Supporting Information

Injectable coacervate hydrogel for delivery of anticancer drug-loaded nanoparticles *in vivo*

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Synthesis of protected guanidinium-functionalized cyclic carbonate (MTC-OCH$_2$BnBocGu)

Synthesis of (4-azidomethyl-phenyl)-methanol

4-(Chloromethyl)-benzylalcohol (5.01 g, 32 mmol) and NaN_3 (2.50 g, 38.4 mmol) were stirred in DMF (25 mL) at 90° C. for 17 h. The solvent was removed under reduced pressure and the residue was partitioned between water (50 mL) and diethyl ether (100 mL). The aqueous layer was extracted with diethyl ether (2x50 mL) and the combined organic layers were dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure to yield (4-azidomethyl-phenyl)-methanol pure enough for further reaction. Yield: 5.57 g; ^{1}H-NMR (400 MHz, $CDCl_3$, 22ºC): δ 1.96 (t, J = 5.2 Hz, 1H), 4.33 (s, 2H), 4.69 (d, J = 5.2 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H).

Synthesis of (4-aminomethyl-phenyl)-methanol

(4-Azidomethyl-phenyl)-methanol (1.96 g, 12.0 mmol) and PPh_3 (6.50g, 24.8 mmol, 2.05 eq) were dissolved in THF (25 ml) and heated at 60°C for 1 hour. Water (4.5 ml, 248 mmol, 20 eq) was added and the reaction was heated overnight. The solvent was evaporated *in vacuo* and the residue obtained was purified by flash chromatography (4:1 DCM:methanol) to yield (4-aminomethyl-phenyl)-methanol as a white solid. Yield: 1.44 g; 1H-NMR (400 MHz, $CDC1_3$, 22ºC): δ 7.35 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 4.67 (s, 2H), 3.85 (s, 2H), 1.68 (bs, 3H)

Synthesis of (4-Boc-guanidinomethyl-phenyl)-methanol

To a solution mixture of (4-aminomethyl-phenyl)-methanol (1.56 g, 11.4 mmol, 2.0 equiv) and N,N-diisopropylethylamine (3.0 mL, 17.2 mmol, 3.0 equiv) was added 1,3-bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (1.65 g, 5.8 mmol, 1.0 equiv) in 10 mL of dry CH_2Cl_2, and the mixture was left to stir overnight at room temperature. Upon reaction completion, a constant stream of nitrogen gas was bubbled through the reaction mixture for approximately 1 h so as to aid in purging of the gaseous by-product, MeSH. After the removal of residual solvent *in vacuo*, the crude product was purified by flash column chromatography using silica gel and a hexane-ethyl acetate solvent system as the eluent (gradient elution up to 50% vol. ethyl acetate) to yield the Boc-protected guanylated alcohol ((4-Boc-guanidinomethyl-phenyl)-methanol) as a white solid (2 g, 5.3 mmol, 91% yield). 1H-NMR (400 MHz, $CDCl_3$, 22° C): δ 11.53 (s, 1H, N*H*), 8.59 (s, 1H, N*H*), 7.37 – 7.27 (m, 4H, phenyl -C*H*), 4.72 – 4.60 (m, 4H, -CH_2-), 1.53 – 1.43 (m, 18H, Boc –CH_3)

Synthesis of MTC-OCH$_2$BnBocGu

Briefly, in a dry three-neck round bottom flask equipped with a stir bar, MTC-OH (1.54 g, 9.65 mmol) was dissolved in dry THF (25 mL) with 2-3 drops of dimethylformamide (DMF). A solution of oxalyl chloride (1.23 mL, 14.3 mmol) in THF (25 mL) was subsequently added from a dropping funnel. Under an inert atmosphere, the solution was stirred for 1 h, after which volatiles were removed under vacuum, yielding an off-white solid (i.e. 5-chlorocarboxy-5-methyl-1,3-dioxan-2-one intermediate). The solid was heated to 60 ºC for a brief 2-3 min to remove any residual solvent, and then re-dissolved in dry CH_2Cl_2 (25 mL) and cooled down to 0 ºC via an ice bath. A mixture of (4-aminomethyl-phenyl)-methanol (2 g, 5.3 mmol) and pyridine (775 µL, 9.65 mmol) dissolved in dry CH_2Cl_2 (25 mL) was then added dropwise over a duration

