Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information for:

Degradation Profiles of Poly(ethylene glycol) Diacrylate (PEGDA)-Based Hydrogel Nanoparticles

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Detailed and Additional Experimental Procedures

General Considerations

All solvents and materials were obtained and used without further purification. Methanol was obtained from Fisher Chemical. Silicone oil AP 1000, PEGDA 700, 2-carboxyethyl acrylate, 1,6-hexanediol dimethacrylate, bis(2-methacryloyl)oxyethyl disulfide, and diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide were obtained from Sigma Aldrich. Rhodamine B and DMSO-d_6 was obtained from ACROS chemicals.

Synthesis of PEGDA-Based Nanoparticles

PEGDA-based nanoparticles were synthesized using the procedure described in the main text with the following UV exposure times and sonication times, shown in **Table S1**. Some samples were synthesized with rhodamine B in the formulation, noted in the table below.

Table S1. PEGDA-based nanoparticle UV exposure and sonication times. The table below includes the UV exposure and sonication times for each of the respective components for PEGDA-based nanoparticle formulations used, including formulations with and without co-monomers, as well as those which encapsulated rhodamine B.

Formulation (wt%)	UV Exposure Time (s)	Sonication Time (s)
30 wt% (●)	25	25
50 wt% (●)	8	30
30 wt% with Rhodamine B (●)	30	30
50 wt% with Rhodamine B (●)	30	30
50 wt% with 10 mol% HDDMA (●)	15	30
50 wt% with 30 mol% HDDMA (●)	20	30
50 wt% with 10 mol% DSDMA (●)	17	30
50 wt% with 30 mol% DSDMA (●)	28	30

Synthesis of PEGDA-Based Bulk Gels

PEGDA-based bulk gels were synthesized using the procedure described in the main text with the following UV exposure times, shown in **Table S2**. Some samples were synthesized with rhodamine B in the formulation, noted in the table below.

Table S2. PEGDA-based bulk gel UV exposure times. The table below includes the UV exposure times for each of the respective components for PEGDA-based bulk gel formulations used, including formulations with and without co-monomers, as well as those which encapsulated rhodamine B.

Formulation (wt%)	UV Exposure Time (s)
30 wt% (■)	30
50 wt% (■)	15
30 wt% with Rhodamine B (■)	60
50 wt% with Rhodamine B (■)	60
50 wt% with 10 mol% HDDMA (■)	30
50 wt% with 30 mol% HDDMA (■)	90
50 wt% with 10 mol% DSDMA (■)	60
50 wt% with 30 mol% DSDMA (■)	90

Thermogravimetric Analysis (TGA) and Salt Correction

PEGDA-based nanoparticle concentrations were determined via TGA through a temperature ramp to 120°C at 10°C/minute, followed by an isothermal step for 10 minutes, a temperature ramp to 430°C at 20°C/minute, and another isothermal step for 30 minutes. This procedure was developed to evaporate the solvent (water) and then determine the mass of PEGDA nanoparticles by decomposing the PEGDA present. This provides a mass difference between the isothermal steps at 120°C and 430°C respectively, which is the mass of PEGDA present.

It was discovered, however, that for samples containing the phosphate salts, that additional mass was lost during the temperature ramp from 120°C to 430°C. This is because of the partial decomposition of said phosphate salts.[1] ¹¹Accordingly, correlations were devmeloped and tested to account for the mass lost because of the loss of mass from salt decomposition. These corrections were based on the final mass present at the end of the

procedure, which can then be transformed to the original amount of salt present and thus accounted for. Additionally, TGA incurs thermal mass drift during the course of the temperature ramp, which was also accounted for in the aforementioned correlations to account for mass loss from the presence of salt.

Rhodamine B Release Studies

Supernatant samples from rhodamine B-encapsulated PEGDA nanoparticles and bulk gels were collected at each timepoint. Bulk gel supernatants were diluted by a 1:5 ratio of DI water or pH 5, pH 7.4, or pH 10 conditions, corresponding to the original sample solution. Rhodamine B fluorescence (λ_{ex} = 555 nm, λ_{ex} = 580 - 600 nm; step size = 10 nm) in nanoparticle and diluted bulk gel supernatants samples was measured by spectral scanning with a Cytation 5 Cell Imaging Multi-Mode Reader, and the maximum relative fluorescence unit (RFU) measurement was recorded for each sample. A master calibration curve for Rhodamine B fluorescence in DI water and each of the pH conditions (n = 3) was made using a 1:2 serial dilution and an initial concentration of 0.01 mg/mL rhodamine B. A linear correlation between RFU and rhodamine B concentration was calculated for each of the solution conditions and an averaged between replicates. Rhodamine B concentration in the sample supernatants was calculated from measured RFU using the corresponding averaged calibration curve for each sample.

NMR measurements

In an NMR tube, ~10 mg of sample (either precursor material or product) was dissolved in 750 μL of DMSO-d_6 and ^{1}H NMR measurements were performed with an AVIII600 NMR at 600 MHz and 16 scans.

Nuclear Magnetic Resonance (NMR) of Starting Materials and Products

^{1}H NMR measurements were performed on the starting material and product nanoparticles to determine if the polymerization was detectable; however, results would suggest that the nanoparticles were not soluble in DMSO and thus only a small number of peaks corresponding to CEA and the PEGDA700 can be seen.

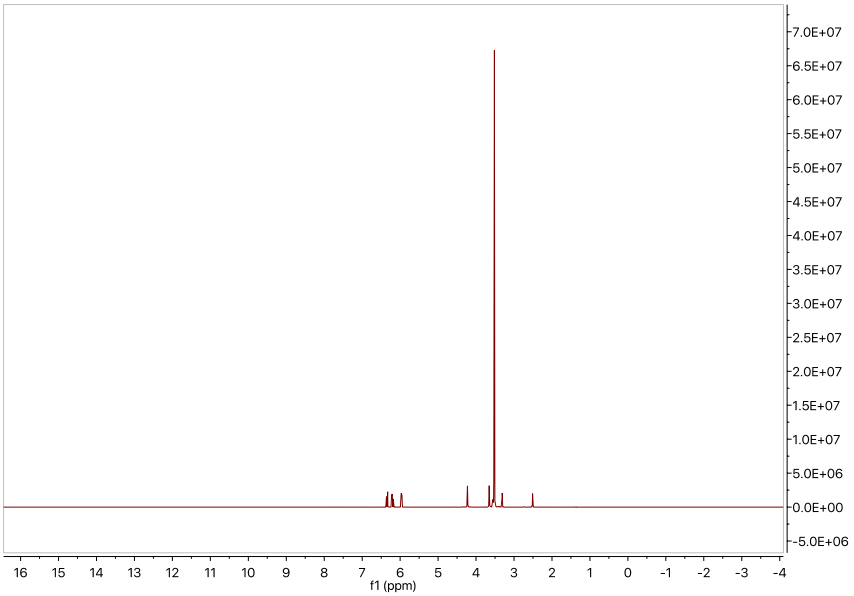

Figure S1 – ^{1}H NMR of PEGDA700 in DMSO-d_6.

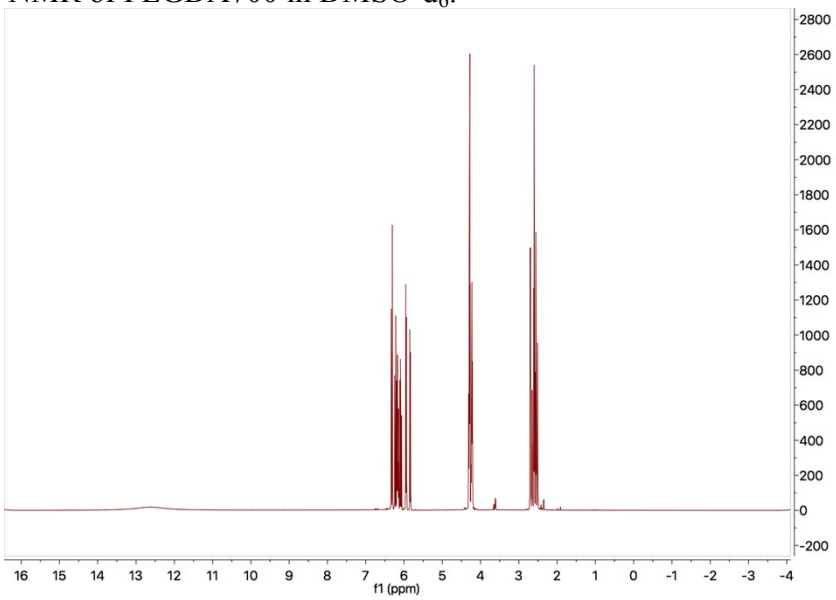

Figure S2 – ^{1}H NMR of CEA in DMSO-d_6.

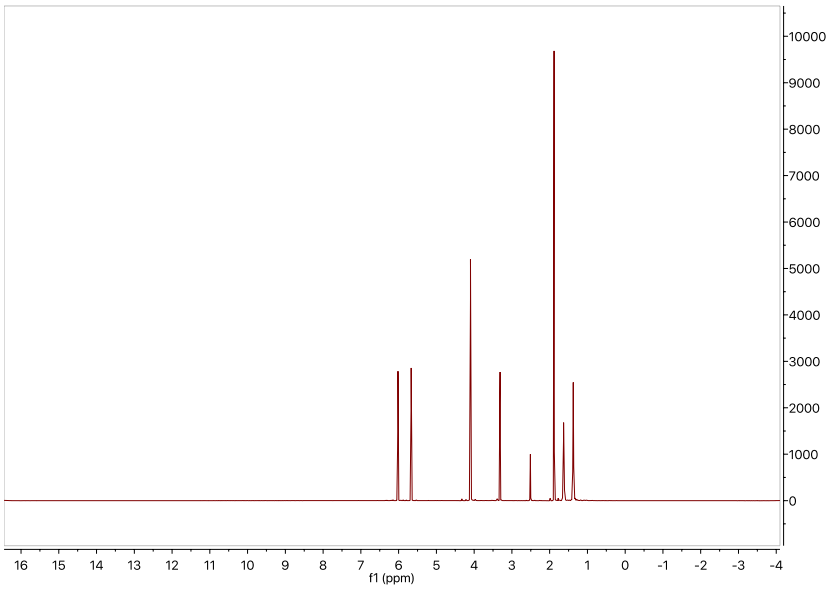

Figure S3 – ^{1}H NMR of HDDMA in DMSO-d_6.

