**Supplementary Information**

**Phosphonium hydrogels for controlled release of ionic cargo**

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General Materials and Procedures

PEGDMA was synthesized as previously reported. Solvents were purchased from Caledon Laboratory Chemicals (Georgetown, ON, Canada). Deuterated solvents were purchased from Cambridge Isotopes Laboratories (Tewskbury, MA, USA). Phosphines were supplied by Cytec Solvay (Niagara Falls, ON, Canada). Igracure 2959, tributylamine, 4-vinylbenzyl chloride and fluorescein sodium salt was purchased from Sigma-Aldrich and used as received. Triethylamine, methacrylic anhydride and diclofenac sodium salt were purchased from Alfa Aesar and used as received. Neutral alumina was purchased from Fisher Chemical and used as received. Nuclear Magnetic Resonance (NMR) spectroscopy was conducted on a Bruker AvIII HD 400 MHz Spectrometer ($^1$H 400.09 MHz, $^{31}$P{$^1$H} 161.82 MHz, $^{13}$C{$^1$H} 100.5 MHz). All $^1$H and $^{13}$C{$^1$H} NMR spectra were referenced relative to the residual solvent peak (H$_2$O: $^1$H d = 4.79). All $^{31}$P{$^1$H} NMR spectra were referenced using an external standard (85% H$_3$PO$_4$: $^{31}$P d = 0).

Coupling constants (J) are expressed in Hertz (Hz). Fourier transform infrared (FTIR) spectroscopy was conducted using a Perkin Elmer FT-IR Spectrum Two Spectrometer (Waltham, MA, USA) in the universal attenuated total reflectance mode (UATR), using a diamond crystal as well as the UATR sampling accessory (part number L1050231). Differential scanning calorimetry (DSC) was performed on a DSC Q20 TA Instruments (Waters, New Castle, DE, USA) at a heating rate of 10 °C per minute, under an N$_2$ atmosphere, in an aluminum Tzero™ pan with approximately 5 mg of sample. The melting temperature ($T_m$) and crystallization temperature ($T_c$) was determined from the second heating cycle. Thermogravimetric analysis (TGA) was completed on a Q600 SDT TA Instruments and analyzed using TA Universal Analysis, under an N$_2$ atmosphere at a heating rate of 10 °C per minute up to 800 °C using a ceramic pan with approximately 5 mg of sample. The onset
degradation temperatures were determined as the temperature at which 3% mass loss had occurred. UV-visible spectroscopy was conducted on a Tecan Infinite M1000 Pro plate reader. Costar 96 well UV plates (#3635) with UV transparent flat bottoms were used. Mass spectrometry was completed using a PE-Sciex API 365 instrument using electrospray ionization in positive mode. Curing of the hydrogels was performed in a UV-box from UV Process Supply Inc. (Chicago, IL, USA) equipped with a Mercury Bulb with an energy density of UVA (0.031 mJ cm\(^{-2}\)) and UVV (164 mJ cm\(^{-2}\)). This was determined by a PP2-H-U Power Puck II purchased from EIT Instrument Markets (Sterling, VA, USA). Scanning electron microscopy (SEM) was performed in the University of Western Ontario’s Nanofabrication Facility using a LEO 1530 instrument, operating at 2.0 kV and a working distance of 4-6 mm. Samples were prepared by soaking them in deionized water for 24 h, freezing them, then lyophilizing. Samples were mounted to stubs covered in carbon tape and coated in osmium using a SPI Supplies, OC-60A plasma coater.

**Monomer Synthesis**

**Synthesis of Trishydroxypropyl(4-vinylbenzyl)phosphonium chloride (T(hp)-P)**

Tris(hydroxypropyl)phosphine (13.9 g, 66.8 mmol), 4-vinylbenzyl chloride (10.8 g, 70.8 mmol), and acetonitrile (30 mL) were combined in a pressure tube with a stir bar under N\(_2\) atmosphere and heated at 60 °C for 16 hours. A solid precipitated out of solution and was filtered and washed with acetonitrile, yielding a white powder (19.0 g, 79%).

