

Supporting Information for:

**Hydrogel Nanoparticle Degradation Influences the Activation and Survival of Primary
Macrophages**

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Table of Contents

| | |
|---|-----|
| Representative Cryo SEM Images | S3 |
| Zeta Potential Measurement in PBS | S3 |
| Full XEDS Spectra | S4 |
| Example Reaction Scheme of GSH-Mediated Degradation | S5 |
| NP Degradation Data Grouped by NP Type (0%, 10%, and 20%) | S6 |
| Mass Spectrometry of Degradation Products of 0%, 10%, and 20% NPs | S7 |
| Normalized BMM Viability for 10 μ g/ml NP Concentrations | S15 |
| Representative Flow Cytometry Gating | S16 |
| CD80 Expression | S17 |
| IL-10 Secretions in BMM Supernatants | S17 |

Representative Cryo-SEM images

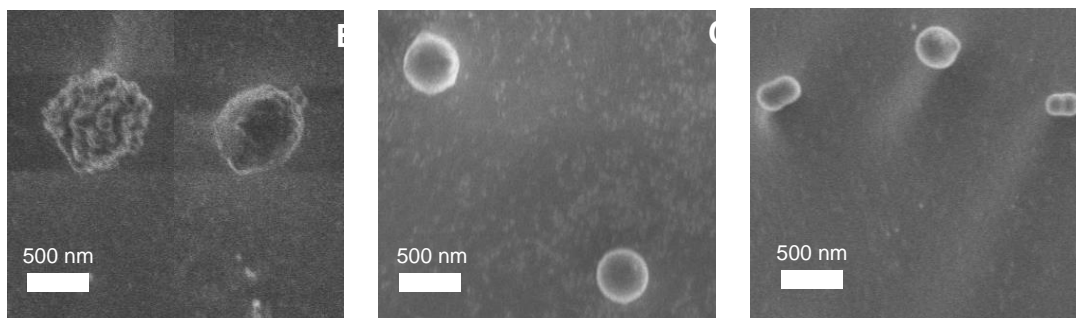


Figure S1: Cryo SEM images of A) 0%, B) 10%, and C) 20% NPs. Note that the 0% image has been cropped to fit multiple particles from the same image because of lower density of the sample.

Zeta Potential Measurement in PBS

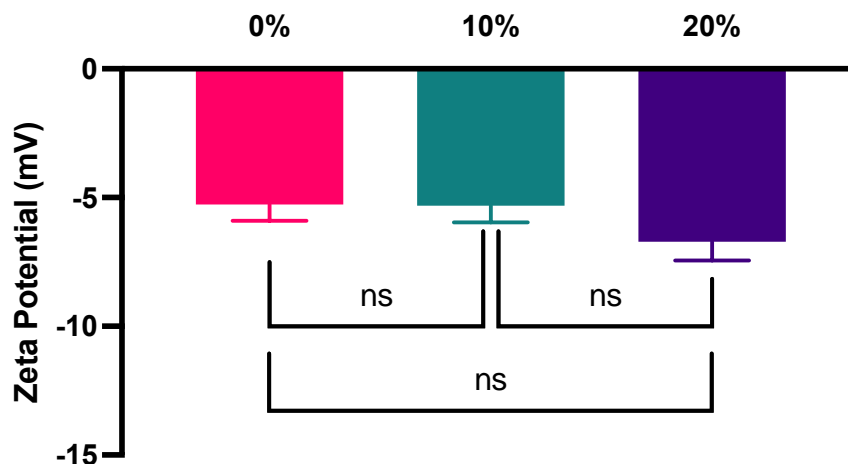


Figure S2: Zeta Potential Measurement in PBS. Zeta potential measurements for the 0%-, 10%, and 20%-SH NPs. The graph shows the mean and SD from two independently synthesized samples measured 3 times each in PBS solution (6 total measurements, $N=2$).

Full XEDS Spectra

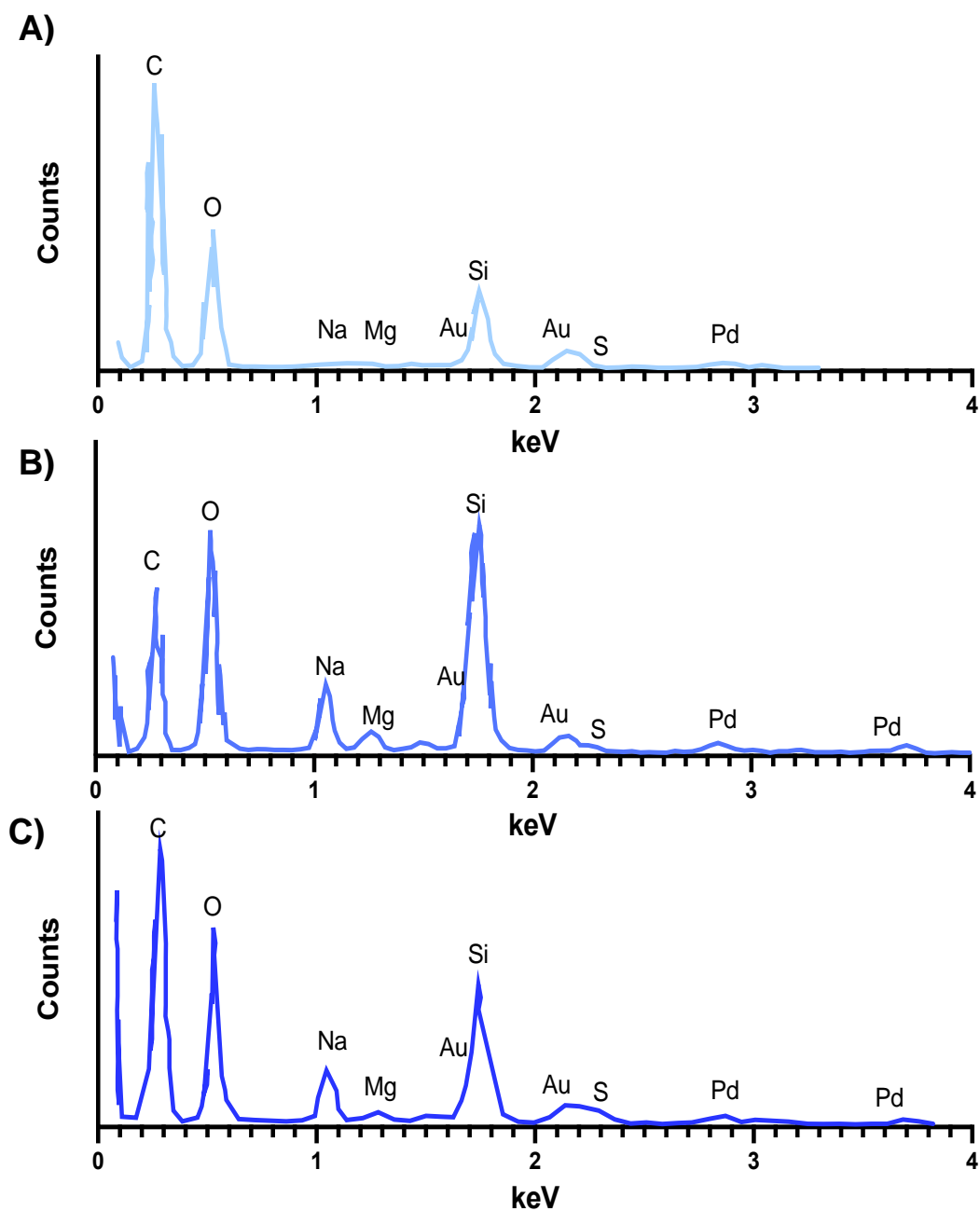
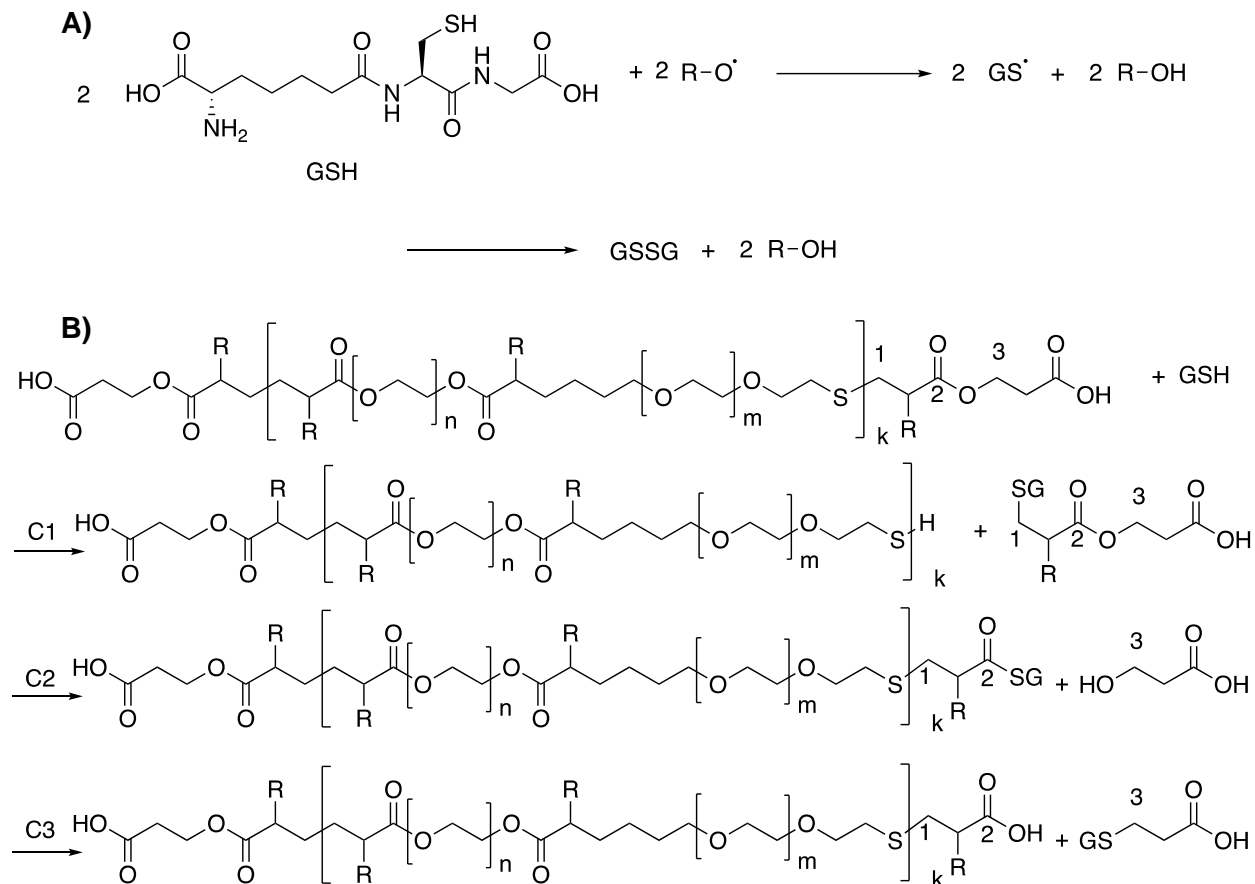


