Synthesis of Poly(Amino Ether) Capped Gold Nanoparticles for Transgene Delivery

Olivia Lambdin
Biological Systems Engineering, University of Nebraska – Lincoln

NNIN REU Site: ASU NanoFab, Arizona State University, Tempe, AZ
NNIN REU Principal Investigator: Dr. Kaushal Rege, Chemical Engineering, Arizona State University
NNIN REU Mentor: James Ramos, Bioengineering, Arizona State University
Contact: olambdin@gmail.com, kaushal.rege@asu.edu, jimr975@gmail.com

Abstract and Introduction:

The search for safe and effective gene delivery agents is at the forefront of biomedical research. Delivering plasmid deoxyribonucleic acid (DNA) to live cells for gene therapy may one day cure diseases from autoimmune disorders to cancer. Polymers, liposomes, viruses, and gene guns have been investigated as potential vehicles for gene delivery [1]. Although these methods show promise, concerns regarding safety and low transfection efficiency remain. Thus, literature reflects a search for competitive, alternative gene therapy agents.

Our lab has created a promising polymer library for use in polymer-mediated gene delivery [2]. We chose lead polymers with high transfection efficacies to determine whether they could be employed for a one pot synthesis and capping of gold nanoparticles (AuNPs) aimed at gene delivery. By acting as a reducing and capping/protecting agent, the poly(amino ether) polymers encapsulate the colloidal nanoparticles, enhance the dispersion's stability, and prevent their aggregation in biologically relevant media. We used leads from our polymer library, 1,4C-1,4Bis and EGDE-3,3' and used 25-kDa poly(ethyl imine) (PEI) as a control.

Our research focused on synthesizing these poly(amino ether) gold nanoparticles (PAE-AuNPs) and characterizing their potential for biomedical applications. The characterization techniques used included: ultraviolet-visible (UV-Vis) and infrared spectroscopy, zeta potential and dynamic light scattering (DLS) measurements, and MTT assay. Our research concluded with a preliminary qualitative analysis of *in vitro* transgene expression.

Experimental Procedure:

Poly(amino ethers) were synthesized as described by Barua, et al. [2].

Generation of Polymer Capped AuNPs. PAE-AuNPs were synthesized at different polymer:gold weight ratios by mixing polymer and 0.001 M $HAuCl_4$. Following formation, excess polymer was removed via centrifugation. The nanoparticles were centrifuged at 800 rpm for 10 min, and the supernatant was discarded. AuNPs were suspended in nanopure water and adjusted to an optical density of 0.25 a.u.

Cytotoxicity Studies with Prostate Cancer Cells. Human PC3 prostate cancer cells (PC3) were seeded in 24-well plates at a density of 50,000 cells/well. Following overnight incubation (37°C, 5% CO_2), the cells were treated with different amounts of PAE-AuNPs and the well volumes were brought to 500 μL using serum free media. The MTT Assay was conducted to determine the cell viability.

Results:

Kinetics of Formation Revealed Unique Qualities of Our Polymers. After mixing gold salt and polymer at various polymer to gold weight ratios, UV-Vis spectroscopy was employed to observe the rate of PAE-AuNP formation. The emergence of a peak at 520 nm indicated formation of spherical AuNPs (Figure 1). It was observed that both 1,4C-1,4Bis and EGDE-3,3' reduced and capped the gold salt faster than PEI. Also, at high polymer to gold ratios, such as 250:1 and 500:1, our lab's polymers successfully made AuNPs, while PEI was unsuccessful.

PAE-AuNPs Exhibit Short-Term Stability in Biologically Relevant Media. Following PAE-AuNP formation, we investigated short-term optical stability of our nanoparticles when dispersed in serum-free media (SFM), and their

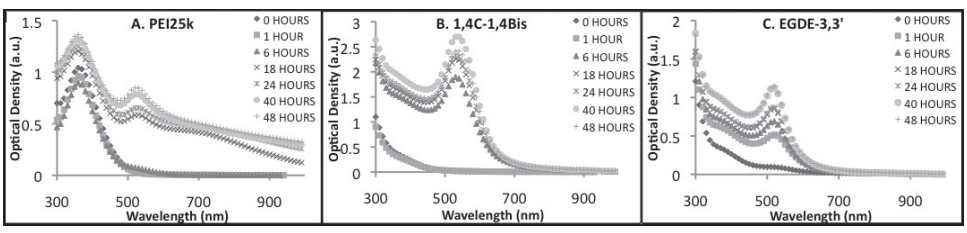

Figure 1: Spectra readings illustrate 50:1 PAE-AuNPs kinetics of formation.

