Controlled Therapeutic Release from a Nucleic Acid Aptamer Functionalized Gold Nanoparticle Platform

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Statement of Purpose: Nucleic acid aptamers can be conjugated to gold nanoparticles (AuNps) by stable thiol-gold covalent bonds and can be highly responsive to various physical and enzymatic triggers (1, 2). Moreover, they can be used for controlled release of therapeutic or multiple therapeutics from a particle surface. In this work, we have developed a nano-drug delivery carrier for the antibiotic neomycin. This novel carrier, designed using the principles of molecular biology, is expected to impact a number of treatment strategies.

Methods: An anchor DNA strands with thiol modification on their 5' terminal were synthesized in-vitro and attached to 15-nm gold nanoparticles (AuNps) via covalent bonds, which were stable at temperatures up to 90°C. The attachment of DNA to AuNps was confirmed by agarose gel electrophoresis. DNA loading was optimized using [^{32}P]-labeled anchor DNA oligonucleotide. We determined that stable conformation of attached DNA on AuNps depended on DNA density and length of the DNA strands. We also determined mean diameter of DNA functionalized AuNps using dynamic light scattering. Neomycin binding affinity to DNA aptamer was investigated using surface plasmon resonance (SPR). The change in response units in SPR were recorded for the calculation of dissociation constant (K_d). Aptamer with 3' tail part complimentary to the anchor DNA were hybridized to anchor DNA oligonucleotides and the resulting complex of the DNA aptamer and DNA anchor was attached to the AuNps (pre hybridization). Alternatively, the anchor DNA was attached to AuNps and then the aptamer was hybridized (post hybridization). Neomycin was attached to aptamer before or after immobilizing the aptamer on AuNps (pre and post binding), respectively.

Neomycin release was investigated using a dialysis approach. Temperature was used to disrupt aptamer-neomycin complexes. In control experiments, aptamers were omitted and free neomycin was used.

Results: The salt and DNA concentration affects the loading of DNA on AuNps. In our work, the loading was maximized at 0.4M NaCl and 4µM thiol-derivatized DNA strands, respectively. An average of 100 strands of anchor DNAs were immobilized per AuNp at optimal conditions. Further increase in salt and DNA concentrations had detrimental effects on loading. As the number of immobilized DNA on AuNps increased, DNA strands adopted different conformation, ranging from coiled to fully elongated. The DNA conformation also changed from fully elongated to partially coiled at maximum loading as DNA length was increased. Comparing both the methods of hybridization of aptamer to anchor, we found that pre-hybridization method yielded more aptamers per AuNp than the post- hybridization method. On average 21 neomycin molecules were attached per gold particle using both pre- and post-method. The change in response unit with neomycin binding SPR was correlated to concentration of neomycin by Burk's equation and the calculated K_d for neomycin attachment to DNA was shown to be 100nM which is approximately equal to the K_d of the RNA aptamer binding neomycin found in literature (3). The neomycin release study showed an increase in drug release rate as temperature was increased due to a decrease in number of hydrogen bonds in the double-stranded segment of the aptamer. Release was extended for approximately 1 day. The unattached neomycin control experiments indicated that the release was slowed due to the interaction of the aptamer and therapeutic and was not due to varying diffusion phenomena. These results strongly indicate that release may be controlled and extended at a given temperature by decorating an affinity spectrum of aptamer on the particle surface.

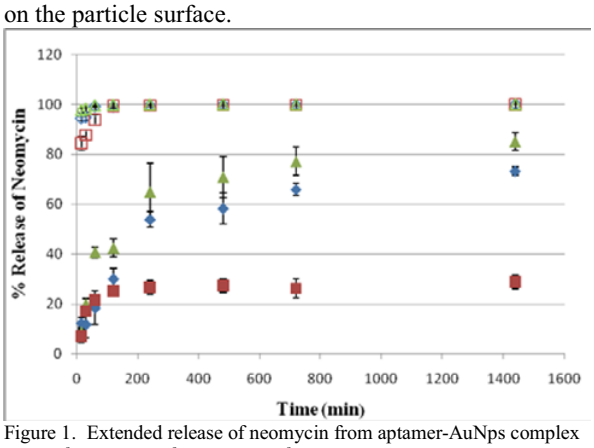

Figure 1. Extended release of neomycin from aptamer-AuNps complex at 3 ± 1°C (■),22 ± 1°C (♦), 40 ± 1°C (▲) and diffusion of unattached neomycin at 3±1°C (□), 22±1°C (◊), 40±1°C (△).

Conclusions: We have synthesized, characterized and optimized nano-drug delivery carrier which utilizes the versatile properties of nucleic acid for programmable and on-demand drug release. A controlled drug release profile from the synthesized carrier was obtained by altering the complexation of the aptamer to drug via temperature changes. Also, for the first time, we have shown that DNA aptamers bind neomycin with the same affinity as RNA aptamers. The way neomycin binds to DNA is currently under investigation as it may explain side effects of neomycin. Lastly, this work highlights the significant potential to design nanoparticle carriers with extended and controlled release by using aptamers.

References:

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