Figure S3: A) Full XEDS spectrum of the 0% NPs with some potentially identified species indicated at their respective energies. B) Full XEDS spectrum of the 10% NPs with some potentially identified species noted. C) Full XEDS spectrum of the 20% NPs with some potentially identified species noted.

Example Reaction Scheme of GSH-Mediated Degradation



Scheme S1: A) Example reaction scheme of GSH functioning as a reducing agent to neutralize reactive oxygen species (in this example, represented as R-O \cdot). B) Examples of GSH functioning as a nucleophile in potential breakdown mechanisms of the HS-PEG-SH-based NPs with potential nucleophilic attack at three different carbons shown.

NP Degradation Data Grouped by NP Type (0%, 10%, and 20%)

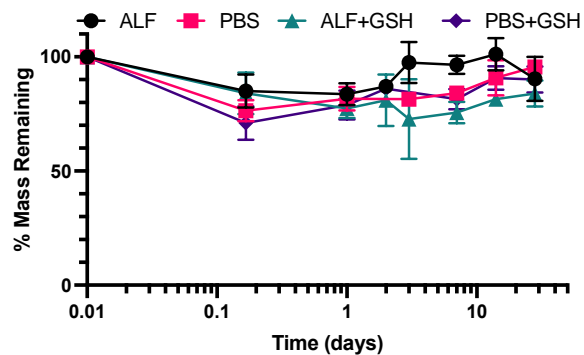


Figure S4: Degradation by mass of 0% NPs in ALF (black circles), PBS (pink squares), ALF+GSH (green triangles), and PBS+GSH (purple diamonds).

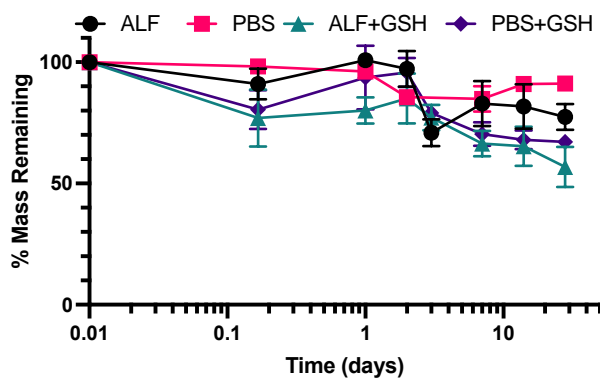


Figure S5: Degradation by mass of 10% NPs in ALF (black circles), PBS (pink squares), ALF+GSH (green triangles), and PBS+GSH (purple diamonds).

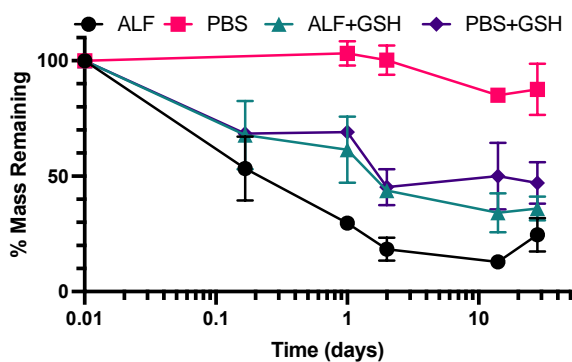


Figure S6: Degradation by mass of 20% NPs in ALF (black circles), PBS (pink squares), ALF+GSH (green triangles), and PBS+GSH (purple diamonds).

