Bio-eliminable Nano-hydrogels for Drug Delivery

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Experimental Details

The surfactant Aerosol OT (AOT; 98%) was purchased from Aldrich. Calcein AM and propidium iodide were purchased from Molecular Probes. Acrylamide (AAm), 3-mercapto-1-propanol, degradable glycerol dimethacrylate, N, N-methylenebis (acrylamide) (MBA), N, N, N', N'-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) were purchased from Sigma. Methanol and hexane were purchased from Fisher. All the above chemicals were used without any further purification. Pierce Coomassie (Bradford) protein assay kit was used for the protein adsorption study.

Rat C6 glioma cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 400 mg L^{-1} D-glucose, 2 mM L-glutamine, 10% fetal bovine serum, 0.3% penicillin, streptomycin and neomycin and incubated at 37 ºC in a 5% CO_2 environment. Cells in the exponential growth phase were plated on uncoated 22-nm glass cover slips 2 days prior to experimentation. Cells of the mouse macrophage-RAW 264.7 line were initially cultured on suspension plastic. Then they were re-plated onto cover-slips for confocal imaging.

Philips XL30 FEG SEM at University of Michigan was used for nanoparticle size determination. Fluorescence was measured in a FluoroMax-2 spectrofluorometer (ISA Jobin Yvon-Spex, Edison, NJ, USA). Cell images were acquired with a Perkin Elmer UltraView confocal microscope system equipped with an argon–krypton laser.

Ultrahydrogel linear column Shodex OHpak SB-800HQ column was used for gel permeation chromatography with Wyatt Mini-Dawn light scattering detector for monitoring

nanoparticle degradation and distribution. The GPC is calibrated with a series of polyacrylamide standards from Polymer Sciences Inc. Waters Millennium software was used for GPC data acquisition and processing.

Nanoparticle Preparation and Characterization: 1.6g of AOT and 3.2ml of Brij 30 were dissolved in 45ml of hexane. 0.6g of acrylamide, 0.225g of degradable glycerol dimethacrylate cross linker and 4.2mg of 3-mercapto-1-propanol in 2ml of water were emulsified into hexane. The solution was purged with argon for 0.5h then the chain transfer polymerization was initiated by 6μl of 10% APS and 8μl of TEMED. The polymerization lasted overnight before the reaction was stopped. The solvent was then evaporated and the residue was washed with ethanol and water thoroughly by Amcon filter. The PI was encapsulated into the nanoparticles by the same microemulsion method. The short chain polyacrylamide was also synthesized by the same way without cross linker. For non degradable nanoparticle synthesis, cross linker N, N-methylenebis (acrylamide) was used without 3-mercapto-1-propanol.

Matrix Cytotoxicity Study: To study cellular viability, the investigation of nanoparticle cytotoxicity was performed by using the MTT assay based on the observation that the mitochondria in living cells can catalyze MTT molecules to a colorimetrically detectable dye. Rat C6 glioma cells were seeded in 96-well plates at a density of 10^4 cells per well. After incubation overnight (37 °C, 5% CO_2), the medium in each well was aspirated off and replaced with 200μl of fresh medium containing 0-5 mg/mL of polymers or 0-5 mg/mL of nanoparticles. These solutions were sterilised by passing through a 200nm membrane prior to incubation. After incubation for 72 h, the nanoparticle containing medium in each well was aspirated off and replaced with 150 μl of serum free medium and 50 μl of a 5 mg/mL MTT solution. The cells were incubated for 4 h, then the medium was removed, and purple crystals were observed. These crystals were dissolved in 200 μl of DMSO and 25 μl of Sorensen's buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5). The absorbance at 560 nm was measured using

a Spectra-MAX 190 microplate reader (Molecular Devices). The percent viability of treated cells relative to non treated cells was calculated and represented as the average of eight measurements.

Nanoparticle Uptake by Macrophages: Macrophages were cultured in 6 well plates containing sterile cover-slips. Confluent cells were incubated with 1mg/ml of PI encapsulated nanoparticles for two hours. After incubation, the cells were carefully washed three times with medium. The cover slip was then placed in a microscope chamber at 37 ºC for confocal imaging.

Nanoparticle in vitro Degradation inside Macrophages: Macrophages were cultured in 6 well plates containing sterile cover-slips. PI encapsulated degradable and non-degradable NPs were introduced into the macrophages by gene gun method. The cells then were carefully washed three times with medium, and the cover slip was placed in a microscope chamber at 37 ºC for confocal imaging. Calcein was used to stain the cells in order to locate the position of cells. For non-degradable nanoparticles, free PI was added after nanoparticles were introduced into cells, in order to confirm the particles indeed were inside the cells.

