



## Team Advisors

[HOME](#)
[ABOUT](#)
[MEDIA ROOM](#)
[RESOURCES](#)
[HELP](#)
[AEOP](#)

Hello, Laura Tenorio

[Advisor Home](#)
[My Info](#)
[Logout](#)
[BACK TO HOME](#)

## Mission Folder: View Mission for 'MemoryTygers'

State	New Mexico
Grade	9th
Mission Challenge	Food, Health and Fitness
Method	Scientific Inquiry using Scientific Practices
Students	Britney Hsu (misaki) Abdul-Rahman Khweis (Nanoball) Mikayla Martinez (sugarwyrn)

### Team Collaboration

**(1) Describe the plan your team used to complete your Mission Folder. Be sure to explain the role of each team member and how you shared and assigned responsibilities. Describe your team's process to ensure that assignments were completed on time and deadlines were met.**

Each member of the team started research by first interviewing each other and their families to determine the extent and commonalities of people we know in our lives that suffer from neurodegenerative diseases. First, we started with Alzheimer's, but then expanded to other areas that had the potential to be connected. We took this information and broke it down to different areas of research. Misaki took Alzheimer's and the Asian remedy heritage. Nanoball took Arabic remedy heritage and Parkinson's and sugarwyrn took Hispanic remedy heritage and Diabetes. We then took this information and joined it together after a series of collaboration sessions to produce an actual problem to focus on and solve, as well as narrowing down the long list of potential remedies. We worked together to determine a media that would be best to test upon. Misaki discovered and took charge of the C. elegans, while sugarwyrn determined methods to induce amyloidosis, and nanoball determined the best dye to detect the amyloidosis and reduce mortality.

We collaborated with our team advisor on constructing the experimentation and in obtaining the materials. Our team advisor also kept us on task, and made sure that we kept on subject during our collaboration sessions. Our team advisor also made sure that all safety precautions were observed during our experimentation. We consulted with the state Neurosciences Institute for research.

Misaki was in charge of caring for and cultivating the C. elegans, sugarwyrn was in charge of all diabetic aspects including obtaining the insulin, and nanoball took charge of collecting the remedies that were settled on, and keeping the solutions fresh. All members were responsible for experimentation and took turns in each aspect of experimentation. The data analysis was done in a fashion similar to the conceptualization of the community problem; each member took the data separately, assessed the data and then joined together in a collaboration session to determine if the results were the same to be able to collaboratively draw the conclusion.

We are currently still meeting for collaborative meetings during weekends to progress future avenues of research and potential development.

### Scientific Inquiry

#### Problem Statement

**(1) What problem in your community did your team investigate? Why is this problem important to your community?**

Recent studies have linked Alzheimer's, Parkinson's and Diabetes acceleration to a cellular dysfunction called amyloidosis, where alpha and beta amyloid plaques build up. These plaques then accumulate in neural and nutrient pathways, that lead to neural diseases and diabetic symptoms. Diseases like this are prevalent world wide and increasing in frequency yearly across all ages. Our research indicated that there are two population centers that have less occurrence in these neurodegenerative and diabetes diseases; Hispanics and Chinese cultures, but there is an acceleration in incidences occurring in insulin injection cases. The problem we are investigating is to determine the insulin threshold on amyloidosis production and to be able to reverse this amyloid production using natural remedies found in Chinese and Hispanic cultures to produce a medication that has the potential of reducing amyloidosis, and therefore reducing neurodegenerative diseases and associated diabetes. By utilizing natural cultural remedies and deriving a medicine to reduce this syndrome could increase a farming and production industry within the community and help those who suffer from these ailments to reduce the impacts of these ailments.

**(2) List at least 10 resources you used to complete your research (e.g., websites, professional journals, periodicals, subject matter experts). Use multiple types of resources and do not limit yourself to only websites.**

See attached.

**(3) Describe what you learned in your research.**

C. elegans are soil nematodes that eat bacteria and other soil microbes, but share similar genetic sequences as human beings and many animals. Insulin injection sites and synthetic insulin have been indicated by research to produce amyloids, both alpha and beta, and enhance the process of amyloidosis, which are contributors to plaque buildups that lead to diseases such as Alzheimer's and Parkinson's. Amyloidosis is an emerging disease in which cells go into hyperactive production of alpha and beta amyloids, which become plaques that build up in the organs, and block nerve and neural signals. When these plaques accumulate in the brain, it triggers neural cell deterioration and can cause organ failure. Natural remedies have a better chance of being able to penetrate the blood/brain barrier, than synthetically produced medications whose molecules are too large. They also contain antioxidants that replace the missing electron that produces free radicals, that are responsible for plaque production and disease. The only drawback to natural remedies are the concept that they can be oxidized quickly or diluted past the point of effectiveness. Congo Red is a dye that colorimetrically reacts with amyloid proteins and can determine reduction or production of amyloids both alpha and beta. Research indicates that injected insulin causes amyloidosis, particularly near the injection sites, but recently research has also indicated that Diabetics have a higher amount of amyloids throughout their bodies. Congo red is the best dye for indicating amyloid production and reduction, with the least potential side effects, although it is capable of being a high level carcinogen.

