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Using nucleic acid-gold nanoparticle conjugate in the fight against bacteria that are resistant to tetracycline antibiotics

Allen T. Livingston
Winthrop University

Using Nucleic Acid-Gold Nanoparticle Conjugates in the Fight Against Bacteria that are Resistant to Tetracycline Antibiotics

Allen Livingston (2023)

Mentor: Dr. Timea Fernandez

Abstract

Antibiotic resistance is major problem in modern medicine. Seventy percent of bacterial strains are resistant to at least one antibiotic, making treatment of bacterial infections ever more expensive and difficult. Currently, we are investigating the therapeutic potency of nucleic acid-gold nanoparticle conjugates as treatments against bacteria that are resistant to the antibiotic tetracycline. We hypothesize that by attaching RNA that binds to tetracycline to silver or gold nanoparticles the resulting conjugates will work as a "Trojan-horse" tetracycline-delivery vehicle that smuggles the antibiotic into the cell without being detected by cellular defense systems. Moreover, we reason that silver or gold ions released by the nanoparticles add to the antimicrobial effects of tetracycline.

To demonstrate the viability of this idea, we set out to generate a nuclease resistant variant of the tetracycline binding RNA ykkCD and attach it to gold nanoparticles. During the summer we furthered the progress of this research in there major ways. First, we optimized conditions used for the polymerase chain reaction that generates the DNA template for RNA synthesis. Second, we found the most efficient way to synthesize RNA containing modified nucleotides. Usage of modified nucleotides is necessary to prevent degradation of the tetracycline-binding RNA by cellular nucleases. Third, we optimized a stability assay to verify that the modified RNAs are stable in cellular environments. Fourth, we attached the tetracycline binding RNA to gold nanoparticles.

The next steps in the process would be to test the potency of this modified RNA-gold nanoparticle conjugate against a tetracycline-resistant strain of the bacteria E. Coli.

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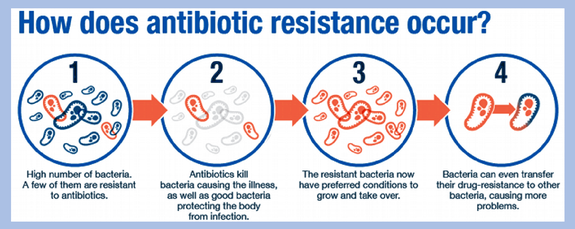

Source: http://modmedmicro.nsms.ox.ac.uk/learn-more-about-antibiotic-resistance/

Figure 1: Mechanism of bacterial antibiotic resistance.

What are nanoparticles?

Nanoparticles are particles that vary in size from 1nm-100nm, they are also tools that detect and deliver small molecules. They are used in our work to transport medicine to bacteria that would normally be resistant to the medicine.

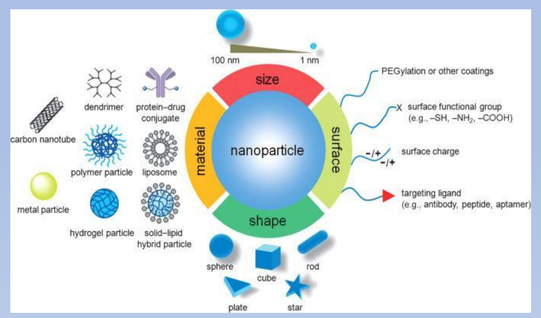

Figure 2: Schematic describing the versatile applications of nanoparticles.

Usage of RNA aptamers in medicine

We propose that using nucleic acid-gold nanoparticle conjugates could be an effective way to treat antibiotic-resistant bacterial infections. We hypothesize that the tet-binding RNA aptamer will deceive tet-resistant cells to uptake tetracycline by acting as a caging compound. The tet-binding aptamer is attached to gold nanoparticles, because bacteria is known to uptake nanoparticles 4-40nm in size using receptor mediated endocytosis. In addition to the cytotoxic affect of tetracycline, the Ag^{+} ions released form the nanoparticle will add to the antimicrobial effect of tetracycline.

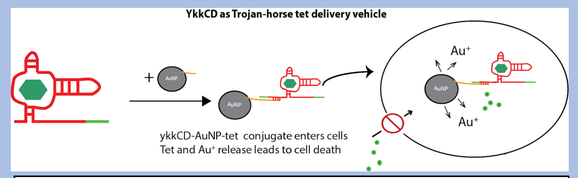

Figure 3: Schematics showing how the tet-binding RNA aptamer is attached to nanoparticles and expected to exert is antibacterial function.

Why is the RNA modified?

RNA aptamers that are used as treatment have to be resistant to cellular nucleases to exert their function. Natural RNA nucleotides are replaced with 2' modified ones to avoid self-cleavage of RNA.

