Supporting Information

Promoting cell adhesion on slippery phosphorylcholine hydrogel surfaces

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Experimental

Materials

2-Hydroxyethyl methacrylate (HEMA) was purchased from Sigma Aldrich and purified by Kugelrohr distillation at 120 °C prior to use. 2-Methacryloyloxyethyl phosphorylcholine (MPC) monomer was purchased from Sigma Aldrich and purified by precipitation prior to use. Lipoic acid, methacrylic acid, dimethylaminopyridine (DMAP), Fmoc-chloride, 6-aminocaproic acid, triisopropylsilane (TIPS), poly(ethylene glycol) diacrylate (M_n 2,000 and 700), methanol (anhydrous), dimethylsulfoxide (anhydrous), and dimethylformamide (anhydrous) were purchased from Sigma Aldrich and used as received. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC HCl) was purchased from TCI America. 2-chlorotrityl chloride resin, Fmoc-Ser(But)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, N,N-diisopropylethylamine (DIPEA), N-hydroxybenzotriazole (HOBt), O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphosphate (HBTU), trifluoroacetic acid and piperidine were purchased from Advanced Chem Tech. Dichloromethane was dried over calcium hydride and tetrahydrofuran was dried over sodium/benzophenone ketyl and freshly distilled before use. All other chemicals were used as received without further purification unless otherwise noted. Dulbecco's Modified Eagles Medium (DMEM), Penicillin and Streptomycin were purchased from Life Technologies. CellTiter-Glo Luminescent Cell Viability Reagent was purchased from Promega. McCoy's 5A medium was purchased from ATCC. Fetal Bovine Serum was purchased from Atlanta Biologicals.

Instrumentation

Nuclear magnetic resonance (NMR) spectroscopy was performed on a Brüker Spectrospin DPX300 machine. Aqueous GPC was performed in 0.1 M sodium nitrate and 0.02 weight percent sodium azide buffer against poly(ethylene oxide) calibration standards, with three Waters Ultrahydrogel columns (7.8 x 300 mm). Dynamic mechanical analysis (DMA) was performed using a Rheometrics Mechanical Spectrometer, with frequency sweeps from 0-10 Hz. Experiments were performed at room temperature on hydrogels in equilibrium swollen state. Optical microscopy was performed on a Nikon CKX41 inverted microscope and cell density measured by plate reader in luminescence mode (BMG Labtech FLUOstar OPTIMA plate reader).

Methods

Synthesis of HEMA-LA (**1**). Lipoic acid (4.00 g, 19.4 mmol) and 2-hydroxyethyl methacrylate (2.50 g, 19.4 mmol) were dissolved in 60 mL of anhydrous CH_2Cl_2 in a dry roundbottom flask. The stirring solution was cooled to 0 °C, and EDC (7.40 g, 38.8 mmol) and DMAP (2.40 g, 19.4 mmol) were added as solids. The reaction mixture was allowed to warm to room temperature, and stirred for 18 hours. The mixture was diluted with dichloromethane, and washed with 1 M $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated by rotary evaporation, to give monomer **1** as a yellow oil (4.9 g, 80 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.06 (s, 1H), 5.36 (s, 1H), 4.26 (s, 4H), 3.5 (m, 1 H), 3.11 (m, 2H), 2.40 (m, 1H), 2.3 (t, 2H), 1.87 (s, 3H), 1.35-1.70 (m, 8H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.31, 24.60, 28.70, 33.87, 34.58, 38.49, 40.21, 56.29, 61.99, 62.43, 126.10, 135.89, 167.08, 173.22 ppm.

Synthesis of N-Fmoc-amidocaproic acid. Fmoc-chloride (21.0 g, 80.2 mmol) was dissolved in 80 mL of dioxane in a roundbottom flask and cooled to 0 °C. Separately, 6-aminocaproic acid (7.0 g, 53 mmol) was dissolved in 100 mL of 5% $NaHCO_3$ aqueous solution, and added

to the sitrring dioxane solution. The reaction was warmed to room temperature, and stirred for 18 hours. The reaction was diluted with 200 mL of water, and the product was extracted with ethyl acetate. The organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by silica column chromatography, eluting with 5-10 % MeOH/CH_2Cl_2, to obtain the desired product in 76% yield (14.3 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (d, 2H), 7.61 (d, 2H), 7.32 (m, 4H), 4.43 (m, 2H), 4.22 (br, 1H), 3.21 (t, 2H), 2.39 (t, 2H), 1.78-1.21 (br, 6H). ^{13}C NMR (75 MHz, DMSO): δ = 174.35, 155.67, 143.53, 140.41, 127.34, 124.85, 119.86, 64.76, 46.39, 33.31, 28.95, 25.53, 23.97.