Hypothesis**(4) State your hypothesis. Describe how your hypothesis could help investigate your problem.**

It is hypothesized that natural remedies with a high level of antioxidants such as goji berry and capsaicin will have a faster and higher impact on the reduction and control of amyloids than the remedies that have lower levels of antioxidants. It is also hypothesized that the synthetic insulin will increase amyloid production in the *C. elegans*, as indicated by a darker colorimetric change in Congo Red. Finally, it is hypothesized that the mortality rate of the *C. elegans* will go up in the untreated insulin specimens than treated specimens because the acceleration of amyloid production will go unchecked. The metabolism of treated specimens will be accelerated as the remedies will accelerate metabolic function due to free radical elimination and the breakdown of the amyloid plaques. This will be indicated by movement, mortality and colorimetric change. This multi-stage hypothesis will account for variables, and will allow for the experimentation to support the focus of the problem.

**(5) Identify the independent variables and the dependent variables in your hypothesis.**

Independent Variables: Impact of natural remedies on amyloid production and formation as indicated through colorimetric change and mortality in the presence of Congo Red.

Dependent Variables: The impact of insulin in varying levels on the amyloid production of *C. elegans*.

**(6) When you developed your hypothesis how did you know it could be tested AND could be proven false by testing?**

Through the use of extensive research, the first problem to be solved was the concept of how to test the theories. We knew that we wanted to test Chinese and Hispanic remedies, but we also know that we couldn't test human subjects. After extensive research, we learned of *C. elegans* and how they can be turned transgenic to produce Amyloid plaques with exposure to synthetically produced insulin. However, it was not guaranteed that there would be excessive amyloid production within the first generation offspring, therefore this could have proven false. We also had to determine the best and least catastrophic method to test the amyloid production, which resulted in the use of the dye Congo Red. However, Congo Red is not meant for living tissues or subjects, so this also could have proven false, impacting the mortality of the *C. elegans*. There was also the distinct possibility that the remedies would interact with the Congo Red producing a false positive, which ensured that many more variables would have to be isolated during testing to be able to develop the hypotheses.

Experimental Design**(7) List the materials you used in your experiment. Include technologies you used (e.g., scientific equipment, internet resources, computer programs, multimedia, etc.).**

See attached.

**(8) Identify the control group and the constants in your experiment.**

Controls were *C. elegans* not exposed to anything, as well as the subjects exposed only to the dye, each solution without insulin, each solution with insulin and without dye. Constants within the experiment were the amount and concentration of each solution given to the *C. elegans*, the amount of time the subjects were monitored, the environment and feeding schedule of the subjects, the magnification and type of microscope used throughout the experiment, and the type of solutions introduced to them. The dye came from the same lot, and was diluted to the same 50/50 level each time. The sterility of the environment was maintained.

**(9) What was your experimental process? Include each of the steps in your experiment. Include all safety precautions used by your team as step one.**

See attached.

Data Collection and Analysis**(10) Present the data you collected and observed in your testing. The use of data tables, charts, and/or graph is encouraged.**

See Attached.

**(11) Analyze the data you collected and observed in your testing. Does your data support or refute your hypothesis? Do not answer with a yes or no. Explain your answer using one of the following prompts: 'Our data supports/refutes the hypothesis because...'**

Our data supports the hypotheses as stated due to information indicated as follows; it was observed that goji berry was the second most effective overall. It lightened the color scale with an average decrease of 1.39 over three days, decreased the amyloid length with an average of 40.49 nanometers over three days, decreased the average total length by 9.84 nanometers over three days, increased the non-amyloid tissue length by an average of 30.65 nanometers, and had a survival rate of 93.3%. The cinnamon and cota were equally effective overall. It was observed that turmeric was the least effective overall. It lightened the color scale with an average decrease of 1.28 over three days, decreased the amyloid length by an average of 38.65 nanometers over three days, decreased the total length by an average of 30.35 nanometers over three days, and increased the non-amyloid tissue length by an average of 8.31 nanometers over three days. This proved that turmeric had anhydrous effects and was detrimental to the survival rate, 46.7%. Therefore, the most effective overall was capsaicin, and the least effective overall was turmeric.

It was also observed that the synthetic insulin did increase amyloid production in *C. elegans*. The synthetic insulin flooded specimen had a colorimetric increase of 1.4 in Congo Red, while the non-synthetic insulin specimen had a colorimetric decrease of 0.02 in Congo Red.

It was observed that the mortality rate of the *C. elegans* did increase in the untreated insulin specimens than the treated specimens. In all specimen that were treated, the color decreased and became lighter, and did decrease in amyloid length while increasing in non-amyloid tissue length, no matter the remedy. Turmeric, the least effective remedy, still had a lighter color, less amyloid, a greater non-amyloid tissue length, and a smaller mortality rate, than the untreated insulin specimens. Turmeric had a color decrease in shade of 1.28, an amyloid decrease of 38.65, a non-amyloid tissue length increases of 8.3, and a survival rate of 46.7%. The untreated specimens had a color increase of 0.49, an amyloid length increase of 8.9, a total length increase of 16.51, and a survival rate of 0%. יריריריר

Also, it was observed that the *C. elegans* were dead if they appeared as a straight segment, and alive if they appeared curved.

**(12) Explain any sources of error and how these could have affected your results.**

Initially the culture media was not easily heated or pourable, producing chunky agar plates that the *C. elegans* could not freely move around in. These experiments were re-started and had no impact on the results. The goji berry solution grew fungus quickly, and had to be redone, having no impact on the results. There were some temperature variations during experimentation which may have had an impact on the mortality, and may have had a slight impact on the results, for which there were extra trials performed.

