Nanotherapeutics for Advanced Cancer Disease

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Introduction:

Plasmonic nanoparticles are gaining increased attention for use in biological systems due to their versatile, tunable optical properties [1]. In particular, gold nanorods (GNR) can be tuned to specifically absorb light in the near infrared region, at wavelengths which do not harm living tissues. The absorbed infrared energy is then dissipated as heat from the nanorods to their surroundings. Sufficient heating within living tissues can induce hyperthermic cell death and damage due to hyperthermic temperatures (> 43°C) [2]. Delivery of these nanoparticles to cancerous cells at concentrations that will be responsive to treatment from an infrared laser is key. This project demonstrates that GNR coated with branched cationic polymers synthesized in our laboratory can be employed to deliver plasmid deoxyribonucleic acid (DNA) to PC3 prostate cancer cells, leading to transgene expression. Ongoing work explores the use of GNR for simultaneously administering laser-induced hyperthermia and delivering therapeutic genes to cancer cells.

Methods:

Hexadecyltrimethylammonium bromide capped GNR were synthesized via seed growth method as described by El-Sayed, et al. [3].

Cationic polymers investigated were synthesized as described previously by the Rege group [4] by reacting equimolar amounts of the desired diglycidyl ethers and poly(amines) shown in Figure 1.

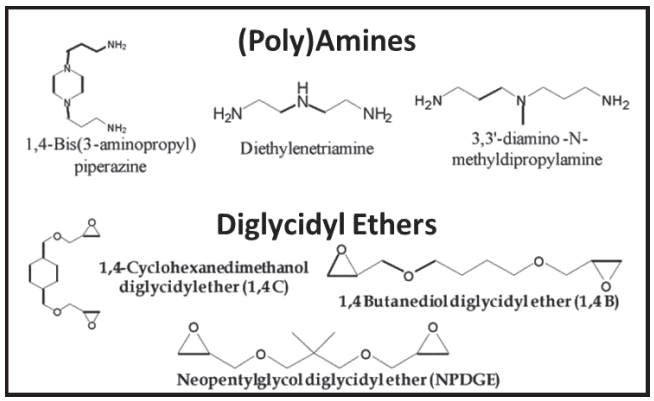

Figure1: Diglycidyl ethers and poly(amines) used to synthesize novel cationic polymers (reproduced from reference 4).

Polyelectrolyte layers were added to the CTAB capped GNR via electrostatic interactions to form polyelectrolyte gold nanorods (PE-GNRs). As-synthesized CTAB-GNRs were centrifuged at 6000 *rcf* for 10 minutes to remove excess water. 100 μL of 10 mg/mL poly(styrene sulfonate) (PSS) (anionic) was added to the collected rods under sonication. The solution was sonicated for 30 minutes to allow a layer of PSS to form on the surface of the GNR and then centrifuged as before. 300 μL of nanopure water and 200 μL of desired cationic polymer were added in the same manner as PSS. Collected cationic polymer coated rods were resuspended in serum free media and their optical density adjusted to 0.25 a.u.

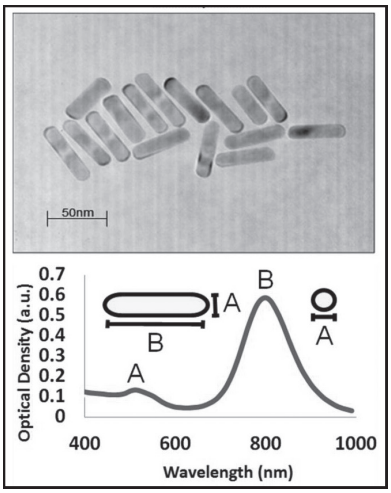

Figure 2: (a) TEM of as-synthesized GNRs, and (b) Typical absorbance spectra of as synthesized GNRs.

PE-GNR cytotoxicity studies were carried out by introducing different concentrations of as described PE-GNRs to PC3 human prostate cancer cells which were seeded at 50,000 cells per well and allowed to incubate overnight. The final well volume was always brought to 500 μL and treatment occurred over six hrs. After six hrs, 20 μL of MTT reagent was added to each well and allowed to incubate for two hrs. At the two hr mark, 100 μL of detergent was added to each well. After an additional two hours and endpoint absorbance reading was taken at 570 nm in a Bio-Tek Synergy 2 plate reader. Cell viability was recorded as percentages of live and dead controls.