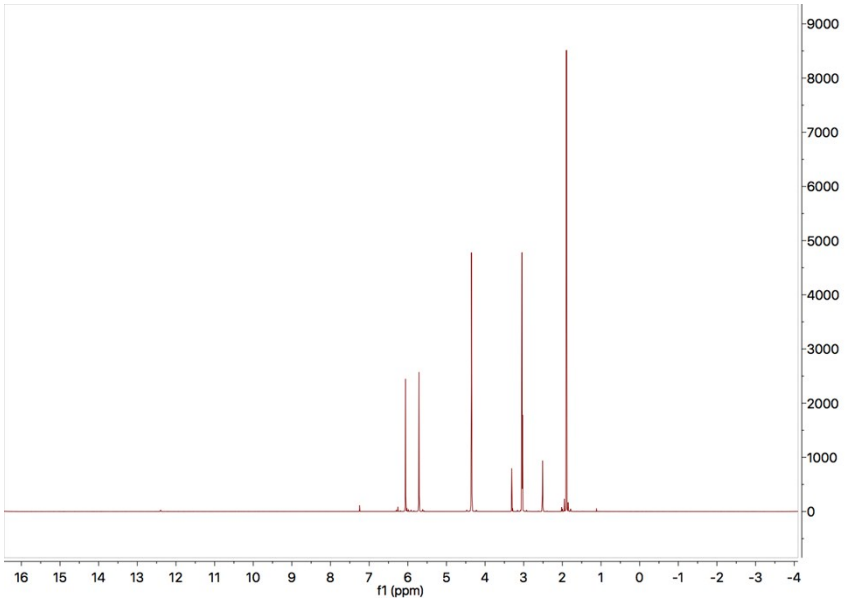

Figure S4 – ^{1}H NMR of DSDMA in DMSO-d_6.

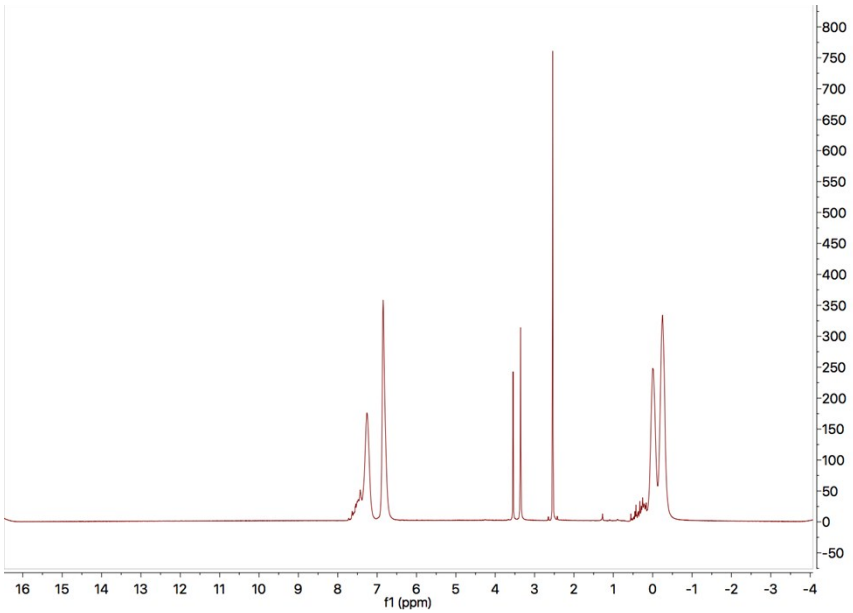

Figure S5 – ^{1}H NMR of 30 wt% PEGDA-based hydrogel nanoparticles in DMSO-d_6.

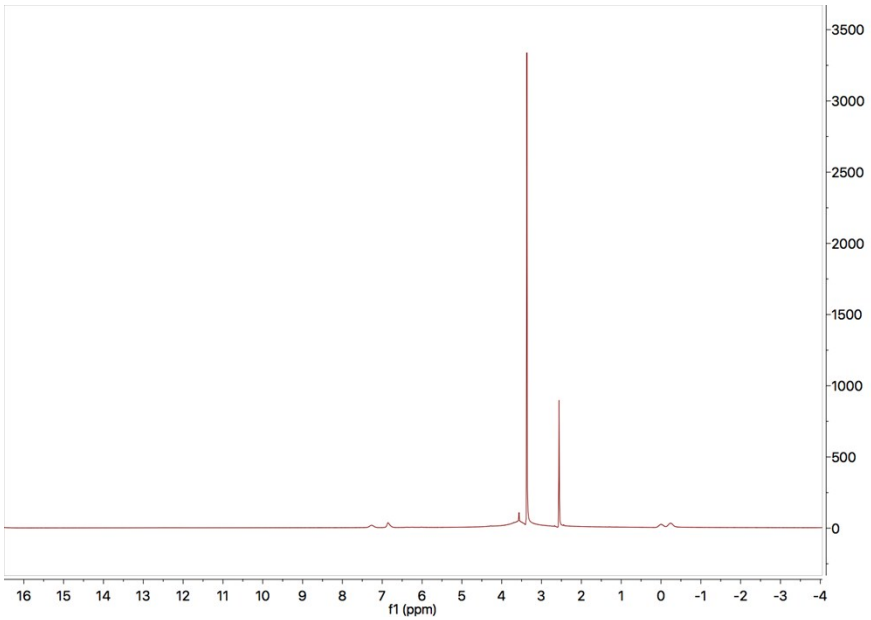

Figure S6 – ^{1}H NMR of 50 wt% PEGDA-based hydrogel nanoparticles in DMSO-d_6.

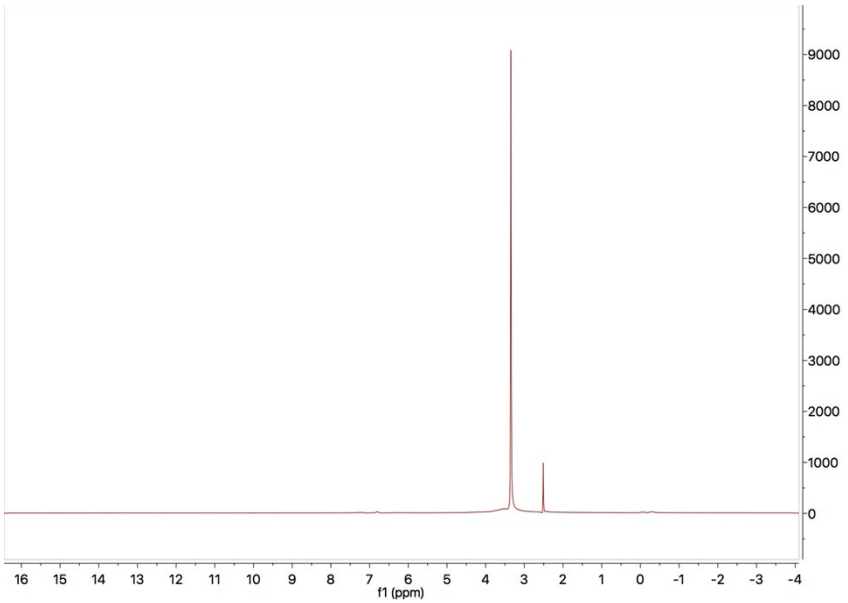

Figure S7 – ^{1}H NMR of 50 wt% PEGDA-based hydrogel nanoparticles with 10% HDDMA in DMSO-d_8.

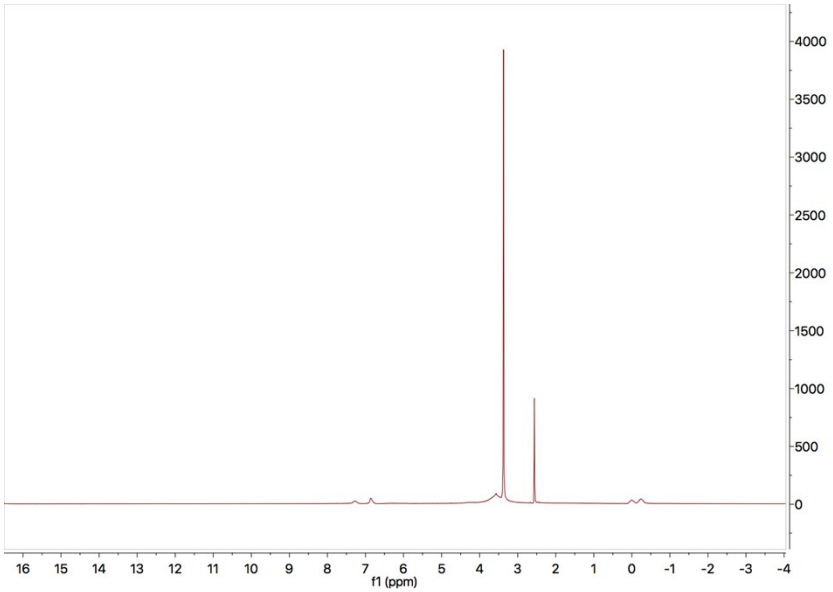

Figure S8 – ^{1}H NMR of 50 wt% PEGDA-based hydrogel nanoparticles with 30% HDDMA in DMSO-d_6.