\(^1\)H NMR (400 MHZ, D\(_2\)O): \(\delta = 7.42\) (d, \(^3\)J\(_{H-H}\) = 8 Hz, 2H; CH) 7.20 (d, \(^3\)J\(_{H-H}\) = 8 Hz, 2H; CH), 6.66 (dd, \(^3\)J\(_{H-H}\) = 17.6 Hz (trans), \(^3\)J\(_{H-H}\) = 10.8 Hz (cis), 1H; CH), 5.77 (d, \(^3\)J\(_{H-H}\) 1H; CH) = 16 Hz, 5.25 (d, \(^3\)J\(_{H-H}\) = 8 Hz, 1H; CH), 3.615 (d, \(^2\)J\(_{P-H}\) = 12 Hz, 2H; CH\(_2\)), 3.55 (t, \(^3\)J\(_{H-H}\) 6H; CH\(_2\)) = 12 Hz, 2.32 (m, 6H, CH\(_2\)), 2.32 (m, 6H, CH\(_2\)), 1.70 (m, 6H, CH\(_2\)).
\[^{31}\text{P}\{^{1}\text{H}\}\text{ NMR (161.82 MHz, D}_2\text{O)}: \delta = 36.41 (s), 34.37 (s), 33.90 (s)\]

\[^{13}\text{C}\{^{1}\text{H}\}\text{ NMR (100.5 MHz, D}_2\text{O):} \delta = 137.55 (d, ^3J_{\text{P-C}} = 4 \text{ Hz}), 135.82 (d, ^5J_{\text{P-C}} = 2 \text{ Hz}), 130.32 (d, ^2J_{\text{P-C}} = 5 \text{ Hz}), 127.33 (d, ^4J_{\text{P-C}} = 9 \text{ Hz}), 127.25 (d, ^6J_{\text{P-C}} = 3 \text{ Hz}), 115.43 (d, ^7J_{\text{P-C}} = 2 \text{ Hz}), 60.94 (d, ^3J_{\text{P-C}} = 17.1 \text{ Hz}), 25.66 (d, ^3J_{\text{P-C}} = 45.2 \text{ Hz}), 23.52 (d, ^2J_{\text{P-C}} = 4 \text{ Hz}), 14.87 (d, ^1J_{\text{P-C}} = 49.2 \text{ Hz}).\]

\text{ATR-FTIR (cm}^{-1} \text{ ranked intensity): 614 (3), 862 (4), 910 (5), 1057 (1), 1410, 1511 (6), 2906 (7), 2938 (8), 3288 (2)\]

\text{ESI-MS (m/z): ESI+: 325.2 ([C}_{18}\text{H}_{29}\text{O}_3\text{P}])^{+}, 685.4 [C_{36}\text{H}_{58}\text{O}_6\text{P}_2\text{Cl}]^{+}\]

**Figure S1.** \(^{1}\text{H}\) NMR spectrum of T(hp)-P monomer (400 MHz, D\textsubscript{2}O).
**Figure S2.** $^{31}P\{^1H\}$ NMR spectrum of $T(hp)$-P monomer (161.8 MHz, D$_2$O).

