

Mixed-Monolayer Gold Nanoparticles for Cancer Therapeutics

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Abstract

This paper describes the development of an efficient transfection vehicle for nucleic acids using surface-functionalized gold nanoparticles (AuNPs) with a mixed-monolayer of biological molecules—a hybrid approach that combines two previously investigated models. The objective is to improve upon previously described nanoparticles used to introduce nucleic acids to cells—oligonucleotide-functionalized AuNPs and liposomal nanoparticles—and tailor the new structures in such a way that they will be more biologically compatible in the context of cellular and subcellular therapeutic delivery. Using several quantitative methods, the goal of creating mixed-monolayer AuNPs with covalently coupled surface oligonucleotides was confirmed. Initial research demonstrates that these unique conjugates interact with human cancer cells. Furthermore, these conjugates may be explored as a robust nanoparticle platform for controlled covalent attachment of biological molecules (e.g., DNA and proteins) to the mixed-lipid monolayer adsorbed to the nanoparticle surface.

Introduction

The surface modification of AuNPs with nucleic acids (e.g., DNA) has generated nanoparticles (DNA AuNPs) with unique and tunable biological properties.¹ DNA AuNPs are being explored as cancer diagnostics and therapeutics by tailoring these properties to optimize their diagnostic capabilities, increase cellular uptake, and affect gene regulation.^{2,3} As potential therapeutics, DNA AuNPs near-universally enter cells via endocytosis.⁴ It is desirable to maximize the number of AuNPs that enter the cytosol of cells so that they can be most effective in targeting messenger RNA (mRNA) and inhibiting protein translation.⁵ In order for this type of therapy to be maximally effective, the AuNPs must escape the endosome. Nanoparticles made of phospholipids, most commonly liposomes, have been shown to be effective therapeutic vehicles for the delivery of nucleic acid cargo and are sold commercially as transfection and drug-delivery agents. These particles permeate the membrane of cells due to their phospholipid surface and

deliver their cargo to the cellular cytoplasm. However, these nanoparticles have been shown to be cytotoxic and require a mechanism to release internalized therapeutic cargo once inside the cell.

Seeking to avoid the pitfalls and capitalize on the benefits of each approach to therapeutic nucleic acid delivery, this project chemically combined both methods on a single platform, potentially providing a more effective approach to cancer therapeutics. This project fabricated AuNPs with a mixed monolayer of thiol-modified phospholipids—either 1,2-bis(11-mercaptoundecanoyl)-*sn*-glycero-3-phosphocholine (C10) or 1,2-bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (C15); and mercaptohexadecanoic acid (MHA). Surface functionalizing the nanoparticles with phospholipids may provide cell permeation and biocompatibility similar to liposomal phospholipid nanoparticles. MHA provides a convenient terminal carboxyl group for the covalent attachment of oligonucleotides. These conjugate structures, having both surface nucleic acids and phospholipids, may provide for avid cellular uptake and allow the nanoparticles to deliver oligonucleotides directly to the cytoplasmic compartment. By using a combined approach, these mixed-monolayer and DNA-functionalized nanoparticles may represent effective cancer therapeutics.

Background

Current approaches to therapeutic gene regulation with oligonucleotide- (DNA- or siRNA-) functionalized AuNPs demonstrate that the nanoparticles are taken up by cells through energy-dependent endocytosis.⁷ The consequence of this process is that many of the nanoparticles are trapped in endosomes and do not maximally concentrate in the cytosol.^{5,7} For therapeutic approaches that require conjugate nanostructures to interact with targeted and pathologically up-regulated intracellular mRNA targets, this can limit therapeutic efficacy. The problem potentially addressed by the mixed-monolayer structure is not cellular uptake, but the subcellular localization of therapeutic nanostructures. Mirkin et al. demonstrated that oligonucleotide-functionalized AuNPs have a transfection efficiency of 99% in cultured cells and may be a universal nucleic acid transfection agent.⁶ These nanoparticles successfully targeted and downregulated gene expression in a model system. In this project, the aim is to improve the delivery of DNA AuNPs to the cytosol within the cell in order to achieve maximal gene regulating capacity.