Synthesis of GRGDS-MA (**2**). Standard solid phase peptide synthesis procedures were used, starting from a 2-chlorotrityl chloride resin containing 1.6 mmol/g active sites. Resin (3.0 g, 4.8 mmol) was added to the reaction vessel, and 30 mL of anhydrous dichloromethane was added. The suspension was agitated with dry nitrogen pressure for 30 minutes to swell the resin. Separately, Fmoc-Ser(But)-OH (3.7 g, 9.6 mmol) was dissolved in 30 mL of anhydrous dichloromethance. DIPEA (2.47 g, 19.2 mmol) was injected to the serine solution immediately prior to addition to the reaction vessel. The peptide-resin mixture was agitated with nitrogen pressure for one hour at room temperature. The reaction mixture was filtered. CH_2Cl_2:MeOH:DIPEA (80:15:5) (30 mL) was added, and agitated with nitrogen pressure for 10 minutes, to block any unreacted active sites. The solution was filtered, and 30 mL of fresh CH_2Cl_2/MeOH/DIPEA solution was added and agitated for 10 minutes. The resin was washed with 30 mL DMF (3 x 1 minute each). The amino acid was deprotected using a 25% piperidine solution in DMF, agitating for three minutes, then exchanging for fresh solution and agitating for 20 minutes. The resin was washed with DMF (6x), CH_2Cl_2 (3x), isopropanol (3x), hexanes (6x), and once with dichloromethane, then dried under vacuum overnight. Serine loading was calculated to be 1.36 mmol/g. Aspartic acid (6.7 g, 16.32 mmol), HBTU (5.3 g, 16.3 mmol), and HOBt (2.20 g, 16.3 mmol) were dissolved in 40 mL

anhydrous DMF. DIPEA (4.20 g, 32.6 mmol) was added, and the solution was quickly transferred to the reaction vessel containing the serine-loaded resin and agitated with nitrogen pressure for 1 hour. The solution was filtered, and washed with DMF (3x), then deprotected with 25% piperidine in DMF. After filtering, the resin was washed with DMF (6x). This procedure was repeated for the additions of glycine, arginine, glycine, N-Fmoc-amidocaproic acid, and methacrylic acid. After the addition of methacrylic acid, the resin was washed with dichloromethane (6x), and then agitated for 1 hour with a 95:2.5:2.5 trifluoroacetic acid:water:triisopropylsilane solution to cleave the peptide from the resin. The solution was filtered into a dry round bottom flask; the cleavage procedure was then repeated twice. The peptide solution was concentrated to a minimal volume using rotary evaporation and precipitated into 1 L diethyl ether. The GRGDS-methacrylamide monomer **2** was recovered as a white solid by filtration and dried under vacuum (1.9 g, 45%). ^{1}H NMR (300 MHz, DMSO): δ = 7.9-8.5 (br, 8H), 5.61 (s, 1H), 5.28 (s, 1H), 4.55-4.75 (br, 3H), 4.2-4.4 (br, 2H), 3.6-3.85 (br, 6H), 3.15 (br, 4H), 3.0 (br, 1H), 2.85 (br, 1H), 2.7 (br, 1H), 2.55 (br, 2H), 2.12 (tr, 2H), 1.84 (s, 3H), 1.5 (br, 8H), 1.25 (br, 2H). ^{13}C NMR (75 MHz, DMSO): δ = 173.19, 172.27, 172.15, 171.97, 171.39, 171.11, 170.23, 169.78, 169.10, 167.85, 157.12, 140.53, 119.21, 67.08, 65.38, 55.28, 52.65, 51.06, 49.65, 42.42, 36.72, 35.53, 29.48, 29.30, 26.56, 25.38, 19.13. ESI-MS [M+H]: calculated, 672.3; found, 672.4.