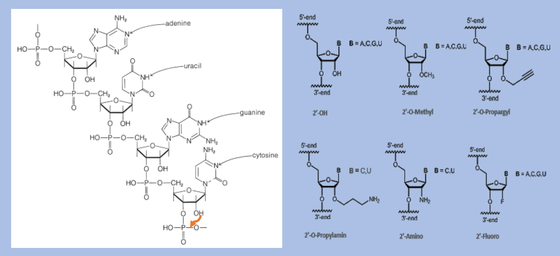

Figure 4: *Left panel:* RNA is prone to degradation because the 2'OH act as nucleophile to attack the adjacent phosphodiester bond and cleave the backbone (red arrow). *Right panel.* Modified RNA nucleotides. 2'-fluoro modified nucleotides are used in this project.

Results

- Optimized the synthesis of template DNA.
- Optimized the conditions used to synthesize RNA.
- Demonstrated that modified RNA is resistant to cellular nucleases.
- Attached gold nanoparticles to RNA.

DNA synthesis was most efficient with high template concentration

To optimize *in vitro* DNA synthesis using PCR we varied the concentration of the DNA template, Mg^{2+} and tested whether the addition of DMSO increases DNA yield. We found that elevated template DNA concertation increased PCR yield but other additives did not.

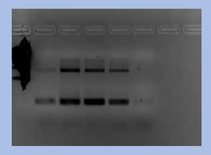

Figure 5: Optimization on template DNA synthesis with PCR. From left to right with (1) DNA ladder, (2) low template mixture, (3) high template mixture, (4) extra Mg^{2+}, (5) DMSO and (6) no enzyme negative control. The samples were resolved on a 1% agarose gel that was ran at 100V for 30 minutes.

RNA synthesis was most efficient with 12ng/µl DNA, excess Mg^{2+} and nucleotides.

To optimize *in vitro* RNA synthesis we varied the following parameters. The first optimization involved how much the concentration of template DNA affected the production of RNA. The second optimization varied the amount of Mg^{2+} and NTP concentration and used modified nucleotides. We found that 12ng/µl DNA, 20mM Mg^{2+} and 4mM NTP produced optimal RNA yield.

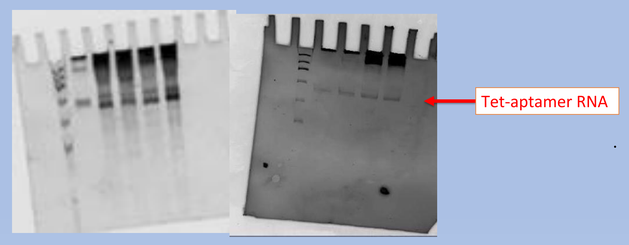

Figure 6: Optimization of RNA synthesis. *Left panel:* (1) DNA Ladder (2) no enzyme, (3) 1mM NTP, 5mM Mg^{2+}, (4) 1mM NTP, 20mM Mg^{2+}, (5) 4mM NTP, 5mM Mg^{2+} and (6) 20 mM Mg^{2+}, 4mM NTP. *Right panel:* (1) DNA Ladder (2) No Enzyme (3) 6ng/µl DNA (4) 12 ng/µl DNA (5) 15 ng/µl DNA. Samples were resolved on a 10% denaturing PAGE run for 30min with 15W.

The tet-binding RNA aptamer is resistant to cellular nucleases

To assess the stability of the tet-binding aptamer in biological fluids we incubated the RNA with 10% bovine serum for 80 minutes at 37°C. Start and end point samples were compared on a denaturing PAGE. Modified RNA samples only show slight degradation as indicated by a single band that co-migrates with the start point samples. Unmodified RNA (last sample) appears completely degraded as it does not appear to be present in the end-point sample.

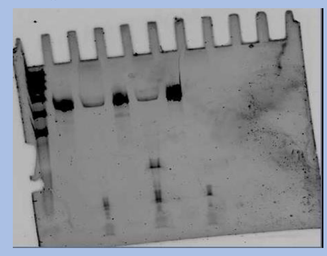

Figure 7. RNA stability assay. From left to right (1) RNA molecular weight ladder, (2) dUTP RNA untreated, (3) dUTP RNA treated, (4) 2'-F-dUTP RNA untreated, (5) 2'-F-dUTP RNA treated.

RNA was successfully attached to gold nanoparticles

A slower migration of the RNA-AuNP conjugate compared to AuNPs alone (gel retardation) indicates that the RNA was attached to the nanoparticles

Figure 8. Attachment of RNA to gold nanoparticles. From left to right. (1) gold nanoparticles, (2) 2-F'-dUTP RNA-gold nanoparticle conjugates and (3) dUTP RNA-gold nanoparticle conjugates. Samples were run on a 1% agarose gel for 60min at 100V.

Future goals

We will make more of this Au-modified RNA nanoparticle conjugate and test its efficacy on tet resistant *E Coli*.

We will try other tet-binding aptamers. We will use secondary structure prediction and optimize the length of the linker between the tet-binding aptamer and the sequence that targets the RNA to the ribosome. We will aim for a setup that maintain the structure both aptamers.

Acknowledgement and Funding

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