of 30 min, and allowed to stir at 0 ºC for an additional 30 min before leaving it at ambient temperature for further stirring overnight. After removal of solvent, the crude product was subjected to purification by flash column chromatography using silica gel and a hexane-ethyl acetate solvent system as the eluent (gradient elution up to 80% vol. ethyl acetate) to yield MTC-OCH_2BnBocGu as a white solid (75% yield). 1H-NMR (400 MHz, CDCl3, 22° C): δ 11.53 (s, 1H, N*H*), 8.60 (s, 1H, N*H*), 7.39 – 7.26 (m, 4H, phenyl -C*H*), 5.18 (s, 2H, -CH_2-), 4.68 (d, J = 10.9 Hz, 2H, -CH_2-), 4.61 (d, J = 5.2 Hz, 2H, -CH_2-), 4.20 (d, J = 10.8 Hz, 2H, -CH_2-), 1.50 – 1.45 (m, 18H, Boc -CH_3), 1.30 (s, 3H, -CH_3).

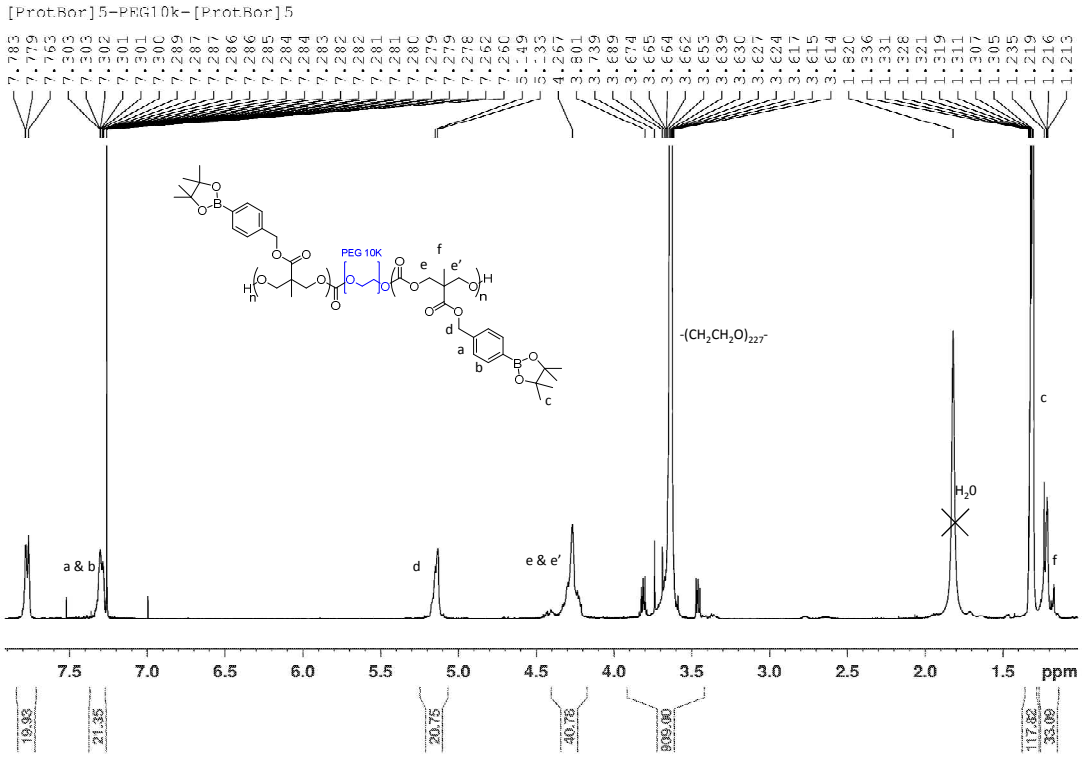

Fig. S1. ^{1}H NMR spectrum of P(ProtBor)$_5$-PEG-P(ProtBor)$_5$ (400 MHz, $CDCl_3$, 22 ºC).

