

Supplementary Materials for

Modular, tissue-specific, and biodegradable hydrogel cross-linkers for tissue engineering

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Fig. S4. GPC standard curve.

Table S1. An overview of PdBT compared to several commonly used in situ hydrogel cross-linkers.

References (38–40)

Supplementary Materials

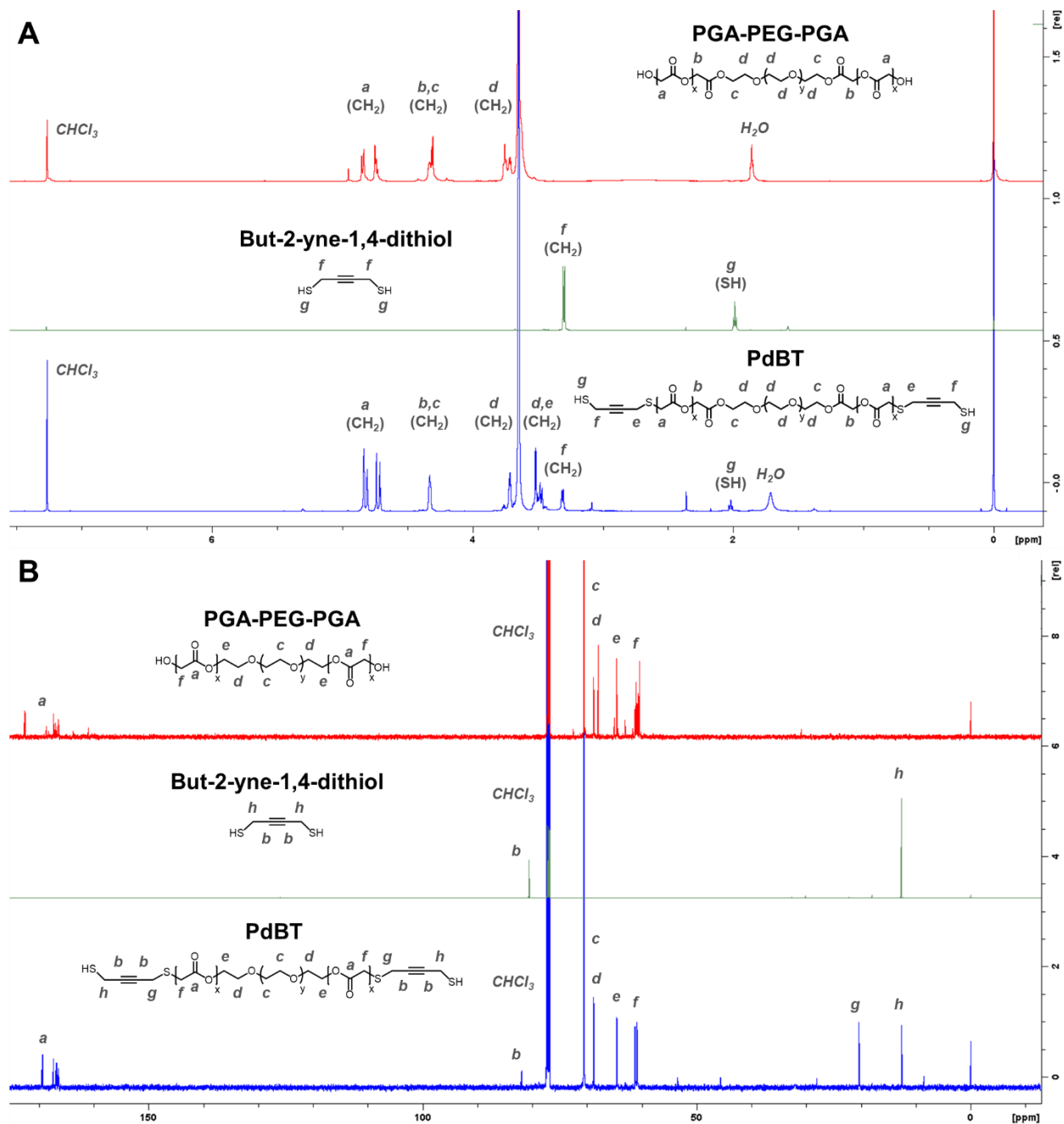
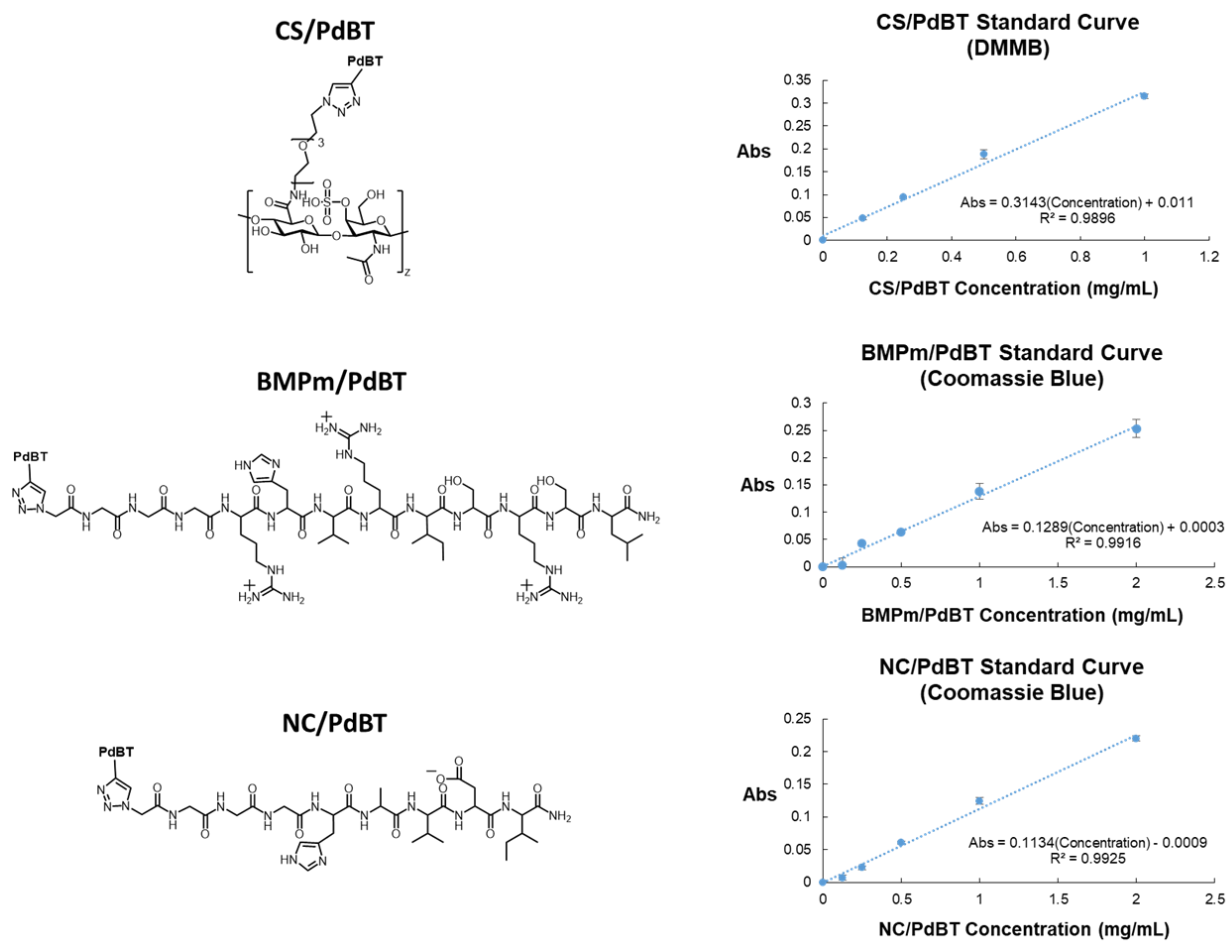