Drawing Conclusions**(13) Interpret and evaluate your results and write a conclusion statement that includes the following: Describe what you would do if you wanted to retest or further test your hypothesis. Evaluate the usefulness of the data your team collected. What changes would you make to your hypothesis and/or experimental design in the future, if any?**

Upon conducting the experiment and analyzing the data it can be concluded that insulin increases potential amyloidosis within two generations and continues to produce it exponentially if it goes unchecked. Capsaicin followed by goji berry were the most effective at reducing and preventing amyloid production in insulin modified *C. elegans*.

Cinnamon and cota were moderately effective, while Turmeric was least effective with the highest mortality and least amyloid neutralization. Capsaicin had the most survivors, the most amyloid neutralization and reduction. Capsaicin in itself is an antioxidant of the flavonoid type, and was also the remedy that was a direct extraction of the antioxidant, while the others had other compounds in addition to the primary antioxidant, therefore Capsaicin had the highest antioxidant value. Goji berry are high in beta-carotene antioxidants, and are amongst the highest rated superfoods, therefore supporting the hypothetical statement that the antioxidants would impact the efficiency of free radical neutralization and therefore reduce plaque formation.

There was significant indication that the introduction of insulin to the *C. elegans* cultures increased transgenesis within two generations, increasing amyloid production, as indicated by the amount and darkness of dyed specimens. It also increased mortality rate of the offspring. The Congo Red was a viable indicator of the increase and presence of proteins that were developing in accordance to the characteristics of Congo Red's reaction with Amyloids. This was indicated in early experimentation of the controls.

The mortality rate of *C. elegans* increased in the untreated insulin specimens than treated specimens. These specimens had unchecked and uncontrollable amyloid plaque growth, and the *C. elegans* died within 24 hours. All solutions extended the lifespan of the insulin enhanced specimens, this data does support the hypotheses as stated. Antioxidants of higher concentration had a significant impact on the growth of amyloid plaques. The plaques did not grow as fast on pre-treated solutions (controls) and the controls had less amyloid presence after solutions were applied.

Overall, capsaicin was the ultimate winner in amyloid deterrence. Capsaicin was the freshest solution obtained, and was the only solution that was a proper extract. Future phases would include using fresh antioxidant extractions from goji berry, cinnamon, cota and turmeric to have more direct antioxidant impact against the amyloid plaques. A different, safer and less negatively impacting dye would also be utilized to reduce impact to the organism. Different capsaicin concentrations (heats) are also a viable method of experimentation. The capsaicin extract in this particular experiment came from the *Capsicum chinense*, or habanero pepper, which is a close relative of the goji berry, so future capsaicin extractions amongst the annum group rather than *chinense* would prove vital. Another eventual stage would be to develop these solutions so that experimentation could eventually move up from *C. elegans* to more clinical type trials. These are the preliminary steps to put out a cost-efficient and effective prevention and medication against amyloidosis, which could then therefore reduce the neurological and diabetic disease occurrences and reduce the pain and suffering caused by these ailments.

#### Uploaded Files:

- [ [View](#) ] **Research Works (Bibliography)** (By: sugarwyrn, 02/02/2018, .pdf)  
*Research information and experimental works cited for experimentation.*
- [ [View](#) ] **Materials and Methodology** (By: misaki, 02/28/2018, .pdf)  
*Procedures used to conduct MemoryTygers' experiment.*
- [ [View](#) ] **Results and Observations** (By: Nanoball, 03/04/2018, .pdf)  
*Experimentation results and observations for MemoryTygers*

## Community Benefit

**(1) How could your experiments and data help solve your problem and benefit your community? Describe next steps for further research/experimentation and how you have or how you could implement your solution in the future.**

Recent studies have linked Alzheimer's, Parkinson's and Diabetes acceleration to a cellular dysfunction called amyloidosis, where alpha and beta amyloid plaques build up. These plaques then accumulate in neural and nutrient pathways, that lead to neural diseases and diabetic symptoms. Diseases like this are prevalent world wide and increasing in frequency yearly across all ages. The problem we are investigating is to determine the insulin threshold on amyloidosis production and to be able to reverse this amyloid production using natural remedies found in Chinese and Hispanic cultures to produce a medication that has the potential of reducing amyloidosis, and therefore reducing neurodegenerative diseases and associated diabetes. By utilizing natural cultural remedies and deriving a medicine to reduce this syndrome could increase a farming and production industry within the community and help those who suffer from these ailments to reduce the impacts of these ailments. Our experiments and data proved that the congo red dye was effective to detect the presence of amyloid production, that amyloid production can be increased by one generational exposure to synthetically produced insulin, and that capsaicin is viable as an amyloid reducer while making sure that the subjects stay alive. Our next concentration will be to focus on getting capsaicin through the blood/brain barrier, particularly utilizing membranes, and using varying heat levels of capsaicin to determine if the results will be similar to different. By being able to hone the capsaicin content against amyloid production, a potentially effective drug could be developed, that would reduce the amyloid production and giving people more time with their memories and loved ones.