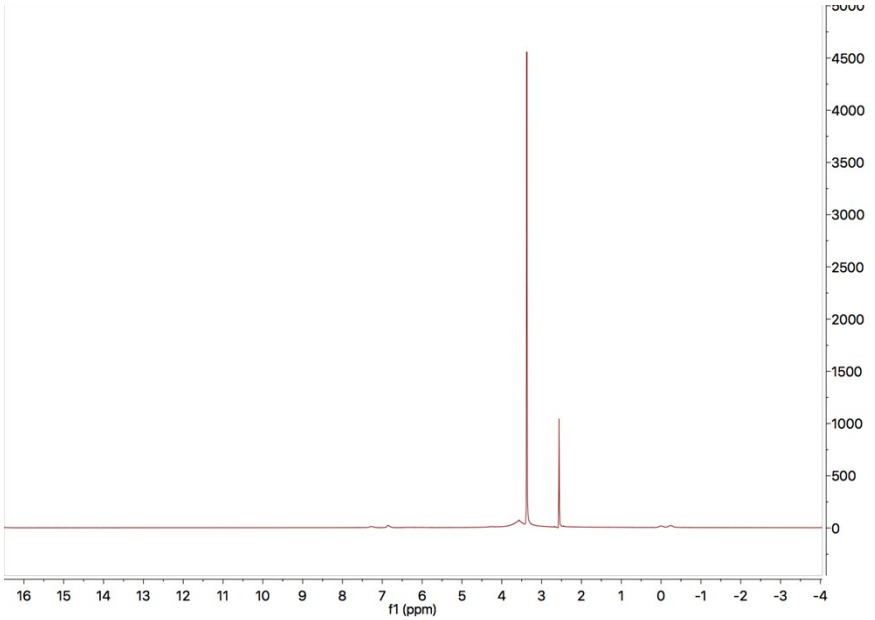

Figure S9 – ^{1}H NMR of 50 wt% PEGDA-based hydrogel nanoparticles with 10% DSDMA in DMSO-d_6.

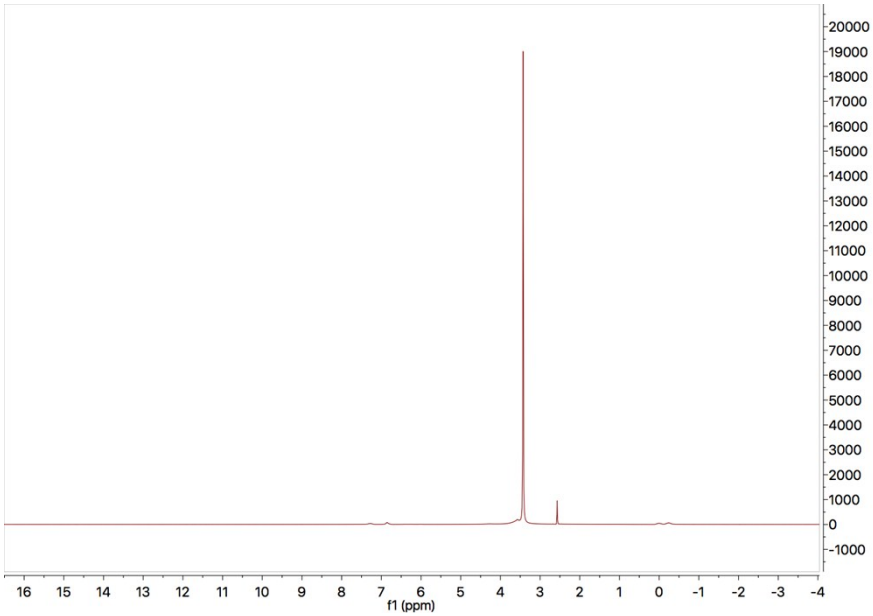

Figure S10 – ^{1}H NMR of 50 wt% PEGDA-based hydrogel nanoparticles with 30% DSDMA in DMSO-d_6.

Polydispersity Indices of PEGDA-Based Nanoparticles Over Time

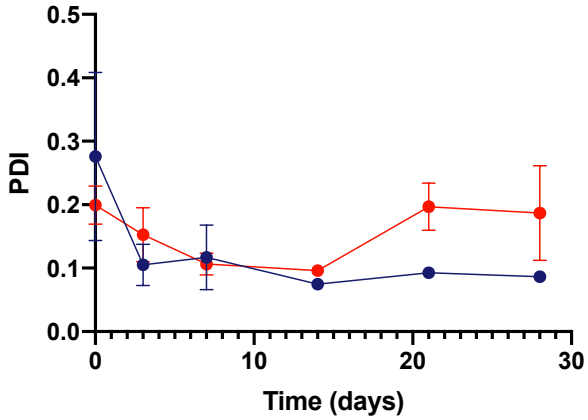

Figure S11 – PDI of the 30 wt% and 50 wt% PEGDA-based nanoparticles incubated in water shown over time.

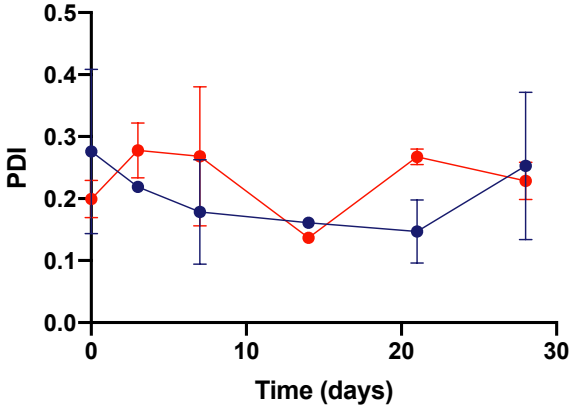

Figure S12 – PDI of the 30 wt% and 50 wt% PEGDA-based nanoparticles incubated in the pH 5 condition shown over time.

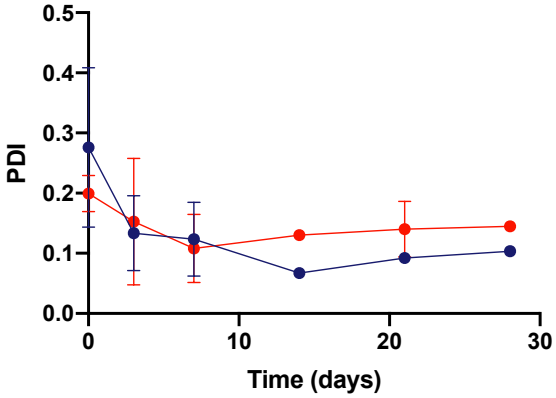

Figure S13 – PDI of the 30 wt% and 50 wt% PEGDA-based nanoparticles incubated in the pH 7.4 condition shown over time.

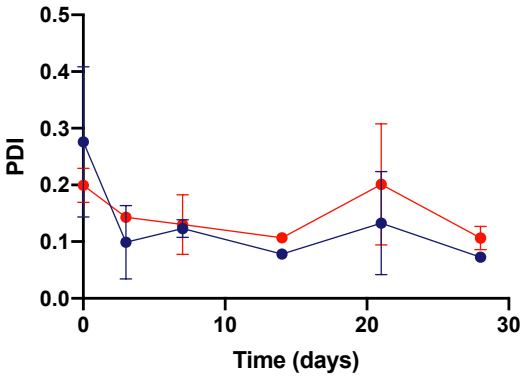

Figure S14 – PDI of the 30 wt% and 50 wt% PEGDA-based nanoparticles incubated in the pH 10 condition shown over time.

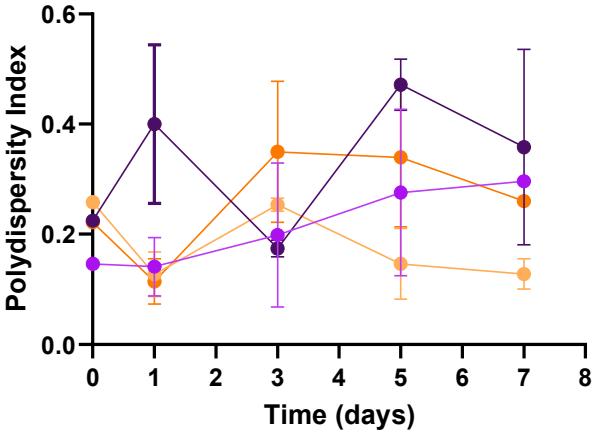

Figure S15 – PDI of co-monomer containing PEGDA-based nanoparticles incubated in the pH 5 condition shown over time.

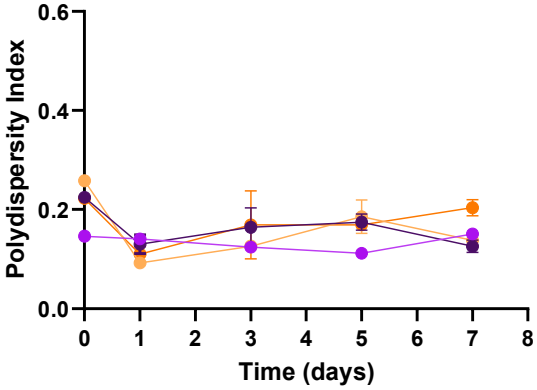

Figure S16 – PDI of co-monomer containing PEGDA-based nanoparticles incubated in the pH 5 condition shown over time.

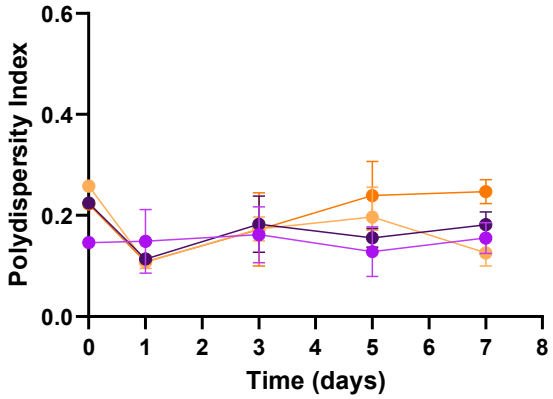

Figure S17 – PDI of co-monomer containing PEGDA-based nanoparticles incubated in the pH 5 condition shown over time.

Additional Cryo-SEM Images

The following cyro-SEM images show PEGDA nanoparticle samples with a ruptured appearance that may be because of their degradation. The images below were taken from images for 30 wt% PEGDA-based nanoparticles left in water for 42 days.