Fig. S1. Correlation of PdBT to starting materials on ^1H and ^{13}C NMR. (A) ^1H NMR spectra are compared between two constituent starting materials, PGA-PEG-PGA and but-2-yne-1,4-dithiol, and the final synthesized crosslinker, PdBT. Peaks are identified consistently between spectra using the letters a-g. (B) ^{13}C NMR spectra are compared between PdBT and starting materials, PGA-PEG-PGA and but-2-yne-1,4-dithiol. Peaks are identified consistently between spectra using the letters a-h.



	Leached Concentration (mg/mL)	% Mass Retention in Crosslinked Gel
CS/PdBT	0.134 ± 0.035	94.9 ± 1.3
BMPm/PdBT	0.348 ± 0.046	90.1 ± 1.3
NC/PdBT	0.223 ± 0.017	88.7 ± 0.9

Fig. S2. Measurement of biomolecule incorporation using DMMB and Coomassie blue assays. Assays were performed for CS/PdBT, BMPm/PdBT, and NC/PdBT using standards of known macromer concentrations in PBS at pH 7.4. Standard curves are shown (top) with points reported as means ± standard deviation for a sample size of n=3. The leached biomolecule concentrations, and their corresponding percentages of mass retention inside the hydrogels (bottom) are reported as means ± standard deviation for a sample size of n=3.

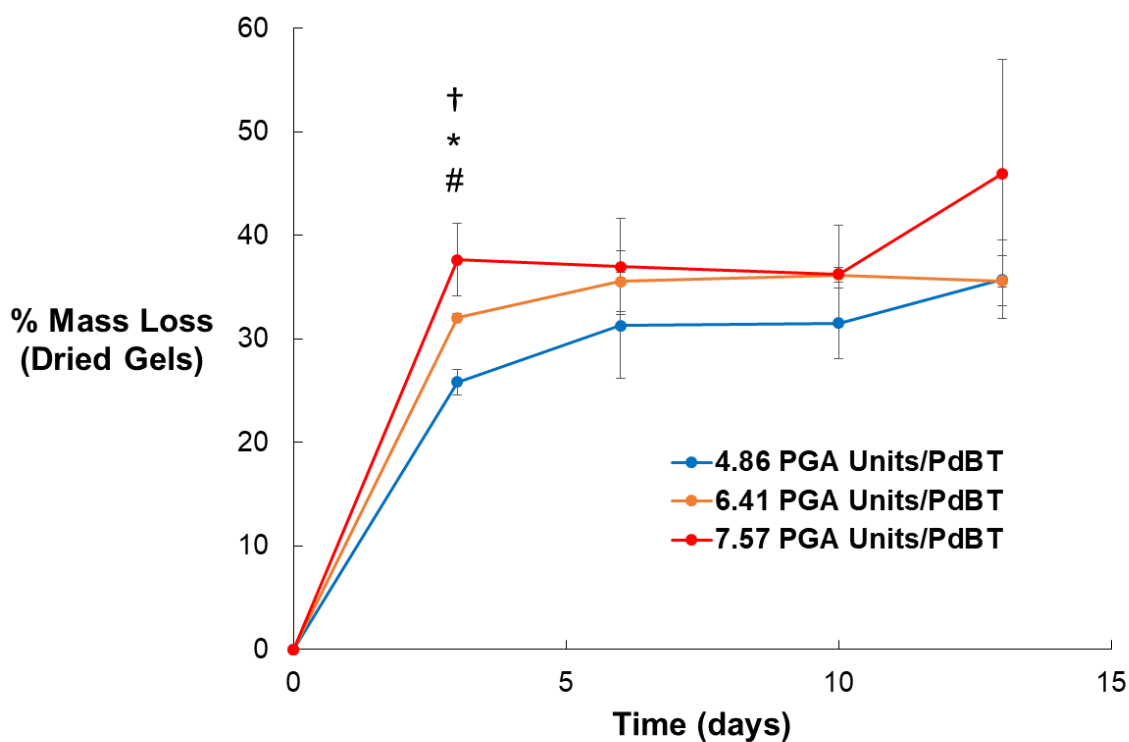


Fig. S3. Modulation of PdBT degradation kinetics by PGA block length. Accelerated degradation over a 14 day period of PdBT-crosslinked hydrogels in 0.1M HCl at 37 °C, with varying average molar number of PGA units per PdBT chain (as quantified by NMR) and 3.5% w/v PdBT. # indicates statistical significance of 4.86 PGA Units/PdBT group vs. 6.41 PGA Units/PdBT group, * indicates statistical significance of 4.86 PGA Units/PdBT group vs. 7.57 PGA Units/PdBT group, and † indicates statistical significance of 6.41 PGA Units/PdBT group vs. 7.57 PGA Units/PdBT group at a given timepoint. Data are reported as means \pm standard deviation for a sample size of n=3.

