and analyzed using a custom MATLAB code. NO release data showed a significantly higher release of NO from 8 mg SNAP particles compared to 4 mg SNAP particles. The increase in NO release is more noticeable at the first minute of the experiment followed by a plateau. The total amount of NO from the particles was calculated by integration over the area under the curve of the 24-hour release data. The total NO release of 4 mg SNAP particles was found to be at 3.913×10^{-9} mol while the 8 mg SNAP particles was found to be at 6.245×10^{-9} mol. The result suggested a 1.6-fold increase in NO release when the weight of the sample was doubled.

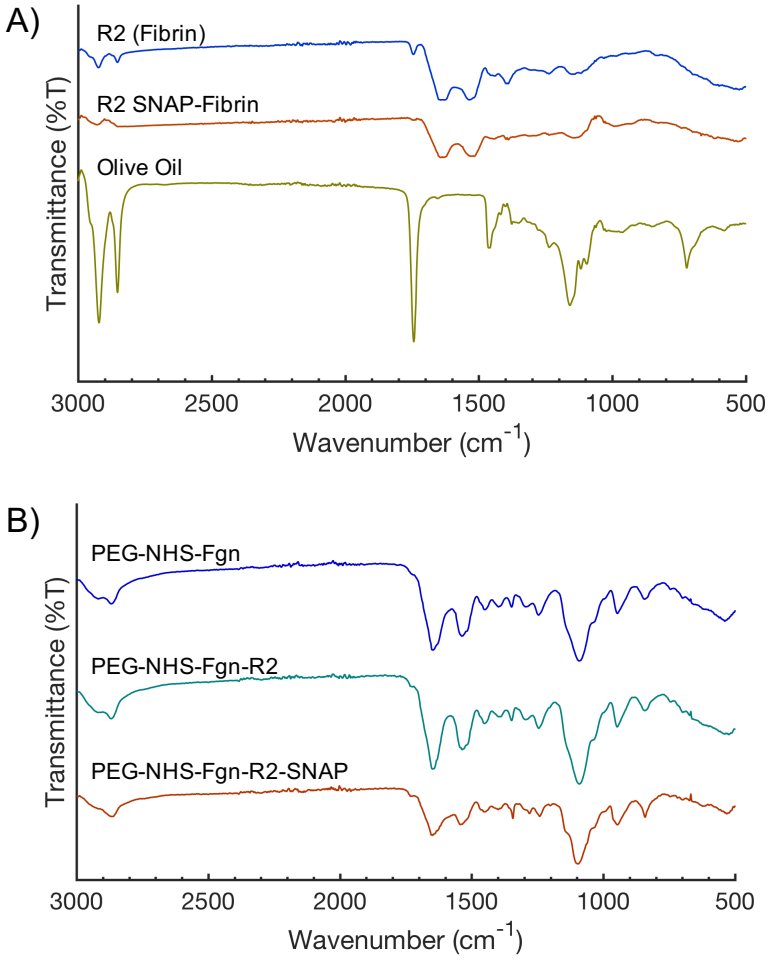

Figure S7. FTIR analysis of microparticles and composite hydrogels. A) microparticles and olive oil; and B) hydrogels. IR data of each microparticle type show expected peaks (1650 and 1550 cm^{-1}) for fibrinogen materials [2]. The similarity of the fibrin particle spectra to that of the SNAP fibrin is likely due to the relatively low amount of SNAP incorporated into the particles during synthesis (20 mg SNAP/2 mL of 200 mg/mL fibrinogen) (see FTIR analysis, A). The spectrum of olive oil exhibited similar peaks to previously published studies [3]. None of the major peaks of the olive oil spectra are indicated in the spectra of the microparticles, which supports that washing removed trace olive oil that may be present at synthesis. There are no major differences in the spectra of hydrogels, which is also expected as the small amount of SNAP present in the final materials is likely overshadowed by the significantly more abundant bonds present in the hydrogel matrix between PEG-NHS and fibrinogen (see FTIR analysis, B).

References

1. Ketchum, A.R.; Wolf, A.K.; Meyerhoff, M.E., Synthesis, Spectroscopy, and Stability of the Nitric Oxide Donor S-nitroso-N-acetylpenicillamine: An Undergraduate Laboratory Experiment. *Journal of Laboratory Chemical Education.* **2017**, 5(4), 79-85. DOI: 10.5923/j.jlce.20170504.03

2. Azpiazu, I.; Chapman, D., Spectroscopic studies of fibrinogen and its plasmin-derived fragments. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology* **1992,** *1119* (3), 268-274. DOI: 10.1016/0167-4838(92)90213-W

3. Rohman, A.; Che Man, Y., Quantification and classification of corn and sunflower oils as adulterants in olive oil using chemometrics and FTIR spectra. *The Scientific World Journal* **2012,** *2012*. DOI: 10.1100/2012/250795