## Mission Verification

**(1) Does your Mission Folder project involve vertebrate testing, defined as animals with backbones and spinal columns (which include humans)? If yes, team must complete and attach an IRB approval form.**

No

**(2) Did your team use a survey for any part of your project? If yes, team must complete and attach a survey approval form.**

No

**(3) You will need to include an abstract of 250 words or less. As part of the abstract you will need to describe your project and explain how you used STEM (Science, Technology, Engineering and Mathematics) to improve your community**

Alzheimer's Disease is a lethal illness caused by amyloid beta plaques which clog neural pathways, blocking cell communication. This and other illnesses can be caused by diabetes and amyloidosis. Synthetically produced medications have reduced efficiency due to the blood brain barrier. This experiment will determine impact of natural plant-derived remedies on reduction of amyloid (Alpha/Beta) formation and accumulation using *C. elegans* as a demonstration media. And to determine how much insulin will impact the progression of amyloidosis.

Natural remedies with high antioxidant levels (goji berry and capsaicin) will reduce more amyloids than lower level antioxidant remedies. Synthetic insulin will increase amyloid production in *C. Elegans*, indicated by darker Congo Red colorimetric change within four generations. *C. Elegans'* mortality rate will increase in untreated insulin specimens because the acceleration of amyloid production will go unchecked. Metabolism of treated specimens will be accelerated as the remedies will accelerate metabolic function via free radical elimination. Results will be indicated by Color/shade, total length, mortality, and clean tissue growth

*C. Elegans* were treated with 1 microdrop (md) insulin in their food supply. 1md of each 1% solution was applied. Synthetic insulin increased mortality rate, and production of amyloid plaques. Capsaicin had the highest amyloid reduction and least plaque formation, followed by goji berry. Turmeric was the least effective with minimal survival and increased plaques. Insulin increased amyloidosis within two generations and therefore extreme amyloidosis within four generation, supporting the hypotheses as stated.



Akhondzadeh, S., & Abbasi, S. H. (2006). *Herbal medicine in the treatment of Alzheimer's disease*. American Journal of Alzheimer's Disease & Other Dementias®, 21(2), 113-118.

Alters, Sandra & Alters, Brian (2008) Introduction to Biology “Chapter 12: Cell Reproduction” Wiley and Sons, NJ pgs. 179-193

Amyloidosis Foundation. 2017. “Facts about Amyloidosis, Symptoms and Treatments” <http://www.amyloidosis.org/facts/>

Centers for Disease Control and Prevention (CDC) (2012) “E. coli –Escherichia coli” <http://www.cdc.gov/ecoli/>

“Diabetes” (2017) <https://www.cdc.gov/diabetes/home/index.html>

Corsi A.K., Wightman B., and Chalfie M. A Transparent window into biology: A primer on *Caenorhabditis elegans* (June 18, 2015), WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.177.1, <http://www.wormbook.org>.

De la Monte, S. M., & Wands, J. R. (2008). Alzheimer’s Disease Is Type 3 Diabetes—Evidence Reviewed. Journal of Diabetes Science and Technology (Online), 2(6), 1101–1113. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2769828/>

Dostal, V., & Link, C. D. (2010). Assaying  $\beta$ -amyloid Toxicity using a Transgenic C. elegans Model. Journal of Visualized Experiments : JoVE, (44), 2252. Advance online publication. <http://doi.org/10.3791/2252>

Drugs.com. 2016. “Novolog” <https://www.drugs.com/novolog.html>

Drugs. Com. 2016. “Lantus” <https://www.drugs.com/lantus.html>

Environmental Protection Agency. “Final Assessment of Escherichia coli Strain K-12” 2012. [http://www.epa.gov/biotech\\_rule/pubs/fra/fra004.htm](http://www.epa.gov/biotech_rule/pubs/fra/fra004.htm)

Ernst, E. (2002). *The risk–benefit profile of commonly used herbal therapies: Ginkgo, St. John's Wort, Ginseng, Echinacea, Saw Palmetto, and Kava*. Annals of internal medicine, 136(1), 42-53.

Food and Drug Administration (FDA). 2016. FDA. NovoLog Insulin Aspart (rDNA origin) Injection.. [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2000/20986lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2000/20986lbl.pdf)

Geun Kim, H., & Sook Oh, M. (2012). *Herbal medicines for the prevention and treatment of Alzheimer's disease*. Current pharmaceutical design, 18(1), 57-75.

Howes, M. J. R., Perry, N. S., & Houghton, P. J. (2003). *Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders*. *Phytotherapy Research*, 17(1), 1-18.

Kroner, Z. (2009). The relationship between Alzheimer's disease and diabetes: Type 3 diabetes?. *Alternative Medicine Review*, 14(4), 373.

Mayo Clinic. 2017. "Amyloidosis" <https://www.mayoclinic.org/diseases-conditions/amyloidosis/symptoms-causes/syc-20353178>

Moerman, Daniel E. Native American Medicinal Plants: An Ethnobotanical Dictionary. Portland,OR: Timber Press, 2009

Novikoff, Alex B. "Mitochondria (chondriosomes)." *The cell* 2 (2014): 299-421.

Perry, E. K., Pickering, A. T., Wang, W. W., Houghton, P. J., & Perry, N. S. (1999). *Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy*. *Journal of Pharmacy and Pharmacology*, 51(5), 527-534.

Riddle DL, Blumenthal T, Meyer BJ, et al., editors. *C. elegans* II. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. Section I, The Biological Model. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK20086/>

Santos-Neto, L. L. D., de Vilhena Toledo, M. A., Medeiros-Souza, P., & de Souza, G. A. (2006). *The use of herbal medicine in Alzheimer's disease—a systematic review*. *Evidence-Based Complementary and Alternative Medicine*, 3(4), 441-445.

