Supporting Information for:

Ultralow protein adsorbing coatings from clickable PEG nanogel solutions: Benefits of attachment under salt-induced phase separation conditions and comparison with PEG/albumin nanogel coatings

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PEG-azide synthesis: Four arm PEG-mesylate was synthesized from four arm PEG-OH MW 10,000 by mesylating the alcohol group with mesyl chloride. This was done by dissolving PEG-OH in dichloromethane (DCM), adding 4 equivalents of triethylamine and 4 equivalents of methanesulfonyl chloride while on ice, and letting it react overnight under constant stirring and nitrogen flow. After removing the salt byproduct, excess DCM was removed by using the rotovap, and the PEG-mesylate was precipitated using cold diethyl ether. The product was dried under vacuum overnight to remove any remaining diethyl ether. The next step was the nucleophilic azidation of the mesylate group with sodium azide. 3 equivalents of sodium azide were dissolved in dimethylformamide (DMF). PEG-mesylate was then dissolved in the DMF mixture and put under nitrogen and constant stirring in a hot water bath at 60°C. The reaction was run overnight. The following day, excess salt was filtered out followed by rotovapping, diethyl ether precipitation, and drying as was done for the PEG-mesylate. The product was dissolved in a basic water solution with a pH between 9 and 12, and then extracted with DCM over anhydrous Na_2SO_4. A standard extraction procedure was done to extract the product into DCM. After 3 extractions, the Na_2SO_4 was filtered out and the process of rotovapping, diethyl ether precipitation, and drying was done as before.

PEG-cyclooctyne synthesis: PEG-mesylate was synthesized as described above. Four arm PEG-amine was then synthesized from PEG-mesylate by an amination reaction with ammonium hydroxide. PEG-mesylate was first dissolved in 400 mL of 28-30% ammonium hydroxide. The PEG-mesylate needed to be constantly stirred in a sealed bottle for 3-4 days. After 4 days, the bottle was uncapped to allow the ammonia to evaporate while still under constant stirring, which took 3-5 days. To collect the product, the pH of the solution needed to be raised to 13 with

sodium hydroxide (NaOH), and then extracted with DCM over anhydrous Na_2SO_4. A standard extraction procedure was done to extract the PEG-amine into the DCM. After 3 extractions, the Na_2SO_4 was filtered out and the process of rotovapping, diethyl ether precipitation, and drying was performed as was done for the PEG-mesylate. PEG-cyclooctyne was finally synthesized from PEG-amine. PEG-amine was dissolved in DCM in a beaker, and 1.5 equivalents of diisopropylcarbodiimide (DIPCDI) was added to a separate spherical flask with DCM while on ice and under nitrogen flow and constant stirring. Next, 1.5 equivalents of hydroxybenzotriazole (HOBt) and 1.5 equivalents of dibenzyocyclooctyne acid (DBCO acid) was added to the mixture in the flask and allowed to stir for 10 minutes. While waiting, 3 equivalents of N,N-diisopropylethylamine (DIPEA) were added to the dissolved PEG-amine. Finally, this mixture was slowly added to the spherical flask, and the reaction was allowed to go for 24 hours in the ice bath, under constant stirring and nitrogen gas. Following that process, the urea precipitate was filtered out, and the process of rotovapping, diethyl ether precipitation, and drying was performed. The product was then dissolved in distilled H_2O and underwent the extraction procedure that was done for the PEG-amine. Further rotovapping, diethyl ether precipitation, and drying were done.