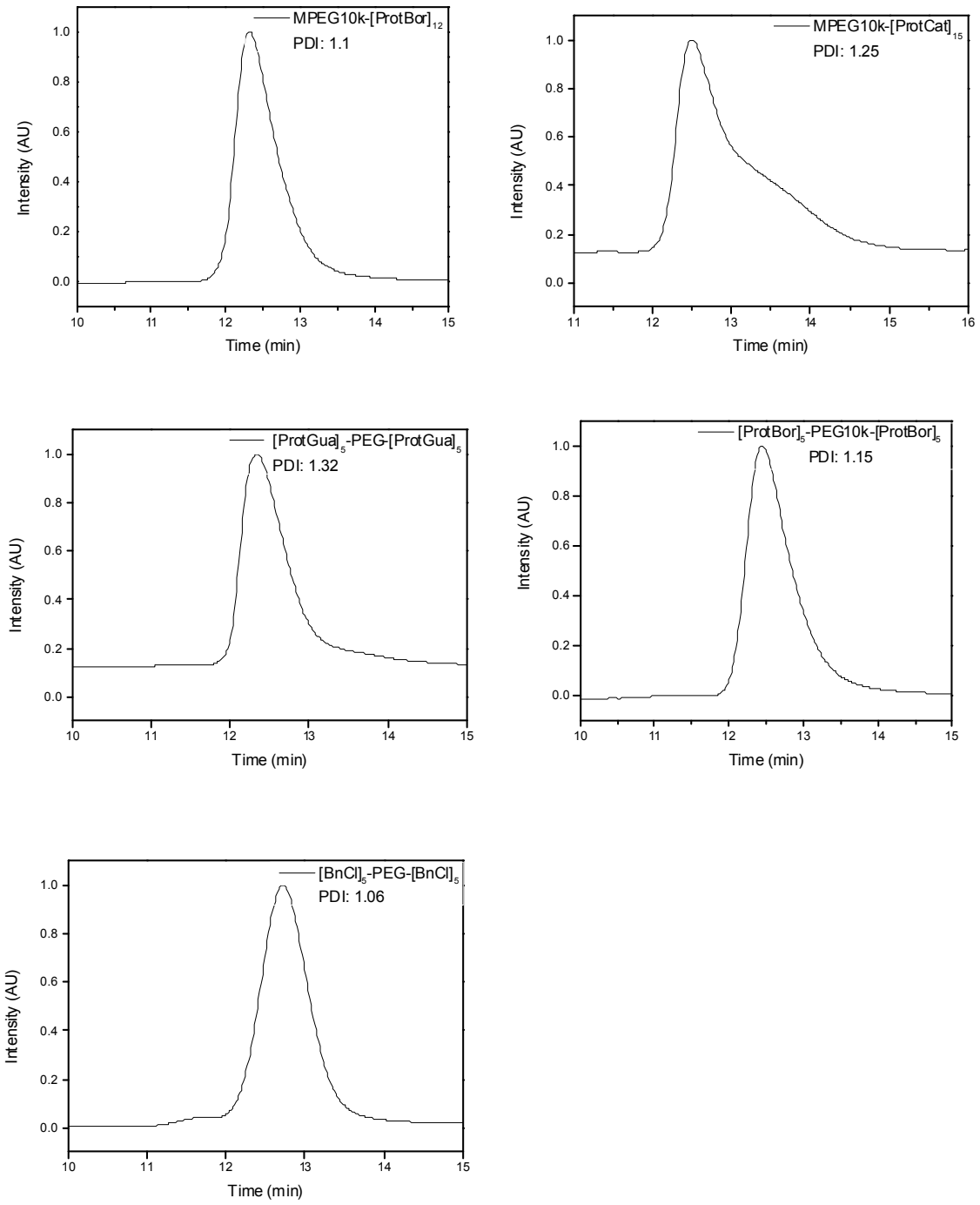

Figure S2. GPC traces of various triblock and diblock copolymers.