Schwiebert, Erik M. "ABC transporter-facilitated ATP conductive transport." *American Journal of Physiology-Cell Physiology* 276.1 (1999): C1-C8.

Steen, E., Terry, B. M., J Rivera, E., Cannon, J. L., Neely, T. R., Tavares, R., ... & de la Monte, S. M. (2005). Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes?. *Journal of Alzheimer's Disease*, 7(1), 63-80.

Tian, J., Shi, J., Zhang, X., & Wang, Y. (2010). Herbal therapy: a new pathway for the treatment of Alzheimer's disease. *Alzheimer's research & therapy*, 2(5), 30.

Tso, Zoncho. New Mexico State University (NMSU). "Thelesperma megapotanicum" Medicinal Plants of the Southwest. 1999, 2008. <http://medplant.nmsu.edu/thelesperma.shtm>

Wormatlas. 2006. "Anatomy of C. elegans" <http://www.wormatlas.org/ver1/handbook/anatomyintro/anatomyintro.htm>

**Materials:**

Equipment	Chemicals	Biological
Hypodermic Needle	Novolog	C. elegans
Digital Microscope	Congo Red	Nutrient Agar
Scalpel	Distilled water	Nutrient Broth
Inoculation Loop	Isopropyl Alcohol	Nematode Growth Agar
Electronic scale	Sodium Chloride	E. coli Strain K-12
Weighing paper		
Micro-test tube		
50 mL Beakers		
150 mL Beakers		
Parafilm		
Hot Plate/Stirrer		
Thermometer		
Aluminum Pan		
Pipettes		
Plastic Cups		
Autoclave		
250 mL Erlenmeyer Flasks		
1000 mL Beakers		
Ceramic Wire Gauze Pad		
Nutrient Agar Plates		
Bunsen Burner		
Inoculation Loop		
Incubator		
Petri Dishes		
Strainer		

**Procedures**

## 1. Experimentation – *C. elegans*

- a. Place insulin adapted *C. elegans* specimens onto test plate.
- b. Under inverted microscope, place 1 md Amyloid presence dye and 1 md of each solution on top of 10 separate areas on the plate, observe reaction.
- c. Incubate 24 hours at 24 deg. C, feeding cells as needed.
- d. Remove plates, observe behavior, morphology and mortality through counts and microscope observation.
- e. Additional experimentation: add 1 md, 2 md... to each *C. elegans* with 1 md Congo Red to determine threshold of amyloid hyperproduction in *C. elegans*. Once this threshold is determined, add to feeder solution for final subject testing.

## Nutrient Agar Plates

- a. Sterilize work area with isopropyl alcohol.
- b. Weigh out 11.5 g of Nutrient Agar powder using electronic scale.
- c. Measure 500 mL of distilled water.
- d. Pour distilled water into 1000 mL beaker.
- e. Place magnetic stirrer in beaker.
- f. Place beaker with distilled water and magnetic stirrer on hot plate.
- g. Turn heat up to setting of 10, turn stirrer up to setting of 5.
- h. Add Nutrient Agar powder to distilled water.
- i. Heat until liquid gently boils and turns slightly clear. (approx. 10 minutes)
- j. Remove from heat using heat resistant glove.

- k. Equally distribute Nutrient Agar solution into three screw cap Erlenmeyer flasks and place in autoclave.
- l. Place 250 mL of tap water in autoclave.
- m. Place 3 screw cap Erlenmeyer flasks in autoclave and seal.
- n. Place pressure cooker onto hotplate, heat for 15 minutes after autoclave has attained 15 psi pressure and 121°C. Remove autoclave from heat and allow to cool.
- o. With heat resistant glove, pull Nutrient Agar solution bottles from autoclave, and pour nutrient agar into Petri dishes. Allow Nutrient Agar to gel, invert and refrigerate until time of use.
- p. Re-sterilize work area with isopropyl alcohol.

#### Solution Preparation

- a. Add 1g of each natural substance to 100 mL distilled water, heat on hot plate to 50 deg. C for 5 minutes, strain, agitate and store in refrigerator until use.