Synthesis of poly(MPC-co-DHLA) (**3**). MPC (1.0 g, 3.4 mmol), HEMA-LA (218 mg, 0.69 mmol), and 2,2'-azobisisobutyrylnitrile (AIBN) (8 mg, 0.05 mmol) were added to a dry roundbottom flask. A 1:1 mixture of MeOH and DMSO (6 mL total volume) was added and the solution was purged with dry nitrogen gas. The reaction mixture was placed in a preheated oil bath at 70 °C and stirred for 4 hours. Propagation was terminated by placing the solution in liquid nitrogen, then allowing the mixture to warm while open to air. The solution was precipitated into THF to afford the polymer product as an off-white solid. This solid was

dissolved in 20 mL of degassed water, and stirred at 0 ºC. Sodium borohydride (104 mg, 2.74 mmol) was added under a stream of nitrogen. The reaction mixture was stirred at 0 ºC for 1 hour, then at 25 ºC for 1 hour. $HCl_{(conc)}$ was added to adjust the pH to ~3, and the polymer was purified by dialysis (MWCO 1,000) against methanol and water at 4 °C. Lyophilization afforded the desired copolymer **3** as a white solid. ^{1}H NMR (300 MHz, MeOD):δ = 4.4 (br, 2H), 4.3 (br, 2H), 4.1 (br, 2H), 3.75 (br, 2H), 3.0 (br, 2H), 2.75 (br, 2H), 2.5 (br, 2H), 1.5-2.1 (br, 5H), 0.8-1.1 (br, 3H). ^{13}C NMR (100 MHz, MeOD/CDCl3): δ = 177.5, 66.1, 62.9, 59.3, 53.8, 45.0, 44.7, 42.7, 39.0, 38.4, 33.6, 26.4, 24.4, 21.8, 18.6, 16.7. Aqueous GPC (0.2 M $NaNO_3$ + 0.01 % NaN_3; PEO standards): M_n, 64,200 g/mole; PDI, 4.4. This general procedure was used for all of the GRGDS-containing polymers, adding the desired amount of oligopeptide comonomer at the outset of the polymerization.

Poly(MPC-co-DHLA) hydrogel preparation (**4**). Stock solutions of poly(MPC-co-DHLA) (with and without the GRGDS peptide) were prepared at a concentration of 100 mg/mL in pH 9 sodium borate buffer. Separately, a stock solution of $PEG_{2000}DA$ cross-linker was prepared at a concentration of 180 mg/mL in sodium borate buffer. The poly(MPC-co-DHLA) and PEGDA solutions were combined to give a [SH]:[acrylate] ratio of 1:1, then heated to 37 °C for 20 minutes. The resulting hydrogels were swelled in pure water or PBS, which was changed several times to remove any uncross-linked material. The equilibrium water content (EWC) was determined by comparing the weight of the gel after swelling in water for 3 days to the weight of the dry gel. Equation **1** was used to determine EWC (as a percent):

$$\text{EWC (\%)} = \left(1 - \left(\frac{Wd}{Ws}\right)\right) \times 100 \qquad \textbf{(1)}$$

where W_s and W_d are the weights of the swollen and dried gels, respectively. Excess water was removed from the hydrogel by gently wicking with filter paper. Dynamic mechanical analysis was used to characterized the physical properties of the hydrogels. PolyMPC-*co*-

DHLA hydrogels were prepared with $PEG_{700}DA$ as the cross-linker, with a polyMPC-*co*-DHLA concentration of 50 mg/mL in pH 9 borate buffer. The hydrogel samples were swelled to equilibrium for 48 hours. Frequency response tests were conducted at room temperature, from 0 - 10 Hz, and the storage (G') and loss (G") moduli were recorded.

Cell culture, cell density and proliferation studies. Mouse skeletal muscle myoblasts C_2C_{12} cells were cultured in growth medium (Dulbecco's Modified Eagles Medium, DMEM), while human ovarian adenocarcinoma SKOV3 cells were cultured in growth medium (McCoy's 5A) supplemented with 10% Fetal Bovine Serum (FBS) and Penicillin and Streptomycin, at 37 °C in a 5% CO_2 incubator. Gels were prepared in a tissue culture 24-well plate, according to the general procedure described previously, with a final solution volume of 200 μL. The 24-well plate was incubated at 37 °C for 20 minutes. The gels were rinsed and swollen in PBS for 18 hours. The hydrogels were washed twice with sterile growth medium, and were incubated with growth medium for 2 hours at 37 °C in 5% CO_2 incubator. The medium was then replaced with 1 mL growth medium containing 10×10^4 proliferating C_2C_{12} or SKOV3 cells and incubated at 37 °C for up to 24 hours. Cell spreading and proliferation were visualized by optical microscopy. Percent cell density was determined using the CellTiter-Glo reagent and a luminescence plate reader. Samples were tested in triplicate, and statistical significance was determined using GraphPad Prism Software.