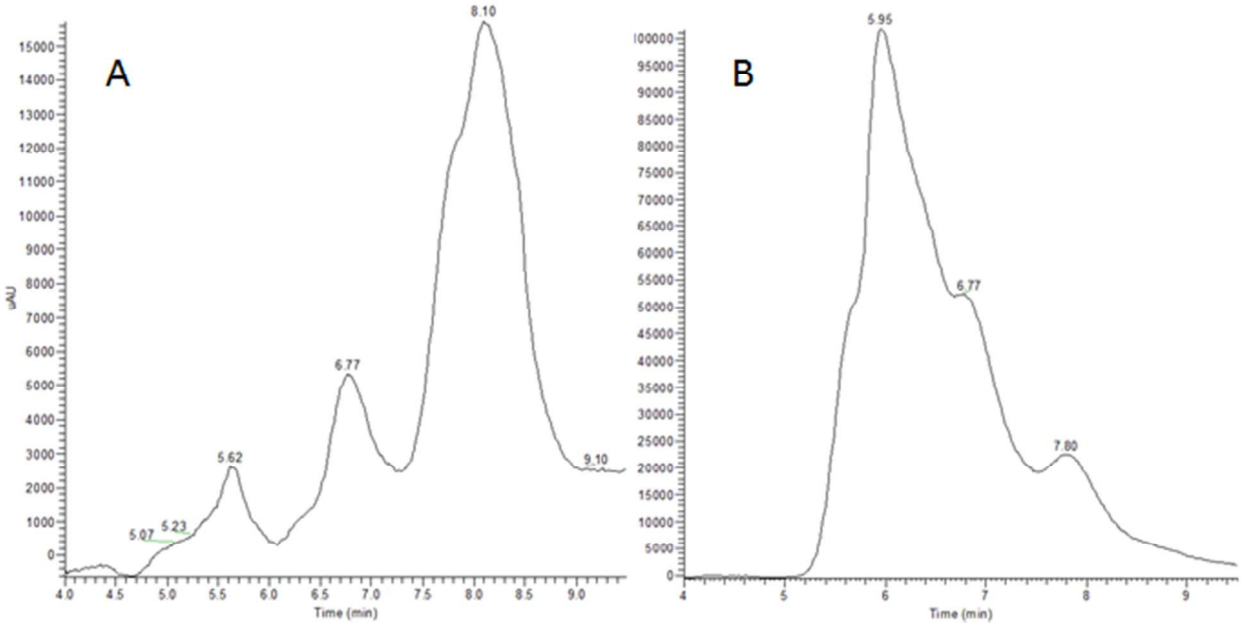

Figure S1. SEC profiles monitored over a ~9 min period with UV detection at 200 nm of A) 10% (w/v) PEG-alkyne and B) 20% (w/v) chelated clickable nanogel (hydrodynamic radius = 120 nm) solutions show a large shift in the molecular weight distribution that results from partial crosslinking of the nanogel solution.