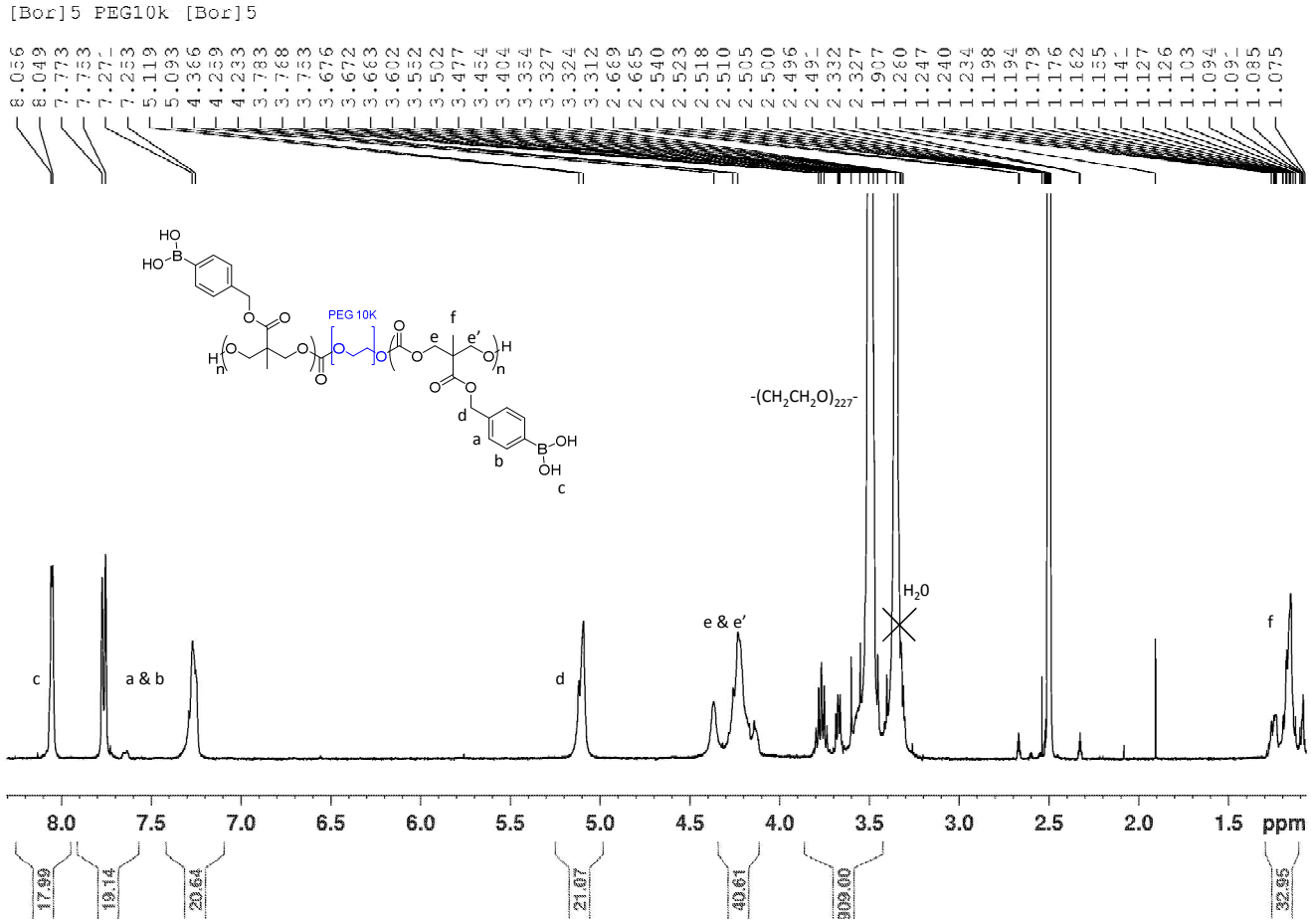

Figure S3. ^{1}H NMR spectrum of P(Bor)$_5$-PEG-P(Bor)$_5$ (400 MHz, DMSO$_d$, 22 ºC).