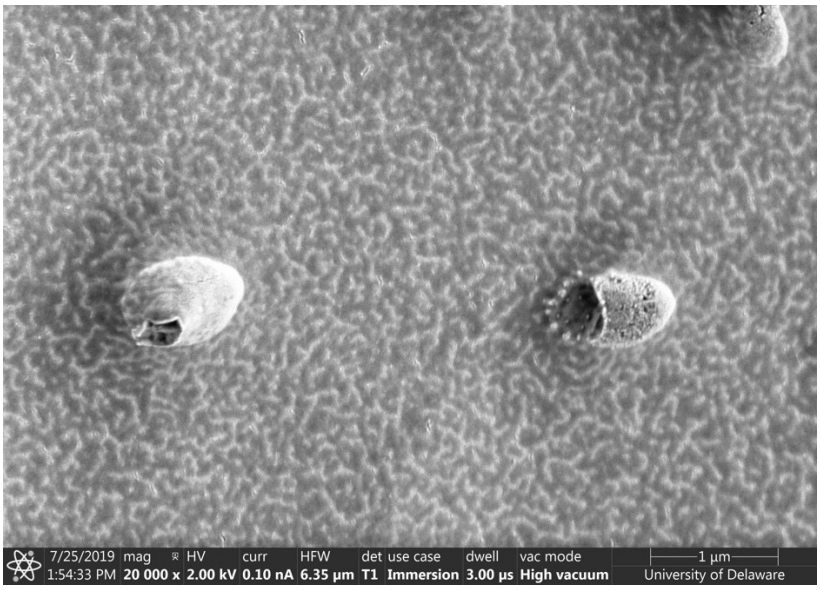

Figure S18 – Cryo-SEM image of 30 wt% PEGDA-based nanoparticles left in water for 42 days.

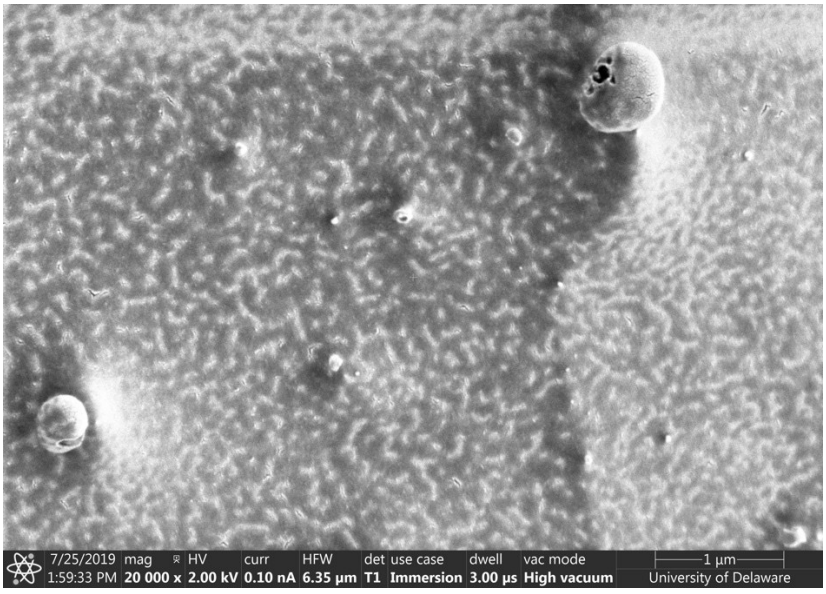

Figure S19 – Cryo-SEM image of 30 wt% PEGDA-based nanoparticles left in water for 42 days.

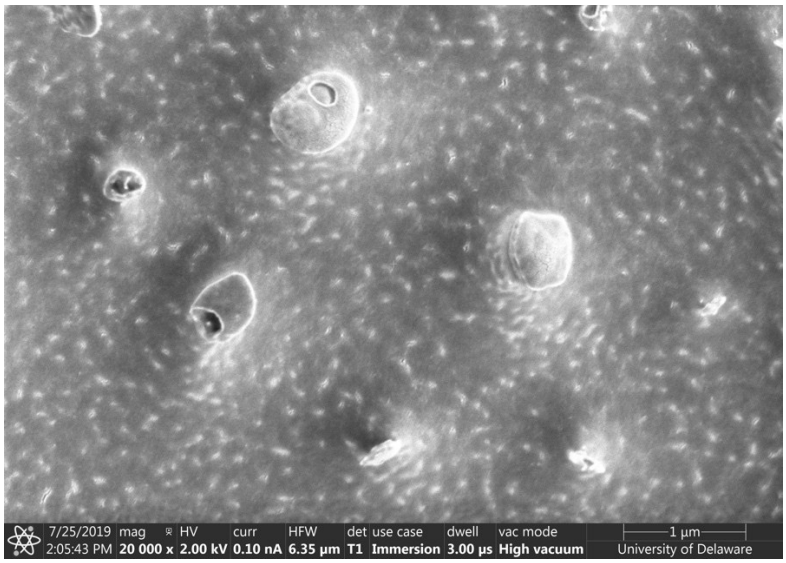

Figure S20 – Cryo-SEM image of 30 wt% PEGDA-based nanoparticles left in water for 42 days.

Degradation Modeling Using Cargo Release of Rhodamine B

Supernatant samples from rhodamine B-encapsulated PEGDA nanoparticles and bulk gels were collected at each timepoint and released rhodamine B measured in each solution. As can be seen in Figure S21 (below) for all four conditions, the rates of rhodamine B release from the 30 wt% (●) and 50 wt% (●) PEGDA nanoparticles are comparable to each other, increasing gradually over the course of one week of incubation. However, after 1 week, these values plateau and do not approach the theoretical maximum released based on the calculated loading amounts (following a mass balance on the supernatant wash). Although time was confirmed as a significant factor for all conditions except water via two-way ANOVA, this release profile results contrasts with the degradation patterns determined for nanoparticle mass loss reported via TGA.

The nanoparticle rhodamine B release results also contrast with the release from bulk PEGDA hydrogels, as seen in Figure S22. Rhodamine B release was much more rapid and complete than that from the nanoparticles of the same formulation. We hypothesize that the poor agreement between the nanoparticle rhodamine release and the mass loss reported by TGA is because of the small length scale of the particle. The characteristic size of the nanogel compared to the size of the bulk gel differs by several orders of magnitude; thus, the release of rhodamine through diffusion occurs over significantly shorter time scales than for the bulk hydrogels. This would agree with theoretical diffusion calculations, which approximate that rhodamine B would diffuse out of a 300 nm nanoparticle on the timescale of milliseconds versus the timescale of days for bulk gels. These diffusion calculations are shown below. However, these calculations assume a constant mesh network density; the PEGDA network is certainly a heterogeneous network that can yield a variable range of rhodamine entrapment at both longer and shorter

diffusion times than calculated here, which is likely responsible for the slight increase in rhodamine diffusion from nanoparticles over the initial week (Figure S21).[2]

In addition to the rapid timescale of diffusion, our results point to variable rhodamine loading efficiency, with differing amounts of rhodamine released during nanoparticle purification steps. This variation in encapsulation also contributed to an issue with the normalization of data and variability in calculated release between samples.

Collectively, these issues fail to reflect the degradation profile of the nanogel itself based on TGA, DLS, and cryo-SEM measurements. While helpful in understanding model drug encapsulation and (rapid) release from the nanoparticle, use of rhodamine to infer particle degradation was not found to be feasible for this system.

Calculated Diffusivity of Rhodamine B

In water at 25 C: $D_0 = 4.50*10^{-6}$ cm^2/s

In water at 37 C: $D_0 = 6.02*10^{-6}$ cm^2/s

$r_s = 0.545$ nm

To calculate D_g, polymer volume fraction (φ, also v_2) and mesh size (ξ) must be known.

$\varphi = 0.373$

$\xi = 1.13\ nm$

Mesh size calculations were performed using the Floy-Rehner calculations of mesh size using equation (1) with prior calculations and procedures described by Zustiak and Leach for PEG.[3] Mass swelling ratios were assumed to be equivalent to those for bulk gel equivalents.

$$\xi = {v_2}^{-1/3}(\bar{r}^2_0)^{1/2} \quad (1)$$

Where ξ is mesh size, $(\bar{r}^2_0)^{1/2}$ is the root mean squared polymer chain end-to-end distance in an unperturbed state, and v_2 is the polymer volume fraction in the hydrogel.

Accordingly, using equation (2) from free volume theory, $D_g = 1.718*10^{-6}$ cm^2/s with Y =1.[4,5]

$$\frac{D_g}{D_0} = \left(1 - \frac{r_s}{\xi}\right) exp\left(-Y\left(\frac{\varphi}{1-\varphi}\right)\right) \quad (2)$$

Thus, characteristic diffusion time for 300 nm particle was determined to be 0.001 s and characteristic diffusion time for gel was determined to be 3.37 days.