Previous research using phospholipid vesicles, or liposomes, have shown that phospholipids are effective drug-delivery agents.⁸ In some cases, liposomes are able to permeate cell membranes. However, studies have also shown that as they pass through the cell membrane, these phospholipid particles (about 100 nm in diameter) generating temporary holes that which can be cytotoxic.⁷ By surface modifying AuNPs with a mixed monolayer of phospholipids and MHA, it is hypothesized that these nanoparticles will interact with cells in a manner that more closely resembles that of phospholipid liposomes, permeate the cell

acid moiety of MHA. A 3' fluorophore label (fluorescein) was used in order to easily quantify conjugate AuNP loading of oligonucleotides and to serve as visual labels for cell-culture experiments. Modified antisense oligonucleotides were purified using high-performance liquid chromatography. Coupling of antisense survivin DNA to the surface of mixed-monolayer AuNPs was determined using a fluorescent plate reader to measure the concentration of fluorescently labeled DNA on the AuNPs. Using a standard dilution series of the labeled oligonucleotide, the approximate number of DNA strands per particle was determined.

Nanoparticle Uptake in Cancer Cells

Mixed-monolayer nanoparticles were added to human prostate cancer cells (LnCaP) grown in culture and imaged using confocal fluorescence microscopy. LnCaP cells were grown in monolayer cell culture to 60–80% confluence in glass-bottom live-cell imaging dishes. Mixed-monolayer DNA-functionalized AuNPs were transfected at a concentration of 100 pM (12 hrs) and compared to a control group of cells incubated with phosphate-buffered saline (PBS). After incubation, the cell monolayers were washed with PBS (three times), and Leibovitz's media was added for confocal microscopy imaging of live cells.

Results

Nanoparticle Characterization

Dynamic light scattering was used to measure the size of the nanoparticles before and after surface modification (Table 1). Unmodified AuNPs have a hydrodynamic radius of 9 ± 1 nm. Upon addition of phospholipids and MHA, the diameter of the nanoparticle increases significantly and supports the presence of the mixed-lipid monolayer on the surface of the particles. Overall, the hydrodynamic diameter of the C15 lipid is greater than the C10 lipid, which agrees with the alkyl tail-length differences between C15 and C10 lipids. Figure 2 displays TEM images of the C10 and C15 lipid-mixed monolayer nanoparticles, each with a 10:1 ratio of phospholipid to MHA. Following negative staining with uranyl acetate a halo-like ring is evident around each nanoparticle, supporting the presence of the mixed monolayer on the nanoparticle surface. The presence of the carboxylic group was confirmed qualitatively via the addition of sodium bicarbonate and the evolution of gas (carbon dioxide) bubbles. The reaction for this experiment is:



Immobilization of DNA

Fluorescence measurements demonstrate the capability of mixed-monolayer phospholipid-functionalized AuNPs to bind DNA. A standard curve of free fluorescently labeled oligonucleotides was employed to determine the concentration and number of DNA sequences bound to the mixed-monolayer nanoparticles (Table 2). In general the results show an increase in the number of oligonucleotides bound to the AuNP surface as the ratio of phospholipids to MHA increases. Optimal binding of oligonucleotides to the mixed-monolayer AuNPs was observed for the C10 versus C15 phospholipid.

Nanoparticle Uptake in Cancer Cells

LnCaP prostate cancer cells were transfected with the mixed-monolayer DNA-functionalized AuNPs and imaged using confocal microscopy (Figure 3). Cells were transfected with AuNPs functionalized with the mixed monolayer of C10 and MHA or C15 lipids and MHA in a 10:1 ratio. In each case the mixed-monolayer AuNPs were surface functionalized with fluorescein-labeled DNA. The images in Figure 3 demonstrate

the lack of fluorescence in the control group of cells—the anticipated result. Co-localization of nanoparticle fluorescent signal and LnCaP cells in phase implies that both conjugates effectively interact with LnCaP cells. The subcellular localization of the mixed-monolayer nanoparticles cannot be verified from these images.

