COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Research and Creative Experience for Undergraduates - Summer 2019

Nanobiotech

Targeted Nanocarriers for Small Molecule Drug Delivery to *Mycobacteria*

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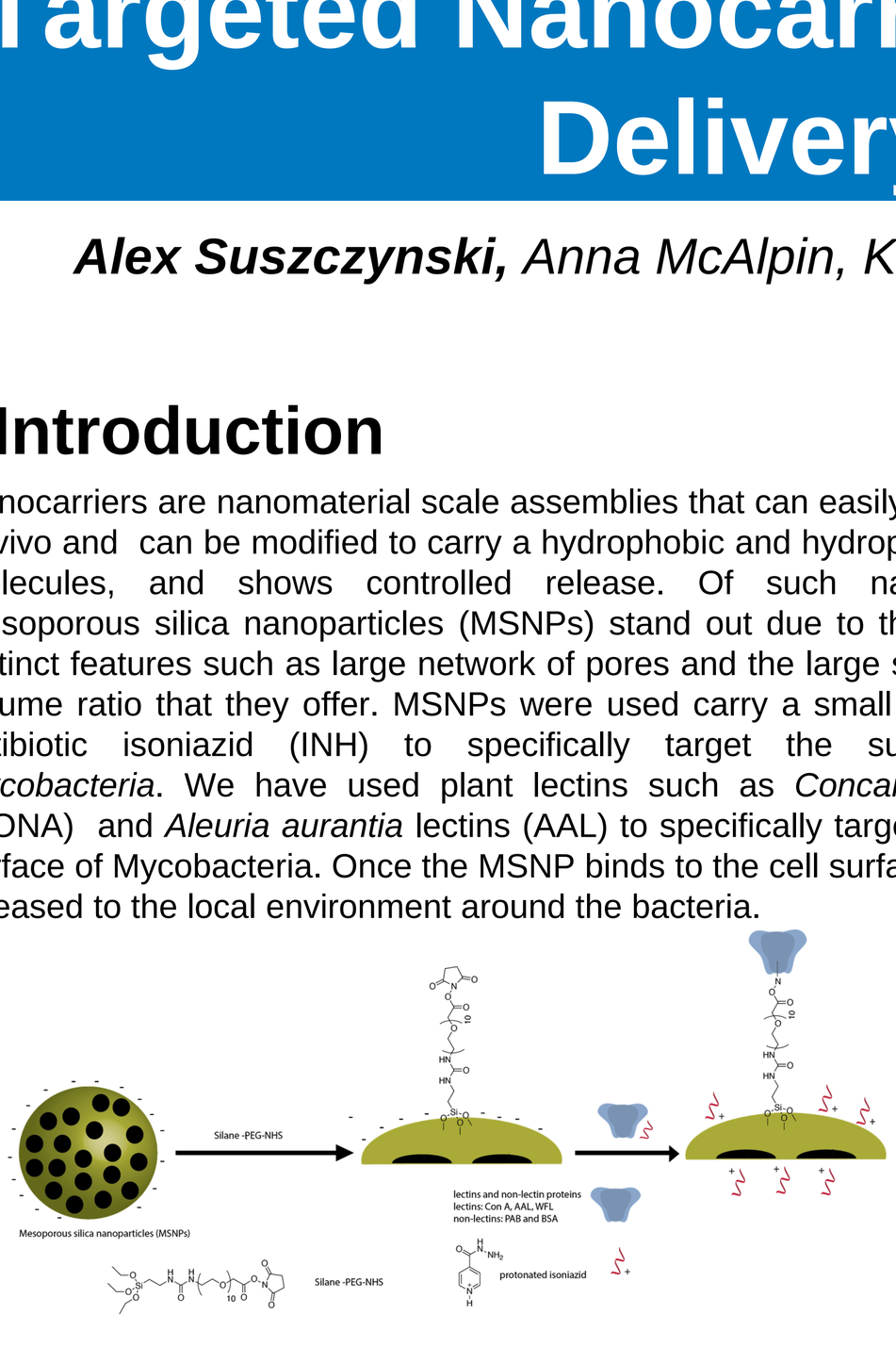
Introduction

Nanocarriers are nanomaterial scale assemblies that can easily circulate in-vivo and can be modified to carry a hydrophobic and hydrophilic drug molecules, and shows controlled release. Of such nanocarrier mesoporous silica nanoparticles (MSNPs) stand out due to their many distinct features such as large network of pores and the large surface to volume ratio that they offer. MSNPs were used carry a small molecule antibiotic isoniazid (INH) to specifically target the surface of *Mycobacteria*. We have used plant lectins such as *Concanavalin* A (CONA) and *Aleuria aurantia* lectins (AAL) to specifically target the cell surface of Mycobacteria. Once the MSNP binds to the cell surface INH is released to the local environment around the bacteria.