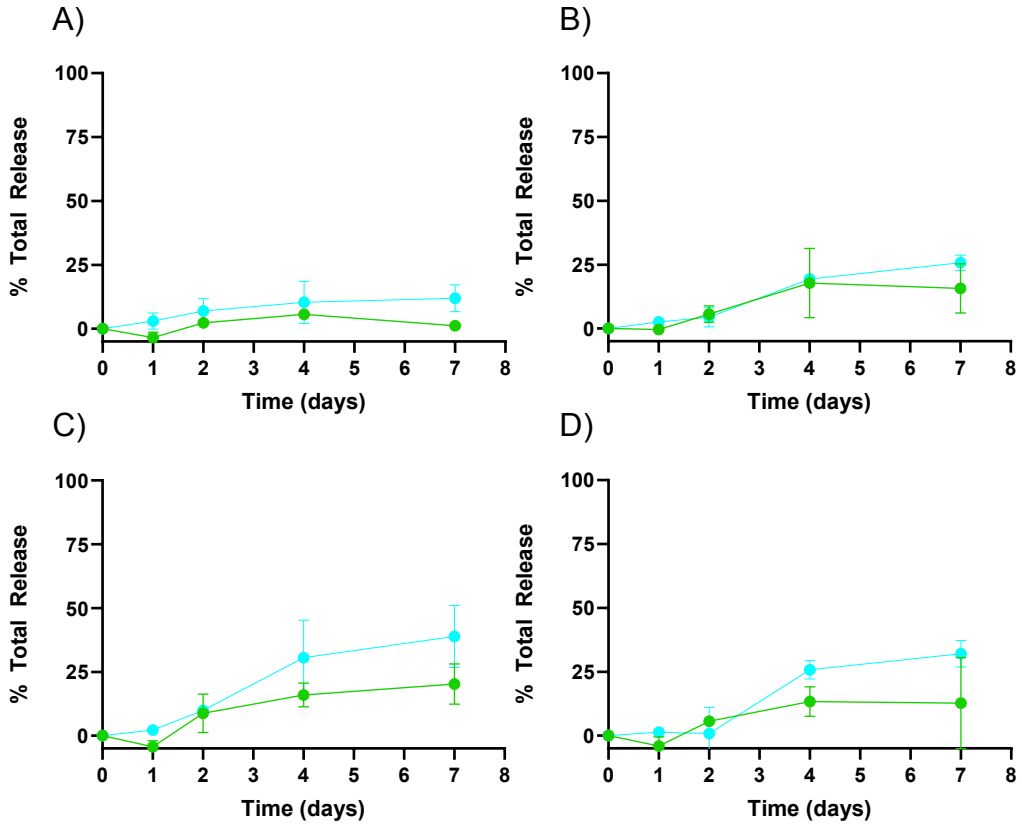

Figure S21 – Rhodamine percent total release profiles for 30% (●) and 50% (●) PEGDA nanoparticles in **A)** DI water, and **B)** pH 5, **C)** pH 7.4, and **D)** pH 10 conditions over a 7-day incubation period.

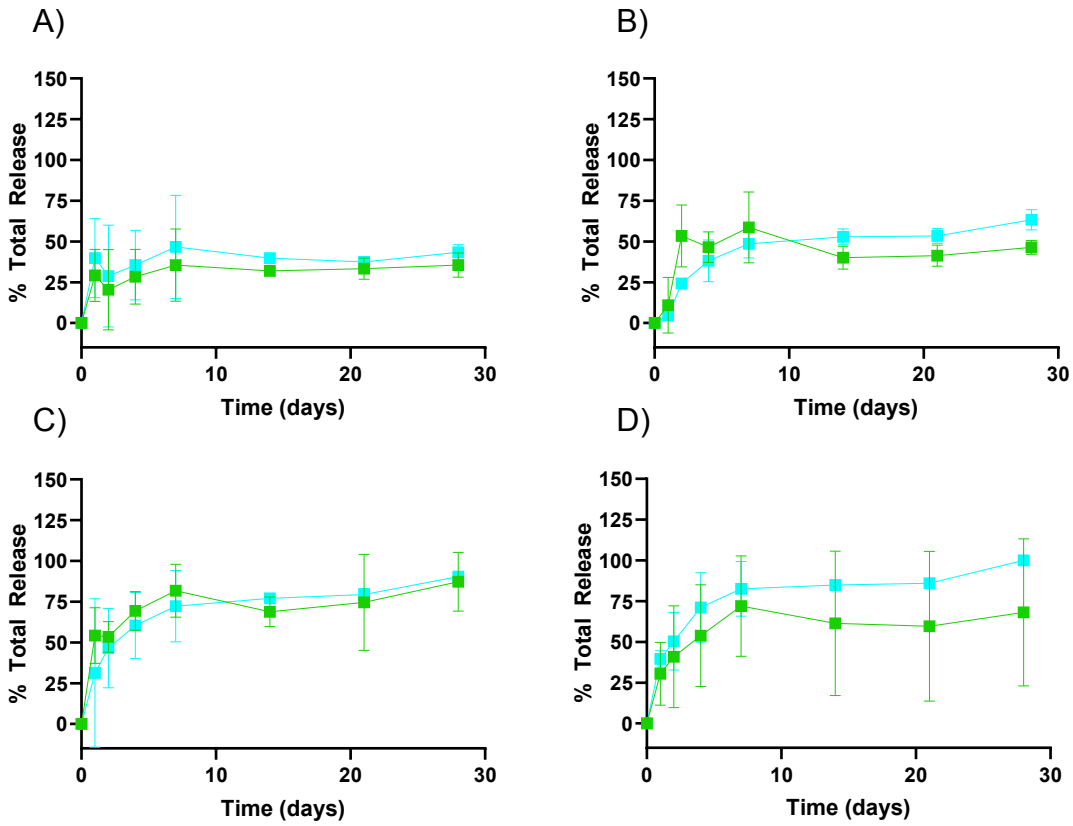

Figure S22. Rhodamine B release profiles from PEGDA bulk gels. Percent rhodamine B release profiles from day 0 – day 28 for 30 wt% (■) and 50 wt% (■) PEGDA hydrogels in **(A)** water, **(B)** pH 5, **(C)** pH 7 and **(D)** pH 10 conditions [Analyzed by two-way ANOVA; n = 4].

MALDI-TOF MS Spectra Over Time for PEGDA-Based Nanoparticles and Bulk Gels

The following data shows the progression of MALDI-TOF MS data different PEGDA-based nanoparticles and bulk gels.

30 wt% PEGDA nanoparticles (Figures S23-S26)

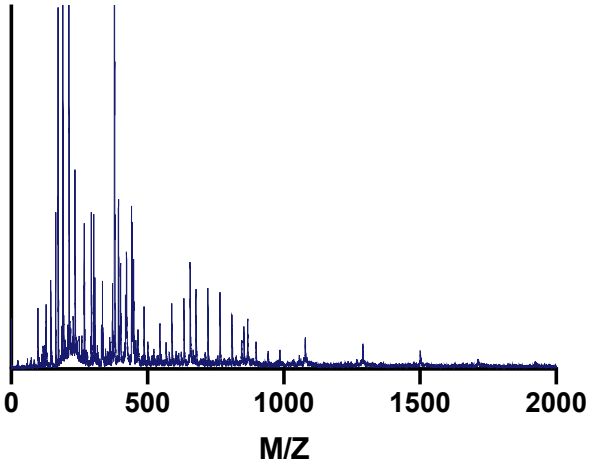

Figure S23 – MALDI-TOF spectrum for 30 wt% nanoparticles incubated for 3 days.

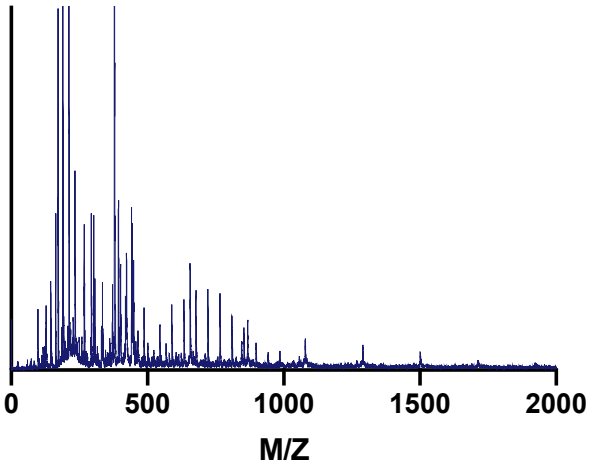

Figure S24 – MALDI-TOF spectrum for 30 wt% nanoparticles incubated for 7 days.

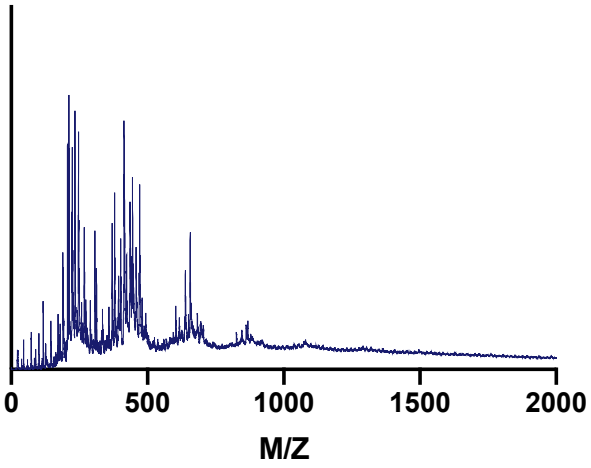

Figure S25 – MALDI-TOF spectrum for 30 wt% nanoparticles incubated for 21 days.

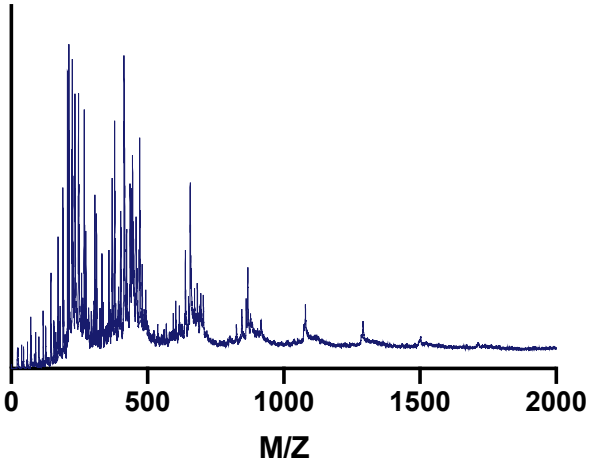

Figure S26 – MALDI-TOF spectrum for 30 wt% nanoparticles incubated for 28 days.