Discussion

This study demonstrates that AuNPs can be fabricated with a mixed monolayer of thiol-modified phospholipids and MHA. By using MHA as a surface component of the AuNPs, covalent coupling of amine-terminated DNA oligonucleotides can be achieved using well-established EDC/NHS coupling chemistry. The mixed-monolayer nanoparticles containing C10 lipids provided a more favorable chemical background for the covalent attachment of amine-terminated oligonucleotides to co-adsorbed MHA molecules. Presumably, the longer

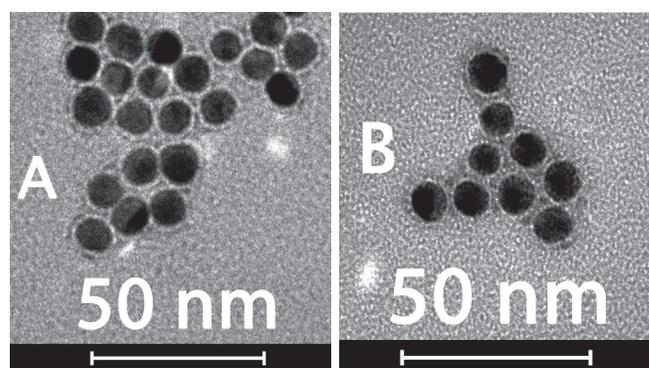


Figure 2. TEM images of mixed-monolayer functionalized AuNPs (stained with uranyl acetate) in a ratio of 10:1 of either a) C10 or b) C15 phospholipid to MHA. (Andrea Luthi, Department of Chemistry, Northwestern University)

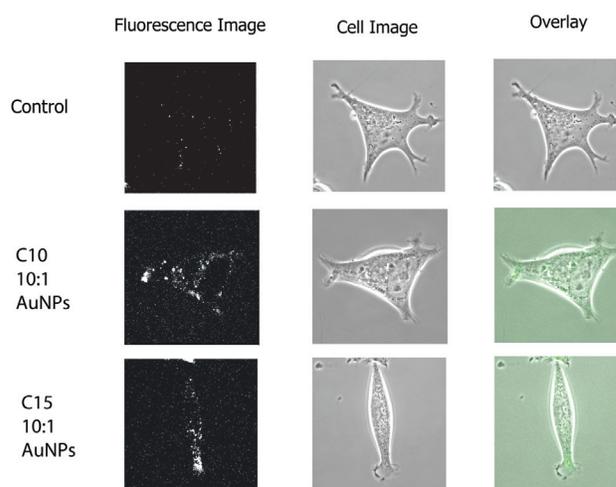


Figure 3. LnCaP prostate cancer cells transfected with mixed-monolayer functionalized AuNPs and imaged using confocal microscopy. (Brian Helfand, Department of Urology, Feinberg School of Medicine)

alkyl tail length of the C15 versus C10 lipid caused increased steric hindrance of productive MHA coupling to incoming amine-terminated DNA sequences.

As Table 2 indicates, an increasing amount of lipid to MHA resulted in an increasing amount of DNA loading per particle. Theoretically, the more MHA available to couple with amine-terminated DNA, the higher expected concentration of coupled oligonucleotides. However, the experimental results indicate that the negatively charged carboxylic acid groups, when present in increasing amounts, may increase electrostatic repulsion of incoming DNA oligonucleotides and lead to a reduction of surface attachment. On the other hand, the positively charged choline head group of the phospholipid may provide for an increasingly favorable electrostatic attraction between the particle surface and the incoming DNA. Further study is required to fully understand and optimize the covalent coupling of amine-terminated DNA to the surface of mixed-monolayer AuNPs. However, by accomplishing DNA loading onto these mixed-monolayer AuNPs, the potential exists for conjugates to be used as cancer therapeutics and for controlling gene expression.

This goal of this project was to assess the ability of DNA-functionalized mixed-monolayer AuNPs to be taken up into prostate cancer cells grown in culture as a means to determine their potential as therapeutic agents. Initial cell-uptake experiments imply that the nanoparticle conjugates interact favorably with cancer cells. Future studies will focus on the interaction of the mixed-monolayer DNA AuNP conjugates with cancer cells and more thoroughly assess their subcellular distribution and biological function.

Conclusions

The results of this project demonstrate a successful approach to surface functionalizing AuNPs with both lipids and DNA in order to potentially realize the benefits of both of these biological molecules in the context of cellular transfection and gene regulation. Further study will be necessary in order to verify the exact mechanism these nanoparticles employ to interact with cells, their ultimate subcellular fate, and their biological function with regard to gene regulation.

Although the primary purpose of this research was geared toward cancer therapeutics, these results also open up the possibility for future work involving other applications with proteins or other biologically important molecules coupled to the surface of the mixed-monolayer AuNPs using facile EDC/NHS coupling chemistry. Certainly, mixed-monolayer AuNPs present an exciting nanoparticle platform for future work.

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