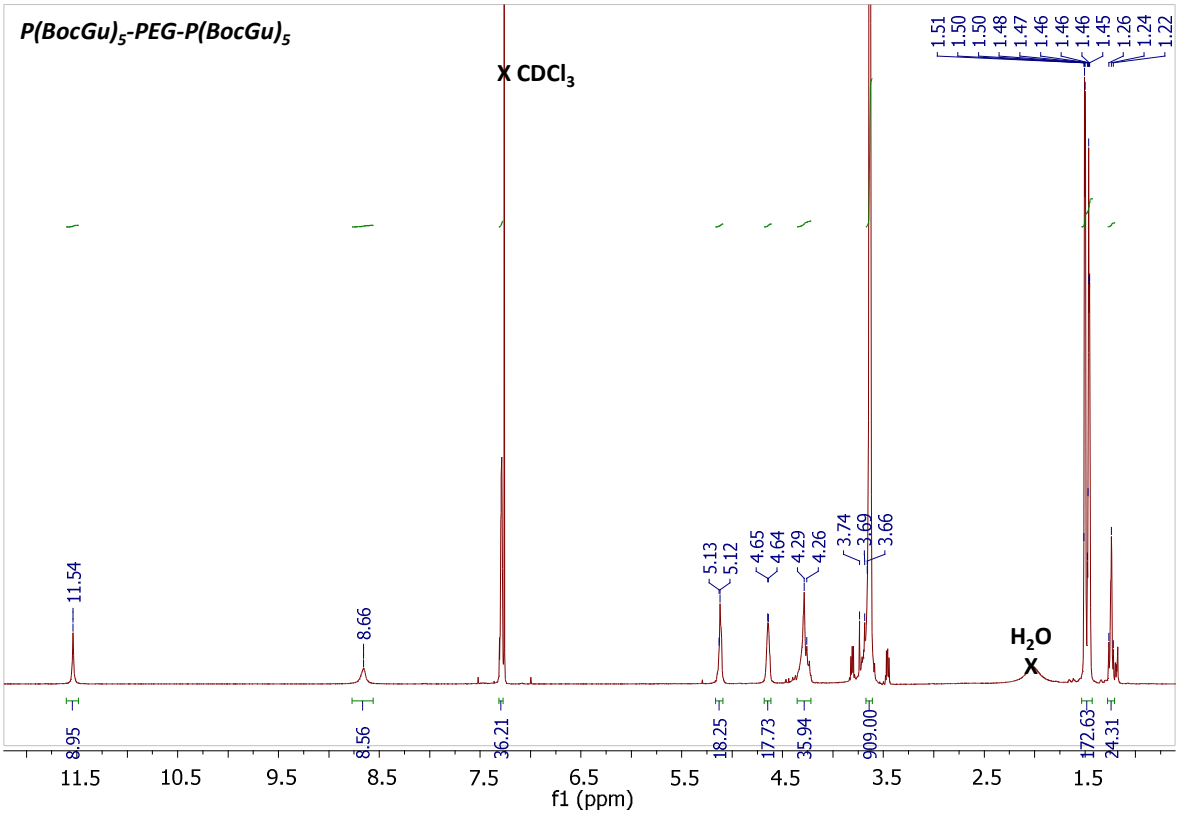

Figure S4. ^{1}H NMR spectrum of P(BocGu)$_5$-PEG-P(BocGu)$_5$ (400 MHz, $CDCl_3$, 22° C).