Mass Spectrometry of Degradation Products of 0%, 10%, and 20% NPs

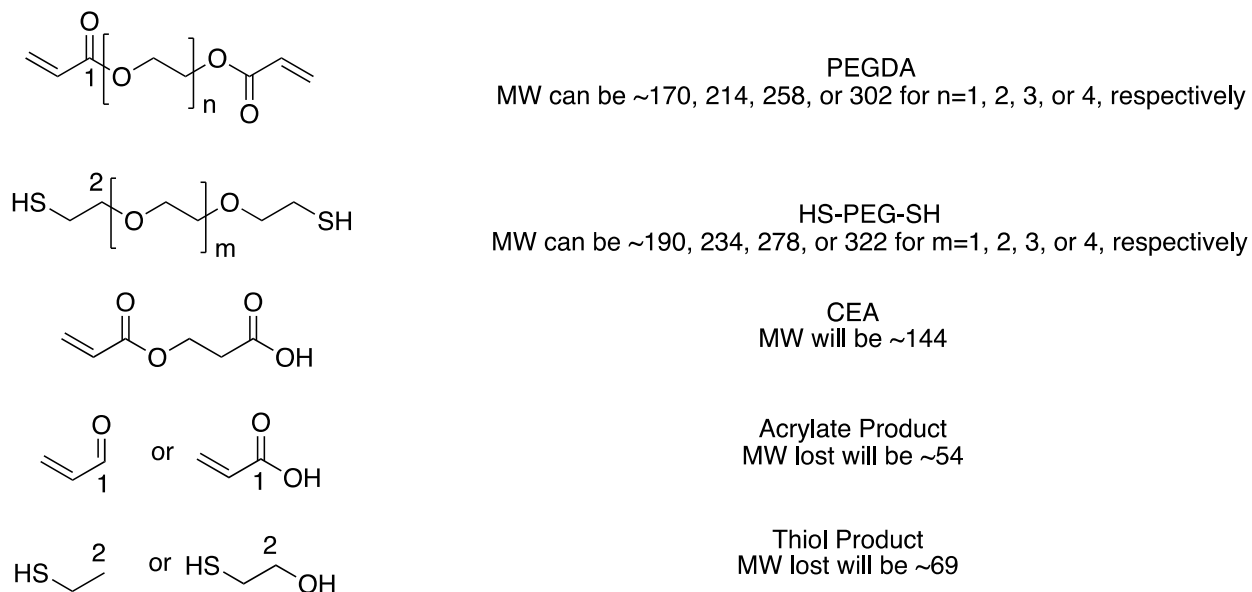


Figure S7: Key structures and molecular weights for compound identification in the table below.

Table S1: Selected list of identified compounds from the degradation of 0%, 10%, and 20% particles along with some notes.

| Approximate Molecular Weight (g/mol) | Possible Compound (with notes) |
|--------------------------------------|--|
| 133 | CEA after hydrolysis (loss of C=C, gain H) (all NPs) |
| 147 | PEGDA with n=2, loss of two acrylate products from hydrolysis or CEA after hydrogenation (all NPs) |
| 160 | PEGDA with n=2 and loss of acrylate product (all NPs) |
| 171 | PEGDA with n=1 (all NPs) |
| 191 | HS-PEG-SH with n=1 (only 10%, 20% NPs) |
| 215 | PEGDA with n=2 (all NPs) |
| 236 | HS-PEG-SH with n=2 (only 10%, 20% NPs) |
| 248 | PEGDA with n=4 after loss of acrylate product from hydrolysis (all NPs) |
| 256 | PEGDA with n=3 (all NPs) |
| 269 | PEGDA with n=1 plus CEA and loss of one acrylate product (all NPs) |
| 289 | PEGDA with n=2 and addition of acrylate after hydrolysis on other end of acrylate (all NPs) |
| 314 | PEGDA with n=1 and CEA or PEGDA with n=1 and the addition of two acrylate groups after hydrolysis (all NPs) |
| 320 | HS-PEG-SH with n=4 |
| 324 | PEGDA with n=4 with addition of one acrylate group after hydrolysis and loss of different acrylate product (all NPs) |

| | |
|-----|---|
| 331 | PEGDA with n=3 and addition of acrylate after hydrolysis on other end of acrylate (all NPs) |
| 358 | PEGDA with n=2 and addition of two acrylate groups after hydrolysis (all NPs) |
| 386 | PEGDA with n=1 and addition of three acrylate groups after hydrolysis (all NPs) |
| 402 | PEGDA with n=3 and addition of two acrylate groups after hydrolysis (all NPs) |
| 446 | PEGDA with n=4 and addition of two acrylate groups after hydrolysis (all NPs) |
| 521 | HS-PEG-SH with PEGDA with n=1 plus CEA and loss of one acrylate product (only 10%, 20% NPs) |
| 589 | HS-PEG-SH with PEGDA with n=1 plus CEA and loss of one acrylate product and loss of one thiol product (only 10%, 20% NPs) |

Selected Plots from Liquid Chromatography-Mass Spectrometry

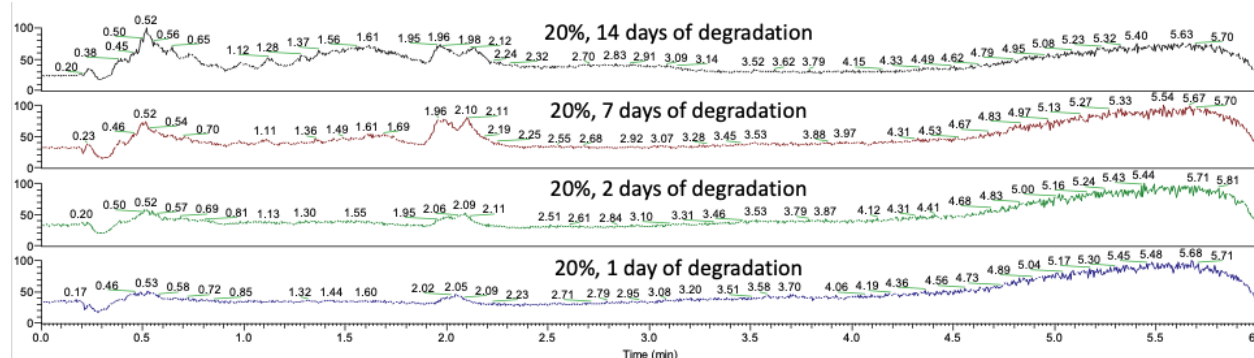


Figure S8: Signal vs elution time for 20% NPs as a function of degradation time. As can be seen from the plots, the peak positions are qualitatively similar, with some increasing in intensity at the later time points. This trend was similar for the 0%, 10%, and 20% particles.

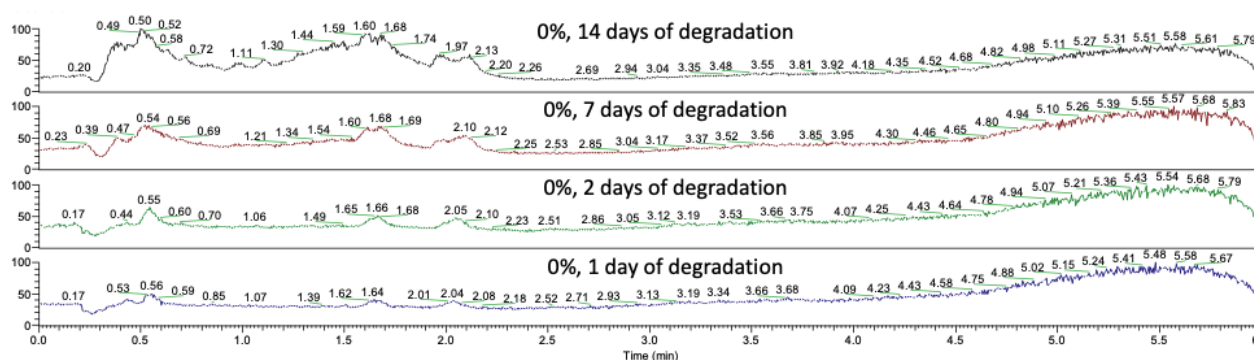


Figure S9: Signal vs elution time for 0% NPs as a function of degradation time. As can be seen from the plots, the peak positions are qualitatively similar, with some increasing in intensity at the later time points. This trend was similar for the 0%, 10%, and 20% particles.

The following plots are demonstrative of the trends observed for the degradation products over time. As time progressed, the products tended to shift toward higher MW.

