Effects of Immobilized Oligoarginine Peptides on Cellular Uptake of Gold Nanoparticles

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Abstract:

Developing ways to deliver cargoes past the lipid bilayer of cells is a significant field of investigation in modern nanobiology. The use of cell-penetrating peptides (CPPs) [1,2] and incubation in the presence of pyrene butyrate [3] are two methods that have gained prominence. The objective of this project was to evaluate the efficiency of oligoarginines (R_n) as a CPP intended to internalize gold nanoparticles (GNPs) into cells. The GNPs were modified with dithiol-ended poly(ethylene glycol) (PEG) and an oligoarginine-conjugated PEG-dithiol (PEG-R_n). Several parameters such as GNP diameter, the ratio of PEG to PEG-R_n, and the length of both PEG and arginine molecules were investigated in detail. Results showed that 30 nm GNPs modified with a 1:1 ratio of PEG_{5k} and PEG_{5k}-R_{12} conjugate had significant interaction with the cell walls, ultimately resulting in cellular uptake through endocytosis.

Experimental Procedure:

A bulk conjugate of dithiobis (succinimidyl undecanoate) with PEG-R_n, PEG-Tx (Texas Red conjugate), or PEG was prepared in a two hour reaction in the presence of triethylamine as a catalyst. This disulfide conjugate was then reacted directly with citrate-capped GNP to immobilize the PEG, PEG-R_n, or PEG-Tx on the nanoparticle surface in an overnight reaction. The sample was then purified through centrifugation and washed into phosphate buffer saline (PBS). After sample preparation, zeta-potential was measured to confirm successful immobilization and absorbance spectra were recorded to check the sample's dispersity.

Cell experiments were then performed to determine the sample's level of interaction with the cell membrane. Because the lissamine rhodamine fluorescent labels on the arginine peptides were found to be quenched through interaction with arginine, confocal reflectance mode microscopy [4] was used to observe the GNP's interaction with cells. HeLa cells were incubated with 7 μM pyrene butyrate in PBS for 5 minutes, followed by a 10 minute incubation with GNPs and pyrene butyrate in PBS. After incubation with GNPs, cells were washed with Hank's Balanced Salt Solution (HBSS) to remove any GNPs that had not interacted with the cells. Cells were then observed on a Leica SP5 confocal in reflectance mode, followed by further incubation and observation.

Four parameters were varied throughout the project: GNP diameter, ratio of PEG to PEG-R_n, length of PEG, and length of arginine. GNPs of 5 nm, 15 nm, and 30 nm diameter were used, and the ratio of PEG to PEG-R_n was varied from 1:1 to 1:9. For cell experiments, all samples had a 1:1 ratio of PEG to PEG-R_n. This ratio was found to be an ideal balance between the cell penetrating properties of oligoarginine and the stabilizing influence of PEG on nanoparticle dispersity. In addition, PEG of molecular weights 5000 (PEG_{5k}) and 2000 (PEG_{2k}), and oligoarginines composed of 8 (R_8) and 12 (R_{12}) arginines, were used.

Results and Discussion:

Because aggregated GNPs change from red to blue, an absorbance spectra is important in determining the stability of the sample. As seen in Figure 1, the absorbance spectra indicated no significant aggregation in the samples used in

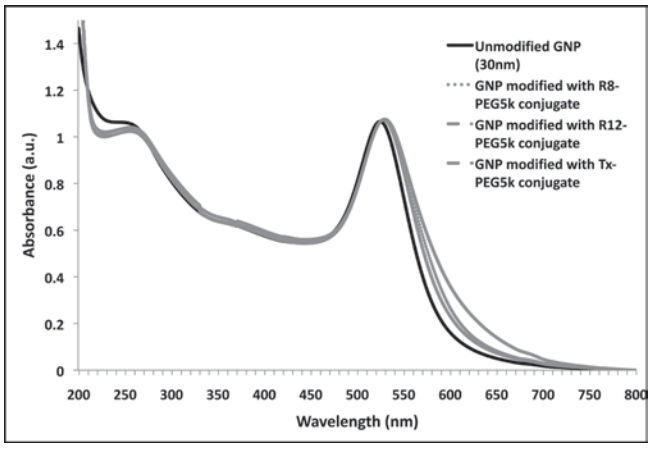

Figure 1: Absorbance spectrum of modified and unmodified particles.

cell experiments. In addition, the successful immobilization of Rn can be inferred from the slight red shift in the spectra of the modified particles due to the color of lissamine rhodamine dye. Immobilization of PEG and PEG-R_n to the GNP surface was also verified from the change in the zeta potential from -27.3 to 0.24 mV.

Of all the parameter permutations attempted, only the 30 nm GNP modified with a 1:1 ratio of PEG_{5k} to PEG_{5k}-R_{12} conjugate successfully interacted with the cells. It is important to note that, if either the particle diameter or the length of the arginine peptide is changed, the GNP ceases to have any interaction with the cells. Thus, a significant conclusion of this work is that particle diameter and peptide length are dependent parameters for inducing cellular uptake.