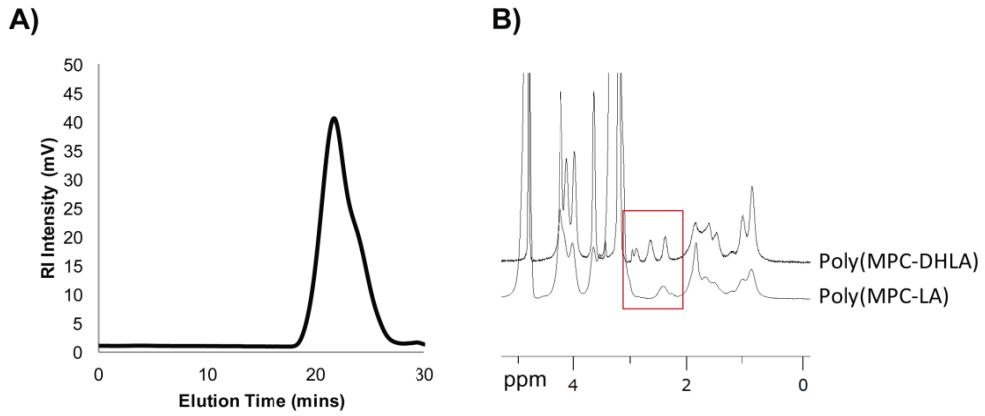

Figure S1. A) Aqueous GPC and B) ^{1}H NMR spectroscopy (in MeOD) of poly(MPC-co-DHLA).

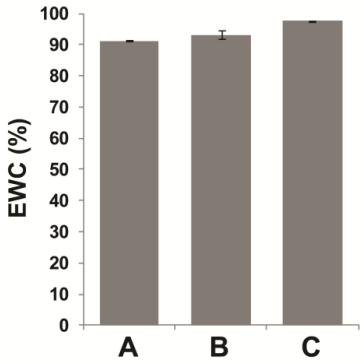

Figure S2. Equilibrium water content (EWC, %) of polyMPC hydrogels: (A) hydrogel cross-linked with $PEG_{700}DA$ (91.3 ± 0.2 %); (B) hydrogel cross-linked with $PEG_{2000}DA$ (93.2 ± 1.5 %); (C) GRGDS-containing hydrogel cross-linked with $PEG_{2000}DA$ (97.6 ± 0.2 %).

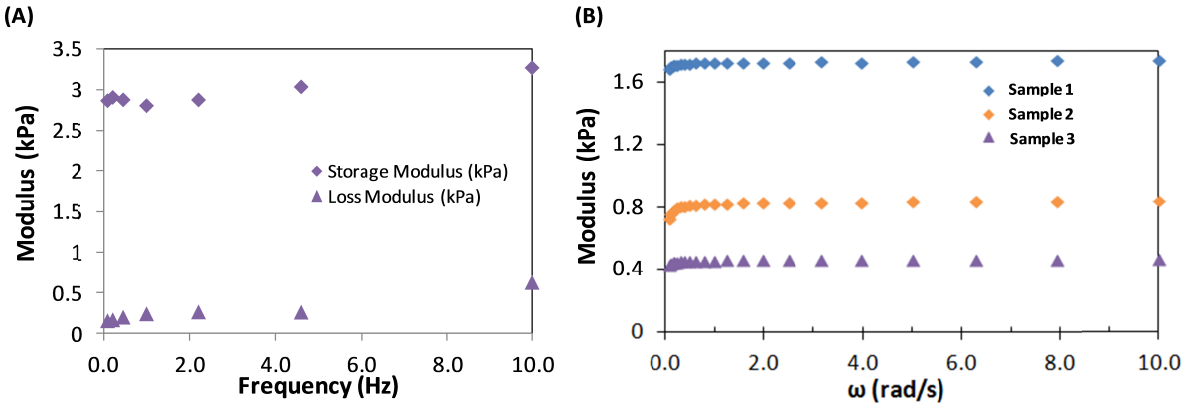

Figure S3. (A) Dynamic mechanical analysis frequency response experiment of hydrogel prepared from polyMPC-DHLA (100 mg/mL), cross-linked with $PEG_{700}DA$. The storage modulus (G') was found to be 2.95 ± 0.16 kPa and the loss modulus was 0.27 ± 0.16 kPa. G' was observed to be greater than G" across all frequencies tested. (B) Shear rheology experiments demonstrate the effect on elastic modulus (G') of varying molecular weight of the polymer components at constant concentration (25 mg/mL): Sample 1 (blue) is composed of polyMPC-DHLA (60 kDa) cross-linked with $PEG_{6000}DA$; Sample 2 (orange) is composed of

polyMPC-DHLA (25 kDa) cross-linked with $PEG_{6000}DA$; Sample 3 (purple) is composed of polyMPC-DHLA (25 kDa) cross-linked with $PEG_{700}DA$. As expected, an increase in modulus was observed for hydrogels prepared from higher molecular weight polymers. Also noted was the effect of polymer concentration: 100 mg/mL polyMPC-DHLA resulted in hydrogels with elastic moduli in the range of 3 kPa (shown in A), whereas 25 mg/mL polyMPC-DHLA caused a decrease in the modulus, to ~0.4 kPa (shown in B).