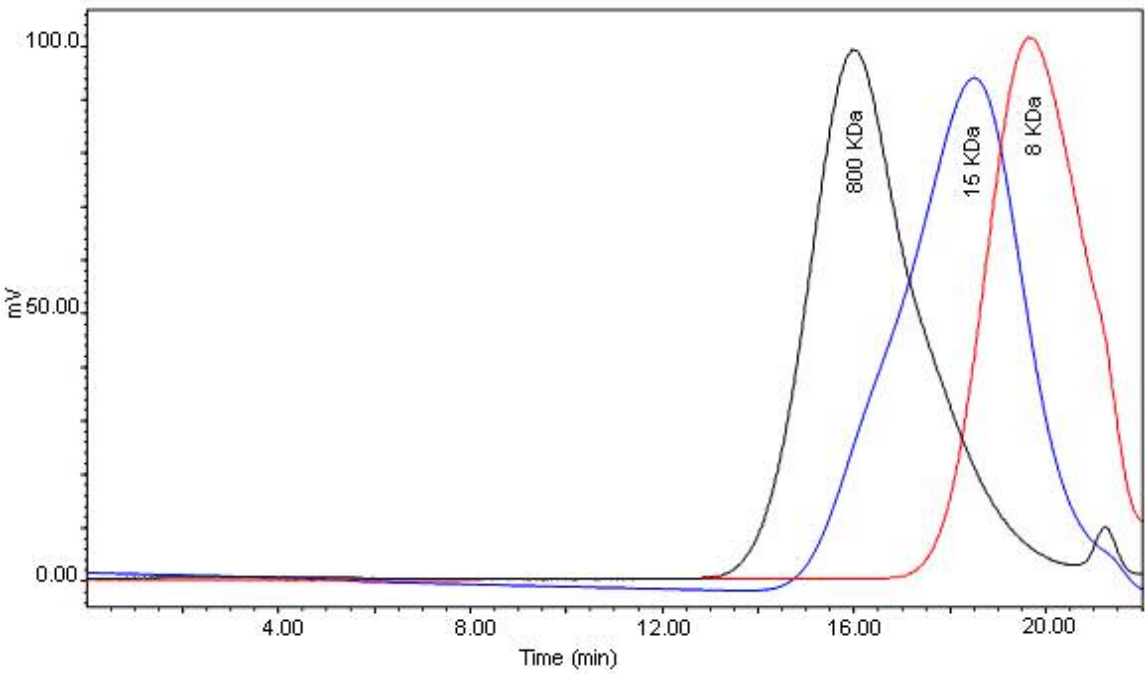

Figure 1S. GPC of linear polyacrylamides synthesized by free radical polymerization (black) and by chain transfer polymerization (blue and red). The molecular weight of polyacrylamide obtained with free radical polymerization is 800 KDa while the lowest molecular weight of polyacrylamide obtained with chain transfer polymerization is 8 KDa

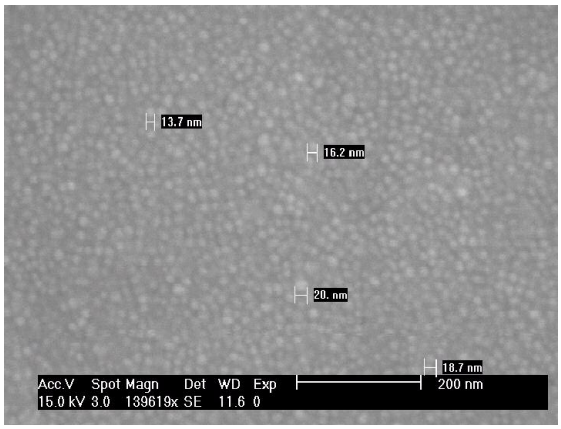

Figure 2S. SEM of synthesized degradable polyacrylamide nanoparticles. The typical diameter is 15- 20 nm.