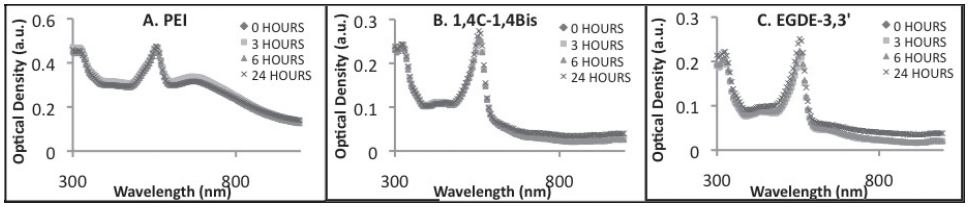

Figure 2: 50:1 PAE-AuNPs dispersed in SFM maintain absorption peak at 520 nm.

UV-Vis spectrum was monitored over a 48-hour period. Short-term optical stability was determined by monitoring any changes in the peak at 520 nm. It was observed that all three polymer capped AuNPs exhibited good stability (Figure 2), indicating a lack of aggregation and potential for biological applications.

1,4C-1,4Bis-AuNPs Demonstrate Lower Cytotoxicity than PEI-AuNPs. Though polymers stabilize gold in biologically relevant media, they are potentially toxic to cells. Utilizing the MTT assay, we investigated the toxicity limits for 50:1 polymer capped AuNPs in the PC3 cell line. Our results revealed that 1,4C-1,4Bis capped AuNPs are less toxic than those capped by PEI at lower optical densities (Figure 3). EGDE-3,3' AuNPs were inconclusive. Our study also showed that optical densities below 0.005 a.u. are desirable for biological applications.

Preliminary Zeta Potential and DLS Measurements Reveal Colloidal Stability and Nanometric Scale of 1,4C-1,4Bis Capped AuNPs. Zeta potential and size measurements were performed for 50:1 1,4C-1,4Bis capped AuNPs with and without EGFP plasmid DNA. Our preliminary findings verified the stability of both polymer coated colloidal systems (Figure 4). The positive charge indicates successful polymer coating on the AuNPs, while the measurement of 20 mV suggest colloidal stability of the complex. DLS showed naked 1,4C-1,4Bis capped AuNPs were approximately 104 nm in diameter and 126 nm after DNA complexing.

Preliminary Fluorescence Microscopy Images Show Successful *in vitro* Transfection of Enhanced Green Fluorescent Protein (EGFP) Plasmid DNA. PC3 cells were treated with different doses of polymer capped AuNPs loaded with various amounts of EGFP plasmid DNA. Preliminary fluorescent microscopy images exhibited cells had produced the green fluorescence protein, indicating successful transfection. The resulting images also showed that PEI and 1,4C-1,4Bis appeared equally effective, while EGDE-3,3' AuNPs did not transfect.

Conclusions and Future Work:

Our results revealed a potential non-viral vector for gene delivery. PEA-AuNPs demonstrated promising characteristics: stability in SFM, colloidal stability, low cytotoxicity, and successful transfection of EGFP plasmid DNA in PC3 cells. We also discovered that our polymer, 1,4C-1,4Bis, exhibited more favorable characteristics than the polymer standard, PEI. Future work will determine whether this class of nanomaterials is a competitive gene delivery vector and will focus on producing a quantitative analysis of transfection efficacies, taking TEM images, and repeating the above experiments to ensure accuracy and precision of results.

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References:

[1] Mohammed, S., et al. Nonviral Gene Delivery: Principle, Limitations, and Recent Progress. The AAPS Journal. 11 (4) 671-681 (2009).

[2] Barua, S. et al. Parallel Synthesis and Screening of Polymers for Nonviral Gene Delivery. Molecular Pharmaceutics. 6 (1) 86-97 (2009).

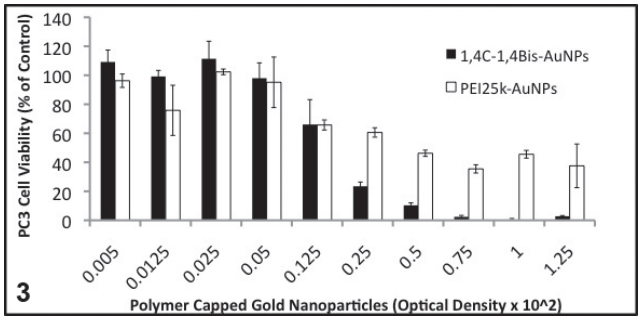

Figure 3: Cytotoxicity comparison between 50:1 1,4C-1,4Bis and PEI capped AuNPs at different optical densities.

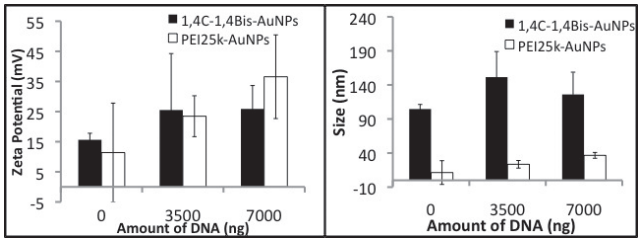

Figure 4: Preliminary zeta potential and DLS measurements for 50:1 1,4C-1,4Bis and PEI capped AuNPs.