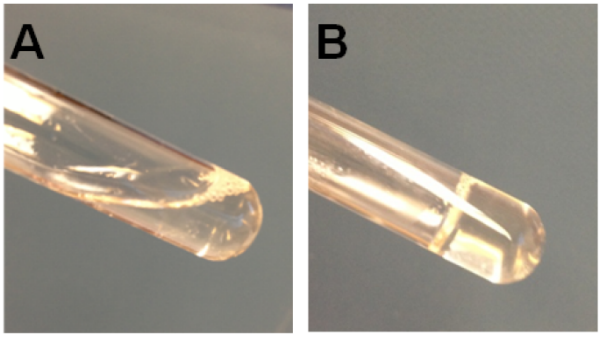

Figure S4. PolyMPC-co-DHLA and $PEG_{2000}DA$: (A) before gelation; (B) hydrogel formation after 10 minutes of heating at 37 °C.

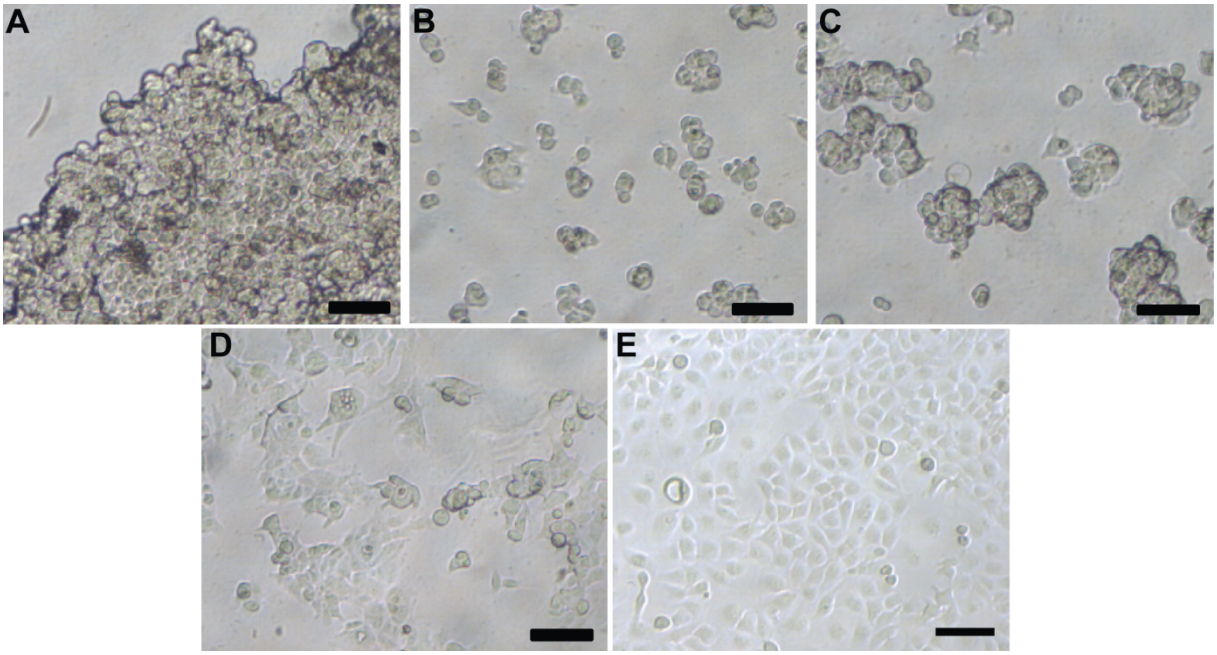

Figure S5. Optical micrographs of SKOV3 cells after 24 hour incubation on hydrogels from polymers containing (A) no GRGDS, (B) 0.25% GRGDS, (C) 1% GRGDS, (D) 5% GRGDS and on (E) polystyrene tissue culture plate. Scale bars are 100 μm.