50 wt% PEGDA nanoparticles (Figures S27-S29)

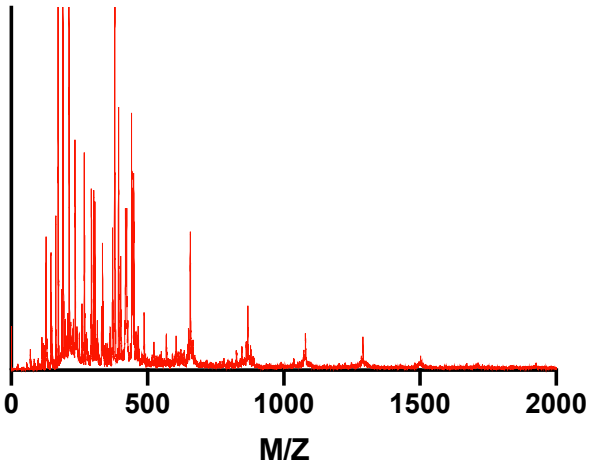

Figure S27 – MALDI-TOF spectrum for 50 wt% nanoparticles incubated for 3 days.

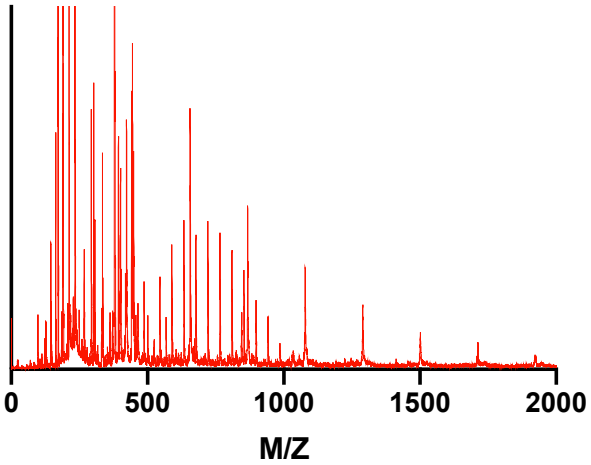

Figure S28 – MALDI-TOF spectrum for 50 wt% nanoparticles incubated for 7 days.

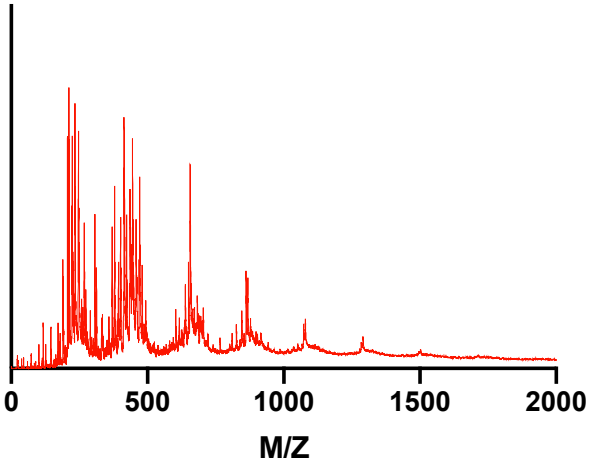

Figure S29 – MALDI-TOF spectrum for 50 wt% nanoparticles incubated for 21 days.

30 wt% PEGDA bulk gels (Figures S30-S31)

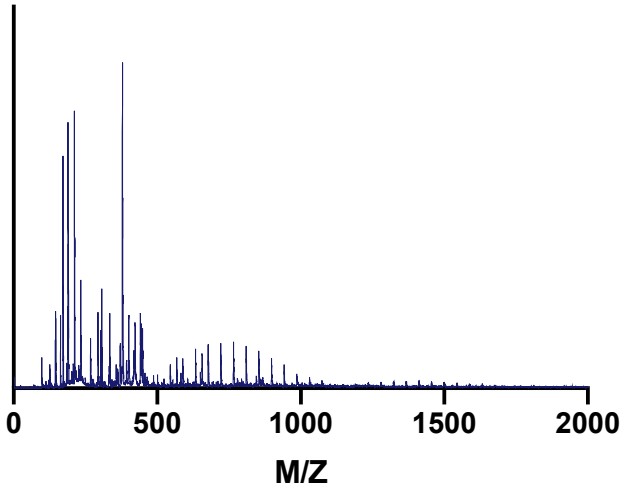

Figure S30 – MALDI-TOF spectrum for 30 wt% bulk gels incubated for 7 days.

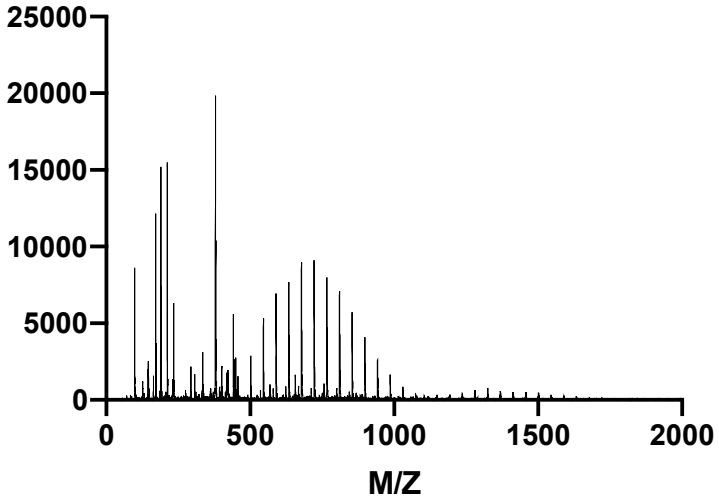

Figure S31 – MALDI-TOF spectrum for 30 wt% bulk gels incubated for 14 days.

50 wt% PEGDA bulk gels (Figures S32-S34)

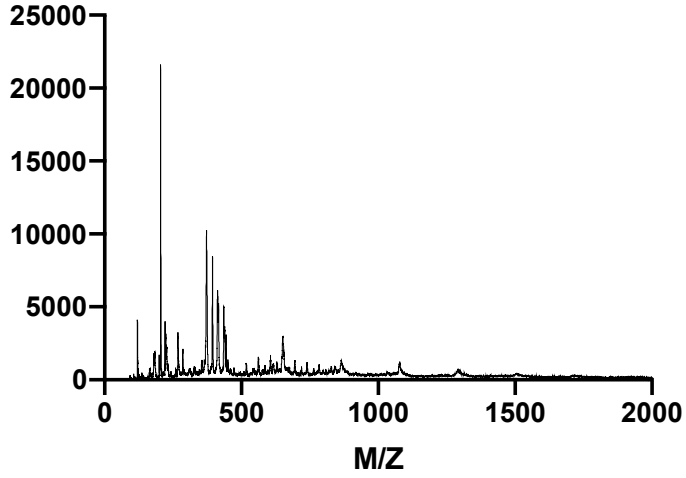

Figure S32 – MALDI-TOF spectrum for 50 wt% bulk gels incubated for 1 day.

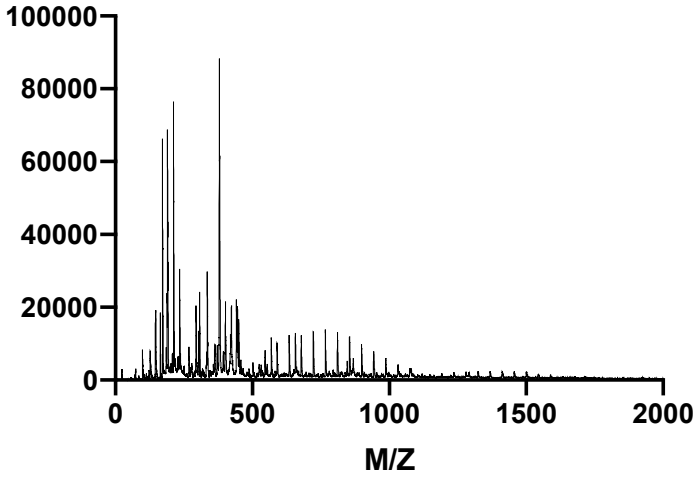

Figure S33 – MALDI-TOF spectrum for 50 wt% bulk gels incubated for 3 days.

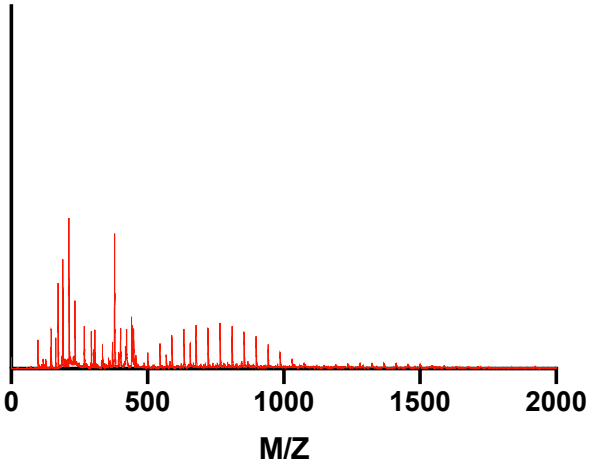

Figure S34 – MALDI-TOF spectrum for 50 wt% bulk gels incubated for 7 days.

50 wt% PEGDA bulk gels with 10% HDDMA co-monomer (Figures S35-S38)

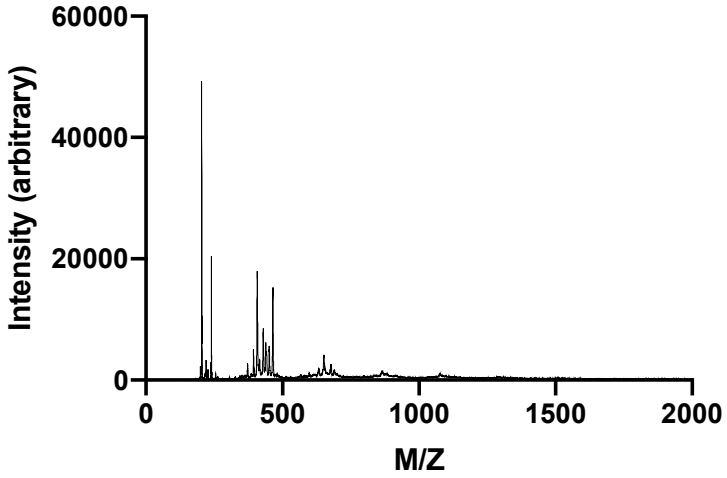

Figure S35 – MALDI-TOF spectrum for 50 wt% bulk gels with 10% HDDMA co-monomer incubated for 1 day.