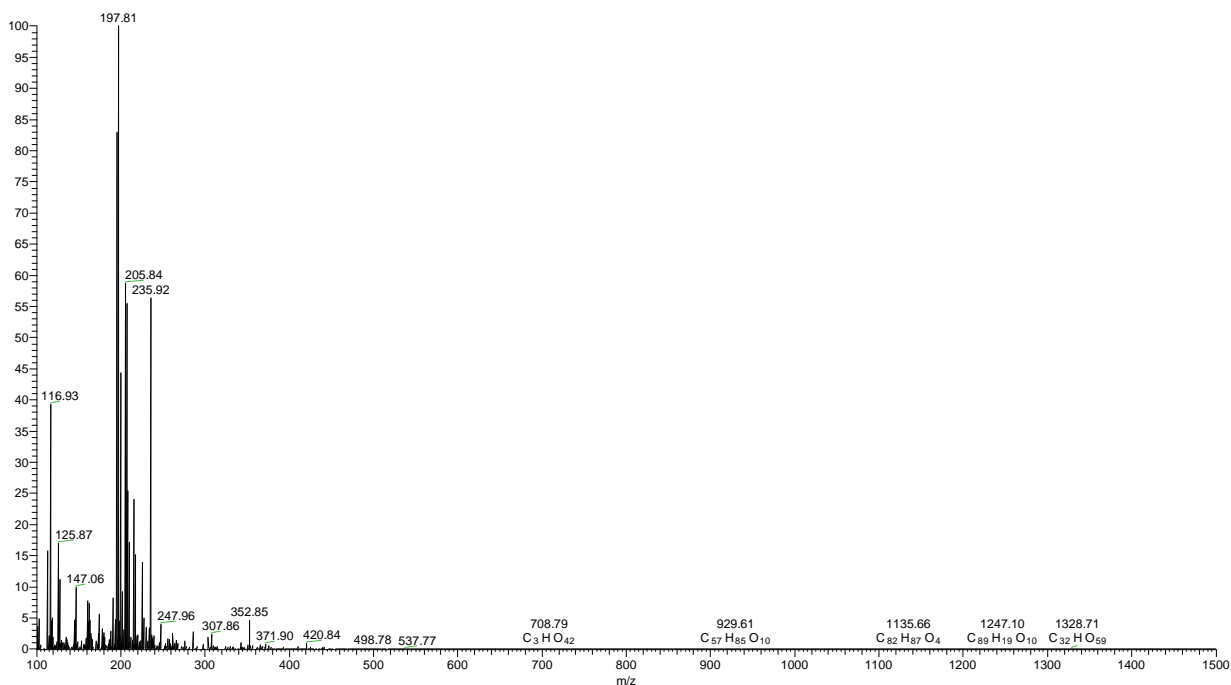


Figure S10: Relative intensity versus molecular weight divided by charge for 0% NPs after 1 day of degradation at an elution time of 0.39 minutes.

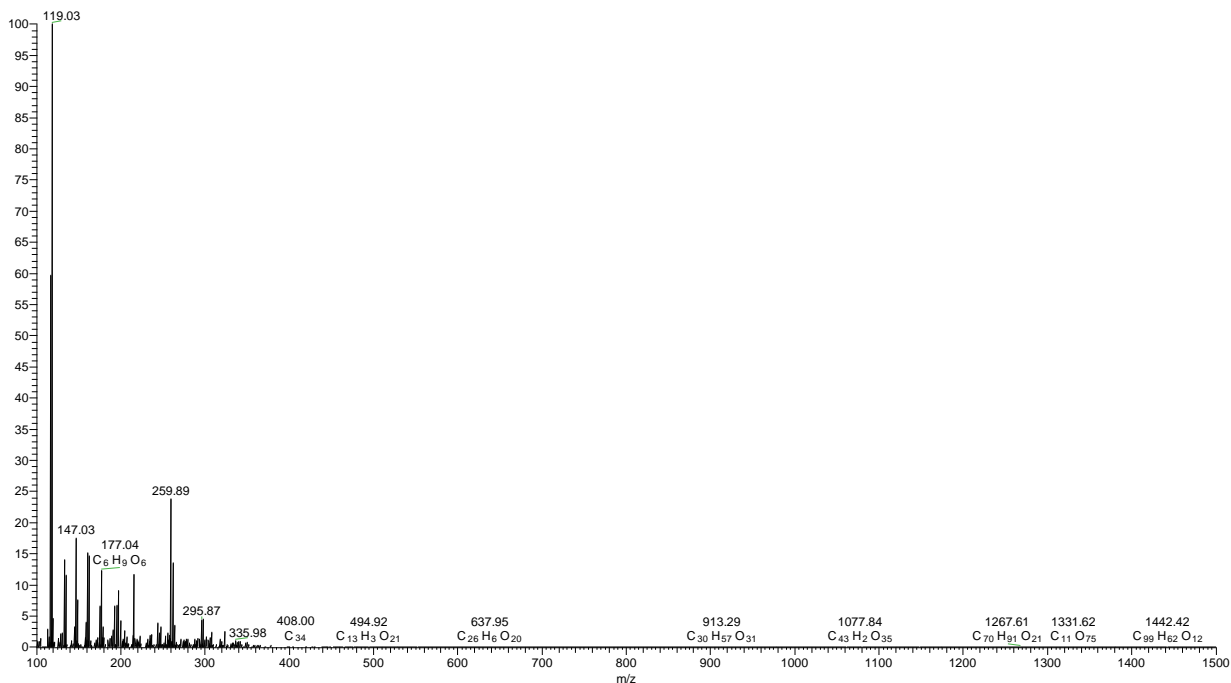


Figure S11: Relative intensity versus molecular weight divided by charge for 0% NPs after 14 days of degradation at an elution time of 0.39 minutes.

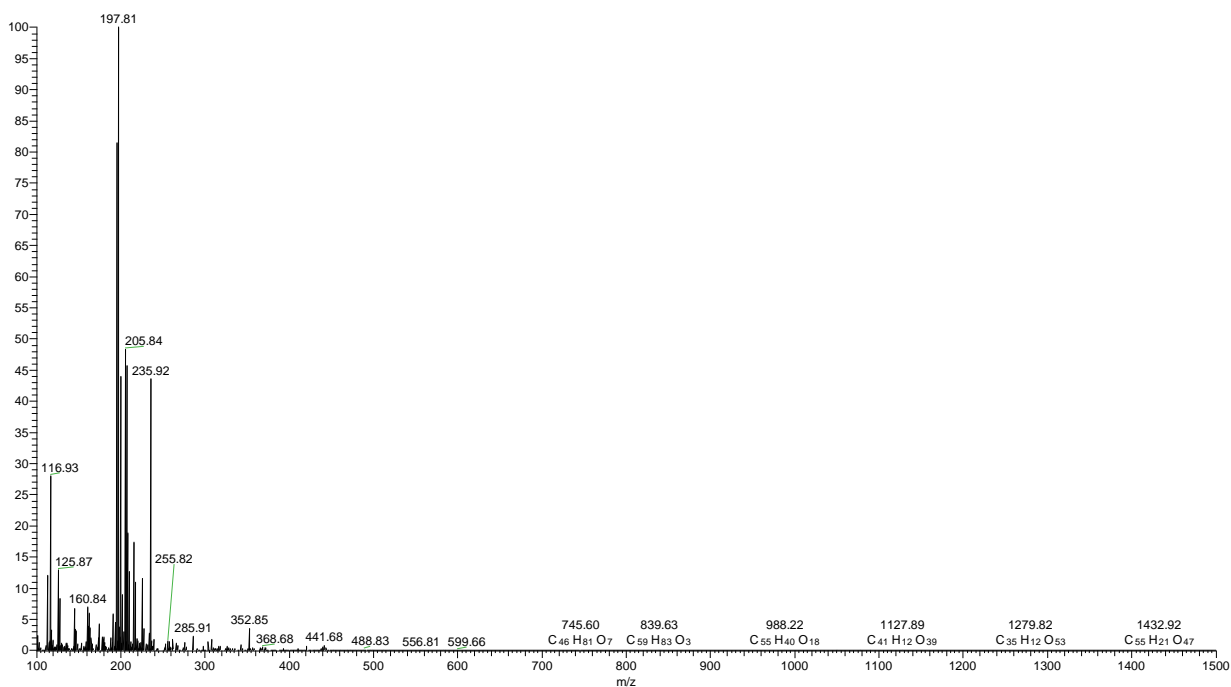


Figure S12: Relative intensity versus molecular weight divided by charge for 0% NPs after 1 day of degradation at an elution time of 0.65 minutes.