When GNPs modified with PEG_{5k}-Tx were incubated with cells, little to no reflectance was observed, indicating low internalizing efficiency of GNPs without oligoarginine, as shown in Figure 2. On the other hand, high reflectance was observed when GNPs modified with PEG_{5k}-R_{12} were incubated with cells (Figures 3 and 4).

In addition, it should be noted that, as seen in Figure 3, the GNPs are only found on the outside of the cell after the initial 10 min incubation. In Figure 4, however, the GNPs can be seen inside the cell after an additional 3 hour incubation. This slow uptake of the particles is most likely due to endocytosis, rather than direct membrane translocation. To achieve direct membrane translocation, the interaction between particles and the cell membrane must be further optimized.

Conclusions:

The combination of 30 nm GNPs with PEG_{5k}-R_{12} creates the strongest interaction with the cell membrane of any other attempted combination. This sample was ultimately internalized through endocytosis.

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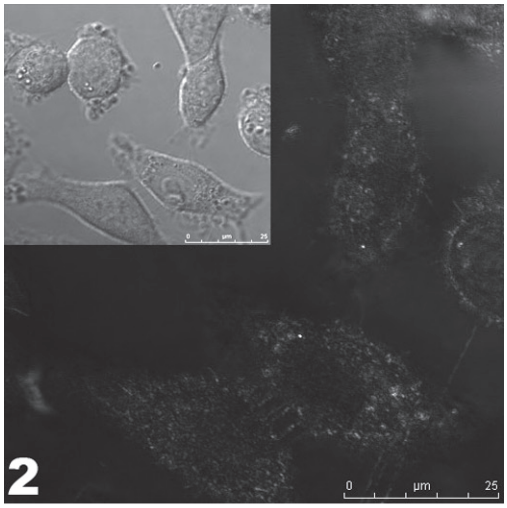
References:

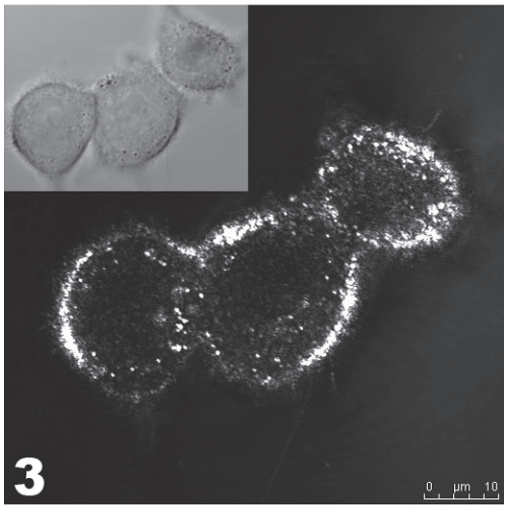
[1] Pujals, S; "Shuttling Gold Nanoparticles into Tumoral Cells with an Amphipathic Proline-Rich Peptide." ChemBioChem, 10, 1025-1031, (2009).

[2] Takayama, K; "Novel System to Acheive One-Pot Modification of Cargo Molecules with Oligoarginine Vectors for Intracellular Delivery." Bioconjugate Chem, 20, 249-257, (2009).

[3] Takeuchi, T. "Direct and Rapid Cytosolic Delivery Using Cell-Penetrating Peptides Mediated by Pyrenebutyrate." ACS Chemical Biology, 5, 299-303, (2006).

[4] Pujals, S. "Shuttling Gold Nanoparticles into Tumoral Cells with an Amphipathic Proline-Rich Peptide." ChemBioChem, 10, 1025-1031 (2009).





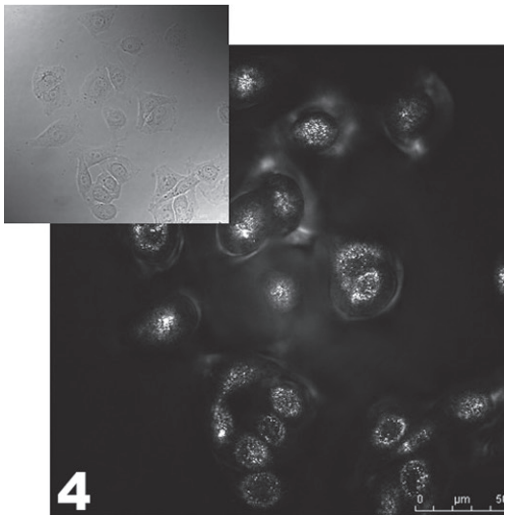


Figure 2, top: Reflectance image of HeLa cells loaded with GNP modified with 1:1 ratio of PEG_{5k} and PEG_{5k}-Tx.

Figure 3, middle: Reflectance image of HeLa cells loaded with 30 nm GNP modified with 1:1 ratio of PEG_{5k} and PEG_{5k}-R_{12} after 10 minute incubation.

Figure 4, bottom: Reflectance image of HeLa cells loaded with 30 nm GNP modified with 1:1 ratio of PEG_{5k} and PEG_{5k}-R_{12}, three hours after initial incubation.