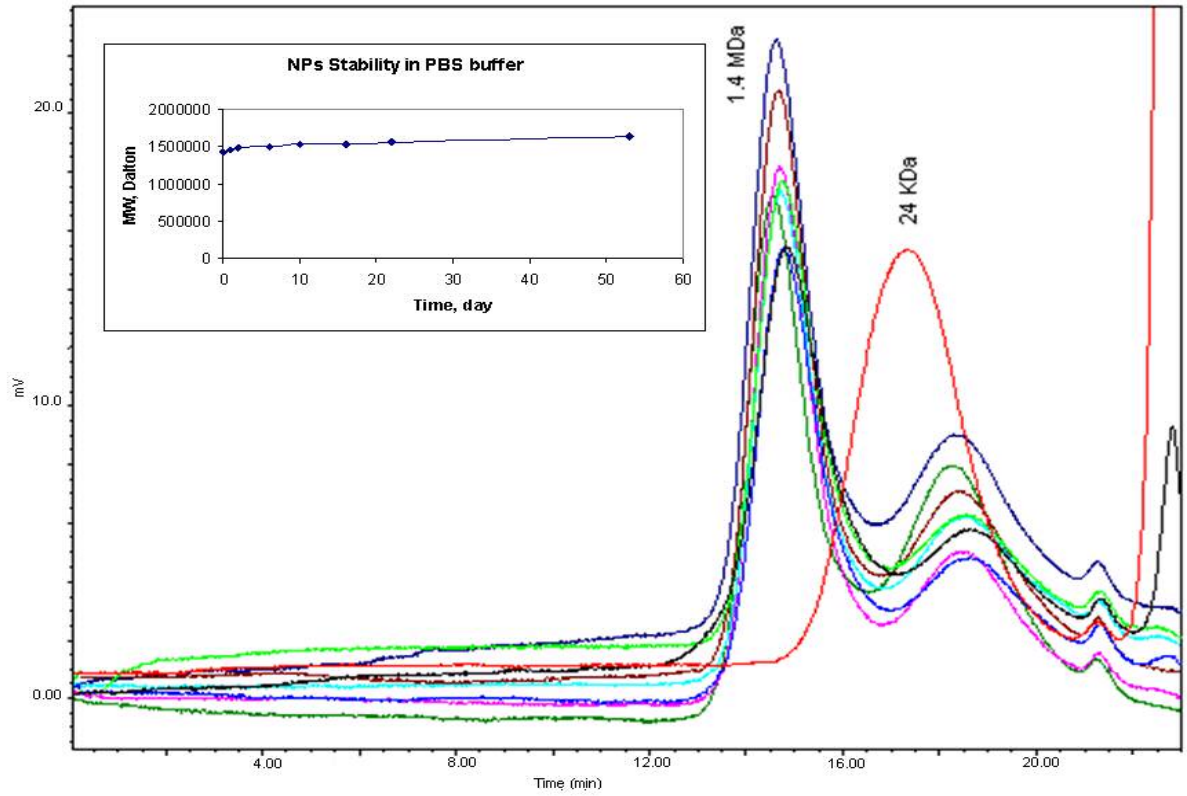

Figure 3S. Nano-hydrogel stability monitored by GPC. The nanohydrogel is stable in PBS buffer and plasma for over two months. However, it quickly degrades to low molecular weight products when mixed with NaOH solution (Red Curve).

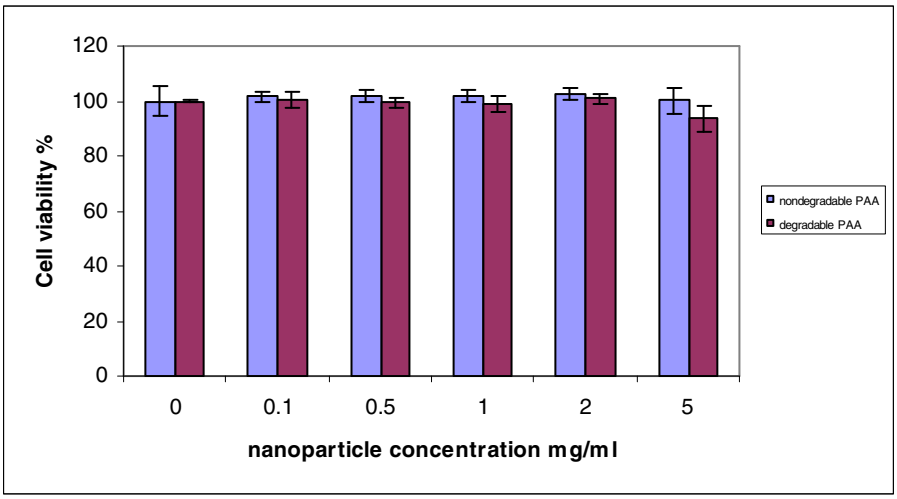

Figure 4S. Toxicity of nondegradable and degradable NPs to cells. Blue: nondegradable polyacrylamide nanoparticle toxicity to Rat C6 glioma cells; Red: degradable polyacrylamide nanoparticle toxicity to Rat C6 glioma cells. Negligible toxicity is shown for all these doses.

a b c

Figure 5S. Non-degradable NPs within cells. PI encapsulated non-degradable NPs were introduced into macrophage cells by gene gun method. (a) Calcein AM stains for non degradable NP loaded cells. (b) NPs within cells do not degrade and no PI nuclei stain was found in these NP loaded cells when the cells were excited at the PI excitation wavelength (568nm). (c) The nuclei stain could be visualized with additional free PI to these NP loaded cells, when the cells were excited at the PI excitation wavelength (568nm). The scale bar is 30µm.