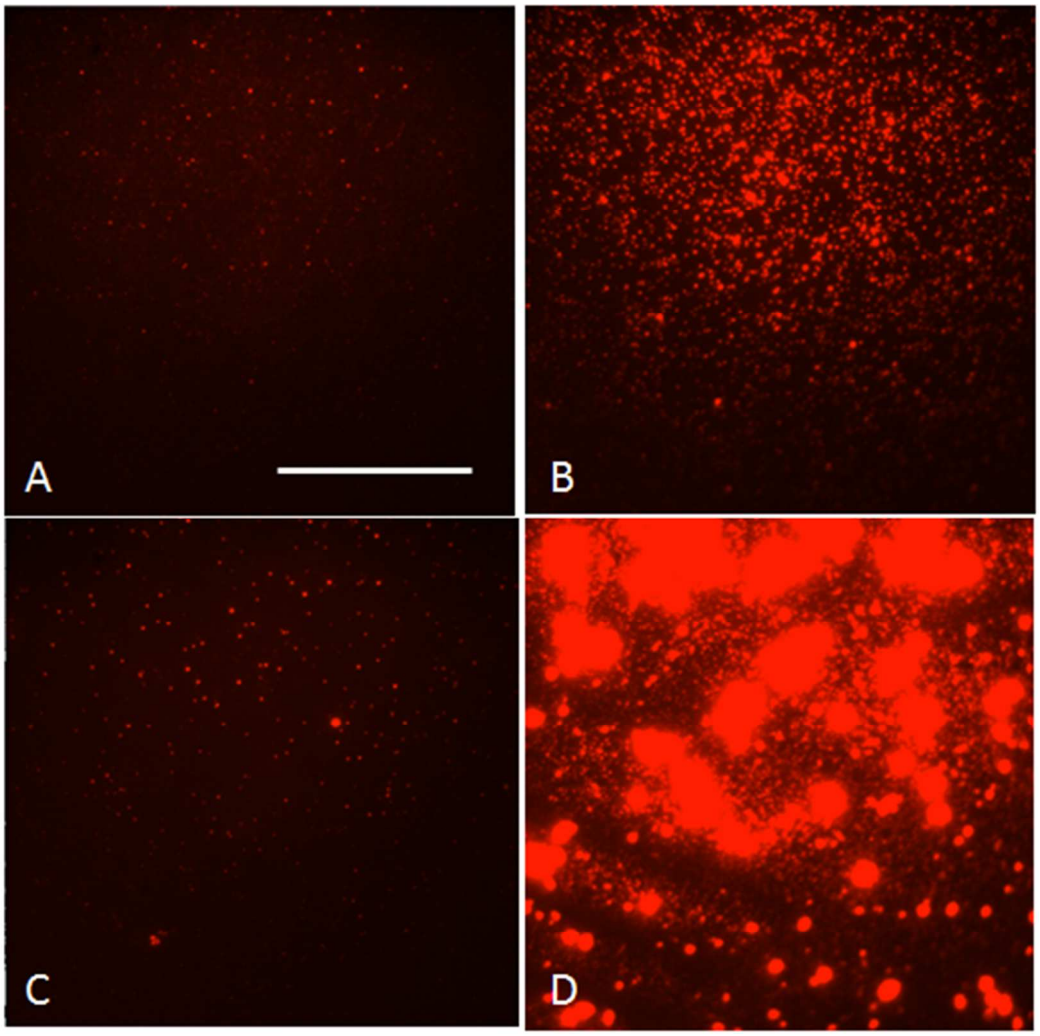

Figure S2. Fluorescent images taken using TIRF microscopy of nanogel coatings after 1 h incubations with 0.25 mg/mL Cy5-labeled fibrinogen, including: (A) VS:BSA without surface crosslinking, (B) clickable attached with salt without surface crosslinking, (C) clickable attached with salt with surface crosslinking by 2mM copper, and (D) clickable attached with salt with surface crosslinking by 8 mM copper. The large fluorescent spots in (D) may result from stress induced damage to the surface. The scale bar represents 100 μm.

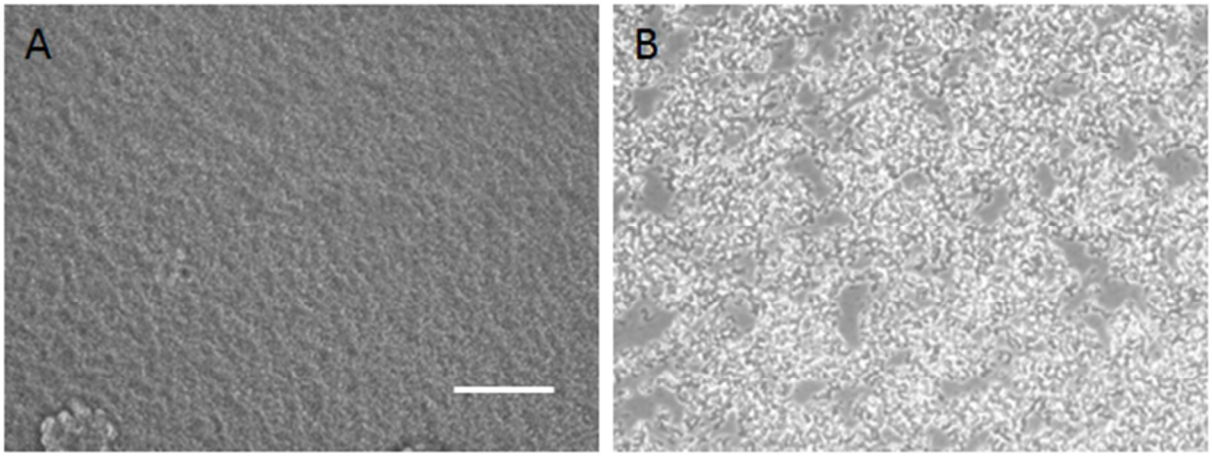

Figure S3. Micrographs of (A) VS:BSA and (B) VS:Am nanogel coatings show distinct surface morphologies after replicating surface crosslinking in 1.5 M salt by overnight incubations in 1.5 M sodium sulfate prior to capping vinyl sulfone groups. Images were taken after fibrinogen incubation and cell seeding. The scale bar represents 100 μm.