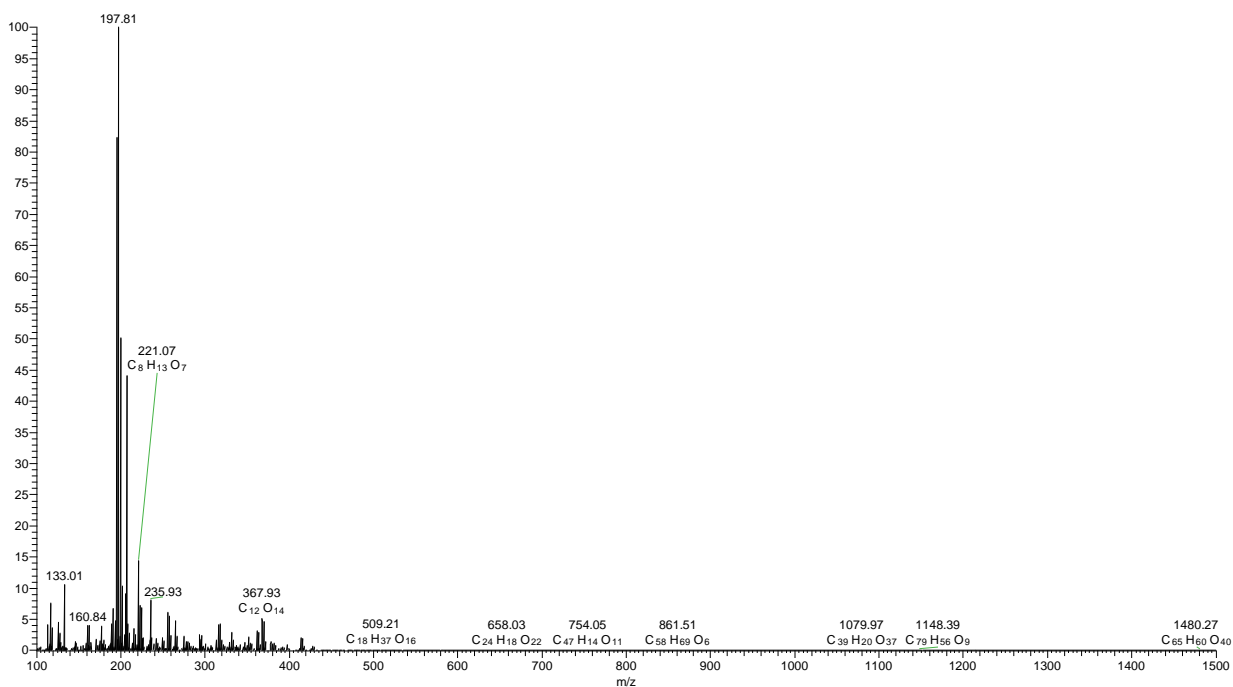


Figure S13: Relative intensity versus molecular weight divided by charge for 0% NPs after 14 days of degradation at an elution time of 0.65 minutes.

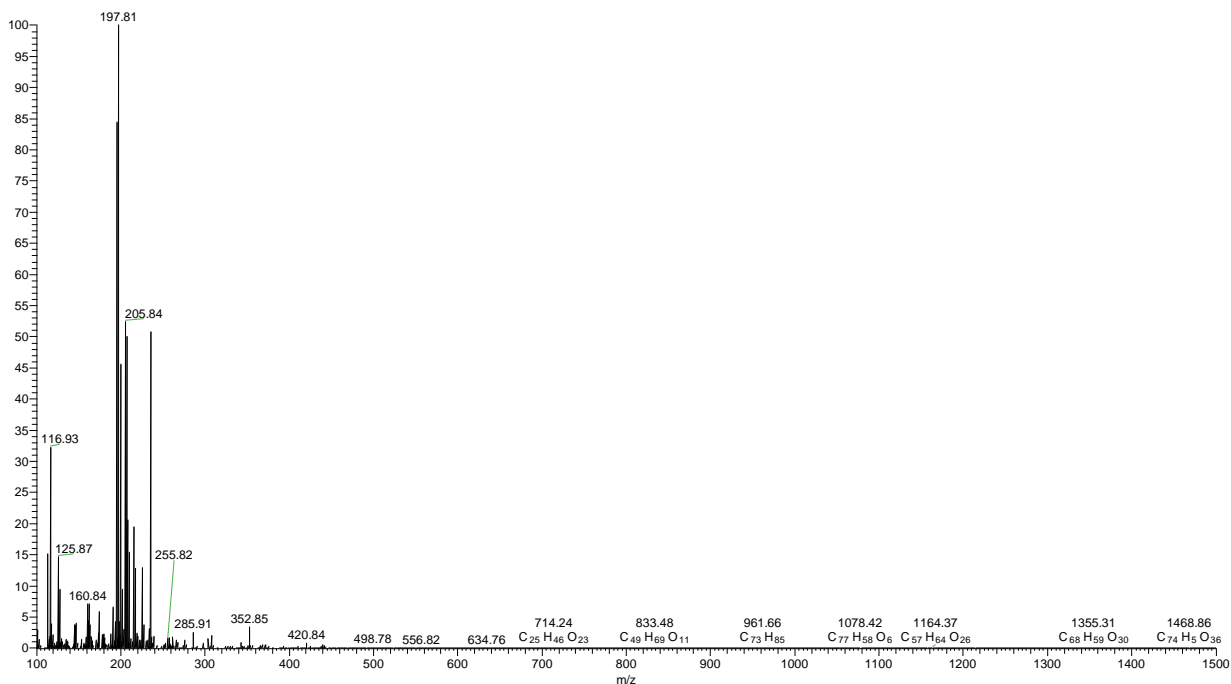


Figure S14: Relative intensity versus molecular weight divided by charge for 0% NPs after 1 day of degradation at an elution time of 0.98 minutes.