**Figure S3.** $^{13}C\{^1H\}$ NMR spectrum of $T(hp)$-P monomer (100.5 MHz, D$_2$O).
Synthesis of Tri-\textit{n}-butyl(4-vinylbenzyl)phosphonium chloride (Bu-P)

Tri-\textit{n}-butylphosphine (10.9 g, 53.9 mmol) and 4-vinylbenzyl chloride (8.63 g, 56.5 mmol) were dissolved in CH\textsubscript{3}CN (50 mL) under an N\textsubscript{2} atmosphere in a pressure tube and stirred at 70 °C for 16 hours. The solvent was then removed \textit{in vacuo}. The resulting oil was dissolved in a minimal amount of CH\textsubscript{2}Cl\textsubscript{2} (15 mL) and then precipitated in cold Et\textsubscript{2}O (500 mL) and left to sit overnight in the freezer. The precipitate was then filtered and dried \textit{in vacuo} yielding a white powder (14.34 g, 75%). Spectral data agreed with those previously reported.\textsuperscript{2} \textsuperscript{1}H and \textsuperscript{31}P\{\textsuperscript{1}H\} NMR spectra are included for reference here.

\textbf{Figure S4.} \textsuperscript{1}H NMR spectrum of Bu-P monomer (400 MHz, D\textsubscript{2}O).
Figure S5. $^{31}$P${}^{1}$H NMR spectrum of Bu-P monomer (161.8 MHz, D$_2$O).

Synthesis of Triphenyl(4-vinylbenzyl)phosphonium chloride (Ph-P)

Triphenylphosphine (3.18 g, 12.1 mmol), 4-vinylbenzyl chloride (2.03 g, 13.3 mmol), and acetonitrile (30 mL) were combined in a pressure tube with a stir bar under N$_2$ atmosphere and heated at 60 °C for 72 hours. The solvent was then removed in vacuo. The resulting solid was dissolved in minimal dichloromethane (10 mL) and precipitated in diethylether (500 mL). The precipitate was filtered, washed with ether, and dried in vacuo yielding a white powder (4.424 g 88 %). Spectral data agreed with those previously reported.$^2$ $^1$H and $^{31}$P${}^{1}$H NMR spectra are included here for reference.
Figure S6. $^1$H NMR spectrum of Ph-P monomer (400 MHz, CDCl$_3$).

Figure S7. $^{31}$P{$^1$H} NMR spectrum of Ph-P monomer (161.8 MHz, CDCl$_3$).
Synthesis of Tri-\(n\)-butyl(4-vinylbenzyl)ammonium chloride (Bu-N)

Tributylamine (15 g, 80.9 mmol) and 4-vinylbenzyl chloride (12.35 g, 80.9 mmol) were dissolved in CH\(_3\)CN (25 mL) in a round bottom and stirred at 50 °C for 48 hours. The solvent was then removed \textit{in vacuo}. The resulting oil was then washed with ethyl acetate to produce a white solid (22.31 g, 82%). Spectral data agreed with those previously reported.\(^3\) A \(^1\)H NMR spectrum is included for reference here.

\[\text{Figure S8.} \quad \text{\(^1\)H NMR spectrum of Bu-N monomer (400 MHz, D}_2\text{O).}\]
Hydrogel Preparation

Synthesis of Tris(hydroxypropyl)(4-vinylbenzyl)phosphonium chloride (T(hp)-P) hydrogel

PEGDMA (1.22 g, 89 wt%), tris(hydroxypropyl)(4-vinylbenzyl)phosphonium chloride (0.140 g, 10 wt %), and Igracure 2959 (0.014 g, 1 wt %) were sonicated and dissolved in 3.75 mL of water. 300 µL of the formulation was then pipetted into round teflon molds with a diameter of 1 cm. The teflon molds were then placed into a closed curing box with a glass lid and purged with nitrogen to create an inert atmosphere. The box containing the teflon molds was then moved into the UV-curing box and irradiated for 30 minutes. The hydrogels were then taken out and cut into pieces. 

\[ T_m = 68 \, ^\circ C; \quad T_c = 31 \, ^\circ C; \quad \text{Onset Decomposition Temperature:} \quad 240 \, ^\circ C.\]

