Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

FINAL REPORT

Final reports will be published on the Cal Poly Digital Commons website (http://digitalcommons.calpoly.edu).

I. **Project Title:** *Influence of Nanosilver Surface Charge on Cytotoxic Efficacy against Cancer Cells*

II. **Project Completion Date:** 7 June, 2017

III. Student(s), Department(s), and Major(s)

(1) Elliot Frey: B.S. Materials Engineering, Biology minor

IV. Faculty Advisor and Department

Amro El Badawy, Civil and Environmental Engineering Department

Team Members:

- Sandra Clement, Cal Poly Biology Department
- Alice Hamrick, Cal Poly Biology Department
- Kevin Dunham, Cal Poly Chemistry Department

V. Cooperating Industry, Agency, Non-Profit, or University Organization(s)

Jennifer Vanderkelen, Cal Poly Center for Applications in Biotechnology (CAB)

VI. Executive Summary

Silver and silver-based products are known to cause cytotoxic effects to both microbes and eukaryotic cells. Because of this property, silver nanoparticles (AgNPs) are being studied for their potential in targeted tumor treatments. Previous studies with microbes suggest that AgNPs with cationic capping agents possess enhanced cytotoxicity by virtue of Coulombic attraction between the nanoparticle and the negatively-charged cell wall. Since animal cells possess similar negatively-charged plasma membranes, this research hypothesized that human cells would be more susceptible to positively-charged AgNPs than to negatively-charged AgNPs. To investigate this hypothesis, cancerous cervical cells (HeLa) and healthy fibroblast cells (3T3) were subjected to treatments of 40 nm diameter AgNP with branched polyethylenimine (AgBPEI, $\zeta = +69$ mV) and citrate (AgCit, $\zeta = -49$ mV) capping agents. $AgNO_3$ was also tested to compare AgNP toxicity to that of ionic silver (Ag^+). An alamarBlue® viability assay was used to quantify the cytotoxicity of the treatments relative to an untreated control group.

AgBPEI displayed a lower LD_{50} (median lethal dose) than both Ag^+ and AgCit to both cell lines. This suggests AgNP toxicity is not solely from Ag^+ dissolution, and also ostensibly supports the initial hypothesis (compared to AgCit, AgBPEI was approximately 84% more cytotoxic to HeLa cells and about 65% more cytotoxic to fibroblast cells). However, significant AgCit aggregation was observed in culture media, which obfuscates surface charge-based toxicity effects because larger diameter AgNPs are less cytotoxic. In this way, size-dependent toxicity must also be considered. While this does not allow for conclusions regarding the

sole influence of surface charge, and therefore the hypothesis cannot be directly supported, results do not negate its validity. Ultimately, since AgBPEI is more stable than AgCit under *in vitro* conditions and is more prone to Coulombically interact with cancer cells, researchers investigating AgNPs for targeted tumor treatments should utilize AgBPEI over AgCit on the premise of enhanced bioavailability.

This research will continue over the summer of 2017 to retest AgBPEI against a negatively-charged AgNP that is stable in test media. To this end, a silver nanoparticle coated with carboxyl (lipoic acid, $\zeta < 0$ mV) has been ordered. This would isolate surface charge as the only independent variable and eliminate aggregation effects as a source of error, thus allowing for verification of the existing hypothesis.

VII. Major Accomplishments

(1) AgBPEI displayed enhanced toxicity over AgCit to both cancerous and healthy mammalian cells.

(2) AgCit agglomerated in test media, revealing AgBPEI is more stable at physiological pH and in *vitro conditions*.

(3) It was found that AgBPEI is a superior nanoparticle candidate over AgCit for targeted tumor research on the premise of enhanced bioavailability.

(4) AgBPEI's LD_{50} was lower than Ag^+ for both cell lines. This suggests AgNP toxicity is not solely from Ag^+ dissolution, and that physical toxicity mechanisms exist between AgNPs and the cell.

VIII. Expenditure of Funds

Prior Expenses

(1) AgCit, 1 mg/mL, 2 mL
(2) AgBPEI, 1 mg/mL, 2 mL
(3) alamarBlue®, 25 mL
(4) Corningstar 96-well plates
(5) Triethylamine, 250 g
(6) Sodium Chlorite (s), 2 kg

Total Expenditures before 6/14/17: $\sum = \$1341.54$

Ordered for continuation of research (6/14/17):

(7) Serological pipets 5 mL individually wrapped 200/case x 1
(8) Serological pipets10 mL individually wrapped 200/case x 1
(9) DMEM Media, 10/500 mL x 1
(10) Trypsin/EDTA Solution, 100 mL
(11) Ag(Lipoic Acid), 1 mg/mL, 1 mL
(12) Ferrite Nanoparticles, 20 mg/mL, 10 mL

Order Estimate (including tax and shipping) : $\sum = \$692.12$

Total Expenditures (approximate): $\sum = \$\mathbf{2033.66}$

IX. Impact on Student Learning

The primary takeaway from this research was first-hand experience in the field of nanotoxicology. The student learned and applied tissue culturing techniques, ran dye-based viability assays, quantified and statistically analyzed cytotoxicity trends, modeled nanoparticle aggregation, and became familiar with

academic grade research protocols. This study reflects the hands-on focus of the Cal Poly undergraduate experience and the interdisciplinary ideals of the Baker and Koob Endowment.