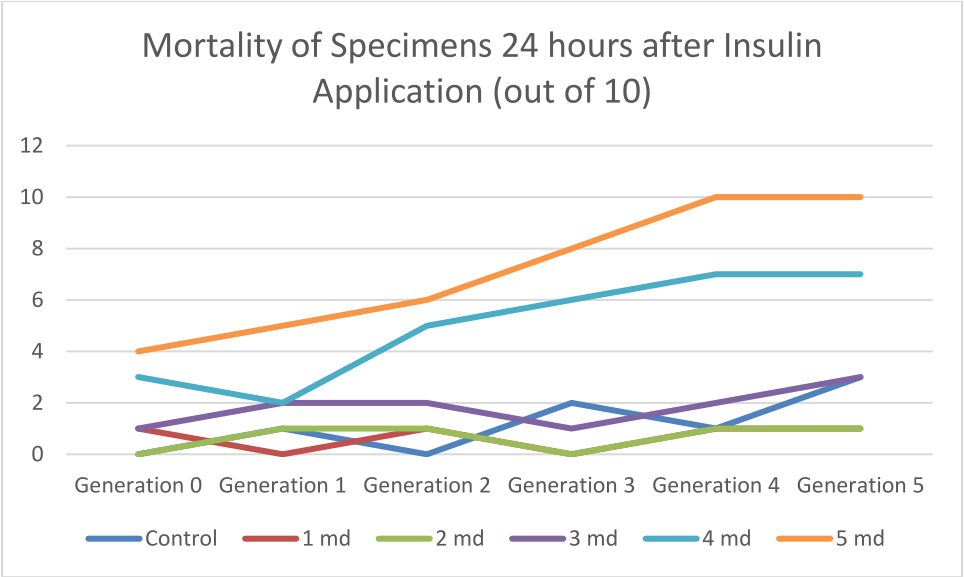
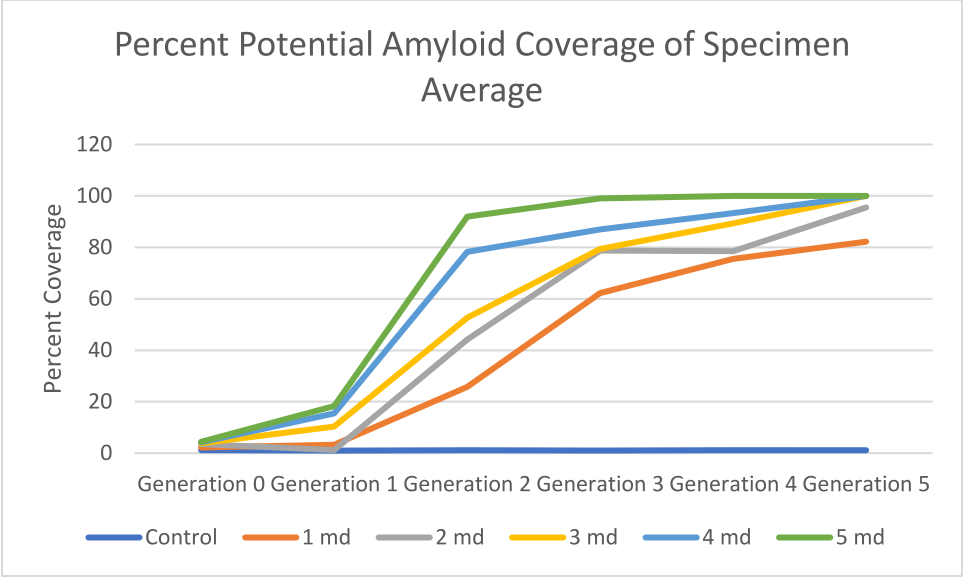
#### *C. elegans* Preparation

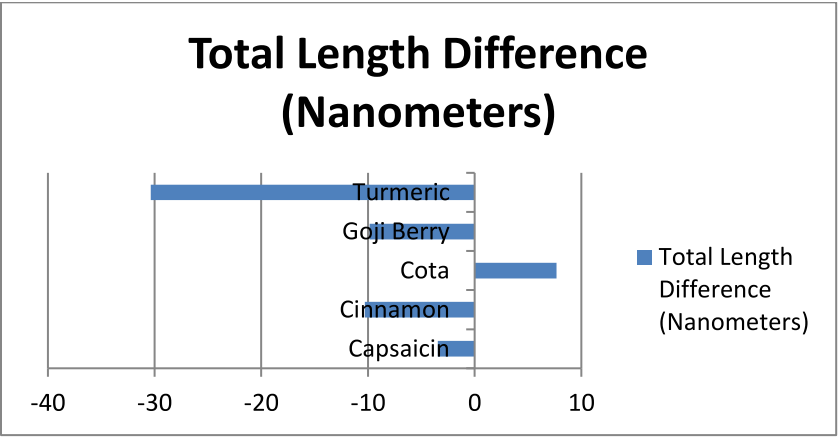
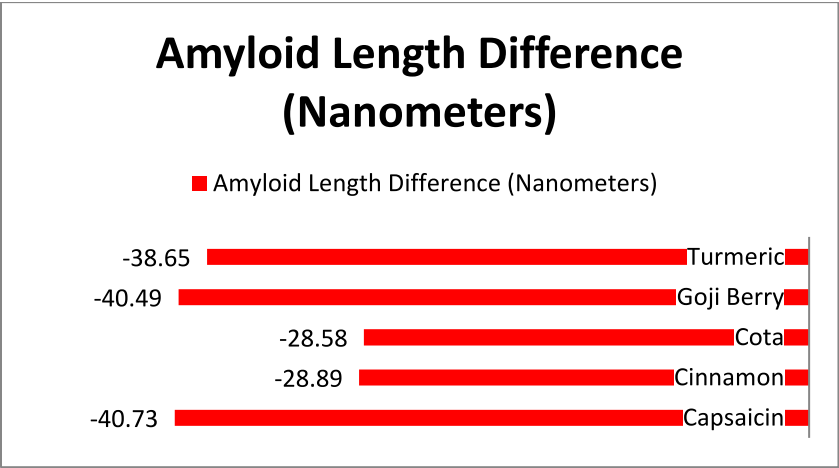
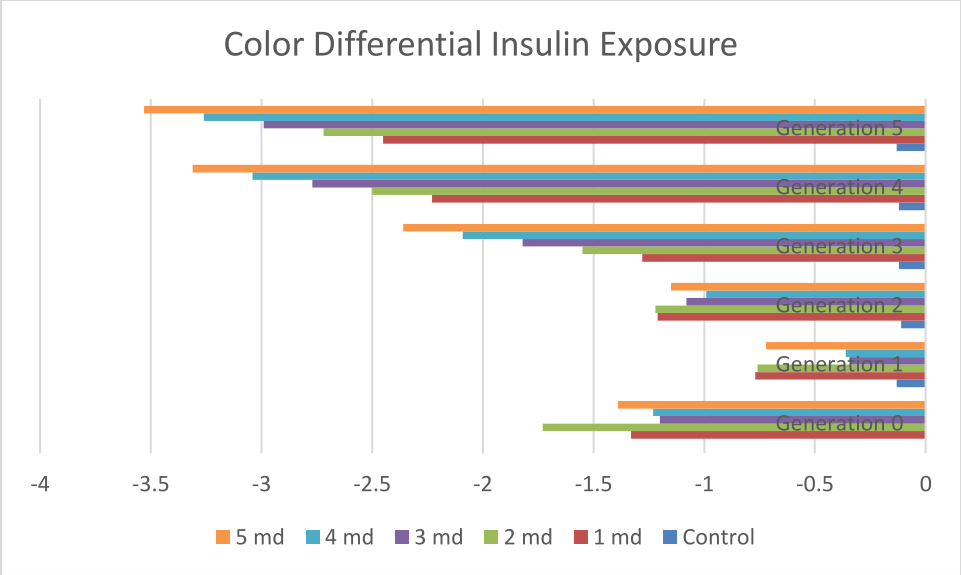
- a. Place pre-prepared Nutrient Media/Agar into water bath at 40°C until melted.
- b. Pour Nutrient Media into petri dishes, allow to set.
- c. Using scalpel, cut 1 cm<sup>2</sup> of culture media and transfer to Nutrient Media plates.
- d. Feed plates with 2 drops *E. coli Strain K-12* and 1 microdrop of Insulin per media transfer.
- e. Place in dark incubator for 24 hours at 24°C.
- f. Remove from incubator and determine growth patterns over 3 days before use in experiment.