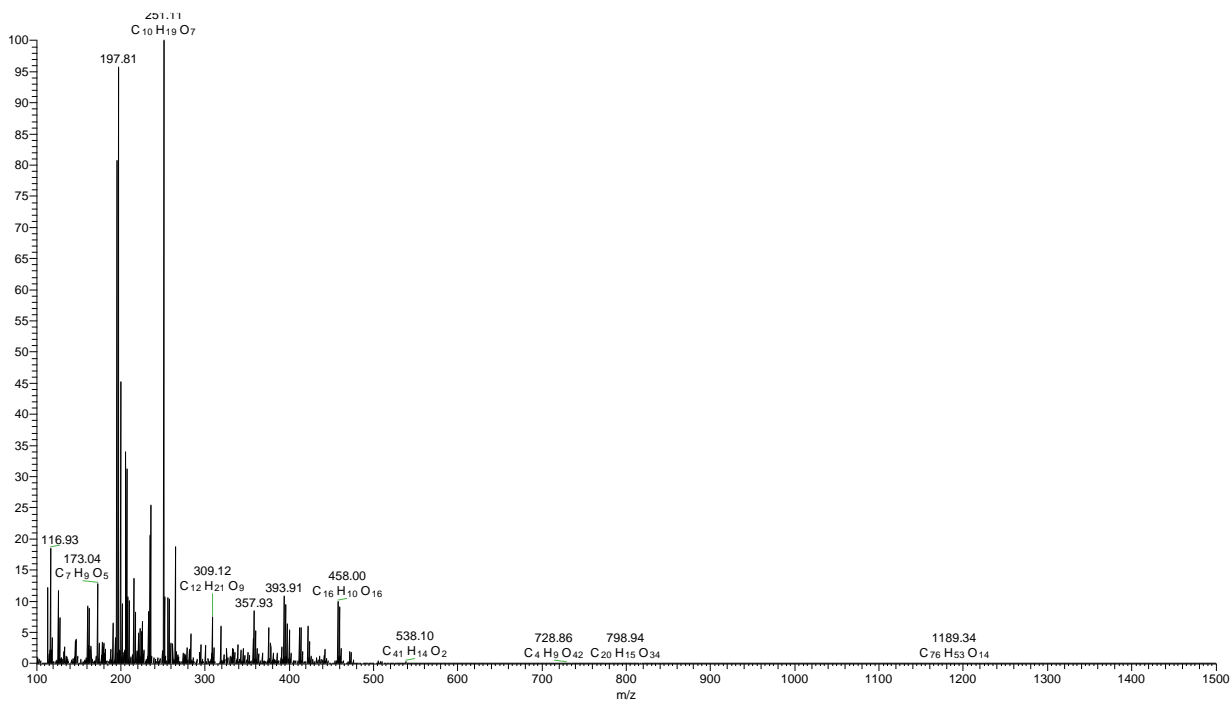


Figure S15: Relative intensity versus molecular weight divided by charge for 0% NPs after 14 days of degradation at an elution time of 0.98 minutes.

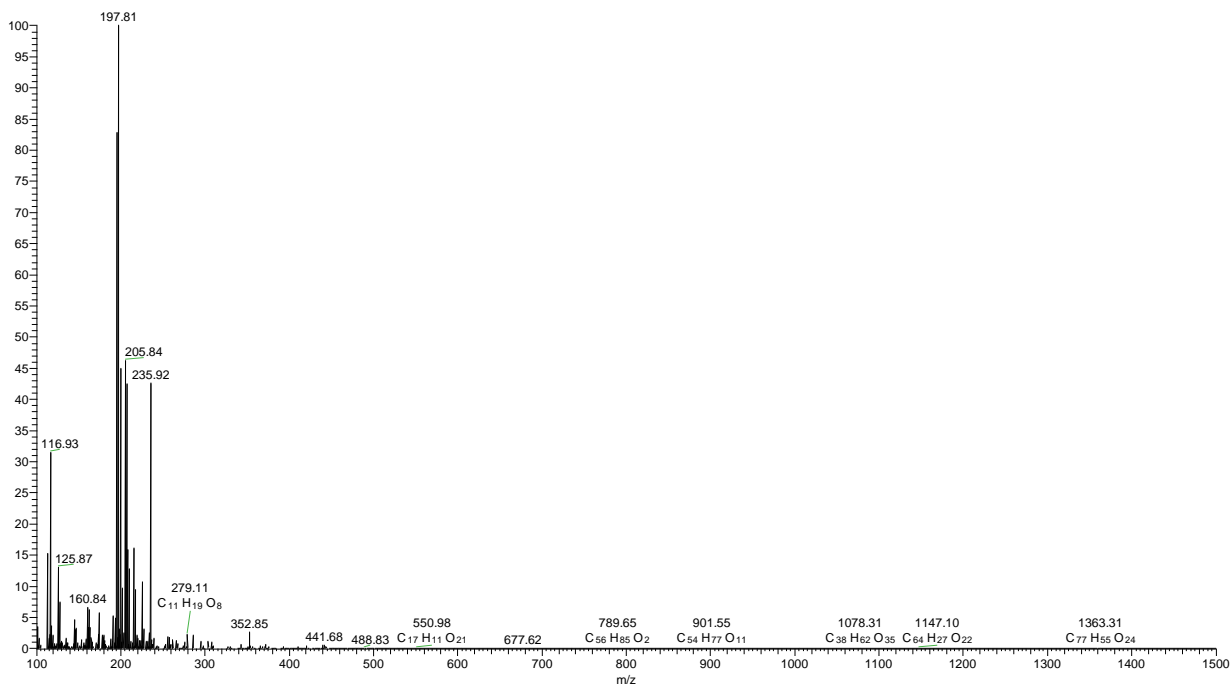


Figure S16: Relative intensity versus molecular weight divided by charge for 0% NPs after 1 day of degradation at an elution time of 1.28 minutes.

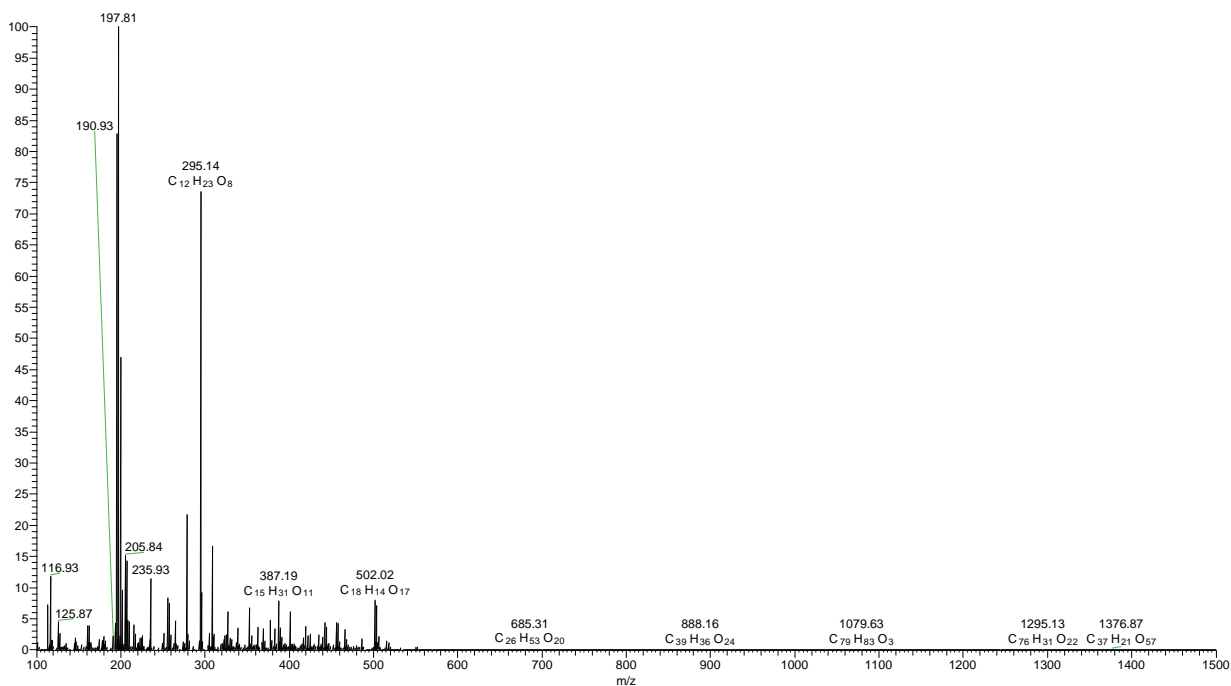


Figure S17: Relative intensity versus molecular weight divided by charge for 0% NPs after 14 days of degradation at an elution time of 1.28 minutes.