Synthesis of Tri-n-butyl(4-vinylbenzyl)phosphonium chloride (Bu-P) hydrogel

PEGDMA (1.50 g, 89 wt%) and tri-n-butyl(4-vinylbenzyl)phosphonium chloride (0.172 g, 10 wt %) and Igracure 2959 (0.017 g, 1 wt %) were sonicated and dissolved in 4.6 mL of water. The hydrogels were then prepared as described above for T(hp)-P. \[ T_m = 60 \, ^\circ C; \quad T_c = 40 \, ^\circ C; \quad \text{Onset Decomposition Temperature:} \quad 242 \, ^\circ C.\]

Synthesis of Triphenyl(4-vinylbenzyl)phosphonium chloride (Ph-P) hydrogel

PEGDMA (1.57 g, 89 wt%) and triphenyl(4-vinylbenzyl)phosphonium chloride (0.182 g, 10 wt %) and Igracure 2959 (0.018 g, 1 wt %) were sonicated an dissolved in 4.8 mL of water. The hydrogels were then prepared as described above for T(hp)-P. 

\[ T_m = 61 \, ^\circ C. \quad T_c = 39 \, ^\circ C. \quad \text{Onset Decomposition Temperature:} \quad 237 \, ^\circ C.\]

Synthesis of Tri-n-butyl(4-vinylbenzyl)ammonium chloride (Bu-N) hydrogel

PEGDMA (0.817 g, 89 wt%) and tributyl(4-vinylbenzyl)ammonium chloride (0.092 g, 10 wt %) and Igracure 2959 (0.009 g, 1 wt %) were sonicated an dissolved in 2.5 mL of water. The hydrogels were then prepared as described above for T(hp)-P. 

\[ T_m = 59 \, ^\circ C. \quad T_c = 45 \, ^\circ C. \quad \text{Onset Decomposition Temperature:} \quad 233 \, ^\circ C.\]
Temperature: 180°C.

**Gel Content Measurements**

Gels were prepared as described above, then cut into pieces with dry weight of 20-30 mg. Each piece was dried immediately under vacuum to remove any water from the initial formulation. The mass of each hydrogel was then recorded and each of the hydrogels was swelled in deionized water (25 mL) for 4 days, replacing the water every 24 h. This was done to remove any unreacted starting material from the hydrogels. The hydrogels were then taken out of solution and dried under vacuum. The hydrogels were weighed again. The percent mass remaining relative to the initial mass was determined to be the gel content. Each hydrogel was measured in triplicate and the results reported as the mean ± standard deviation.

**Buffer Preparation**

A 0.1 M citric acid/ 0.2 M disodium phosphate buffer was chosen as it covers a wide range from pH 2.2 to pH 8.0. Citric Acid (0.1 M) and 0.2 M disodium phosphate were combined in such volumes to make 20 mL of buffered solutions. To make buffered solutions at pH 5, 6 and 7.4 the following volumes of each were added:

**Table S1.** Volumes required of 0.1 M citric acid/ 0.2 M disodium phosphate to create the buffer

<table>
<thead>
<tr>
<th>pH required</th>
<th>0.2 M Na₂HPO₄ (mL)</th>
<th>0.1 M citric acid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10.30</td>
<td>9.70</td>
</tr>
<tr>
<td>6</td>
<td>12.63</td>
<td>7.37</td>
</tr>
<tr>
<td>7.4</td>
<td>18.17</td>
<td>1.83</td>
</tr>
</tbody>
</table>
Hydrogel Swelling Measurements

The swelling of each of the hydrogels was characterized. The swelling of each was measured by comparing the swelled weight ($m_s$) to the dried weight ($m_i$; Eqn. 1).

$$\text{Swelling} \% = \left( \frac{m_s - m_i}{m_i} \right) \times 100\%$$

Each hydrogel was initially dried to remove any residual water and weighed. Each respective hydrogel was then placed in either the buffered solutions (pH 5, 6, 7.4) or DI water. Hydrogels were taken out at specific time intervals, placed in-between two paper towels to remove any residual water on the surface and weighed.