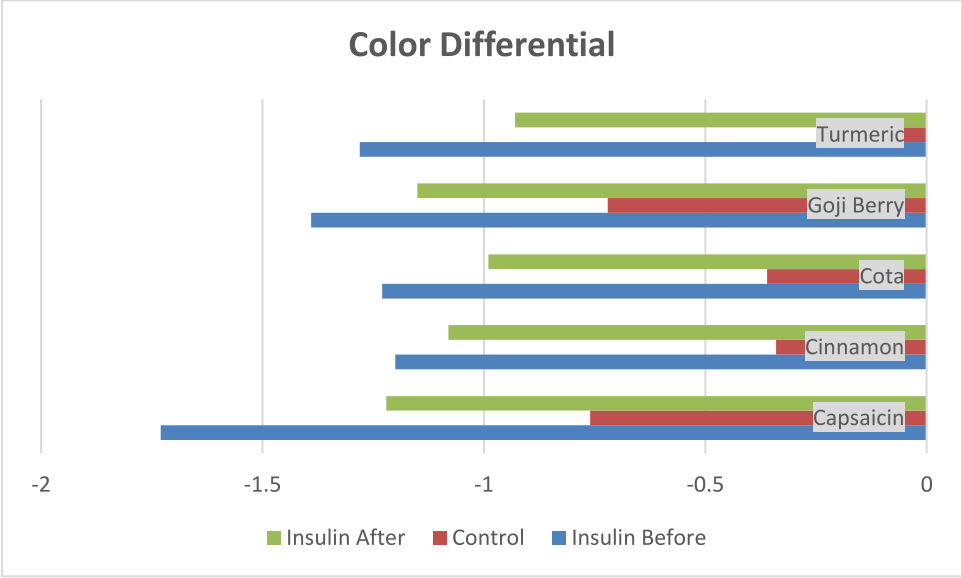
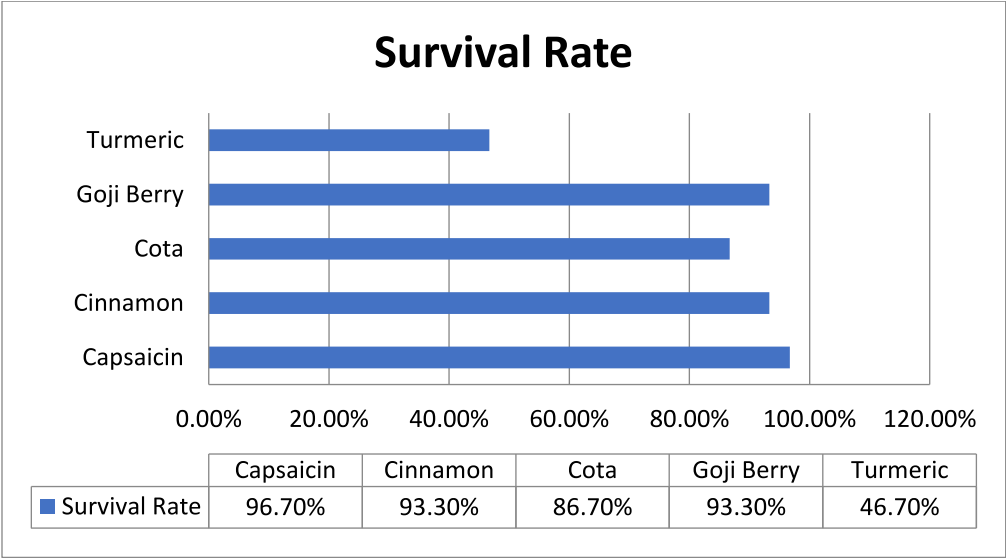
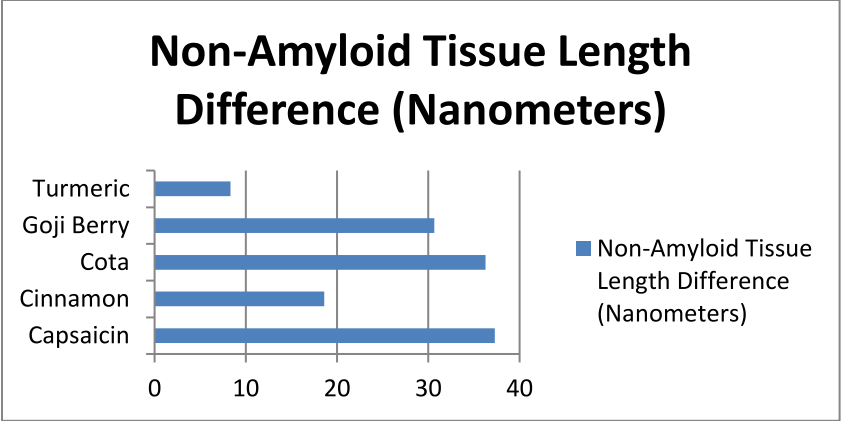
**Procedure Rationale:** The focus of this experimentation is to determine the impact of synthetically produced insulin and herbal remedies on the mortality, morphology and amyloid production of *C. elegans* as a model for amyloidosis in humans. The Nutrient Agar and support procedures are for the feeding process for the *C. elegans*, who process *E. coli* Strain K-12. The *C. elegans* will be cultured on Nematode Growth Agar supplied by Carolina Biological Supply Company, then be transferred to a test plate to be have one microdrop of insulin placed directly on them, and will then be isolated under a microscope and flooded with one microdrop of remedies to determine impact of the remedies. Congo red diluted by 80% will be introduced to their environment to track potential amyloidosis, and to determine the reversal of amyloid production. The data will be assessed on behavioral changes of the *C. elegans*, colorimetric changes, mortality and morphology.

