COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

DNA Origami Nanostructures For Effective Targeted Intracellular Delivery Of Small Molecule Drugs.

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Background and purpose: Finding novel methods of increasing intracellular efficacy of antibiotics is crucial in the fight against biothreats. Here, we are developing a technology that will transform how the military manages and treats infectious diseases. This work would drastically increase drug efficacy due to lowered effective dose and targeted drug delivery and would help overcome antibiotic resistance by repurposing existing antimicrobial drugs.

During the course of infection, Y. pestis is known to hide intracellularly within macrophage cells to evade the immune system. These intracellular infections are particularly challenging to treat, partially because the therapeutic must be delivered inside the host cell at an effective concentration. This often results in patients being treated with high doses of antibiotics in attempt to get to the bacteria which results in side-effects and often leads to antibiotic resistance.

Objective and rationale: In this project, we investigated the intracellular antibacterial efficacy of the antibiotic Doxycycline loaded onto DNA origami (DNAO) nanostructures against Yersinia pestis A1122 as compared to the free drug. The goal for this research was to demonstrate that encapsulation and delivery of the antibiotic, Doxycycline, using DNA origami nanostructures increases the intracellular efficacy of the antibiotic against Y pestis as compared to the efficacy of the free drug.

Relationship to other areas of study: This work is being adapted to deliver antivirals, small molecule immune modulators and host directed proteins. DNA origami is also amenable to artifical intelligence guided learning and can be adapted as a universal drug delivery platform.

Methods: Cuboid shaped DNA origami nanostructures (50 nm x 21 nm x 16 nm) were synthesized, purified, characterized and loaded with Doxycycline hyclate. RAW.267 macrophage cells were infected with Y. pestis and treated with three concentrations—10ug/mL, 100ug/mL, or 1000ug/mL—of either DNAO + doxycycline or free doxycycline at 12 hours post infection to determine intracellular bacterial survival rates at 4, 12, and 24 hours post treatment.

Preliminary results and conclusions: ·DNAO loaded doxycycline was able to control intracellular infection similarly to free doxycycline despite a 5X lower concentration of drug at 24 hr post-infection. These results, which are the first ever demonstration of delivering Doxycyline using DNA origami nanostructures, have tremendous potential for (1) increasing drug efficacy and decreasing side-effects due to dose sparing phenomena and (2) drug repurposing. We also observed controlled drug release of the DNAO loaded drug which can be tuned by modulating DNAO shape and structure.

Impact to DTRA JSTO mission and the Joint Force: Developing targeted delivery systems to increase the efficacy of existing antibacterial drugs which can be stored stably and administered systemically can improve therapy outcomes by preventing the need for repeat rounds of antibiotics due to treatment failure, which increases healthcare costs, raises the risk of side effects, and contributes to antibiotic resistance. As no single treatment or approach is likely to target and treat all pathogens, investing in combination therapies to combat multi-drug resistance is crucial. This research is relevant to the DTRA JSTO mission as it increases the nation's preparedness in fighting off biothreats.