Figure S9. Swelling of each of the hydrogels in buffered solutions at pH= 5, 6, 7.4 and DI water

A) Bu-P; B) T(hp)-P; C) Ph-P; D) Bu-N.
Figure S10: SEM images of hydrogels prepared from A) T(hp)-P; B) Ph-P; C) Bu-P D) Bu-N.

Samples were prepared by the lyophilisation of gels that were swelled in deionized water, followed by coating with osmium.

**Loading/Release Studies**

Hydrogels were initially placed in either 1 wt% (in DI water) fluorescein sodium salt, diclofenac sodium salt or tryptophan for 24 hours to allow time for anion exchange to occur. Hydrogels were then placed into deionized water to release any unbound molecules. When no further release occurred (as monitored by UV-visible spectroscopy, Figure S11), the washing of unbound molecules was deemed complete.
Hydrogels were placed into solutions containing 25 mL of water, 0.1 citric acid/ 0.2 M sodium phosphate buffer (pH= 5, 6, 7.4), 0.1 M NaCl or a 1 wt% solution of cetytrimethylammonium chloride (CTAC) at 37 °C. Aliquots of 150 µL were then taken at specific time points and pipetted into the 96 well plates. The solutions were changed at each time point. To determine the total number of molecules in the hydrogel, CTAC was used to anion exchange with fluorescein sodium salt and 0.1 M NaCl was used to anion exchange with diclofenac and tryptophan. Calibration curves were prepared for each drug in each release medium (Figure S18-S20).

**Figure S11.** Release of anionic molecules during washing with deionized water to confirm that any non-ionically bound molecules had been removed prior to the release studies. When no further release occurred, the washing of unbound molecules was deemed complete. A) fluorescein sodium salt B) diclofenac sodium salt C) tryptophan in DI water.
**Figure S12.** Bu-P hydrogel (top) and PEGDMA hydrogel (bottom). Hydrogel Bu-P retained its deep red colour after washing, whereas the control PEGDMA gel did not retain any detectable dye.

**Figure S13.** Release rates of fluorescein from hydrogels: A) T(hp)-P; B) Bu-P; C) Ph-P; D) Bu-N.
Figure S14. Release rates of diclofenac from hydrogels: A) T(hp)-P; B) Bu-P; C) Ph-P; D) Bu-N.

Drug Loading Capacity Measurements

Drug loading capacity (%) was calculated by the equation:

\[
\text{Drug Loading Capacity (\%)} = \frac{\text{mass of encapsulated drug}}{\text{dry mass of hydrogel used for encapsulation}} \times 100\%
\]

This procedure was based on a previously reported method. Each hydrogel was measured in triplicate and the results reported as the mean ± standard deviation.
Evaluation of Cell Growth on Films

C2C12 mouse myoblast cells were cultured in growth medium composed of Dulbecco’s Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and supplemented with 1% Glutamax (100×) solution and antibiotics (Penicillin and Streptomycin, 100 units/mL each). Glass coverslips were placed into each well of a 12-well culture plate. The glass coverslips and hydrogels, which had a thickness of 170 µm and surface area of 2.25 cm², were then sterilized with 70% ethanol for 30 minutes. The ethanol was aspirated and the hydrogels were exposed to UV light for a 2-3 hours. The hydrogels were conditioned overnight in Hank’s Balanced Salt Solution (HBSS, 0.5 mL). The HBSS was then aspirated and the cells (2000 per well) were added directly onto the surfaces or control coverslip, and placed in the incubator for 2 hours. 0.5 mL of media were added to each well and the systems were incubated for 72 hours.

To mount and fix the cells after the 72-hour incubation, the media was removed, and each well was washed with phosphate buffered saline (PBS) (3 x 0.5 mL). Neutralized paraformaldehyde was prepared and 0.5 mL was added to each well and let stand for 10 minutes. The wells were then washed with PBS (3 x 0.5 mL) and 0.5 mL of cold acetone at -20 °C was then added to each well and let stand for 3-5 minutes. The wells were then washed with PBS (3 x 0.5 mL).