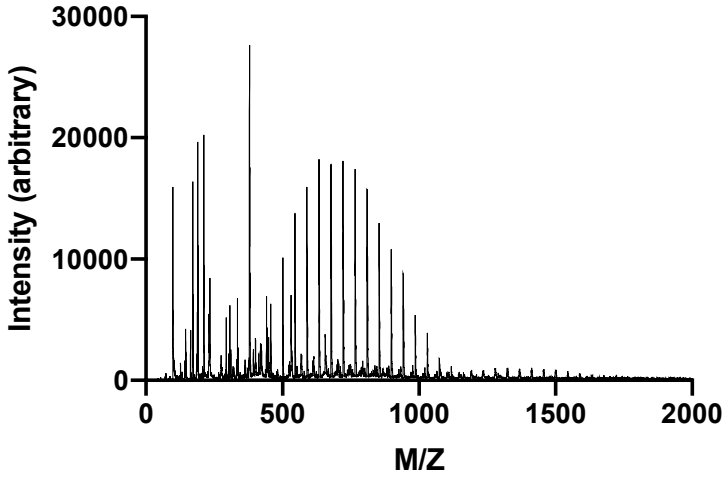

Figure S36 – MALDI-TOF spectrum for 50 wt% bulk gels with 10% HDDMA co-monomer incubated for 3 days.

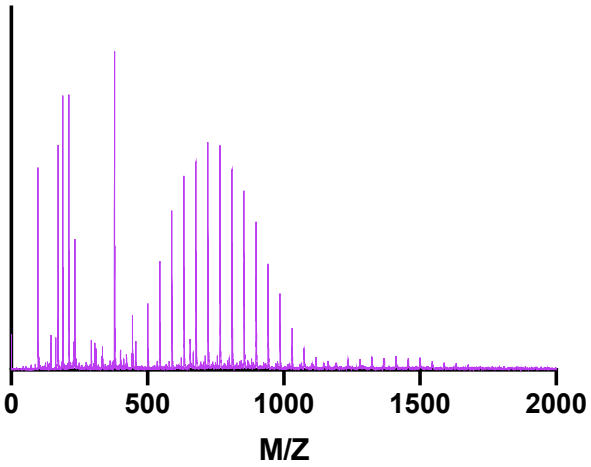

Figure S37 – MALDI-TOF spectrum for 50 wt% bulk gels with 10% HDDMA co-monomer incubated for 7 days.

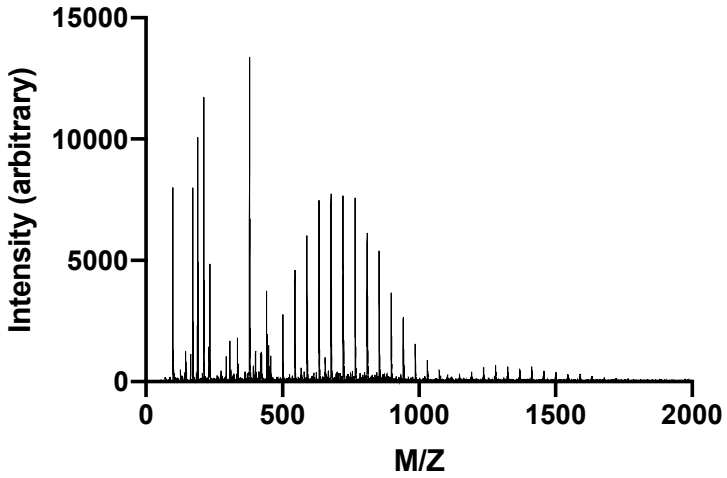

Figure S38 – MALDI-TOF spectrum for 50 wt% bulk gels with 10% HDDMA co-monomer incubated for 14 days.

50 wt% PEGDA bulk gels with 30% HDDMA co-monomer (Figures S39-S42)

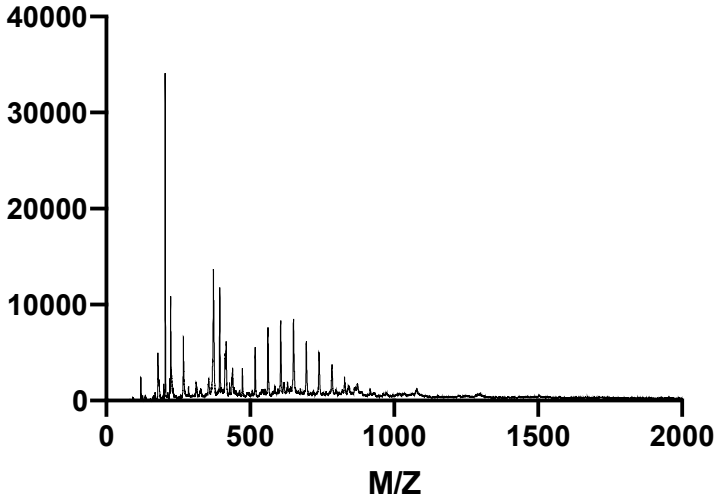

Figure S39 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% HDDMA co-monomer incubated for 1 day.

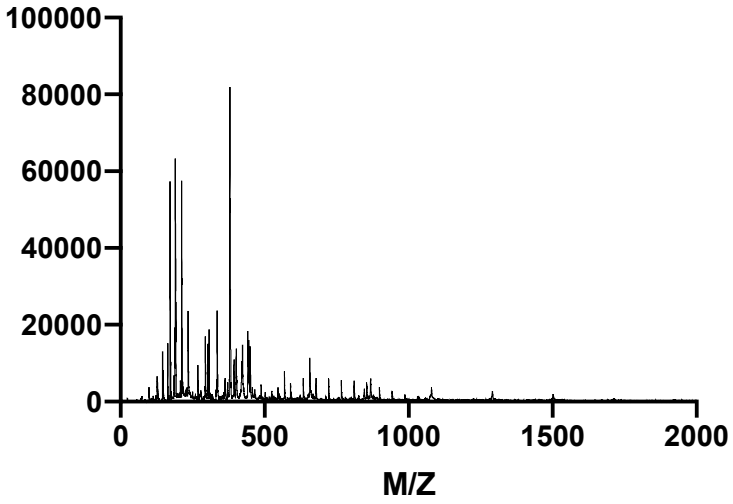

Figure S40 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% HDDMA co-monomer incubated for 3 days.

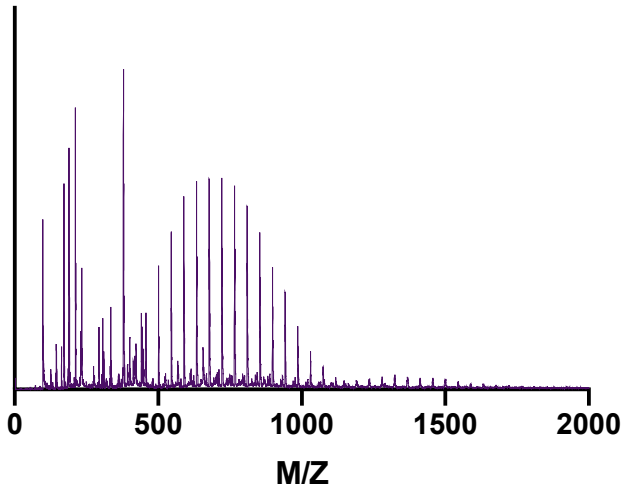

Figure S41 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% HDDMA co-monomer incubated for 7 days.

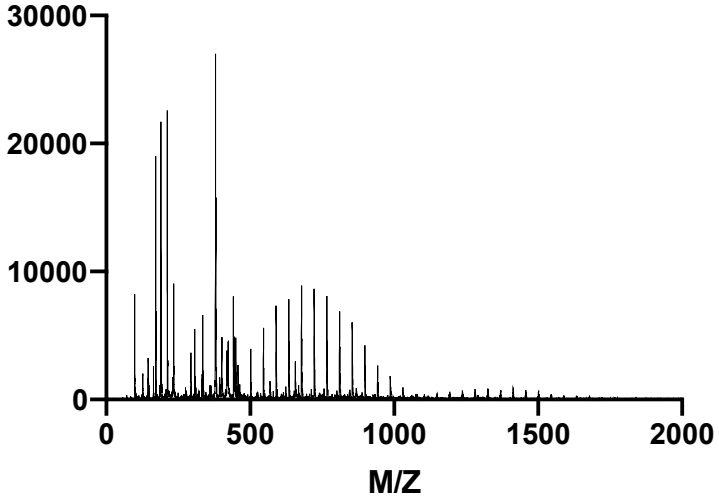

Figure S42 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% HDDMA co-monomer incubated for 14 days.

50 wt% PEGDA bulk gels with 10% DSDMA co-monomer (Figures S43-S46)

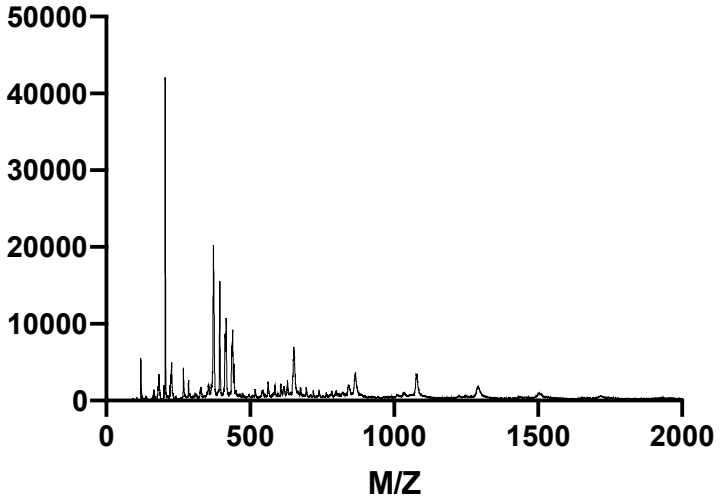

Figure S43– MALDI-TOF spectrum for 50 wt% bulk gels with 10% DSDMA co-monomer incubated for 1 day.