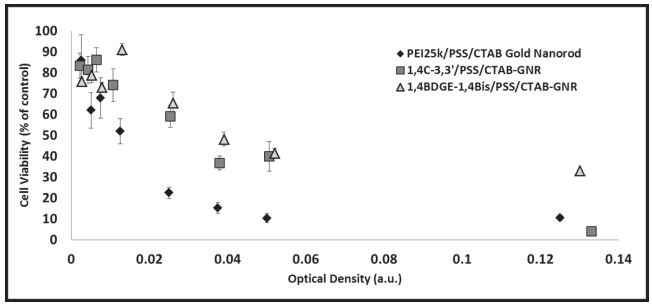

Figure 3: Cytotoxicity data of PE-GNRs made from investigated polymers.

25 μL of as-described PE-GNRs were placed into wells in 96 well plates. Addition of the plasmid DNA layer was accomplished by adding the desired mass of plasmid DNA to each well. After ensuring that the solution was well dispersed, the plasmid DNA layer was allowed to form via electrostatic interaction at room temperature for 30minutes.

Transfection studies were carried out as described previously by the Rege group [4].

Results and Discussion:

Figure 3 illustrates the results of the PE-GNR cytotoxicity studies for two of the polymers used, 1,4C-3,3' and 1,4BDGE-1,4C as compared to poly(ethyl imine) (PEI), which is regarded as the current standard in polymeric gene delivery. The figure illustrates that at the lowest concentrations of PE-GNRs the investigated polymers showed cytotoxicity that was similar to that of PEI. As concentrations increased however; it is clear that both of the investigated polymers are less cytotoxic than PEI.

Successful delivery of PE-GNRs to PC3 cells was quantified through transfection study. In order for the plasmid DNA to be expressed by the cell, the assumption made was that the entire PE-GNR complex had to be up taken by the cell. Figure 4 illustrates the results of transfection studies with all four investigated polymers. Results are reported as relative to PEI. Greater luminescence indicates higher expression of the luciferase protein in cells following delivery of the plasmid DNA. Investigated polymers 1,4C-1,4Bis and 1,4C 3,3' show a clear advantage over PEI, while the 1,4BDGE-1,4Bis protein approaches the efficacy of PEI at most loadings.

Conclusions and Future Work:

These studies have illustrated that delivery of GNRs to PC3 cancer cells can be achieved at high levels as compared to PEI by utilizing certain novel cationic polymers investigated. Investigated polymers are less cytotoxic and more effective in transfection as compared to PEI. Future work includes additional trials to reduce error and inconsistencies between trials. Investigating transfection at higher treatment concentrations may yield even further increases in expression of desired protein as compared to PEI based on cytotoxicity data. Zeta potential studies are required to determine if accumulated negative charge as a result of higher amounts of DNA is having a detrimental effect on cellular uptake.

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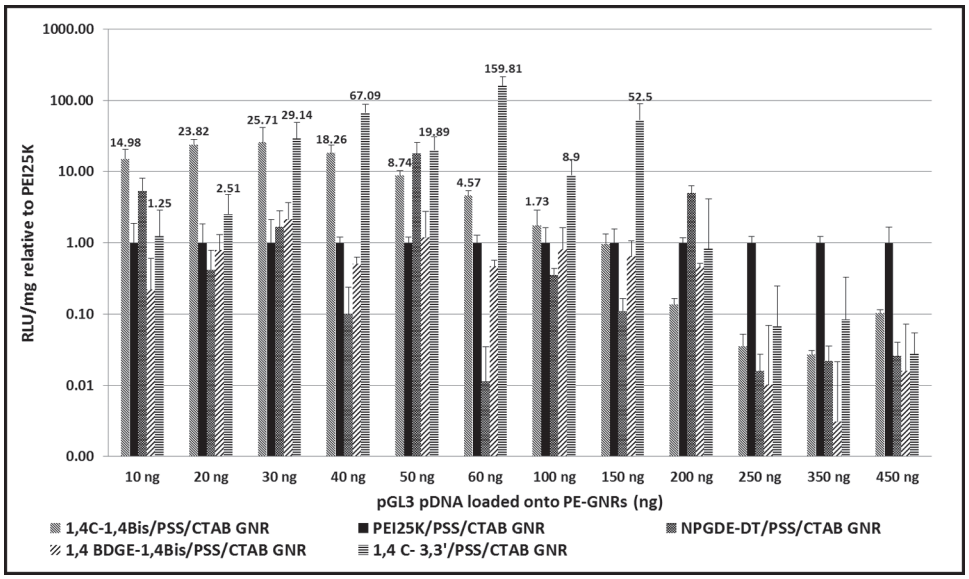

Figure 4: Transfection efficacy of investigated polymers as compared to PEI.

References:

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