Scheme 1. Synthesis of lectin conjugated isoniazid (INH) loaded mesoporous silica nanoparticles (MSNP-INH-Lectin)

Materials and Methods

MSNP Synthesis - 204 mg of Cetrimonium bromide (0.00056 mol) were mixed with 140 mL of filtered water and allowed to stir. 1.688 mL of tetraethyl orthosilicate (TEOS, 0.008 mol) was added dropwise to the mixture. 1.688 mL of ammonium hydroxide (NH_4OH, 0.043 mol) was additionally added to the mixture and added dropwise. The solution was allowed to stir overnight. The next day, the solution was centrifuged at 7830 rpm for 10 minutes. The supernatant was removed, and 15 mL of water was added to the precipitate. The solution was sonicated and added to a round bottom flask. 100 mL of ethanol (1.714 mol) was added to the solution. Additionally, 0.2 mL of hydrochloric acid (HCl, 0.0065 mol) was added to the solution. The solution was heated at 60℃ and stirred at 400 rpm overnight. The next day, the solution was centrifuged for 10 minutes at 7830 rpm. The supernatant was removed, and 15 mL of water was added to the precipitate. The solution was sonicated, and the solution was centrifuged for 10 minutes at 7830 rpm. This step was repeated. Once the final solution was prepared, the zeta and size were measured.

MSNP-INH Synthesis - After the purification of the MSNP, 10 mg of isoniazid (INH) was dissolved in 5 mL of filtered water. This mixture was added to the MSNP solution. Afterwards, 60 µL of a diluted Hydrochloric acid (1 mL Hydrochloric acid 9 mL water) was added to the solution. The solution was allowed to stir overnight. The next day the mixture was centrifuged at 7830 rpm for 10 minutes. After being the centrifuged, the size, zeta, and concentration of the solution was measured.

MSNP-PEG-NHS Synthesis - After the purification of the MSNP, 1 mg of the ligand NHS-PEG-Silane (0.0002 mol) was added to the solution of MSNP and, it was allowed to stir overnight. The next day the mixture was centrifuged at 7830 rpm for 10 minutes. After being the centrifuged, the size, zeta, and concentration of the solution was measured.

MSNP-Protein Synthesis - After the addition of the ligand (NHS-PEG-Silane) 1 mg of the protein (lectin and non-lectin) being tested (CON A, bovine serum albumin (BSA), AAL, polyclonal antibody (PAB), or *Wisteria floribunda* lectin (WFL) was added to the MSNP solution. The solution was allowed to stir overnight. The next day the mixture was centrifuged at 7830 rpm for 10 minutes. After being the centrifuged, the size, zeta, and concentration of the solution was measured.

Characterization - Size (diameter) of the spherical particles were measured using dynamic light scattering (DLS, Malvern NanoZS). The surface charge was measure using zeta potential. Particles were imaged using transmission electron microscope (TEM, 200 kV FEI Tecnai Osiris)

Antibacterial Assays - *Mycobacterium smegmatis (Msmeg)* was grown to a absorbance of 0.5 (10^5 CFU/mL), 10 µL was incubated at 37 ^oC overnight with, 20 µL of varying concentrations (1 µg/mL - 1 mg/mL) of nanoparticles. The following day 20 µLof the incubated solution was incubated with 10 µL of water soluble tetrazolium (WST-8) reagent and 170 µL of Mueller Hinton broth for 2 hours at 37 ^oC. After incubation the absorbance was recorded at 650 nm.

Determining Antibacterial Activity - The antibacterial activity of the particles were determined by the results of WST-8 assay. The absorbance at 650 nm was compared to the control of live untreated bacteria to determine percentage (%) viability.