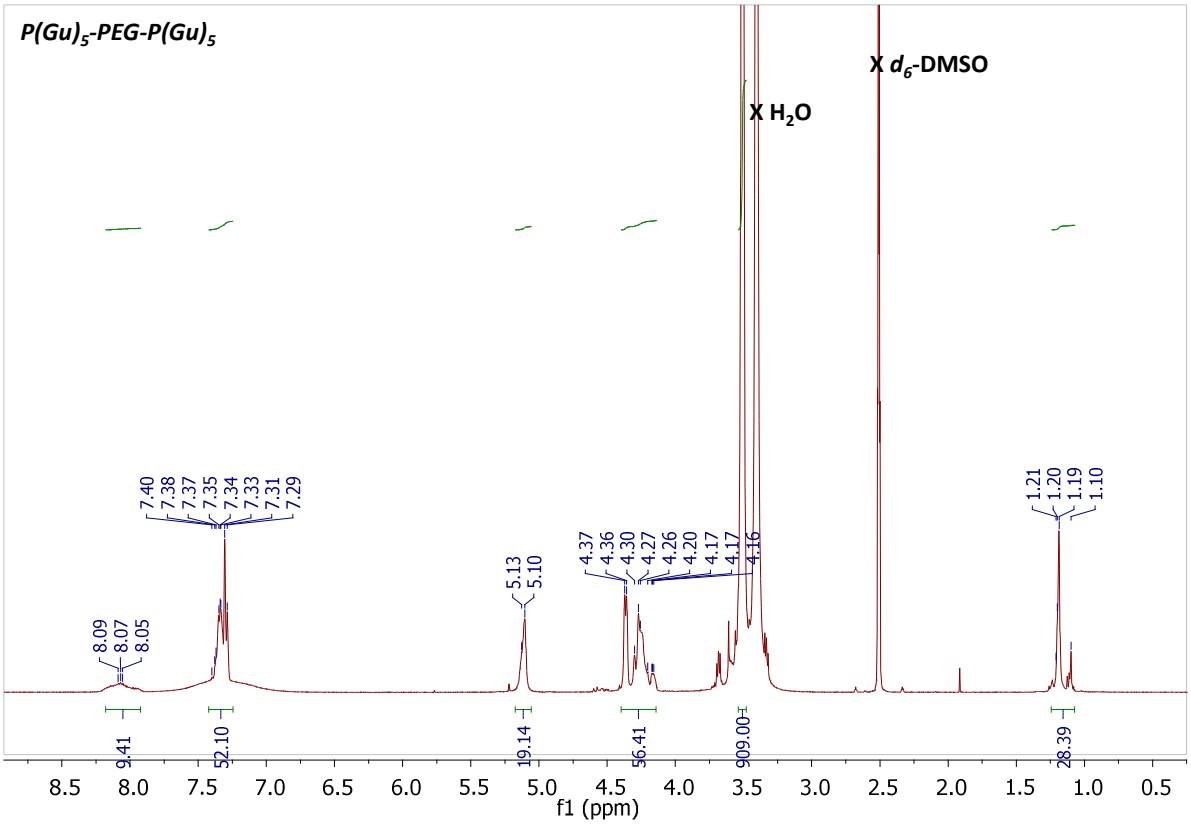

Figure S5. ^{1}H NMR spectrum of P(Gu)$_5$-PEG-P(Gu)$_5$ (400 MHz, DMSO, 22° C).

