ND*nano* Undergraduate Research Fellowship (NURF)
2015 Project Summary

1. Student name: Jennifer Diamond

2. Faculty mentor name: Dr. Paul Huber

3. Project title: Toxicity Studies of Nanoparticles

4. Briefly describe any new skills you acquired during your summer research:

Microinjector was used to inject the nanoparticles into the embryos of Xenopus.

Plasmid Prep was used to purify plasmid DNA.

Dynamic light scattering was used to measure the size of the nanoparticles of interest.

5. Briefly share a practical application/end use of your research:

Nanoparticles are present in everyday life from medicine to cosmetics to food. Since they are so commonly found in products it is important to figure out their effects on the world. As of right now there is relatively little knowledge on the health risks of these products which is why studying them is so important.

Project Summary:

Nanoparticles are microscopic particles that are classified by the size of their dimensions and come in many different forms. A common nanoparticle is TiO_2 which is a metal oxide nanoparticle that is found in a large number of consumer products, and has been found in recent experiments to have toxic effects. The cause of these toxic effects has yet to be revealed and could be caused by composition of the TiO_2 (the chemical and physical characteristics of the particles) or by the size of the particle. The cause of these toxic effects has yet to be revealed and

could be caused by composition of the TiO2 (the chemical and physical characteristics of the particles) or by the size of the particle. Polystyrene beads were chosen as an inert control for experiments to characterize the apparent toxicity of TiO2 nanoparticles. Two different types of polystyrene beads were selected: 1.) surfactant free polystyrene beads and 2.) polystyrene beads coated with the protein BSA..

The two different polystyrene beads were injected directly into the embryo over multiple trials. This was done by a microinjector allowing direct injection into the embryos. The BSA coated polystyrene beads and the surfactant free polystyrene beads were each injected at the following concentrations of 225 pg, 45 pg and 9 pg with water injection serves as a negative control that serves as a base of comparison. By using TiO_2 we were able to look at a known toxic nanoparticle and compare it to the two different types of coated polystyrene beads in terms of toxicity. Overall both types of polystyrene beads had an effect on the embryo's overall development with a higher mortality and adverse phenotypes when compared to the controls. The BSA coated polystyrene beads had the most significant mortality with consistently over half of the embryos at each concentration dying but not a noticeable dose dependence. This can be seen in Table 1 as a summary of the results take over a 4 day period.

Injected	24 hours past inj.	48 hours past inj.	72 hours past inj.	96 hours past inj.
225 pg BSA Coated Beads	11 out of 40 alive	9 out of 40 alive	9 out of 40 alive	9 out of 40 alive
45 pg BSA Coated Beads	12 out of 40 alive	11 out of 40 alive	11 out of 40 alive	11 out of 40 alive
9 pg BSA Coated Beads	13 out of 40 alive	12 out of 40 alive	11 out of 40 alive	11 out of 40 alive
Water	34 out of 40 alive	33 out of 40 alive	33 out of 40 alive	33 out of 40 alive
Noninjected	38 out of 40 alive	37 out of 40 alive	37 out of 40 alive	37 out of 40 alive
.498 mg/nl TiO_2	8 out 20 alive	6 out of 20 alive	6 out of 20 alive	6 out of 20 alive

Table 1: BSA coated polystyrene beads death count over a 96 hour period.

The surfactant free polystyrene beads had a less lethality but there was still a difference compared to water controls. Again with the surfactant free polystyrene beads there was not a noticeable difference in the mortality across concentrations as can be seen in Table 2.

Injected	24 hours past inj.	48 hours past inj.	72 hour past inj.	96 hours past inj.
225 pg Surfactant Free Beads	23 out of 40 alive	21 out of 40 alive	19 out of 40 alive	19 out of 40 alive
45 pg Surfactant Free Beads	25 out of 40 alive	23 out of 40 alive	21 out of 40 alive	21 out of 40 alive
9 pg Surfactant Free Beads	28 out of 40 alive	26 out of 40 alive	25 out of 40 alive	25 out of 40 alive
Water	35 out of 40 alive	34 out of 40 alive	34 out of 40 alive	34 out of 40 alive
Noninjected	36 out of 40 alive	36 out of 40 alive	36 out of 40 alive	36 out of 40 alive
.498 mg/ml TiO_2	7 out of 20 alive	6 out of 20 alive	5 out of 20 alive	5 out of 20 alive

Table 2: Surfactant free polystyrene beads death count over a 96 hour period.

With both of the polystyrene beads there was a difference in the severity of adverse phenotypes compared to the water controls that can be a shorten tail or deformities in the head. These results seem to indicate that the polystyrene beads are not as inert as anticipated. While the TiO_2 nanoparticles have the greatest amount of embryo death, the BSA coated polystyrene beads had a nearly similar lethality. One factor that could be leading to such toxicity of the BSA coated polystyrene bead is whether contaminants from the attachment process are present. Future studies could look at how injecting the BSA coated polystyrene beads is different from injecting BSA itself. The results could indicate whether or not there are other factors that are on the BSA coated polystyrene beads that are affecting their toxicity. The results of the surfactant

free polystyrene beads indicate that there is some toxicity with the uncoated polystyrene beads but not to the same degree of the TiO_2 nanoparticles. This preliminary result suggests that the toxicity of microinjected TiO2 nanoparticles does not arise solely from the physical presence of the particle within the cell, but that other properties of the metal oxide, such as redox activity, also contribute to its adverse biological activity.