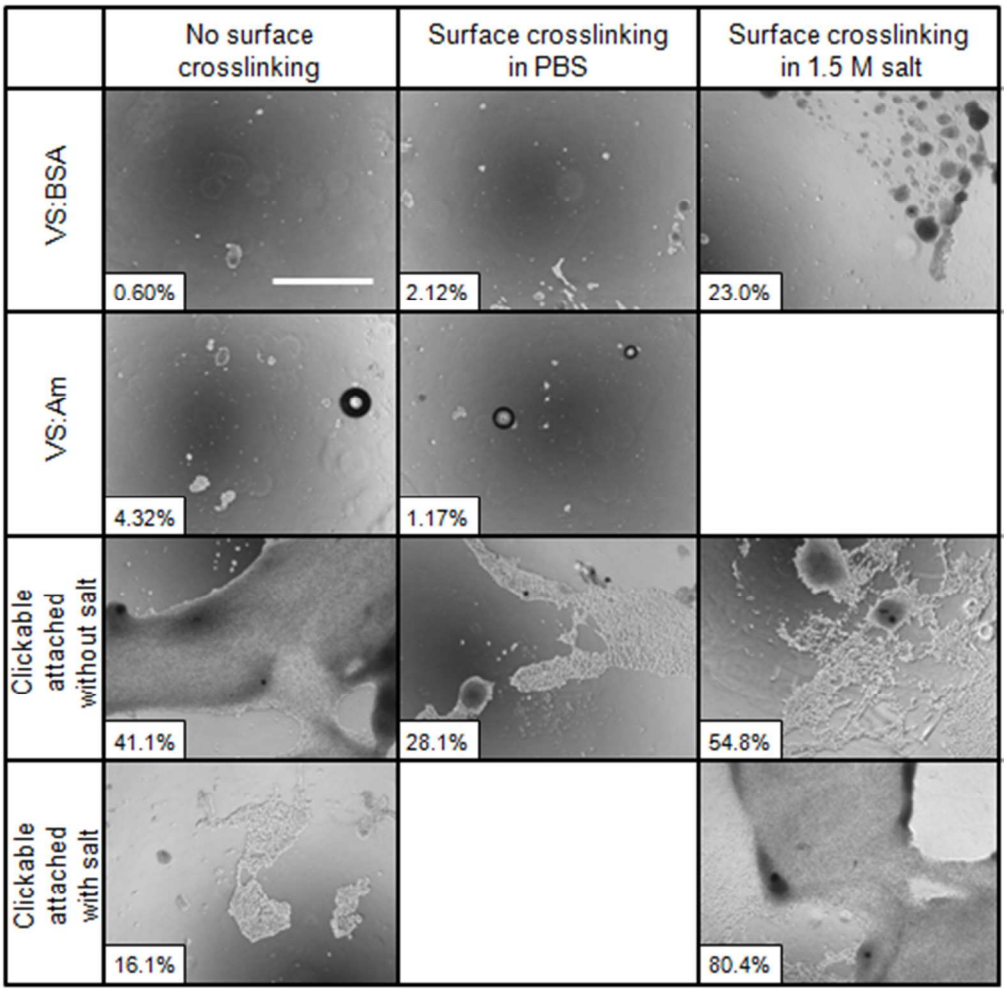

Figure S4. Cell adhesion of 3T3 fibroblasts was examined one day after the fifth seeding at 100,000 cells/cm^2 on several surfaces that were initially incubated with 2.5 mg/mL fibrinogen for 2 h at 37°C. Surface crosslinking had been allowed overnight on some VS:BSA and VS:Am nanogel coatings before capping and implemented with 2 mM copper overnight on some clickable nanogel coatings both at 37°C. Approximate percentage of surface coverage by adhered cells is indicated in the insets. The scale bar represents 1 mm.

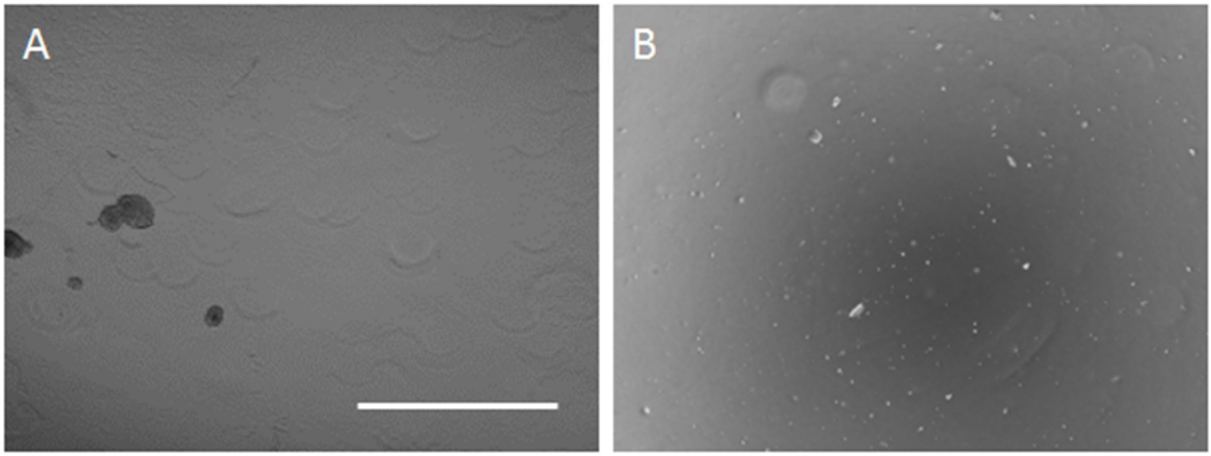

Figure S5. Cell adhesion of 3T3 fibroblasts was examined one day after the initial seeding at 100,000 cells/cm^2 after incubation with 2.5 mg/mL fibrinogen for 2 h at 37°C. Surfaces include clickable nanogel coatings attached with salt via: (A) SPAAC to a PEG-cycloctyne interface on azidosilane, or (B) UV thiol-yne to mercaptosilane, both without surface crosslinking. The attachment chemistries appear to perform comparably in terms of cell adhesion. The scale bar represents 1 mm.