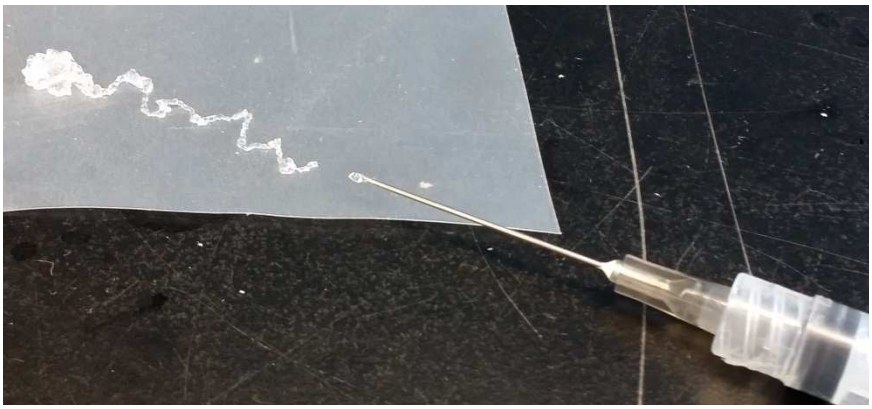

Figure S6. An image showing the injectability of BTZ-loaded micelle/(B7+G3) hydrogel composite through a 22G needle.

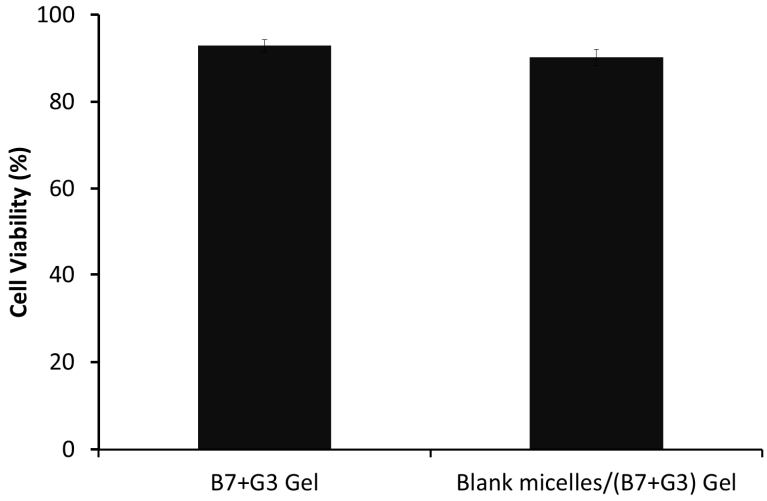

Figure S7. Viability of human dermal fibroblast (HDF) cells after 48-h incubation with blank (B7+G3) hydrogel and blank micelles/(B7+G3) hydrogel formulations at 37°C.

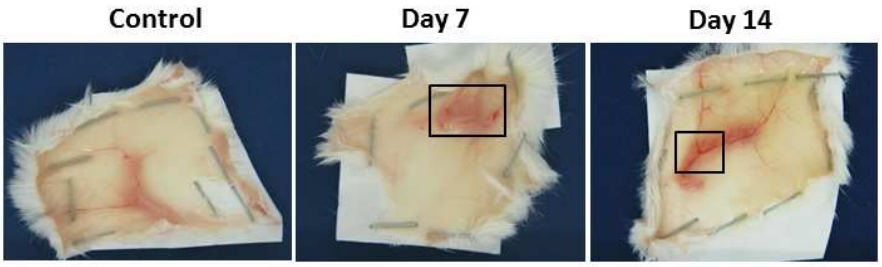

Figure S8. Images of excised skin from mice on the 7th and 14th day post injection. Boxed areas indicate the presence of the blank micelles/(B7+G3) hydrogels. With time, the gels became gradually smaller in size, thereby showing the biodegradability of the hydrogel.

Table S1. Critical aggregation concentrations (CACs) of PEG-$(Cat)_{13}$ and ABA-type triblock copolymers.

Polymer	CAC (mg/L)
$P(Bor)_5$-PEG-$P(Bor)_5$	14
$P(Th)_5$-PEG-$P(Th)_5$	66
$P(Gu)_5$-PEG-$P(Gu)_5$	490
PEG-$P(Cat)_{13}$	21