#### ***Risk Assessment***

Risk assessment for the procedures within this project is minimal. All safety precautions for chemical and biological safety will be observed, ranging from personal protective gear including goggles, aprons/jackets, nitrile gloves, and face shields/masks to laboratory protective gear ranging from chemical (alcohol) and UV sterilization. The methods are all in practice with BSL-1 laboratory practices and proper thermal disposal in an autoclave will be observed for discarding all final products. Proper handling and disposal procedures for sharps will also be observed, as will all sterility practices.







It was observed that the insulin increased amyloid production within the specimens immediately within the first generation and then increased exponentially within two generations and up through four generations, covering up the most of the specimens with amyloid plaques. This increased the mortality of the specimens and also reduced the metabolic activities of the *C. elegans*. This metabolic reduction eventually leads to mortality in nearly all samples.

It was observed that out of the materials tested, capsaicin, cinnamon, cota, goji berry, and turmeric, that capsaicin was the most effective overall, especially at lightening the color scale with an average decrease of 1.73 over the three days of testing, decreasing the amyloid length with an average of 40.73 nanometers over three days, decreasing the total length by an average of 3.44 nanometers over three days, increasing the non-amyloid tissue length by an average of 37.29 nanometers over three days, and having an average survival rate of 96.7%.

It was observed that goji berry was the second most effective overall. It lightened the color scale with an average decrease of 1.39 over three days, decreased the amyloid length with an average of 40.49 nanometers over three days, decreased the average total length by 9.84 nanometers over three days, increased the non-amyloid tissue length by an average of 30.65 nanometers, and had a survival rate of 93.3%. The cinnamon and cota were equally effective overall. It was observed that turmeric was the least effective overall. It lightened the color scale with an average decrease of 1.28 over three days, decreased the amyloid length by an average of 38.65 nanometers over three days, decreased the total length by an average of 30.35 nanometers over three days, and increased the non-amyloid tissue length by an average of 8.31 nanometers over three days. This proved that turmeric had anhydrous effects and was detrimental to the survival rate, 46.7%. Therefore, the most effective overall was capsaicin, and the least effective overall was turmeric.

It was also observed that the synthetic insulin did increase amyloid production in *C. elegans*. The synthetic insulin flooded specimen had a colorimetric increase of 1.4 in Congo Red, while the non-synthetic insulin specimen had a colorimetric decrease of 0.02 in Congo Red.

It was observed that the mortality rate of the *C. elegans* did increase in the untreated insulin specimens than the treated specimens. In all specimen that were treated, the color decreased and became lighter, and did decrease in amyloid length while increasing in non-

amyloid tissue length, no matter the remedy. Turmeric, the least effective remedy, still had a lighter color, less amyloid, a greater non-amyloid tissue length, and a smaller mortality rate, than the untreated insulin specimens. Turmeric had a color decrease in shade of 1.28, an amyloid decrease of 38.65, a non-amyloid tissue length increases of 8.3, and a survival rate of 46.7%. The untreated specimens had a color increase of 0.49, an amyloid length increase of 8.9, a total length increase of 16.51, and a survival rate of 0%.

Also, it was observed that the *C. elegans* were dead if they appeared as a straight segment, and alive if they appeared curved.

### Capsaicin



## Cinnamon:



## Cota



## Goji Berry



## Turmeric