Results and Discussion

Particles	Diameter (nm)	Zeta Potential (mV)	Concentration (mg/mL)
MSNP	492.7 nm	-40.8	0.8 mg/mL
MSNP-INH	763.6 nm	12.8	1.4 mg/mL
MSNP-PEG-NHS	432 nm	-26.2	1.1 mg/mL
MSNP-CONA	441.2 nm	-39.3	1.2 mg/mL
MSNP-BSA	413.1 nm	-34.0	2.7 mg/mL
MSNP-AAL	401.7 nm	-34.3	1.3 mg/mL
MSNP-PAB	391.1 nm	-32.0	2.0 mg/mL
MSNP-WFL	365.3 nm	-33.8	0.9 mg/mL
MSNP-INH-CONA	637.3 nm	26.4	2.7 mg/mL

Table 1. The diameter and zeta potential was measured for all synthesized mesoporous silica particles (MSNs).

MSNPs are negatively charged as can be seen with the zeta potential of -40 mV. But as the surface is conjugated with proteins and antibiotics the surface charge has changed to +26 mV.

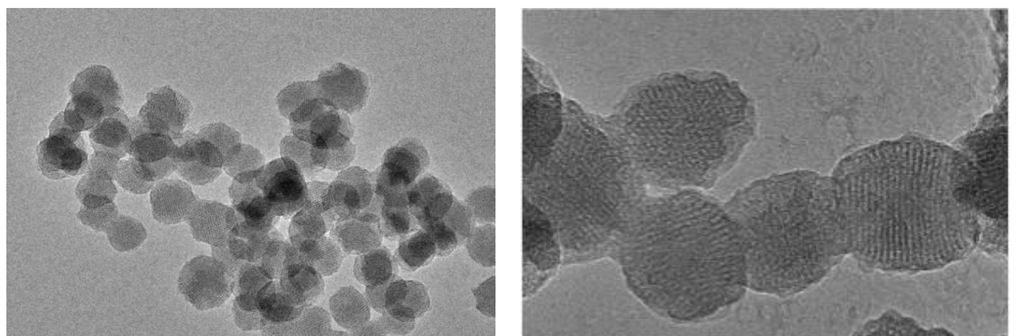


Figure 1. Transmission electron microscopy image of (left) mesoporous nanoparticles (MSNPs). (right) image magnified to show the porous network of MSNPs.

Porosity of MSNPs measured by Brunauer–Emmett–Teller method. Total pore volume was found to be 2.4 cm^3/g and surface area was measure to be 8.8 m^2/g. The antibacterial assay shows that both MSNP-INH-CONA, and MSNP-INH has antibacterial activity.

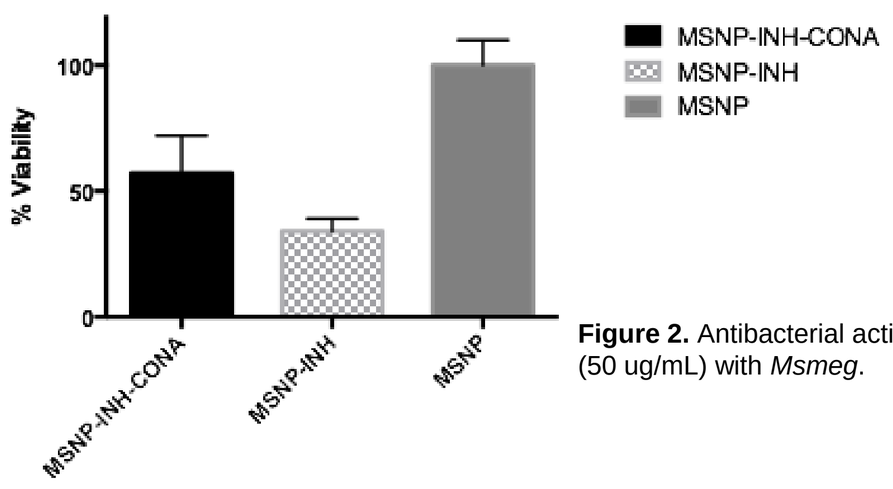


Figure 2. Antibacterial activity of the particles (50 ug/mL) with *Msmeg*.

Conclusions and Future Work

MSNP-INH-CONA has lower antibacterial activity than MSNP-INH. For future work, INH will be quantified on MSNP-NIH-CONA and the assays will be repeated.