Supporting Information for

Polybasic Nanomatrices Prepared By UV-initiated Photopolymerization

Authored by

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Gel Permeation Chromatography

To obtain the molecular weight of uncrosslinked PDGP, gel permeation chromatography (GPC) was performed using a Waters 2695 high performance liquid chromatography (HPLC) solvent pump attached serially to four Ultrahydrogel columns (300 × 7.8 mm; Waters Technology Corp.), with a Waters 2414 refractive index detector. A 4:1 v/v mixture of 0.1 M aqueous sodium nitrate solution and acetonitrile was used as solvent. The flow rate was 1 mL/min and the columns were maintained at 40° C. A series of near monodisperse poly(ethylene oxide) (PEO), standards were used for calibration (M_p = 500-2,740,000, Sigma-Aldrich, St. Louis, MO).

^{1}H-NMR Spectroscopy

The steric stabilizer content was determined using ^{1}H NMR spectroscopy. Dried, uncrosslinked PDGP was dissolved in D_2O with 0.1 N DCl. NMR spectra were recorded using a 300 MHz Varian UNITY+ 300s spectrometer. The integrated intensity of the methylamine proton peak at 3.2 ppm was compared to that of the oxyethylene proton signal from the PEGMMA grafts at 3.6 ppm.

Scanning Electron Microscopy

Particle morphology and size in the dry state were determined using a LEO 1530 scanning electron microscope (SEM). Dried particles were resuspended in either 0.1 N HCL or 0.1 N NaOH and a small drop was allowed to adsorb onto a glass coverslip. The coverslips were rinsed once with ddH_2O and allowed to air dry. They were then sputter coated with gold before imaging.

Light Scattering

The hydrodynamic diameter and zeta-potential of the polymer networks was obtained using a Brookhaven ZetaPlus instrument (Brookhaven Instruments Corp., Holtsville, NY) operating with a 635

nm diode laser source. Dynamic light scattering measurements (DLS) of particle size were conducted as follows: Dry particles were first resuspended in ddH_2O then brought up to final volume in 1x PBS with a 10x PBS concentrate to 0.01-0.5 mg/mL. The suspension was then adjusted to a starting pH of 10 using microliter amounts of 1 N NaOH. Measurements were taken at 25^{o} C, with detection at a 90° scattering angle, at set pH values by lowering the pH with similar amounts of 1 N HCl. Each measurement was repeated six times.

Electrophoretic light scattering measurements of the zeta-potential were also measured using the Brookhaven ZetaPlus. Dried particles were resuspended the same as for DLS measurements except that 5 mM sodium phosphate was used as a background buffer instead of PBS. All measurements were repeated 10 times, at 25^{o} C with scattering detected at a 15° angle.

Insulin Loading

The previously prepared nanomatrices were resuspended in PBS at a final concentration of 0.5 mg/mL as follows: To prevent flocculation, dried particles were resuspended in ddH_2O at 90% of the final volume by a combination of stirring and vortexing. This was brought up to final volume using 10x PBS concentrate and the pH was adjusted to 6.5 the same way as above. Insulin was dissolved in PBS at 0.5 mg/mL, pH adjusted to 6.5, with 76.9 μM ethylenediaminetetraacetic acid (EDTA) to keep insulin in its monomer form. The particle suspensions and insulin stock were combined at a 1:1 ratio and stirred for 30 minutes. The polymer networks were then collapsed by adjusting the pH to 7.5. Samples of the suspension were taken before and after collapsing the particles and filtered through 0.020 μm Whatman Anotop syringe filters. The insulin content in the filtrates were analyzed by HPLC using a Waters 2695 Separations Module (Waters Corp., Milford, MA). Briefly, a solvent of 80% water/20% acetonitrile with 0.1% trifluoroacetic acid was used as the mobile phase through a Symmetry300TM C4 column, also from Waters Corp. A sample injection volume of 20 μL and flow rate of 1 mL/min was used.

Colloidal Gold Loading

To determine if gold nanoparticle loading into nanomatrices could be controlled by mesh size, we used colloidal gold 2-5 nm in diameter prepared using the method of Duff and coworkers.[1] All reagents were prepared fresh and filtered through a 0.45 μm syringe filter before use. Briefly, 45.5 mL ddH_2O, 1.5 mL of 0.2 N NaOH and 1 mL of 0.96% THPC were combined in a Pirhana cleaned (sulfuric acid and 30% hydrogen peroxide in water, 3:1 ratio) round bottom flask while stirring. After 2 minutes, 2 mL of 1 mg/mL solution tetrachloroauric(III) acid was added. After a visible change from clear to orange-brown, the colloid was covered and stored at 4^{o}C until use. Dried polymer particles at 4 different crosslinking mole feed ratios were resuspended in ddH2O and combined with the colloid at a 7:1 polymer/gold mass ratio. In order to entrap the gold particles inside the matrices, their aggregation was induced by dropping the pH to 5 in 0.03 N HCl, then raising it back up to 8 using an equivalent amount of base. Particles were prepared for transmission electron microscopy (TEM) studies by adsorbing onto a formvar coated 300 mesh copper grid (Ted Pella Inc., Redding, CA).

Cell Viability

NIH/3T3 mouse fibroblasts were maintained in DMEM with 4 mM L-glutamine, 4.5 g/L glucose, supplemented with 10% bovine calf serum. The cells types were kept in a humid environment of 95% air and 5% carbon dioxide until use. For viability studies cells were seeded in 96-well plates at a cell density of 2500 cells/cm^2 and grown to 95-100% confluence. A volume of 200 μL of fresh media was placed in each well 1 hr before experimentation. Particle suspensions were prepared in HBSS. 50 μL volumes of each suspension were placed in each cell culture well, mixed by gentle rocking, and incubated for 2 hr. The media was then removed and replaced with a standard MTS assay solution (CellTiter 96 AQueous, Promega) diluted in HBSS. After incubating for 90 minutes the absorbance was measured at 490 nm using a microplate reader (Synergy HT, BioTek Instruments, Inc., Winooski, VT).

References

1. Duff, D. G.; Baiker, A.; Edwards, P. P. *Langmuir* **1993,** 9, 2301.