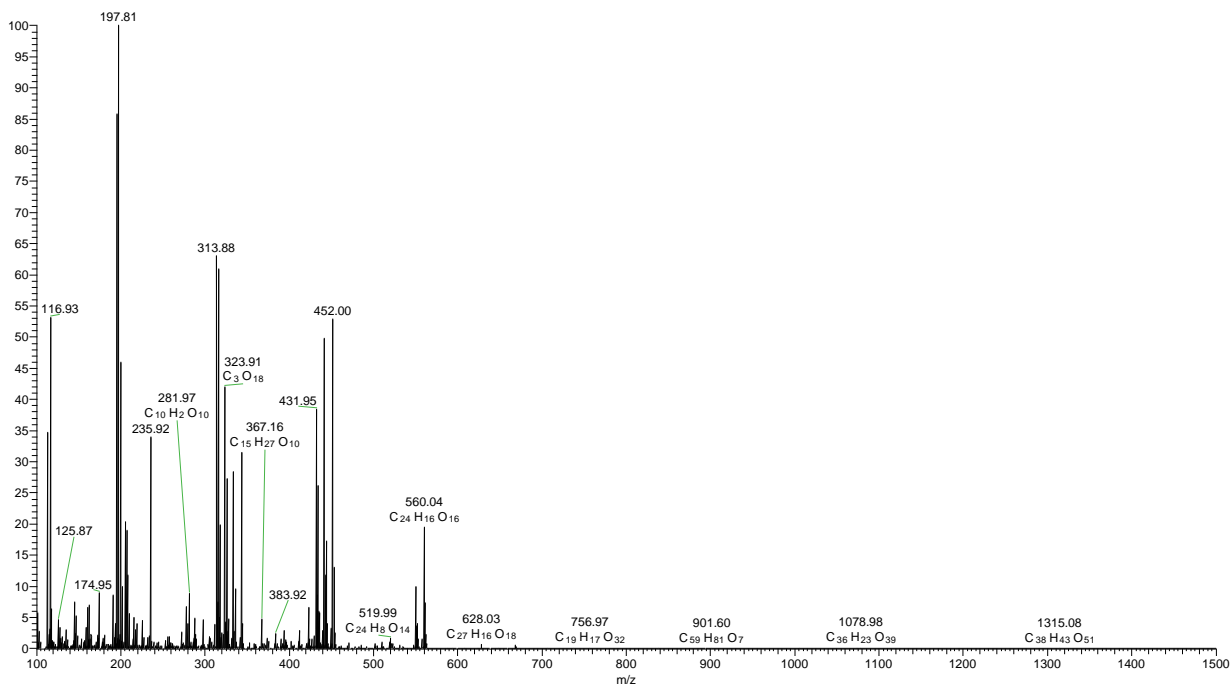


Figure S18: Relative intensity versus molecular weight divided by charge for 0% NPs after 1 day of degradation at an elution time of 1.61 minutes.

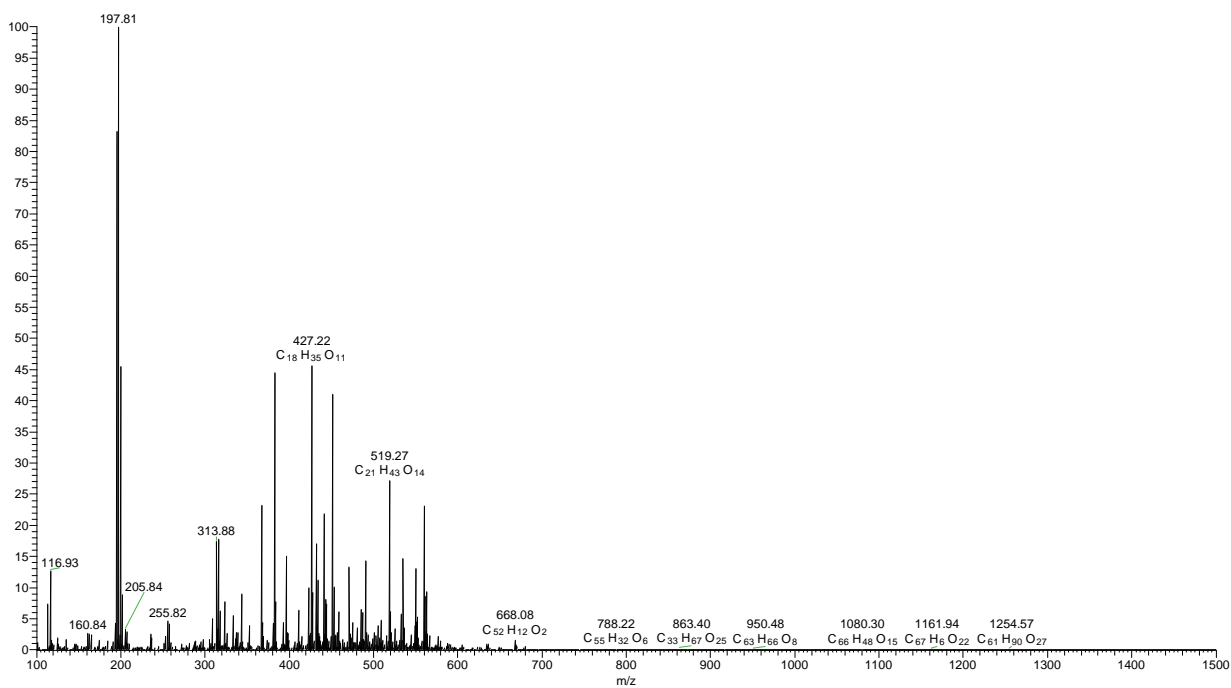


Figure S19: Relative intensity versus molecular weight divided by charge for 0% NPs after 14 days of degradation at an elution time of 1.61 minutes.

The degradation products of the 10% NPs were very similar to those from the 20% NPs, but generally were generated at a later time point. This is demonstrated qualitatively from the comparison of their elution curves from the 7-day time point from the 20% NPs and the 14-day time point from the 10% NPs, shown below.

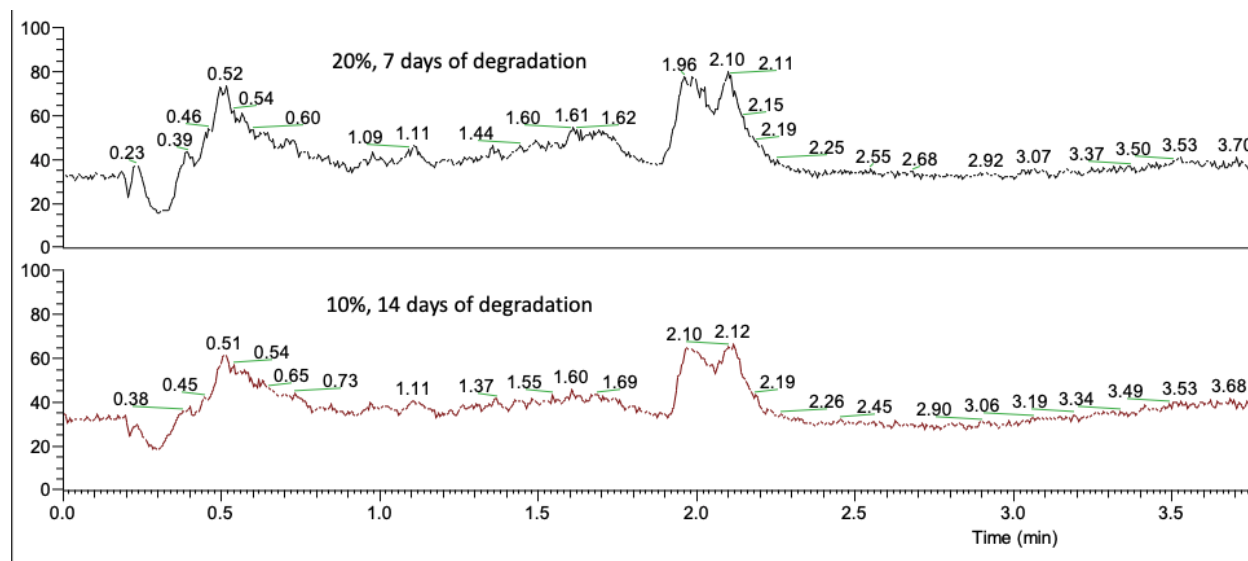


Figure S20: Signal vs elution time for 20% NPs after 7 days and for the 10% NPs after 14 days of degradation.