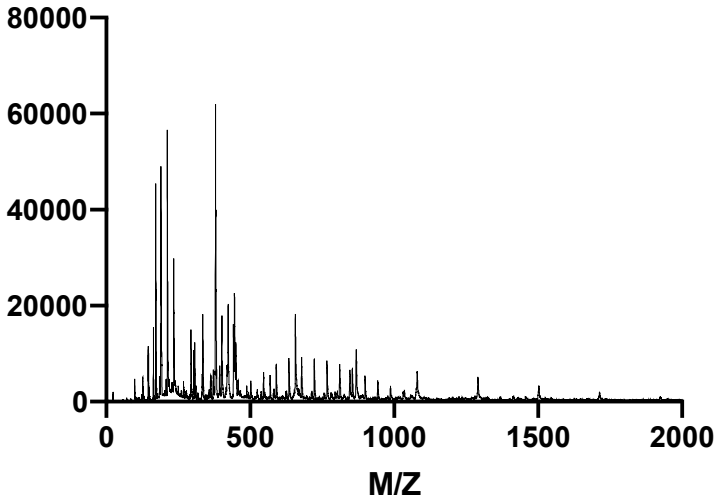

Figure S44 – MALDI-TOF spectrum for 50 wt% bulk gels with 10% DSDMA co-monomer incubated for 3 days.

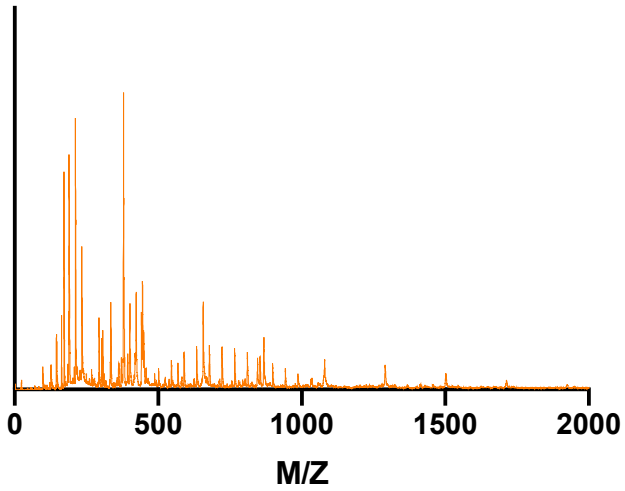

Figure S45 – MALDI-TOF spectrum for 50 wt% bulk gels with 10% DSDMA co-monomer incubated for 7 days.

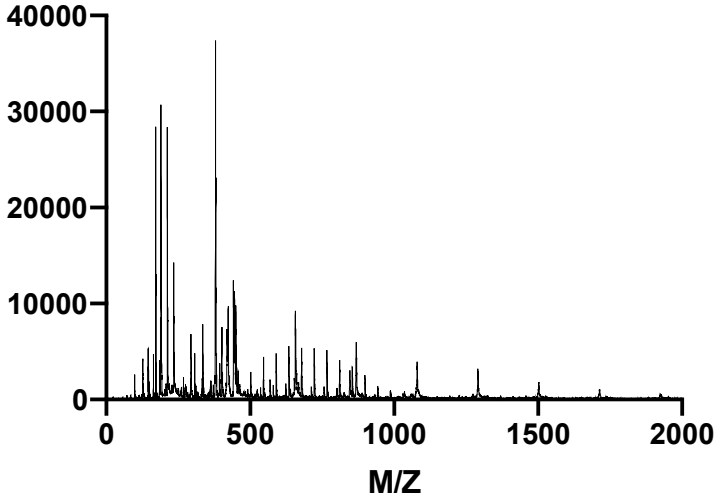

Figure S46 – MALDI-TOF spectrum for 50 wt% bulk gels with 10% DSDMA co-monomer incubated for 14 days.

50 wt% PEGDA bulk gels with 30% DSDMA co-monomer (Figures S47-S50)

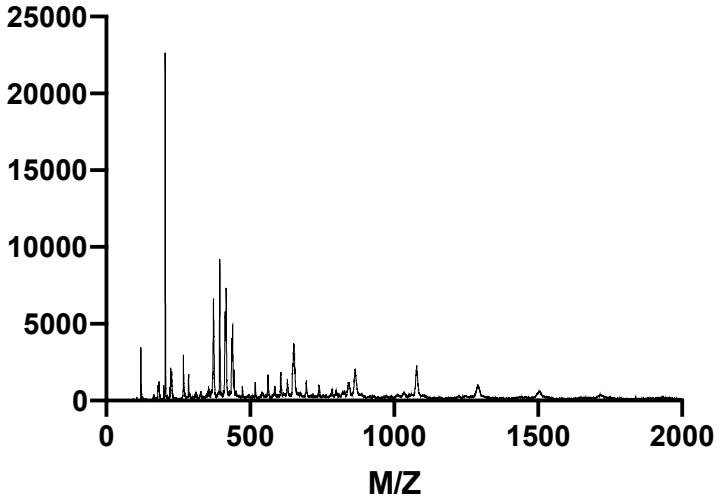

Figure S47 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% DSDMA co-monomer incubated for 1 day.

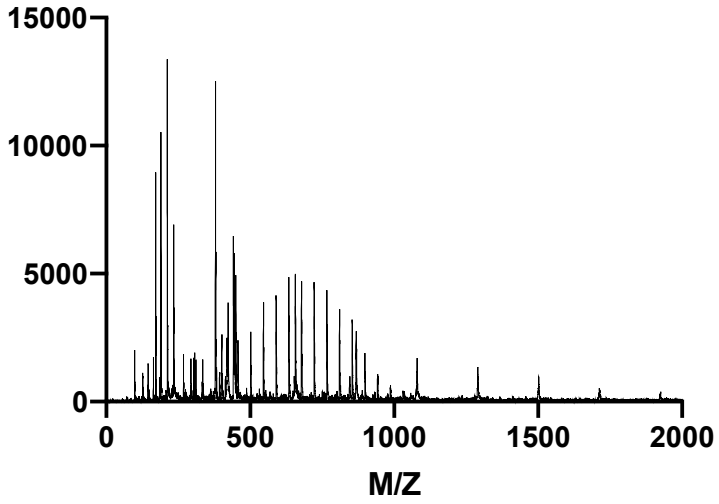

Figure S48 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% DSDMA co-monomer incubated for 3 days.

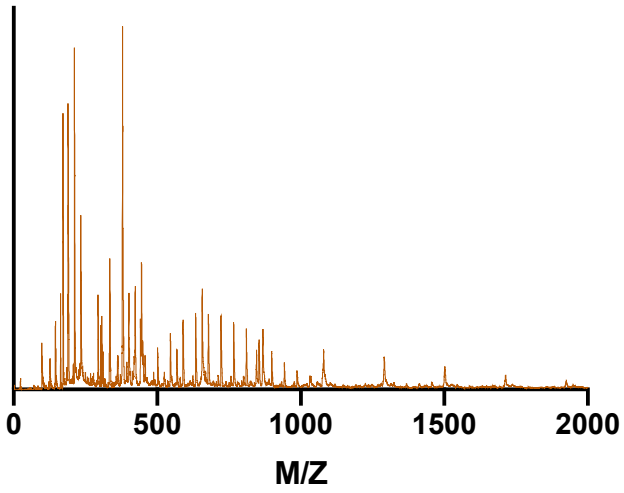

Figure S49 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% DSDMA co-monomer incubated for 7 days.

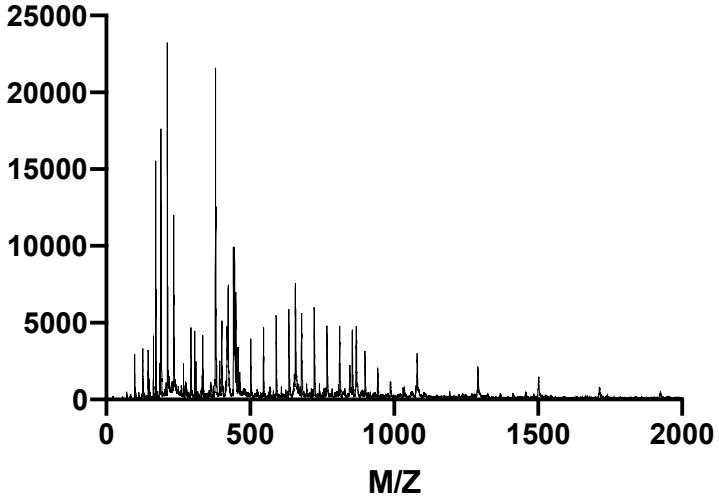

Figure S50 – MALDI-TOF spectrum for 50 wt% bulk gels with 30% DSDMA co-monomer incubated for 14 days.

Gel Permeation Chromatography (GPC)

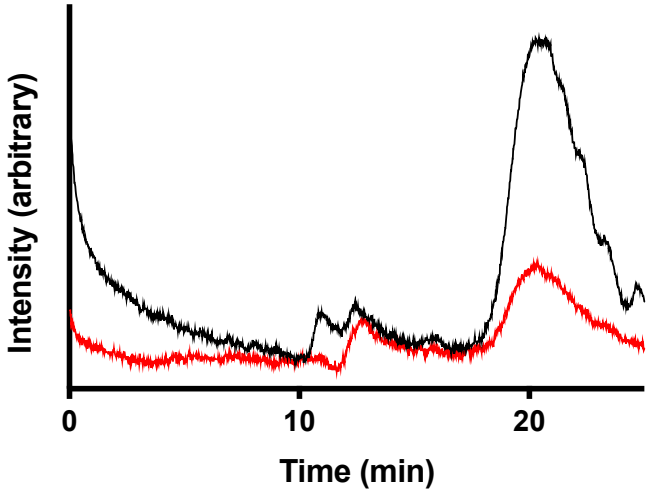

Figure S51 – Representative gel permeation chromatography (GPC) results from the degradation of 50 wt% PEGDA nanoparticles (red) and the control of PEGDA 700 (black).

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