To stain the F-actin (cytoskeleton), phalloidin-Alex fluor 568 (Molecular Probes) was prepared at 20x dilution with PBS. 200 µL was added into each well and let sit for approximately 20 minutes. To stain the nucleus, 500x dilution DAPI (Molecular Probes) was added (150 µL) to each well and let stand for approximately 5 minutes. Each well was then washed with PBS (3 x 0.5 mL) and immersed in PBS for 1 hour. The hydrogels were removed with tweezers and placed face up on the slide. A drop of Prolong was the added directly on the hydrogel and coverslip and the
A coverslip was placed on top of the hydrogel. The hydrogels and coverslips were let to sit in darkness for 24 hours and the sides were sealed with nail polish.

**Figure S15.** Confocal microscopy images of C2C12 cells adhered to A) glass slide (control); B) A hydrogel containing Bu-P. C) A hydrogel containing Ph-P. The cell nuclei are stained blue with DAPI and cytoskeletons are stained red with Alexa Flour (568). Each image represents an area of 0.45 x 0.45 mm.
Cell Toxicity Assays

C2C12 mouse myoblast cells were cultured as above and then seeded in a Nunclon 96-well U bottom transparent polystyrol plate to obtain approximately 10 000 cells/well in 100 µL of DMEM containing serum, glutamax, and antibiotics as described above. The cells were allowed to adhere to the plate in a 5% CO₂ incubator at 37 °C for 24 h. Meanwhile, for the leaching assays, samples of dry hydrogel (10 mg) were immersed in cell culture medium and incubated overnight at 37 °C to enable the leaching of potentially toxic species from the hydrogels over a period of 24 h.

The growth medium was then aspirated from the cells and replaced with either solutions of sodium dodecyl sulfate (SDS) in the cell culture medium at concentrations of 0.2, 0.15, 0.10, or 0.05 mg/mL, which were used as positive controls, serial 2-fold dilutions of the leachate in culture medium, serial 2-fold dilutions of the monomer in culture medium, or fresh medium as a negative control. The cells were then incubated at 37 °C (5% CO₂) for 24 h. The medium was again aspirated and replaced with 110 µL of fresh medium containing 0.5 mg/mL (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) (MTT). After 4 h of incubation (37 °C, 5% CO₂), the MTT solution was carefully aspirated and the purple crystals were dissolved by addition of 50 µL of spectroscopic grade dimethyl sulfoxide (DMSO). After shaking (1 s, 2 mm amp, 654 rpm), the absorbance of the wells at 540 nm was read using an M1000-Pro plate reader (Tecan). The absorbance of wells prepared in the same way but without cells was subtracted as a background and the cell viability was calculated relative to wells containing cells that were exposed only to the culture medium. No (0%) cell viability was detected for cells exposed to the highest concentrations of SDS, confirming the sensitivity of the assay.
Figure S16. In vitro cytotoxicity of leachate obtained from A) \textbf{T(h)p}-P hydrogel, B) \textbf{Bu-P} hydrogel, C) \textbf{Ph-P} hydrogel, and D) \textbf{Bu-N} hydrogel as measured by MTT assays following 24 h incubations with C2C12 mouse myoblast cells. Data represent the mean and standard deviation of six replicates per concentration.
Figure S17. In vitro cytotoxicity of A) monomer T(hp)-P, B) monomer Bu-P, C) monomer Ph-P, and D) monomer Bu-N as measured by MTT assays following 24 h incubations with C2C12 mouse myoblast cells. Data represent the mean and standard deviation of six replicates per concentration.
Calibration Curves

**Figure S18.** Fluorescein sodium salt calibration curve.

**Figure S19.** Diclofenac sodium salt calibration curve.
Figure S20. Tryptophan calibration curve.
References