Normalized BMM Viability for 10 μ g/ml NP Concentrations

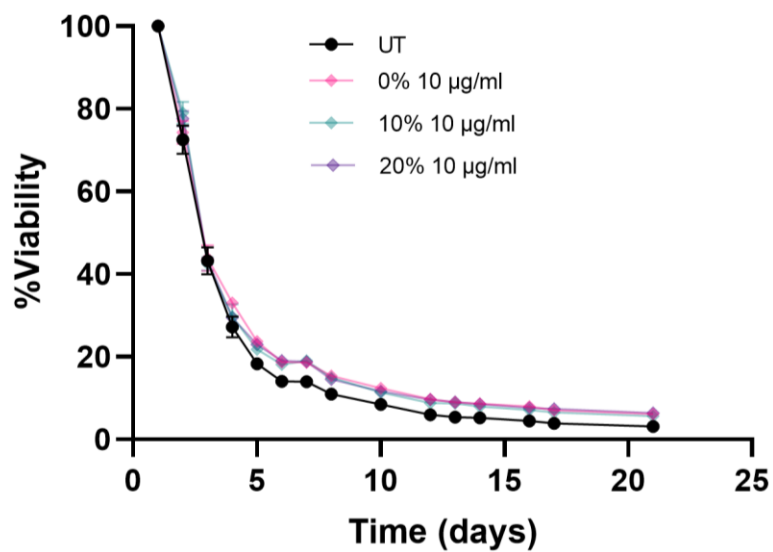


Figure S21: Normalized cell counts over time of BMMs treated with 10 μ g/ml of 0%, 10%, and 20% NPs ($N=8$).

Representative Flow Cytometry Gating

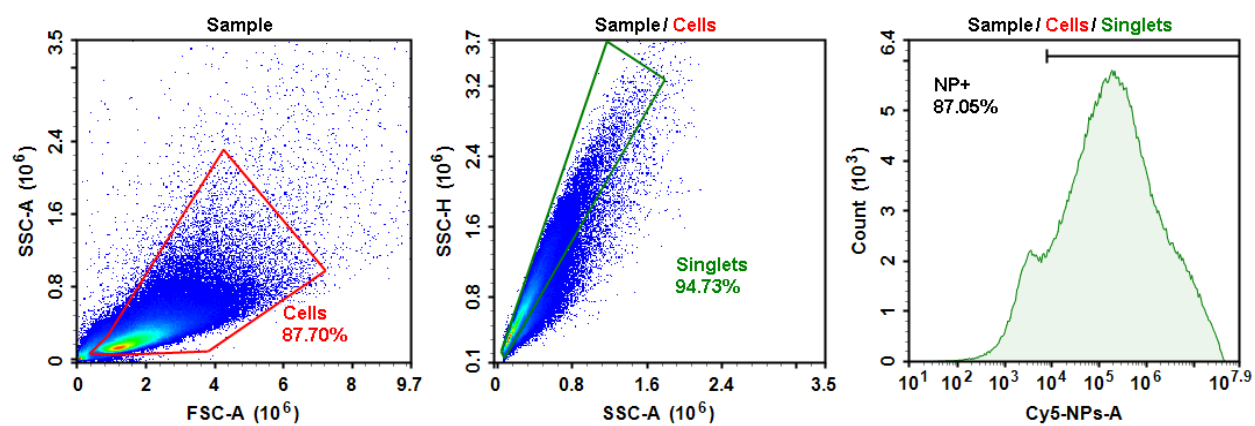


Figure S22: Representative Flow Cytometry Gating. Events are gated to remove debris and isolate singlet populations. Cy5 gating was performed to identify %NP+ cells for subsequent uptake analysis.

CD80 Expression

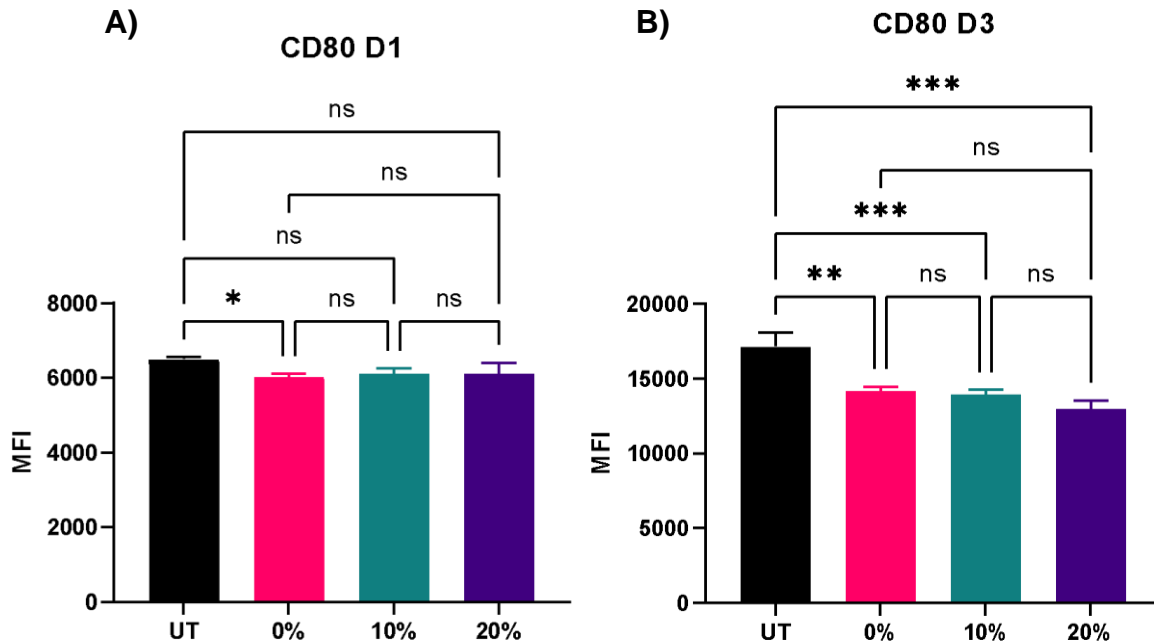


Figure S23: Expression of CD80 costimulatory of BMMs treated with 100 $\mu\text{g/ml}$ of 0%, 10%, and 20% NPs **A)** 24 h **B)** 72 h following treatment. * $p<0.01$, ** $p<0.01$, *** $p<0.001$, ns=not significant using Tukey's multiple comparisons tests as part of a one-way ANOVA ($N=3$). Error bars represent SEM.

IL-10 Secretions in BMM Supernatants

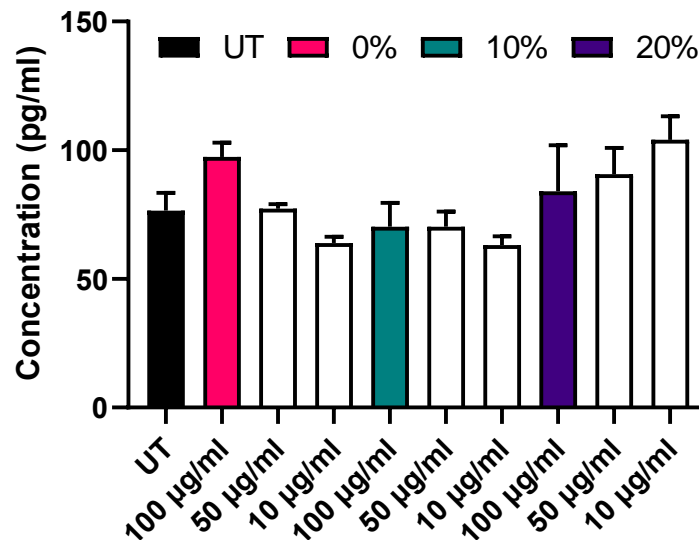


Figure S24: IL-10 concentrations of BMM supernatants 72 h following treatment with 0%, 10%, and 20% NP formulations. $p>0.05$ compared to untreated using Tukey's multiple comparisons tests as part of a one-way ANOVA ($N=3$). Error bars represent SEM.