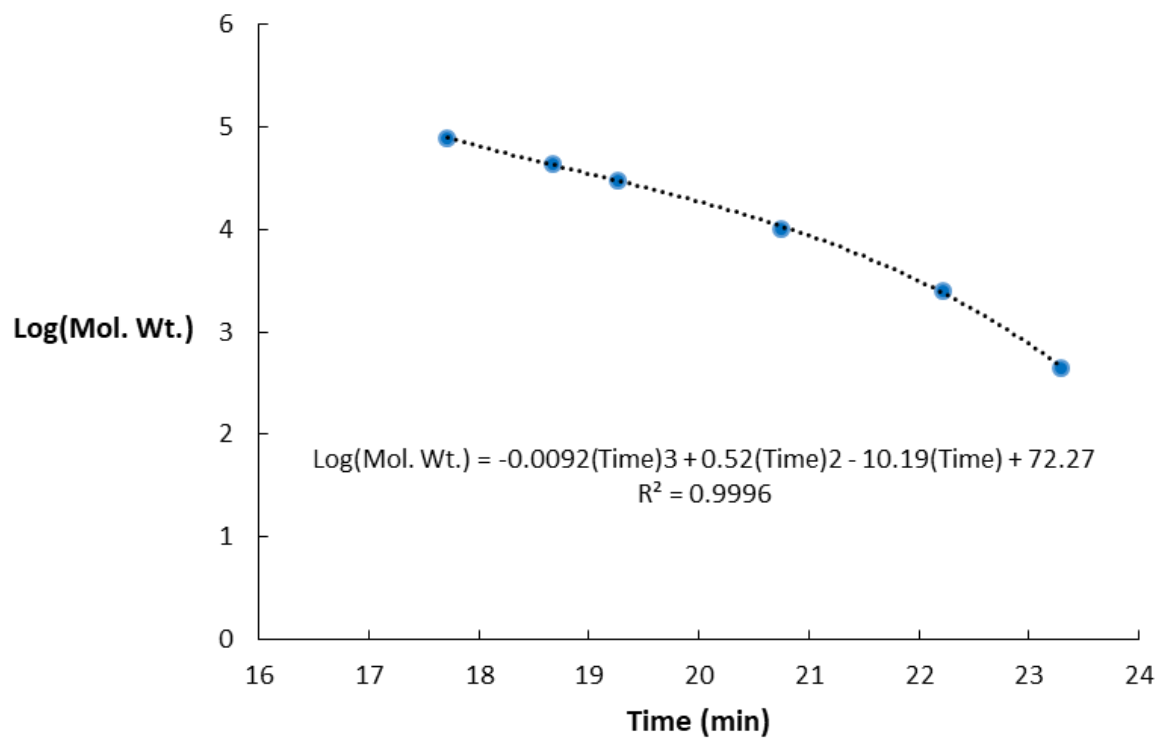


Fig. S4. GPC standard curve. Curve was obtained using narrowly dispersed 450, 2500, 10225, 30250, 44000, and 78300 Da PEG standards dissolved in 100mM NaNO₃. Points are reported as means ± standard deviation for a sample size of n=3.

Table S1. An overview of PdBT compared to several commonly used *in situ* hydrogel crosslinkers. PdBT possesses many of the favorable characteristics and requirements of traditional crosslinkers, such as cytocompatibility, hydrolytic degradability, and fast reaction kinetics, while novelly enabling tissue-specific biological cues from the crosslinker itself by its prefunctionalization with tissue-specific biomolecules such as peptides and glycosaminoglycans.

Crosslinker/ Crosslinking Method	Cytocompatibility	Degradability	Crosslinking Kinetics	Biological Cues
Linear poly(ethylene glycol) (PEG) (21, 38)	Excellent	Afforded by co- polymerization with hydrolysable units	Dependent on functional termini (usually < 60 min)	None (requires additional delivery of biomolecules)
Branched/ Multi-arm PEG (21, 38)	Excellent	Afforded by co- polymerization with hydrolysable units	Dependent on multiple functional termini (usually < 60 min)	None (requires additional delivery/entrapment of biomolecules)
Polyelectrolytes (38, 39)	Potentially cytotoxic (concentration- dependent)	Afforded if polyelectrolyte contains hydrolysable units	Fast (often < 30 min)	Occasionally, depending on base polymer
UV induced self-crosslinking (38, 40)	Dependent on selection of photoinitiators	Limited, depending on degradability of base polymer	Dependent on photoinitiator and functional groups (usually < 60 min)	Occasionally, depending on base polymer
Chemically induced self- crosslinking (38, 40)	Dependent on selection of initiators	Limited, depending on degradability of base polymer	Dependent on initiator and functional groups (usually < 60 min)	Occasionally, depending on base polymer
PdBT	Excellent	Provided by poly(glycolic acid) units	< 60 min	Provided <i>in situ</i> by conjugated tissue- specific biomolecules